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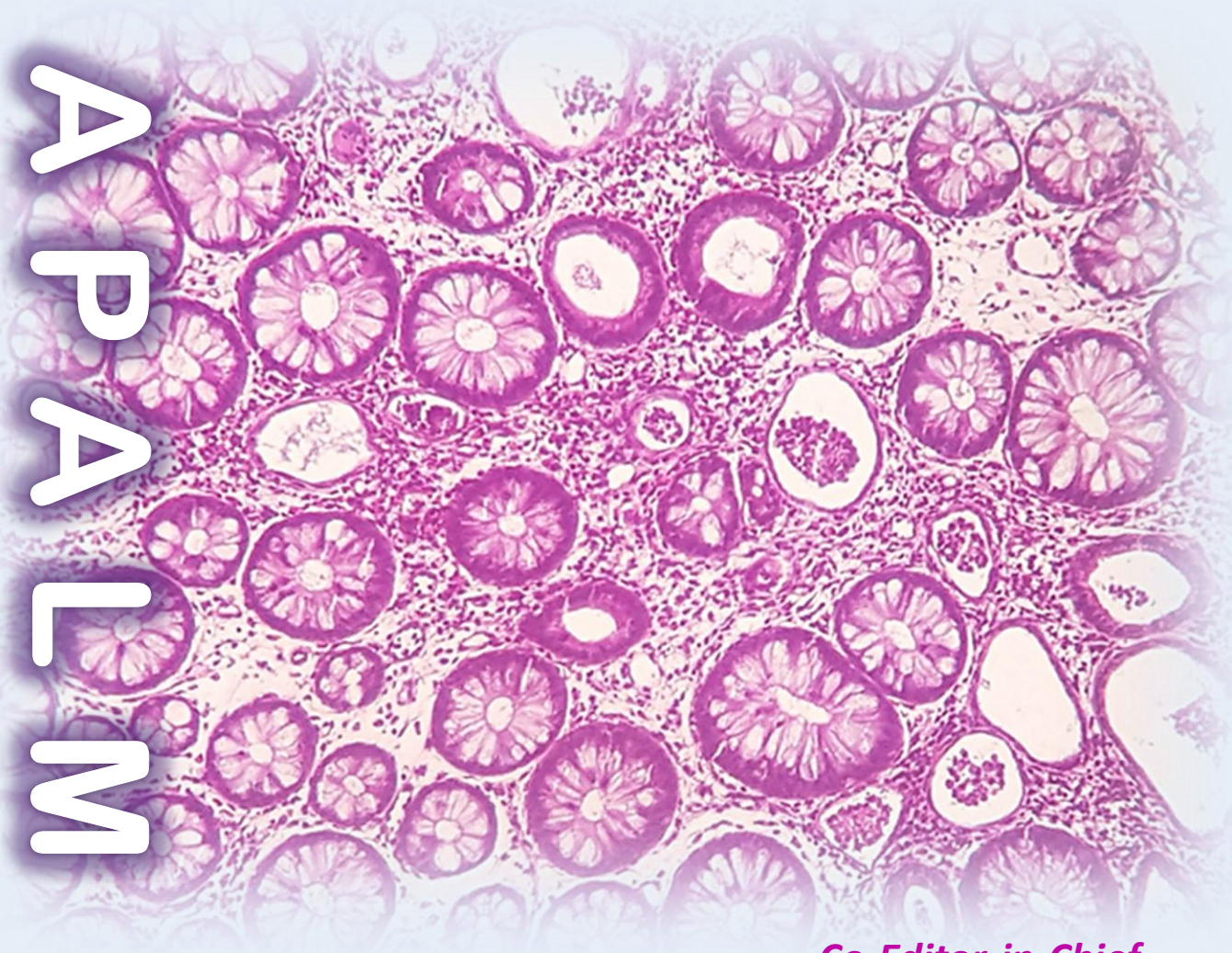

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Original Article

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Multiple Myeloma: Clinico-hematological Profile in A Tertiary Care Hospital: A Three Years Study

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ABSTRACT

Background: To analyze the clinical findings and haematological profile of multiple myeloma patients.

Material and methods: All newly diagnosed patients of multiple myeloma between January' 2013 to December' 2015 in the department of pathology, Indira Gandhi Medical College, Shimla were the study subjects. The history and clinical findings were recorded. All relevant blood, radiological investigations including peripheral smears, bone marrow aspiration and biopsy were done.

Results: Fifty one patients were diagnosed with multiple myeloma and they comprised 19% of all haematological malignancies. Out of these, 30 were males and 21 females with a mean age of presentation 58.38 years. The commonest presenting complaint was bone pain followed by fever, CKD and bleeding disorder. Common clinical findings were anemia, osteolytic lesions and renal insufficiency. M band was seen in 83% of patients on serum protein electrophoresis. On bone marrow examination, majority (53%) of patients had plasmablastic morphology while, 47% had plasmacytic features. Diffuse pattern of infiltration on bone marrow biopsy was observed most commonly in 68.3%patients.

Conclusion: The present study concluded that multiple myeloma is a disease of middle age and elderly with male preponderance. The clinical presentation varies with bone pain being the most common presenting complaint. Morphologically,plasmablastic morphology predominated on bone marrow aspiration while diffuse pattern of infiltration was observed on bone marrow biopsy.

Keywords: *Multiple Myeloma, Bone Marrow.*

Introduction

"Multiple myeloma" a term coined by Von Rustizky in 1873, is a bone marrow based clonal expansion of B lineage plasma cells associated with production of monoclonal immunoglobulins (Ig) or Ig fragments in serum and/or urine. [1] It is included in the category of mature B-cell neoplasms in WHO classification of hematopoietic and lymphoid neoplasms.[2, 3]

It accounts for 1% of all neoplastic disorders and 10 – 15% of hematological malignancies. Multiple myeloma is seen in all races, with a higher incidence in African Americans in comparison to Asian. Multiple myeloma (MM) is a disease predominantly of middle aged and elderly.[1]The median age of presentation in India is a decade lesser around 55 -62 years [4-7]in comparison to Western countries. [1, 8]Incidence increases with age and the disease has slight male predilection. [1]

Though the etiology of the disease is largely unknown various factors like environmental, occupational, radiation exposure, metal industries, benzene exposure and pre-existing medical conditions have been found to increase the incidence of MM.[1]The biological behaviour of myeloma cells depends upon complex and dynamic interplay between myeloma cells and BM microenvironment. This interaction is mediated by a variety of adhesion molecules, cytokines and receptors. The IL-6 is the most important proliferation and survival factor in myeloma.[9]

The clinical manifestations arise as a result of bone marrow infiltration by clonal plasma cells and secretion of M proteins. These are characterized by a clinical pentad: anemia, M protein in the serum/ urine or both, abnormal bone radiographs and bone pain, hypercalcemia, and renal insufficiency/ failure.[1]Bone pain is the commonest presenting symptom with most of the patients having punched out lytic lesions, osteoporosis, osteopenia or fractures on radiological examination.[1, 8]

Bleeding manifestations are a result of adsorption of clotting factors by M protein, decrease in clotting factors II, V, VII, VIII, X, and fibrinogen, hyperviscosity and thrombocytopenia. Thrombocytopenia is uncommon in early phases of myeloma, possibly because of the thrombopoietic activity of IL-6. However, thrombocytopenia may develop subsequent to therapy or from autoimmune mechanisms.[8]There is increased tendency to acquire infections due to immune deficiency resulting from diffuse hypogammaglobulinemia.[8] Renal involvement is most common in patients having Bence Jones (free light chain only) myeloma and IgD type of myeloma.[10]

Majority of patients show normocytic normochromic anemia and rouleaux formation. Sometimes, leukoerythroblastic blood picture or plasma cell leukemia is also seen.[11] Bone marrow examination along with clinical, laboratory and radiological parametersis essential for establishing the diagnosis of Multiple myeloma. Plasma cell morphology, percentage and pattern of infiltration of plasma cells in marrow have significant correlation with the clinical stage and survival. Higher percentage of Plasma cell fraction is a reliable predictor of relapse in treated MM patients and it also helps to evaluate morphological remission and minimal residual disease in MM patients. [12,13]

The present study was undertakenwith the aim to analyze the clinico-hematological profile of multiple myeloma patients in IGMC Shimla, a tertiary care hospital of Himachal Pradesh.

Material and Methods

The study included all newly diagnosed patients of multiple myeloma from Jan 2013 to Dec 2015. The patients on treatment and follow up were excluded. Patients were diagnosed as per the WHO criteria. Relevant history, clinical and radiological findings of the patients were recorded. Complete hemogram, peripheral smears, biochemical investigations, serum protein electrophoresis and urine protein electrophoresis were done. A written, informed consent was taken from the patients. Bone marrow aspiration and biopsy were performed in all the cases suspected of having multiple myeloma.

Results

Out of total 952 bone marrow examinations performed from Jan 2013 to Dec 2015, 264 cases had hematological malignancies. Out of these51 were multiple myeloma cases which accounted for 19% of hematological malignancies. The age of patients ranged from 23 to 90 years with a mean of 58.38 years. The male: female ratio was 1.4:1.

The commonest presenting complaintwas bone pain comprising 62.74 % of patients followed by generalized weakness (23.52%), fever (15.68%), renal involvement (15.68%) and bleeding disorders in (8%) patients. Majority (94%) of the patients had anemia, followed by bony tenderness (47%), splenomegaly (9.8%), hepatomegaly (7.8%) and lymphadenopathy (6%). Bone lesions included lytic lesions (53%), osteoporosis (19.6%), pathological fractures (15.6%)and osteosclerotic lesions (4%).

The results of lab investigations revealed that, majority of the patients had moderate (8-10g/dl) to severe anemia (< 8g/dl) in 45% each and mild anemia in 4% patients. Six percent patients did not have anemia. On peripheral smear findings normocytic, normochromic anemia was observed in majority i.e. 70.59% patients followed by macrocytic in 23.53% and microcytic in 5.85%. Short rouleaux formation was seen in 70% patients. Twelve percent patients presented with leukoerythroblastic blood picture. ESR was raised in all the patients however, ESR (>100 mm in 1st hour) was observed in 51% patients. Majority i.e. 72% patients had total leukocyte count WNL. However leucopenia was seen in 22% and leucocytosis in 6% patients. Thrombocytopenia was seen in 23.53% of patients, rest of them had normal platelet count. Serum Calcium levels >11mg/dl (hypercalcemia) were observed in 11.76% patients. On Serum protein electrophoresis (SPEP), M band was detected in 83% patients whereas 17% patients did not have M band.

Plasma cell morphology on bone marrow aspiration was predominantly plasmablastic (poorly differentiated type of myeloma cells) in 53% followed by plasmacytic (well differentiated and intermediately differentiated) in 47% cases (Figure 1, 2).

Majority of the patients i.e. 82.35% had stage III (>50% of plasma cells on bone marrow biopsy) disease at the time of presentation (Table 1). Patterns of infiltration on BM biopsy in decreasing order of frequency were diffuse in 68.62% patients followed by mixed 21.57%, interstitial 7.85% and paratrabecular in 1.96% patients (Table 2, Figure 3-5).

Discussion

Multiple myeloma comprises 10-15% ofall hematological malignancies.[1] In the present study we observed multiple myeloma patients accounting for 19% of all the

Table 1: Percentage of plasma cells on BM biopsy & staging

% of plasma cells	Stage	Number of patients (n=51)	(%)
<20	I	2	3.92
20-50	II	7	13.73
>50	III	42	82.35

Table 2: Pattern of infiltration of bone marrow

Pattern of infiltration	Number of patients(n=51)	(%)
Interstitial	04	7.85
Nodular	-	-
Mixed	11	21.57
Diffuse	35	68.62
Paratrabecular	01	1.96

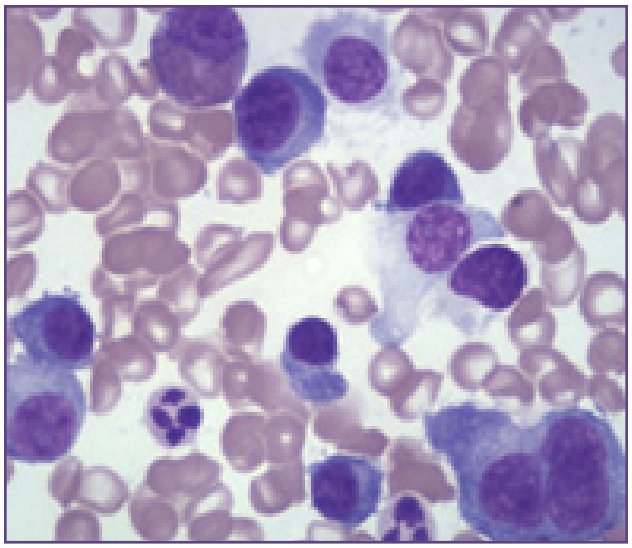


Fig. 1: Bone Marrow aspiration(Giemsa stain, 100X): Mature and immatureneoplastic plasma (myeloma) cells.

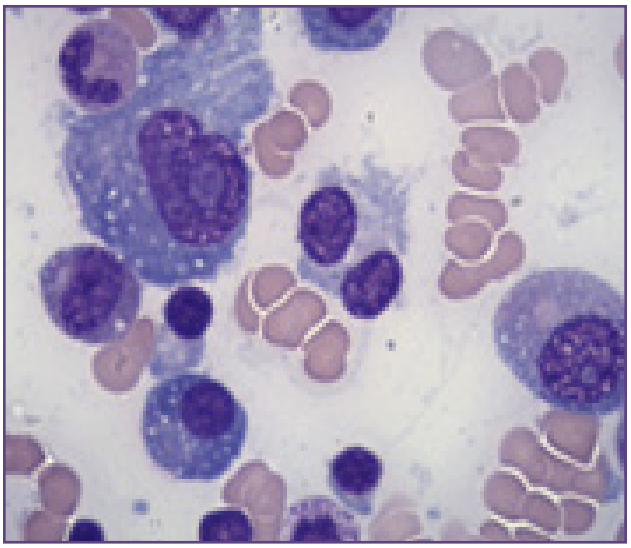


Fig. 2: Bone Marrow aspiration (Giemsa stain, 100X): Plasmablastic plasma cell morphology.

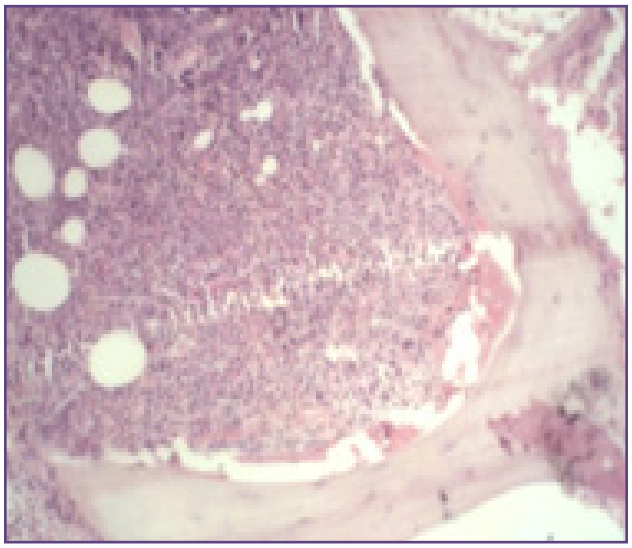
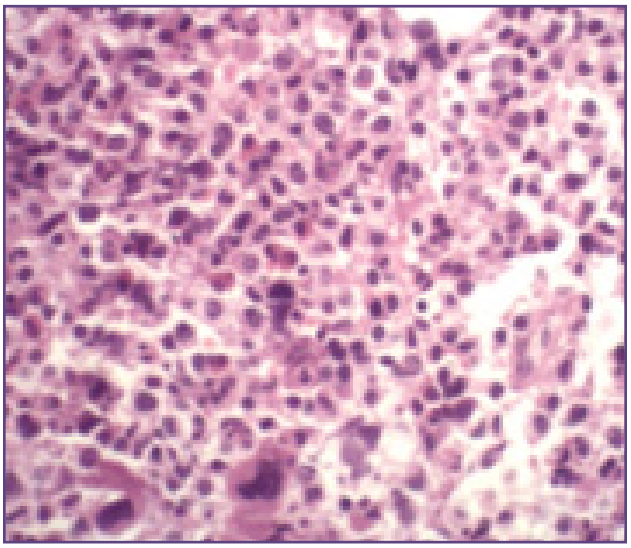


Fig. 3: BM trephine biopsy sections (Paraffin - embedded,H &E stain, 10X) showing Diffuse infiltration (packedmarrow).



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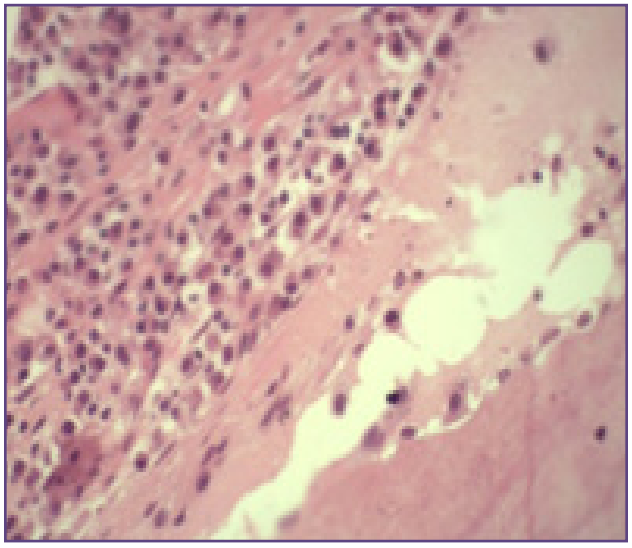

Fig. 5: BM trephine biopsy sections (Paraffin - embedded,H &E stain, 40X) showing Paratrabecular pattern of infiltration.

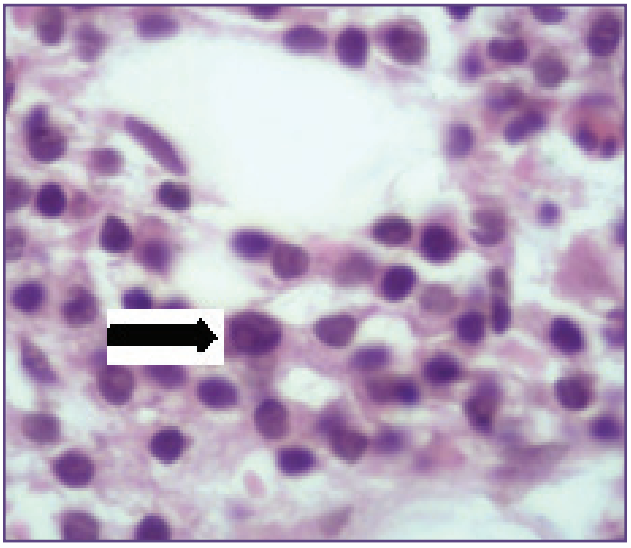

Fig. 6: BM trephine biopsy sections (Paraffin - embedded,H &E stain, 100X) Dutcher body shown by (➜)

hematological malignancieswhich is higher in comparison to reports by Diwan G et al (13%),[14] Kaur et al (11%)[5] and Rajkumar S.V. (10%).[15] The higher percentage in our study may be due to our institute being the only Regional cancer treatment center and tertiary care referral hospital in our state.

The disease predominantly affects middle aged and elderly and is unusual in young patients with approximately 2% beingless than 40 years and still rarer in patients younger than 30years.[16]We also observed only one patient less than 40 years. The mean age of presentation was 58.38 years which is in accordance with other studies[4-7, 15] whereas it is lower than that observed by Diwan G et al (62 years)[14] and Rajkumar S.V. (65 years).[15]

Present study revealed male preponderance (M:F- 1.4:1) as seen in other studies ranging from 1.5: 1 to 2: 1[4,5,7] whereas both sexes were equally involved in a study by Diwan et al.[14]

These patients have variable clinical presentation. We observed bone painas chief presenting complaint in 63%patients. These are higher than observed by Kyle et al and Kaur et al. 58% and 50% respectively[5,18]however lower than observed by Diwan et al (85%) and Subramanian R et al. (82%).[4,14] Bone pain was followed by generalized weakness (23.52%) which was lower than Diwan et al (55%) and Kyle et al (32%).[4,18] Sixteen percent patients came with fever which was higher than Kyle et al (0.7%) whereas lower than that observed by Diwan et al (35%). Renal involvement in our study (16%) was quite lower than Subramanian R et al. (36%) and Diwan et al. (30%). [4,14]Bleeding manifestations in present study (8%) were comparable to Diwan et al (10%).[14]

We found splenomegaly in 9.8%, hepatomegaly in 7.8% and lymphadenopathy in 6% of myeloma patients. It was quite higher in comparison to study by Kyle et al who observed splenomegaly (1%), hepatomegaly (4%) and lymphadenopathy in (1%) of patients however Diwan et al did not encounter organomegaly.[14,18]

Lytic lesions which are the hallmark of myeloma patients, were seen in 53% patients under study and these are well in accordance with other studies in literature.[6,18] Osteoporosis/osteopenia (20%) the next frequentobservation in thisstudy was comparable to study by Kyle et al (20%) while Kalita et al had reported a very high incidence (52%) of osteoporosis.[6,18]We found myeloma patients presenting with pathological fracturesin 16% cases well in tune with literature report of 6 - 20%.[6,18] However none of the studies have reported osteosclerotic lesions which we found in 4% of our patients and have been mentioned in literature also.[1,8]

Multiple myeloma patients have increased ESR due to anemia and increased paraproteins. ESR >100 mm in 1st hour is reported in different series from 33- 100%.[4,5,14,18] Our observation (51% patients) is similar to these studies.

Anemia in myelomais caused due to replacement of marrow by myeloma cells and decreased production of erythropoietin due to accompanying renal involvement. In some cases it may be associated with cytokine mediated

BM suppression.[1]Severe anemia (Hb level < 8g/ dl)[19]was seen in 45% patients in the present study. Though lower number of patients having severe anemia were observed as compared to Kaur et al (75%) and Subramanian R et al (71%) but higher than Kalita L K et al (33%) and Diwan et al (25%).[4-6,14]

In most patients there is increased rouleaux formation and increased background basophilic staining due to the presence of paraproteinin the blood.[8] Rouleaux formation was seen in 70% of our patients which is lower in comparison to observations by Subramanian R et al (91%)and Kaur et al (82%), though higher than Diwan et al (35%).[4,5,14]

Thrombocytopenia is uncommon in early phases of myeloma, possibly because of thrombopoietic activity of IL-6. However, thrombocytopenia may develop subsequently due to therapy or from autoimmune mechanisms.[8] Thrombocytopenia in our patients (24%) was comparable to observations made by Kaur et al (25%) [5], however higher than Diwan et al and Kyle et al 10% and 5% respectively.[14,18]

M band is detectable in 97% of myeloma patients they may have either intact immunoglobulin or fragment or a free light chain on serum / urine protein electrophoresis whereas cases with non-detectable monoclonal proteins are referred to as non-secretors (1-3%) [1]. M band was detectable in 83% of our patients which is slightly higher than that observed by Kaur et al (75.4%)while lower than Diwan et al who found it in all the patients and Kyle et al in (93%) patients.[5,14,18]

The bone marrow aspiration revealstwo types of plasma cell morphology (plasmacytic and plasmablastic), the plasmablastic morphology correlates well with poor prognosis.[12]We found predominantly plasmablastic morphology in majority (53%) where as plasmacytic morphology was observed in (47%) myeloma patients. The observation was in concordance with study by Kaur et al [plasmacytic in (39%) and plasmablastic in (61%)] and Subramanian R et al [plasmacytic in (44%) and plasmablastic in (56%)] who also found plasmablastic morphology predominantly.[4,5]

Another indicator of high prognostic significance is percent infiltration of myeloma cells in bone marrow biopsy (more than 50% plasma cell infiltration stage III).[12] Most of our patients (82%) were in stage III, which is higher than that observed by Subramanian R et al (71%),Kaur et al (64.3%) and Kalita LK et al (26%).[4-6]

Various patterns of infiltration of marrow like interstitial, focal/nodular, diffuse, mixed and paratrabecular are observed in myeloma patients.A packed/diffuse marrow indicates worse prognosis.[10]In our study majority of cases had diffuse involvement (68.62%) followed by mixed (21.57%), interstitial (7.85%) and paratrabecular (1.96%). Similarly diffuse infiltration was also the predominant pattern of infiltration in other studies.[4, 5]

Conclusion

The present study concluded that multiple myeloma is a disease of middle aged and elderly with male preponderance. They accounted for slightly higher percentage of haematological malignancies in present study in comparison to literature.This may be due to our institute being the only Regional cancer treatment center and tertiary care referral hospital in our state. Bone pain was the most common presenting complaint along with lytic lesions on skeletal survey. Osteosclerotic lesions were also observed. Majority of our patients had plasmablastic morphology on bone marrow aspiration while bone marrow biopsy revealed majority of patients in stage III with predominantly diffuse pattern of infiltration.Hence most of our patients presented in advanced clinical stage.

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Epidemiological Study on Human, Cattle and Rodent Leptospirosis in South Gujarat Region of India

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ABSTRACT

Background: Surat district and neighbouring districts like Valsad, Navsari in South Gujarat region are considered as an endemic area for leptosprirosis. Types of leptospira serovars are depend on geographical area so it was important to find serovars which are predominant in humans, cattle and environment (soil, water) of this area. So aim of the study was to establish the transmission cycle of leprospirosis in this area by Isolation techniques and serology.

Material and Methods: 207 human, 92 rodent and 258 animal blood samples were subjected to PCR, MAT test and culture in EMJH media. In addition, 550 animal urine, 7 rodent urine, 19 animal kidney and 571 rodent kidney were also tested by PCR.

Results: Out of total 207 suspected cases, 161 samples were positive by MAT and 94(45%) positive by Real time PCR. 51(24%) samples were positive by MAT and PCR both tests. In cattle and rodent, 65 and 9 serum samples were positive by MAT test for leptospirosis. Predominant serovars observed in human cases, cattle and rodents were L. Autumnalis, L. Canicola, L. Pomona and L. Icterohaemorrahgiae.

Conclusion: The current study reveals the presence of common leptospiral serovars, Autumnalis and Pomona infecting man, animals, and rodents, by serology and isolation. Hence this study reiterates the need for a strong and highly detailed control program for leptospirosis in this region, which should begin from the rodents and encompass the domestic animals as well.

Keywords: *Leptospirosis; MAT (Microscopic Agglutination Test); PCR (Polymerize Chain Reaction)*

Introduction

Leptospirosis is common bacterial zoonosis worldwide, caused by spirochetes of genus Leptospira. Wild rodents serve as a natural reservoirs of infection, human and few others domesticated animals are accidental hosts in the transmission cycle of leptospirosis [1, 2] which can lead to abortion, stillbirth, infertility, mastitis, weak progeny and decreased milk production in them. [3, 4] The key feature in the transmission of Leptospirosis between animals, and between animals and man, is infection of renal tubules and excretion of infectious leptospires in the urine of carrier animals. Urine shed from carrier animal can result in direct transmission of the infection via contamination of mucous membranes of another animal, or in indirect transmission via contamination of the environment. The scenario of leptospiral infection is different in developed and developing countries. In developed countries, infection is increasingly being associated with outdoor recreational exposure and international travel. In rural areas of developing countries, transmission is usually associated with farming and livestock. In urban areas, infection is associated with overcrowding, poor hygiene standards, inadequate sanitation and poverty, all of which typically takes place in urban slums of developing countries. [5]

Suitability of environmental condition for survival of leptospires appears to be critical factor in maintaining the infection. Factors like tropical climates with heavy rainfall, stagnant waters, poor level of sanitation, occupational or recreational exposure and close proximity of mammalian reservoir to human population are associated with endemic leptospirosis.[6,7] Increased temperature may lengthen the survival rate of leptospires in the environment and can result in expansion of these reservoir species into higher elevation and latitudes. Hence, these all global climatic changes are contributing to leptospirosis as an emerging disease. [8]

A basic knowledge of serovars and their maintenance hosts is required to understand the epidemiology of leptospirosis in a region. Though there is distinct variation in maintenance hosts and the serovars they carry can occur throughout the world.[9] Generally dairy cattle have a role as a natural host of serovars Hardjo, Pomona and Gripphotyphosa, while pigs may harbor Pomona, Tarassovi, and Bratislava. Sheep may harbor serovars Hardjo and Pomona, and dogs may harbor serovar Canicola. [10]Diagnosis of leptospirosis in animals is done by three different methods which include the isolation from samples, detection of leptospiral DNA by real time polymerase chain reaction and detection

of anti- leptospiral antibodies. Isolation by culture is very time consuming, laborious and depends upon the presence of live leptospira in sample, so PCR and serology are the only method used for diagnosis. The detection of antileptospiral antibodies can be done with MAT (Microscopic Agglutination Test) and ELISA (enzyme linked immunosorbent assay). [11] MAT test can be used qualitatively and quantitatively to detect infecting serovars as well as give the titer of individual serovars. Furthermore the sensitivity and specificity of MAT in reported study were 91.94% and 73.77% respectively. [12]It is therefore important to have knowledge of the serovars present and their reservoir host.

Surat district and area around Surat district including neighbouring districts like Valsad and Navsari are considered as endemic area of leptosprirosis. Reporting of cases coincide with the rainy season as well as amount of rainfall. Case fatality due to leptospirosis in Surat district remains below 15% except in 1996 and 2006 when it was 22% and 28% respectively. As the leptospirosis is common in this area, present study was designed to establish the transmission cycle of leprospirosis. Types of serovars are depending on geographical area so it is important to find serovars which are predominant in humans, animals and environment (soil, water) of this area. Understanding serovar host dynamics and transmission cycle may help in better designing of preventive and treatment strategies so that mortality and complications of this infection can be decreased.

Materials and Methods

A retrospective study was conducted during the period of July 2008 to November 2008.207 samples were collected from suspected leptospirosis patients admitted in New Civil Hospital, Surat, South Gujarat. Serum samples were subjected MAT and PCR. The informed consent was taken from all suspected leptospirosis patients. Clinical suspicion of acute Leptospirosis was defined as fever and/or myalgia, tender liver, jaundice, acute renal failure, bleeding tendency, meningism and radiological lung infiltrates which accounted in the first week of fever. Patients confirmed for other diseases like hepatitis, malaria, dengue etc. were excluded from the study. Cattle (n=258) and rodents (n=92) serum samples were received at Microbiology laboratory in cold chain from different endemic areas of south Gujarat region and tested by Microscopic Agglutination Test and PCR for leptospirosis. The cattle included in present study were from various sources representing the diverse livestock production system e.g. rural subsistence, periurban, semi commercial and organized commercial dairy farms, where human leptospirosis cases were known to occur. 550 cattle urine and 7 rodents' urine samples were also received to the laboratory and were subjected to PCR. In addition, 19 cattle kidney and 571 rat kidney samples were screened by PCR.

Microscopic Agglutination Tests (MAT): The MAT test was performed using standard procedure.[13]Serogroups included in the antigen panel *L. australis*, *L. autumnalis*, *L. grippotyphosa*, *L. canicola*, *L. hebdomadis*, *L. pomona*, *L. semeranga* (patoc), *L. pyrogen* (Pyrogen) and *L. icterohaemorrhagiea*. All the strains were obtained from the National Leptospirosis Reference Centre, Regional Medical Research Centre (World Health Organization collaborating centre for diagnosis in leptospirosis, ICMR) in Port Blair, Andaman and Nicobar islands. The cultures used as antigens should be checked by MAT against homologous antisera frequently for quality control. These serovars were maintained in 0.1% semisolid EMJH agar by using Leptospira medium base supplemented with 10% enrichment (Diffco, USA) at 28-30°C in screw-capped test tubes.

Preparation of antigens: A 0.5 ml of each representative strain from the panel of 12serovars was inoculated into 10 ml of liquid EMJH medium. A loopful of culture was checked under dark field microscopy to confirm the absence of contamination or clumps and presence of viable leptospires. Incubation was done at 30°C for five to seven days. A density of approximately 2-3x 10^8leptospira/ml of media was used as an antigen.

Procedure: Doubling dilutions from 1 in 10 to 1 in 640were prepared by using phosphate buffer saline as a diluents. 50ul of the specific serovar was added to all the wells. One of the wells included only the antigen without addition of antibody and served as the antigen control. The final dilutions after adding the antigen were from 1 in 20 to 1 in 1280. The plates were covered with aluminum foil and incubated at 37 °C for 2 h. The highest serum dilution showing approximately 50% agglutinated leptospires or a reduction in the number of leptospiral cells as compared to the antigen control was taken as end point titer. A titer of 1 in 80 or more was considered positive.

Isolation Procedure of Leptospira Spp: Whole blood samples collected upon admission of patients were cultured in Ellinghausen-McCullough-Johnson-Harris liquid medium supplemented with enrichment medium and 5-fluorouracil (200µg/ml) and incubated aerobically at 28°-30°C. A drop from each culture medium was examined weekly, for 3 months by dark field microscopy. In case of contamination, cultures were filtered through 0.22 um pores to remove contaminants. [14]

Serogrouping: Serological characterization of clinical isolates was performed at the National Reference Center ICMR, Portblair, India for Leptospira. A Microscopic agglutination test (MAT) was performed to determine the serogroup of Leptospira isolates using rabbit antisera against reference serovars representing a standard battery of 24 serogroups. High rates of agglutination of the serum with one particular antigen were used to identify the presumptive serogroup of the infecting bacterium. [15]

Real Time PCR Assay: Total DNA from cattle serum (200 µl) was prepared using QIAamp DNA Mini Kits (QIAGEN, USA) according to the manufacturer's instructions. The primers and probes were designed from alignments of available Leptospira spp. LipL41 sequences obtained from the GenBank nucleotide sequence database. The program used was Primer Express™ (Applied Biosystems, USA). For real time PCR, 5 µl of DNA was added to the 45 µlTaqMan Universal PCR Master Mix (Applied Biosystems, USA) in final concentrations of 3 pmol/µl of each primer and 2 pmol/µl of the FAM-TAMRA labelled probe. A negative control without added template in the above reaction mixture was used as a control to detect the presence of contaminating DNA. Amplification and fluorescence detection was conducted in an ABI Prism 7300 sequence detector (Applied Biosystems, USA) with a program of 40 cycles, each cycle consisting of 95°C for 15 seconds and 60°C for one minute as per the manufacturer's instructions.

Result

Total 207 human cases of suspected leptospirosis were referred to New Civil Hospital, Surat for the treatment. Of 207 cases; 108 (52%) were from Surat district and 99 (48%) were from neighboring Valsad district. Of the 207, 108 (52%) of patients were of middle age group (20-39 year), 79 (38%) were having age above 40 years and 20 (10%) were below 19 years. Majority of cases were males as compared to females (82% versus 18%). Out of total 207 suspected cases, 161 (77.7%) samples were positive by MAT and 94(45%) positive by Real time PCR. 51(24%) samples were positive by MAT and PCR both tests.

Different samples from cattle [urine (n=550), serum (n=258), kidney (n=19)] and rodent [urine (n=7), serum (n=92), kidney (n=571)] were also received at Microbiology department. In cattle, 27 urine samples, 31 serum and 2 kidney samples were positive by PCR for leptospirosis. In rodent, 36 kidney samples were positive by PCR for leptospirosis and all urine and serum samples were negative. In cattle, out of 258 serum samples, 65 (25.1%, 95% CI 20.2% to 30.8%) were positive by MAT test for leptospirosis and out of 92 rodent serum samples 9 (33.3%, 95% CI 18.6% to 52.1%) were positive by MAT test for leptospirosis. MAT and PCR results of different samples from cattle and rodent had also shown in table.1.

Predominant serovars observed in human cases, cattle and rodents by doing MAT test are shown in table 2. Serovars common to all three species (Human, Cattle and rodent) were L. autumnalis, L. Canicola, L. Pomona and L. Icterohaemorrhagiea. Three other species L. Australis, L. Hebdomadis and L. Pyrogen were common only in human and cattle. *3 Leptospira species* were isolated from three human patients in EMJH media with 10% rabbit sera. The results of serotyping from National Leptospirosis Reference Centre, Port Blair, suggested that two of the isolates belonged to serogroup Autumnalis and one was from Pomona.

Table 1: shows PCR and MAT results of different samples from Cattle and Rodents.

Test	Cattle urine (N= 550)	Cattle serum (N= 258)	Cattle kidney (N= 19)	Rodent urine (N= 7)	Rodent Serum (N= 92)	Rodent Kidney (N= 571)
PCR Positive	27	31	2	0	0	36
PCR Negative	523	227	17	7	92	535
MAT positive	--	65	--	--	9	--
MAT negative	--	193	--	--	83	--

Table 2: Predominant serovars from serum of human, cattle and rodents by MAT test.

Serovars	Human (n = 161)	Cattle (n = 65)	Rodent (n=9)
L. Pyrogen	32 (20)	1 (1)	0
L. Australis	31 (19)	2 (3)	0
L. Autumnalis	73 (45)	21 (34)	6 (66)

Serovars	Human (n = 161)	Cattle (n = 65)	Rodent (n=9)
L. Gripphotyphosa	2 (1)	0	0
L. Canicola	15 (9)	12 (18)	1 (11)
L. Pomona	3 (2)	7 (11)	1 (11)
L. Icterohaemorrahgiae	4 (2)	13 (20)	1 (11)
L. Hebdomadis	1 (0.6)	9 (14)	0

(Value in parenthesis is percentages)

Discussion

The present study was carried out to detect the presence of common serovars of leptospira infecting the cattle and human beings in South Gujarat region by MAT and culture methods and also to understand transmission cycle of leptospirosis in this geographical area. Heavy rainfall and flooding increase the risk of leptospirosis by bringing bacteria and their animal hosts into closer contact with humans. Numerous outbreaks of leptospirosis have been reported following extreme weather events around the world, in geographically diverse areas including India, Laos, Indonesia, Italy, Brazil, Guyana Nicaragua, Puerto Rico, the USA, New Caledonia and Australia. [16]

Surat district and area around Surat district including neighbouring districts like Valsad and Navsari in South Gujarat are considered as endemic areas for leptosprirosis. Each year the area from where the cases come is expanding. First case was reported in 1994 and peak was observed in 1998 with fluctuations thereafter. Reporting of cases coincide with the rainy season (Between June to October) as well as amount of rainfall (heavy rainfall more cases). Case fatality due to leptospirosis in Surat district remains below 15% except in 1996 and 2006 when it was 22% and 28% respectively. Majority of cases came from laborers working in sugar cane and rice field. As transmission cycle varies with the host, agent and environment so it was necessary to explore transmission cycle in this area which was yet to be explored as done in the case of other areas of India and world. [6, 17, 18, 19]

In present study, 36 rodent kidney samples were PCR positive and in 9 rodent serum samples were showing agglutinating antibodies against different serovars of leptospira in MAT test. Rodents are susceptible to acute infection only in the early days of their life. Later, the immune system develops, and surviving ones become resistant to further infection. [20] This could be a probable reason for the low levels of antibodies detected in rodents. As the leptospires get lodged in the renal tubules of the rodents, rodent urine becomes a source of leptospiral infection in grazing animals like cattle, which in turn contribute to infection in human beings. [21]*Leptospira* could be isolated from three human patients in EMJH media with 10% rabbit sera. The inability to isolate *Leptospira* from other infected human beings could be attributed to administration of antibiotics by local practitioners before the patients were referred to the hospitals. [22] The results of serotyping from National Leptospirosis Reference Centre, Port Blair, suggested that two of the isolates belonged to serogroup L.Autumnalis and one was from L.Pomona. The MAT conducted on human, cattle and rodent's sera also revealed that predominant serovars common to all three species were L. autumnalis, L. Canicola, L. Pomona and L. Icterohaemorrhagiea. L.Autumnalis and L.Pomona as the predominant serovars infecting man, cattle and rodents. These strains from domestic and peridomestic animals play a major role in the epidemiology of human leptospirosis. Similar observation was also found in other studies suggested the antileptospiral antibodies belonging to serovars L.Autumnalis. [23, 24, 25] As this serovar had been included as an antigen for MAT in this study, the possibility of L.Autumnalis being the predominant serovar in this region. MAT should ideally be done on paired sera samples, for detection of a fourfold rise in the antibody titer. Since agglutinins to MAT tend to remain in the body for a prolonged period of time following infection, detectable MAT titers may be present even in healthy animals and man. But in an acute infection, MAT titers develop very late in the body usually by the second to fourth week of infection. These two factors may account for the false Positive and the false negative results by MAT. [24] In present study, the cutoff titer for MAT in humans and cattle were kept at 1: 80 dilutions as per results cited by various authors. [13, 26, 27]

The MAT has many disadvantages which indicate the need for an alternative test for routine diagnosis of leptospirosis. One major problem with the MAT is its use of live organisms as antigens. This requires the continuous culture and handling of these hazardous bacteria in laboratories and the subjective assessment of results can

also make quality assurance of the MAT difficult. Another problem associated with the MAT is that it only detects agglutinating antibodies and non-agglutinating antibodies may go undetected.

Conclusion

Control measures for leptospirosis should begin from rodents, which are the main reservoir hosts of *Leptospira,* and domestic animals, which are the carrier hosts of the infection. The current study reveals the presence of common leptospiral serovars L.Autumnalis and L.Pomona infecting man, cattle and rodents, by serology and isolation. Hence this study reiterates the need for a strong and highly detailed control program for leptospirosis in this region, which should begin from the rodents and encompass the domestic animals as well.

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Diagnostic Significance of Serum Ascites Cholesterol to Differentiate Malignant and Non Malignant Ascites

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ABSTRACT

Background: Ascitic fluid usually forms slowly as a result of obstruction of proximal vascular systems (Venous, lymphatic). It may also form directly in response to disease involving the peritoneum. Differentiation between malignancy related and non-malignant ascites is a challenge that is not always met satisfactorily. Both malignant and tuberculous ascites are exudative in nature with lymphocytic predominance and low Serum Ascitic Fluid Albumin Gradient values can not be differentiated easily form each other. Studies have shown that parameters like ascitic fluid cholesterol have been superior to the conventional method of ascitic fluid analysis in discriminating ascites caused by malignancy from others.

Method: This study was conducted in the Department of Pathology, Shyam Shah Medical College, Rewa during the period from May 2009 to October 2011. The study comprised of 100 patients with different causes of ascites admitted to wards of S.G.M.H. Rewa.

Result: Cases were divided in to 3 groups. Groups I consists of patents (39 male and 31 female) with ascites due to chronic liver disease and other non-tubercular and non neoplastic diseases, Group II consists of 20 patients (4 male and 16 female) with ascites due to tuberculosis, Group III consists of 10 patients (4 male and 6 female) with ascites due to malignant diseases. Ascitic fluid cholesterol level was found to be 32.9571±7.1183mg%, 0.05±9.047gm%, 74.1±16.1707mg% in Group I, Group II and Group III respectively. On comparing Group III with I and II values were found to be highly significant ($p<0.005$) and Group I with II was found to be insignificant ($p>0.05$). The value of ascitic fluid cholesterol level found in Group III was comparatively higher (>54.5mg%) than Group I and Group II with an exception in one case, where we found a higher level of cholesterol.

Conclusion: In our study we found a significant raised cholesterol level (74.1 ±16.1707 mg%) well above the cut off value (54.5mg%) and it has got a good differentiating potential for determining malignant ascites from non-malignant ascites.

Keywords: *Malignant Ascitis, Portal Hypertension, Serum Ascites Cholesterol, Serum Ascitic Fluid Albumin Gradient*

Introduction

Ascitis defined as accumulation of free fluid in the peritoneal cavity . Ascitic fluid usually forms slowly as a result of obstruction of proximal vascular systems (Venous, lymphatic). It may also form directly in response to disease involving the peritoneum. The commonest cause of ascites is liver cirrhosis (80%) followed by malignancy (10%), tuberculous peritonitis (2%), congestive cardiac failure, nephrotic syndrome, and others (3%). [1,2]

Various parameters like ascitic fluid physical examination, cell count, total protein concentration, Serum Ascitic Fluid Albumin Gradient [SAAG], cytology, cholesterol, amylase, lactic acid dehydrogenase, adenosine deaminase, and fibronectin levels have been used to differentiate exudative (ascitic fluid total protein>2.5 gm %) and transudative (ascitic fluid total protein ≤ 2.5 gm %) ascites of different etiologies.[3,4] .

The physiologically based approach to classify ascites by Serum Ascites Albumin Gradient (SAAG) has completely replaced the traditional way of classification as transudate and exudates. [5,6,7] The serum-ascites albumin gradient (SAAG), based on oncotic-hydrostatic balance, is the subtraction of ascitic fluid concentration from the serum albumin concentration. It has been found to categorize ascites much better than total protein concentration. However, albumin gradient does not explain the etiology of ascites, if SAAG is more>1.1, the patient is diagnosed to be having portal hypertension. Cirrhosis, cardiac failure, Budd-Chiari syndrome are some examples of high SAAG. Lesser values indicate that portal hypertension is minimal or absent and therefore that and exudative peritoneal lesion is present. A low gradient (<1.1gm%), in conditions where ascites is not related to portal hypertension, but due to peritoneal cause as in peritoneal malignancy, tuberculous peritonitis, metastatic peritoneal deposits. [5,7,8] .Differentiation between malignancy related and non-malignant ascites is a challenge that is not always met satisfactorily. Both malignant and tuberculous ascites are

exudative in nature with lymphocytic predominance and low SAAG values and can not be differentiated easily form each other. Fluid cytology has low sensitivity for malignancy as the differentiation between reactive atypical mesothelial cells and malignant cells is sometimes difficult. [9,10] Most of the time, diagnosis in not possible without invasive and expensive investigations like CT abdomen, Biopsy and FNAC of peritoneal nodes and diagnostic laparotomy/laparoscopy. So there is a need for more specific and a highly sensitive new marker in presumptive diagnosis of ascites. Studies have shown that parameters like ascitic fluid fibronectin and cholesterol have been superior to the conventional method of ascitic fluid analysis in discriminating ascites caused by malignancy from others.

A study found that fibronectin levels yielded 79% diagnostic accuracy in differentiating malignant from non malignant ascites. When compared, ascitic cholesterol has a higher sensitivity than fibronectin levels (100% Vs. 93%) in diagnosis of malignant ascites, therefore it is preferred test because of its simplicity and cost effectiveness. [11]

Materials and Method

This study was conducted in the Department of Pathology, Shyam Shah Medical College, Rewa during the period from May 2009 to October 2011. The study comprised of 100 patients with different causes of ascites admitted to wards of S.G.M.H. Rewa.

Cases were divided in to 3 groups. Groups I consists of patents (39 male and 31 female) with ascites due to chronic liver disease and other non-tubercular and non neoplastic diseases. Chronic liver disease cases included alcoholic cirrhosis, post necrotic cirrhosis and hepatitis progressing to cirrhosis.

Group II consists of 20 patients(4 male and 16 female) with ascites due to tuberculosis.

Group III consists of 10 patients (4 male and 6 female) with ascites due to malignant disease.

The diagnosis was done on the basis of clinical diagnosis along with radiological, haematological, biochemical, histopathological examinations and ascitic fluid findings.

Ascitic fluid and blood samples for biochemical and cytological examination were collected simultaneously. Serum and Ascitic fluid Albumin were estimated in autoanalyser by Bromocresol green. Total Protein were estimated in autoanalyser by Biuret methods. The serum cholesterol and Ascitic fluid cholesterol were also estimated.

Result

In our present study, we had found that serum ascitic albumin gradient is a better parameter reflecting the oncotio pressure gradient between the vascular bed and the interstitial splanchnic or ascitic fluid. The value more than 1.1 gm% is highly suggestive of higher oncotic pressure gradient even in the high protein ascites. In our study, we found higher value of SAAG (1.66±0.3063gm%) in the non tubercular and non malignant cases. Thus its high value (>1.1.gm%) is a good parameter to rule out the cause of ascites due to tubercular and malignant diseases and it should be included along with ascites total protein evaluation.

Total protein and SAAG have no differentiating characteristics between tubercular and malignant ascites, both of them show low value of SAAG and higher value of total protein and the differential diagnosis between these two groups is of problem. Tuberculosis is one of the important cause of ascites in our country. Direct smear of ascitic fluid for AFB gives poor results and invasive procedure for biopsy is generally not done.

In the present study total protein concentration in ascitic fluid in Group I was found to be 1.654±0.6274, is significantly lower than in Group II 3.71±0.4426 and Group III 4.09±0.7245. But the difference between the Group II and III was not significant. When we compared Group I and II, we found't' value 16.60 and 'p' value < 0.0005 which is highly significant, but when we compared Group II and III we found 't' value 1.2821 and 'p' value > 0.05 which is highly insignificant, thus the total protein value is not useful in differentiating tubercular from malignant ascites.

In this study we found protein concentration in ascitic fluid was more than 2.5g/dL or more in 11 out of 70 patients in Group I and 3g/dL or more than 3g/dL in 4 patients which in accordance with Sampliner RE's study in 1974. In Group III we found less than 2.5g/dL in 1 out of 10 patients.

A total of 100 patients were taken for study, which included 70 from Group I, 20 from Group II (Tubercular Group) and 10 from Group III (Malignant Group).

In tubercular group, the incidence in males was 4 (20%) and that in females was 16 (80%). And in the malignant group, the incidence in males was 4 (40%) and that in females was 6 (60%). Table No. 1 shows total protein values is significantly raised in Group II and Group III in comparison to Group I, but the difference between Group II and Group III was not significant. Table No. 2 shows that SAAG value is significantly high in Group I as compared to Group II and Group III. Comparison between Group II and group III was insignificant. Table No. 3 shows that ascitic cholesterol is significantly raised in Group III as compared to Group I and Group II. Table No. 4 shows that serum A/G ratio was low in Group I as compared to Group II and Group III. Serum Cholesterol does not show significant different amongst these groups.

Table 1a: Showing Mean S. D. and statistical interpretation of total protein in ascitic fluid.

	Group I (gm%)	Group II (gm%)	Group III (gm%)
Total Protein	1.6514	3.71	4.09
S.D.	0.6274	0.4426	0.7245

Table 1b: Comparison of values of total protein in ascitic fluid in various groups.

	Group I Vs II	Group I Vs III	Group II Vs III
t value	16.60	9.8697	1.2821
p value	<0.0005	<0.0005	>0.05
Significance, protein	H.S.,	H.S.,	H.S.,

Table 2a: Showing Mean S. D. and statistical interpretation of SAAG in ascitic fluid.

	Group I (gm%)	Group II (gm%)	Group III (gm%)
SAAG	1.66	0.655	0.53
S.D.	0.3063	0.2312	0.2532

Table 2b: Comparison of values of SAAG in ascitic fluid in various groups.

	Group I Vs II	Group I Vs III	Group II Vs III
t value	15.9	12.8848	1.3061
p value	<0.0005	<0.0005	> 0.10
Significance	H.S., ↓	H.S., ↓	Ins. ↓

Table 3a: Showing Mean S. D. and statistical interpretation of Cholesterol in ascitic fluid.

	Group I (gm%)	Group II (gm%)	Group III (gm%)
Cholesterol	32.9571	30.05	74.1
S.D.	7.1183	9.047	16.1707

Table 3b: Comparison of values of cholesterol in ascitic fluid in various groups.

	Group I Vs II	Group I Vs III	Group II Vs III
t value	1.3247	8.0473	7.9652
p value	>0.05	<0.0005	<0.0005
Significance	Ins., ↓	H.S.,	H.S.,

Table 4: Showing Mean and S. D. values of serum.

	Group I (gm%)		Group II (gm%)		Group III (gm%)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Albumin (gm%)	2.7386	0.2319	2.93	0.3114	2.83	0.2213
Globulin (gm%)	3.1586	0.2851	3.085	0.2978	2.93	0.2908
A/G Ratio	0.8701	0.072	0.9565	0.1345	0.976	0.1348
Chole. (mg%)	168.86	20.7394	161.0	17.7408	166.0	20.1108

Discussion

This study was carried out on 100 patients and they were divided into three groups. Group I consists of 70 patients with ascites due to chronic liver disease and other non-tubercular and non neoplastic diseases. Chronic liver disease cases included alcoholic cirrhosis, post necrotic cirrhosis and hepatitis progressing to cirrhosis. The diagnosis was done on the basis of clinical diagnosis and ascitic fluid findings. In this group the diagnosis of patient no. 3, was confirmed as hydatid cyst on the basis of radiological and histopathological examination. The diagnosis of patient no. 6 was confirmed as sickle cell anaemia with hypoproteinemia by haematological and biochemical examination. Diagnosis of patient no. 68, was confirmed as CRF by clinical and biochemical examination. Of the total number of cases, 39 were males and 31 were

females, with maximum number of patients falling in the age group 13-60 years with mean age 45.87 years in case of males and 41.8 years in females.

Group II consists of 20 patients (4 male and 16 female) with ascites due to tuberculosis, the patients were diagnosed on the basis of clinical diagnosis, ascitic fluid findings and the response to anti-tubercular drugs. Most of the patients fall in the age group 13-40 years with an average age of 37 years in case of males and 28.12 years in females.

Group III consists of 10 patients with ascites due to malignant diseases. The primary malignancy was already diagnosed by clinical and histological examination. Of these cases, 4 were males and 6 females. Most of the patients fall in the age group 41-60 years, with an average age of 59 years in case of males and 52-16 years in females. In the present study total protein concentration in ascitic fluid in Group I was found to be 1.654±0.6274, is significantly lower than in Group II 3.71±0.4426 and Group III 4.09±0.7245. But the difference between the Group II and III was not significant. When we compared Group I and II, we found't' value 16.60 and 'p' value < 0.0005 which is highly significant, but when we compared Group II and III we found 't' value 1.2821 and 'p' value > 0.05 which is highly insignificant, thus the total protein value is not useful in differentiating tubercular from malignant ascites.

In this study we found protein concentration in ascitic fluid was more than 2.5g/dL or more in 11 out of 70 patients in Group I and 3g/dL or more than 3g/dL in 4 patients which in accordance with Sampliner RE's [6] study in 1974. In Group III we found less than 2.5g/dL in 1 out of 10 patients.

In the present study, SAAG was found in Group I (1.66±0.3063), it was significantly higher than those found in Group II (0.655±0.2312) and Group III (1.53±0.2532). And we compared Group I with II, we found 't' value 15.9 and 'p' value<0.0005 which is highly significant, on comparing Good I with III, we found 't' value 12.8848 and 'p' value <0.0005 which is highly significant, but when we compared Group II with III, we found 't' value 1.3061 and 'p' value > 0.05 which is insignificant, thus value is not useful in differentiating tubercular from malignant ascites.

SAAG value as found in our study are in accordance with the studies of Pare P., Talbot J, Hoefs JC (1983) [5] , Runyon BA et al (1988) [14] , Goyal AK et al (1989) [15], Alba D. et al (1995) [16].

Another Study: have evaluated the diagnostic value of ascitic fluid cholesterol in differentiating between tuberculous and malignant ascites. They look 54.4mg/dl as the cut off value for ascitic cholesterol. The sensitivity, specificity, positive and negative predictive value and overall diagnostic accuracy in differentiating malignant from tuberculous ascites being 89.65%, 100%, 83.3% and 93.18% respectively. [12]

Again a study by Vyakaranam et al shows cholesterol has been found to clearly differentiate between tuberculous and malignant ascites. [13] The elevated cholesterol levels in malignancy is due to the increased vascular permeability, increased cholesterol synthesis and release from malignant cells implanted on peritoneum. [10,12] As studies on this are very less, hence the present study has been undertaken to evaluate sensitivity and diagnostic accuracy of ascitic fluid cholesterol level in diagnosing malignant ascites.

In our study, ascitic cholesterol level was found to be 32.957±7.1183 in Group I and 30.05±9.047 and 74.1±16.1707 in Group II and Group III respectively. When we compared Group I and II, we found 't' value 1.3247, 'p' value > 0.05 which shows highly insignificance, on comparing Group I and III, we found 't' value 8.0473, 'p' value < 0.0005 which is highly significant, and again on comparing Group II and III, we found 't' value 7.9652, 'p' value <0.0005 which is highly significant. Thus, ascitic cholesterol level was found significantly raised in malignant group as compared to Group I and Group II. Our results are in concordance with the studies of Sood A, Garg R et al (1995) [12].

Our results were in accordance, in chronic liver disease and malignant diseases, with that of Prieto M. et al (1988) [17] , Barbare JC, Diab G. et al (1989) [18] and Castaldo G., Oriani G. et al (1994) [19] .

Conclusion

Total protein and SAAG have no differentiating characteristics between tubercular and malignant ascites, both of them show low value of SAAG and higher value of total protein and the differential diagnosis between these two groups is of problem. In our study we found a significant raised cholesterol level (74.1±16.1707mg%) well above the cut off value (54.5mg%) and it has got a good differentiating potential for determining malignant ascites from non-malignant ascites.

The lower value of cholesterol is a good indicator to rule out malignancy. This technique being simple, cost effective and easily available, it should be included alongwith other examinations (SAAG, ascitic fluid total and differential WBC count, and cytology), at least in cases of ascites with suspected malignancy.

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Ethical Approval

The study was approved by the Institutional Ethics Committee.

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EGFR Mutational Status of Primary Lung Adenocarcinoma in an Indian Cohort Based on 2015 WHO Classification of Lung tumors

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ABSTRACT

Background: Epidermal growth factor receptor (EGFR) mutations have been known to be associated with adenocarcinoma, women, non- smokers and East-Asian ethnicity. This study was aimed to characterize the frequency of EGFR mutations and their association with histologic subtypes in primary lung adenocarcinoma in an Indian cohort.

Methods: Two seventy-four cases were categorized using 2015 WHO classification of lung tumors. The frequency of each histologic subtype and cell type was correlated with EGFR exon sequences in a subset of 120 cases using polymerase chain reaction (PCR) gene sequencing.

Results: The predominant biopsy categories in 274 cases were acinar 167(61%), solid 63(23%), mucinous 19(7%), lepidic 11(4%) and others 14(5%). EGFR mutations were detected in 49/120 (40.8%) including 3/5(60%) lepidic, 4/9(44.4%) papillary, 29/68(42.7%) acinar, 10/24(41.7%) solid and 1/13(7.7%) mucinous subtypes and were significantly associated with the cuboidal cell type (p=0.01). These mutations were common in women and non-smokers, although not statistically significant. Exon 19 mutations predominated in 36/49(73.4%). The majority, 213/263 (81%), were thyroid transcription factor 1(TTF-1) positive. The polygonal cell type and the solid subtype were frequent amongst stage IV tumors and smokers.

Conclusions: EGFR mutations were most frequently seen with the lepidic and papillary subtypes, not associated with the mucinous subtype, more common in women and non-smokers and significantly associated with the cuboidal cell type.

Keywords: *EGFR Mutation, Lung Cancer, Histology, Adenocarcinoma, Who Classification, India*

Introduction

Non-small cell lung cancer (NSCLC) accounts for 75-80% of all lung cancers. Histologically, NSCLC is classified into squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. In India, squamous cell carcinoma has been the most common histological type of NSCLC with a growing predominance of adenocarcinoma[1]. Lung adenocarcinomas are well known to display inter and intra-tumoral heterogeneity with profound implications for exact histological classification by pathologistsaccounting for more deaths than breast, prostate and colon cancer combined.[2] While treatment decisions are determined primarily by stage, therapeutically non small cell lung cancer (NSCLC]. The New International classification (IASLC/ATS/ERS)[3,4] and 2015 World Health Organization (WHO) classification[5]"defined the adenocarcinoma in situ(AIS), minimally invasive adenocarcinoma (MIA) and invasive lepidic adenocarcinoma, replaced the 'mixed subtype category' in 2004 WHO classification[6] with the predominant subtype, included the micropapillary subtype and replaced the term "mucinous bronchioloalveolar carcinoma (BAC)" with invasive mucinous adenocarcinoma.

Over the past decade, introduction of targeted therapy with epidermal growth factor receptor- tyrosine kinase inhibitors (EGFR-TKI) has revolutionized the treatment of adenocarcinoma. EGFR activating mutations are associated with clinical phenotype like females, non-smokers, East-Asian ethnicity, and adenocarcinoma, demonstrating responsiveness to tyrosine kinase inhibitors like gefitinib or erlotinib[7]. Although a histological correlation has been well established between lung adenocarcinoma and EGFR mutations, there is limited information regarding the correlation between the predominant histological subtype of adenocarcinoma and EGFR mutations in an Indian cohort. Therefore, this analytic and descriptive

study was aimed at comprehensive histological subtyping and analysis of the frequency of EGFR mutations in primary lung adenocarcinomas based on 2015 WHO classification of lung tumors and correlating this data with clinicopathological features in an Indian cohort.

Materials and Methods

This retrospective study was conducted in a referral tertiary care center in the Department of Pathology, after the approval from the institutional review board.

Patient Selection and Histologic Analysis: This study evaluated all cases of primary lung adenocarcinomas from June 2011- December 2012, using the archival stained Haematoxylin and Eosin (H&E) slides and formalin fixed paraffin embedded blocks following strict inclusion and exclusion criteria.

Inclusion Criteria: 1) All cases of primary lung adenocarcinoma biopsied at our institution during June 2011- December 2013.

Exclusion Criteria: 1) Cases with no definite morphological features and/or no immunohistochemical evidence of adenocarcinoma, 2) Squamous cell carcinoma by morphology or by Immunohistochemistry, 3) Non-small cell carcinoma, not otherwise specified (NSCC-NOS); 4) Large cell carcinoma and 5) Inadequate biopsy material for definite categorization.

Cases were categorized using 2015 WHO classification of lung tumors[5]" incorporated from the IASLC/ATS/ ERS classification for small biopsies[3]. Data regarding the relevant history, clinical diagnosis, and radiological findings were obtained from hospital records. A detailed histopathological analysis was performed independently by two pathologists (A.K and P.R). Individual features including the predominant cell type, presence of mucin (PAS-Diastase), desmoplasia, stromal elastosis, lymphovascular invasion, and necrosis were studied. Comprehensive histologic subtyping was done to assess histologic patterns semi-quantitatively in 5% increments, choosing a single predominant pattern. These features were correlated with one or more immunohistochemical markers namely Thyroid Transcription Factor-1 (TTF-1) (Clone 8G7G3-1, Dako, Glostrup, Denmark), cytokeratin 7(CK7) (Clone OV-TL12/30, Dako, Glostrup, Denmark), and Ep-CAM (BerEp4) (Clone Ber-EP4, Dako, Glostrup, Denmark) where available, using the automated Ventana system. CK7 was used to differentiate between primary and metastatic lung adenocarcinoma, while BerEp4 immunohistochemistry was used to exclude pleural tumors. Details, where available, for stage of disease and smoking status, were included. Patients were staged according to the American Joint Committee on Cancer (AJCC) TNM staging manual, seventh edition[8]. EGFR mutational analyses were performed using polymerase chain reaction (PCR) sequencing of exons 18, 19, 20 and 21.

EGFR Mutational Analysis: A subset of 120 cases based on sample size calculation were randomly selected from the pool of cases of primary lung adenocarcinoma for EGFR mutational analysis. EGFR mutational analysis was performed by PCR on formalin fixed and paraffin embedded blocks. The area with tumor cellularity of > 50% was identified. The tumor was further manually microdissected and 5 paraffin sections at 3-4 µm thickness were used for DNA extraction. Extraction was carried out using the DNA tissue extraction kit (QIAGEN India Pvt. Ltd, New Delhi, India). The DNA was quantitated using the Nano-drop (Nano-Drop Technologies, Wilmington, Delaware, USA) and the 260/280 ratio was determined.

Molecular Analysis: The PCR for four exons, namely 18, 19, 20 and 21 was performed using the primer sequences by Lynch et al[9]" (EGFR. All reactions were carried in 25 µl volume. The following thermal cycling profile was used for all PCRs:95^{0}C for 8 min, 95^{0}C for 30 secs, optimized anneal for 30 secs, 72^{0}C for 1 min and final extension of 72^{0}C for 10 min. The PCR product was detected using a 1.5% agarose gel. Sequencing of both the sense and antisense strands for all exons was performed with an automated DNA sequencer (ABI PRISM 310 genetic analyzer) using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). Mutational analysis was performed by comparing the sequence with the wild type and by identifying all known mutations in these exons.

Statistical Analysis: Descriptive statistics for continuous data was analyzed using mean with standard deviation or median with inter-quartile range. Categorical data were described using frequencies and percentages. Histopathological subtypes with EGFR mutational analysis were associated using Fischer's exact test and Chi-square test. For all analyses, a $p<0.05$ was considered statistically significant. Data were analyzed using the SPSS Statistics software, version 16.

Results

Patient Characteristics: The study included 274 cases of primary lung adenocarcinoma. Of the 274 cases, 186 (67.88 %) were males and 88(32.12 %) were females with the mean age being 58 (range 30-76 years) and 55 years (range 32–78 years) respectively. As per the geographical distribution, 148 (54%) were from the east,93 (33.90 %) from the south, 6 (2.2%) from the north, 2 (2.2%) from the west of India. Twenty-five (9.1%) cases were from Bangladesh. Smoking history was obtained in 165 cases, and a majority of

patients were non-smokers 117(70.9%), while 48 (29.1%) were smokers. The commonest location of tumors was the right lung in 125(45.6%), while in 76(27.7%) they were bilateral and 69 (26.6%) in the left lung. Although the right lung had a predilection for tumors, the right upper and lower lobes were involved in equal frequencies. The hilum was infrequently involved. The tumor dimension of >3cm documented on CT scan was seen in 178/206 (86.4%) cases. Amongst 237 cases where staging data were available, a significant proportion 195 (82.2%) were stage IV at diagnosis, 31(13.5%) were stage III and 11(4.7%) were Stage II and none with Stage I of disease.

Histologic Findings and Immunohistochemistry: Of the 274 cases, the predominant subtypes were 167 (61%) acinar, 63(23%) solid, 19 (7%) invasive mucinous, 11(4%) lepidic, 9 (3.30%) papillary, 2 (0.7%) colloid and 2(0.7%) non-small cell carcinoma, not otherwise specified (NSCC-NOS) and 1micropapillary (0.3%) subtype(Figure 1A-1D; Figures 2A-2D).

The polygonal cell type was the most frequent comprising of 160(58.40%) cases followed by columnar 69(25.2%), cuboidal 38(13.9%), signet ring 4 (1.4%), hobnail 2 (0.7%) and clear cell 1(0.4%) types. A significant percentage of tumors were associated with mucin production, desmoplasia, stromal elastosis, lymphovascular invasion and necrosis (Table 1). Lymphovascular invasion was most commonly seen amongst stage III/ stage IV disease in 187/226 (82.7%). The majority of tumors, 213/263 (81%) were TTF-1 positive. Almost all cases, demonstrated positivity for CK7 134/135 (99.3%) and Ber-EP4 63/65 (97%).

Correlation of Histologic Subtypes with Smoking history: The papillary subtype in 7/7 (100%) followed by the lepidic predominant in 4/5(80%), acinar in 75/102 (73.6%) and solid in 16/36 (44.4%) subtypes were frequent with non-smokers. The solid subtype was relatively more frequent among smokers, 20/36 (55.6%)as compared to the other subtypes.

EGFR Mutational Analysis: EGFR mutational analysis was performed on a subset of 120 cases and 49 (40.8%) were found to harbor a mutation in any one of the four exons (18, 19, 20, 21) that are known to be hotspots of mutations, except one case which had combined exon 20 and 21 mutations. Exon 19 mutations predominated in 36/49 (73.4%) cases with del E746-A750, as the commonest form with a few other uncommon deletions (Figure 3).There were 12(24.5%) cases with exon 21 mutation, all with the L858R mutations.

Correlation of EGFR Mutations with Histologic Subtype and Clinicopathological Features:

A comparison of the mutational pattern with the histological subtype indicated that mutations were most commonly seen in the lepidic subtype 3/5 (60%), followed by papillary4/9 (44.4%), acinar 29/68 (42.7%) and solid subtypes10/24 (41.7%) in decreasing frequencies. Mucinous adenocarcinomas were infrequently associated with EGFR mutations 1/13(7.7%), with statistical significance **p=0**.01 (Table 2).

Although EGFR mutations were more common amongst females 25/52 (48.1%) and non-smokers 30/66 (45.5%), they were also noted amongst smokers 5/18 (27.8%) and males 22/68 (32.4%). There was no statistical association of EGFR mutational status with the geographical distribution of patients, residing in India and outside of India. TTF-1 positivity was significantly associated with EGFR mutational status**(p = 0**.007). Regression analysis of patients with mutation and TTF-1 positivity showed a relative risk of 5. (Table 3)

Correlation of Cell Type with EGFR Mutations, Smoking Status and Stage of Disease: Although the polygonal cell type 21/47(44.6%) was more frequent with EGFR mutations, there was no statistical significance between the two. However, there was a significant association between the cuboidal cell type and EGFR mutations **(p**=0.01). The polygonal cell type was more common in smokers 27/48 (60.4%) and in stage IV disease 112/195(57.4%).

Discussion

The prevalence of EGFR mutations in this study was 40.8% similar to a study published from our center, where a prevalence of 39.6% of EGFR mutations was reported

Table 1: Associated histopathological features with lung adenocarcinoma.

Associated features	N=	Present (%)	Absent (%)	p value
Mucin production	274	226(82.5)	48(17.5)	0.002
Desmoplasia	274	267(97.4)	7(2.6)	0.02
Stromal elastosis	274	252(92)	22(8)	0.05
Lymphovascular invasion	274	227(82.8)	47(17.2)	0.01
Necrosis	274	175(63.9)	99(36.1)	0.18

Table 2:Association of EGFR mutations with predominant histological subtype of lung adenocarcinoma.

Subtype	N=	Negative EGFR	Mutated EGFR	p value
Lepidic	5	2(40%)	3(60%)	0.40
Papillary	9	5 (55.6%)	4(44.4%)	0.73
Acinar	68	39(57.3%)	29(42.7%)	0.34
Solid	24	14(58.3%)	10(41.7%)	>.99
Mucinous	13	12(92.3%)	1(7.7%)	0.01
Micropapillary	1	1(100%)	-	
Abbreviation: EGFR, Epidermal Growth Factor Receptor.				

Table 3: Correlation of EGFR mutational status with clinicopathological features and TTF-1 immunohistochemistry.

Characteristics	Number (%)	Wild type (EGFR)	Mutated (EGFR)	p value
Location	N=120(%)			
North	4 (3.3%)	3(75%)	1(25%)	0.78
West	2 (1.7%)	1(50%)	1(50%)	
East	45(37.5%)	30(66.7%)	15(33.3%)	
South	61(50.8%)	34(55.7%)	27(44.3%)	
Outside India	8(6.7%)	5(62.5%)	3(37.5%)	
Gender	N=120(%)			
Male	68(56.7%)	46(67.6%)	22(32.4%)	0.08
Female	52(43.3%)	27(51.9%)	25(48.1%)	
Smoking status	N=84(%)			
Smoker	18(21.4%)	13(72.2%)	5(27.8%)	0.51
Non-smoker	66(78.6%)	36(54.5%)	30(45.5%)	
TTF-1	N=112(%)			
Positive	96(85.7%)	52(78.8%)	44(45.8%)	0.01
Negative	16(14.3%)	14(21.2%)	2(4.3%)	
Abbreviations: Epidermal Growth Factor Receptors, TTF: Thyroid Transcription Factor				
IHC: Immunohistochemistry				

Table 4: Details of studies correlating EGFR mutations and predominant subtypes.

Author	Number	TNM stage	Specimen type	Predominant subtype associated with EGFR
Motoi et al[16]	100	Stage I-III	Resections	1.Papillary 35.1% (13/37)
				2.Micropapillary (NA)

Yoshizawa et al[17]"	167	Stage I-III	Resections	1.Lepidic (71.4%)
				2.Papilllary (68.5%)
Sun et al[18]"	382	NA	Resections and biopsies	1.Papillary 21/30(70%)
				2.Acinar 98/173 (56.6%)
				3.MP 2/4(50%)
				4.Infrequent with mucinous subtype 1/3 (33.3%)
Present study	120	Stage III-IV	Small biopsies	1. Lepidic 3/5(60%)
				2. Papillary 4/9(44.4%)
				3.Acinar 10/24(41.7%)
				4. Infrequent with mucinous subtype 1/13(7.7%)
Abbreviations: EGFR: Epidermal Growth Factor Receptor, NA: Not available.				

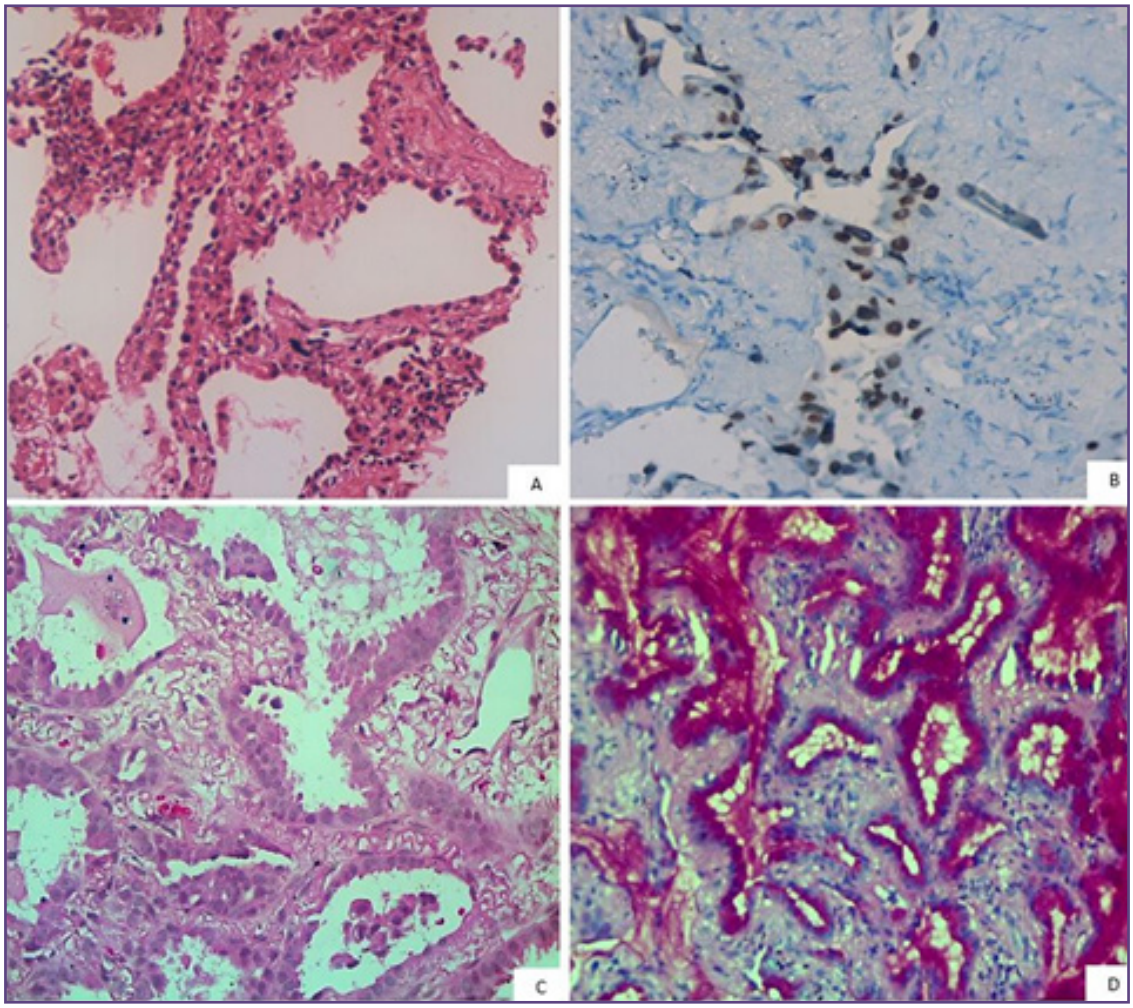

Fig. 1A): Lepidic predominant adenocarcinoma (H&E,200x); 1B) TTF-1 positive lepidic adenocarcinoma (200x); 1C) Acinar adenocarcinoma with hobnail cell type and elastosis (H&E 400x); 1D)Invasive mucinous adenocarcinoma (PASD, 200x).

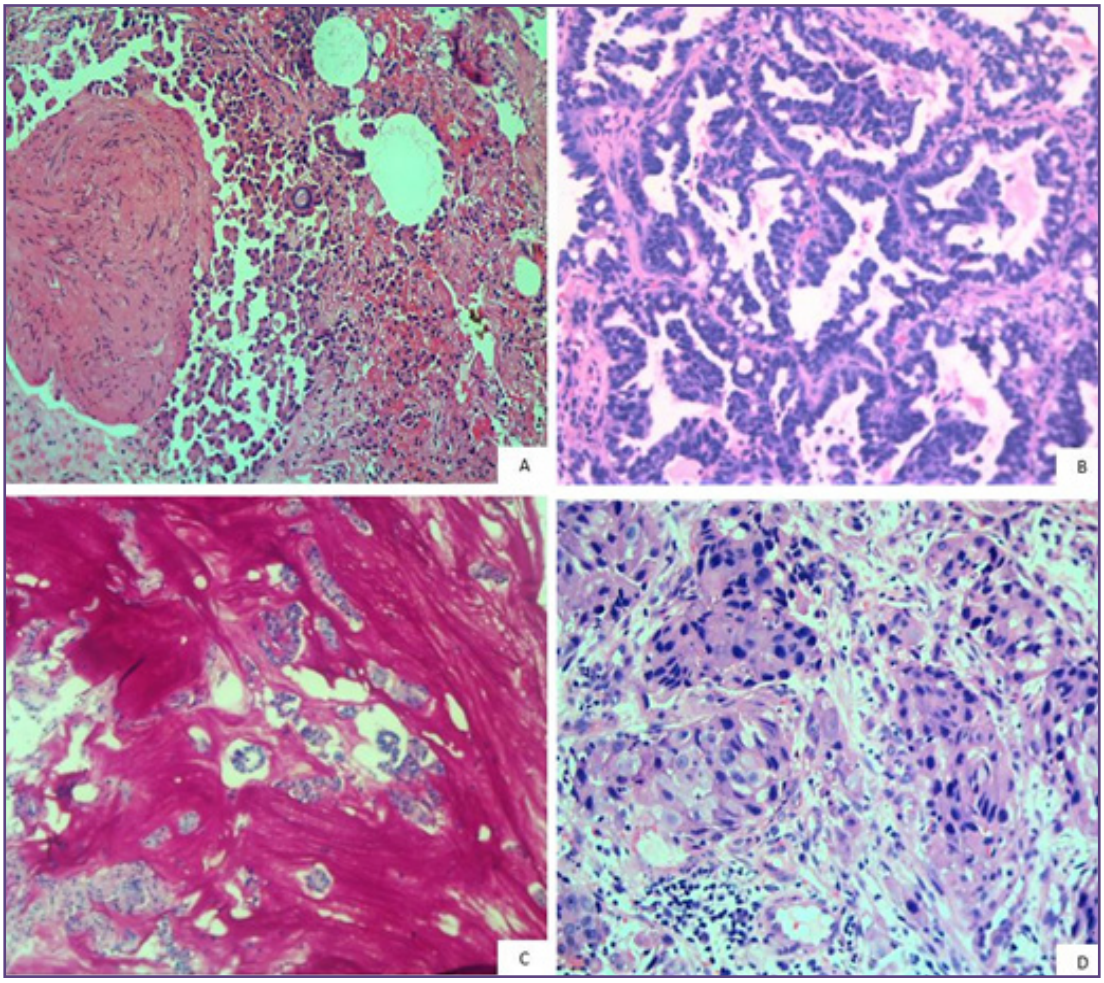

Fig. 2A) Micropapillary adenocarcinoma (H&E, 200x); 2B) Papillary adenocarcinoma (H&E, 200x); 2C) Colloid adenocarcinoma (PASD, 200x); 2D) Solid adenocarcinoma (H&E 200x).

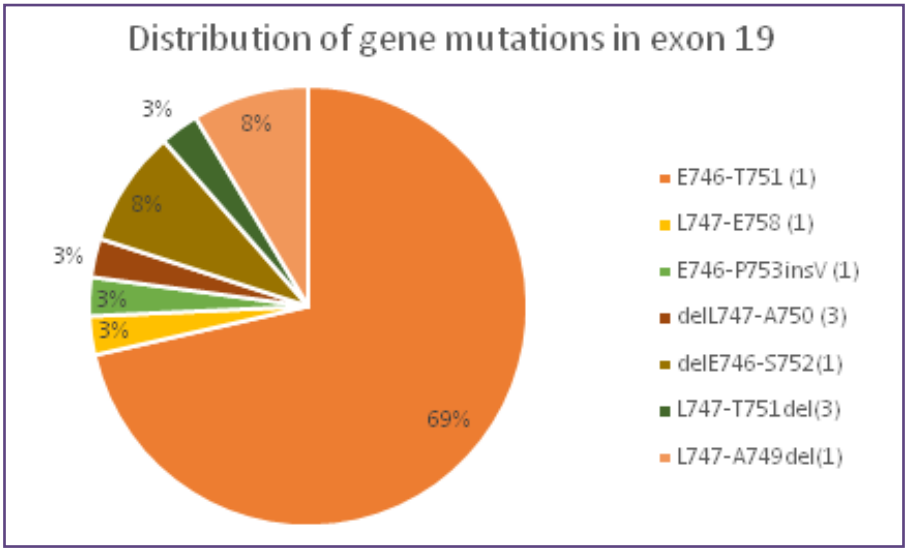

Fig. 3: Distribution of gene mutations in exon 19.

with favorableprogression-free survival response with chemotherapy followed by tyrosine kinase inhibitors (TKI's)[10].Studies from India have reported the prevalence of EGFR mutations ranging from 16.6%- 44%[10–13]. While this fluctuation in values could be attributed to the technique used and the type of histologic diagnosis selected for the study, the role of genetic heterogeneity within India, contributing to variations in mutation frequencies, cannot be entirely ruled out [14].

In the present study, there was a predominance of exon 19 mutations in 73.4% cases, with 15 bp del (E746-A750) being the commonest form amongst exon 19 mutations(69.4%). Exon 21 mutations were seen in 24.4%, all being the L858R type. Exon 18 mutation was not seen in any of the cases. Molecular analysis has shown that short in-frame deletions of exon 19 and a point mutation in exon 21 at base pair 2573, resulting in the substitution of leucine by arginine in codon 858 (L858R) make up for ~90% of all mutations. Point mutations in exons 18 and exon 20 account for 5% of EGFR mutations in non-small cell lung cancer[15]. In the present study, there was one case with a combined exon 20(T790M) and 21(L858R) mutation. T790M mutations can occur in 50-60% patients with resistance to TKI's,largely due to a conformational change in the EGFR molecule that prevents drug binding. It is also reported that the T790M mutation may confer primary resistance to TKI's and possibly co-exist with L858R mutations[15]. This finding was noted in the present study. The study did not find any significant association between mutational status and age, gender, smoking status and TTF-1 positivity.

Several studies have shown that EGFR mutations are associated with the lepidic, papillary, micropapillary and acinar subtypes (Table 4). In the present study, EGFR mutations were most common in the lepidic subtype (60%) followed by the papillary (44.4%), and acinar subtypes (42.7%) and were rare with the invasive mucinous subtype (7.7%). These findings are consistent with the reports of Motoi et al[16], Yoshizawa et al[17]and Sun et al[18]. We did not find an association between EGFR mutations and the micropapillary subtype, probably due to a very small sample number of this particular subtype. Subtyping of tumors in patients undergoing complete resection, using the WHO 2015 classification appears to be very important, as highlighted by Tsao and colleagues since some subtypes like the solid and micropapillary tumors that have a poorer survival would significantly benefit from adjuvant chemotherapy. In contrast, this may not be as beneficial for acinar and papillary predominant tumors, that tend to have a better survival[19] and subtyping tumors might be able to guide therapy.

In this study, EGFR mutations were more common in females (48.1%) and non-smokers (45.5%), although a proportion of smokers (27.8%) and males (32.4%) also were positive for these. Similar results were reported by Sun et al[18], where the incidence of EGFR mutations in men who smoked with adenocarcinomas was 29.7% (35/118). As per the study by D'Angelo and colleagues in 2,142 patients with lung adenocarcinoma, 57% of EGFR mutations would be missed if testing were restricted to women who never smoked cigarettes[20]. In the present study, we found the solid predominant subtype was more common among smokers, which is similar to the finding by Motoi et al[16].

The current set guidelines recommend mutational analysis of all lung tumors with a diagnosis of adenocarcinoma or with an adenocarcinoma component, irrespective of the age, gender, ethnicity, smoking status, and histological subtype. In a setting of small biopsies and cytology specimens where an adenocarcinoma component cannot be completely excluded, testing for EGFR mutations can be considered in cases showing a small cell or squamous histology. However, in this scenario, clinical criteria like female gender, the absence of smoking history and age, may be useful indicators in the selection of a subset for testing[21].

In the present study, EGFR mutations were significantly more common in tumors expressing TTF-1, hence TTF-1 positivity could be used as a predictive marker for EGFR mutations as shown in several other studies [18,22]. By regression analysis, cases that were positive for TTF-1 were 5 times more likely to harbor an EGFR mutation. However, there was no statistical significance established with TTF-1 and any particular histological subtype.

We also compared the predominant cell type with EGFR mutations, smoking status, and stage of the disease. Although the polygonal cell type was the commonest, the cuboidal cell type was significantly associated with EGFR mutations. A similar study by Okada et al[23] proposed the five-cell type classification and found the hobnail cell type to be significantly associated with EGFR mutations ($p<0.001$), followed by mixed, columnar/ cuboidal, polygonal and goblet cells. The study also found a higher percentage of smokers to be significantly associated with the cuboidal/ columnar and polygonal cell type, similar to findings in this study where the polygonal cell type was associated with smokers.

Although survival analysis was not done in the present study, it is worthwhile to note that several clinicopathological parameters have been associated with poor disease-free

survival, which includes male gender, gross and invasive tumor component, vascular invasion, necrosis and higher stage of disease. The AIS and MIA have a 100% disease-free survival (DFS) at 5yrs, followed by the lepidic, acinar and papillary subtypes with a 5-year DFS between 83-90%. The solid, micropapillary, colloid and invasive mucinous subtypes that belong to the poor prognostic group have a 5-year DFS between 67-76% [24].

A limitation of this study is that the histologic and molecular profile have not been correlated with progression-free survival and overall survival, as was done previously for a cohort of 106 cases at our center[9].

Conclusion

This study has helped to highlight that EGFR mutations were most frequently seen with the lepidic and papillary subtypes, not associated with the mucinous subtype, were more common in women and non-smokers and were significantly associated with the cuboidal cell type. This data will be of value to overcome the lacunae in an Indian setting.

Acknowledgments

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Histopathological Spectrum of Breast Lesions in Association with Histopathological Grade Versus Estrogen Receptor and Progesterone Receptor Status in Breast Cancers : A Hospital Based Study

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ABSTRACT

Background: A retrospective study of 2-years duration from January 2015 to December 2016 was undertaken to evaluate the histopathological spectrum of breast lesions including both the benign breast lesions and malignant breast lesions. To evaluate the Estrogen receptor and Progesterone receptor status in breast cancer cases by using immunohistochemistry and is to correlate the histopathological grade with ER, PR status of breast cancer.

Methods: The histopathological findings of the 132 biopsied Specimens of all age groups and both sexes were studied and most of them were lumpectomy specimens and few were mastectomy specimens. IHC markers were applied on breast cancer cases.

Results: Out of the 132 cases benign breast lesions constituted 78.78% and malignant breast lesions 21.22%. Among the benign breast lesions fibro adenoma was the commonest and among the malignant breast lesion, infiltrating ductal carcinoma was commonest. The peak incidence of benign breast lesion was in 11 to 40 years and malignant lesion in 41 to 70years. Out of 22 cases of infiltrating ductal carcinoma, 27.27% cases showed both ER, PR positivity and 63.64% cases showed both ER, PR negativity. A significant association was seen between histologic grade and ER, PR status.

Conclusion: In our study fibro adenoma was the commonest benign breast lesion and infiltrating ductal carcinoma was the predominant malignant breast lesion. Among the breast cancers, both ER and PR negativity cases were predominant and low grade tumors were showing high ER, PR expression and high grade tumors were showing low ER, PR expression in our study.

Keywords: *Breast Lesions, Fibro Adenoma, Infiltrating Ductal Carcinoma, Histopathological Grade, Immunohistochemistry.*

Introduction

According to WHO statistics 2008,[1] malignant breast lesions comprises 1.38 million cases (10.9% of total cancer cases). According to Indian statistics malignant breast lesion is the second most common malignancy in woman after carcinoma of cervix and is detected in 20 per 1,00,000 women.[2,3] Fortunately, most of the breast lesions are diagnosed as benign breast lesions.[4] Main aim of our study is to evaluate the Histopathological spectrum of breast lesions in patient attending the NMCH, Nellore Andhra Pradesh, India,3

The spectrum of breast lesions consists of benign lesions including fibro adenoma , phyllodes tumor, Gynaecomastia, Breast abscess & chronic mastitis, malignant lesions including ductal carcinoma, lobular carcinoma, colloid carcinoma and medullary carcinoma. Benign breast lesions incidence begins during the second decade of life and peaks in the fourth and fifth decades. But malignant breast lesions incidence increase after menopause. [5]

Material and Methods

The study was conducted in the department of pathology, NMCH, Nellore, Andhra Pradesh, India from January 2015 to December 2016. 132 biopsy specimens were received. Among the 132 biopsy specimens most of biopsy specimen's were lumpectomy specimens and few were mastectomy specimens. These biopsy specimens were fixed in 10% formalin solution for 24 hours. The tissue was processed routinely, and paraffin embedded tissue were cut on microtome to the thickness of 4 microns. The sections were stained with Haematoxylin and Eosin stain and reported. Out of 28 cases of breast cancers, 22 cases were infiltrating ductal carcinomas which were histologically

graded according to Modified Bloom-Richardson-Elston grading system. After that, breast cancer tissue block were selected for IHC evaluation (ER and PR status).

Statistical Analysis: Data collected was entered in MS Excel and analyzed using SPSS-Version 22.0. Percentages and chi-square values were calculated. A P value of 0.05 was taken as significance.

Result

132 cases of breast lesions were studied over a period of Two years from January 2015 to December 2016. The commonest presenting symptom in breast lesion was lump in the breast. Out of 132 cases, 20 cases were males and 112 cases were females. Out of 20 cases of males, majority of cases were Gynaecomastia. Among 132 cases, benign breast lesions consistituted 104 cases (78.78%) and malignant breast lesions 28 cases (21.22%).The youngest male (14 years) was diagnosed with gynaecomastia and the youngest female (12 years) was diagnosed with fibro adenoma breast. The ages of the cases ranged between 11 years to 80 years. The peak age of occurrence to the benign lesions was found to be in between 11-40 years, youngest case detected as fibro adenoma at 12 years of age. The peak age of occurrence of the malignant lesion was found to be in between 41-60 years, youngest case diagnosed as medullary carcinoma at 35 years of age, and oldest case diagnosed as a mucinous carcinoma at 80 years of age.

Among the 104 cases of benign breast lesions, 78 cases (75%) were of fibro adenoma, 2 cases (1.92%) were of benign phyllodes, 14 cases (13.46%) were of Gynaecomastia, 4 cases (3.85%) were of breast abscess and 6 cases (5.77%) were of chronic inflammatory pathology. Among the benign breast lesion, fibro adenoma was the most common lesion [Table 1].

Among the 28 cases of malignant breast lesions, 22 cases (78.57%) were of infiltrating ductal carcinoma [Figure 1], 2 cases (7.14%) were of medullary carcinoma, 2 cases (7.14%) were of lobular carcinoma and 2 cases (7.14%) were of mucinous carcinoma. Among the malignant breast lesions, infiltrating ductal carcinoma was the most common lesion [Table 1].

Among the 22 cases of infiltrating ductal carcinoma, the commonest grade was grade 2 accounting to 72.73% followed by grade 3 and grade 1 with 18.18% and 9.09% respectively. Among the 22 cases of infiltrating ductal carcinoma, 10 cases of grade 2 breast carcinoma were in age group of 41-50 years, 2 cases of grade 2 breast carcinoma were in age group of 51-60 years, 2 cases of grade 1, 4 cases of grade 2 and 4 cases of grade 3 breast carcinoma were in age group of 61-70 years [Table 2] Out of 22 cases of infiltrating ductal carcinoma lesions were studied for the ER, PR expression. The number of tumors positive for both ER and PR [Figure 2 & 3] were 6 (27.27%). The number of tumors for ER positive and PR negative was two cases (9.09%). The number of tumors negative for both ER and PR were 14 (63.64%).

Among the 22 cases of infiltrating ductal carcinoma 50% of grade 1 cases were ER positive and PR positive, 25% of grade 2 and 25% grade 3 were also ER positive and PR positive. 68.75% of grade 2 and 75% of grade 3 tumors were both ER negative and PR negative [Table 3].

Table 1: Different histopathological lesions in Benign Breast lesions &malignant breast lesions

Benign Breast lesions		
Histopathological diagnosis	**Number of cases**	**Percentage of benign Breast lesions**
1.Fibroadenoma	78	75%
2.Benign Phyllodes	2	1.92%
3.Gynaecomastia	14	13.46%
4.Breast abscess	4	3.85%
5.Chronic inflammatory pathology	6	5.77%
Total	**104**	**100%**
Malignant breast lesions		
Histopathological diagnosis	Number of cases	Percentage of Malignant breast lesions
1.Infiltrating ductal carcinoma	22	78.57%
2.lobular carcinoma	2	7.14%
3.Medullary carcinoma	2	7.14%
4.Mucinous carcinoma	2	7.14%
Total	**28**	**100%**

Table 2: Correlations between age wise distribution and grade of breast carcinomas

s.no	Age	Grade 1	Grade 2	Grade 3	Total
1	41-50	-(0%)	10 (62.5%)	-(0%)	10(45.5%)
2	51-60	- (0%)	2 12.5%	-(0%)	2(9%)
3	61-70	2 (100%)	4 (25%)	4 (100%)	10(45.5%)
Total		**2 (100%)**	**16 (100%)**	**4 (100%)**	**22 (100%)**

There is statistically significant difference among the grades when compared to the different agent age groups (p=0.042).

Table 3: Association of IHC Hormone receptor status with grade of Breast carcinomas

IHC Hormone receptor status	Grade 1(%)	Grade 2(%)	Grade 3(%)
ER+/PR+	1(50%)	4(25%)	1(25%)
ER+/PR-	1(50%)	1(6.25%)	0
ER-/PR+	0	0	0
ER-/PR- 0		11(68.75%)	3(75%)
Total	2(100%)	16(100%)	4(100%)

Table 4: Comparative study of spectrum of Benign breast lesions & malignant breast lesions

Benign breast lesions				
s.no	**Benign lesions**	**Malik et al (2003)(% benign).[14]**	**Kulkarni S.et al (2009)(% benign).[15]**	**Our study (% benign)**
1	Fibro adenoma	55.0	62.32	75
2	Benign phyllodes	1.27	1.45	1.92
3	Gynaecomastia	-	-	13.46
4	Breast abscess	-	1.45	3.85
5	Chronic inflammatory lesion	-	-	5.77
Malignant breast lesions				
S.no	**Malignant lesions**	**Malik et al (2003) (%malignant).[14]**	**Kulkarni S.et al (2009) (%malignant).[15]**	**Our study (%malignant)**
1	Infiltrating Ductal carcinoma	88.20	84.85	78.57
2	Infiltrating Lobular carcinoma	3.21	3.03	7.14
3	Mucinous carcinoma	0.64	3.03	7.14
4	Medullary carcinoma	2.57	-	7.14

Table 5: Comparative incidence of frequency of grade of Breast carcinomas

Grade of the tumor	Azizun-Nisa et al (2008).[17]	Adedayo et al (2009).[18]	Suvarchala et al (2011).[19]	Ambroise et al (2011).[20]	Ghosh et al (2011).[21]	Geethamala k et al (2014).[22]	Present study
1	6.7	21.2	28.12	9.4	0.3	19	9.09%
2	55.3	38.4	42.18	57.4	15.9	54	72.73%
3	38.0	35.9	29.69	33.3	75.4	27	18.18%

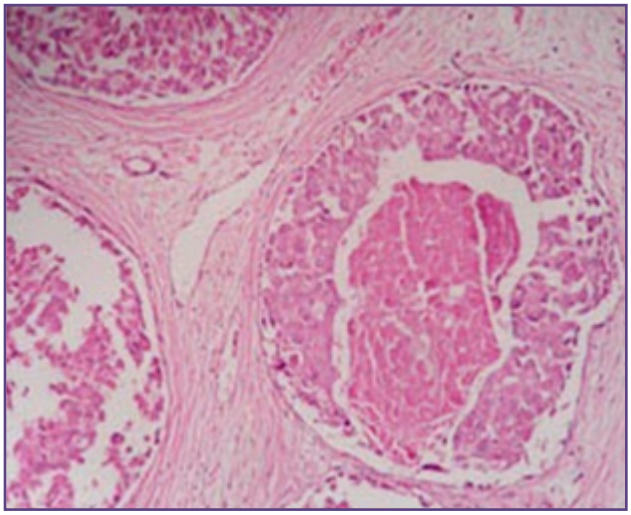

Fig. 1: Photomicroscopic picture of Infiltrating of ductal carcinoma (H&E, 400X).

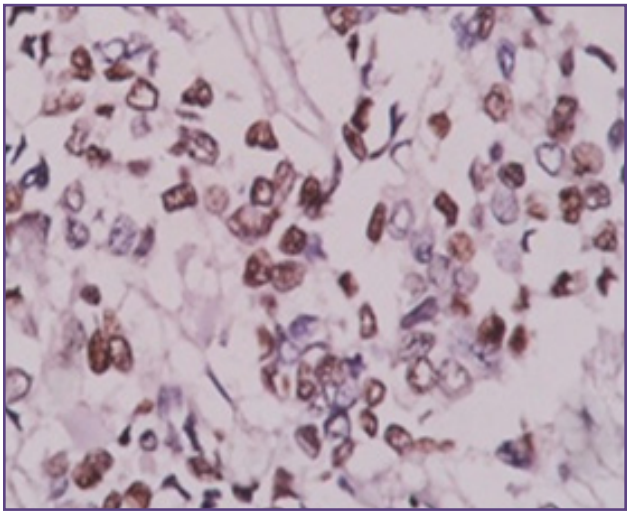

Fig. 2: Photomicroscopic picture of ER positive breast carcinoma (400X).

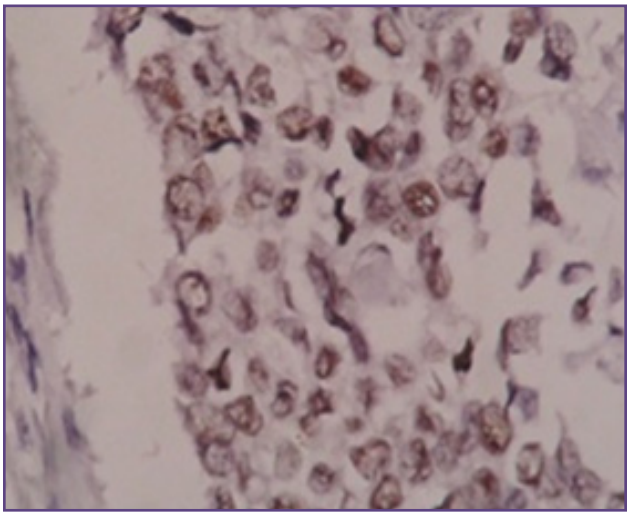

Fig. 3: Photomicroscopic picture of PR positive breast carcinoma (400X)

Discussion:

The breasts are composed of specialized epithelium and stroma that may give rise to both benign and malignant lesions. The human breast contains six to ten major ductal systems. The keratinizing squamous epithelium of the overlying skin dips into the orifices at the nipple and then abruptly changes to a double layered cuboidal epithelium lining the ducts. Successive branching of the large ducts eventually leads to the terminal duct lobular unit.

Two cell types lining the ducts and lobules are luminal epithelial cells overlaying the epithelial cells. Spectrums of breast lesions consist of benign lesions and malignant lesions. Most common benign breast lesions are fibro adenoma, phyllodes tumor, mastitis and breast abscess. Most common malignant lesion are ductal carcinoma, lobular carcinoma, tubular carcinoma, mucinous carcinoma, medullary carcinoma, papillary carcinoma and metaplastic carcinoma.

Breast lesions are more predominance among females when compared to males and histopathological spectrum of breast lesion and their etiology varies among different countries and ethnic group. [6] Benign breast lesions are more predominant as compared to malignant breast lesion as seen throughout the world. [7] Risk factors for both benign breast lesions and malignant breast lesions include multiparty, low parity, low age at first child birth and late menopause, highlighting the fact towards excessive circulating estrogen. [8, 9]

In our study, benign breast lesions comprised 78.78% of the total lesions and malignant lesion 21.22%. The percentage of malignant breast lesions appears to be higher than that in the west (10%) and nearer to Africa (21%). [10, 11]

In our study most common benign breast lesion was found to be fibro adenoma and most common malignant breast lesion was infiltrating ductal carcinoma. Similar results were noted in the other studies. [14, 15]

In our study, among the benign breast lesions, incidence of fibro adenoma is 75% which is higher than the Malik R study (55.0%) and Kulkarni s study (62.32%). [14, 15] The incidence of benign phyllodes is 1.92% which is nearly compatible with Malik R study (1.27%) and Kulkarni S study (1.45%) [14, 15] The incidence of gynaecomastia is 13.46% among benign breast lesion. The incidence of Breast abscess is 3.85% which is higher than the Kulkarini S study (1.45%). [15] Incidence of chronic inflammatory lesion is 5.77 % [Table 4].

In our study, among the malignant breast lesion, incidence of infiltrating ductal carcinoma is 78.57% which is lower than the Malik R study (88.20%) and Kulkarini S study (84.85%). [14, 15] Incidence of medullary carcinoma is 7.14% which is higher than the Malik R study (2.75%).Incidence of lobular carcinoma is 7.14% which is higher than the Malik R study (3.21%) and Kulkarini S study (3.03%). [14, 15] Incidence of mucinous carcinoma is 7.14% which is higher than the Malik R study (0.64%) and Kulkarini S study (3.03%). [14, 15] [Table 4].

In our study incidence of benign breast lesion is 78.78% and malignant breast lesion is 21.22% which is nearly similar to U R Singh et al 2009, Rasheed A et al 2009-2011, and Malik et al 2003 studies. [12, 13, 14,]

In our study, the number of tumors positive for both ER and PR was 27.27% which is nearly similar to Thakral et al 2016 study (25.64%).[16] In our study the number of tumors for ER positive and PR negative was 9.09% which is higher than the Thakral et al 2016 study (5.98%). [16] In our study, the number of tumors negative for both ER and PR was 63.64% which is nearly similar to Thakral et al 2016 study (63.25%).[16]

In the present study majority of breast cancers were grade 2 (72.73%) followed by grade 3 (18.18%) and grade 1(9.09%) which is in concordance with Azizun- Nisa et al 2008 study,[17] Adebayo et al 2009 study,[18] Suvarchala et al 2011 study,[19] Ambroise et al 2011 study,[20] and Geethamala k et al 2014 studies, [22] except for one study by Ghosh et al 2011 having more of grade 3 tumors (75.4%) [Table 5]. [21]

In the present study immunohistochemistry revealed 27.27% of ER positive / PR positive and 63.64% of ER negative / PR negative. These results were in concordance with Survachala et al 2011 study. [19] In the present study significant co-relation was established between ER/PR hormone receptor statuses and grading of tumor. 50% of grade 1, 25% of grade 2 and 25% grade 3 tumors were ER positive /PR positive. 68.75% of grade 2 and 75% grade 3 tumors were ER negative/PR negative. Our study was in concordance with Geethamala k et al 2014 study. [22]

Conclusion

Our study comprised 132 cases of breast lesions. The cases presented to Narayana Medical College & Hospital, Nellore. The biopsy specimens were then evaluated histopathologically, and 22 cases were analyzed by immunohistochemistry. In our study the most common histopathological type of benign breast lesion was fibro adenoma (78 cases, 75% of the total benign breast lesion). The most common histopathological subtype of breast malignancy was infiltrating ductal carcinoma-NOS type (22 cases, 78.57% of the total cases with malignant lesion). Grade 1 tumors (low grade) were showing higher ER, PR expression .Grade 3 tumors (high grade) were showing lower ER, PR expression. Tumor grading highly correlates with the survivor rate and receptor status predicts the response to hormonal therapy.

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Study on Mean Platelet Volume and Platelet Count in Diabetes Mellitus Type 2

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ABSTRACT

Background: Most Diabetes Mellitus related deaths are due to increased risk of micro- and macrovascular complications of the disease. Larger platelets with higher mean platelet volume increase the propensity to thrombosis. Therefore, increased MPV is emerging as a risk factor for vascular complications.

Objectives: To determine mean platelet volume (MPV) and platelet count (PC) in diabetics (type2) and non-diabetics and to determine correlation of MPV and platelet count with fasting blood sugar.

Methods: This prospective study was conducted on 100 subjects with type II DM and 50 non-diabetic controls over a period of 3 months.

Results: Significant increase in value of MPV was seen in diabetics.

Conclusion: MPV being a simple, economical test, can serve as an effective tool in monitoring the patients for thrombogenicity especially in patients with altered blood glucose metabolism.

Keywords: *Diabetes Mellitus, Fasting Blood Sugar, Mean Platelet Volume, Platelet Count*

Introduction

Diabetes mellitus (DM) is a global health problem. Most DM related deaths are due to the increased risk of developing atherosclerosis and micro- and macrovascular complications related to diabetes mellitus. Larger platelets with higher mean platelet volume (MPV) are hemostatically more reactive and produce higher amounts of the prothrombotic factor -Thromboxane A2, increasing the propensity to thrombosis. Therefore, increased MPV is emerging as an independent risk factor for thromboembolism, stroke and myocardial infarction. Activity of platelets is usually determined by very simple platelet indices like platelet count(PC) and MPV. Amongst that MPV is a proven marker of the platelet function and activation. In patients with diabetes, MPV was higher compared with the normal glycemic control.[1] It has been shown that diabetic patients have increased thrombotic adhesion, aggregation, thromboxane synthesis and platelet factor 4 plasma levels.[2] Aim of the present study **is** to determine mean platelet volume and platelet count in diabetics (type2) and non-diabetics and**, t**o determine correlation of mean platelet volume and platelet count with fasting blood sugar.

Materials and Methods:

The present study is a prospective study which included type II diabetic patients who were screened from outpatient clinic of a medical college hospital in South Karnataka, over a period of three months. Institutional Ethical Committee gave approval for the study A total of 100 patients with type II DM and 50 non-diabetic controls belonging to the age-group of 35 years and older constituted the study population. Subjects on anti-platelet medications, subjects with leucocytic or platelet disorders and subjects with Hb<13g/dl in males and Hb<11.5g/dl in females were excluded from the study. Blood samples were collected from these patients after overnight fasting(8 hours) in EDTA and Fluoride vacutainers for measurement of complete blood count and blood glucose levels. All samples were run for blood glucose and complete blood counts within half an hour to avoid variations relating to old samples. Blood glucose levels were tested using automated biochemistry analyzer. The platelet count and MPV estimated using automated blood cell counter.

Results

Initially the MPV and PC of diabetics and non diabetics were compared(Table 1). Independent sample t test was used for the same. In diabetics, MPV had a mean value of 9.64 with standard deviation of 0.81.In non diabetics the mean value was 8.66 with standard deviation of 0.58 . In diabetics, PC had a mean value of 284.21 with standard deviation of 79.41.In non diabetics the mean value was 298.18 with

standard deviation of 78.11. It was concluded that the mean platelet volume showed a statistically significant difference between the groups (p value <0.001) whereas there was no statistically significant difference with respect to platelet count between the groups (p value=0.309)

To examine the correlation of the two groups with respect to MPV and PC, scatter plot was used. When MPV and FBS among diabetics was correlated(Figure 1), it was found that there was significant correlation between the MPV and FBS with Pearson's correlation coefficient of 0.2 and p value = 0.03. Correlation between PC and FBS among diabetics was done (Figure 2). It showed significant correlation between the PC and FBS with Pearson's correlation coefficient of 0.19 and p value = 0.04.

On correlating MPV and FBS among non diabetics(Figure 3), there was no significant correlation between the MPV and FBS with Pearson's correlation coefficient of 0.263 and p value = 0.06. On correlating PC and FBS among non diabetics (Figure 4),there was no significant correlation between the PC and FBS with Pearson's correlation coefficient of 0.06 and p value = 0.65.

Discussion

DM is a complex syndrome characterized by chronic hyperglycaemia responsible for complications affecting the peripheral nerves, kidneys, eyes, and micro- and macrovascular systems. The prevalence of all types of diagnosed diabetes in most western societies is 3–7%. Countries with highest number of diabetics are India(19 million),China(16 million) and United States(14 million) [3,4].The increased morbidity and mortality in DM is due to its vascular complications. Diabetic patients are at risk of increased thrombosis and atherogenesis. Changes in hemostatic balance constitute a pathogenetic factor with a role in vascular complication in DM.

Platelets play a major role in hemostatic balance. Changes in platelets in diabetic patients have been studied extensively

Table 1: Comparison of mean platelet volume and platelet count in diabetics and non diabetics.

	Diabetics		Non diabetics		P value
	Mean	Standard deviation	Mean	Standard deviation	
Mean Platelet volume(fL)	9.64	0.81	8.66	0.58	<0.001
Platelet count (Lakhs/cu.mm)	284.21	79.41	298.18	78.11	0.309

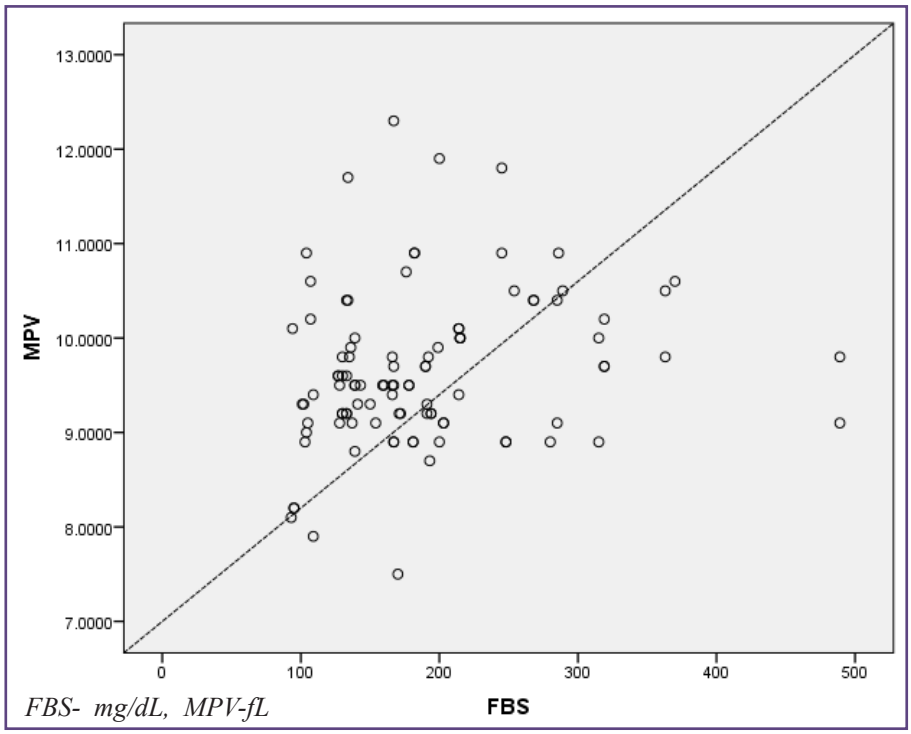

Fig. 1: Scatter plot showing the Correlation between MPV and FBS among diabetics

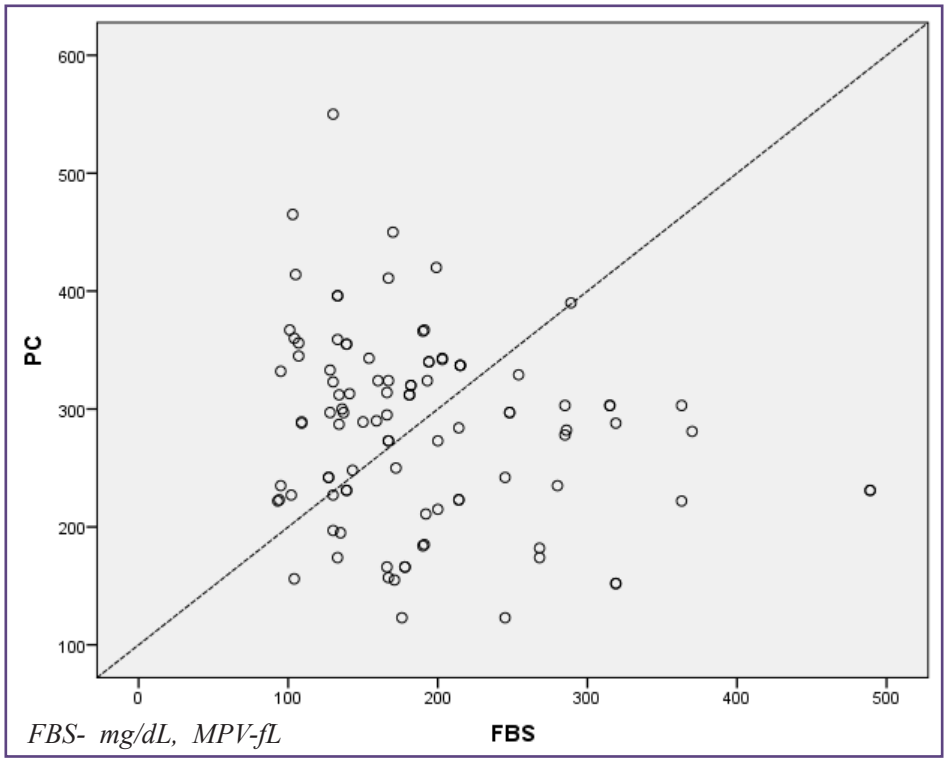

Fig. 2: Scatter plot showing the Correlation between PC and FBS among diabetics.

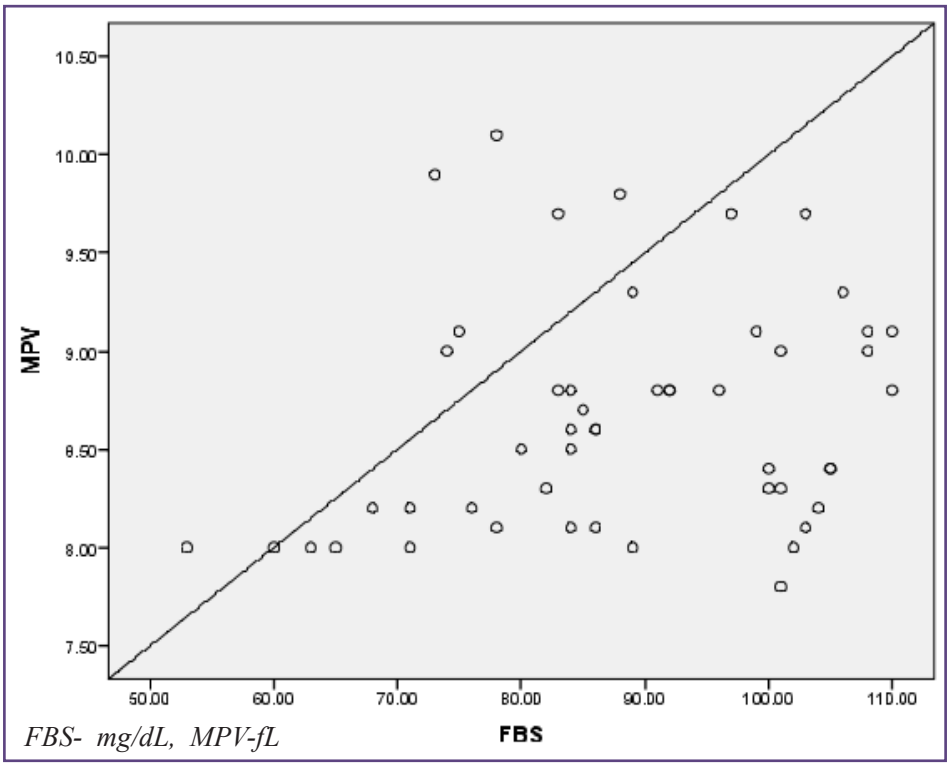

Fig. 3: Scatter plot showing the Correlation between MPV and FBS among non diabetics.

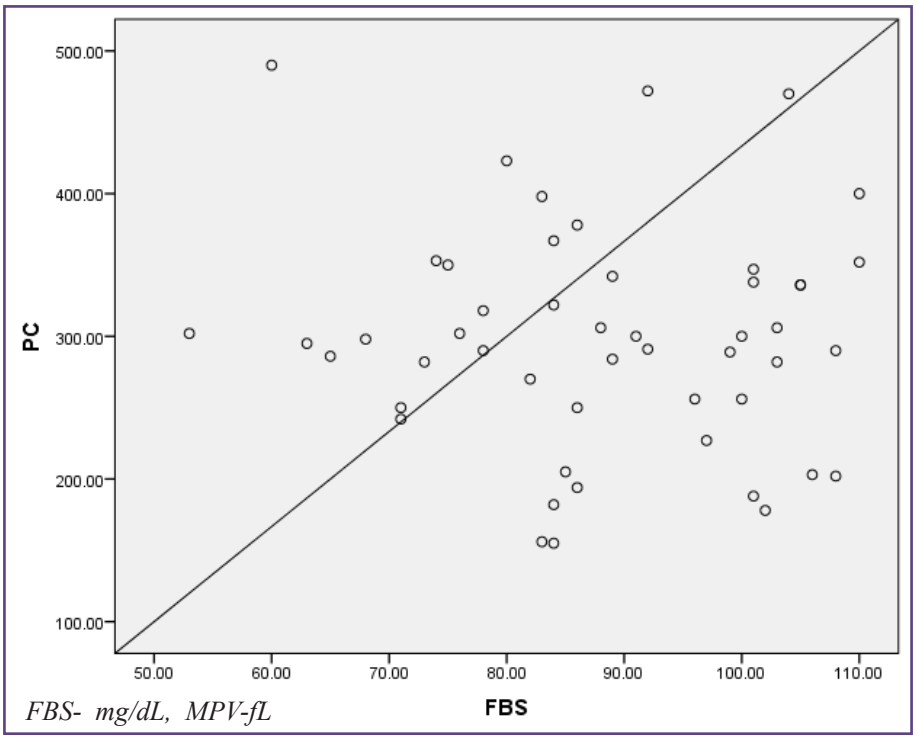

Fig. 4: Scatter plot showing the correlation between PC and FBS among non diabetics

and an increase in thrombotic adhesion, aggregation and secretion has been shown in many of these.

Mean platelet volume is an indicator of platelet function and activation. Large platelets have metabolically and enzymatically denser granules than smaller ones, and display high thrombotic potential [5,6]. This suggests a relationship between platelet functions, mainly MPV and vascular complications of DM. Mean platelet volume, a determinant of platelet activation, is an emerging risk factor for atherothrombosis. Hyperglycemia increases platelet reactivity directly and by promoting glycation of platelet proteins.

Various pathogenic mechanisms were proposed, of which significant ones are:

1. Persistent hyperglycaemia and its metabolic products causing osmotic swelling of platelets
2. Shorter life span of platelets in diabetics leading to higher platelet turn over with younger platelets being larger, more active and thrombogenic.
3. Platelets from patients with altered fasting glucose also have dysregulated signalling pathways as well as have significantly higher von-Willebrand factor levels in serum, resulting in an increased tendency to aggregate and activate. [7,8]

In a study conducted by Kapoor et al 3471 subjects subjects were taken for evaluation of fasting blood glucose (FBG) levels, PC and MPV, they found progressive increase in value of MPV with the increasing FBG levels. The platelet count however, did not show much statistical significance with rising glucose levels.[1] On the contrary ,in a study conducted by Yenigün et al, there was no association found between MPV, and HbA1c and fasting blood glucose, but they found an association between higher MPV and macrovascular complications.[2]

In a study conducted by Akinsegun et al on diabetics, a positive statistical Pearson's correlation was seen between MPV and fasting blood sugar and duration of diabetes. While a negative correlation was seen between platelet count and fasting blood sugar, and duration of diabetes.[7]

Kodiatte et al conducted a study which showed that, in diabetes mellitus platelets become more reactive and aggregable and their mean volume(MPV) is increased. The increased platelet size may be one of the factors in the increased risk of atherosclerosis associated with diabetes mellitus and associated vascular complications. And they concluded that MPV would be a useful prognostic marker of cardiovascular complications in diabetes.[8]

A study by Ulutas et al showed a relationship between MPV and HbA1c. They suggested that platelets of diabetic patients become more aggregable and reactive due to increased MPV. Increased risk of atherosclerosis in type 2

DM may be a result of high MPV. Therefore, MPV might be a useful prognostic marker of cardio-vascular complications in patients with type 2 DM.[9]

In a study conducted by Ozder et al it was shown that in diabetes mellitus, MPV is increased and it is indicative of worsening glycemic control. The increased platelet size may be one of the factors in the increased risk of atherosclerosis associated with diabetes mellitus and associated micro- and macro-vascular complications. Hence, MPV would be a useful prognostic marker of cardio-vascular complications in diabetes. They also proposed that increase in HbA1c was directly proportional to increase in MPV.[10]

In a study by Coban et al, results showed that subjects with impaired glucose tolerance(IGT) have higher MPV, which suggests increased platelet activation. Increased platelet activity could contribute to increasing the risk of atherothrombotic complications in IGT. Thus, due to the positive correlation between MPV and 2 h plasma glucose levels during oral glucose tolerance test, MPV may have clinical implications.[11] According to a study conducted by Dayal etal mean MPV was significantly higher in the diabetic group compared to the controls[12] .

Shah et al. reported a significant correlation between MPV and the degree of glycemic control in diabetic patients. They suggested that the positive relationship between an increased glucose level and increased MPV is a unique phenomenon of diabetes.[13] Although Kim et al mentioned negative correlation between MPV and FBG with normal glucose tolerance and intermittent hyperglycemia in Korean subjects, there was positive relationship between an increased glucose level and increased MPV in diabetes .[14]

In our study ,MPV showed significant correlation with FBS among diabetics similar to the results obtained in study by Kapoor et al,Kodiatte et al,Ulutas et al, and Ozder et al.[1,8,9,10]

In our study, the mean platelet count in the diabetic group was lower than that of the nondiabetic group that was similar to the studies done by Hekimsoy et al[15]. It is however in contrast with the findings of a study conducted by Thomas et al[8]. This suggests that the platelet count is the net result of the interplay of platelet survival and platelet production rate.Hence the platelet count could be dependent on several variables,that is, mean platelet survival, platelet production rate, and turnover rate in DM.

Keeping in view the uncertainties regarding results of various studies conducted in past further large prospective studies should be conducted to confirm the clinical utility of this marker and also to elucidate the association between MPV and platelet hyper-reactivity in relation with fasting plasma glucose levels. Therefore, it may be concluded that glycemic control decreases the hyperactivity of the platelet function and thus may prevent or delay possible diabetic vascular complications.However all these data has to be confirmed with larger studies.

Conclusion

Our study supports the possibility of increase in MPV with rise in fasting plasma glucose levels. It is emphasized that MPV can be quick ,cost effective and very simple tool especially in developing country like India with limited resources to predict early vascular complications in patient with altered glucose metabolism.

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Incidental Findings on Liver Autopsy with Specific Emphasis on Hepatitis B

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ABSTRACT

Background: Silent liver disease is a major cause of morbidity and mortality in the population due to its silent progression to end stage liver disease without significant symptoms. These have to be detected at an earlier stage to reduce the morbidity. This study is aims at correlating the gross and microscopic morphology of 100 liver specimens obtained by autopsy and to correlate with the clinical parameters.

Methods: 100 liver specimens obtained by the autopsy of 100 patients, who died of causes like road traffic accidents, poisoning etc. with no medical history during the period from 2007 to 2012, were taken for the study. Histological samples of liver specimens were stained with the routine heamatoxylin and eosin staining, along with special stains like PAS, Reticulin, Perl's Prussian blue and Orcein staining.

Result: The most common pathology was steatosis (25%) following normal histology (49%), which was followed by chronic venous congestion (12%), chronic hepatitis (6%), cirrhosis (4%), liver cell dysplasia (2%) and metastases (2%). These findings have been correlated with age, sex and special staining. Orcein staining identified ground-glass hepatocytes with detection of Hepatitis B surface antigen in a background of chronic hepatitis and cirrhosis, indication the most probable etiology of Hepatitis B.

Conclusion: This study reiterates the unexpected higher prevalence of NASH among the younger generation and the importance of clinical autopsy in understanding the magnitude of clinically silent liver lesions, mainly Hepatitis B.

Keywords: *Hepatitis, Cirrhosis, Orcein Stain, Steatohepatitis, Liver Autopsy.*

Introduction

Silent liver disorders, similar to disease processes in other organs of the body may be symptomatic or may not sometimes produce evident phenotypic features to aid in early diagnosis and treatment. The disease process may progress and then later produce symptoms that may be brought to the purview of the clinician. Though early diagnosis has been made possible with the advent of latest technological and radiological advances, a major portion of disorders remain underdiagnosed which can even progress silently to death. Thorough postmortem examination identifies the cause of death or validates the clinical diagnosis that helps in unleashing the underlying disorders. Pathological processes like fatty liver disease, alcoholic and non-alcoholic fatty liver disease, chronic hepatitis and carriers of the same, etc. produce an indolent course which fails detection through the regularly applied tests until the process becomes more severe. With the increasing prevalence of Hepatitis B in the world, failure to diagnose it in the absence of symptoms increases the chain of transmission thereby producing a vicious cycle. Through this study we would like to study the presence of various diseases in the absence of clinical history by examining the gross and microscopic morphologies of liver specimens correlated with their age and sex prevalence obtained from the autopsy of 100 patients. Also, to identify the pathology of viral hepatitis, especially hepatitis B using the special technique of Orcein staining and to associate its clinical implication in undiagnosed hepatitis B cases which pose a threat to the community.

Materials and Methods

The material for study, which included liver specimens, was obtained from the autopsy of 100 patients during the period from 2007 to 2012. The cases were of road/railway accidents, poisonings, etc with no known history of liver disease. The specimens were weighed and examined for gross abnormalities and fixed with 10% formalin, followed by taking small bits of tissue, 2 each from both the lobes of the liver. Histological samples were stained with the routine heamatoxylin and eosin staining, along with special stains like PAS, Reticulin, Perls Prussian blue and Orcein staining.

Orcein staining is done by preparing Shikatas orcein solution is prepared by dissolving 1 g of orcein powder in 100 ml of 70 % alcohol. 1 ml of concentrated nitric acid is added to it and kept aside at room temperature for 24 hours. The tissue is then deparaffinized and hydrated with distilled

water. It is oxidized in freshly prepared acidified potassium permanganate for 5 minutes followed by being bleached in 2 % aqueous solution of oxalic acid for 1 minute or until the sections turn white. The tissues are washed well with water and rinsed briefly with 70 % alcohol. It is now placed in modified Shikatas solution for 10 minutes. Excess stain is removed by placing in 70% alcohol. The tissues are then studied under the microscope to look for positively stained ground glass hepatocytes.

The samples were studied and analyzed according to the criteria as follows:

Steatosis: presence of fatty change; graded as 1+ to 4+ depending on the percentage of cells containing fat.

Steatohepatitis: presence of pericellular fibrosis, portal and acinar inflammation, ballooning degeneration, hepatocyte necrosis associated with fatty change.[1]

Chronic venous congestion: presence of sinusoidal dilatation, congestion and presence or absence of centrilobular necrosis.

Chronic hepatitis: interface hepatitis, portal inflammation and spotty necrosis.

Cirrhosis: presence of regenerative nodules and fibrosis.

Liver cell dysplasia: cellular enlargement, nuclear pleomorphism, multinucleation with normal nuclear: cytoplasmic ratio.

Ground glass hepatocytes identified by Orcein staining are graded as Type I- marginal GGH (positive staining of cytoplasmic periphery), Type II- diffuse GGH (diffuse staining), Type III- Globular GGH (globular staining of hepatocytes), Type IV- spotty drop like positive structures, Type V- stained GGH with fatty changes.[2]

Each of the above said morphological features were analyzed with respect to various parameters such age, sex, weight of the liver, special stains, etc.

Result

This study dealt with the morphological and histological examination of liver biopsy specimens obtained through the autopsy of 100 patients, who died of road/railway accidents, poisoning, etc., in which no history of any medical ailment was available. The liver tissue was obtained, sectioned, stained and observed under the microscope following the gross examination of the same.

Out of the 100 cases studied, 49 cases (49%) showed normal histology [Figure 1]. Fatty change was the most commonly seen morphology in 25% of cases among which 8% were observed to be associated with hepatitis [Figure 2]. Chronic venous congestion was seen in 12% of cases, which was the second most common. This was followed by chronic hepatitis in 6%, and 4% with cirrhosis and 2% each of liver cell dysplasia and metastases. Each of these histological findings were correlated with respect to age, sex and findings on using special stains. This was done by categorizing the cases into 7 groups based on the age, in intervals of 10 years like Group A- 21 to 30 years, Group B-31 to 40 years and so on till Group F- 71 to 80 years.

The finding of steatosis was the most predominantly observed histology in 17% of cases of which, 88.2% were males and 11.8% females. Its incidence was maximum in the age group of 31-40 years (41.1%) followed by 31.3% in 51-60 years. It was not very evident in cases aged older than 60 years.

In 8% of the cases with steatohepatitis, 62.5% were males and the rest, females. Equal incidence was seen in the ages between 31-40 years and 41-50 years, 37.5% each. No cases were noted with this change in the ages between 21-30 years and 71-80 years.

Chronic venous congestion was seen in 12% of cases, of which 83.3% were males and 16.7% females. 41.7% of these cases were seen in the age group of 41-50 and least observed between 61-80 years and not seen in cases aged between 21-30 years.

Among the 4% of cases with cirrhosis, all the 4 were males (100%) with 3 cases (75%) aged between 61-70 years and 1 case (25%) between 41-50 years [Figure 3]. Grossly, two were of micronodular variety, and one each of macronodular and mixed types.

Chronic hepatitis was noted in 6% of cases among which, the male:female ratio was equal. The major bulk was seen in the age group of 41-50years (66.6%) [Figure 4].

Liver cell dysplasia was observed in 2% of cases of which 1 was a male and the other female. Both were noted in the age range of 71-80 years.

Liver metastases were observed in 2% of cases of which one was male and the other female in the age groups of 51-60 years and 41-50 years respectively.

Special stains were applied to the specimens which gave the following results: PAS for estimating the presence of glycogen and Perl's stain for iron to assess the random prevalence of hemochromatosis did not show significant positivity except for Grade 2+ positivity for increased iron stores in one case in the age group of 61-70 years which was seen in association with micronodular cirrhosis, probably implicating alcohol etiology. Orcein staining was

done [Figure 5], the technique of which was as proposed by Borchard and Gussman.[3] Among which the maximum positivity was observed in the age range of 41-50 years (5 cases). All of these were seen along side normal histologies, steatosis, cirrhosis or chronic hepatitis. One case was seen with chronic hepatitis and one with cirrhosis (7.14% each) which implicated a viral etiology in these cases most probably that of hepatitis B. The other cases were seen with steatosis (42.8%), cirrhosis (14.3%), chronic hepatitis (7.14%) and with normal histology in 35.7% of cases [Figure 6]. Among these, 71.4% were males and the rest females. Of the five cases with normal histology, four showed type I and one type II. Of the 6 cases with steatosis, type I and type II were shown by 50% each of the cases.

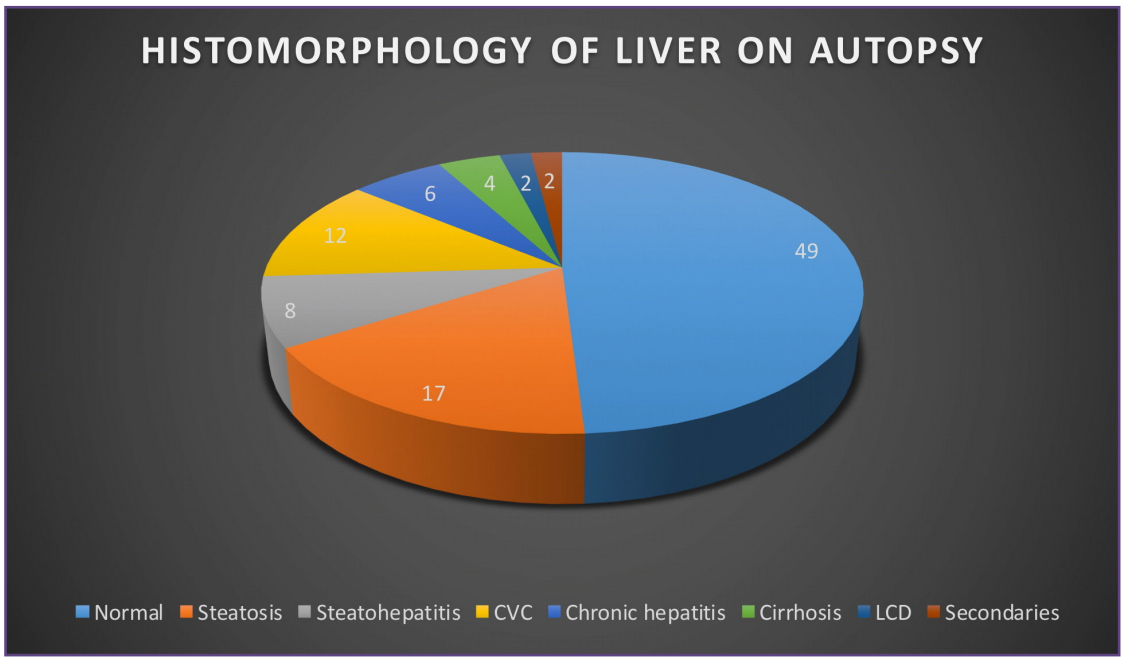

Fig. 1: Histomorphology of liver on autopsy.

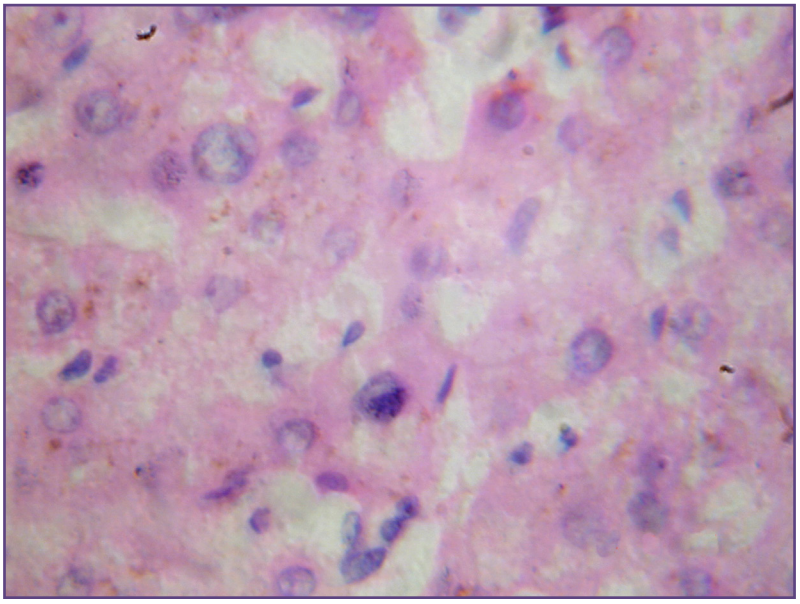

Fig 2: Ground glass hepatocytes H and E x400.

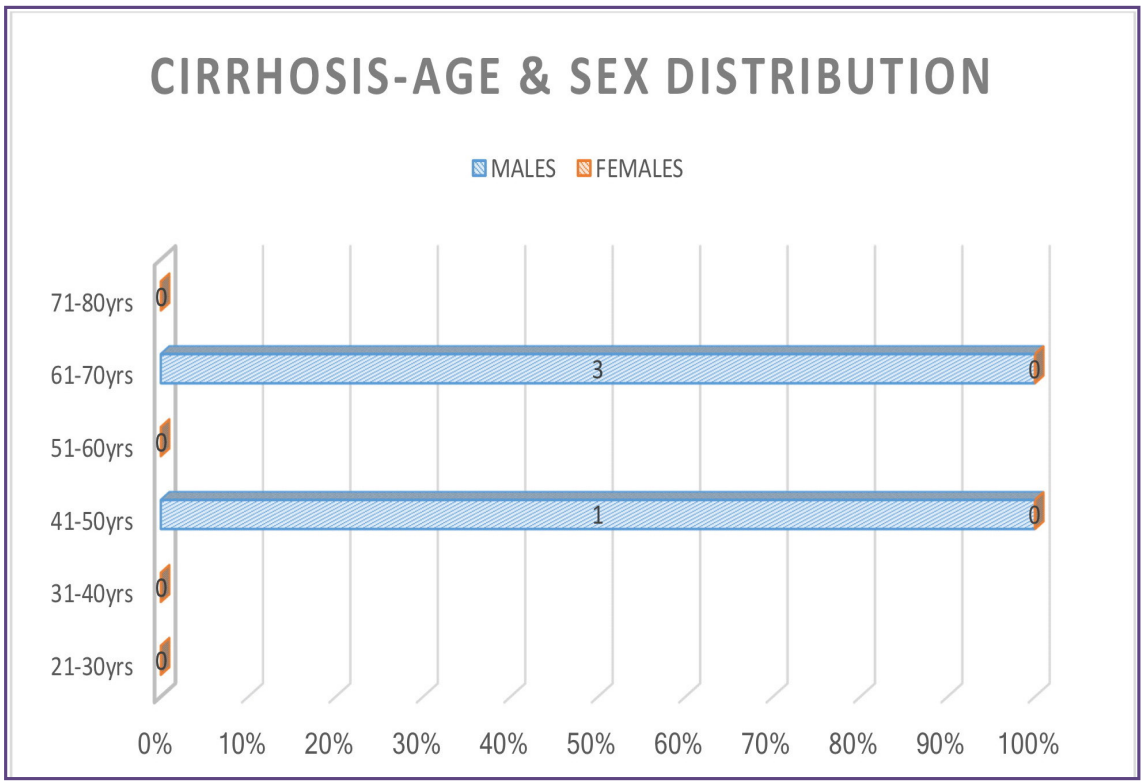

Fig. 3: Cirrhosis - Age and sex distribution.

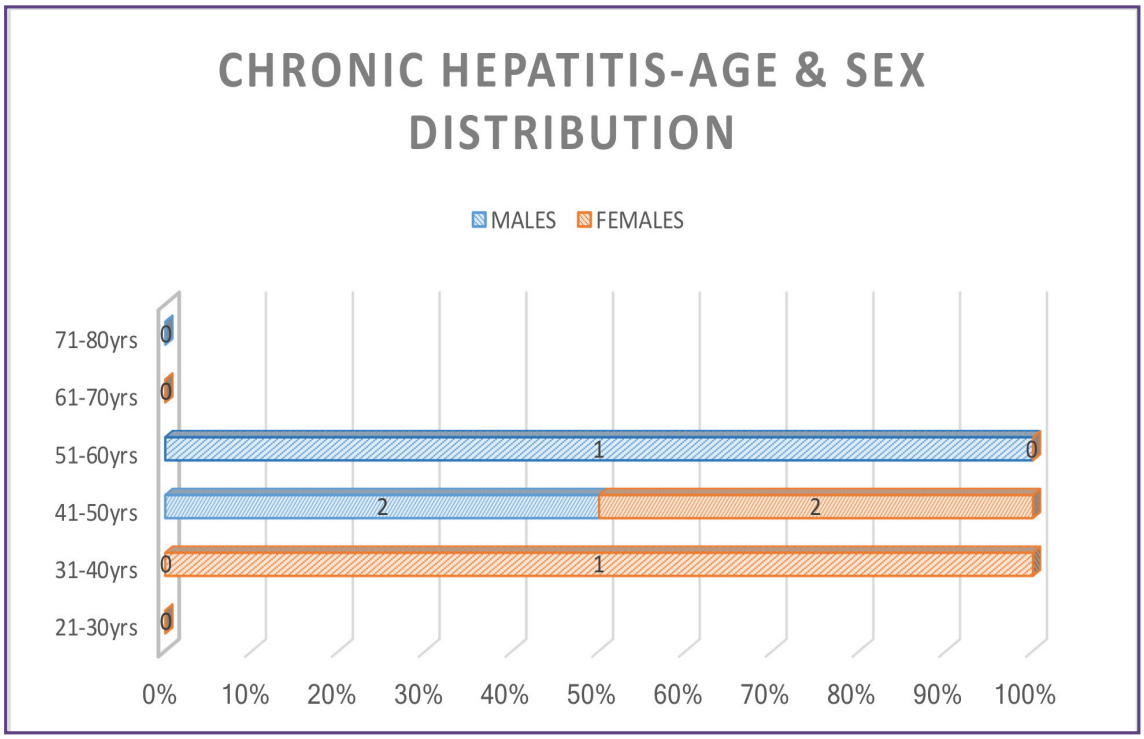

Fig. 4: Chronic hepatitis - Age and sex distribution.

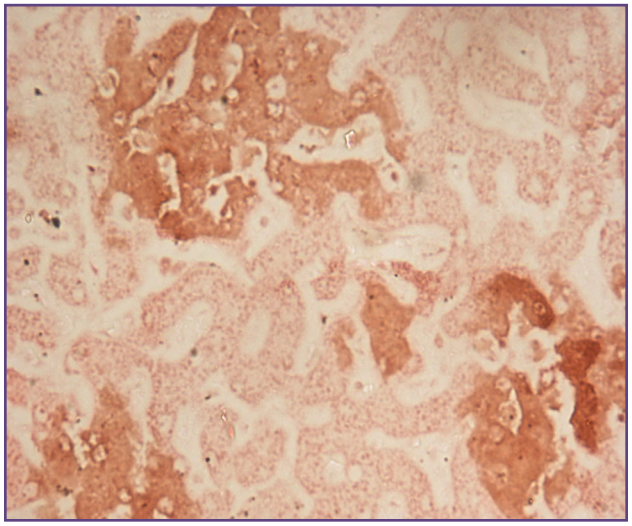

Fig. 5: Positive staining Ground glass hepatocytes - Orcein stain H and E x100.

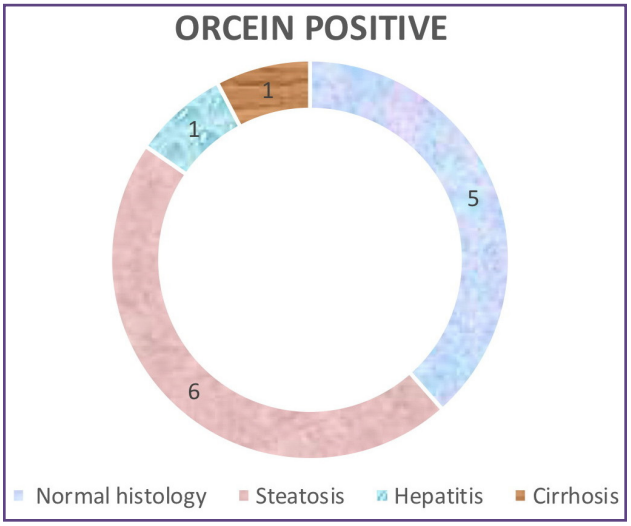

Fig. 6: Orcein positivity in relation with the histopathology.

Discussion

Postmortem examination dates back to nearly third century BC and is being performed till date for various reasons. Though it has been performed for thousands of years, it was during the eighteenth century that it came to be recognized as being fundamental to medical practice. It is one of the best tools to validate clinical diagnosis.[3] Gall in his write-ups in the years 1912, 1937,1960 and 1968 has mentioned that "when autopsy records were used to measure the range of clinical error, there was approximately an identical percentage of diagnostic error". Though the reasons for this finding are many, it remains undoubted that clinical diagnosis needs surveillance, the best tool being autopsy.[4] Later, Bauer and Robbins have also suggested that there exists a discrepancy between the clinical diagnosis and anatomical findings, all of which cumulatively suggest the indispensable nature of autopsy to clarify the clinical diagnosis to help in better disease detection and hence in treatment of the cases that follow.[5]

Liver is the site of many diseases, many of which become symptomatic, while some are diagnosed only during autopsy. Silent liver diseases, which are becoming an emerging threat to health have to be studied in detail to identify them earlier and provide appropriate treatment. The exact incidence or prevalence of these diseases in unknown because most of these need an invasive investigation in the form of liver biopsy, which is usually not done on a routine basis. In the USA, Steatosis is identified to be the major silent liver disease, while non-alcoholic steatohepatitis constitutes a major minority, whose incidence is increasing. A meta analytic study to estimate the prevalence of NASH showed that the global prevalence is around 25.24%.[6]

The term NASH has been coined by Ludwig et al, with the disease process ranging from uncomplicated steatosis to steatohepatitis to steatonecrosis to liver cell failure.[1] In India, alcohol related liver disease is the major health problem. Alcoholic liver disease has been reviewed by many and mentioned in great detail by McSween, Maddrey WC, Rabin and Ishak, et al, however the true incidence of alcoholic hepatits is not knowing owing to the late presentation to the clinician, ending up in cirrhosis. The susceptibility of patients to alcohol related liver disease has been studied by Saunders et al and Sorensons et al.

The other common disease being hepatitis, where extensive research has been done. With the increasing prevalence of Hepatitis B worldwide, its diagnosis and detection of carrier states is needed to reduce the transmission. This is done through serum tests or if asymptomatic, usually diagnosed only on autopsy. Cirrhosis, which is on the extreme end of the spectrum of liver disorders due to various etiologies, can be diagnosed usually on biopsy only, which is seldom done. Morphological classification of the cirrhotic liver helps in establishing the etiology.

Further, using special stains aid in more specific detection of the diseases. Perl's stain for the detection of iron stores in the form of both ferritin or hemosiderin. A definitive grading system was established by Searle JW, Ken JFR, et al. A mild increase in the levels of iron has been observed in cases with non-alcoholic steatohepatitis. The detection of glycogen has been made easier with PAS staining, which was reviewed by Sheehan DC and Luna et al. A special stain called Orcein was developed and later modified by Shikata, which was used to stain hepatocytes for the detection of Hepatitis B surface antigen.[2] Positively staining ground

glass hepatocytes were graded in five grades.[2] In acute viral hepatitis, no ground glass hepatocytes were found but a few positively staining phagocytes were seen. Types I and II are usually seen in carriers and patients with minimal hepatitis B. in cases with chronic hepatitis, type II and III were observed, while type IV was seen in patients with chronic active hepatitis. Kostich ND, Ingham CD et al, used the orcein and modified Shikata's orcein staining to diagnose carriers and chronic hepatitis.

In our study, steatosis which is the most common histological finding which was mostly seen in the 3rd to 5th decades of life and also more among the males than females. This was in similarity with the study done by M.S.Bal, S.P. Singh et al, which included patients aged more than 40 years, where in steatosis was seen in 53.85% cases in the 5th decade falling to 35.9% in the 6th decade.[7]

In 8% of the cases with steatohepatitis, maximum incidence was seen in the third and fourth decades and males are more frequently affected while the female proportion of these cases may implicate non alcoholic steato-hepatitis, which is one of the more common liver pathologies in non-alcoholics. In the study of Soutoudehmanesh, Soutoudeh et al, the prevalence was found to be 2.1% which compared well with our study, though in lower proportions, which may be attributed to the racial and social differences.[8]

Among the cases diagnosed with chronic venous congestion, a higher proportion of males was again noticed with more number of them clustered in the 41-50 years age group. Bal et al, reported a higher percentage of cases in the fifth decade, while the study by Ghazala anif, Hannan et al, reported a much lower proportion of 2.7% of the same.[7,9]

Maximum incidence of cirrhosis was noted among males between the age group of 61-70 yrs. this could probably be attributed to alcoholism. Ghazala anif, Hannan et al, showed a similar incidence of around 4.5%, while, Bal et al, reported a much higher rate of 42.85% in the 41-50 years age group. However, Sotoudehmanesh, Soutodeh et al, observed only 0.8% of cases to be of cirrhosis liver.[7,8,9]

Chronic hepatitis was more common among the 4th decade with an equal incidence among males and females. Bal et al, and Soutoudehmanesh et al, reported similar incidences of 3% and 2.6% respectively while, Ghazala anif, Hannan et al, reported chronic hepatitis in 12.7% of cases.[7,8,9]

Liver cell dysplasia was observed in only 2% of cases and this finding was not reported in most of the earlier studies. However Anthony et al reported its presence of 1% in normal appearing livers. A few cases were positively associated with Hepatitis B along with its usual correlation with hepatocellular carcinoma. Liver is a common site for metastases, which has been observed in 2% of cases in our study. Borja ER, Hori et al, reported 25 cases of carcinomatous cirrhosis with secondaries from breast carcinoma.[10] The diagnosis of the primary from liver examination can be done by immunohistochemical methods and detection by histopathology is not possible.

Orcein stain, which is specific for Hepatitis B surface antigen in the ground glass hepatocytes, was done to assess the random prevalence of Hepatitis B surface antigen with a sensitivity of around 80% in cases with minimal changes, chronic hepatitis and cirrhosis. It stains the viral coat better than the core antigen. It therefore cannot be used as a test for primary detection of Hepatitis B, but nevertheless, its use in detection of carriers has been proven to be effective. Shikata's stain also shows positivity with elastic fibrous tissue, thereby contributing to its findings in those lesions apart from those seen in Hepatitis B. It also stains positively with copper inclusions, which needs to be excluded if significant. Cases of chronic venous congestion showed type I positivity. In the patients with cirrhosis, one showed type I and the other type III, whereas in cases of chronic hepatitis, type III was observed. Types I and II indicate carrier state and minimal hepatitis, whereas, types II and III are observed in chronic hepatitis. In a study done by N C Nayak and R. Sachdeva, The effectiveness of orcein staining has been compared with immunoperoxidase methods and was found been to be as effective as the latter and less time consuming, though the sensitivity is more.[11] This shows that orcein staining is a good tool to identify Hepatitis B surface antigen, though few false negative or positive cases may occur.

Conclusion

This study has helped in identifying hepatitis B in the autopsy specimens through special staining, which could have had far reaching consequences both clinically and socially in the form of spread of the disease from asymptomatic cases or carriers. This has also been felt to be a major health hazard to the health care professionals, which can be reduced by its earlier detection. Steatosis, though a normal finding in many aged people, has been seen as early as 22 years of age. In our population of study, chronic venous congestion, chronic hepatitis, steatohepatitis and cirrhosis were seen as early as around 40 years of age and clustered more in the 4th and 5th decades of life. Steatohepatitis was found among the females which points to the probable possibility of non-alcoholic steatohepatitis, also implicating the unexpected higher prevalence of NASH among the younger generation and that metabolic syndrome continues to be underdiagnosed among the female population. This study thus reiterates the importance of clinical autopsy in understanding the magnitude of clinically silent liver lesions, mainly Hepatitis B.

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Utility of a Serum *Aspergillus* Galactomannan Assay in Diagnosis of Invasive Pulmonary Aspergillosis in HIV/AIDS Patients: A Prospective Analysis

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ABSTRACT

Background: Invasive pulmonary aspergillosis (IPA) is a life threatening infection among immunocompromised patients. A timely and precise diagnosis of this condition is often precluded by lack of sensitive and specific noninvasive diagnostic tools. Detection of galactomannan has emerged as a promising tool in this regard. While the analytical performance of serum galactomannan assay has been extensively evaluated in various high risk groups, its possible utility in HIV/AIDS patients is yet to be explored. The present study was undertaken to evaluate the yield of a serum galactomannan assay for diagnosis of IPA in a cohort of HIV/AIDS patients.

Methods: 49 febrile HIV reactive patients with co-existent symptomatic AIDS were prospectively enrolled for the evaluation. Blood samples were drawn from each patient and serum galactomannan screening was performed employing the commercially available serum galactomannan assay, Platelia™ Aspergillus EIA. An optical density index >0.5 for at least two serum samples was considered the defining criteria for a positive result. Furthermore, sputum samples were collected and direct microscopic examination (10% potassium hydroxide wet mount) and fungal cultures were performed.

Result: In accordance with the definitions laid down under EORTC/MSG (European Organization for Research and Treatment of Cancer / Mycoses Study Group) criteria, ten cases of probable and 23 cases of possible IPA were diagnosed. The sensitivity, specificity, and positive and negative predictive values of Platelia™ Aspergillus EIA for patients with probable IPA were 90%, 100%, 100% and 94.12 % respectively.

Conclusion: Serum galactomannan assay has a high sensitivity and specificity and when complemented with relevant lower respiratory tract symptomatology and imaging findings, can serve as a useful adjunct to conventional modalities for the diagnosis of IPA in HIV/AIDS patients.

Keywords: *Galactomannan, HIV/AIDS, Invasive Pulmonary Aspergillosis, Serum*

Introduction

Invasive mycoses such as invasive pulmonary aspergillosis (IPA) are a significant cause of mortality and morbidity in the immunosuppressed population.[1,2] In human immunodeficiency virus (HIV) infected persons in particular, the median survival is nearly 3 months following a diagnosis of IPA. [3]

An accurate diagnosis of IPA is a challenge till date. The clinical signs and symptoms are generally non-specific and chest roentgenographic features are frequently absent. [4, 5] High resolution computed tomography (HRCT) findings are also not independently diagnostic and fail to differentiate IPA from candidiasis, cryptococcosis and bacterial infections. [6, 7, 8] A histopathological demonstration of invasion of lung tissue with fungal hyphae, in conjunction with isolation of *Aspergillus* species from tissue samples is generally considered the reference standard for a diagnosis. [9, 10] However, the diagnostic yield of routine mycological procedures such as microscopy and culture is generally low. [8, 11] Moreover, interpretation is often complicated by difficulty in discriminating invasive disease from colonization. [11] Tissue biopsy, too, is invasive and seldom not feasible due to serious underlying condition of the patients and frequent presence of hypoxia, cytopenias, coagulation abnormalities and hemodynamic disturbances. [4, 8, 12]

Keeping in view the limitations of the currently available diagnostic modalities and with the understanding that early diagnosis of IPA would result in better patient outcomes, the significance of newer and rapid diagnostic platforms

in guiding prompt therapeutic management cannot be undermined. This has led to a search for non-invasive and non-culture- based approaches for an early and rapid diagnosis of invasive aspergillosis. Advent of biomarker assays has been a major breakthrough in this regard. These tests detect circulating *Aspergillus* antigens, metabolites or nucleic acid targets as surrogate markers for diagnosing this invasive condition. [13] While molecular methods such as polymerase chain reaction have been evaluated for this purpose, lack of standardization and validation of these procedures has limited their diagnostic utility. [14]

The second approach, that is, detection of cell wall components such as galactomannan and 1, 3-ß-D-glucan offers a promising alternative. Galactomannan, a cell wall component, is not released into the circulation till the fungus invades the endothelial compartment and is thus a marker of angioinvasion by the fungus. [15] Initial assays for detection of this antigen included latex agglutination test and suffered from low sensitivity. [4, 5] The currently available technique is a double sandwich enzyme linked immunosorbent assay (ELISA), that has been extensively evaluated and has also been approved by the United States Food and Drug Administration for diagnostic use. [4, 16]

While assays for galactomannan detection are widely available in India and have been used in high risk population groups such as stem cell transplant recipients and patients with hematological malignancies, there is virtually no published data regarding its possible use for diagnosis of IPA in HIV positive patients. [17, 18] The present study was undertaken to detect circulating serum galactomannan antigen in a group of febrile HIV reactive patients with co-existent symptomatic acquired immunodeficiency syndrome (AIDS) and to assess its yield and diagnostic performance for diagnosis of IPA.

Materials and Methods

The present, cross-sectional, single institutional and non-interventional study was conducted in a cohort of HIV reactive patients seeking medical care at a tertiary care teaching hospital in New Delhi, India. From October 2008 to September 2011, HIV positive patients from various inpatients units as well as those attending anti-retroviral treatment clinic were prospectively analyzed. Patients were eligible for enrollment if they had a body temperature >38°C with co-existent symptomatic AIDS (a host factor criterion consistent with invasive pulmonary aspergillosis). [10] A convenient sample of 234 consecutive HIV positive subjects was chosen from which 49 subjects fulfilling the inclusion criteria were selected. HIV reactive patients on long term steroids or other immunosuppressive agents; those receiving piperacillin-tazobactam or amoxycillin-clavulanate; and those with a concurrent neutropenia were excluded from the analysis. The study protocol was approved by the institutional ethics committee and written informed consent was requested from all enrolled subjects before recruiting them for the study.

A standardized, pre-designed, structured proforma was used to collect information regarding patients' sociodemographic characteristics, clinical profile, radiological findings and various laboratory parameters. All the patient data were anonymized in order to maintain confidentiality.

Blood samples were collected following standard precautions and transported to the laboratory. Sera were separated and stored at -70 °C till the serum galactomannan assay was performed. All patients were screened for serum galactomannan employing the Platelia™ *Aspergillus* EIA (Bio- Rad Laboratories). The test was performed and analyzed in accordance with the procedural and interpretative details as mentioned in the manufacturer's guidelines. An optical density index (ODI) >0.5 was considered as positive. While a patient with a negative result with Platelia™ *Aspergillus* EIA was considered as a galactomannan negative case, a repeat sample was tested in patients with a positive result. The test result was considered positive only when the second sample yielded the same result. Furthermore, 20 healthy HIV negative individuals with no clinical, radiological or microbiological evidence of invasive pulmonary aspergillosis were recruited as controls to establish the validity of the Platelia™ *Aspergillus* EIA kit.

In addition, sputum as a respiratory source for mycological assessment was collected from each patient and subjected to direct microscopic examination (10% potassium hydroxide wet mount) and fungal culture on Sabouraud's dextrose agar. The cultures were examined every second day for up to six weeks before they were reported as sterile. Any suspected fungal growth during this period was examined for gross colony morphology including color, texture, topography and the underside or reverse. Further confirmation of the isolates was done by slide culture technique, preparing lactophenol cotton blue mount and examining with the aid of 10 X and 40 X objective of the microscope. Speciation of *Aspergillus* isolates was done based on characteristic morphology of their hyphae and spores under microscopy and employing tests as per standard protocol. [19] Any positive result on microscopy and/or culture was confirmed by subsequent sampling and repeat demonstration/isolation.

Each patient was classified as probable, possible and non-IPA as per definitions laid down under EORTC/MSG (European Organization for Research and Treatment of

Cancer / Mycoses Study Group) criteria. [10] While probable cases required a compulsory microbiological proof in addition to host factors and clinical criteria, possible cases required compatible host factors in conjunction with either clinical features or mycological findings.

Descriptive statistics were performed with arithmetic mean and standard deviation calculated for central tendencies and median for non-normal/skewed distributions. The analysis was performed using the Epi info software, version 3.5.3, CDC, Atlanta, GA, USA. In addition, serum galactomannan assay and lower respiratory tract *Aspergillus* isolation were assessed with respect to sensitivity, specificity, positive predictive value, and negative predictive value of these tests. The values were calculated with their respective confidence interval of 95%. For performing the calculations, possible IPA cases were not included in the analysis.

Results

Thirty three (67.3%) of our study participants were drawn from the antiretroviral treatment clinic while 16 (32.7%) were recruited from the various inpatient units. The mean and median age of the study subjects was 35.24±9.43 years and 34 years respectively (range=18-61 years). The study population comprised of 36 (73.5%) males and 13 (26.5%) females with a male to female ratio of 2.8:1. The study group included 42 (85.7%) married and seven (14.3%) unmarried participants. Heterosexual promiscuous was the most commonly reported mode of HIV transmission documented in 43 (87.8%) study subjects followed by intravenous drug abuse in four (8.1%) and blood transfusion in two (4.1%). Fever, cough and dyspnea were the most common clinical manifestations noted in 49 (100%), 32 (65.3%) and 15 (30.6 %) patients respectively. A high proportion of patients (29; 59.2 %) had tuberculosis as an underlying predisposing condition and ten (20.4%) patients had a *Pneumocystis carinii* pneumonia. The mean and median CD4 count of the study population was 196.57±109.62 cells/µl and 178 cells/µl respectively with a range of 16-423 cells/µl. In addition, while a normal chest radiograph was documented in 25 (51%) patients, the remaining had radiographic abnormalities as follows: pleural effusion in 15 (30.6%); infiltrates in five (10.2%); opacity in three (6.1%) and hyperinflation in one (2%).

Employing the EORTC/MSG criteria, ten cases of probable and 23 cases of possible invasive pulmonary aspergillosis were diagnosed. The characteristics of HIV reactive patients diagnosed with a probable IPA are summarized in Table 1. Chest radiography of eight (80%) of these patients revealed a pleural effusion, while infiltrates were seen in two (20%). HRCT scan revealed the characteristic air-crescent sign in only one of these patients. Furthermore, CT confirmed the presence of pleural effusion in the eight cases and documented the presence of new infiltrates not fulfilling major criteria in another patient.

The diagnostic yield of sputum culture, direct microscopy and Platelia™ *Aspergillus* EIA in HIV reactive patients with probable IPA was 30% (3/10), 30% (3/10) and 90% (9/10) respectively. The sensitivity, specificity and positive and negative predictive values of Platelia™ *Aspergillus* EIA were 90% (55.50- 99.75%), 100% (79.41- 100%), 100% (66.37- 100%) and 94.12 % (71.31- 99.85%) respectively, while the corresponding performance characteristics of lower respiratory tract *Aspergillus* isolation were 30% (6.67- 65.25%), 100% (79.41- 100%), 100% (29.24- 100%) and 69.57% (47.08-86.79%) respectively.

Further, it is noteworthy to know that two healthy HIV negative controls with no signs and symptoms of invasive pulmonary aspergillosis gave positive results with Platelia™ *Aspergillus* EIA, but only on one occasion. A repeat test performed on a fresh blood specimen drawn from these patients did not yield a positive result, and thus these could not be considered as confirmed false positives.

Table 1: Characteristics of HIV*/ AIDS† patients with probable IPA‡ (n=10)

S. No.	Age	Sex	Clinical presentation	Mode of HIV transmission	Underlying lung disease	CD4 count	Direct microscopy	Fungal culture	Aspergillus species isolated	Aspergillus galactomannan antigen
1.	28	M§	Fever, cough	Heterosexual contact	TB‖	107	Fungal hyphae seen	Positive	Aspergillus flavus	Negative
2.	52	M	Fever, cough, dyspnea	Heterosexual contact	-	85	Fungal hyphae not seen	Negative	_	Positive
3.	49	M	Fever, dyspnea	Heterosexual contact	TB	168	Fungal hyphae not seen	Negative	_	Positive

S. No.	Age	Sex	Clinical presentation	Mode of HIV transmission	Underlying lung disease	CD4 count	Direct microscopy	Fungal culture	Aspergillus species isolated	Aspergillus galactomannan antigen
4.	38	M	Fever, dyspnea	Heterosexual contact	TB	85	Fungal hyphae not seen	Negative	_	Positive
5.	42	M	Fever, dyspnea	Heterosexual contact	TB, PCP**	54	Fungal hyphae not seen	Negative	_	Positive
6.	19	M	Fever, cough	Intravenous drug abuse	TB	174	Fungal hyphae not seen	Negative	_	Positive
7.	18	M	Fever. Cough	Heterosexual contact	TB	317	Fungal hyphae seen	Positive	Aspergillus flavus	Positive
8.	42	M	Fever, cough	Heterosexual contact	-	54	Fungal hyphae not seen	Negative	_	Positive
9.	26	M	Fever, dyspnea	Blood transfusion	TB	120	Fungal hyphae not seen	Negative	_	Positive
10.	32	M	Fever, cough	Heterosexual contact	_-	83	Fungal hyphae seen	Positive	Aspergillus niger	Positive

**HIV, Human immunodeficiency virus; †AIDS, Acquired immunodeficiency syndrome; ‡IPA, Invasive pulmonary aspergillosis; §M, Male; ||TB, Tuberculosis; **PCP, Pneumocystis carinii pneumonia*

Discussion

The ever increasing number of immunosuppressed conditions has expanded the spectrum of patients at risk of developing invasive fungal diseases such as IPA. [20, 21] Typically, a definite diagnosis is rarely established and diagnosis frequently relies on a combination of clinical features and radiological abnormalities in a patient with high-risk predisposing conditions. While an accurate diagnosis relies on histopathology, a lung biopsy is seldom performed and even in cases where it is feasible the diagnostic accuracy rarely exceeds 80%, more so in event of false negative results due to failure of the invasive method to reach the area infected by *Aspergillus* species. [22, 23] Additionally, while *Aspergillus* hyphae can be readily differentiated from the wide, pauciseptate hyphae of mucoraceous moulds, distinguishing them from the narrow, septate hyphae of *Fusarium* and *Scedosporium* is often not possible. [11] Culture is time consuming and may delay the diagnosis of IPA. Furthermore, its clinical utility is limited by low sensitivity. [24] Poor sensitivity of mycological cultures for diagnosis of IPA is also evident from our study.

Biomarkers such as serum galactomannan provide a potential adjunct in the diagnosis of IPA. In 2002, galactomannan antigen detection was included as an indirect evidence of a probable invasive fungal disease and has been retained as such in the definition of mycological criteria as described by the updated EORTC/MSG guidelines published in 2008. [10, 25] The sensitivity and specificity of serum galactomannan assay has been reported to vary across different studies. In a meta-analysis by Pffeifer, including pediatric as well as adult population, the sensitivity has been reported to vary between 38-100% and the specificity between 17-100%. [4] As postulated by some researchers, serum galactomannan is rapidly cleared by circulating neutrophils in non-neutropenic patients and generally less angioinvasive forms of *Aspergillus* infection are seen in them that further restricts the access of galactomannan to the blood stream. [26, 27, 28] Contrary to the expected, we report a high sensitivity of 90 % for serum galactomannan assay in non-neutropenic HIV reactive patients with a probable IPA.

In addition, we found serum galactomannan assay to exhibit 100% specificity in HIV reactive patients with a probable IPA. Previous published peer-reviewed studies report false positive galactomannan results as high as 5.7% to 14% with serum samples. [5, 29] Administration of antibiotic preparations containing piperacillin-tazobactam; amoxycillin-clavulanate parenteral preparations; administration of cyclophosphamide; consumption

of milk preparations containing high concentrations of galactomannan; intravenous administration of the electrolyte solution, Plasmalyte®; cross reactions with other moulds such as *Pencillium* species, *Paecilomyces*, *Fusarium*, zygomycetes, *Trychophyton* and dematiaceous fungi such as *Alternaria* species that contain cross-reacting epitopes; cross-reactions with antigens from *Cryptococcus neoformans* and *Geotrichum capitatum*; and presence of bacteremia, are among the many reasons cited for false reactivity with adult sera. [30, 31, 32, 33, 34, 35, 36, 37] However, serial sampling has been stated to improve the accuracy of serum galactomannan as a screening tool and as per one of the studies, false positivity rates declined from 54.2% to 11.2% if positivity for at least two samples was taken as a defining criterion. [18] The authors of the present study have also followed the same criterion and thus false reactivity seems less likely.

In our study, CT scans were only performed in patients with a probable invasive pulmonary aspergillosis and hallmark imaging findings were noted in only one of them. While the performance and utility of CT scans in diagnosis of IPA is beyond the scope of this study, we would surely like to emphasize upon the risk of radiation exposure associated with frequent CT scans and the difficulty encountered in moving unwell patients from protective isolation for this purpose. On the other hand, blood for serum galactomannan assay can be drawn more often and the test can be repeated at frequent intervals. Thus, biomarker assays such as serum galactomannan test have provided an alternative diagnostic approach to accomplish an early and specific diagnosis of IPA.

Our study is fraught with certain limitations. The number of patients included in the analysis was relatively small. Secondly, due to ethical concerns, lung biopsy for a histomorphological demonstration of invasive disease was not performed. As a consequence, galactomannan assay test results could not be verified by the invasive reference test and the true performance characteristics of Platelia™ *Aspergillus* EIA could not be determined. Thirdly, thoracic CT scans which are clearly more sensitive than plain chest radiographs, were not performed in all patients. Finally, as with few other studies, a possibility of incorporation bias cannot be ruled out. [38] We have included a positive serum galactomannan result as a microbiological criterion to define probable IPA, and by doing so, we have included the test under evaluation in the reference standard. This accounts for the higher than usual sensitivity and specificity values of serum galactomannan assay in our study. We suggest that larger, well designed, prospective and multi-center studies are undertaken to assess the analytical performance and evaluate the utility of serum galactomannan antigen for diagnosis of IPA in non-neutropenic patient populations such as HIV reactive patients.

We would also like to discuss upon the utility of EORTC/MSG criteria for diagnosis of IPA in our study group. These definitions were primarily devised for patients with malignant diseases and allogeneic hematopoietic stem cell transplant recipients. [10, 38] Their application to non-cancer populations is questionable in view of lack of conclusive evidences to substantiate their utility. Furthermore, the authors of the present study have employed the original EORTC/MSG definitions and not the revised criteria, for categorizing IPA cases into probable and possible. This is because fever, which was included as a host factor in the original definition, was removed in the subsequent revision due to its non-specificity for invasive fungal disease and the plausible reason by consensus group that presence of fever represents a clinical manifestation and not host factor. Thus, while inherited severe immunodeficiency disorders were included in the revised host criteria, febrile illness in an AIDS patient was no longer retained in the definition and therefore the revised EORTC/MSG guidelines could not be applied to our study group. [10, 25] Also, the concept of two levels-major and minor evidences, that was originally proposed for the definition of clinical criterion, was abandoned in the revised guidelines, and a diagnosis of probable IPA now relied on documentation of at least one of the three highly specific radiological criteria (dense, well-circumscribed lesions(s) with or without halo sign, air crescent sign or cavity). [10, 25] Thus, while the revised EORTC/MSG definitions are more specific, they are also definitely more restrictive and it is quite possible that in the wake of highly specific but less sensitive eligibility criteria the clinical significance of IPA may be underestimated and awareness and consciousness of this entity would soon be lost. Thus, clinicians should keep in mind that these criteria are just meant to stratify the certainty and risk of invasive pulmonary aspergillosis, and to serve as a clinical and epidemiological research guide, and every patient need not fulfill these criteria in order to warrant treatment for IPA.

Conclusion

To summarize, in the era of diagnostic deficiencies of conventional modalities, serum galactomannan antigen offers a key approach to provide a supportive evidence of IPA. Our study has shown that the assay has a high sensitivity and specificity. However, positive findings need to be cautiously interpreted in the context of clinical and radiological evidences. All positive results should be validated by repeat testing and causes of false positive reactivity such as galactomannan contamination and cross-reactive mycoses be ruled out.

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Immunohistochemical Expression of IDH1R132H in Astrocytic Tumours and its Association with Histopathological Grade, TP53 and EGFR Protein Expression.

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ABSTRACT

Background: Molecular studies have uncovered that histopathologically diagnosed Astrocytomas are genetically heterogeneous. Majority of Diffuse Astrocytomas (DA), Anaplastic Astrocytomas (AA) and secondary Glioblastoma multiforme (GBM) have concurrent mutations of IDH, TP53 and ATRX. Astrocytomas without IDH mutation have distinct genotype and poor prognosis. Primary GBM show EGFR amplification and lack IDH mutation.

Methods: We studied 95 Astrocytic tumours including 15 Pilocytic Astrocytoma (PA), 21 Diffuse Astrocytomas, 7 Anaplastic Astrocytomas and 52 GBM using tissue-microarray (TMA) to assess immunohistochemical expression of IDH1R132H, TP53 and EGFR protein in all four grades and the association between these three immunohistochemical markers. TMA blocks with core size 3.0 mm were constructed using manual tissue-microarrayer. IHC for IDH1R132H, TP53 and EGFR was performed.

Results: A 13%(2/15) of PA showed IDH1R132H expression. TP53 and EGFR expression was not seen in any case. 52%(11/21), 71%(15/21) and 9%(2/21) of DA showed expression of IDH1, TP53 and EGFR respectively. Frequency of mutant IDH1, TP53 and EGFR in Anaplastic astrocytoma was 85%(6/7), 85%(6/7) and 0%(0/7). Among GBMs there was significant difference in IHC expression of IDH1 and EGFR. Primary GBMs show high EGFR expression of 70%(26/37) and low IDH1 expression of 21%(8/37). Secondary GBM in contrast show higher IDH1 expression 80%(12/15) and low EGFR expression of 13%(2/15).

Conclusion: IDH and TP53 mutations are seen in majority of DA, AA and secondary GBM and are hallmarks of these tumours. Primary GBMs have distinct molecular pathway. They lack IDH, TP53 mutation and overexpress EGFR.

Keywords: *IDH, Glioma, EGFR, TP53, Immunohistochemistry, Genotype*

Introduction

Gliomas account for 70% of primary brain tumours in adults with a yearly incidence of 6 cases per 100,000. [1] The 2007 WHO classification of CNS tumours was based on histopathological features, histogenesis and immunohistochemical expression of lineage-specific proteins. This approach has changed in the 2016 update, which has brought in the concept of integrated diagnosis. Integrated diagnosis uses molecular pathology in addition to light microscopy to classify CNS tumours.[2]

Glial tumours are divided into two major classes on the basis of invasion into surrounding brain tissue. Diffuse gliomas infiltrate into the adjacent brain parenchyma and recur even after gross total resection. These tumours respond poorly to conventional chemotherapy and radiation and are thus incurable. Therefore, understanding of their molecular pathways to develop more effective targeted therapy is urgently needed. Targeting driver molecular aberrations has been a very promising advance in recent years in cancer therapeutics and development of targeted therapies is much needed for gliomas.

Advances in molecular genetics have revealed that histopathologically diagnosed diffusely infiltrating astrocytomas consist of two genetically different groups of tumours. The majority have concurrent mutations of IDH1/IDH2, TP53 and ATRX.Concurrent IDH1/2 and TP53 mutations are now considered to be a genetic hallmark of diffuse astrocytomas. [3]

The molecular pathogenesis of Glioblastomas is different and majority (>90%) arise de-novo without any evidence of a pre-existing lower-grade astrocytoma. These GBMs are known as primary GBMs and show a higher prevalence of EGFR gene amplification (>40%) and EGFR overexpression (>60%). Secondary GBM, in contrast, arise from lower grade gliomas and are seen in younger patients in comparison to primary GBMs. [4]

EGFR gene amplification is thus one of the genetic hallmarks of GBM and a tumour showing EGFR amplification should be treated like GBM even if the WHO histologic criteria of necrosis and microvascular proliferation are not met.[5] EGFR signalling pathway, therefore, is a potential target for the development of targeted therapies using antibodies, tyrosine kinase inhibitors (TKIs) or vaccines. [6]

With this knowledge, we studied the immunohistochemical expression of IDH1R132H, TP53 and EGFR protein as a surrogate marker of genetic mutations among all four grades of astrocytoma and the association between these mutations. This study will help to better understand the pathways of gliomagenesis and the utility of immunohistochemistry to diagnose, classify, prognosticate astrocytomas and predict response to therapy.

Materials and Methods

In this study, cases of astrocytomas diagnosed at our institute from 2011to 2016 were studied. FFPE tissue blocks were retrieved from the archives. H&E slides were evaluated to ascertain the diagnosis and WHO grade of astrocytomas by an experienced neuropathologist. The clinical data pertaining to the cases was collected from the histopathology database and requisition forms sent with the specimen.

To construct tissue microarray (TMA) H&E slide from each tissue donor block was used to identify the most representative tumour area. H&E slide was overlaid on the donor block and the area of interest was marked.3 mm diameter cylindrical tissue cores were taken from donor tissue blocks using a manual tissue microarrayer (IHC world, USA).These cylindrical tissue cores were arrayed on an empty steel mould containing a small amount of molten paraffin in a 4 x 3 grid with the tissue cores facing down. First tissue core from a different tissue (Liver, spleen) served as an orientation marker. When the cylindrical cores got adhered to the mould additional molten wax at 60 degrees C was poured from the side and a paraffin block containing eleven tissue cores from astrocytic tumours and one core for orientation was prepared.

For the purpose of immunostaining, 3-4 micron thick sections from TMA blocks were obtained on poly-L-lysine coated glass slides and were incubated overnight at 37^0C. High-temperature antigen retrieval was performed by autoclaving the sections in 0.01 M citrate buffer (pH 6.0) at 121°C for 10 minutes. Sections were treated with two drops of 3% hydrogen peroxide of the universal staining kit for 15-20 minutes to block the endogenous peroxidases, followed by protein blocking using ultra block of the universal staining kit for 5 minutes.

Immunohistochemical staining was performed by LSAB technique (LSAB Kit, M/s Abcam, Germany) using mouse monoclonal antibodies to IDH1 2H9 in a dilution of 1:100. (Clone-ab117976; M/s Abcam, Germany). Subsequently, the biotinylated anti-mouse secondary of the universal staining kit followed by streptavidin/horse radish peroxidases was applied to the sections. Finally, DAB chromogen concentrate diluted in the substrate were applied, and slides were continuously seen under the microscope till desired colour intensity was reached. Counterstaining was done by Harris hematoxylin for a period of 1 minute. A visual semi-quantitative grading scale was applied to assess the immunoreactivity. A strong cytoplasmic staining in >10% oftumour cells for IDHR132Hwas scored positive.

For studying EGFR protein expressionprimary polyclonal rabbit anti-EGFR antibody in 1:50 dilution (M/s Biogenex, Fremont CA) was used and a strong cytoplasmic staining in >50%tumour cells for *EGFR* was considered positive. A weak diffuse background staining was taken as negative.

Primary ready to use mouse monoclonal anti-human p53 protein antibody; clone DO-7 (M/s Dako, Denmark) was used for studying TP53 protein expression and a strong nuclear staining of tumour cell nuclei in >10% of tumour cells was taken as positive.

Result

A total of 95 cases of astrocytic tumours were studied which included 15 pilocytic astrocytomas (15.8%), 21 diffuse astrocytomas (22.1%), 07 anaplastic astrocytomas (7.4%) and 52 glioblastomas (54.7%). Among glioblastomas 37 (38.9%) were de-novo or primary glioblastomas and 15 (15.8%) were secondary glioblastomas.

62 patients were male (65%) and 33 were female (34.7%). The age range was 10-75 years and the mean age was 20.9 years for pilocytic astrocytoma (PA), 40.6 years for diffuse astrocytoma (DA), 46 years for anaplastic astrocytoma (AA), 53.4 years for primary GBM and 49 years for secondary GBM.

Isocitrate dehydrogenase 1 (IDHR132H) expression: When IDH1R132Hexpression was compared among different grades of astrocytoma it was observed that only 2/15 cases (13%) of PAwere IDH1 positive. 11/21 (52%) cases of DA and 6/7 cases (85%) of AA showed IDH1 expression.Among glioblastomas, there was a significant difference in the pattern of IDH1 expression among primary and secondary glioblastomas. IDH1 expression was significantly higher in secondary GBM and 12/15 cases (80%) showed positive immunostaining. In contrast, only

8/37 cases (21%) of primary GBM were IDH1 positive (Table 1, figure 1A).There was a significant association between grade of astrocytoma and IDH1R132H expression (Fisher's exact test, p-value <0.001).

TP53 expression: The wild type or unmutated p53 gene is easily degraded and is therefore not detectable by IHC. On the other hand, mutant p53, which has lost its normal function of tumour suppressor gene resists degradation and accumulates in the nucleus. This mutated TP53 which is accumulated in the nucleus is detected as strong nuclear positivity in tumour cells. It was observed that none of the 15 cases of PA expressed TP53 (0%). Among DA and AA TP53 expression was high and 15/21 cases (71%) of DA and 6/7 (86%) of AA were positive for TP53 expression. In contrast to grade II and III astrocytic tumours, the expression of TP53 was significantly lower in primary GBM with only 3/37 cases (8.1%) cases showing positivity. Secondary glioblastomas also had a lower percentage of TP53 positive cases with 4/15 (26.6%) showing nuclear positivity (Table 1, figure 1B).

EGFR protein expression: EGFR expression was significantly higher in primary glioblastomas with 26/37 (70.3%) cases expressing EGFR protein. In contrast, only 2/21 cases (9.5%) of DA and 2/15 (13.3%) cases of secondary glioblastoma expressed EGFR protein. None of the PA or AA showed EGFR expression (Table 1, figure 1C). A significant association was found between tumour grade and EGFR protein expression using Fisher's exact test with a p-value of < 0.05.

Association of IDH1 with EGFR and TP53 protein expression: When expression of IDH1 was compared with EGFR, it was observed that there is an inverse relationship between IDH1 and EGFR expression (figure 3D). Among the subgroup of 39 astrocytomas which were IDH1 positive only 6 cases (15.4%) showed EGFR protein expression. Whereas, among 30 astrocytomas which were positive for EGFR protein; IDH1 expression was seen in 6 cases (20%). Using chi-square test a significant inverse association was found between IDH1 and EGFR protein expression.

In contrast to EGFR it was noted that there is a positive association between IDH1 and TP53 expression. In 39 astrocytomas which were IDH1 positive 17 cases (43.6%) showed TP53 expression. Whereas, among the subgroup of 56 astrocytomas which were negative for IDH1, TP53 expression was seen in 6 cases (10.7%).A significant association was seen between IDH1 and TP53 expression (chi-square test, p-value 0.021)

Discussion

Previous studies have noted that IDH mutation is an early event in the pathogenesis of diffusely infiltrating gliomas (Grade II and III). [7] Normal cellular function of IDH is to protect cells from oxidative stress by producing NADPH. NADPH is used to generate reduced glutathione (GSH), which is the main antioxidant in the central nervous system. [8,9]R132H mutation in IDH1 reduces its enzymatic activity to convert Isocitrate to **α**-KG. [10]Mutated IDH1 also acquires a new enzymatic activity and converts**α**-KG to D-2-hydroxyglutarate (D-2HG) and utilises NADPH in the process. [11]This in turn reduces the concentration of reduced glutathione and makes the cells more susceptible to free radical induced injury and DNA damage. There is evidence to suggest that D-2HG is also an oncometabolite and is directly involved in the pathogenesis of IDH mutated tumours. [12]Astrocytomas further acquire mutations in tumour suppressor gene TP53 and ATRX gene during the course of malignancy. [13]

Secondary GBM progress from pre-existing lower grade gliomas and thus also tend to express these molecular alterations. [14] Primary GBM, on the contrary arise from a different genetic pathway and often show EGFR amplification. [15]This difference in molecular pathogenesis among various grades of astrocytomas manifests as differential immunohistochemical profile among various grades.

Table 1: Table showing immunohistochemical expression of IDH1R132H, TP53 and EGFR protein among different grades of astrocytomas.

WHO Grade	Total cases	IDH +	% IDH +	TP53 +	%TP53 +	EGFR +	%EGFR +
GRADE I	15	2	13%	0	0%	0	0%
GRADE II	21	11	52%	15	71%	2	9%
GRADE III	7	6	85%	6	85%	0	0%
Primary GBM	37	8	21%	3	8%	26	70%
Secondary GBM	15	12	80%	4	27%	2	13%

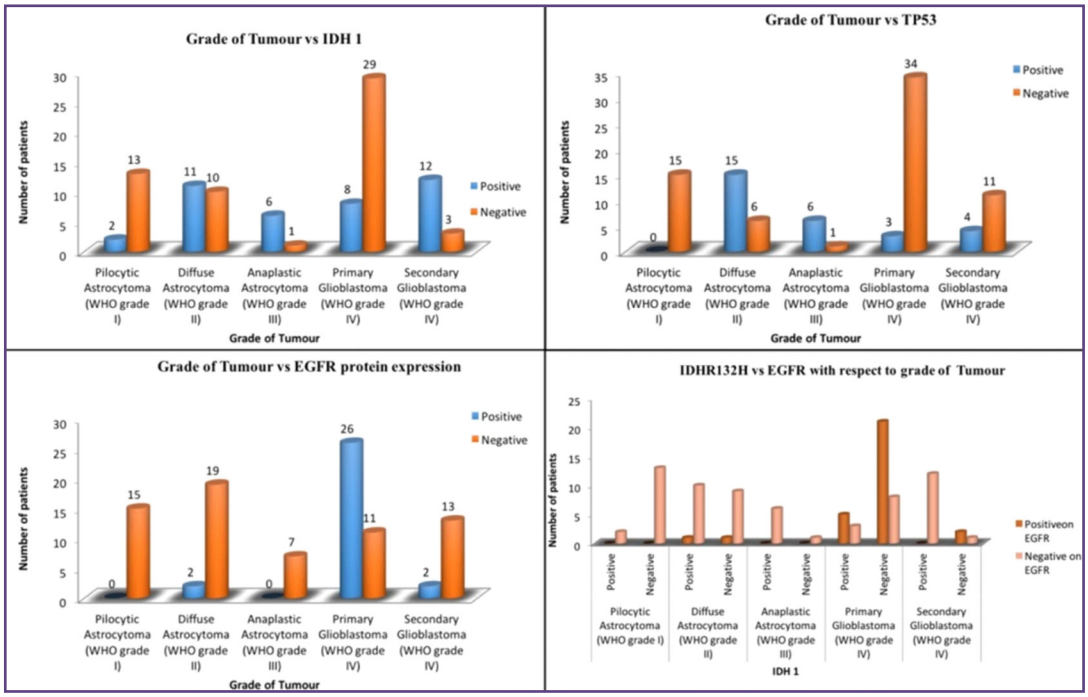

Fig. 1: Bar diagrams showing IDH1R132H (1A), TP53 (1B), EGFR (1C) expression among different grades of astrocytoma. Figure 1D shows association of IDH1 and EGFR expression.

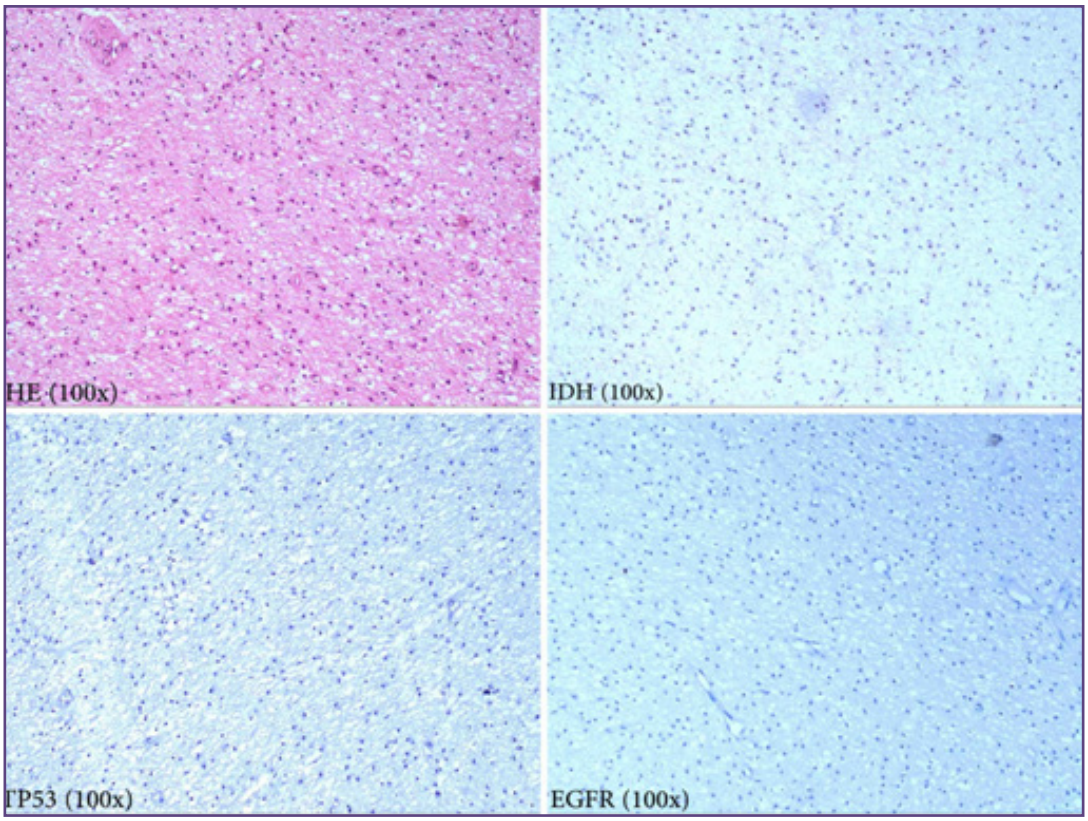

Fig. 2: Photomicrograph showing H&E stained section of Pilocytic Astrocytoma (WHO grade I) with IDH1R132H, TP53 and EGFR protein expression. All three IHC markers are negative.

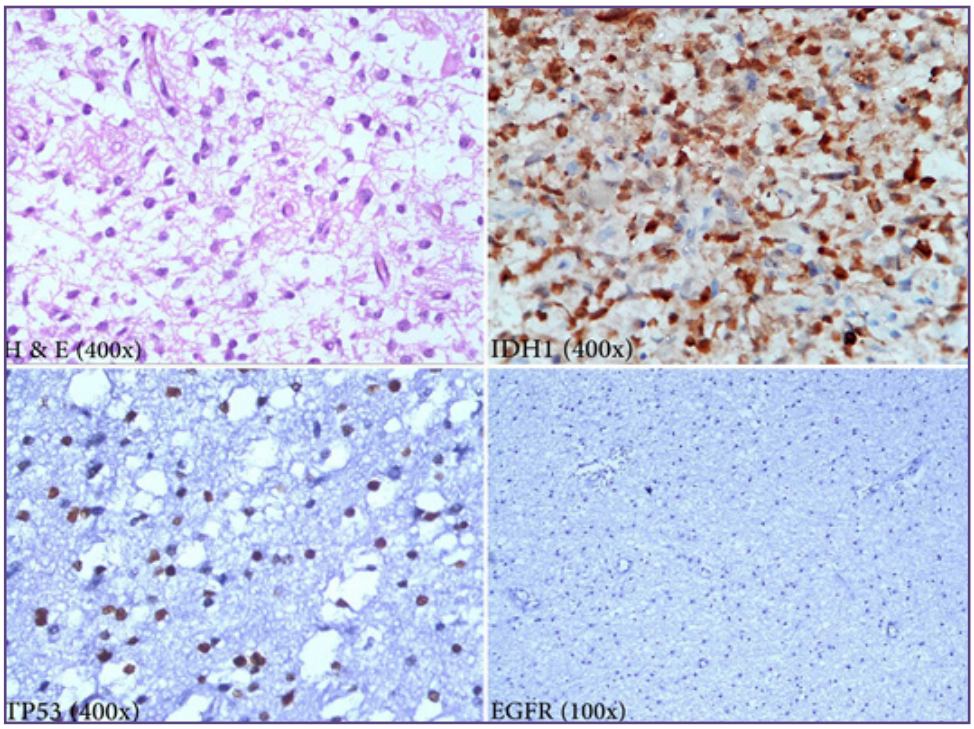

Fig. 3: Photomicrograph showing H & E stained section of Diffuse Astrocytoma (WHO grade II) with IDH1R132H, TP53 and EGFR protein expression. IDH1R132H (cytoplasmic) and TP53 (Nuclear) are positive in tumour cells. EGFR protein expression is negative.

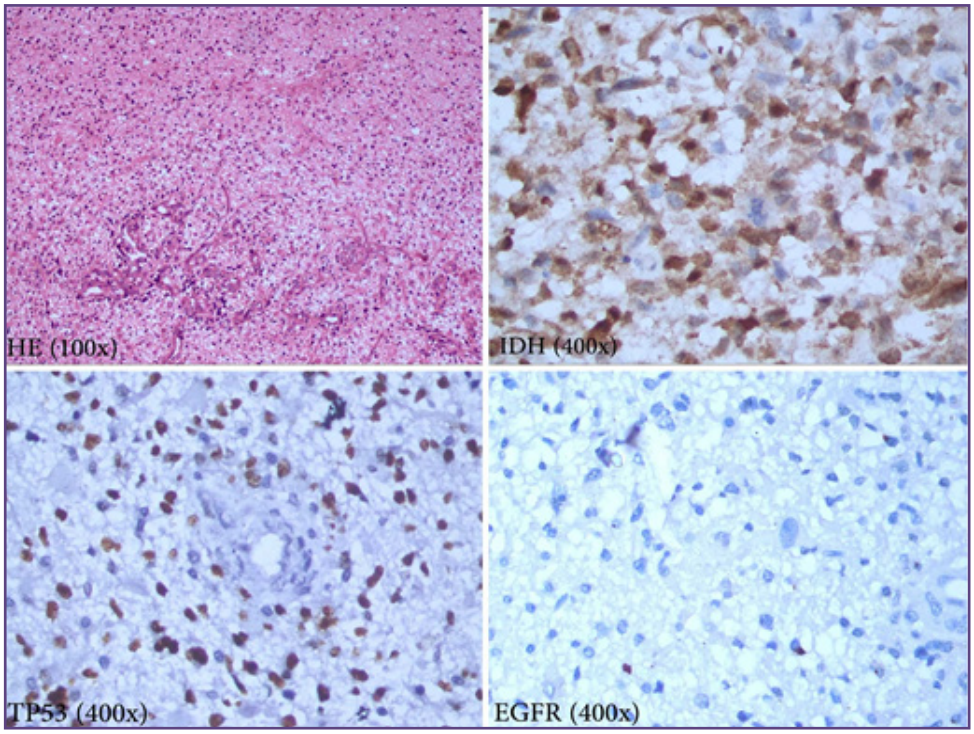

Fig. 4: Photomicrograph showing H & E stained section of Anaplastic Astrocytoma (WHO grade III) with IDH1R132H, TP53 and EGFR protein expression. IDH1R132H (cytoplasmic) and TP53 (Nuclear) are positive in tumour cells. EGFR protein expression is negative.

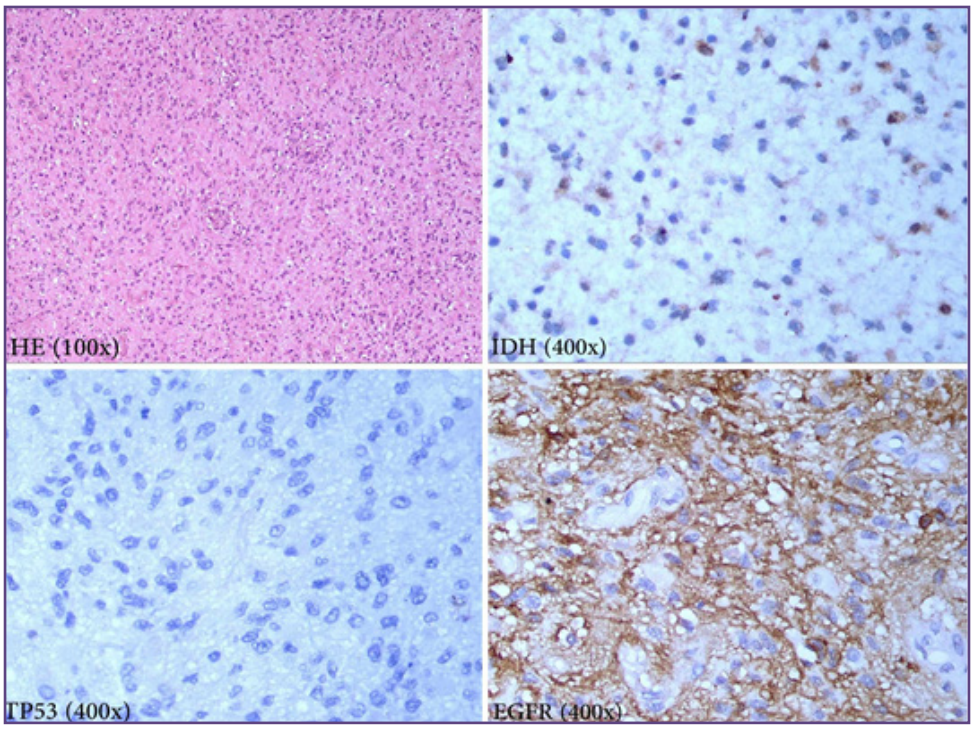

Fig. 5: Photomicrograph showing H & E stained section of Primary GBM (WHO grade IV) with IDHR132H, TP53 and EGFR protein expression. EGFR protein overexpression (cytoplasmic) is seen in tumour cells. IDH1R132H and TP53 expression is negative.

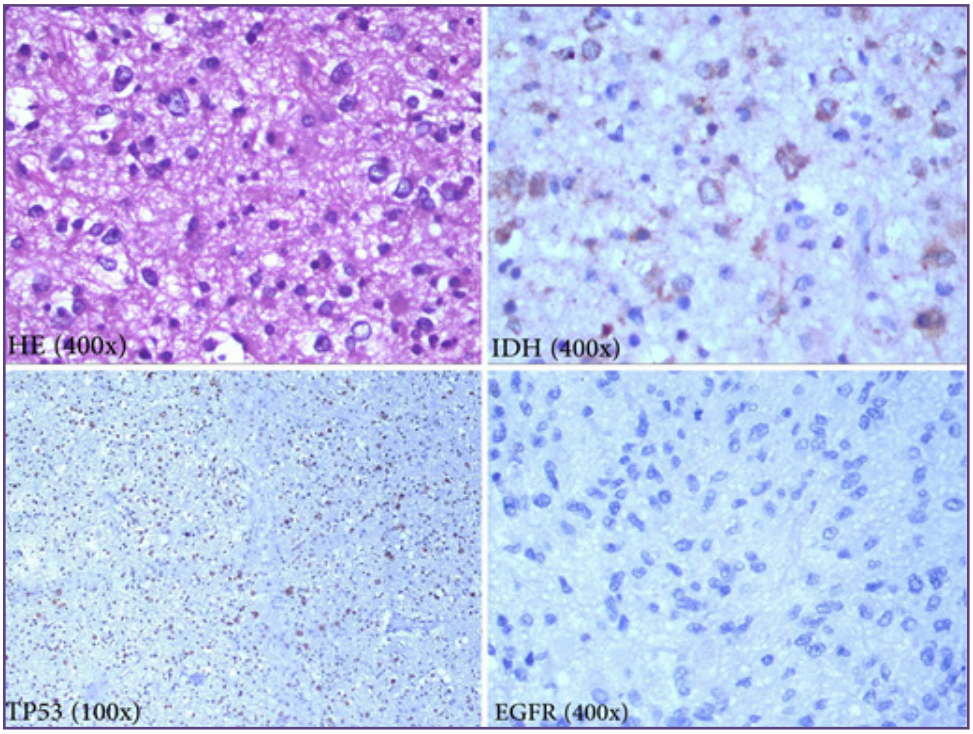

Fig. 6: Photomicrograph showing H & E stained section of Secondary GBM (WHO grade IV) with IDHR132H, TP53 and EGFR protein expression. IDHR132H (cytoplasmic) and TP53 (Nuclear) are positive in tumour cells. EGFR protein expression is negative.

In previous studies 59–90 % of diffuse astrocytoma, 28–82 % of anaplastic astrocytoma and 70-75% of secondary GBM showed IDH1R132H positivity. Whereas IDH1 mutations are less frequent in primary GBM (<10%) and rare in PA. [3,16]

In the present study, we have studied the immunohistochemical expression of IDH1R132H mutations across various grades of astrocytomas and found that 13% of pilocytic astrocytoma, 52% of DA, 85% of AA, 80% of secondary GBM and 21% of primary GBM expressed IDH1R132H. We found IDH mutations are significantly associated with astrocytoma grade.

p53 is a tumour suppressor gene which is mutated in a vast majority of human tumours. [17]Mutated p53 encodes for a mutated nuclear protein which cannot be degraded easily and accumulates in the nucleus. [18]This mutated TP53 can be detected by IHC and so overexpression of p53 is considered to be a surrogate marker for p53 mutation. In our study, it was observed that TP53 expression was significantly higher in grade II (71%) and grade III (86%) astrocytomas. Grade I astrocytomas arise from different molecular alterations and thus do not exhibit p53 mutation (figure 2). Among grade IV astrocytomas the expression was variable among primary GBM (8.1%) and secondary GBM (26.6%). A significant association was found between the grade of tumour and TP53 expression (p value of <0.001 using Fisher's exact test). Tumours with mutated p53 cannot tolerate genotoxic stress and as a result, undergo apoptosis when chemotherapeutic drugs and radiotherapy is used against them. [19]

Previous studies have shown that the frequency of EGFR gene amplification increases with increasing tumour grade. [20]EGFR amplification is rare in grade II astrocytomas and is seen in only 4% of cases in some studies. Glioblastomas show the highest expression of EGFR among all four grades and EGFR amplification is seen in around 60% of cases. [21,22] In our study, EGFR over-expression was not seen in any case of PA. Only 2/21 cases (9.5%) of DA showed EGFR over-expression. Primary GBM show the highest percentage of EGFR protein expression with 26/37 cases showing EGFR positivity (70.3%). Secondary GBM, in contrast, show an expression profile similar to low grade gliomas with only 2/15 cases showing EGFR over-expression (13.3%). In the present study, EGFR overexpression was associated with the higher-grade gliomas, suggesting that EGFR overexpression was associated with tumour aggressiveness and invasion.

Association of IDHR132H with TP53 and EGFR Protein Expression: The results of our study show that IDHR132H mutation is significantly associated with TP53 expression. On the other hand, a reverse relationship was found between IDH1 mutation and EGFR protein expression. TP53 and IDH1 expression are found in grade II (figure 3) and III (figure 4) astrocytomas and younger age group patients. [4] These tumours also acquire mutation of TP53 gene along their natural history and thus both mutations are often found in these group of tumours. The inverse relationship between IDH1 mutation and EGFR expression and the preferential expression of EGFR in glioblastomas shows that glioblastomas arise from a genetic mechanism which is different from lower grade gliomas (figure 5). This view is further strengthened by the fact that secondary glioblastomas arise from lower grade astrocytomasand show an immunohistochemical profile similar to grade II and III astrocytomas (figure 6).

To our knowledge studies showing a functional relationship between IDH1, p53 and EGFR expression in various grades of astrocytomas are few. Our study shows a strong association between IDH1R132Hwith TP53 and an inverse association with EGFR protein expression.[23]Other studies probing new pathways involved in gliomagenesis are underway. A better understanding of these mutations will help investigators develop newer targeted therapies against high-grade gliomas, which as of date are incurable. Targeted therapies which act as competitive antagonists of mutant Isocitrate dehydrogenase enzyme are in development. [24] Glioma patients harbouring IDH mutation may benefit from these therapies in future. [25]

Conclusion

IDHR132H and TP53 mutations are seen in the majority of DA, AA and secondary GBM. Primary GBM, in contrast, were found to overexpress EGFR protein and thus arise from a molecular pathway that is different from that of lower grade gliomas.IDHR132H and EGFR protein expression was found to be mutually exclusive and there is a significant inverse association between these two IHC markers.

Further studies need to be done to better understand the pathogenesis of gliomas so that new molecular targets are identified against which targeted therapy can be developed to treat high-grade gliomas which are incurable and have a poor prognosis and survival despite treatment.

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Multi Drug Resistant Bacteria: Prevalence and Associated Risk Factors Amongst ICU Health Care Workers of a Tertiary Care Hospital

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ABSTRACT

Background: Multi Drug Resistant bacteria pose serious threat to patient safety worldwide. Health Care Workers are an important source of dissemination and transmission of these organisms to patients especially in intensive care units (ICU).Although many studies have been carried out in India which have determined the prevalence of either gram positive or gram negative MDR bacteria colonizing the HCW separately, no study so far has determined the prevalence and risk factors for acquisition of MRSA, VRE, MDR Acinetobacter baumanii & Pseudomonas aeruginosa simultaneously in the same HCW population

Methods: Hand swabs from 198 HCW were obtained, processed and isolates identified by automated method using Vitek II (Biomerieux, Durham, NC).Risk factor assessment was done based on a questionnaire using Fischer's exact /Chi square test.

Result: A total of 24 HCWs (12.1%) were found positive for MDR bacteria. At 5.1% (n=10) MDR Acinetobacter baumanii was the most common isolate obtained. Majority of MDR bacteria (16.1%) were isolated from hands of doctors. Male sex, presence of chronic /open wound and close contact with patients were factors found significantly associated with colonization of hands of HCW.

Conclusion: Health Care Associated Infections in the vulnerable ICU patient population can be linked to the MDR bacterial flora of the HCWs. A.baumanii has been found to be most frequently contaminating the hands of HCW. Compliance with contact precautions, proper hand hygiene and adequate environmental cleaning may decrease this transmission.

Keywords: *Health Care Worker, Multi drug resistant Bacteria, hand hygiene, bacterial colonization*

Introduction

Multi drug resistant (MDR) bacteria pose serious threat to patient safety worldwide adversely affecting their mortality and morbidity despite antimicrobial therapy and advances in supportive care [1,2] This is especially true for patients admitted in Intensive Care Units (ICU) who are more likely to be immunocompromised and suffering from serious underlying diseases[3,4]. Health care workers (HCW) are an important source of dissemination and transmission of these multidrug resistant organisms to patients[5].

Although many studies, conducted in India and globally, have determined the prevalence of bacterial colonization and risk factors for their acquisition by HCWs including contact with wound dressing, linen, artificial airways, infusion pumps, catheters or drains or performing physical examination among others. Very few studies and to the best of our knowledge none so far in India, have accessed risk factors for contamination with the most common MDR bacteria(MRSA,ESBL,VRE, MDR non fermenters etc) simultaneously and on the same HCW population.

Globally studies have shown that 10 to 70% of nosocomial infections are preventable depending on setting, study design, baseline infection rate and type of infection [1] Understanding the factors that lead to contamination of HCW hands in a particular setting is likely to help develop strategies unique to each institution to prevent transmission of MDR bacteria from HCW to patients and vice versa. Also it would help curb the menace of prescribing inadequate empirical antibiotic therapy to patients leading to increased morbidity and mortality, development of antimicrobial resistance and leading to unnecessary hospitalization.

The study was therefore carried out with the objective of determining the prevalence of MDR bacteria in the hand of ICU health care workers and the risk factors associated with the acquisition of these pathogens.

Materials and Methods

Study Design and Population: A prospective study was conducted at the 1000 bedded Shri Mahant Indresh Hospital (Dehradun, Uttrakhand) between June 2016 and December 2016. All samples were collected from the Medical ICU (26 bed unit), Surgical ICU (21 bed unit), Cardiac ICU (14

bed Unit) and Pediatric ICU (28 bed unit).The study was approved by the Institutional Ethics committee.

Sample Collection: HCWs (nurses, technicians and doctors) were approached for participation in the study before engaging in routine, clinical care activities for patients. Samples were taken at the beginning of the day to determine the contamination that the HCWs were carrying with them and not which they may acquire during that particular days activity. Sterile rayon-tipped applicators were moistened with sterile normal saline and hand samples were obtained with a standardized process by swabbing the dorsum of each finger three times and the palm of each hand two times with a twirling motion of the swab with a single swab for both hands[1].

Data Collection: Potential risk factors for MDR bacteria colonization were identified using a questionnaire. This included demographic data such as their age, sex, time for which they have been working in ICUs since prolonged exposure to ICU patients makes them more likely to acquire bacterial contamination), close contact with patient either in form of changing dressing, assisting mobility or carrying out an invasive procedure, presence of open/chronic wounds on exposed areas of the body in the last six months, chronic disorders, chronic skin condition(which are indicative of an immunocompromised state), contact with animals since house hold pets are a known source of infection of MDR bacteria of zoonotic origin such as MRSA, and Extended Spectrum Beta Lactamases producing E.coli[6], their own hospital stay which is indicative of a illness severe enough to have compromised their immunity and making them an easier target for bacterial contamination and infection and use of antibiotics(usage of antibiotics especially the broad spectrum ones can favor colonization with MDR bacteria). The staff was asked to complete this questionnaire on their own.

Microbiological Processing*:* The moistened swabs were then transported to the Microbiology laboratory as soon as possible, dipped in sterile normal saline . Here, each sample was inoculated within an hour of collection in brain heart infusion broth and incubated for 24 hours at 37◦c. After incubation the broth was sub-cultured on 5% sheep blood agar and Mac Conkey agar. All isolates were then identified and their antibiotic resistance profile determined by automated method using Vitek II (Biomerieux, Durham, NC) based on Clinical Laboratories and Standards Institute Guidelines (2016). Further, statistical analysis of data and risk factorestimation was done for isolates positive for MRSA, VRE, MDR *Acinetobacter baumanii*, MDR *Pseusdomonas aeruginosa*. MRSA and VRE were defined as per CLSI 2016 guidelines [7].There are various definitions available in literature. for MDR *Acinetobacter baumanii* and MDR *Pseusdomonas aeruginosa*t For this study, we have defined MDR *Acinetobacter baumanii* and MDR *Pseusdomonas aeruginosa* as, non susceptibility to at least one agent in three or more antimicrobial categories [8].

Statistical Analysis*:* Risk factor analysis was conducted using the Fisher exact test to measure the significance of associations between binary variables and the dependent variable of MDR contamination of hands. We report adjusted odds ratios (aORs) and 95% confidence intervals (CIs) from the multivariate logistic regression model. All statistical tests were 2-sided; P <0.05 was considered to be statistically significant. All analyses were performed using statistical software SPSS, version 22

Result

A total of 198 HCWs (140 females, 58 males) participated in the study. The health care staff included was between 21-65 years of age, with median age of 36. About half of them had worked in this field for at least 1 year while 21% had worked for more than 15 years. [Table 1]

Almost two thirds (72.7%)of them mentioned that as part of their professional duties they had close contact with patients requiring care such as facilitating mobility, changing bandages or treatment of bed sores while close to half (52%) reported to have close contact with animals. A total of 34.3% HCW gave history of use of antibiotics in last six months while 11.1% were admitted in hospital for varied ailments in last one year. Chronic skin disease and chronic wounds were present in 3.6% HCW respectively while 1.6% were found suffering from diabetes mellitus.

Mdr Bacteria Prevalence: Hand swabs were taken from 123 nurses, 44 patient care technicians and 31 doctors. Bacterial isolates were recovered from hand swabs of 148(74.75%) HCWs. These included both commensals as well as known pathogens. A total of 56(28.28%) HCW showed growth of more than one bacteria. Of these 133(68.18%) were established pathogens namely Acinetobacter spp 36.48%, Pseudomonas spp 12.19%, Klebsiella spp 7.31%, S.aureus 7.31%, Enterococcus spp 4.89% while rest 15 were commensals (Table 1).

A total of 24 HCWs (12.1%) were found to be carrying MDR bacteria on their hands distribution of which is as follows: MDR *Acinetobacter baumanii* 10(5.1%), MDR *Pseudomonas aeruginosa* 7(3.4%) MRSA were 5(3.2%), and Vancomycin Resistant Enterococcus 2(0.6%) (Table 2).

Majority of MDR (n=13/24, 54.16%) were isolated from hands of doctors followed by technicians(n=6/24, 25%) and least number was recovered from hands of nurses(n=5/24,

20%) (Chart 1).The most commonly isolated MDR from both doctors and nurses was *Acinetobacter baumanii* (n=7 and 3 respectively) while it was *Pseudomonas aeruginosa* (n=3) from Technicians (Fig1).

Risk factors significant for MDR bacterial contamination of HCWs hands were evaluated with bivariate analysis (Table 3) followed by multiple logistic regression analysis

The number of males affected was significantly higher than females (50% vs 2.5% respectively; p<0.05; Table 3).No difference in susceptibility to MDR bacteria was found in terms of age, occupation and time spent in health care (p>0.05). Time spent in ICU health care (aOR 4.856;95%CI 0.495-47.652), Occpation(aOR 1.912; 95%CI 0.959–3.812),Contact with animals (aOR 4.663; 95%CI 0.289 -75.329), Use of antibiotics in last six months (aOR 4.856;95%CI 0.495-47.652), Hospital admission in last 1 year(aOR 2.610;95%CI 1.339 – 5.088), Chronic skin disease (aOR 0.905; 95% CI 0.905 – 5.654), Dibetes mellitus (aOR 9.938; 95% CI 1.617 – 61.069), were factors not found to be significantly associated with colonization of hands of HCW with MDR bacteria (p > 0.05)

However close contact with ICU patients (aOR 1.125; 95% CI 0.475- 2.667) and having acquired askin ulcer / wound in last six months (aOR 0.677; 95% CI 0.383–1.196) was found to be significantly associated with carriage of MDR bacteria by HCW (p<0.05).

Discussion

The study on MDR bacteria in ICU health workers marks the first time such data has been made available for the state of Uttrakhand and one of the very few studies in India which have determined the risk factors for colonization of hands of HCW with four types of MDR bacteria simultaneously in the same population. The risk factors found to be statistically associated with MDR bacteria colonization include male sex, chronic skin disease and close contact with ICU patient.

Many studies are available from India which have separately determined the prevalence of MRSA 1-3%[9,10], *Acinetobacter baumanii* 5%[11] *Pseudomonas aeruginosa* 2.5% (11) but very few have determined the prevalence of all four MDR bacteria (MRSA, VRE, MDR *Pseudomonas aeruginosa*, MDR *Acinetobacter baumanii*) simultaneously on the same health care population along with their associated risk factors. The prevalence rates in these studies are however similar to those found in our study: *Acinetobacter baumanii* 5.1%, *Pseudomonas aeruginosa* 3.4%, MRSA 3.2%, VRE(0.6%).

We have found that hands of HCW were more frequently contaminated with MDR *Acinetobacter baumanii*(5.1%) than with other MDR bacteria. MDR *Acinetobacter baumanii* has also been detected with higher frequency as compared to other MDR bacteria in a study by Morgan et al[1].This relatively high frequency suggests that *A. baumannii* has a higher propensity to be transmitted to HCWs than do other MDR bacteria. This may be a factor in nosocomial spread, explaining in part the recent worldwide emergence of MDR *A. baumannii*[13]

Also hands of doctors were more frequently contaminated (16.1%) than those of technicians (13.6%) and nurses

Table 1: Organism isolated from hand swabs of HCW

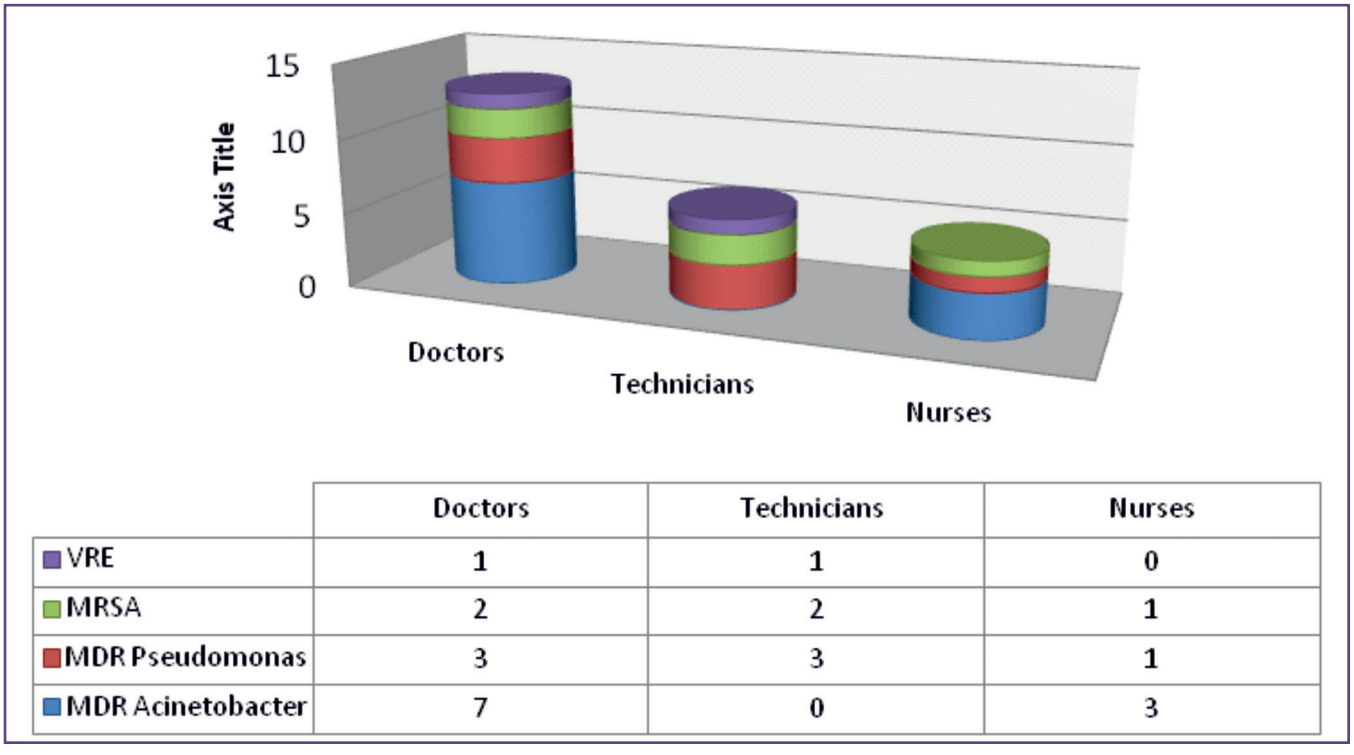
ORGANISM ISOLATED	NUMBER	PERCENTAGE
Staphylococcus aureus	14	7.31
Klebsiella spp	14	7.31
Enterococcus spp	9	4.89
Acinetobacter spp	72	36.48
Pseudomonas spp	24	12.19
Total	133	68.18

Table 2: Prevalence of MDR bacteria amongst HCW

MDR	Number	Percentage
VRE	2	0.6
MRSA	5	3.2
MDR *Pseudomonas aeruginosa*	7	3.4
MDR *Acinetobacter baumanii*	10	5.1
Total	24	12.3

Table 3: Description of study population and MDR positive cases among Health Care workers.

Variable		HCW total N = 198	MDRO staff N = 24 #	p value ##	aOR	95%CI
Sex	female	158(79.8)	4(2.5)			
	male	40(20.2)	20(50)	0.02		
Age in years	<30	43(21.7)	9(20.9)			
	30-39	42(21.2)	4(9.5)			
	40-49	47(23.7)	3(6.4)			
	50-59	52(26.3)	5(9.6)			
	>60	14(7.1)	3(21.4)	0.25	4.228	2.058–8.686
Time spent in ICU health care	<1 yr	30(15.1)	3(10)			
	1-5yr	58(29.3)	5(8.6)			
	6-10 yr	38(19.2)	4(10.5)			
	11-15 yr	31(15.6)	2(6.4)			
	>15 yr	41(20.7)	1(2.4)	0.96	4.856	0.495-47.652
Occupation	Nurse	123(62.1)	13(10.6)			
	Technician	44(22.2)	6(13.6)			
	Doctor	31(15.6)	5(16.1)	0.47	1.912	0.959–3.812
Close contact with patients		144(72.7)	12(8.3)	0.02	1.125	0.475–2.667
Contact with animals		103(52)	2(1.9)	1	4.663	0.289–75.329
Use of antibiotics last six months		68(34.3)	2(2.9)	0.18	4.856	0.495-47.652
Hospital admission last one year		22(11.1)	7(31.8)	0.17	2.610	1.339–5.088
Chronic skin disease		7(3.5)	1(14.2)	0.06	2.263	0.905–5.654
Diabetes mellitus		21(10.6)	1(4.7)	1	9.938	1.617–61.069
Wound or ulcer in last six months		7(3.6)	2(20)	0.04	0.677	0.383–1.196
# Row percentage; ##Comparison of MDR positive against negative tested HCW						



	Doctors	Technicians	Nurses
VRE	1	1	0
MRSA	2	2	1
MDR Pseudomonas	3	3	1
MDR Acinetobacter	7	0	3

Fig. 1: Distribution of MDR bacteria amongst HCW

(10.6%).A higher incidence of contamination of doctors compared with the incidence of contamination of nurses and technicians, is especially concerning, because physicians typically see more patients and have lower rates of hand hygiene than other HCWs [13].

Our analysis showed more frequent MDR colonization amongst the male staff. This finding is similar to that reported in previous studies[14,15]). Also significant colonization was observed in HCW who had sufferd from a wound or ulcer on an exposed part of the body in last six months, corroborated by a previous study [14]].This can be adquetely explained by the fact that break in integrity of skin makes it easier for organisms to gain access to the body.

Also a significant association was found between MDR colonization and close contact with ICU patients which is different from that reported by Petres et al[15,16] For the purpose of the study close contact with patient was defined as changing dressing, or assisting mobility or carrying out an invasive procedure. These procedures make the HCW at risk of acquiring bacterial flora because of contact with contaminated wound, pus, blood or other body fluids. Other factors did not show significant association with colonization of HCW.

We did not collect patient-specific information, so were unable to assess whether the ICU patients were actually the cause of this contamination of hands of HCW .However, as is evident from the demographic data collected, most HCW have been working in close contact with ICU patients for a considerable period of time and many of them had one or more known risk factor for acquiring bacteria flora. Therefore, they have more chances of having acquired the bacterial pathogens from patients either through direct contact or indirectly from contaminated environment than from any other source. Nevertheless, for confirmation of whether the same isolate has been transferred between HCW and patients, molecular characterization is needed and this remains a limitation of our study.

Conclusion

Health Care Associated Infections especially in the vulnerable ICU patient population can be linked to the bacterial flora colonizing the HCWs. This flora is acquired during routine patient care activity either due to direct patient contactor indirectly through contaminated environment or surfaces. This contaminating flora is more likely to be MDR in ICU patients due to risk factors associated with these patients such a prolonged use multiple antibiotics. Determining the prevalence of MDR bacteria on the hands of HCW and the associated risk factors can help the hospital in preparing an effective strategy for curbing the menace of Health care associated infections. Further, investigation of the isolates from nasal swabs and or hands of healthcare workers and simultaneously form ICU patients by molecular studies and genotyping is desirable, in order to establish the authenticity, identity and inter- relationship of the isolated organisms, and prove their roles in infection causation.

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Significance of Spe rm Characteristics in the Evaluation of Male Infertility in a Tertiary Care Centre

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ABSTRACT

Background: Infertility is both a clinical and a public problem. Standard semen analysis is the surrogate measure of male fertility in clinical practice to determine prevalence of low sperm count including oligozoospermia and azoospermia and to assess the pattern and distribution of abnormal semen parameters in infertile men.

Methods: The retrospective study was conducted with compiling of the data from archival record over a period of three years from June 2013 to June 2016. A total of 933 male partners of women attending the fertility clinic of hospital between the ages of 20 and 50 years were recruited. The samples taken were primary infertility cases using simple random sampling technique. Semen analysis was performed according to the standards outlined by the World Health Organization (5th edition 2010). Parameters outlined included: Appearance, Volume, pH, Sperm concentration, Motility, Morphology, Viability and White cell count.

Result: Out of 933 samples, normozoospermia was observed in 659 (70.6%) males, oligozoospermia 170 (18.2%), and azoospermia 104 (11.1%). The azoospermic and oligozoospermic samples had low ejaculated volume, but significantly higher percentage of pus cells in comparison to normozoospermic samples. The oligozoospermic samples had higher percentage of immotile sperms and abnormal morphology in comparison to normozoospermic samples. Asthenozoospermia was observed in 118 (14.2%), teratozoospermia in 24 (2.9%), and oligoteratozoospermia in 11 (1.3%) of samples.

Conclusion: Majority of cases of infertility in males show normal sperm count. Oligozoospermia followed by azoospermia is seen in rest of the cases while less sperm motility or less amount of semen are also responsible in some cases.

Keywords: *Semen Analysis, Male Infertility, Sperm Motility, Morphology, Azoospermia.*

Introduction

Infertility is a comprehensive issue affecting approximately 13-15% of the population all over the world.[1] A host of factors have been implicated in the causation of infertility. [1] The female factor is responsible for 35% of cases whereas the male factor is seen in 45% of cases. The remaining 20% of the couples either have non-identified infertility or mixture of factors. [2]

The role of semen analysis in the assessment of male fertility is paramount and remains the most fundamental and primary investigation. [3] The use of standardized and objective procedures ensures satisfactory categorization of cases of infertility. [3] The results of the test provide vital information regarding the concentration, motility and morphology of the spermatozoa in a semen sample. [1] The sensitivity value of standard semen analysis is 89.6%, which implies that it has the ability to identify 9 out of 10 men having a genuine problem. [4] Semen analysis allows for a better understanding of the structural and dynamical parameters involved in sperm function. [2] The analysis also helps to elucidate the pathological causes for decreased sperm count thereby classifying the issue into a pre-testicular, testicular or post testicular phase. [5]

The majority of male infertility (90%) is due to low sperm number, poor semen quality or a combination of both. Worldwide collected data suggest that there has been a continuous decline in semen quality and quantity. [6] This alarming finding can be attributed to increased prevalence of sexually transmitted diseases (STDs), urogenital infections and modern lifestyle influences. [7] In our population where infertility is considered to be a social stigma and the female is often held responsible for the inability to conceive, screening by semen analysis to rule out the male factor is imperative before subjecting the couple to extensive investigations. [8]

In this study we determine the frequency of low sperm count in Indian population and semen parameters include sperm motility and morphological details to identify abnormalities in semen.

Materials and Methods

The retrospective study was carried out in the Department of Pathology, Government Medical College and Hospital,

Chandigarh, from June 2013 to June 2016. All these couples were unable to conceive for at least 12 months. All the cases, which included the study, were archived from hospital records.

933 Samples of male partners (933) of women attending the fertility clinic of the hospital between the ages of 20 and 50 years were evaluated. The samples taken were primary infertility cases. Cases of secondary infertility were excluded from the study.

Analysis of semen was performed according to the standard methods outlined by the World Health Organization (WHO laboratory manual for the examination and processing of human semen 5th edition 2010). Parameters outlined included: Appearance: grey/opalescent; Volume: 2.0ml or more; pH: alkaline i.e. 7.2-7.8; Sperm concentration: $>15x10^6$ spermatozoa/ml; Total sperm count: $39x10^6$ per ejaculate or more; Motility: 40% or more including progressive and non progressive motility; Morphology: 4% or more with normal forms; Viability: 58% live spermatozoa; White cell count: $<1x10^9$/ml.

Complete sample collection and analysis was done by the same lab technician to avoid inter-laboratory variation. Within 60 minutes of collection, semen analysis was performed and parameters included appearance, morphology, motility, volume, liquefaction, pH, concentration, viability and the occurrence of pus cells. Disposable pipette (graduated) were used to measure semen volume; pH test was done with the help of pH paper. After liquefaction, sperm motility was assessed by microscopic evaluation of 200 spermatozoa from different fields. Counting of spermatozoa was done using improved Neubauer's chamber. Viability was assessed with eosin stain. The semen samples were categorized on the basis of sperm count/mL of semen in accordance with WHO normal and pathological ranges i.e. normozoospermia (normal sperm count), oligozoospermia, (total number (or concentration, depending on outcome reported) of spermatozoa below the lower reference limit) and azoospermia (no spermatozoa in the ejaculate). The different samples were categorized and compared for ejaculated volume, sperm count, viability, pus cells, motility and morphology.

The following operational definitions were used: Normozoospermia: Sperm count 15 million/ml to 150 million/ml; Oligozoospermia: Sperm count below 15 million/ml; Azoospermia: Complete absence of spermatozoa in the ejaculation; Asthenozoospermia: Reduced sperm motility below the lower reference limit; Teratozoospermia: Abnormal sperm morphology; Oligoasthenoteratozoospermia: All sperm variables abnormal; Hypospermia: Volume <2ml; Normospermia: Volume 2-5 ml; and Hyperspermia: Volume >5ml.

The data was analysed using SPSS software (version 15). Mean ± Standard deviation (SD) were calculated for sperm count, volume, motility, morphology and pus cells; 95% confidence interval was calculated for proportions and for means. Mean values were also compared for statistical significance using t-value with level of significance <0.05 (p value).

Result

A total 933 semen analysis reports of male partners of infertile couples were analyzed over a period of 3 years. Among the 933 males, the mean age was 30.02±4.72 years. Using WHO standard for semen normality, 933 semen samples were analysed, out of these 659 (70.6%) had normozoospermia, 170 (18.2%) had oligozoospermia and 104 (11.1%) azoospermia, as depicted in Table 1. On the basis of semen volume, samples were categorized as normospermia (2-5ml), hypospermia (<2ml), hyperspermia (>5ml). The distribution of semen volume is shown in Table 2.

After excluding 104 samples with azoospermia, semen parameters were compared in oligozoospermic and normozoospermic samples for count/sperm concentration (million/ml), volume (ml), liquefaction time (min), viability (%), motile sperms (including progressive motile and non-progressive motile sperms), immotile sperms, morphologically normal sperms and abnormal sperms (including head, neck and tail abnormalities) and pus cell (per HPF). The oligozoospermic samples had significantly higher percentage of immotile sperms 55.79±28.00 and abnormal morphology 23.64±27.28 compared to normozoospermia in which non-motile sperms were 33.32±19.40, and abnormal morphology was 11.45±9.74 respectively (p <0.001).

Comparison of volume showed mean volume of 2.64±1.38ml in normozoospermia vs 2.25±1.17ml in oligozoospermia (p 0.002), and pus cells 4.33±4.38/HPF in normozoospermia vs 5.71±6.56/HPF in oligozoospermia. This was statistically significant (p 0.009) (Table 3). Normal motility was observed in 66.66±19.99 of normozoospermic vs 44.38±28.20 of oligozoospermic samples, and normal morphology of sperms was observed in 88.54±9.74 of normozoospermic vs 76.43±27.27 of oligozoospermic samples (p < 0.001).

Comparison of sperm viability showed mean viability of 69.87±26.69 in normozoospermia vs 51.78±24.15 in oligozoospermia (p <0.001) and liquefaction time

42.76±10.20 min in normozoospermia vs 44.25±10.67min in oligozoospermia. This was not statistically significant (p 0.093) (Table 3).

The proportion of multiple factor abnormalities defects were seen in 253 cases out of 829 cases of both normozoospermia and oligozoospermia as given in Table 4.

Table 1: Frequency of sperm concentration

Category	Frequency (N=933)	Percentage (%)
Normozoospermia	659	70.6
Oligozoospermia	170	18.2
Azoospermia	104	11.1

Table 2: Distribution of semen volume

Volume	Frequency (N=933)	Percentage (%)
Normospermia (2-5ml)	658	70.5
Hypospermia (<2ml)	252	27.0
Hyperspermia (>5ml)	23	2.4

Table 3: Comparison of semen parameters between normozoo-spermia and oligozoo-spermia

Category	Count mean±SD	Volume mean±SD	Viability mean±SD	Pus cells mean±SD median±IR	Motile sperm mean±SD	Immotile sperm mean±SD	Normal sperm mean±SD	Abnormal sperm mean±SD
Normozoo -spermia	84.98±32.87	2.64±1.38	69.87±26.69	4.33±4.38	66.66±19.99	33.32±19.40	88.54±9.74	11.45±9.74
95%CI	82.46-87.49	2.53±2.74	67.83-71.91	4.00-4.67	65.13-68.19	31.84±34.80	87.79-89.28	10.71±12.20
Oligozoo-spermia	9.78±3.82	2.25±1.17	51.78±24.15	5.71±6.56	44.38±28.20	55.79±28.00	76.43±27.27	23.64±27.28
95%CI	9.20-10.36	2.08±2.43	48.12-55.44	4.72-6.71	40.11-48.65	51.55±60.03	72.29-80.55	19.50±27.77
P value	<0.001	0.002	<0.001	0.009	<0.001	<0.001	<0.001	<0.001

Table 4: Proportion of multiple factor abnormalities defect

Pattern of abnormalities	Frequency	Percentage (%)
Asthenozoospermia	118	14.2
Teratozoospermia	24	2.9
Asthenoteratozoospermia	13	1.39
Oligoasthenozoospermia	68	8.2
Oligoteratozoospermia	19	2.3
Oligoasthenoteratozoospermia	11	1.3

Discussion

Infertility has long been a subject of debate and the females have always had to bear the brunt of the socio-cultural connotations of this multifaceted issue. [1,10] Advancements and progress of novel assisted reproductive techniques establish males to be an equal, if not higher contributor to this complex problem.[2] Despite education and enlightenment, the social attitude towards infertility results in much trauma, emotional instability and psychological stress, which in turn has an adverse bearing on the physiology and psychology of the individual, particularly in a social set-up such as ours, where there has been a strong emphasis on child-bearing. [11] Semen analysis provides some insight about the pathology of epidemiological problems occurring in the male genital tract.[1,4] As high as majority (90%) of male infertility problems are connected to sperm count and a positive association between abnormal semen parameters and sperm number, has been observed. The problem of sperm count, motility and morphology stems from disarray in

control mechanisms, including pre-testicular, testicular and post-testicular factors. [12]

In our study it was found that of total 933 cases 659 (70.6%) males had normal sperm count and rest 274 (29.3%) males had abnormal semen analysis report. This is similar to a study done in 2012 which reported the incidence of male infertility as 62%.[7] The reported prevalence of oligozoospermic, azoospermic, asthenozoospermic and asthenoteratozoospermic in cases of primary infertility in same study was 33.17%, 9.89%, 1.83 and 1.08% respectively, [13] which were similar to our study results. The prevalence of azoospermia in our study population was 10.70%, oligozoospermia 34.14%, asthenozoospemia 14.2%, and of asthenoteratozoospermia 1.39% respectively. [7] The results are comparable to study which reported the occurrence of azoospermia as 14.28% and that of oligozoospermia 21.43%,[14] in another study, the incidence rate of azoospermia was 16%.[15]

Mean ejaculated volume in normozoospermia was 2.64±1.38 ml vs 2.25±1.17 ml in oligozoospermia and 2.20±1.30 ml in azoospermic samples respectively. Majority of our patients had normal semen volume 70.5%, while 27.0% showed hypospermia (<2ml), and hyperspermia in 2.4%, these results can be comparable to a study conducted in Sudan where majority of the subjects (89.7%) had adequate semen volume, while only 10.3% had abnormal semen volume. [16] Moreover, these results are also analogous to a study conducted in Nigeria in which majority of the subjects (91%) had adequate semen volume, while only 9% had abnormal semen volume i.e 7.3% hypospermia and 1.7% hyperspermia. [17] The adequate semen volume obtained in our study may be a result of the 3-6 days of sexual abstinence.

In normozoospermia samples, the mean percentage of normal motile sperms was 57%±0.18 as compared to oligozoospermia in which motile sperms were 38%±23%. However, advancing techniques to some extent overcome the problems of sperm motility in infertile couples, but asthenozoospermia is still a common cause of human male infertility. In our study, asthenozoospermia was observed in 14.2% of samples and the results were comparable to a study conducted at the National Institute of Heath, Islamabad, in which the prevalence was around 21.42%. [18] In another study, the prevalence of asthenozoospermia was 18%.[19]

Morphology of a sperm i.e. the differential development of the head, mid-piece and tail is a function of testes as well as the epididymis. In this study in normozoospermia samples, mean normal morphology was found to be 88.54±9.74% vs. 76.43±27.27% in oligozoospermic samples. The oligozoospermic samples had significantly higher abnormal motility 55.79±28.00% and abnormal morphology 23.64±27.28% as compared to normozoospermic samples with 33.32±19.40% abnormal motility and 11.45±9.74% abnormal morphology. These results are comparable to a study in which abnormal morphology was observed in 53% and abnormal motility in 60% oligozoospermic males.[20] So sperm motility and morphology are changing parameters and their relative levels depend on the existing sperm count in an individual. [20]

Oligozoospermic samples were found to be associated with significant higher abnormal motility 62%±0.239 and abnormal morphology 55%±0.156 as compared to normozoospermic samples although we did not specify the type of abnormal morphology. The results are comparable to a study in which abnormal morphology was observed in 53% and abnormal motility in 60% oligozoospermic males. So sperm motility and morphology are changing parameters and their relative levels depend on the existing sperm count in an individual. [20]

The prognosis of the infertile couple is inversely proportional to the number of abnormal patterns so one pattern of abnormality is better than two-pattern abnormality, and two is better than three-factor abnormality. [21,22] When three-pattern abnormalities were identified in oligozoospermic sample population, the prevalence of oligoasthenoteratozoospermia was 1.3%. The results were comparable to a study in which prevalence of oligoasthenoteratozoospermia was 11%. [18] The prevalence of teratozoospermia in our study population was 2.9%.

Conclusion

Semen analysis is primary tool to investigate male infertility which is more useful in developing countries like India. It comes under basic investigation done at minimal rates for infertility cases. It is a cost effective, more reproducible and gives robust information of male reproductive function. The use of conventional parameters, such as sperm count, viability, sperm morphology and motility are markers of male reproductive function. Thus, semen analysis serves as a preliminary investigation to rule out male cause of infertility.

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Competing Interests

There are no conflicts of interest.

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Expression of PSMA in Thyroid Follicular Neoplasms: Utility for Differentiating Between Benign from Malignant Lesions

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ABSTRACT

Background: Prostate-Specific Membrane Antigen (PSMA) has been known as a tumor associated neovasculature marker in some solid malignant tumors. Targeting of PSMA by cytotoxic- conjugated antibody can represent powerful tool for vascular targeted therapy. In thyroid, it appears that angiogenesis factors are involved in neoplastic growth and aggressiveness of tumors.

This study was conducted to evaluate the expression of PSMA as an angiogenesis factor in neovasculature of thyroid follicular neoplasms by immunohistochemistry to determine its usefulness for distinguishing between adenoma and carcinoma.

Methods: Paraffin Blocks of formalin fixed samples representing 48 cases of Follicular Thyroid Adenoma (FTA) and 15 cases of Follicular Thyroid Carcinoma (FTC) were evaluated.

Results: Among 15 cases of FTC, 11 cases revealed both capsular and vascular invasion. In one case only capsular and in two cases only vascular invasions were observed. One case also revealed suspicious vascular invasion with further bone metastasis.

The intensity of PSMA staining in vascular structures of carcinomas were significantly higher in comparison with thyroid follicular adenomas (p=0.028). However, we observed no significant difference in the extent of PSMA expression in adenoma and carcinoma groups (p=0.239).Thyroid follicular cells were negative for PSMA in adenoma, carcinoma and in non-neoplastic area.

Conclusion: According to our findings, significant higher intensity of PSMA expression in FTC could be useful in differentiating between FTA and FTC. However, more importantly, expression in neovasculature of differentiated thyroid tumors can represent a potential utility for PSMA-targeted radionuclide therapy in advanced differentiated thyroid cancers.

Keywords: *Angiogenesis, Prostate-Specific Membrane Antigen, Thyroid Follicular Adenoma, Thyroid Follicular Carcinoma*

Introduction

Thyroid cancers are the most common endocrine malignancies that have increased recently.[1] The number of cases with thyroid nodule requiring further evaluation even is higher, probably due to use of more sensitive detection techniques. [2] Thyroid lesions with follicular growth pattern are composed of a wide range including benign adenomatous nodule, Follicular Thyroid Adenoma (FTA), Follicular Thyroid carcinoma (FTC) and Follicular variant of Papillary Thyroid Carcinoma (FVPTC).[3-5]

FTA, FTC and FVPTC are well encapsulated lesions sharing many imaging, cytologic and histologic features[5-8].

Difficulties are also not uncommon while reporting these follicular pattern neoplasms based on histomorphological findings.[9] Thus, diagnosis of a solitary encapsulated nodule with follicular histologic pattern is sometimes problematic for pathologists.[5, 10]

One of the most challenging tasks in pathologic evaluation of thyroid follicular pattern lesion is distinction between FTC and FTA as a common benign thyroid nodule. [4]

Diagnosis of carcinoma is based on the presence of capsular, vascular or extra thyroid tissue invasion or nodal or distant metastasis.[5, 8, 10-12]

Different tools such as IHC and molecular profiling have been used to differentiate between benign and malignant follicular neoplasms or differentiating malignant tumor subtypes, [4, 9, 13] including some angiogenesis factors. [9]Thyroid is rich in vascular network[14]and shows increased vascularity in both non neoplastic and malignant conditions. [9]

On the other hand, a system of nutrient vessels is an essential component of tumor growth. [15] For example, expression of VEGF, a hypoxia induced angiogenic factor, concomitant with hyper vascularity have been induced more strongly in differentiated malignant thyroid tumors than benign tumors. [16] Micro-vessel Density (MVD) as an indicator of angiogenesis is shown to be correlated with disease free survival in PTC and intra thyroid tumor spread in FTC.[9, 14, 17]

These data suggest that angiogenesis factors are involved in neoplastic growth and aggressiveness of thyroid tumors.

[14] Thus, monitoring the process of angiogenesis may represent an early tool for diagnosis of malignancy.[18]

Prostate Specific Membrane Antigen (PSMA) is type II integral membrane glycoprotein initially expressed in prostate cancer cells but then it had been discovered that it also is expressed in neovasculature of the various solid tumors, including some carcinomas, neuroendocrine tumors, sarcomas and melanomas[15, 19-21], but neither normal endothelium [15] nor endothelial cells of benign tissue are positive.[19] Recently, it has been suggested that PSMA expression has been evident in some thyroid neoplasms using [^{68}Ga]PSMA-HBED-ccPET/CT.[22-26]

This study is conducted to evaluate the expression of PSMA by Immunohistochemistry in neovasculature of thyroid follicular neoplasms to determine its usefulness for distinguishing between adenoma and carcinoma. Also, to evaluate the potential capability of radio labeled Anti PSMA antibody as complementary target therapy in advanced thyroid malignancies.

Materials & Methods

Paraffin Blocks of formalin fixed samples representing 48 cases of FTA and 15 cases of FTC were retrieved from archive of pathology department .The questionable cases were excluded. Blocks with minimal necrosis or hemorrhage and representative amount of tumor tissue were selected for IHC study. IHC was performed using monoclonal liquid NCL-L-PSMA mouse antibody (Clone 1D6, Novocastra) according to the manufacturers' protocol. Prostate tissue was considered as positive control. Also we performed CD31 staining (JC/70A,Biogenex) to confirm the localization of neovasculature.

The stained sections were assessed for the extent and intensity of endothelial cell staining in tumor micro-vessels and scored semi-quantitatively.(table-1)

After data collection, all quantitative and qualitative data were analyzed using statistical package for Social Sciences (SPSS) version 19. P-value less than 0.05 was considered significant.

Results

Totally, 63 cases of thyroid follicular neoplasms were evaluated including 48 cases of follicular thyroid adenoma and 15 cases of follicular thyroid carcinomas. Among FTCs, 11 cases revealed both capsular and vascular invasion. In one case only capsular and in two cases only vascular invasions were observed. One case also revealed suspicious vascular invasion with further bone metastasis.

Female: male ratio in adenoma and carcinoma groups were 37:11 and 9:6, respectively. The average age of the patients in adenoma and carcinoma groups was 35.8± 10.1 and 43.8 ± 13.4 years, respectively. Thus, the patients with carcinoma were significantly older than adenoma group. (p=0.029). Regarding to the size of the tumor, we did not find any significant relationship. (3.3 ± 1.7 cm in adenoma versus 3.7±2.4cm in carcinoma; p=0.597).

We evaluated the extent and intensity of PSMA staining in vascular endothelial cells and scored semi-quantitatively (Table-1). The results for PSMA IHC staining are summarized in table 2 and 3. The intensity of PSMA staining was significantly higher in carcinoma rather than adenoma cases (p=0.028) (Table-2). However, we did not observe any significant difference in the extent of PSMA expression in vascular structures of thyroid adenoma and carcinoma groups (p=0.239)(Table-3). Thyroid follicular cells in adenoma, carcinoma also non neoplastic area were negative for PSMA.

Discussion

Reliable determination of thyroid tumor pathology could reduce the cost of management of thyroid nodules by eliminating many of the diagnostic thyroidectomies

Table 1: Scoring system for the Extent and Intensity of PSMA in IHC staining

Percentage of stained endothelial cells(%)	Interpretation	Intensity	Interpretation
0-9	Negative	No reaction	0
10-39	Minimal	Faint reaction visible only at high power	1+
40-69	Moderate	Moderate intensity at low power	2+
≥70	Diffuse	Strong reaction easily visible at low power	3+

Table 2: The intensity of PSMA staining by immunohistochemistry in FTA and FTC

Intensity Score, Histologic Dx	0	1	2	3	Total
Follicular Thyroid Adenoma	39(81.25%)	6(12.50%)	1(2.08%)	2(4.17%)	**48**
Follicular Thyroid Carcinoma	10(66.67%)	1(6.67%)	4(26.66%)	0(0.00%)	**15**

Table 3: The extent of PSMA staining by immunohistochemistry in FTA and FTC

Extent Score, Histologic Dx	Negative	Minimal	Moderate	Diffuse	Total
Follicular Thyroid Adenoma	41(85.42%)	3(6.25%)	3(6.25%)	1(2.08%)	**48**
Follicular Thyroid Carcinoma	11(73.33%)	1(6.67%)	1(6.67%)	2(13.33%)	**15**

being performed for benign thyroid nodules or morbidity associated with these operations.[2] Some angiogenesis modulating genes such as VEGF have been suggested as a marker of more aggressive differentiated thyroid cancers[2] or more strongly induced in differentiated malignant tumors in comparison with benign tumors.[16, 27, 28]

PSMA also known as folate hydrolase , is type II transmembrane glycoprotein that located on chromosome II.[15, 29] It was first described to be expressed in benign prostate acinar epithelium [15] but further studies have shown that PSMA is also expressed in non-prostate tissue including luminal/apical aspect of duodenum, kidney proximal tubules and neuroendocrine cells in colonic crypt.[29]

Also, PSMA has been known as a tumor associated neovasculature marker in some solid malignant tumors[19] which targeting of that by cytotoxic- conjugated antibody can represent powerful tool for vascular targeted therapy in malignant tumors.[15] We did not find significant difference between extent of expression of PSMA between benign and malignant follicular neoplasms which limits its ability as a differentiating marker. In a study by Karl Sgal et al,[14, 30] a marginal difference was noted in the degree of vascularity between FTA and FTC using VWF by IHC method. An important finding of the study was increased density of vessels adjacent to capsule (especially in area of invasion) in FTC which was not the same in FTA(1 vessel/Two thyroid follicular cells versus 1 vessel /10 thyroid follicular cells, respectively). Also areas with solid proliferation showed increased vascularity.[14, 30] In another study by Frigugliett et al,[14, 31] using CD34 to estimate the angiogenesis, there was no significant difference between FTC and FTA(p=0.388). Also there was no significance regarding to sex, size, or age. However, AcSDKP as an angiogenic active marker showed five times higher mean tissue concentration in PTC rather than benign nodular goiter by Kusinski et al.[18]Working on the value of angiogenesis-modulating genes in various thyroid lesions by Kebebew et al,[2] some independent markers of malignancy of follicular cell origin were observed including ANGPT2 (Angiopeptin2)and TIMP1 (Tissue inhibitor of metalloproteinase I) mRNA. They recommended that they could be helpful adjunct to FNA cytology.[2]

Recently, there has been some evidences regarding to increased uptake of PSMA using [^{68}Ga] PSMA-HBED-ccPET/CT in different thyroid tumors.[22-26] For example, in a report by Kanthan et al[23] a known case of prostate cancer underwent [^{68}Ga] PSMA-HBED-ccPET/CT for staging. A PSMA –Avid thyroid lesion was identified which on further histologic exam was diagnosed as FTA.[23]

Incidental FTC with increased uptake also reported by Sager et al,[24]in a known case of prostate cancer. In another report by Tawade et al[25] they believes that it was more effective in detection of multiple mediastinal, lung and supra-clavicular lymph nodes and brain metastatic lesions in a patient with rising thyroglobulin and negative whole body radioiodine scan.[25]

In a tissue microarray based study by Mhawech-Fauceglia P et al[32, 33] they observed positive PSMA staining in some isolated thyroid cancers. However no PSMA positivity was reported by their team in 846 benign tumors.[32, 33]

On histology, However; there are limited data regarding to expression of PSMA in thyroid neoplasms. In a study by Silver et al[29] and Mhawech-Fauceglia et al., [33] they found thyroid is negative for PSMA using clone mouse monoclonal antibody CYT-351(clone 7E11-C5;Cytogen, Princeton, NJ) and monoclonal antibody YPSMA-1,1:50;Genetex,Inc. , San Antonio, Tx, USA), respectively. We also did not find any reaction of thyroid follicular cell using monoclonal liquid NCL-L-PSMA mouse antibody (Clone 1D6, Novocastra). However regarding to tumor neovasculature, seven out of 48 and four out of 15cases of FTA and FTC were positive for PSMA, respectively.

Conclusion

To our knowledge, this is the first study focusing on expression of PSMA in thyroid follicular neoplasms by IHC method. The intensity of PSMA in vascular structures of follicular thyroid carcinomas was significantly stronger than adenomas. However, Respect to thyroid follicular cell staining, our findings do not support usefulness of PSMA in differentiating between FTA and FTC. More importantly, expression of PSMA in neovasculature of differentiated thyroid follicular neoplasms can represent a potential utility for PSMA-targeted radionuclide therapy in advanced ^{131}I-resistant/negative differentiated thyroid cancers.

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Histopathological and Histomorphometric Analysis of Pancreas and Liver of Diabetic Rats Treated with Mucuna Pruriens Seed Extract

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ABSTRACT

Background: Medicinal plants play a major role in controlling diabetes by producing microstructural changes in liver and pancreas. Quantification of islets and hepatocytes by using histomorphometric tools are effective in evaluating the antidiabetic action of drugs.

Aims and Objectives: To assess the anti diabetic properties of alcoholic extracts of Mucuna Pruriens seeds (200 mg/kg b.w) by means of histological and histomorphometric analysis of pancreas and liver .

Methods: Seventy two neonatal wistar rats were randomly divided into four groups, i.e. Normal , Diabetic control, diabetic rats treated with Mucuna and Glibenclamide. Diabetes was induced by a single intraperitoneal administration of streptozotocin (65mg/kg b.w) in 5 day old neonatal rats (n =54).12 weeks after the injection, animals were divided in to four groups with 18 animals in each. The study groups were as follows. Group A -Normal control, Group B – STZ untreated, Group C - Mucuna Pruriens 200mg/kg, Group D -Glibenclamide (1mg/kg). Fasting blood sugar levels were monitored once a week during the drug study .i.e. 12 weeks. At the end of every 4th week, 6 animals from each group were sacrificed for histological and histomorphometric studies on liver and Pancreas.

Results: From the histological studies, the diabetic group showed reduction of cell population in the pancreas and depletion of the number of hepatocytes in the liver. After 12 weeks of Drug treatment, These changes were markedly reduced in Mucuna Pruriens treated group. The obtained results were further quantified by means of Histomorphometric analysis and the results showed reduction in the number and diameter of islets, number of beta cells and number of healthy hepatocytes in diabetic control group and treatment with Mucuna Pruriens seed extract significantly reversed these changes ($p < 0.05$)

Keywords: *Histomorphometric Analysis, Mucuna Pruiens, Pancreas, Liver, Diabetes*

Introduction

Diabetes is on the rise. No longer a disease of predominantly rich nations, the prevalence of diabetes is steadily increasing everywhere, most markedly in the world's middle-income countries. Based on a recent systematic review, it has been estimated that the direct annual cost of diabetes to the world is more than US$ 827 billion[1]. The International Diabetes Federation (IDF) estimates that total global health-care spending on diabetes more than tripled over the period 2003 to 2013 – the result of increases in the number of people with diabetes and increases in per capita diabetes spending[2].

Diabetes mainly affects the metabolic functions of liver and pancreas; primarily beta cells in islets of Langerhans. Streptozotocin is derived from a soil bacterium called *Streptomyces Achromogenes*, produces permanent damage to the beta cells by DNA fragmentation and oxidative stress, which leads to a reduction in the beta cell mass and pancreatic islets. Furthermore it elicits microstructural damage in hepatocytes, that adversely affects the glucose and lipid metabolism[3].The effectiveness of the antidiabetic agents like biguinaides, sulphonylureas and metformins are mostly dose dependent and prone to side effects[4].

In Indian system of Medicine, many plants are effectively used in diabetes treatment[5].The *Mucuna Pruriens* Linn. (Fabaceae), generally known as cowhage , is used for the treatment of Erecile Dysfunction and Parkinsonism in Ayurveda. Several research studies have been conducted on the antidiabetic activities of the seeds of MP through biochemical and hematological studies. However, most of this research was short term and no histopathological and histomorphometric parameters were incorporated [6]. Hence

the present study was undertaken to analyze the effects of *Mucuna Pruriens* seed extract in diabetic wistar rats by histological and histomorphometric analysis.

Materials and Method

The research protocol has been duly approved by IAEC of Mahatma Gandhi Medical College and Research Institute, SBV University, Puducherry and CPCSEA (686/02/a/CPCSEA). Five-day old neonate wistar rats (n-72) were received from the Central Animal House of Mahatma Gandhi Medical College and Research Institute. The rats were made diabetic by a single dose i.p. injection of streptozotocin (Sigma Aldrich, U.S.A) (65 mg/kg b.w) dissolved in ice cold citrate buffer (0.1M, pH-4.5). After 4 weeks, blood sugar levels [BGL] were measured by using a one - touch glucometer (Accu Chek, Roche Diagnostics, USA) and animals showing value > 150mg/dl of BGL were selected for the drug study[7].The animals were kept under standard laboratory conditions, fed with standard pellet diet and water ad libitum. 12 weeks after the injection, the rats were classified into four groups of eighteen in each. The groups are as follows

Group A – Normal control (Non diabetic rats); Group B – Diabetic control; Group C – Experimental drug (Mucuna Pruriens Seed extract 200mg/kg b.w); Group D-Standard drug (Glibenclamide 1 mg/kg b.w).

The alcoholic extract of *Mucuna Pruriens* (200mg/kg body weight) and glibenclamide (1 mg/kg body weight) were administered orally for 12 weeks. The Fasting Blood Glucose Level [FBGL] was measured once a week during the treatment period with the help of a one - touch glucometer (Accu Chek, Roche Diagnostics, USA). After every four weeks of drug treatment, six animals from each group were sacrificed by painless cervical dislocation under mild chloroform anesthesia.

Liver and pancreas were carefully dissected out and transferred to normal saline solution in a petridish. After wiping out the water content with a tissue paper, the organs were weighed by using a digital weighing machine .Then the liver specimens were transferred to 10% neutral buffer formalin and the pancreas to freshly prepared bouin's fluid. After 48 hours of fixation, the tissues were processed for histological studies. Pancreatic and liver Sections were stained with haematoxylin and eosin (H&E) and Gomori's chrome alum haematoxylin phloxine stain. The tissue sections were analyzed under a trinocular research microscope (Olympus CX 41) fitted with camera (Olympus E420).

Histomorphometry: Conventional stereological principles and accepted morphometric procedures[8,9] were followed to obtain quantitative information about the pancreatic islets and hepatic parenchyma. 100 serial sections from each group were examined with an ocular micrometer and a calibrated graticule. The parameters analyzed were The number of beta cells per unit area, the diameter of the islets per unit area, the number islets per unit area, the number of healthy and necrotic hepatocytes per unit area and number of binucleated hepatocytes per unit area

The islet area was calculated in each pancreatic section at 400X magnification with the help of an area calibrated ocular grid. The number of islets and the area of pancreatic tissue were counted at 40X magnification using the ocular grid. The total number of islets was expressed as N/10 mm^2 of the pancreatic parenchyma. The number of beta cells and hepatocytes were determined by direct counting method at 1000X magnification using the ocular grid.

To analyze the diameter of islets, the major axis (a) and minor axis at right angles to the major axis of the islets (b) were measured ,and the diameter of the islet (Di) is calculated using the formula (Di) = $\sqrt{}$ab(10)but fall into two basic categories: images of sections through the structure and projection images viewed through it. The most intensive use of stereology has been in conjunction with microscope images, which includes light microscopes (conventional and confocal.

Statistical Analysis: The observed data were subjected to one-way ANOVA and the significance was determined using a "Tukey's post-hoc" with $P < 0.05$ for statistical significance. The statistical tests were performed using software SPSS 15.

Results

Fasting blood glucose level In the diabetic group, a significant rise in the blood glucose level was noted (P value <0.05). Treatment with *MP* seed extract resulted in statistically significant reduction of blood glucose level when compared to diabetic control (Table 1). The oral administration of *Mucuna Pruriens* produced statistically significant decrease in FBGL (65.4% reduction) ($P < 0.01$).

In the diabetic pancreas, decrease in pancreatic islet's size and number, atrophy and vacoulations, cellular infiltrations, clumping of beta cells and moderate to heavy beta cell destruction were detected. While these abnormal histological signs were significantly decreased as in *Mucuna Pruriens* treated rats. The inflammatory cells and necrotic changes gradually decreased with mild beta cell clumping and increased number of beta cells in the islets.

The structural changes in the pancreas in the glibenclamide group showed similar changes as observed in mucuna group.

In the liver, hydropic dystrophy necroses of hepatocytes and lymphatic infiltration were observed in diabetic rats. In addition to this, dilated sinusoidal space and damaged periportal areas were also visible. Whereas, regenerative changes were observed with the *Mucuna Pruriens* group. i.e, increased number of binucleated hepatocytes, reduced necrotic cells and lymphatic infiltrations.

The results of the histomorphometric assessmentshows that total Number of pancreatic islets was markedly decreased in diabetic control group when compared to the control group (Table no 2.).Whereas a significant increase was observed with *Mucuna Pruriens* and glibenclamide group (P<0.05).The diameter is significantly reduced in the diabetic control group and the shape of the islets was altered (Table. 3). Treatment with *Mucuna Pruriens* resulted in the partial recuperation of the shape and the diameter (P < 0.05). Streptozotocin primarily targets beta cells of the islets, which comprises 75% of islet population. The beta cell population was drastically decreased in diabetic group when compared to the Normal control group (table no -4). The *Mucuna Pruriens* treatment resulted in increased number of beta cells in the islet. The obtained data was statistically significant by 12th week (P <0.05).

In diabetic control, the number of hepatocytes was significantly decreased, and the number of necrotic cells was increased (Table. 5). In *Mucuna Pruriens* treated group, a statistically significant decrease in the necrotic cells and in increase in the number of healthy hepatocytes were noted. Towards the end the drug study the number of active hepatocytes and binucleated hepatocytes were markedly improved in the *Mucuna Pruriens* group when compared to diabetic. P <0.05.

Table 1: The effect of*Mucuna Pruriens* seed extract on Fasting Blood glucose level (in mg/dL).

Period (weeks)	Control	Diabetic	Mucuna	Glibenclamide
1st	83.1±2.3	186±2.9*	179±2.76*	175±3.12*
2nd	83.4±2.1	187.9±2.99*	172±2.65*	169.2±2.93*
3rd	82.9±2.3	190±2.95*	168.6±2.65*	160.1±2.54*
4th	83.5±2.2	195±3.53*	163.6±2.33*	156.8±2.98*
5th	84.6±2.4	201.5±3.62*	158.2±2.43*	152±2.94*
6th	85.1±2.2	206.2±4.02*	154.4±2.78*	150.2±2.87*
7th	83.2±2.6	208.2±3.59*	150.6±2.54*	148.4±2.87*
8th	84.2±2.3	210.3±3.92*	146.5±2.22*	147.1±2.80*
9th	83.6±2.3	211.1±4.45*	143.3±2.11*	146.6±2.44*
10th	82.1±2.6	211.4±4.52*	140.3±2.32*	145±2.55*
11th	81.2±2.7	210±4.22*	134.4±2.98*	143.5±2.65*
12th	83.1±2.1	209.5±4.3*	126.8±2.99*	142.5±2.71*

*Mean±SD, *= P< 0.05.Normal compared with diabetic, and diabetic compared with MP and GC*

Table 2: The number of islets per unit area of pancreas[N/10 mm^2].

Group	4th week	8th week	12th week
Control	15±3.01	14±3.23	14 ±3.32
Diabetic	5 ±1.34*	4±1.54*	4±1.43*
MP extract	8±2.74*	10±3.45*	11±2.26*
Glibenclamide	8±2.43	9±2.45	10±2.12

*Mean ±SD, * =P< 0.05, Normal control compared with diabetic, and diabetic compared with MP and GC.*

Table 3: The Mean diameter of islets [µm].

Group	4th week	8th week	12th week
Control	132.4±14.32	130±14.8	129±12.3
Diabetic	61.3±72*	59±6.44	59±6.33
Mucuna	92.3±9.26 *	95.9±9.87*	97.7±6.64*
Glibenclamide	96.1±8.46 *	95±5.58 *	96.1±5.13*

*Mean ±SD, * =P< 0.05, Normal compared with diabetic, and diabetic compared with MP and GC.*

Table 4: Number of beta cells per unit area of Islets[N/1000 µm^2].

Group	4th week	8th week	12th week
Control	11±2.34	12±3.3	11±3.32
Diabetic	4 ±1.12*	3±1*	3±1.34*
Mucuna	6±2.23 *	7±2*	8±2.45*
Glibenclamide	6±2.13	6±2*	7±3.34*

*Mean ±SD, * =P< 0.05, Normal compared with diabetic, and diabetic compared with MP and GC.*

Table 5. Histomorphometric analysis of the liver [N/1000 µm2].

Parameter	Group	4th week	8th week	12th week
Number of normal hepatocytes	Control	21±03.21	22±3.31	20±3.32
	Diabetic	6±2.32*	6 ±3.15*	7 ±3.43*
	Mucuna	10 ±.13*	12±4.22*	15 ±3.21*
	Glibenclamide	9 ± 2.14*	12±3.64*	13± 2.32*
Number of necrotic hepatocytes	Control	1±1.87	2±1.62*	1 ±1.37*
	Diabetic	18 ±2.24*	17±3.83*	17 ±1.75*
	Mucuna	10 ±3.11*	09 ±4.42*	6 ±03.32*
	Glibenclamide	9 ±3.04*	10 ±3.12*	11± 03.2*
Number of binucleated hepatocytes	Control	3±1.5	3±1.54	3±1.2*
	Diabetic	2±1.54*	2±1.34*	2±1.43*
	Mucuna	5 ±3.5*	6 ±3.65*	6 ± 3.43*
	Glibenclamide	6 ±3.9	4 ±3.9*	3± 3.43*

*Mean ±SD, * =P< 0.05, Normal compared with diabetic, and diabetic compared with MP*

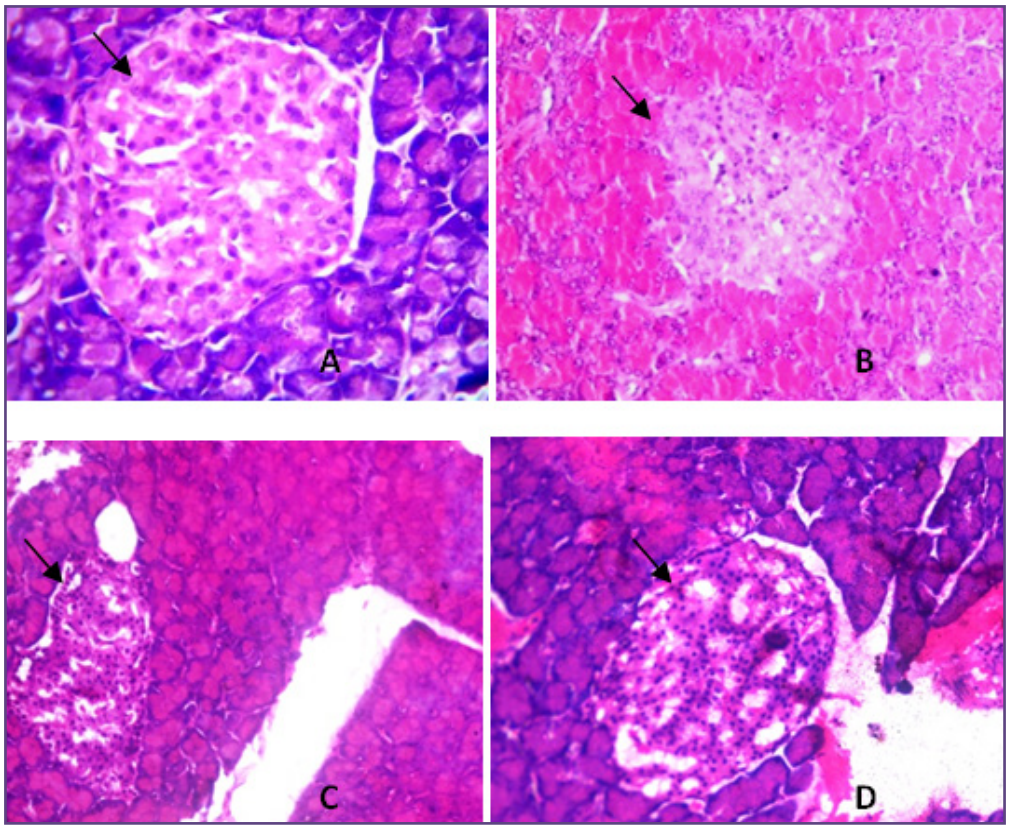

Fig. 1: Pancreas 12th week - H&E Staining : 400x magnification A - Normal islets showing plates of beta cells with sinusoidal spaces. B. Diabetic pancreas showing severe destruction of islets with necrotic beta cells .C & D. Mucuna pruriens and Glibenclamide group showing active regeneration of beta cells.

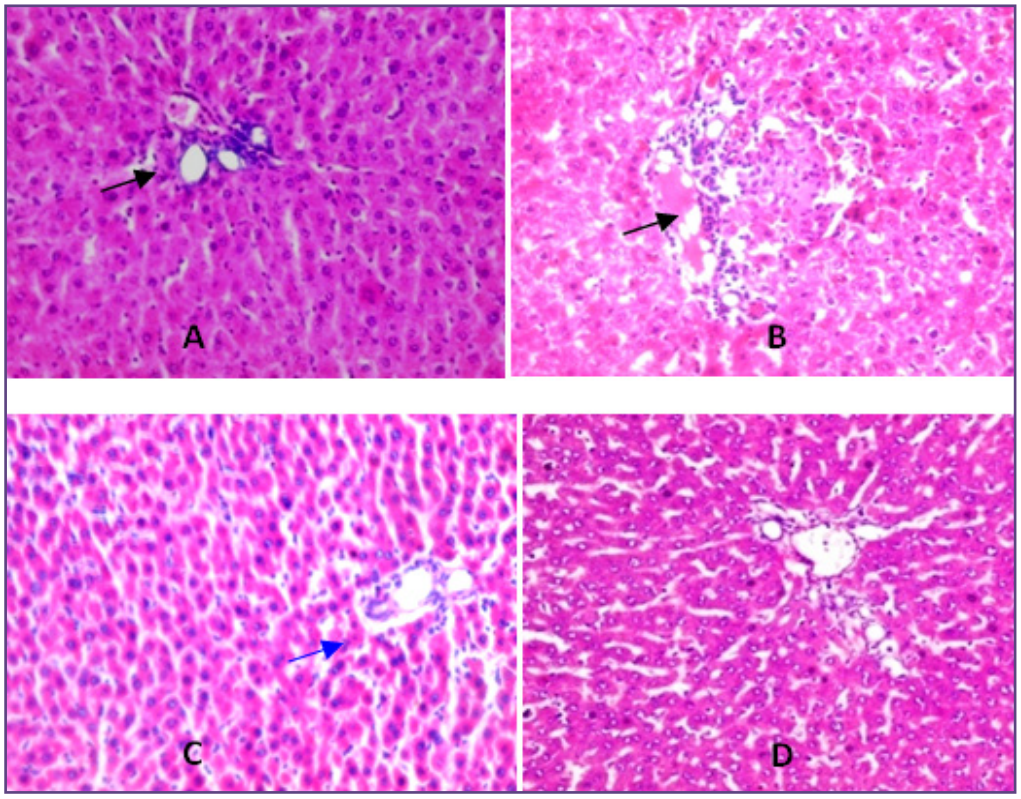

Fig. 2: Liver - 12th week - H&E Staining : 400x magnification A - Normal liver showing portal area and normal hepatocytes with sinusoidal space. B – Diabetic control Liver showing rigorous damage to the portal area with necrotic hepatocytes and infiltrations. C – Mucuna pruriens group showing near normal portal area with few binucleated hepatocytes. D - Glibenclamide group showing chords of hepatocytes with minimal necrosis of hepatocytes.

Discussion

Diabetes mellitus comprises a group of heterogeneous disorders which affects almost all systems of the body. Apart from the currently available drugs for the treatment of diabetes, a wide spectrum of drugs extracted from plant species were studied for their anti-diabetic properties. On these backgrounds, the present study was designed to evaluate the antidiabetic properties of *Mucuna Pruriens* seed extract on the structural changes liver and pancreas of Streptozotocin-induced Type 2 diabetic rats by quantitative and qualitative methods.

Chemical induction of diabetes by injection of a single dose of streptozotocin is a widely accepted model for diabetic experimental studies in rodents [10] consequences and treatment of diabetes. There are different ways to induce experimental diabetes, chemically induced experimental diabetes has been widely used. Alloxan and streptozotocin are the chemicals most used. A comparative study of these two agents was performed in order to determine their effectiveness. Twenty-seven Sprague-Dawley male adult rats were intraperitoneally (i.p.] .High doses of STZ injection in adult rats produces type1 diabetes, whereas injection of STZ at the neonatal period produces features more or less similar to type 2 diabetes in adult rats.[11] In the present study, 5-day old neonate wistar rats were made diabetic by 65 mg/kg body weight of STZ. The drug study started after twelve weeks after injection ie, once the animals reaches adult period.

Results of this study shows that the oral treatment of *Mucuna Pruriens*produced a significant 65% reduction of blood glucose level after 12 weeks of drug treatment ($p<0.05$). previous researchers like, Anusha et al reported that 21 days oral treatment of water extract of *Mucuna Pruriens*200mg/kg b.w is capable of reducing hyperglycemia by 40.7%[12]. These variations may be attributed to the climatic conditions, the maturity of the plant and soil composition, which leads to significant changes the pharmacological properties of herbs and actual dose of active ingredients[13] Mucuna spp. (Fabaceae).

Streptozotocin (STZ) is a naturally occurring compound, produced by the bacterium Streptomyces achromogenes, that produces diabetes in rodents through severe oxidative stress by free radical formation and through inhibition of DNA synthesis. It involves partial to complete destruction

of the islets and reduction of the diameter of islets, vacuolation and necrosis of the beta cells[14, 15]. In liver, STZ damages the hepatic parenchyma leading to vacuolations and necrosis of hepatocytes, dilation of sinusoidal spaces, periportal inflammation, congestion and hemorrhage[16,17] etc.All these changes were evident in the diabetic control group. The oral treatment of *Mucuna Pruriens* seed extract resulted in regeneration of beta cells of the islets, and increase in the number of hepatocytes in the liver. Anusha et al suggested that the dietary fibers and essential minerals present in the Mucuna Pruriens exerting the hypoglycemic effects [12]. It has been suggested that bioactive phytochemical compounds, having antihyperglycaemic activities, might act through several mechanisms such as stimulating insulin secretion, increasing repair, or proliferation of beta cells and enhancing the effects of insulin.

The reduction in the number of beta cells, size and number of islets in the diabetic group may be attributed to the direct cytotoxic effects of streptozotocin on the beta cells of the islets. Statistically significant increase ($p < 0.05$) in the number and diameter of islets were observed with MP group. This indicates that mucuna Pruriens produced regeneration of beta cells and islets. The regeneration of the STZ-destructed islets is probably due to the fact that the ductal epithelium of the pancreas, from which the pancreatic islets of Langerhans develops, presents dormant stem cells possess the ability of regeneration, replace the lost cells by transforming the surviving cells to proliferate into active cells under favorable conditions[18].

Polyploidation of hepatocytes is believedas an original physiological mechanism in which the cells pass through a binucleation stage which helps them to elevate metabolic yield and constitutes a substitute to cell division [19]. The outcome of the histomorphometric analysis of liver is supporting the suggestions put forwarded by the previous researchers. The reduction of necrotic cells, increased healthy hepatocytes and increased binucleated cells per unit area may be the indications of dynamic metabolic activity of hepatocytes due to the ameliorative effects of *Mucuna* on liver[20,21]. Circulating free radicals are suggested as the mechanism of heaptotoxicity in STZ-induced diabetes. Presence of antioxidant bioactive components might be protecting the hepatocytes from the free radicals.

Conclusion

Results suggests that the alcoholic extract of the test drug is effective against the degenerative changes in the liver and pancreas in the diabetic rats. Further histomorphometric analysison the hepatocytes and pancreatic beta cells were in concordance with the histological and hematological findings. It is concluded that *Mucuna Pruriens* seed has favorable effect to protect from the hyperglycemia and the microstructural changes in liver and pancreas by STZ induced diabetes.

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A Study of Giant Cell Lesions of Bone

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ABSTRACT

Introduction: Giant cell lesions of bone include true giant cell tumors & numerous benign osteoclasts and pseudo-anaplastic-appearing giant cells containing variants. Many times it is difficult to differentiate between true giant cell tumor and other tumor like conditions.

Aims: To study histopathology of various giant cell rich tumour and tumour like conditions of bone.

Methods: Retrospective analysis of 50 cases of giant-cell rich lesions of bone diagnosed and treated at Smt. N.H.L. Municipal Medical College Ahmedabad, Gujarat during 1st January 2002 to 1st January 2003 included in the study. Patients' clinical, radiological details, histopathological examination were studied using structured proforma. The cases were classified in different categories according to age groups, types of tumour, benign versus malignant category.

Results: The most common giant cell containing benign tumor is giant cell tumor (19 cases) followed by Aneurysmal bone cyst (5), Most of the giant cell containing tumors of bone are found in younger age group and are located in epiphysis The common giant cell containing malignant tumor is osteogenic sarcoma (7 cases) followed by Talangiectatic O.S (01). The majority of cases found in age between 15 - 28 years and most common sites are epiphysis of long bones.

Conclusion: The most common giant cell rich benign bone tumour is giant cell tumour and most common giant cell rich malignant bone tumour is osteosarcoma commonly occurs in younger age groups in the epiphysis region long bones.

Keywords: *Osteoclast, Giant cell, Malignant*

Introduction

Giant cell lesions of bone include true giant cell tumors & numerous benign as well as malignant conditions having osteoclasts and pseudo-anaplastic-appearing giant cells containing variants.[1] The approach to any bony lesion should be established by clinical, radiological and pathological investigations, supplemented when necessary by biochemical and hematological studies.[2] The five basic parameters of importance are the age of the patient, bone and specific areas involved within the bone, radiographic appearance and microscopic appearance. Histological study is essential for the precise diagnosis of bony lesions. It usually involves examinations of a biopsy specimen, either open surgical biopsy or needle biopsy. In this study, true giant cell tumor as well as other giant cell containing bony lesions are included.[3]

Osteoclast like giant cells may dominate the histological pattern not only in the giant cell tumour but also a variety of bone lesions namely aneurysmal bone cyst, giant cell-rich osteosarcoma, chondroblastoma[4]

Most of these occurs in adult life (2nd -3rd decade), except for giant cell tumour and chondroblastoma. In young patient, chondroblastoma and in patient older than 20 years, giant cell tumor should be included in differential diagnosis.[5]

However, to avoid confusion and to reach to a definitive diagnosis in such cases, it is necessary to take into account histological features, Clinico- radiologic correlation, and age of patient and site of lesions. [6]

Material and Methods

A retrospective study of 50 cases of giant cell rich lesions was done. Each case was investigated according to age, sex, clinical examination & type of specimen. Radiological findings (X ray, CT scan, MRI, etc.) of all patients are obtained from patient medical records. The specimens & biopsies were fixed in 10% neutral formalin, bony bits transferred to a large volume of 10% nitric acid, decalcified and after proper decalcification embedded in paraffin wax, stained with Haematoxylin and eosin (H & E) & mounted with DPX which were examined for growth pattern, cell size, cell shape, nuclear characteristics, pleomorphism, mitosis, stroma & necrosis.

The cases categorized into different groups according to age, sex, site of origin, type and benign versus malignant categories.

Results

Most of the giant cell containing tumors of bone is found in younger age group in second and third decade while aneurysmal bone cyst is found in third and fourth decade. Most of the giant cell tumors are found between 15 to 40 years of age.

In present study, giant cell tumor of bone is located in epiphysis in most of the cases. Aneurysmal bone cyst is located in the epiphysis as well as metaphysis of long bones. Telangiectatic osteosarcoma is located near shaft of long bones. Langerhan's cell histiocytosis is located in the skull. Chondromyxoid fibroma has been detected in small bones of hands and foot.

The osteoclastoma is maximum of all benign lesions.

Statistical analysis: T test between age and type of lesion has been applied The mean for benign giant cell leson is 24.21 and for malignant tumor the mean is 19.50. There is no statistical significant difference seen between the ages and the nature of lesion (Table-1)

The giant cell tumor of bone and other giant cell rich lesions of bone are is more common in male. (Table -2 & Table 3)

The giant cell rich lesion are most common in tibia 17(34.0) followed by femur 14(28.0) Fishers exact test= 5.821, p value= 1.00 (Table-4) According to present study, the most common giant cell rich lesion is true giant cell tumor (19) followed by osteosarcoma (07) (Table-5)

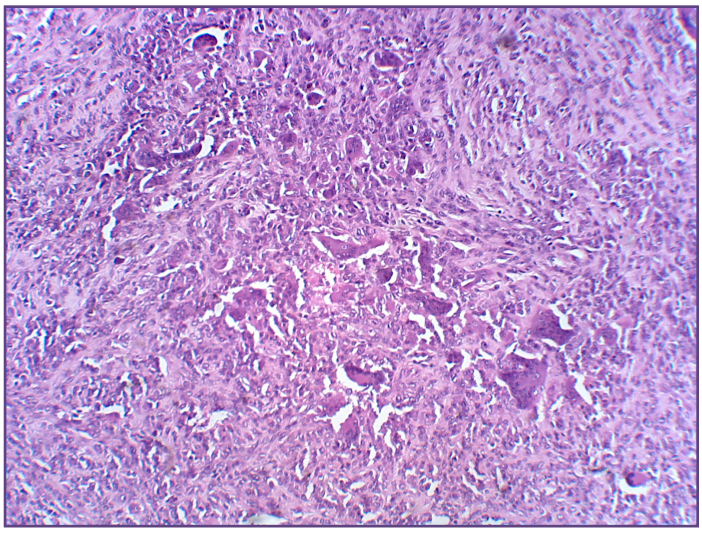

Fig. 1: shows numerous giant cells in Giant cell tumor of bone (Low power view)

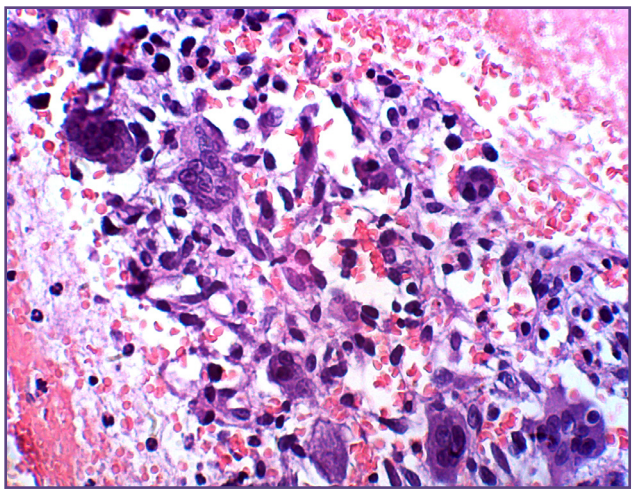

Fig. 2: Shows giant cells in wall of Aneurysmal bone cyst fillwd with RBC (High Power)

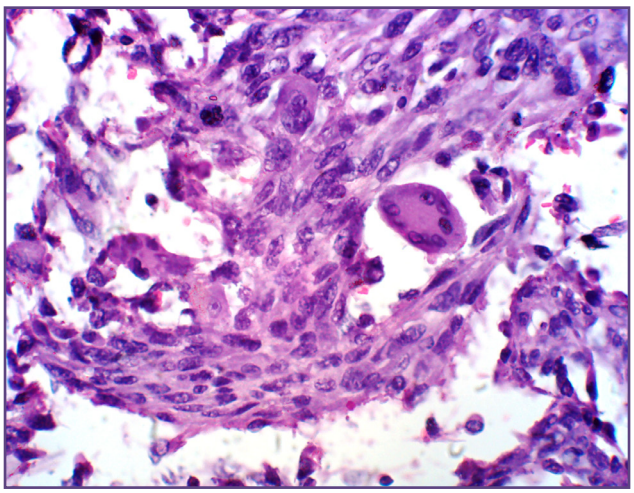

Fig. 3: Shows spindle shaped tumor cells with giant cells in Osteosarcoma (high power)

Table 1: Shows T test between age and type of lesion

Nature of lesion	N	Mean	Std. Deviation	Std. Error Mean
Benign	42	24.21	12.436	1.919
Malignant	8	19.50	5.182	1.832

There is no statistical significant difference seen between the ages and the nature of lesion

Table 2: Sex wise distribution of giant cell rich lesion

Bone	Sex		Total
	Female	Male	
Aneurysmal bone cyst	2(40.0)	3(60.0)	5(100.0)
Chondroblastoma	0(0.0)	1(100.0)	1(100.0)
Chondromyxoid fibroma	0(0.0)	1(100.0)	1(100.0)
Eosinophilic granuloma	0(0.0)	2(100.0)	2(100.0)
Giant cell tumor	9(47.4)	10(52.6)	19(100.0)
Langerhans' cell histiocytosis	0(0.0)	1(100.0)	1(100.0)
Nonossifying fibroma	2(40.0)	3(60.0)	5(100.0)
Osteogenic sarcoma	0(0.0)	7(100.0)	7(100.0)
Osteoid Osteoma	2(40.0)	3(60.0)	5(100.0)
Simple bone cyst	1(33.3)	2(66.7)	3(100.0)
Telangiectatic osteosarcoma	0(0.0)	1(100.0)	1(100.0)
Total	16(32.0)	34(68.0)	50(100.0)

Fisher's Exact Test= 8.844 p=0.566

Table 3: Sex wise distribution of benign and malignant lesion

Nature of lesion	Sex		Total
	Female	Male	
Benign	16(38.1)	26(61.9)	42(100.0)
Malignant	0(0.0)	8(100.0)	8(100.0)
Total	16(32.0)	34(68.0)	50(100.0)

Fisher's Exact Test p=0.043; The difference is significant

Table 4: Shows location of bone of giant cell rich lesion

Bones involved	Nature of lesion		Total
	Benign	Malignant	
Calcaneum	1(2.4)	0(0.0)	1(2.0)
Femur	11(26.2)	3(37.5)	14(28.0)
Fibula	1(2.4)	0(0.0)	1(2.0)
Frontal	1(2.4)	0(0.0)	1(2.0)
Humerus	6(14.3)	2(25.0)	8(16.0)
MC Bone	1(2.4)	0(0.0)	1(2.0)
Phalanx	1(2.4)	0(0.0)	1(2.0)
Radius	3(7.1)	0(0.0)	3(6.0)
Sacrum	1(2.4)	0(0.0)	1(2.0)
Skull	1(2.4)	0(0.0)	1(2.0)
Tibia	14(33.3)	3(37.5)	17(34.0)
Ulna	1(2.4)	0(0.0)	1(2.0)
Total	42(100.0)	8(100.0)	50(100.0)

Fishers exact test= 5.821, p value= 1.00

Table 5: Shows distribution of giant cell rich lesions

Histopathological Diagnosis	Nature of lesion		Total
	Benign	Malignant	
Aneurysmal bone cyst	5(11.90)	0(0.0)	5(10.0)
Chondroblastoma	1(2.3)	0(0.0)	1(2.0)
Chondromyxoid fibroma	1(2.3)	0(0.0)	1(2.0)
Eosinophilic granuloma	2(4.6)	0(0.0)	2(4.0)
Giant cell tumor	19(45.23)	0(0.0)	19(38.0)
Langerhans' cell histiocytosis	1(2.3)	0(0.0)	1(2.0)
Nonossifying fibroma	5(11.90)	0(0.0)	5(10.0)
Osteogenic sarcoma	0(0.0)	7(87.5)	7(14.0)
Osteoid Osteoma	5(11.90)	0(0.0)	5(10.0)
Simple bone cyst	3(7.14)	0(0.0)	3(6.0)
Telangiectatic osteosarcoma	0(0.0)	1(12.5)	1(2.0)
Total	42(100.0)	8(100.0)	50(100.0)

Discussion

The diagnosis of giant cell-rich lesions of bone is often problematic even for the experienced pathologist. The diagnostic key lies in multinucleated osteoclast-like giant cells and a mononuclear stroma.[7] From the histological picture alone it is often difficult to distinguish between individual entities such as conventional giant-cell tumor of bone, non-ossifying fibroma, giant-cell tumor in hyperparathyroidism or an aneurysmal bone cyst.[8]

Total 50 bone lesions were studied and divided into benign and malignant tumors. The incidence of true giant cell tumor (osteoclastoma) is maximum of all lesions in present study which is higher than Goldenberg and Dahlin et al. [9]Although giant cell tumor is considered as potentially malignant tumor, it is considered in benign because all giant cell tumors in this study show no atypical features in stroma. [10]Most of the giant cell containing tumors of bone are found in younger age group in second and third decade between 15 to 40 years of age while aneurysmal bone cyst is found in third and fourth decade. [11] In present study, giant cell tumor of bone is located in epiphysis in most of the cases. Giant cell tumors have higher incidence in male population in present study. Aneurysmal bone cyst is more common in males in the present study. Aneurysmal bone cyst is located in the epiphysis as well as metaphysis of long bones. According to Modi et al [12] osteoclast like giant cells may dominate the histologic pattern not only in the giant cell tumor but also a variety of bone lesions namely aneurysmal bone cyst, giant cell-rich osteosarcoma, chondroblastoma, giant cell reparative granuloma and fibrous dysplasia. Most of these occurs in adult life (2nd -3rd decade), except for giant cell tumor and chondroblastoma, Giant cell-rich lesions don't affect the epiphysis on primarily level. In young patient, chondroblastoma and in patient older than 20 years, giant cell tumor should be included in differential diagnosis.

According to Modi et al, the giant cell rich lesion of bone inclides giant cell tumour of bone (41), aneurysmal bone cyst (04), giant cell-rich osteosarcoma (02), giant cell reparative granuloma (02), and fibrous dysplasia (01) noted at their institute. While in present study, the giant cell rich lesions includes giant cell tumor (19 cases), Aneurysmal bone cyst (5), osteogenic sarcoma (7 cases) and Talangiectatic osteosarcoma (01). Most of the giant cell containing tumors of bone are found in younger age group and are located in epiphysis The majority of cases found in age between 15 - 28 years and most common sites are epiphysis of long bones.

According to Kumavat et al [13]out of 216 cases of bone tumors Out of 216 cases, primary bone tumors were 151 (69.91%), metastatic tumors were 40 cases (18.52%) and tumor like conditions were 25 cases (11.58%). According to Sunita A.Bamanikar et. Al [14].The most common benign tumour is osteochondroma followed by giant cell tumour of all benign tumours. osteosarcoma is commonest malignant tumour.

Conclusion

Detailed histopathological study and clinico-radiological correlation is very helpful to arrive at precise and accurate diagnosis in giant cell rich lesions. The common giant cell rich bone lesion are true giant cell tumor

(osteoclastoma) and osteosarcoma which is common in younger age group.

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Secondary Haematological Cancers in Adults: A Single Centre Experience

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ABSTRACT

Introduction: Advances in early detection and treatment mean that more and more people are surviving cancer today. Increased long-term survival seen in patients with solid and hematologic cancers achieved as a result of aggressive chemo radiotherapy has come at a price. Some cancer survivors may develop a new, unrelated cancer later. This is called a second cancer. Reasons for second cancers are varied. Field cancerization, shared environment, familial syndromes, Radiation and Chemotherapy are among the few risk factors affecting the risk of secondary cancers. Therapy-related acute myeloid leukaemia and secondary non-Hodgkin lymphoma has been frequently documented in these patient cohorts. We aim to study the prevalence as well as analyse the factors involved in secondary haematological cancers in our subset of patients.

Methods: This Cross sectional descriptive study was taken up in Apollo Institute of Medical Sciences and Research, Hyderabad. This is a 5 year observational study. We analysed all patients presenting to the Department of pathology, Apollo Hospitals during the period 2010 to 2015.

Results: Total 4 cases of Second cancers were documented in our study. Out of which 2 cases were acute myeloid leukaemias and both of them had history of treatment with alkylating agents. The other 2 cases were diagnosed to have secondary Multiple myeloma. Of the patient presenting with multiple myeloma one of them had history of prior Renal cell carcinoma

Conclusion: Assessment of the risk of second leukaemia should become part of any therapeutic plan for cancer patients. Chemo is known to be a greater risk factor then radiation. Avoidance of drugs with more leukemogenic potential will reduce the occurrence of second leukaemias. It is also important to understand the possibility of a correlation between renal cell carcinoma and multiple myeloma. Field cancerization and increased cytokine expression probably could play an important role in these second cancers.

Keywords: *Second primary malignancy, Second cancers, Second Leukaemia's, Secondary haematological cancers*

Introduction

Advances in early detection and treatment mean that more people are surviving cancer today. Increased long term survival seen in patients with solid and hematologic cancers - as a result of aggressive chemo-radiotherapy has come at a price. Some cancer survivors may develop a new unrelated cancer later - called as second cancer. Second cancer is a second neoplasm which differs anatomically, histologically and genetically from the primary neoplasm. Second primary cancers have become an increasingly important concern in oncology during the last two decades, as they now comprise the sixth most common group of malignancies after skin, prostate, breast, lung, and colorectal cancers. [1, 2]

Survivors of all cancers are living for longer periods, partly because of the more frequent use of effective therapy. The formerly unacceptable toxicities of therapy are more readily controlled with better supportive care. Other reasons for an increase in multiple cancers include fewer competing causes of mortality and consequently more naturally occurring cancers in an increasingly aging population. Reasons for second cancers are varied. Field cancerisation, Shared environment, Familial syndromes, Radiation and Chemotherapy are among the few risk factors affecting the risk of secondary cancers. [1, 2, 3, 4, 5]

Secondary Cancers are known to result from the radiation therapy and chemotherapy used to treat primary cancers. The Childhood Cancer Survivor Study, a cohort of more than 14,000 childhood cancer survivors with detailed exposure data and long-term follow-up, has substantially contributed to our understanding of the roles of radiotherapy and chemotherapy in second cancer occurrence. In particular, dose-related risks have been demonstrated for second cancers of the breast, thyroid, central nervous system, gastrointestinal tract, and sarcomas following radiation. Cytotoxic chemotherapy, which has long been known to be leukemogenic, also appears to contribute to risk for a range of other second cancer types. Individuals who develop a second cancer are at particularly high risk for developing additional second cancers. [6]

Among adults, risk of second cancers varies substantially by type of first and second cancer, patient age, and

prevalence of second cancer risk factors, including primary cancer treatments, environmental and lifestyle exposures, and genetic susceptibility. Further research is needed to quantify second cancer risks associated with specific etiologic factors and to identify the patients at highest risk of developing a second cancer to target prevention and screening efforts. Being a tertiary centre, we regularly encounter quite a variety of Haematological cancers. We intend to review and analyse the cases of haematological cancer registered in the last five years in our centre and study the prevalence as well as analyse the factors involved in secondary haematological cancers in our subset of patients.

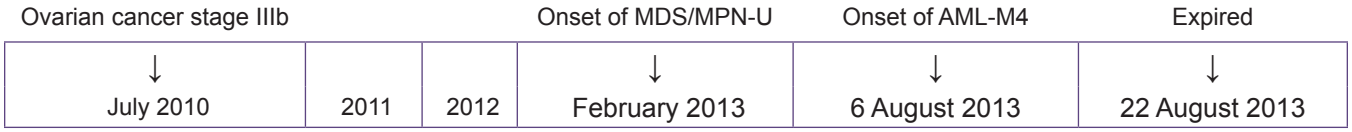
Materials and Methods

This Cross sectional descriptive study was taken up in Apollo Institute of Medical Sciences and Research, Hyderabad. This is a 5 year observational study. The present study was done to evaluate the prevalence and pattern of Secondary haematological cancers in Adult patients based upon the haematological specimen received in Department of Haematology, Apollo Institute of Medical Sciences and Research, Hyderabad. All adult patients diagnosed with haematological cancers during the period of 2010-2015 were included in the study. All the cases were reviewed in detail, history and clinical details were retrieved form Hospital medical records and Laboratory electronic records. Data sheet was completed for each patient detailing the Age, Sex, Clinical presentation, History and pertinent Investigations. The various Secondary Haematological cancers were analysed and grouped according the age, sex and presentation. Data was entered into excel data sheet and appropriate statistical analysis was performed.

Results

From July 2010 to June 2015, total 450 cases were diagnosed to have hematological cancers. Out of them only 4 were diagnosed to have Secondary Hematological Cancers (2.2% of cases).

Case I: A 66-year old postmenopausal woman came with a complaint of lower abdominal mass in July 2010, ultrasound detected left adnexal mass with elevated CA-125 levels (366IU/ml/ RR= <21/U/ml). A total abdominal hysterectomy with bilateral salpingo-oophorectomy, pelvic lymph node dissection and omentectomy were performed. The histopathology diagnosis was poorly differentiated carcinoma. She was staged as FIGO stage IIIb and submitted to six courses of systemic chemotherapy with paclitaxel and carboplatin (total cumulative dose-1200mg and 2700mg respectively). Following completion of chemotherapy course in October 2010, her CA-125 levels were normalized with normal haematological parameters. Computed tomography and Magnetic resonance imaging showed no residual diseases. Based on these findings she achieved in remission and was followed with routine surveillance. Following 27months of disease free survival, she was admitted on February 2013 with anaemia and received 2units of blood transfusion. Bone marrow aspiration and biopsy revealed Myelodysplastic Syndrome (MDS) with trilineage dysplasia. FISH analyses on bone marrow aspirate showed 5q31 and 7q31 deletion whereas a mutation of JAK2V617F was present by qualitative PCR analysis. Based on these findings a diagnosis of therapy related MDS/MPN-U was rendered. Subsequently 6 months later leukemic transformation with Acute Myeloid Leukaemia (AML) -M4 was noted. Patient refused for further treatment and expired within 2 week's time.



Case II: A 30year old male patient presented with Burkitts Lymphoma (February 2008). He was evaluated and was treated with Cyclophosphamide and Cytarabine based chemotherapy with which he achieved remission and was on follow up. Subsequently in on follow up he went on to develop Acute Leukaemia (AML M5) in June 2013. He went on to develop second cancer 5 years after achieving remission from Burkitts Lymphoma.

Case III: A 66 Year old male patient presented with case of Papillary Renal Cell carcinoma (RCC). He underwent Left partial Nephrectomy (14/6/11). Subsequently he went on to develop symptoms of easy fatigability associated with anaemia and deranged renal parameters in January 2014. On evaluation he was diagnosed to have Multiple Myeloma (MM). He developed this inspite of not receiving any chemotherapy.

Case IV: A 55year old female patient is a case of Carcinoma Breast. She underwent Radical Mastectemy in March 2007. Post surgery she received Chemotherapy (Cytarabine + Adriamycin + 5 FU + Cyclophosphamide). Later she went on to develop Multiple myeloma in November 2014, for which she underwent Autologous Stem cell transplant.

Out of the 4 cases diagnosed to have Secondary Haematological cancers, 3 of them had history of Cytotoxic

Chemotherapy. One patient developed Secondary Haematological cancer, in spite of not receiving any chemotherapy or radiotherapy. None of them received any radiotherapy.

Among those developing Secondary Haematological cancers secondary to Chemotherapy, one patient received carboplatin based Chemotherapy and two patients received Alkylating agents (Cyclophosphamide) based Chemotherapy. Among the patients receiving Alkylating agent one patient developed AML-M5 and other developed multiple myeloma. The patient who received Carboplatin based chemotherapy developed MDS which progressed to AML-M4.

Discussion

Therapy-related myeloid neoplasm's (t-MN) are noted in approximately 10 to 20 % of all cases of AML, MDS & MDS/MPN. [3,6,7] More than 50% of patients with secondary AML have breast cancer, Non Hodgkin's Lymphoma (NHL), and Hodgkin's Disease(HD). In the present study, 3 out of 4 cases (75%) diagnosed as Secondary Haematological cancers had history of prior chemotherapy. Both the patients (2/4) who developed secondary acute myeloid leukaemias received Chemotherapy.

Some types of chemotherapy (chemo) drugs have been linked with different kinds of cancer. The cancers most often linked to chemo are Myelodysplastic syndrome (MDS) and

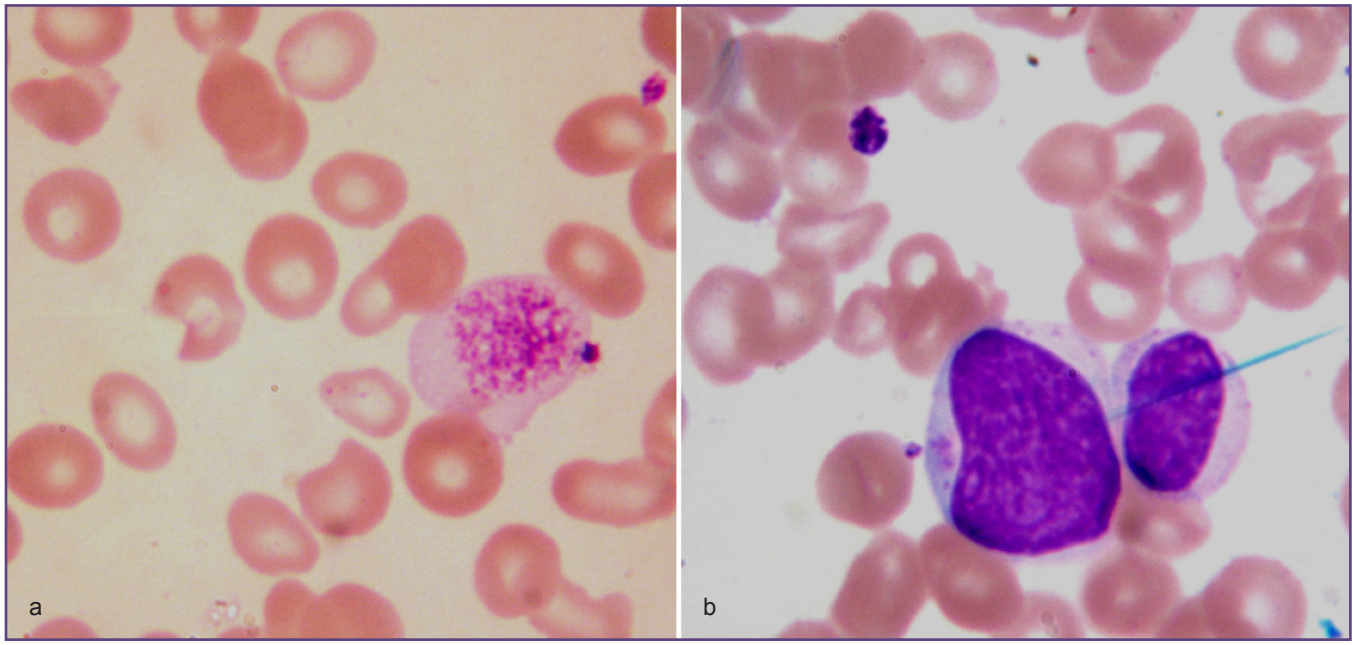


Fig. 1: (Case I) Peripheral smear shows MDS with trilineage dysplasia; (1a) Macroovalocytes with macrohypogranular dyspoietic platelets; (1b)Blast & hypogranular metamyelocyte (Giemsa stain, x100)

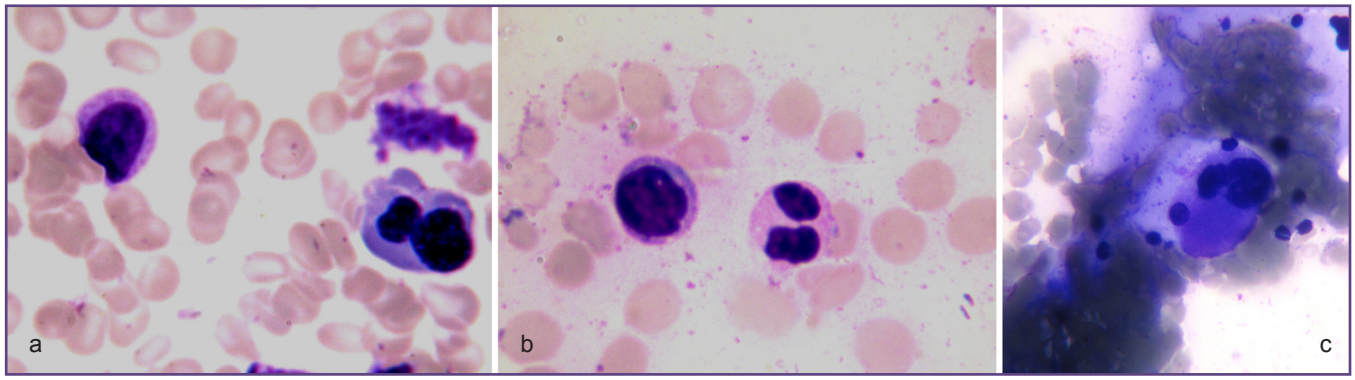


Fig. 2: (Case I) Bone marrow aspiration cytology shows MDS with trilineage dysplasia (a) Binucleate normoblast (b) Pelgeroid neutrophil with hypogranular myelocyte (c) Micromegakaryocyte with hypolobated partially detached nucleus (Giemsa stain, x100)

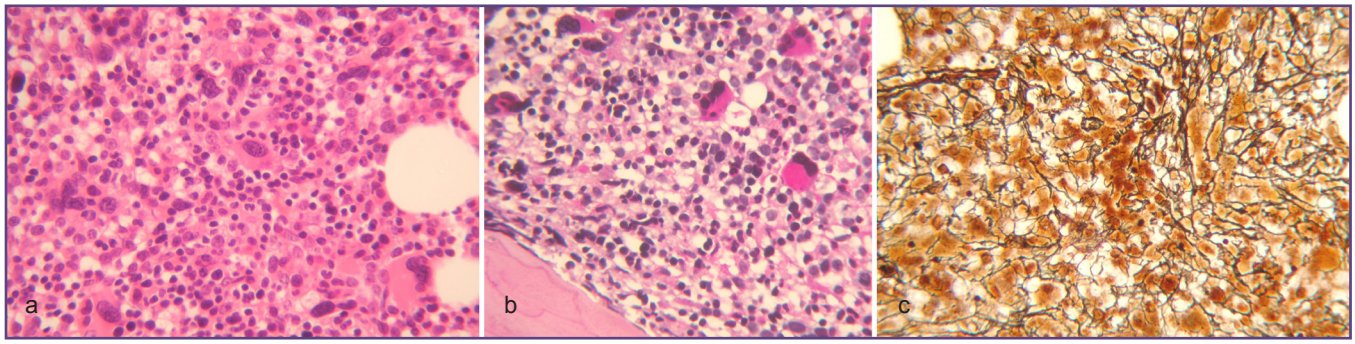


Fig. 3: (Case I) Bone marrow biopsy shows MDS with trilineage dysplasia (a)Hyperplastic and dyspoietic erythropoiesis, hyperplastic granulopoiesis with prominence of blasts, hyperplastic megakaryoctes with hypolobated micromegakaryocytes; (H and E, x400) (b)Hypolobated micromegakaryocytes, (PAS, x400) (c) Grade 3 on scale of 0-3(Reticulin, x400).

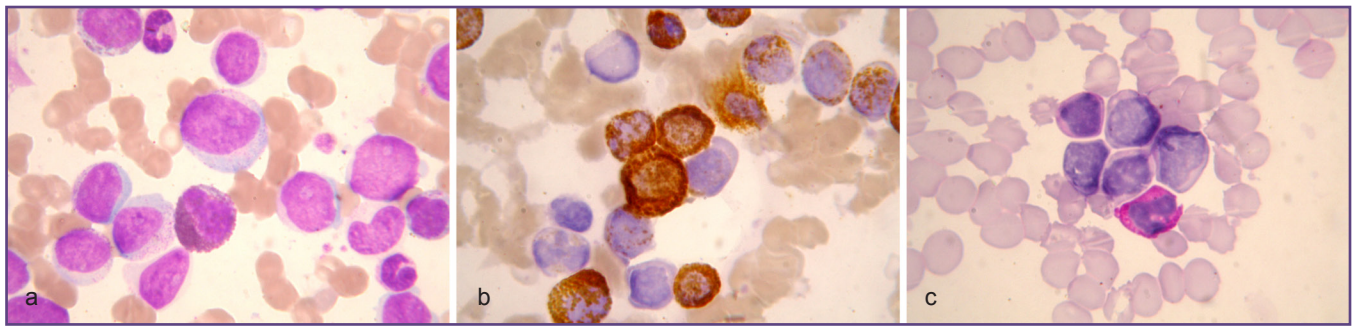


Fig. 4: (Case I) Peripheral smear shows acute leukaemic transformation (a)Immature leucocytes with prominence of blasts (Giemsa stain, x100)(b)Some of the blasts show myeloperoxidase granules (MPO, x100)(c)Blasts show negative staining on PAS (PAS, x100).

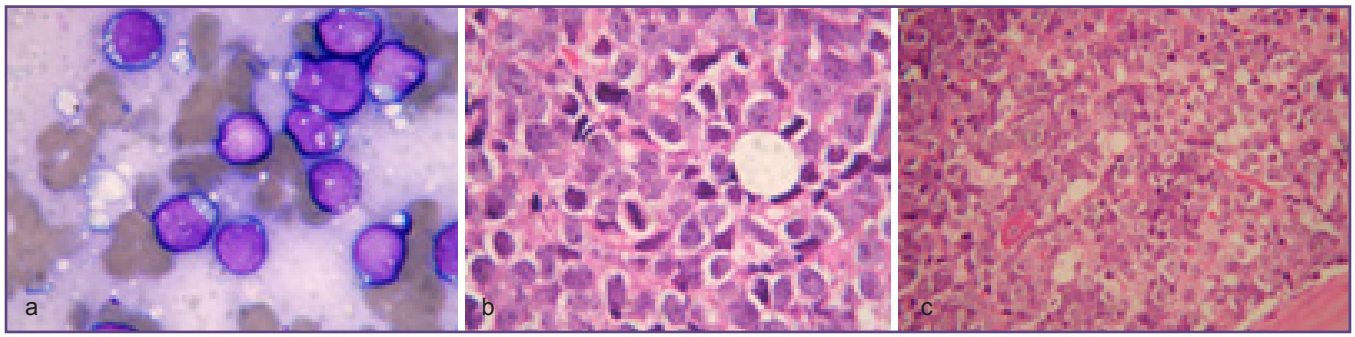


Fig. 5: (Case II) (a) Peripheral smear shows sheets of Monoblasts in high power field (Giemsa stain, x100) (b) & (c) Bone marrow biopsy sections showing blasts with increased mitotic activity (high and low power).

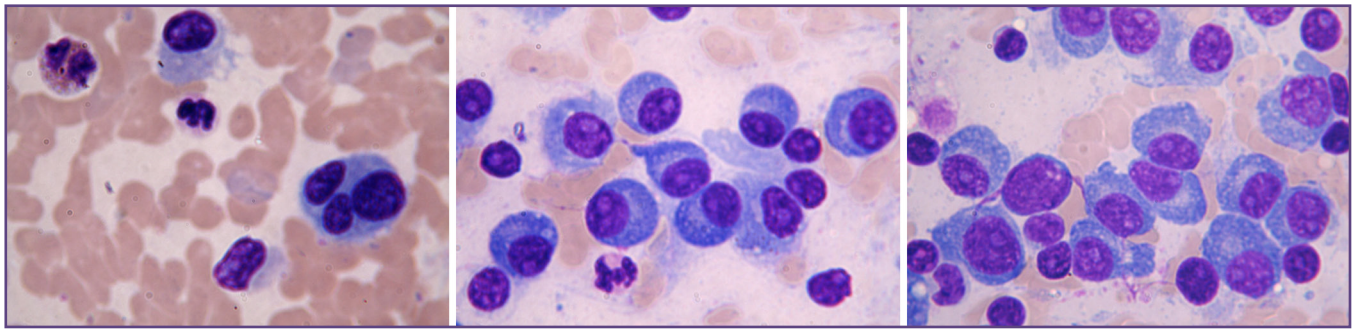


Fig. 6: (Case III): Bone marrow aspirate sections showing sheets of plasmaytoblasts .(high and low power).

acute Myelogenous leukaemia (AML). Sometimes, MDS occurs first, and then turns into AML. Acute lymphocytic leukaemia (ALL) has also been linked to chemo. Chemo is known to be a greater risk factor than radiation therapy in causing leukaemia. Alkylating agents, nitrosureas and procarbazine appear to have the highest leukemogenic potential. [6, 8]

1. Alkylating Agents:
    a. Mechlorethamine
    b. Chlorambucil
    c. Cyclophosphamide (Cytoxan)
    d. Melphalan
    e. Lomustine (CCNU)
    f. Carmustine (BCNU)
    g. Busulfan
2. Topoisomerase II inhibitors
    a. Etoposide
    b. Teniposide
    c. Mitoxantrone
    d. Anthracyclines
        i. Doxorubicin (Adriamycin)
        ii. Daunorubicin
        iii. Epirubicin
        iv. Idarubicin
3. Others
    a. Cisplatin
    b. Carboplatin

One of the patient who developed AML-M5 received Alkylating agent (Cyclophosphamide) and another Patient who developed MDS which progressed to AML-M4 received Cisplatin based chemotherapy. Alkylating agents are chemo drugs that interfere with a cell's DNA in a certain way. These drugs can sometimes cause AML and MDS. Often MDS develops first, which then progresses to AML. Alkylating agents related leukaemias are similar to post MDS leukaemia's with preleukemic phase and trilineage dysplasia. 5 and 7 chromosomal cytogenetic abnormalities are seen, with a poor prognosis. The risk gets higher with higher drug doses, longer treatment time, and higher dose-intensity (more drugs given over a short period of time). Studies have shown that leukaemia risk begins to rise about 2 years after treatment with alkylating agents, becomes highest after 5 to 10 years, and then declines. Unfortunately, MDS and leukaemia that develop after treatment with alkylating agents tend to be hard to treat and have a poor outcome with a median survival of 8 months. [2, 3, 6, 8]

The chemo drugs cisplatin and carboplatin are not alkylating agents, but they attack cancer cells in much the same way. These drugs seem to increase the risk of leukaemia (mainly AML), too, but the risk is not as great as with the alkylating agents. This leukaemia is hard to treat and tends to have a poor outcome, much like the leukaemia linked to the alkylating agents. The risk of leukaemia rises as the amount of drug used gets higher. The risk of developing leukaemia increases even more if radiation is given along with cisplatin or carboplatin. Both the patients in our study had poor outcomes with early mortality. [2, 3, 6, 8]

The class of chemo drugs called topoisomerase II inhibitors stop cells from being able to repair DNA. These drugs can also cause leukaemia, mainly AML. Leukaemia develops sooner after treatment with these drugs than the leukaemia from alkylating agents. Most cases are found within 2 or 3 years of treatment and without MDS occurring first. Balanced translocations involving chromosome 11 q23 are usually seen. Leukaemia from topoisomerase II inhibitors tends to respond to better to treatment and has a better outlook than the leukaemia from alkylating agents. Drugs called anthracyclines are also topoisomerase II inhibitors. Anthracyclines are less likely to cause leukaemia than the other topoisomerase II inhibitors. [6, 8]

Most kinds of leukaemia, including AML, CML and ALL can be caused by past radiation exposure. Myelodysplastic syndrome (MDS), a bone marrow cancer that can turn into acute leukaemia, has also been linked to past radiation exposure. The risk of these diseases after radiation treatment depends on a number of factors such as:

1. How much of the bone marrow was exposed to radiation
2. The amount of radiation that reached the bone marrow
3. The radiation dose rate (how much was given in each dose, how long it took to give the dose, and how often it was given)

The person's age when they were treated with radiation does not seem to be a risk factor. Most often, these cancers develop within several years of radiation treatment, peaking at 5 to 9 years after exposure. [2, 3, 6, 8] None of the patients in our study had an history of prior radiotherapy. This could be due to the referral bias.

Multiple myeloma is a hematologic disorder characterized by monoclonal proliferation of plasma cells in the bone marrow that secrete immunoglobulin's. For the diagnosis of myeloma it is necessary to detect >10 % plasma cells in bone marrow or tissue biopsy. Its incidence is approximately 3-4/100.000 and responsible for about 1% of all malignancy related deaths. Not uncommonly

this is seen as a second cancer. [9,10] In the present study two cases (2/4- 50%) were diagnosed to have secondary Multiple myelomas.

One patient developed Multiple myeloma after chemotherapy for Breast carcinoma (Alkylating agent, Cyclophosphamide). In the literature there are reports of Multiple myeloma cases secondary to chemotherapy and it has long been recognized. [9, 10, 11] Marinopoulos et al reported a case of M.M after chemotherapy for non small cell lung cancer (cisplatin based). [11] Radiotherapy and alkylating agents used in the treatment of carcinoma breast pose the risk of myelodysplasia and secondary leukaemia's. Plasma cell dyscrasias, plasmacytoma and multiple myeloma have rarely been reported in the literature coexisting with carcinoma breast. [10] Multiple myeloma was reported in 5 out of 443 second neoplasms after treatment for Breast cancer in a study by Levi F et al. [7]

Another developed M.M after partial nephrectomy for papillary renal cell carcinoma, with history of neither Radiation nor Chemotherapy. These tumours may represent coincidence of two not so uncommon tumours in elderly population or may show a true association. [13] Number of case reports and a small case series has hypothesized an association between renal cell carcinoma (RCC), and multiple myeloma (MM). [14,15,16,17] This hypothesis has been confirmed in a large population-based study from the USA and the association is supposed to be bidirectional, pointing to shared risk factors rather than treatment related factors. Patients with RCC have higher relative risk of developing MM during follow-up and vice versa. [18]

Although not necessarily specific to this association, certain genetic risk factors may be shared between renal cell carcinoma and multiple myeloma that contribute to the observed bidirectional association. For example, *c-met* oncogene mutations are well recognized in hereditary papillary renal cell carcinoma. [19, 20] Hereditary papillary renal cell carcinoma is most often observed in older age, an attribute that is consistent with multiple myeloma incidences. More importantly, *c-met* expression was recently implicated in myeloma cell proliferation through feedback loops with interleukin-6, an inflammatory cytokine that has a critical role in the development and growth of myeloma cells. [20] Therefore, *c-met* expression may be a candidate for elucidating the association between renal cell carcinoma and multiple myeloma.

Lifestyle-related risk factors such as obesity may also contribute to the observed association. Obesity is a strong risk factor for renal cell carcinoma and has modest but consistent effects on multiple myeloma incidences. [21, 22] Furthermore, obesity is characterized by marked changes in adipose tissue, such as increased number and size of adipocytes. Adipose tissue is a major source of inflammatory mediators, particularly interleukin-6. [23] Therefore, obesity may facilitate the creation of a microenvironment that supports the development of renal cell carcinoma and multiple myeloma. [23, 24, 25]

Shared environmental risk factors are another factor which plays important role in second cancers. Sometimes the second cancer isn't nearby, but is still linked to the same cancer-causing agent. Lifestyle factors, such as smoking, alcohol, exercise, sun exposure, and diet, clearly play individual roles in a long list of cancers, such as those of the head and neck, lung, bladder, skin and gastrointestinal tract, which are often seen as secondary cancers following treatment for a primary neoplasm. Environmental risk factors continue to raise the probability for a new cancer after the diagnosis and treatment of an initial malignancy. [2, 3, 5, 26]

For some cancers, having that cancer means you are at an increased risk of getting another cancer in the same organ or nearby. This may be because the whole organ (and sometimes nearby organs and tissues) were exposed to the same cancer-causing agents that led to the first cancer. This means that the entire area could already have early changes that can lead to cancer. This is called *field cancerization*. Although cancer survivors often reduce their exposures, the effects of earlier exposure can continue to influence their risks for years. Individuals with a genetic predisposition to multiple neoplasm's are another group at risk. Polymorphisms for metabolizing enzymes are another potentially critical category of etiologic factors that cannot yet be evaluated. [26]

Conclusion

Assessment of risk of secondary leukemia should be a part of therapeutic plan for cancer patients. Chemotherapy is a greater risk factor than radiation. Avoidance of drugs with more leukomegenic potential will be beneficial. Correlation between Renal cell carcinoma and Multiple myeloma needs to be further studied. Shared risk factors, Field cancerisation and increased cytokine expression could probably play an important role in these cancers.

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Histopathological Interpretation Of Colonic Mucosal Biopsies With Clinical Correlation: A Study In A Tertiary Care Hospital Kerala

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ABSTRACT

Background: Colon is the most common site for various diseases. Diseases affecting the colon may cause abdominal pain, constipation, diarrhea and bleeding per rectum. Colonoscopy is an endoscopic examination of the colon with a flexible optic fibers, this method allows visual examination of colonic mucosa and provides opportunity for mucosal biopsies. This study was carried out to study and correlate histopathological spectrum of colonic mucosal biopsies with clinical findings.

Methods: This two years study was conducted during the period of April 2015 to March 2017. 250 colonoscopic biopsies were included in the study. All biopsies were fixed in formalin, processed as per routine histopathology processing, stained with Hematoxylin & Eosin stains and studied.

Result: Out of 250 colonoscopic biopsies, 152 were non-neoplastic and 98 were neoplastic. Non neoplastic lesions were found to affect the colon most commonly. Most of the cases with non-neoplastic lesions presented with colicky abdominal pain, diarrhea and constipation. Among neoplastic lesions, 44 cases were benign and 54 cases were malignant. Majority of cases with neoplastic lesions presented with bleeding per rectum and constipation.

Conclusion: Non neoplastic lesions were found to affect the colon most commonly. This study emphasize the increase in incidence of ulcerative colitis and colorectal carcinomas. Histopathological interpretation on colonic mucosal biopsies has taken a cornerstone in the diagnosis and management of patients with colonic lesions.

Keywords: *Colonoscopy, Colitis, Adenoma, Adenocarcinoma*

Introduction

Colon is the primary site for various non neoplastic and neoplastic diseases. The spectrum of colonic lesions range from congenital diseases, Infections, Inflammatory conditions, Vascular diseases, Polyps and Colorectal tumours. Colorectal carcinoma is one of the leading cause of morbidity and mortality with an overall cancer incidence rate of 9%.[1,2]

Development of flexible fibreoptic sigmoidoscopy and colonoscopy revolutionized the diagnosis and management of colorectal diseases, for the reason that the procedure is safe with no serious complications. Application of therapeutic colonoscopy like colonoscopic polypectomy has replaced the open surgical procedure to a great extent.[3]

In developing countries like India, where tuberculosis and infective colitis are more prevalent, the diagnosis of Inflammatory Bowel disease is a great challenge. Colonic mucosal biopsies procured from colonoscopy plays a crucial role in specific diagnosis of patients with Inflammatory Bowel disease [4, 5] and early detection of colonic epithelial tumours.[6] Histopathological interpretation of colonic mucosal biopsies when correlated with clinical findings help in definitive diagnosis and early treatment of patients with colonic lesions.

The current study was carried out with an aim to study histopathological pattern of colonic mucosal biopsies and correlate them with clinical findings.

Materials and Methods

The present study was a two year retrospective study, conducted in the department of pathology DM Wayand Institute of Medical Sciences, Kerala during the period from April 2015 to March 2017. 250 cases presented with symptoms were selected for the study. Patient's age, sex, presenting complaints, relevant past history were noted and colonoscopy was performed.

All biopsies received were immediately fixed in 10% formalin. The tissue bits were counted, measured and processed completely as per routine histopathology processing. From each paraffin block 3 to 5 micron thick

section were prepared by using rotary microtome. All slides were then stained with Hematoxylin & Eosin stains. Special stains like Giemsa and Periodic Acid Schiff were done wherever necessary. Detailed study of all the slides were done under light microscope by the pathologists and diagnosis was furnished.

The study was approved and permitted by Institutional Ethical Committee.

Result

A total of 250 colonoscopic biopsies were examined during the stipulated period of time. Out of 250 cases, 167 were males and 83 were females. Male female ratio was found to be 2.1:1. Age of patients ranged from 20 to 89 years. Majority of the cases 121 (48.4%) were presented with colicky abdominal pain and loose stools, followed by bleeding Per Rectum in 77 cases (30.8%), 34 cases (13.6%) presented with diarrhea and constipation in 18 cases (7.2%)

Among all the colonic biopsies, histologically 152 cases (60.8%) were diagnosed as non-neoplastic lesions and 98 cases (39.2%) were diagnosed as neoplastic lesions. Out of 152 non-neoplastic lesions, Non Specific colitis was found to be the commonest lesion in 71 cases (46.71%) followed by ulcerative colitis in 38 cases (25%). Non neoplastic lesions were found in all age groups with male:female ratio of 2.8:1. [Table 1]

Among 98 neoplastic lesions, Benign lesions were in seen in 44 cases (44.9%) and Malignant lesions were observed in 54 cases (55.1%). All benign lesions were diagnosed as colonic adenomas and all the malignant lesions were diagnosed as adenocarcinomas. Colonic Adenomas were seen between 50 to 80 years of age group with a male to female ratio of 1.6:1 showing a slight male preponderance. Adenocarcinomas were common in 5th to 7th decade with a male female ratio of 1.1:1. The most common histological subtype diagnosed was well differentiated adenocarcinoma.

Histopathology of Non-Neoplastic Lesions: In the present study, non-specific colitis was observed in 71 cases, characterized by unremarkable colonic mucosal glands and expansion of lamina propria by lymphoplasmcytic infiltrate with no cryptitis or crypt abscess. Ulcerative colitis was seen in 38 cases, characterized by cryptitis, crypt abscess, crypt distortion, variation in inter crypt distance, basal plasmacytosis and mixed inflammatory cell infiltrate in lamina propria[Fig 1]. 3 cases of Chron's disease showed well-defined non-caseating granulomas and transmural lymphoplasmacytic infiltrate. Hyperplastic polyp was seen in 12 cases, characterized by elongated glands and crypts with serrated appearance. 5 cases of inflammatory polyp showed infiltration of lamina propria by inflammatory cells with focal mucosal erosions and hemorrhage. Solitary Rectal Ulcer was seen in 5 cases, characterized by cystic dilatation of glands with fibromuscular hyperplasia.

Histopathology of Neoplastic Lesions: Among 44 cases of colonic adenomas diagnosed in our study, tubular adenoma was seen in 38 cases, characterized by increased number of tubular glands and these glands showed nuclear stratification with hyperchromatic nuclei limited to the lower one third of glandular epithelium [Fig 2]. Remaining 6 cases showed tubulovillous adenoma, characterized by tubular and elongated glands with nuclear features same as that of tubular adenoma. High grade dysplasia was observed in 6 cases of adenomatous polyp [Fig 3].

Among 54 cases of adenocarcinomas, well differentiated adenocarcinomas were seen in 29 cases, characterized by well-formed malignant glands infiltrating into submucosa [Fig 4]. 21 cases showed moderately differentiated adenocarcinomas, characterized by ill formed malignant glands with poor outlines. Poorly differentiated carcinomas were observed in 3 cases, characterized by malignant cells in groups or in cords with no glandular structures. Single case showed Signet ring carcinoma characterized by signet shaped malignant cells infiltrating in to the submucosa [Fig 5].

Table 1: Distribution of Non-neoplastic lesions.

S.No	Lesions	Number of cases (Percentage)
1	Non-specific colitis	71 (46.71%)
2	Infective colitis	06 (3.95%)
3	Ulcerative colitis	38 (25%)
4	Chron's Disease	03 (1.97%)
5	Hyperplastic Polyp	12 (7.89%)
6	Inflammatory Polyp	05 (3.29%)
7	Solitary Rectal Ulcer	05 (3.29%)
8	Melanosis coli	04 (2.63%)
9	Pseudomembranous colitis	01 (0.66%)
10	Tuberculosis	01 (0.66%)
11	No significant pathology	06 (3.95%)
	Total	**152 (100%)**

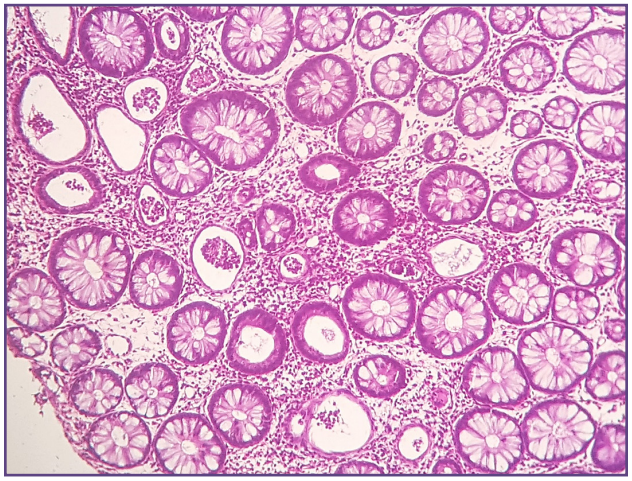

Fig. 1: Photomicrograph of Ulcerative Colitis showing cryptitis, crypt abscess and crypt distortion (H&E; 4X).

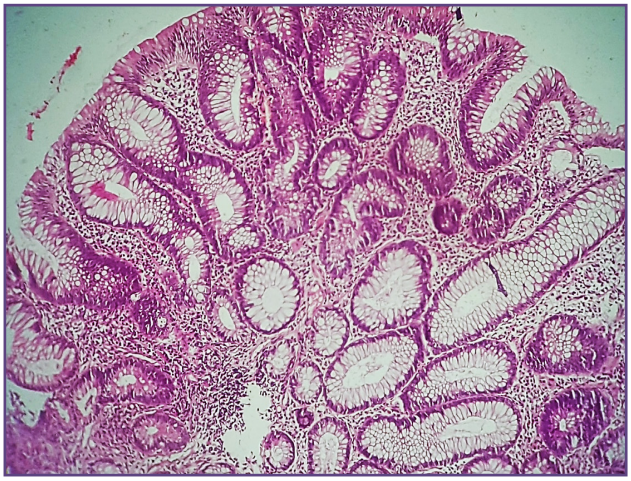

Fig. 2: Photomicrograph of Tubular Adenoma without High Grade Dysplasia (H&E; 10X).

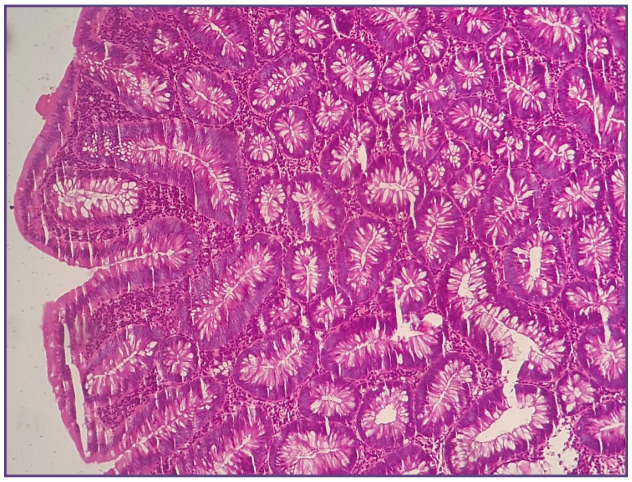

Fig. 3: Photomicrograph of Tubular Adenoma with High Grade Dysplasia (H&E; 10X).

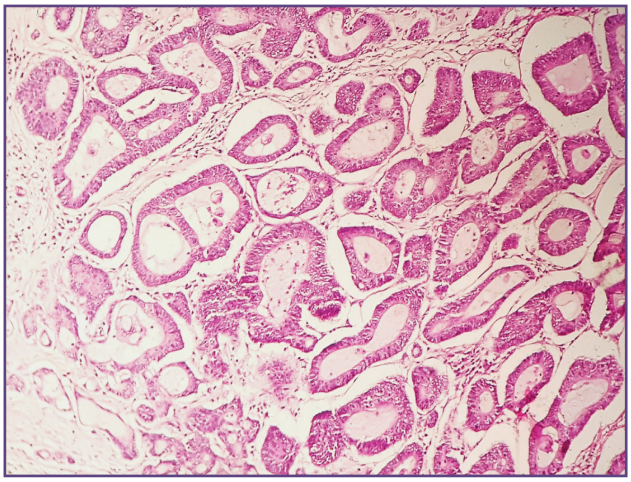

Fig. 4: Photomicrograph of Well Differentiated Adenocarcinoma (H&E; 10X).

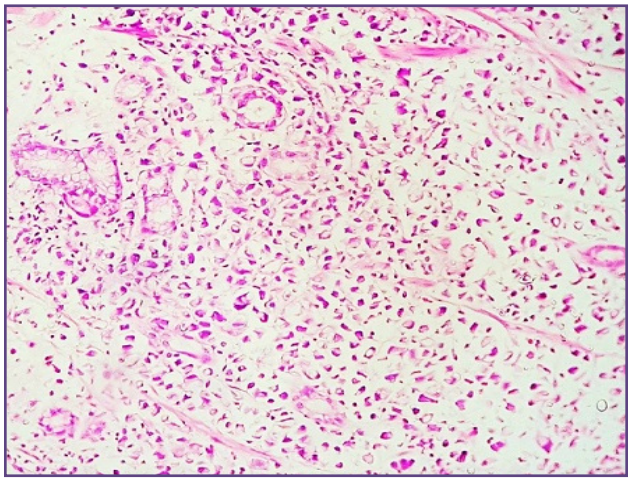

Fig. 5: Photomicrograph of Signet Ring Carcinoma (H&E; 4X).

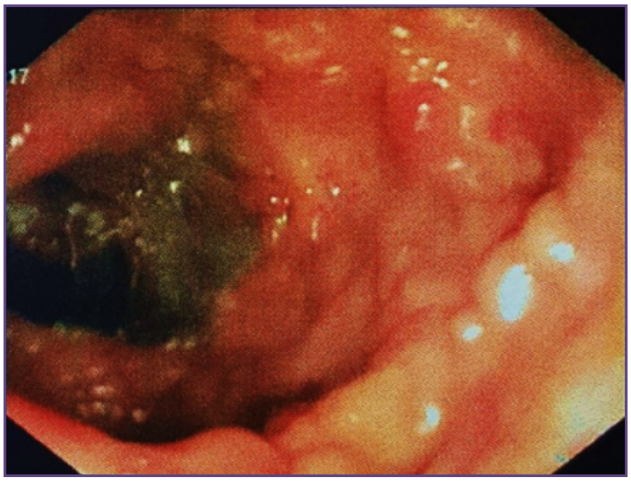

Fig. 6: Colonoscopic image from a case of ulcerative colitis showing multiple pseudopolyps in descending colon.

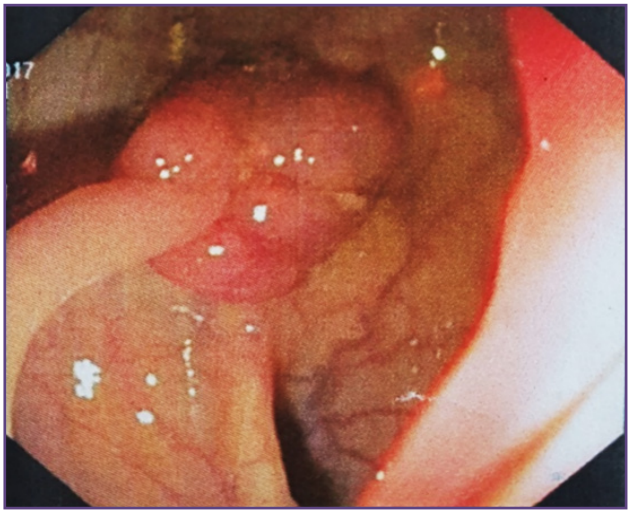

Fig. 5: Photomicrograph of Signet Ring Carcinoma (H&E; 4X).

Discussion

Non-Neoplastic lesions: In the present study, among the non-neoplastic lesions, nonspecific colitis was found to be commonest lesion seen in 71 cases (46.71%), studies done by Deshpande V etal, [7] Rajbhanderr M etal[8] and shefali H leave et al [9] showed the same findings.

Ulcerative colitis though rare is an emerging disease in India, this condition portrays remissions and relapses. [10] In the present study ulcerative colitis was found to be the second commonest non neoplastic lesion observed in 38 cases (25%), which is comparable with the studies done by Qayyers et al,[11] Shama VK et al[12] and is contradictory to the findings of Rajbhandari M et al. [13] The prevalence rate of Ulcerative colitis was high among 20 to 40 years of age with loose stools and colicky abdominal pain being the commonest presenting symptom. These are congruent with the studies done by Megha Shukla Pandey et al, [14] Badmapriya et al [15] and Sood A et al.[16] [Fig 6] Hyperplastic polyp was seen in 12 cases (7.89%) and all these cases were in between 40 to 80 years of age, which is in agreement with the study done by Williams GT et al. [17]

Chron's disease was seen in 3 cases (1.97%) and mean age of presentation was 29 years. Loose stools and bleeding per rectum was found to be commonest symptom. The findings are akin to the studies done by Price AB et al[18] and Sood A et al.[19]

Benign Lesions: All cases of benign neoplastic lesions were diagnosed as colonic adenomas. Tubular adenoma (86.36%) outnumbered all colonic adenomas and remaining cases (13.64%) were tubulovillous adenoma.

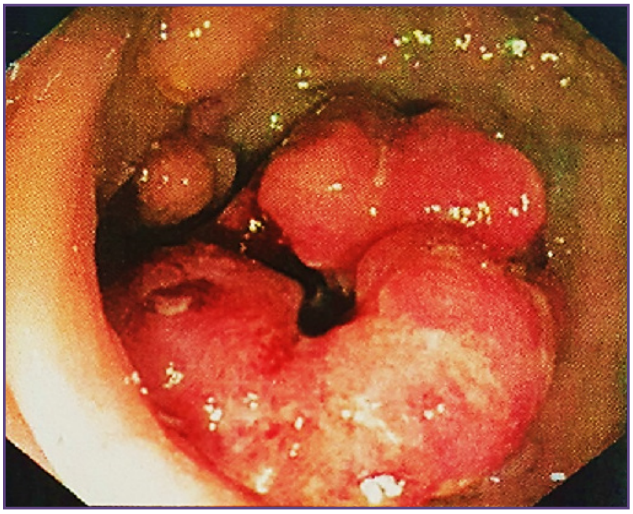

Fig. 8: Colonoscopic image showing a polypodal growth with ulceration seen at rectosigmoid region occupying 80% of lumen.

High grade dysplasia was observed in 13.63% of colorectal polyp. Study done by Konishi Fet al[20] also showed similar findings.

Colonic Adenomas were common in the age range of 50 – 80 years and the commonest site of occurrence was Recto-sigmoid region. The M: F ratio was found to be 2.4:1. These findings were similar to the studies done by Tony J et al[21] and Ritesh sulegaon et al.[22] [Fig 7]

Malignant Lesions: In the present study, all malignant lesions were diagnosed as adenocarcinoma. Majority of the cases presented with bleeding per rectum (80.24%) followed by abdominal pain (10.13%) and constipation (9.63%). Rectum being the most common site of occurrence of adenocarcinoma followed by sigmoid colon. These findings were in concordance with other studies by Yawe KT et al,[23] Saidi HS et al[24] and Gurjeet K et al.[29]

Maximum number of cases (53.7%) were observed in the age group of 60-80 years and about 30% of cases were seen in the age group of 30-50 years, exhibiting a shift in tendency of occurrence of colorectal adenocarcinoma in younger and middle aged adults. In our study the male:female ratio was found to be 1.1:1, showing almost equal incidence among both the sexes. Studies done by Caliskan C et al,[25] Rasool A et al,[26] Sudarshan V et al[27] and Laishram RS et al[28] also showed similar findings. [Fig 8]

Among adenocarcinomas diagnosed in our study, the most common histological subtype was found to be well differentiated adenocarcinoma (53.7%), followed by moderately differentiated (38.89%) and poorly

differentiated type (5.56%). The findings were similar to the study done by Shyamal Kumar Halder et al[30] and in contrast to the studies done by Laishram RS et al,[28] Shefali. H. Karve et al,[9] Caliskan C et al[25] and Ritesh sulegaon et al.[22]

Conclusion

Large intestine is affected by a long array of non-neoplastic and neoplastic lesions. Our study unveils that, ulcerative colitis which was considered to be rare, is now on the rise in India. It was observed that there is increased incidence of colorectal carcinomas among young and middle aged adults. This study emphasize that histopathological interpretation of colonic mucosal biopsies with clinical correlation play a pivotal role in the diagnosis, management and follow up of patients with colonic lesions.

Acknowledgements

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Histopathological Spectrum of Nephrectomy Specimen in a tertiary Care Centre: With an Emphasis on Chronic Pyelonephritis

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ABSTRACT

Introduction: Nephrectomy is a common procedure in urological practice done for various conditions like calculi, chronic pyelonephritis, malignancy, obstruction, injury, etc with wide range of morbidity and mortality. In our country there is increased in number of end stage renal disease treated with dialysis and transplantation. So it is mandatory to study the spectrum of renal disease with special emphasis on Chronic pyelonephritis.

Methods: This study was carried out in the Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidhyapeeth University, Pondicherry. All the nephrectomy specimen received in the department over a period of three years (January, 2013 to December 2015) were included. A total of 34 cases of nephrectomy specimen were studied during this period.

Results: Out of 34 cases of nephrectomy, 24 were done for inflammatory lesions, 8 cases for tumor and 2 cases shows cystic lesion. Out of 24 cases of pyelonephritis, 6 cases were associated with nephrolithiasis. Out of 8 tumor cases, 6 cases were Renal cell carcinoma.

Conclusion: To conclude, Nephrectomies is mostly done for non-neoplastic lesion. CPN also seems to be associated with various findings like pyonephrosis, calculi, malignancies etc. Hence it is mandatory to study each CPN case in detail along with clinical and radiological findings.

Keywords: *Nephrectomy, Renal Cell Carcinoma, Chronic Pyelonephritis*

Introduction

Kidneys are vital organs of the body with multiple functions like excretory function, acid base balance and maintenance of salt and water metabolism. Simple nephrectomy is a common procedure in urological practice and it is indicated in patients with an irreversible damaged kidney resulting from symptomatic chronic infections, obstruction, calculus, severe traumatic injury and renal dysplasia[1].Kidney can be affected by various pathological conditions like cystic disease, glomerulonephritis, pyelonephritis, renovascular hypertension, obstruction, calculous disease, benign and malignant tumor, etc some of which may requires surgical treatment and some may requires medical treatment[2].

Chronic pyelonephritis with hydronephrosis is the most common type of nephrectomy specimen for non-neoplastic conditions due to increase in the incidence of pelvi-ureteric junction obstruction by upper ureteric calculi, whereas in the neoplastic group, renal cell carcinoma is the most common due to increase in the incidence in chronic smokers[3]. Most of the patients with chronic pyelonephritis leads to permanent and progressive damage to the renal parenchyma ending up with Non-functioning kidney. Most of the patients reach hospital with either obstructive symptoms or with acute on chronic renal failure. In our country there is increased in number of end stage renal disease treated with dialysis and transplantation. So it is mandatory to study the spectrum of renal disease with special emphasis on Chronic pyelonephritis.Radical or Partial nephrectomy is the treatment of choice for a greater proportion of patients with renal tumors[4].

The aim of this article was to study the histopathological spectrum of nephrecteomy specimen with an emphasis on pyelonephritis.

Materials and Methods

This study was carried out in the Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidhyapeeth University, Pondicherry. All the nephrectomy specimen received in the department over a period of three years (January, 2013 to December 2015) were included. A total of 34 cases of nephrectomy specimen were studied during this period.Patients details were noted, which includes age, sex, clinical findings and radiological investigations. This is a retrospective study, so all nephrectomy cases were taken out from the records

and slides were reviewed. All these specimens were fixed in 10% buffered formalin, then processed into paraffin embedded sections & stained with haematoxylin & eosin.

Results

The present study includes 34 nephrectomy cases analyzed during the period of January 2013 to December 2015. Among 34 nephrectomy cases, higher incidence were observed in fifth and sixth decades of life with equal preponderance of male and female as shown in [Table 1] and [Table 2].

Loin pain (28 cases) was the most common clinical presenting complaints noted, followed by burning micturition, fever and hematuria. 5 cases presented with abdominal lump and four cases presented with oliguria as shown in [Table 3].

From these 34 nephrectomy cases, 75% of the cases were non-neoplastic lesions and 25% of the cases were neoplastic lesions as shown in [Table 4]. In the histopathological spectrum of nephrectomy specimen with 34 nephrectomy cases, maximum number (24 cases) of cases were found to be chronic pyelonephritis (CPN). Out of this 24 CPN cases, maximum number of cases were found to be associated with pyonephrosis (PN) and Nephrolithiasis as shown in [Table 5]. Granuloma s/o Tuberculosis, Mycormycosis and Hydatid cyst covers minimum number of cases each. Out of 34 cases 6 cases of renal cell carcinoma, 1 case of oncocytoma and 1 case of angiomyolipoma were documented.

In Gross findings in pyelonephritis, predominant number of cases was found to have loss of cortico medullary junction with 91.7% and Dilatation of pelvi-calyceal system with 95.8%. Also 41.7% of cases were found to be cortical scarring and 25% of cases have calculi as shown in [Fig 1].

In microscopic findings of nephrectomy cases, Tubular atrophy and Thyroidisation of tubules were seen in 21 cases each. Maximum number of cases i.e. 22 cases (91.7%) were found to be interstitial fibrosis as shown in [Table 6]. Glomerular sclerosis with hyalinization and periglomerular fibrosis were seen in 62.5% and 66.7% respectively.

In this spectrum, benign tumor accounted for two cases and malignant tumor accounted for 6 cases and Renal cell carcinoma-clear cell type is the most common malignant tumor seen in this study as shown in [Table 7].

Table 1: Distribution of nephrectomy specimens according to age.

Age	Number of Cases	Percentage
0-20	1	3%
21-30	3	9%
31-40	3	9%
41-50	7	20.5%
51-60	10	29.5%
61-70	9	26%
71-80	1	3%
Total	34	100%

Table 2: Distribution of nephrectomyspecimens according to gender

Type of lesion	Male	Female	Total
Non –neoplastic	14	12	26
Neoplastic	3	5	8
Total	**17**	**17**	**34**

Table 3: Clinical presentation.

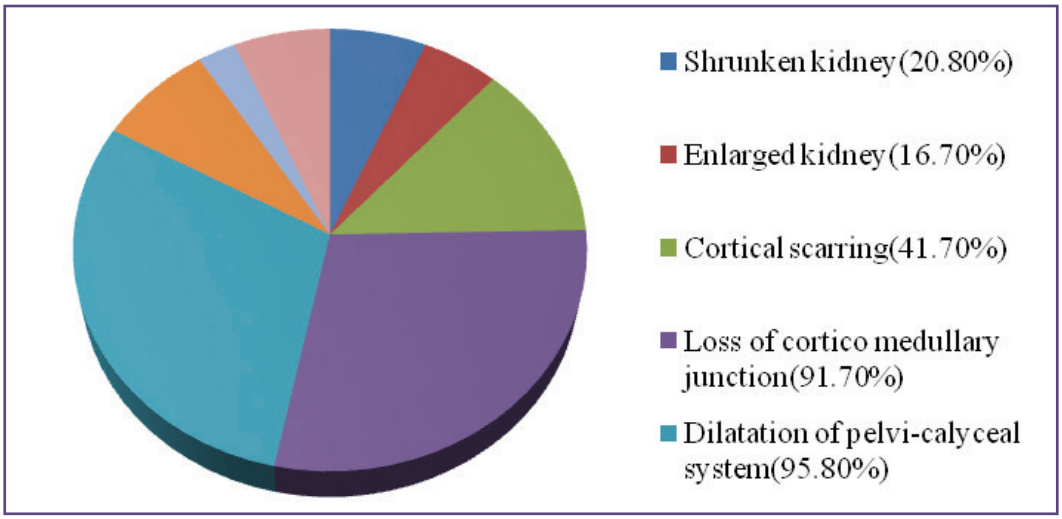
S.No	Symptoms	Number of cases
1	Abdomen lump	5
2	Flank pain	28
3	Hematuria	20
4	Burning micturition	26
5	Fever	22
6	Oliguria	4

Table 4: Spectrum of histopathological diagnosis in nephrectomy specimen.

Lesion	Number of Cases	Percentage
Cystic lesion	2	6%
Chronic pyelonephritis	24	70.5%
Benign	2	6.0%
Renal cell carcinoma	6	17.5%
Total	**34**	**100%**

Table 5: Chronic pyelonephritis (CPN) with associated findings.

S.No	Associated findings	Number of Cases	Percentage
1.	CPN	5	21%
2.	Acute on chronic CPN	3	12.5%
3.	CPN with PN and nephrolithiasis	6	25%
4.	CPN with xantho-granulomatous change	5	21%
5.	CPN with lipomatous change	1	4%
6.	CPN with granuloma s/o Tuberculosis	2	8.5%
7.	CPN with Mucormycosis	1	4%
8.	CPN with Hydatid cyst	1	4%
	Total	**24**	**100%**



Graph 1: Gross findings.

Table 6: Microscopic findings in Pyelonephritis.

Microscopic findings	Number of cases	Percentage
Glomerular sclerosis with hyalinization	15	62.5%
Periglomerular fibrosis	16	66.7%
Tubular atrophy	21	87.5%
Thyroidisation of tubules	21	87.5%
Interstitial inflammation Acute Chronic Granulomas Foamy macrophages	 2 16 3 1	 8.3% 66.7% 12.5% 4.2%
Interstitial fibrosis	22	91.7%
Intimal sclerosis of arcuate vessels	15	62.5%

Microscopic findings	Number of cases	Percentage
Hyaline Arteriosclerosis	14	58.3%

Table 7: Distribution of tumors.

Tumors	Number of cases	Percentage
Angiomyolipoma	1	12.5%
Renal Oncocytoma	1	12.5%
RCC- Clear cell type	5	62.5%
RCC- Papillary type	1	12.5%
TOTAL	**8**	**100%**

Table 8: Furhman nuclear grading of Renal cell carcinoma.

S.No	Furhman nuclear grade	Number of cases	Percentage
1	Grade I	1	16.5%
2	Grade II	4	67%
3	Grade III	1	16.5%
4	Grade IV	-	-

Discussion

In our study, 34 nephrectomy cases were analyzed. Out of 34 nephrectomy cases 26 cases were non-neoplastic and 8 cases were neoplastic. Similar findings were observed by AifaAimanet al[3] and Shaila et al[8]

In the present study majority of nephrectomy were done in sixth decade (29.5%) followed by seventh decade(26%). However it is variable with study of Muhammad et al[9], who observed in third decade (30%) followed by fourth decade(20%).Among the nephrectomy cases 17 cases were male and 17 case were female. This finding is variable to study done by Divyashreeet al[6] and Nusrat et al[7].

In our study majority of the cases presented with loin pain (28 cases) followed by urinary symptoms like burning micturition, hematuria and fever. A majority of patients who presented with hematuria had malignant lesions. These findings are in concordance with study conducted by A. Aiman[3] and Popat et al[10].

In our study the most common lesion observed was chronic pyelonephritis(70.5%), which is followed by Renal cell carcinoma as studied by Popat et al[10].

Radiologically cystic lesions and calculi are identified. Among five cases of Xanthogranulomatous pyelonephritis, all five cases radiologically showed calculi. Out of two cases of tuberculosis one showed calculi, which was in discordance with study conducted by A. Aiman et al[3].

On critical evaluation majority of the chronic pyelonephritis cases calculi. It is due to various factors like dietary, genetic and sedentary life styles. Commonly patient develops calcium oxalate stones which occurs due to consumption of hard water, supersaturation of calcium oxalate and lower urinary citrate concentration[14][15].

Most of the renal stones tends to recur, which also depends upon stone type. Decreased supersaturation of the urinary filtrate and increased urinary citrate concentration will reduce the incidence of stone recurrence [15, 16]. Apart from renal calculi other risk factors for chronic pyelonephritis are vesico-ureteral reflux, benign prostatic hyperplasia and urinary tract infection in pregnancy.

In the present study, two cystic lesions are documented, one case is simple renal cyst and another case is cystic renal dysplasia which is similar to the study conducted by Divyashree et al[6]. Among 24 cases of chronic pyelonephritis(CPN), grossly majority of cases shows dilatation of pelvi-calyceal system(23 cases) and loss of cortico-medullary junction(22 cases), which is concordance with study conducted by Datta et al[11].

Out of 24 cases of CPN [Figure-1], microscopically 6 cases were diagnosed to CPN with nephrolithiasis five cases with Xanthogranulomatous pyelonephritis and 2 cases shows tubercular changes and other associated findings include pyonephrosis, lipomatous change, mucormycosis and hydatid cyst as similar to study done by Shikhaet al[5]. Globally the incidence of xanthogranulomatous pyelonephritis is 0.6 to 1% with female preponderance[13], but in our study 5 cases have been reported which is found to be discordance with the other study.

In the present study nephrectomy done for 8 tumor cases, out of which 6 cases are renal cell carcinoma [Figure-2], 1 case is angiomyolipoma and 1 case is oncocytoma [Figure-3] which is in concordance with study conducted by EI Malick EM et al[12].

Conclusion

Our study reveals that nephrectomies were done most commonly for non-neoplastic lesions when compared to

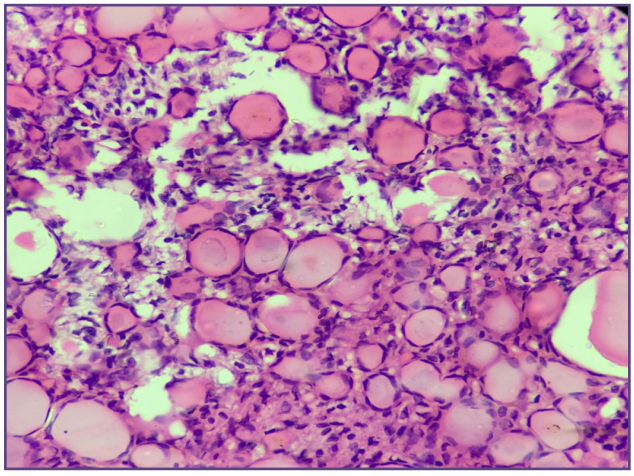


FIG. 1: Chronic Pyelonephritis. Microphotograph showing thyroidisation of tubules. (H&E, 10X)

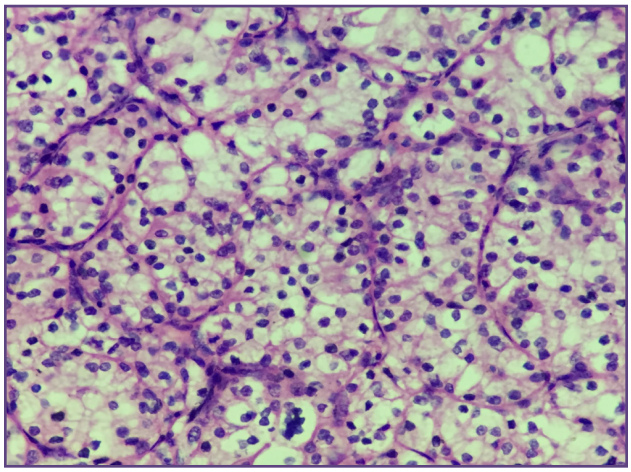


Fig. 2: Renal Cell Carcinoma- Clear Cell Type. Microphotograph showing clear cells with prominent delicate vasculature. (H&E, 40 X)

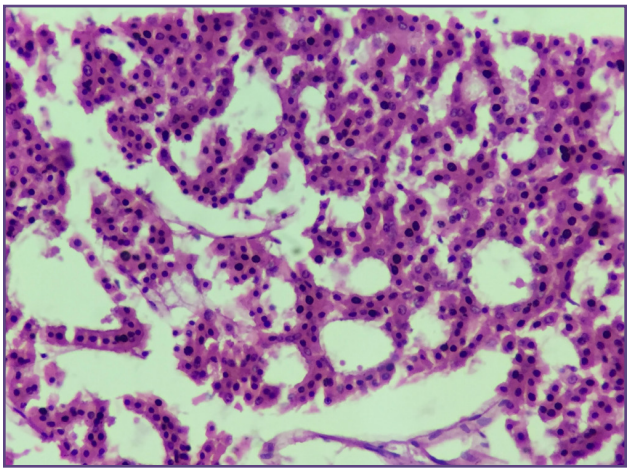


Fig. 3: Renal Oncocytoma- Microphotographs showing oncocytes. (H&E, 10X).

neoplastic lesions. Renal cell carcinoma- Clear cell type is the most common malignant tumor. Chronic pyelonephritis (CPN) is the most common non-neoplastic lesion with series of microscopic changes like thyroidisation of tubules, tubular atrophy, interstitial fibrosis, etc. CPN also seems to be associated with various findings like pyonephrosis, calculi, malignancies etc. Hence it is mandatory to study each CPN case in detail along with clinical and radiological findings.

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Significance of Ki-67 in Prognostication of Soft Tissue Tumors

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ABSTRACT

Background: Histological grading is the most important prognostic factors and the best indicator of metastatic risk in adult soft tissue sarcomas. It has been shown that Ki67 immunohistochemical (IHC) staining is an effective method of assessing the prognosis in a number of tumor types. Ki67 is a cell cycle antigen which is elevated in proliferation states. High level of Ki67 expression is an independent prognostic indicator that correlates with poor outcome in patients with sarcomas.

Methods: In this study 53 cases of soft tissue tumor were selected and were classified according to FNCLCC system. IHC staining of Ki-67 was done in all the 53 samples. Diagnosis was made mainly based on histopathological pattern analysis and with the use of IHC.

Results: By using FNCLCC grading soft tissue tumor cases were graded as grade I, II and III. The correlation between the FNCLCC grading and Ki67 was done and it was observed that there was statistically significant positive correlation between Ki67 and the grade of the tumor, as evidenced by P- values of 0.002, 0.005 and 0.004. The correlation between the FNCLCC grading and Ki67 index was also assessed. Statistically significant positive correlation between Ki67 index and the grade of the tumor was observed.

Conclusion: There was significant correlation noted between FNCLCC grading and Ki-67 index. Thus it can be recommended that Ki-67 IHC stain should be done on routine basis to accurately grade the sarcomas so that it will be beneficial for the management of the patient.

Keywords: *FNCLCC Grading, Immunohistochemical Staining, Ki-67 Index, Soft Tissue Tumors*

Introduction

Soft tissue tumors are composed of numerous and complex diagnostic entities. Because of this complexity and the recognition of an intermediate malignancy category including some tumors with a deceptive bland histological appearance, soft tissue tumors represent a major diagnostic challenge to the general practicing pathologist. Despite the rapid development of molecular genetic technique, immunohistochemistry plays an important role in the diagnosis of soft tissue tumors.

The histological type of sarcomas does not always provide sufficient information for predicting the clinical course and therefore for planning therapy. The two most widely used systems are the NCI (United States National Cancer Institute) system[1,2] and the FNCLCC (French Federation Nationale des Centres de Lutte Contre le Cancer) system.[3-7]

The FNCLCC system is based on a score obtained by evaluating three parameters selected after multivariate analysis of several histological features: tumour differentiation, mitotic rate and amount of tumour necrosis. [7]A score is attributed independently to each parameter and the grade (G) is obtained by adding the three attributed scores. Tumour differentiation is highly dependent on histological type and subtype.[7]

Histological grading is the most important prognostic factors and the best indicator of metastatic risk in adult soft tissue sarcomas. Sarcomas are also characterized by deregulated proliferation. Ki67 is a cell cycle antigen which is elevated in proliferation states. High level of Ki67 expression is an independent prognostic indicator that correlates with poor outcome in patients with sarcomas.

Current classification schemes may require revision where biological behavior and prognostic significance of these tumors is concerned, as an increasing number of studies have suggested that Ki67 may be an important factor in cancer grading and prognostic evaluation. It has been shown that Ki67 immunohistochemical (IHC) staining is an effective method of assessing the prognosis in a number of tumor types.[8,9]

Although pKi67 is a key marker associated with proliferating cancer cells and a poor prognosis, its full potential in increasing proliferation has not been evaluated. In syngeneic animal models with subcutaneous or orthotopic bladder cancer, prostate cancer or renal cell carcinoma, antisense oligonucleotides induced tumor

growth inhibition[10,11], potentially through the inhibition of Ki-67, indicating the involvement of Ki67 in tumor cell proliferation.

Materials and Methods

The present study was conducted in the department of Pathology of Sri Ramachandra Medical College and Research Institute. All soft tissue tumor diagnosed between January 2006 to June 2011 were retrieved from the surgical pathology files. A total of 513 cases were collected and reviewed. Out these cases benign, intermediate and malignant cases were identified. The malignant cases were taken out of which small biopsy specimens and patients who lacked details of important pathological features were excluded from the study.

All the soft tissue tumor specimens which were surgically resected and subsequently diagnosed as one of malignant soft tissue tumor by histopathological examination with Hematoxylin and Eosin (H&E) stain were included in the study.

The clinical features such as age, sex of the patient and location of the tumor were collected. The gross characteristics of the tumor which included the tumor location, size, necrosis, circumscription, cut section and secondary changes were obtained from the pathology report registers. Paraffin blocks of concerned cases were recovered and histological sections (5 to 6 um) were routinely stained with H&E stains.

H&E stained sections were reviewed along with the grading of the tumours according to FNCLCC grading system. The microscopic features were assessed after reviewing all the available slides. These included the histological pattern, cellular features, pleomorphism, mitosis, vascularity and secondary changes. Diagnosis was made mainly based on histopatholgical pattern analysis and with the use of Ki67 analysis.

For doing Ki67 staining 53 cases of sarcomas were selected. Representative slides were selected after looking for the areas of maximum mitosis and absence of necrosis.

Immunostaining of Ki-67 antigen was done using Ki-67 monoclonal antibody (antihuman Ki-67 antigen, AM297-5M, Biogenex, at a dilution of 1:50). Sections from lymph node were included as positive control. Negative control (without adding primary antibody) was included in all the batches. Sections were examined under high power field to observe the immunoreactivity. Hot spot (area with highest density of immunostained nuclei) was selected and adjacent fields counted to include 1000 nuclei . Distinct nuclear staining of the tumor cells was recorded as positive. Ki-67 Labeling Index (LI) was recorded as percentage of positively stained tumor nuclei in 1000 tumor cells. Vascular components and inflammatory cells were excluded. Necrotic, degenerated and poorly preserved areas were also excluded.

Result

FNCLCC grading was used to grade the study cases and it was observed that 35.8% of soft tissue tumours cases were of grade I, 26.4% were of grade II and 37.7% were of grade III. This table (Table no:1) shows the percentage of various grades of sarcomas which are included in the study of 53 cases. The percentage of grade III sarcomas (37.7%) is more compared with other grades.

The correlation between the FNCLCC grading and Ki67 was done. It was observed that there was statistically significant positive correlation between Ki67 and the grade of the tumor, as evidenced by P- values of 0.002, 0.005 and 0.004 (Table no:2). The mean of ki-67 index in different grades were assessed (figure:1) and the mean of ki-67 for 1000 nuclei in different grades were also assessed (figure:2) which showed that ki index is comparatively more in grade III sarcomas with percentage of 10.86. The grade I and grade II shows 1.32% & 6.33%.

The overall comparison of values between H&E mitosis per 10 HPF with Ki67/ 10 HPF showed significant correlation with P value of 0.0005(Table:3).The correlation between the FNCLCC grading and Ki67 index was also assessed. Statistically significant positive correlation between Ki67 index and the grade of the tumors was observed (Table no:4).

Table No.1: Distribution of tumor according to FNCLCC grading.

FNCLCC Grade	N	%
I	19	35.8
II	14	26.4
III	20	37.7
Total	**53**	**100**

Table No. 2: Comparison of FNCLCC grading and Ki67 index.

FNCLCC grading	N	Mean	SD	SE	95% confidence interval for mean	
					Lower bound	Upper bound
I	19	20.15	23.36	5.36	8.89	31.42
II	14	63.28	50.38	13.46	34.19	92.37
III	20	108.6	54.78	12.25	82.96	134.23
Total	**53**	**64.92**	**58.19**	**7.99**	**48.88**	**80.96**

Dependent variable	FNCLCC grading (i)	FNCLCC grading (j)	mean difference (i-j)	Std Error	Significance
Ki 1000	I	II	-43.13	15.73	0.008
		III	-88.44	14.31	0.000
	II	I	43.13	15.73	0.008
		III	-45.31	15.57	0.005
	III	I	88.44	14.31	0.000
		II	45.31	15.57	0.005

Table No. 3 :Comparison between H&E mitosis/ 10 hpf and Ki 67/10 hpf.

	Ki/ 10 HPF	
H & E mitosis/ 10 HPF	No	P value
	53	0.0005

Table No. 4: Comparison of FNCLCC grading and Ki67 index.

FNCLCC grading	N	Ki Index Mean	SD	SE	95% confidence interval for mean	
					Lower bound	Upper bound
I	19	1.31	1.56	0.36	0.57	2.07
II	14	6.33	5.04	1.35	3.42	9.24
III	20	10.86	5.48	1.22	8.29	13.42
Total	**53**	**6.24**	**5.93**	**0.81**	**4.61**	**7.89**

Dependent variable	FNCLCC grading	FNCLCC grading	Mean difference	Std Error	Significance
Ki Index	I	II	-5.01	1.53	0.002
		III	-9.53	1.39	0.000
	II	I	5.01	1.53	0.002
		III	-4.53	1.51	0.004
	III	I	9.53	1.39	0.000
		II	4.53	1.51	0.004

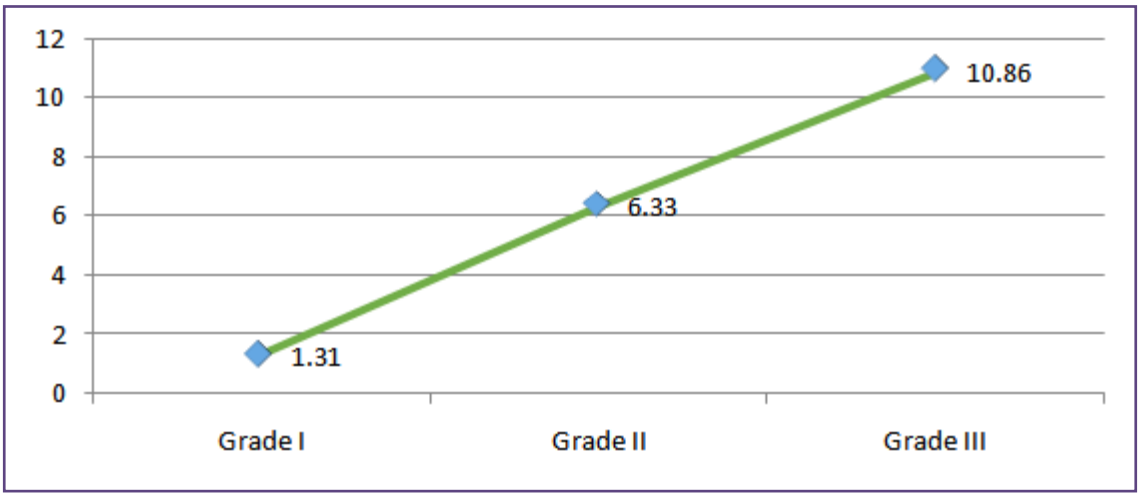

Fig.1: Comparison of FNCLCC grading and Ki67 index.

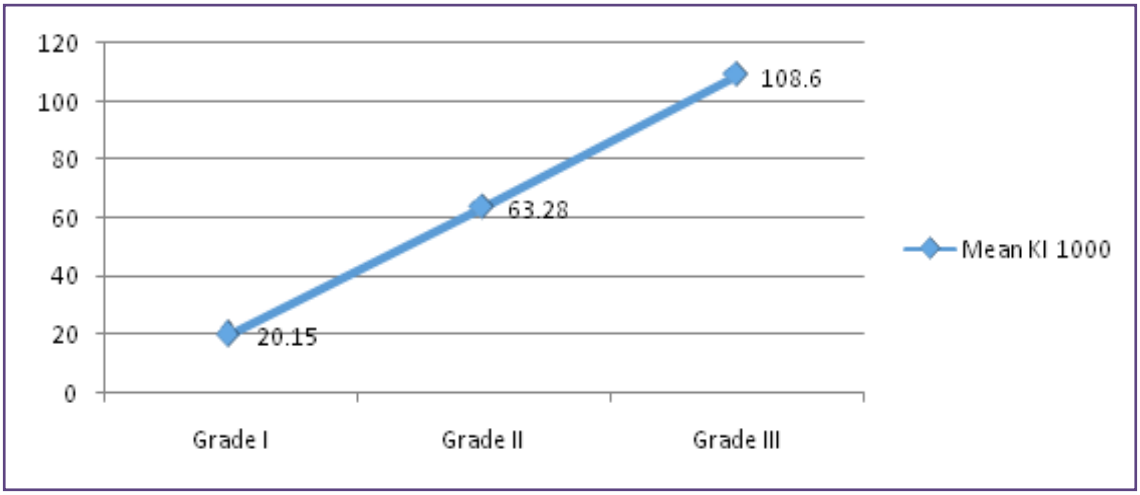

Fig. 2: Comparison of FNCLCC grading and Ki67 score for 1000 nuclei.

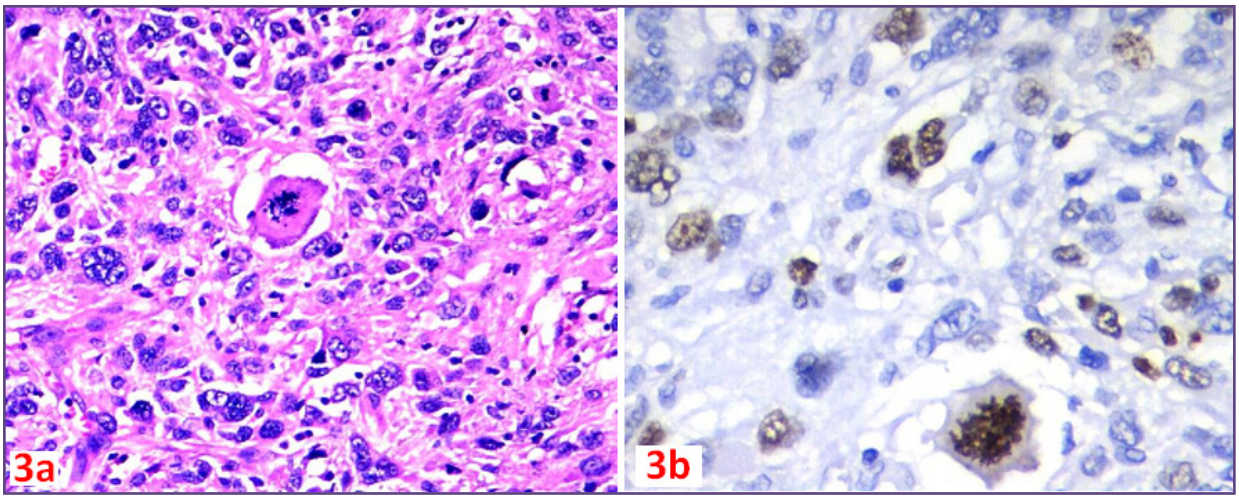

Fig. 3a: H&E 40X- pleomorphic sarcoma sarcoma(G III): shows highly pleomorphic cells with atypical mitosis. Fig:3b: 40XKi67 immunostain: mitosis highlighted.

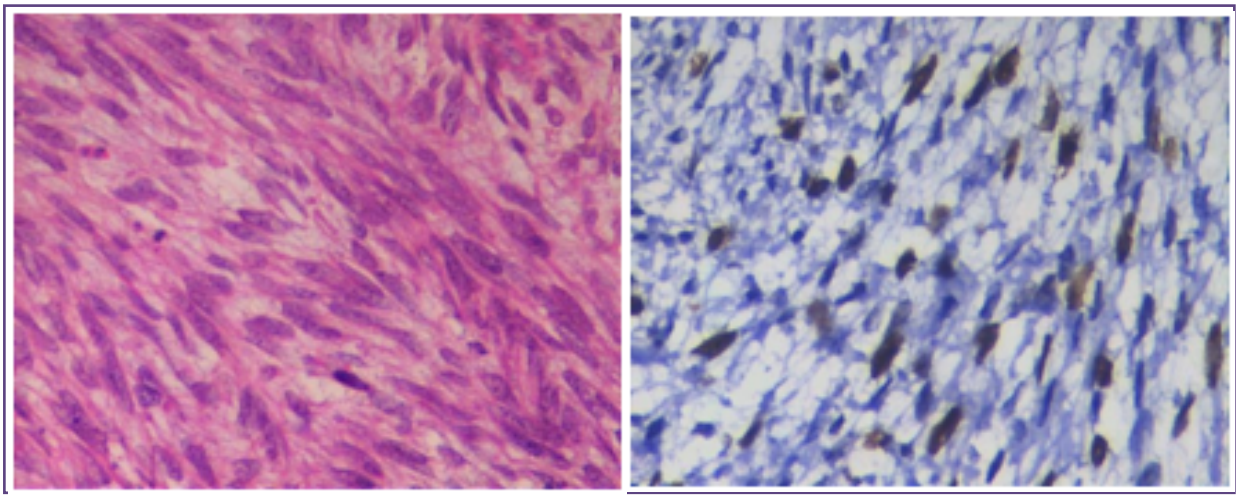

Fig. 4a:H&E 40X-fibrosarcoma (GII): spindle shaped cells arranged in herringbone pattern. Fig:4b: 40X Ki67 immunostain.

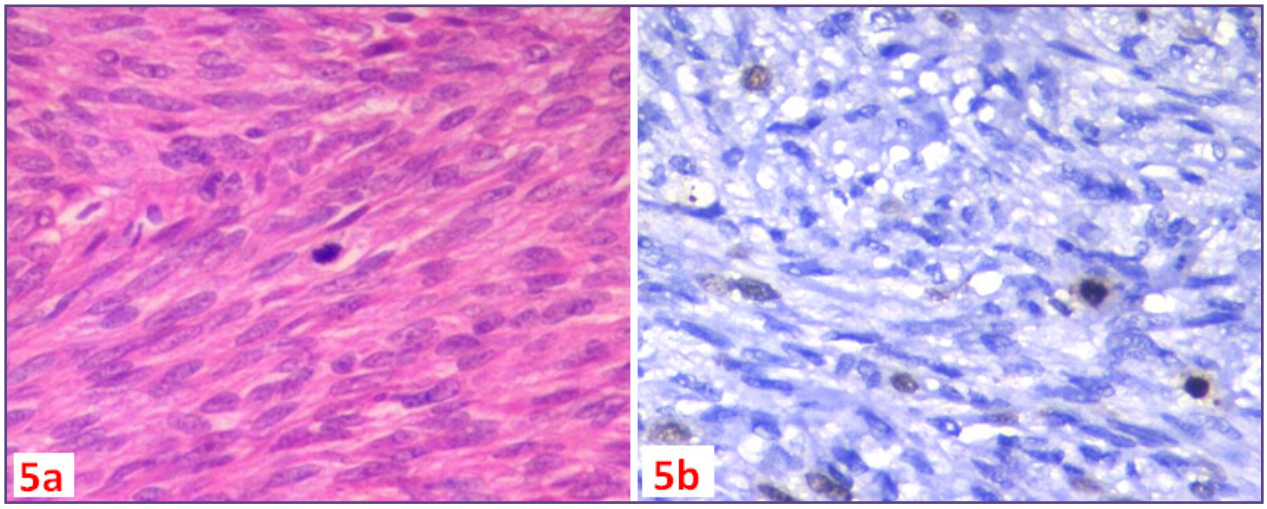

Fig. 5a: H&E 40X- DFSP(GI) shows spindle shaped cells. Fig5b: 40X Ki67immunostainhighlights mitosis.

Discussion

The present study was conducted to analyze the importance of Ki67 index in soft tissue tumours and to see the correlation between FNCLCC grading and Ki67analysis in 53 cases of sarcomas. According to FNCLCC grading it was observed that 35.8% of soft tissue tumours cases were of grade I, 26.4% were of grade II and 37.7% were of grade III.

Immunohistochemistry is the most valuable adjuvant to H&E staining in diagnostic histopathology. It is important in diagnosis of STT because of their variety with several lines of differentiation and the frequent difficulty of diagnosis with numerous pseudosarcomatous benign lesion and non mesenchymal tumors.

Ki67 is frequently used as an indicator of cell proliferation.[12,13] Ki67 was significantly more highly expressed in malignant than in normal tissues.[14,15]The nuclear proliferative antigen Ki67 is identified to correlate with the prognosis of the patients to predict the outcome and distant metastasis of sarcoma patients. A significant correlation has been reported between Ki67 expression and mitotic rate in sarcomas.[16,17] A correlation between ki67 reactivity and tumour grade in sarcomas has been detected in different retrospective studies.

Ki67 IHC staining was done on 53 cases of various sarcomas which had different grades. The correlation between the FNCLCC grading and Ki67 index was assessed. Statistically significant positive correlation between Ki67 index and the grade of the tumor was observed(Fig 3-5). Sahil et al have reported Ki67 index to be very low in benign and G-I tumour and high in G-II and G-III tumor. Sarcomas in our study with G-III also had high Ki index compared to GI tumours which correlated with the study.

The Ki67% was less than 10% in G-I sarcomas in our study which correlated well with Aydin etal study which also showed Ki67 % <10% in all of the G-I tumours (100%). In his study all the G-III tumours had Ki67% more than 10%. But in our study few of the G-III sarcomas like Leiomyosarcoma, MFH, synovial sarcoma had <10% which did not correlate with Aydin et al study. But it correlated with the study of Swanson et al. They have also observed a disparity between Ki67% and histologic grade in some tumor. The Ki67 index strongly correlated with various histological grade with p value of 0.002 with G-I and G-II, 0.0005 in G-I and G-III, 0.004 in G-II and G-III sarcomas.

Comparing Ki67 for 10 HPF and mitotic index which was used in FNCLCC grading showed that more number of mitosis is counted with Ki67 than routine mitotic index using H&E stain. This will help to increase the grade of the sarcomas perfectly and the treatment is also improved. Takafumi veda et al showed positive correlation between Ki67 per 10 HPF and number of mitotic figure per 10 HPF. The present study also showed positive correlation with P value of 0.0005 between Ki67 per 10 HPF and number of mitotic figure per 10 HPF.

The findings in present study indicate that precise assessment of the proliferative index using Ki-67 immunohistochemical stain can be a valuable adjunct to routine histopathology and in treatment of patients. The data on Ki67 as a diagnostic marker is scarce and based on varying laboratory and statistical methods. Cancer has a complex pathogenesis and reliable early diagnosis is difficult.[18]Symptoms usually do not appear until the disease has progressed to an advanced stage. Therefore, further research into diagnostic and prognostic markers may aid early diagnosis.

Conclusion

Thus we conclude that there was significant correlation noted between FNCLCC grading and Ki-67 Index. Thus it can be recommended that Ki-67 IHC stain should be done on routine basis to accurately grade the sarcomas so that it will be beneficial for the management of the patient.

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Efficacy of Pulse Co-oximeter in Hemoglobin Estimation: A Non Invasive Method

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ABSTRACT

Background: Total Hemoglobin(Hb) measurement is one of the most common and important parameter that is investigated in the laboratory. This non invasive method allows pain free continuous online patient monitoring with minimum risk of infection and facilitates real time data monitoring allowing immediate clinical reaction to the measured data. The objective of the study is to test the efficacy of pulse co-oximeter .

Methods: Present study is a prospective comparative study. A total of 261 patients enrolled for the study after their consent. Non invasively measured SpHb values and invasively measured Hb values were then compared by using Bland Altman statistical analysis .

Result: Pulse co-oximeter recorded/displayed SpHb in 90.8% patients and did not display SpHb in 9.2% of patients. Out of which 73-75% patients Hb showed variation of less than or equal to 2gms and 20-24% patients Hb showed variation of more than 2 gms. On Bland Altmann comparative analysis of SpHb and Hb revealed bias and limits of agreement was 0.2+3.3gm/d and 95% of the measurements fell within two standard deviation of the mean difference and P value < 0.001 represents good correlation between SpHb and Hb of Lab1 and Lab 2.

Conclusion: Pulse co-oximeter can be used as a screening tool for Hb measurement . It determines Hb instantly and non invasively with 73-75% of the values show variation of less than or equal to 2gms. Further studies are needed to determine financial aspects and needs upgradation in terms of accuracy of the instrument.

Keywords: *Pulse Co-oximeter, Non Invasive Method, Bland Altmann Analysis*

Introduction

Hemoglobin(Hb) can be measured on a variety of devices using different principles of operation. Non invasive pulse co-oximeter represents the latest development in hemoglobin measuring technology. The technology uses principles similar to pulse oximetry in measuring total hemoglobin, oxyhemoglobin, carboxyhemoglobin and methhemoglobin. The pulse oximeter works by illuminating light into the tissues and sensing the amount of light absorbed. The same methodology is used by laboratory hemoglobinometers to measure hemoglobin concentration. Because both devices work in the same way, efforts were made to modify the pulse oximeter to also measure hemoglobin concentration. Currently there are two commercial pulse co-oximeters (Masimo Rainbow SET and OrSense NBM-200MP) that measure total hemoglobin concentration and one (Masimo) that also measures methemoglobin and carboxyhemoglobin[1]. The technology is noninvasive and provides continuous monitoring in comparison to invasive and discrete techniques used in other methods, pulse co-oximeter purportedly provides an advantage in patient care. In present scenario invasive methods are used to measure the Hb concentration, Hb measurement using an automated analyzer in a clinical laboratory is the gold standard method [2]. Apart from the discomfort of ejecting blood samples an added disadvantage of this method is delay between blood collection and its analyses which does not allow real time patient monitoring in critical situations. The purpose of this research is threefold: a) To study accuracy of pulse co-oximeter (Masimo Rainbow SET) by comparing its results with hematology analyzer b) To review the various underlying principles used in measuring Hb c)To discuss issues in implementing pulse co-oximeter into a laboratory or hospital

Materials and Methods

The present study is a prospective comparative study done over a period of two months during October and November 2016. The study was conducted in central laboratory district hospital VIMS Ballari. After approval from ethical committee VIMS, informed consent was obtained from patients prior to their enrollment. On the basis of previous study performed by Allard [3] et al, we did hemoglobin estimation on 261 patients by pulse co-oximeter(SpHb), Lab1-central lab Sysmex hematology analyzer(Hb) and Lab 2-Medall laboratory hematology analyzer(Hb). SpHb estimation by pulse co-oximeter was done while subjects were quiet and sitting upright. Sensor

of pulse co-oximeter was covered with an opaque shield to prevent optical interference.

Immediately following the noninvasive testing, a venous blood sample was obtained by venipuncture of the median cubital vein of the non dominant arm with a disposable syringe and then transferred to 2ml vacuum tube containing EDTA. Venous blood samples were transported at room temperature and analyzed for reference hemoglobin value with Lab1-central laboratory Sysmex hematology analyzer and Lab 2-Medall laboratory hematology analyzer as per clinical and Laboratory Standard Institute guidelines and manufactures directions for use with in 24 hr of collection. The laboratory analyzer was calibrated daily as per the manufacturer's recommendations and good laboratory practice.

This newly developed pulse co-oximeter (Masimo Rad 57) is an optical sensor system uses multiple wavelengths of light for Hb measurement [2]. The Hb sensor developed for this research is fully integrated into a wearable finger clip. The devise is based on technology known as occlusion spectroscopy which uses an optical measurement platform combined with a ring shaped pneumatic probe that fits on the finger [3] (Figure 1).

Statistical Analysis: Statistical analysis was performed to determine the relationship between SpHb and the standard laboratory Hb. We calculated the correlation coefficient (r) and coefficient of determination (r2). Agreement between the laboratory Hb and SpHb was evaluated as described by Bland and Altman [5]. The accuracy of the SpHb compared with that of the laboratory Hb was calculated using the accuracy root mean square (Arms) with the formula Square root of(mean bias square + SD square) [6,7]. All statistical analysis was performed with SPSS Version 19.0, with the statistical significance set at $P<0.005$.

Results

Conducted prospective study on 261 out patients visiting laboratory for routine hemoglobin estimation . Patients belonged to varied age group and males were 70.9%, females were 29.1%. Hb estimation was done by 3 different methods on 261 patients

1) SpHb by pulse co-oximeter (Masimo)
2) Lab1- central lab district hospital by sysmex hematology analyzer
3) Lab 2- Medall lab hematology analyzer

These 3 different methods were compared and statistically analyzed. Among 261 patients pulse co-oximeter did not record SpHb values in 24 patients. Pulse co-oximeter can record/display SpHb in 90.8% patients and did not display SpHb in 9.2% of patients due to unknown reasons. Hence a total of n=237 cases were statistically analyzed and 24 patients were excluded from the study. On comparison of SpHb values with Hb of lab1, variation of less than or equal to 2gms is seen in 75% of patients and variation of more than 2gms is seen in 20% patients(Table 1). On comparison of SpHb values with Hb of Lab2, variation of less than or equal to 2gm was seen in 73% patients and more than 2gm in 24.1% patients(Table 2).

The mean laboratory Hb value was 11.9 ± 2 for lab 1 and 11.5 ± 2.1 for lab 2, mean SpHb was 12.1 ± 2.1 which is greater than lab1 and lab 2(Table 3). The correlation coefficient (r) was 0.588(Figure 2) and 0.616 for lab1 and 2 against SpHb and the p value < 0.001 represents good correlation between SpHb and Hb of Lab1 and Lab 2(Table 4). The calculated coefficient of determination (r2) was 0.58%. To assess the agreement between the laboratory analyzer and the pulse cooximetry a Bland Altmann plot was applied(Figure 3). The bias and limits of agreement was 0.2 ± 3.3gm/d, using this method 95% of the measurements fell within two standard deviation of the mean difference. The high accuracy(Low ARMS-Accuracy root mean square) was obtained for hemoglobin levels less than 12gm/dl with an ARMS of 2.52gm/dl. For hemoglobin between 12-18gm accuracy was low with an ARMS of 4.5.

Table1: Comparison of SpHb(Pulse co-oximeter) with Hb of Lab1(Sysmex analyzer).

Hemoglobin	Percentage of patients
Difference of >2gms	20%
Difference of ≤ 2gms	75%
Both showed same values	4.2%

Table 2: Comparison of SpHb(Pulse co-oximeter) with Hb of Lab 2(Medall laboratory analyzer).

Difference of >2gm	24.9%
Difference of ≤ 2gm	73%
Both showed same values	2.1%

Table 3: Showing Standard deviation of SpHb, Hb of Lab 1 and Lab2.

	Mean	SD	Number
SpHb	12.1	1.39	237
Hb Lab1	11.9	2.0	237
Hb Lab2	11.5	2.1	237

Table 4: Showing correlative values of SpHb, Lab1, Lab2.

Correlation Values among the Hb of Lab1 and Lab2 with SpHb			
Methods	**R**	**R2**	**P value**
Lab 1	0.589	0.347	<0.001
Lab 2	0.618	0.382	<0.001

Table 5: Showing different studies and their Bias values.

	Number of cases	Bias
Weinstein et al[12]	710	0.03
Hadar et al[13]	63	0.1
Vora et al[14]	76	0.2
Macknet et al[8]		0.15
Present study	237	0.2

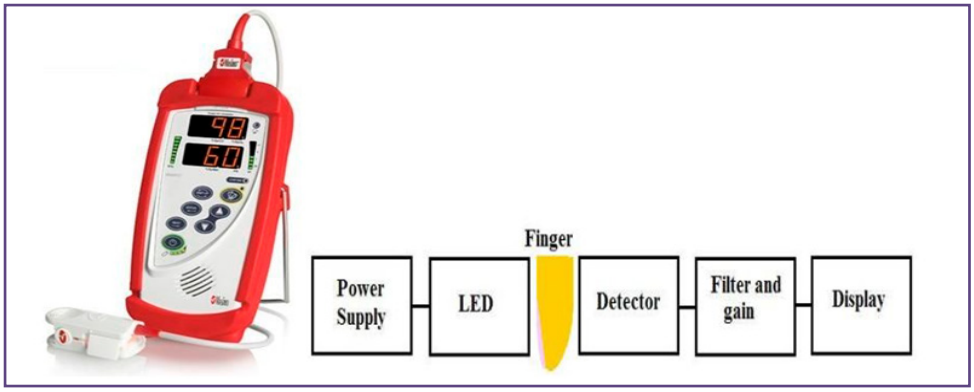

Fig. 1: Pulse Co-oximeter Masimo Rad 57 with displa, figure probe measures SpHb non invasively and basic block disgram[4].

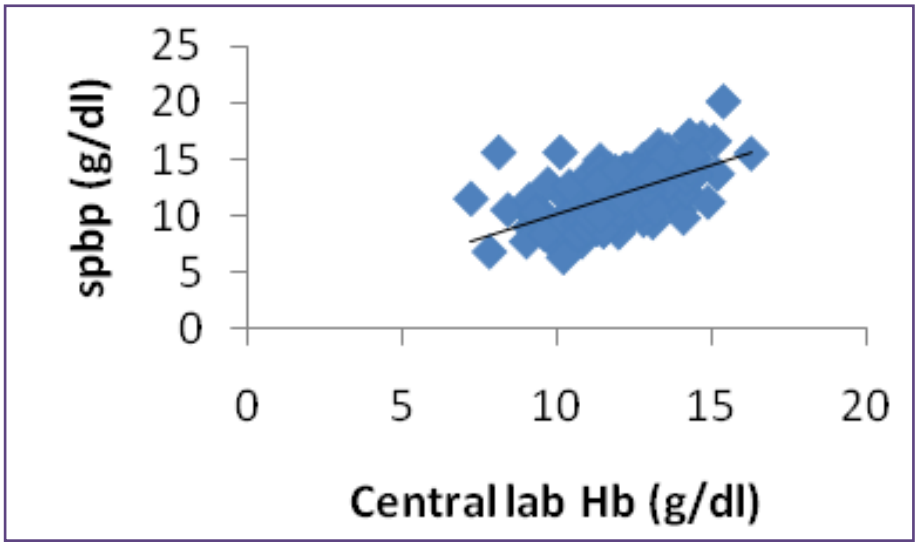

Fig. 2: Scatter plot of hemoglobin values measured by hematology analyzer (lab 1) and pulse co-oximeter. (Correlation co-efficient r =0.588).

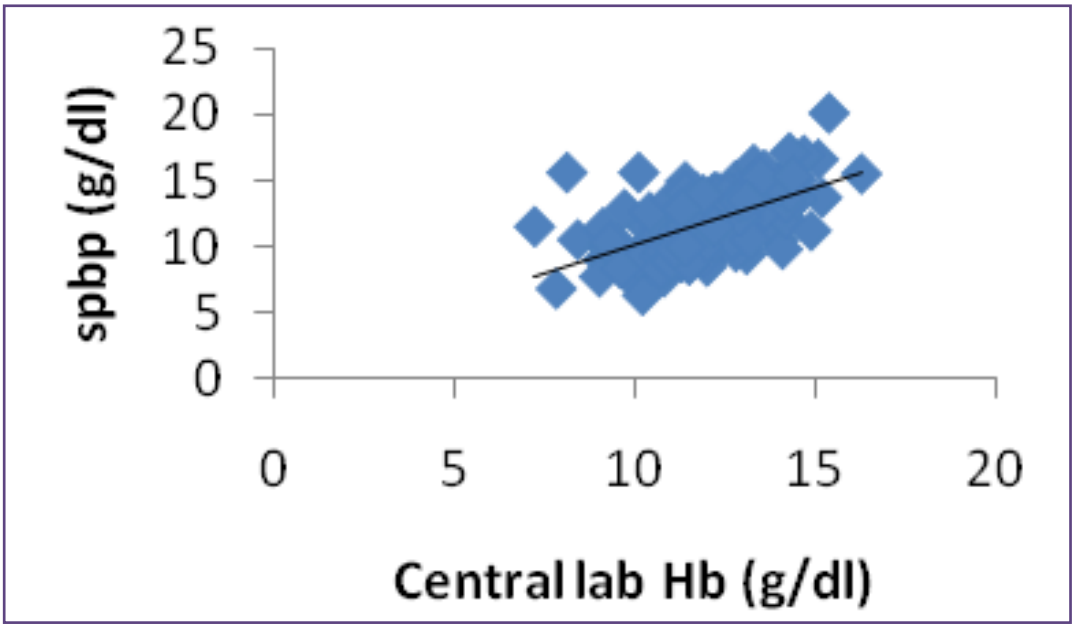

Fig. 3: Bland Altmann representation of comparison analysis between hemoglobin measurement by pulse co-oximeter and hematology analyzer (Lab1). The bias(solid line, o.2) and the limits of agreement (dotted line, bias+1.96SD) are represented on the graph.

Discussion

The accuracy of pulse co-oximeter device in measuring Hb was first evaluated in 2007 by Macknet et al [8], SpHb provided clinically acceptable accuracy compared with the laboratory Hb and they were well correlated. In addition there were several reports suggesting that the SpHb was significantly correlated with the laboratory Hb during surgical procedures with substantial blood loss [9,10]. The present study revealed that noninvasive SpHb measurement with pulse co-oximetry was significantly correlated with laboratory measurement of Hb.

In 1996, the SET (Signal Extraction Technology) was introduced by Masimo (Irvine, CA) to increase the accuracy of 2 wavelength pulse oximetry under motion and low perfusion conditions [11]. Masimo's Rainbow SET technology was later introduced (addition of numerous wavelengths) to measure total Hb, COHb, and MetHb concentrations: CO-Hb in 2005, MetHb in 2006, and noninvasive Hb (SpHb) in 2008 [8]. It measures SpHb using up to 12 wavelengths. Studies have been published evaluating the performance of the Rainbow SET in measuring SpHb relative to laboratory co-oximeters. The Bland-Altman bias and precision analyses are used to compare 2 technologies. Bias is the mean of the measurement differences between methods, and describes systematic error between measurements (i.e., how closely do results of a new monitor compare to measurements in the laboratory (Table 5). The limits of agreement are defined as the differences between two methods approximately 95% of the time. The clinically acceptable limits of agreement depend on the variable of interest, the accuracy of the reference standard and what matters clinically.

Nicholas et al [15] studied twenty seven newborn with weight less than 3000 gms. His study showed good correlation between SpHb and tHb (r=0.75, p=0.0001). The bias and precision for the Hb and SpHb values were 0.10±1.56g/dl. A study by Van Woerkom et al [16] using a diffuse optical spectroscopy instrument demonstrated a reliable correlation between tissue haemoglobin and venous haemoglobin before and after a red blood cell transfusion in preterm infants. Torp et al (n=471) tested the correlation between the Beckman Coulter lab analyzer and the co-oximeter, he found the correlation between the two devices to be 0.93(r) with a bias of 0.97gm/dl.

Beyond the accuracy of SpHb measurement, another concerning issue is the frequency of events where the SpHb monitor did not yield data at all or yielded data of low quality. In present study it did not record SpHb in 9.2% patients. In his study, Macknet [8] reported the inability to measure SpHb in 2.4% of the SpHb measurements. Gayat et al [17] estimated failure rate to be about 9% (although the investigators of this study did not adhere to the manufacturer directions or use in the conduct of the

study). Miller [18] noticed reduced accuracy when the pulse oximeter indicated a low perfusion index. This finding is supported by Gayat et al [17] study where low blood pressure was associated with reduced accuracy. Similar to pulse oximetry, pulse co-oximetry is susceptible to measurement error from the following sources : ambient light interference, low peripheral perfusion, motion artifact, incorrect sensor positioning, nail polish [19]. Shielding around the finger probe or photodetector helps to minimize this interference. If there is no detectable peripheral pulsation, the pulse co-oximeter cannot function. Hypotension, cold extremities and sever vascular disease are all factors that reduce peripheral pulsations [19]. The association between monitor accuracy and peripheral perfusion should not be a surprise, because all pulse oximeters fail to some degree when the patient is peripherally vasoconstricted or hypotensive.

Pulse co-oximeters are good alternate toward reducing iatrogenic blood loss by venipuncture to obtain a blood count. Other draw backs of traditional method are painful needle stick operational inefficiency, delayed Hb results, potential injury to patient. Additional studies to establish whether the use of this method will potentially reduce iatrogenic blood loss are required. Pulse co-oximeter is a costly instrument and studies are needed to compare both methods on financial perspective also.

Noninvasive pulse co-oximeters are classified as monitoring devices by the FDA(Food and drug administratin) and subsequently do not fall under the CAP(College of American pathologist) and CMS(Centre for medicad services) accreditation of laboratory medicine. Like pulse oximeters, these devices use either a disposable or reusable finger probe. The reusable or "reposable" finger probes are guaranteed for approximately 500 uses. Preventative maintenance is carried out annually using a simulator to verify performance. Routine quality control is not necessary. The device can be operated by a respiratory therapist, registered nurse, certified nursing assistant, or doctor.

Conclusion

Pulse co-oximeter can be best used as a screening tool for Hb measurement at out patient departments, blood camps, casuality, ICU and labor rooms. It determines Hb instantly and non invasively. Further studies are needed to determine financial aspects and needs upgradation in terms of accuracy of the instrument.

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Study of Platelet Indices in Type 2 Diabetic Patients and Its Correlation with Vascular Complications

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ABSTRACT

Background: Diabetes Mellitus is a metabolic syndrome characterized by hyperglycemia resulting in macrovascular and microvascular complications. Altered platelet morphology and functions have been linked with the pathological processes and high risk of vascular disease. Platelet indices (Mean platelet volume-MPV, Platelet distribution width-PDW, and Platelet large cell ratio-PLCR) are determinants of platelet functionality.

Methods: The aim of this study was to study the platelet indices (MPV, PDW, P-LCR) in type - 2 diabetic patients with vascular complications and compare them in diabetic patients without vascular complications. The present study was conducted on 70 diabetic patients with vascular complications and 70 diabetic patients without vascular complications for a period of one year and eight months in department of pathology, JSS hospital, Mysore. Patients were divided into cases and controls depending on the presence or absence of macrovascular complications (Myocardial infarction , stroke, peripheral arterial disease) and microvascular complications (retinopathy, nephropathy and neuropathy). Platelet indices (MPV, PDW, P-LCR) were measured using an Automated Blood Counter. FBS and HbA1C levels were collected from the clinical proforma.

Result: Platelet indices were significantly higher in diabetic patients with vascular complications compared to those without complications [11.37±1.19 fL vs 10.17±0.71 fL (P=0.0001), 13.90±2.99 fL vs 11.28±1.55 fL (P=0.0001), 35.61±9.35% vs 26.14±5.79% (P=0.0001) respectively].

Conclusion: The present study showed a significantly higher MPV, PDW and P-LCR in diabetic patients with vascular complications compared to those without complications. This indicates that elevated platelet indices could be the cause for vascular complications. Hence MPV, PDW, P-LCR can be used as simple and cost effective predictive parameters of platelet activation to monitor and predict the risk of vascular complications.

Keywords: *Diabetes Mellitus, Platelet Indices, Mean Platelet Volume, Platelet Distribution Width, Platelet Large Cell Ratio*

Introduction

Diabetes Mellitus is characterized by hyperglycemia resulting in micro and macrovascular complications affecting the nerves, kidneys, eyes, CVS etc.[1] It is associated with varying degree of hyperglycemia accompanied with the biochemical alterations in carbohydrate, protein and lipid metabolism.[2] The injurious effects of hyperglycemia are characterized as macrovascular and microvascular complications. Altered platelet morphology and functions have been linked with the pathological processes and high risk of vascular disease.[3] The platelet indices - (Platelet - PLT, Mean platelet volume - MPV, Platelet distribution width - PDW and Platelet large cell ratio - PLCR) are the determinants of platelet functionality, among which increased mean platelet volume (MPV) and platelet distribution width (PDW) were found to be attributed in the causation of thromboembolic complications.[4,5] It is also noted that the platelets with increased number and size possibly affect the platelet distribution width contributing in the pathogenesis of vascular complications.[6] Hyperactivity of platelets have an important role in the initiation of thrombosis and atherosclerotic lesions. Larger platelets are more active enzymatically and metabolically and have a higher thrombotic ability as compared to the small sized platelets.[7]

Sustained hyperglycemia leads to alterations in the vessel wall leading to endothelial dysfunction and vascular lesions in diabetic complications.[8] Formation of advanced glycation end products, activation of protein kinase C and disturbances in polyol pathways are the possible mechanisms by which increased glucose induces vascular abnormalities.[9]

Large platelets are younger, more active, aggregable, have denser granules and secrete

more pro - aggregatory molecules.[10] Platelet activation triggers thrombus formation and causes thromboembolism with release of PDGF and VEGF that accelerate the

progression of vascular lesions.[11] Increased platelet size may be one of the factor causing increased risk of atherosclerosis associated with diabetes mellitus and vascular complications.[12]

The aim of our study was to determine the hyperactivity of platelets in type 2 diabetic patients and its association with vascular complications. This was done by comparing the platelet indices- MPV, PDW and P-LCR, FBS and HbA1C levels among diabetic patients with vascular complications and diabetic patients without vascular complications.

Materials and Methods

This was an analytical study carried out on 70 type 2 diabetic patients having vascular complications and 70 type 2 diabetic patients without vascular complications for a period of one year and eight months in the department of pathology, JSS hospital, mysore. Data was collected fulfilling the inclusion and exclusion criteria.

Inclusion Criteria: Diabetic patients with vascular complications and diabetic patients without vascular

Exclusion Criteria : 1.) Non-diabetic patients with vascular complications & 2.) Diabetics on antiplatelet drugs such as aspirin and clopidogrel.

Patients were divided into cases and controls depending on the presence or absence of macrovascular complications (Myocardial infarction , stroke, peripheral arterial disease) and microvascular complications (retinopathy, nephropathy and neuropathy). Platelet indices like MPV, PDW, P-LCR were measured in the above target groups using Automatic Blood Counter (SYSMEX , XN-1000). Venous blood samples collected in a vacutainer containing di-potassium EDTA were used. Samples were processed within one hour of collection and were maintained at room temperature. Plasma glucose levels and HbA1c levels of the patient were collected from the clinical data.

Statistical analysis was done using Statistical package for social sciences (SPSS version 22) software. Descriptive statistics such as numbers and percentages were used to describe categorical variables. Mean and standard deviations were used to describe continuous variables like MPV, PDW and P-LCR. Independent sample t-test was applied to find out the significant difference in MPV, PDW and P-LCR between the cases and controls. Pearsons correlation was used to analyse association between different variables. Statistical significance was determined at 5% level of significance (ie. < 0.05 is significant). Microsoft word and Excel have been used to generate graphs, tables etc.

Result

Age of the diabetic patients who had complications ranged from 45 years 90 years with mean of 63.30 $\pm$ 10.04) years and age of the diabetic patients who did not have complications ranged from 48 years 86 years with mean of 61.29$\pm$ 8.89 years. (table 2) There were 26 females and 44 males among cases and 32 females and 38 males among controls with Male to female ratio of 1.7 : 1. Duration of diabetes mellitus ranged from 5 years to >20 years in both the groups (cases and controls) with the mean duration being 15.97±4.42. (table 2)

Among 70 cases, 55 (78.6%) had macrovascular complications and 15 (21.4%) had microvascular complications and among the patients with macrovascular complications, 29 (41.4%) patients had cardiovascular complications, 18 (25.7%) patients had Peripheral arterial diseases and 8 (11.4%) patients had cerebrovascular complications. Among patients with microvascular complications, 11(15.7%) patients had Diabetic nephropathy, 3 (4.2%) patients had Diabetic neuropathy and 1 (1.4%) patient had Diabetic retinopathy. 70 controls did not have any complications. Among cases, 57 out of 70 patients (81.4%) had FBS of > 126 mg/dl, 11 out of 70 patients (15.7%) had FBS between 100-126 mg/dl and 2 out of 70 patients (2.8%) had FBS < 100 mg/dl with a mean of 208.53±79.67. Among the controls, 43 out of 70 patients (61.4%) had FBS of > 126 mg/dl, 16 out of 70 patients (22.8%) had FBS between 100-126 mg/dl and 11 out of 70 patients (15.7%) had FBS < 100 mg/dl with a mean of 174.31±79.83. (table 1)

Among cases 68 out of 70 patients (97.1%) had HbA1C levels of ≥ 6.5% and 2 out of 70 patients (2.8%) had HbA1C levels of < 6.5% with a mean HbA1C levels of 9.58±2.0. Among controls, 58 out of 70 (82.8%) had HbA1C levels of ≥ 6.5% and 12 out of 70 patients (17.1%) had HbA1C levels of < 6.5% with a mean HbA1C levels of 8.31±2.22. (table 1)

Among the cases, 55 out of 70 patients (78.5%) had a mean platelet volume of ≥10.5 fL and 15 out of 70 patients (21.4%) had an MPV of <10.5 fL with a mean of 11.377±1.1969. Among the controls, 46 out of 70 patients (65.7%) had MPV of < 10.5 fL and 24 out of 70 (24.2%) had MPV of ≥10.5 fL with a mean of 10.173±0.7134. (table 2). Among the cases, 49 out of 70 patients (70%) had PDW of ≥12.5 fL and 21 out of 70 patients (30%) had a PDW of < 12.5 fL with a mean PDW of 13.90±2.99. Among the controls, 53 out of 70 patients (75.7%) had PDW < 12.5 fL and 17 out of 70 patients (24.2%) had PDW of ≥12.5 fL with a mean of 11.283±1.5501. Among the cases, 54 out of

70 patients (77.1%) had P-LCR of ≥30.5% and 16 out of 70 patients (22.8%) had a P-LCR of <30.5% with a mean P-LCR of 35.617±9.3589. Among the controls, 51 out of 70 patients (72.8%) had P-LCR < 30.5% and 19 out of 70 patients (27.1%) had P-LCR of ≥30.5% with a mean of 26.144±5.7915.

A positive statistical Pearson correlation was seen among cases between PDW and Duration of diabetes mellitus (P - 0.035) and FBS and HbA1c levels (P - 0.004). However, no statistical correlation was noted between MPV and age, duration of DM, FBS, HbA1C; PDW and age, FBS, HbA1C; P-LCR and age, duration of DM, FBS, HbA1C levels. (table 3) (Graph 1,2)

Among the controls, a positive statistical Pearson correlation was seen between MPV and HbA1c (P - 0.047), PDW and HbA1c (P - 0.003), P-LCR and HbA1c (P - 0.026) and FBS and HbA1c levels (P - 0.0001) (Graph 3). However, no statistical correlation was noted between MPV and age, duration of DM, FBS; PDW and age, duration of DM, FBS; P-LCR and age, duration of DM, FBS.

Table 1: Blood Glucose Parameters in Two Groups of Patients Studied.

	CASES		CONTROLS	
	No.	%	No.	%
FBS (mg/dl)				
<100	2	2.8	11	15.7
100-126	11	15.7	16	22.8
>126	57	81.4	43	61.4
HbA1c				
<6.5	2	2.8	12	17.1
≥6.5	68	97.1	58	82.8

Table 2: MPV in Two Groups of Patients Studied.

MPV	Cases		Controls	
	No.	%	No.	%
<10.5	15	21.4	46	65.7
≥10.5	55	78.5	24	34.2
Total	70	100.0	70	100.0

Table 3: Comparison of Mean Platelet Volume (MPV)

STUDY	MEAN MPV (fL) - CASES	MEAN MPV (fL) - CONTROLS	P – VALUE
Agarwal BK et al[21]	11±2.2	7.8±1.3	0.0001
Khandekar MM et al[15]	10.43±1.03	9.2±0.91	0.001
Sharma M et al[22]	17.60±2.04	9.93±0.64	<0.001
Khode V et al[23]	9.54 ± 0.9	9.21 ± 0.6	0.018
Present study	11.377±1.1969	10.173±0.7134	0.0001

Table 4 : PDW in Two Groups of Patients Studied.

PDW	Cases		Controls	
	No.	%	No.	%
<12.5	21	30	53	75.7
≥12.5	49	70	17	24.2
Total	**70**	**100.0**	**70**	**100.0**

Table 5: Comparison of Platelet Distribution Width (PDW).

STUDY	MEAN PDW (fL) – CASES	MEAN PDW (fL) – CONTROLS	P – VALUE
Khode V et al[23]	10.77 ± 2.0	10.35 ± 1.3	0.182
Jabeen F et al[2]	14.71±0.21	13.86±0.297	0.0269
Khandekar M et al[15]	13.19±2.34	10.75±1.42	0.001
Present study	13.901±2.9995	11.283±1.5501	<0.0001

TABLE 6 : P-LCR in Two Groups of Patients Studied

P-LCR	Cases		Controls	
	No.	%	No.	%
<30.5	16	22.8	51	72.8
≥30.5	54	77.1	19	27.1
Total	**70**	**100.0**	**70**	**100.0**

Table 7: Comparison of Platelet Large Cell Ratio (P-LCR).

STUDY	MEAN PLCR – CASES	MEAN P-LCR – CONTROLS	P – VALUE
Khode V et al[23]	21.33 ± 6.1	19.93 ± 4.6	0.147
Khandekar M et al[15]	29.4±7.38	20.65±6.14	0.001
Yilmaz T et al[24]	31.71 ± 2.16	28.59 ± 2.28	>0.05
Present study	35.617±9.3589	26.144±5.7915	<0.0001

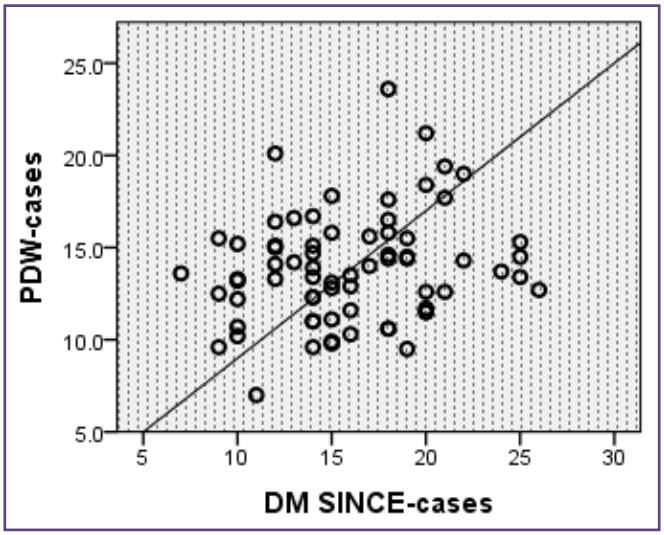
Table 8: Comparison of Study Variables in Cases and Controls Studied.

	DIABETIC PATIENTS WITH COMPLICATIONS	DIABETIC PATIENTS WITHOUT COMPLICATIONS	P – VALUE
Age in years	63.30±10.04	61.29±8.88	0.05
Duration of DM	15.97±4.423	12.36±3.04	0.448
FBS (mg/dl)	208.53±79.67	174.31±79.83	0.009
HbA1C in %	9.58±2.0	8.31±2.22	0.001
MPV (fL)	11.377±1.1969	10.173±0.7134	0.0001
PWD (fL)	13.901±2.9995	11.283±1.5501	0.0001
P- LCR (%)	35.617±9.3589	26.144±5.7915	0.0001

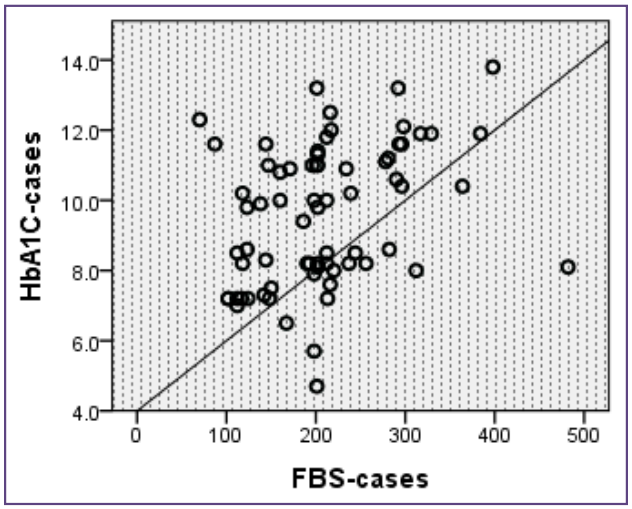
Table 9: Pearson Correlation of MPV, PDW, PLCR and FBS with Study Variables in Cases and Controls.

Pair	CASES (P – VALUE)	CONTROLS (P – VALUE)
MPV vs Age in years	0.388	0.176
MPV vs Duration of DM	0.087	0.344
MPV vs FBS	0.218	0.062
MPV vs HbA1c	0.483	0.047*
PDW vs Age in years	0.113	0.157
PDW vs Duration of DM	0.035*	0.271
PDW vs FBS	0.351	0.087
PDW vs HbA1c	0.326	0.003*
P-LCR vs Age in years	0.412	0.120
P-LCR vs Duration of DM	0.115	0.270
P-LCR vs FBS	0.309	0.077
P-LCR vs HbA1c	0.474	0.026*
FBS vs HbA1c	0.004*	0.0001*

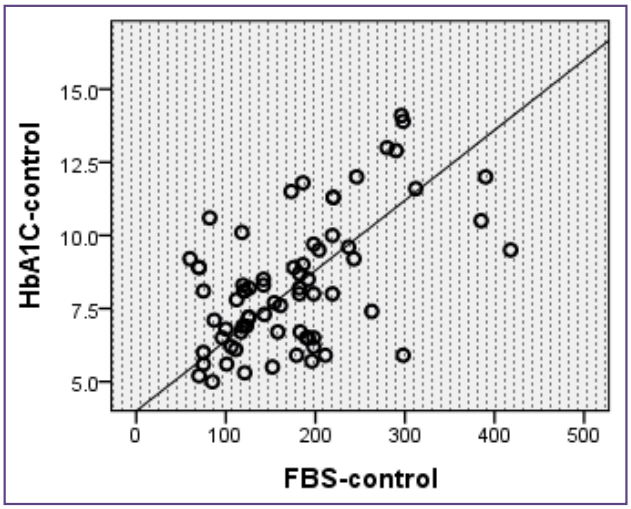
** Statistically significant*



Graph 1: Scatterplot showing a positive correlation between platelet distribution width (PDW) and duration of DM (P -0.035)



Graph 2: Scatterplot showing a positive correlation between FBS & HbA1c (P -0.004) - cases.



Graph 3: Scatterplot showing a positive correlation between FBS &z HbA1c (P-0.0001) - controls.

Discussion

Diabetes Mellitus is a metabolic syndrome characterized by hyperglycemia resulting in macrovascular and microvascular complications. Platelet hyperactivity has been linked with the pathological processes and high risk of vascular disease.

Platelet activation → platelet hyperactivity → thrombus formation → vascular complications

Platelet indices (MPV, PDW, PLCR) have been investigated as prospective platelet activation markers.

MPV is an indicator of average size and activity of platelets. Larger platelets are more active enzymatically and metabolically and have higher thrombotic ability as compared to small sized platelets which are depicted by increased MPV. Normal range - 7.5-11.5 fL. P-LCR is the increased percentage of large platelets. It is the ratio of large platelets from the 12 fL discriminator or larger. Normal range - 11.9-66.9%. PDW reflects how uniform the platelets are in size. Activated Platelets with increased number and size of pseudopodia differ in size, leading to alterations in platelet distribution width. Normal range of PDW is 8.3-25.0 fL.

Platelets play a pivotal role in atherothrombosis.[13] Central to the pathogenesis of occlusive arterial disease is the activation of platelets at sites of vascular injury via pathologically exaggerated and dysregulated protective mechanisms of hemostasis.[14] Platelets secrete and express a large number of substances that are crucial mediators of coagulation, inflammation, thrombosis and atherosclerosis.

[15,16] Inadequate glycemic control, protein glycation and oxidative stress cause endothelial injury and platelet activation with altered platelet morphology and function leading to chronic complications in diabetics.[17]

The present study was done to determine if platelets are activated in diabetes and in its association with micro and macro-vascular complications.

Demographic Data: In the present study, the patients age ranged from 40 years to 90 years. The mean age of diabetic patients with vascular complications (cases) was higher compared to those without complications (controls) [63.30±10.04 vs 61.29±8.88] which correlated with the studies conducted by Jabeen F et al(2) on various population. The maximum number of cases in this study were seen between the age of 50-69 years. Among the cases, 44 out of 70 (62.9%) patients were males and 26 out of 70 (37.1%) were females with male to female ratio of 1.7 : 1. This indicates that there was male preponderance in our study which correlated with the study conducted by Bath P et al[16].

Duration of Diabetes: Duration of diabetes mellitus ranged from 5 years to >20 years in both the groups (cases and controls) in the present study with the mean duration being 15.97±4.42. However other studies conducted by Alex kodiatte T et al[1] and Dindar S et al[14] showed a lesser mean duration.

Complications: Out of 70 cases, 55 (78.6%) had macrovascular complications and 15 (21.4%) had microvascular complications and among the patients with macrovascular complications, 29 (41.4%) patients had cardiovascular complications, 18 (25.7%) patients had Peripheral arterial diseases and 8 (11.4%) patients had cerebrovascular complications.

Among patients with microvascular complications, 11(15.7%) had Diabetic nephropathy, 3 (4.2%) had Diabetic neuropathy and 1 (1.4%) had Diabetic retinopathy. 70 controls did not have any complications.

Blood Glucose Parameters: Among cases, 57 out of 70 patients (81.4%) had FBS of > 126 mg/dl, 11 out of 70 patients (15.7%) had FBS between 100-126 mg/dl and 2 out of 70 patients (2.8%) had FBS < 100 mg/dl with a mean of 208.53±79.67 which correlated with a study conducted by Ozder A et al(18) and Ulutas et al.(19) . Among controls, 43 out of 70 patients (61.4%) had FBS of > 126 mg/dl, 16 out of 70 patients (22.8%) had FBS between 100-126 mg/dl and 11 out of 70 patients (15.7%) had FBS < 100 mg/dl with a mean of 174.31±79.83. In the present study, fasting blood sugar was significantly higher in cases compared to that of controls.

Among cases, 68 out of 70 patients (97.1%) had HbA1C levels of ≥ 6.5% and 2 out of 70 patients (2.8%) had HbA1C levels of < 6.5% with a mean HbA1C levels of 9.58±2.0 which correlated with the studies conducted by Alex kodiatte T et al(1), Sari M et al(20) and Ozder A et al.(18). Among controls, 58 out of 70 (82.8%) had HbA1C levels of ≥ 6.5% and 12 out of 70 (17.1%) had HbA1C levels of < 6.5% with a mean HbA1C levels of 8.31±2.22. In the present study the HbA1C levels were significantly higher in cases compared to that of controls.

Platelet Indices: Among the cases, 55 out of 70 patients (78.5%) had a mean platelet volume of ≥10.5 fL and 15 out of 70 patients (21.4%) had an MPV of <10.5 fL with a mean of 11.377±1.1969 which correlated with the studies conducted by Agarwal BK et al(21). Other studies showed a mean MPV lesser than the present study whereas one study conducted by Sharma M et al(22) showed higher MPV than our study (table 2,3). Among controls, 46 out of 70 patients (65.7%) had MPV of < 10.5 fL and 24 out of 70 (24.2%) had MPV of ≥10.5 fL with a mean of 10.173±0.7134. Hence, the present study showed a significantly higher MPV in diabetic patients with vascular complications compared to diabetic patients without vascular complications (P <0.0001).

Activated platelets are larger, younger, more reactive and aggregable, have denser granules, secrete more pro - aggregatory molecules (serotonin, β- thromboglobulin, thromboxane A2) which leads to thrombosis. This hypothesis has been well proved in our result which has shown an incease in MPV in diabetic patients with complications.

Among the cases, 49 out of 70 patients (70%) had PDW of ≥12.5 fL and 21 out of 70 patients (30%) had a PDW of < 12.5 fL with a mean PDW of 13.901±2.9995 which correlated with studies conducted by Khandekar M et al(15). However, study conducted by Khode V et al(23) showed PDW values lower than the present study (table 4,5). Among controls, 53 out of 70 patients (75.7%) had PDW < 12.5 fL and 17 out of 70 patients (24.2%) had PDW of ≥12.5 fL with a mean of 11.283±1.5501. Hence, the present study showed a significantly higher PDW in diabetic patients with vascular complications compared to diabetic patients without vascular complications (P <0.0001). Activated Platelets with increased number and size of pseudopodia differ in size, leading to increase in platelet distribution width.

Among the cases, 54 out of 70 patients (77.1%) had P-LCR of ≥30.5% and 16 out of 70 patients (22.8%) had a P-LCR of <30.5% with a mean P-LCR of 35.617±9.3589 but the other studies showed a lesser P-LCR than the present study (table 6,7). Among controls, 51 out of 70 patients (72.8%) had P-LCR < 30.5% and 19 out of 70 patients (27.1%) had P-LCR of ≥30.5% with a mean of 26.144±5.7915. Hence, the present study showed a significantly higher P-LCR in diabetic patients with vascular complications compared to diabetic patients without vascular complications (P <0.0001).

Pearsons Correlation: A positive statistical Pearson correlation was seen among cases between PDW and Duration of diabetes mellitus (P - 0.035) and FBS and HbA1c levels (P - 0.004). However, no statistical correlation was noted between other parameters. Among the controls, a positive statistical Pearson correlation was seen between MPV and HbA1c (P - 0.047), PDW and HbA1c (P - 0.003), P-LCR and HbA1c (P - 0.026) and FBS and HbA1c levels (P-0.0001) (table 9).

Conclusion

Mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) are considered the important markers of platelet activation which can be easily measured as part of whole blood count. Hence MPV, PDW, P-LCR can be used as a simple and cost effective predictive parameters of platelet activation to monitor and predict the risk of vascular complications.

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Seroprevalence of Hepatitis E IgG Antibodies Among Voluntary Blood Donors in a Tertiary Hospital

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ABSTRACT

Background: Transfusion safety is a major concern in medical practice. Hepatitis E is a potential threat among transfusion-transmitted infections. In resource poor settings and in endemic countries, parenteral transmission can occur at an increased rate and leads to subclinical infection. Currently Hepatitis E virus screening is not mandatory in many countries. This can possibly endanger the population.

Materials and Methods: We aimed at estimating the seroprevalence of HEV antibodies (IgG) among healthy blood donors in Chennai, South India. A prospective study was conducted among 142 blood donors from January 2016 to November 2016 in a tertiary care teaching hospital. Serum anti HEV IgG antibody was detected by Enzyme Linked Immuno Sorbent Assay (ELISA).

Results: Out of 142 samples, 19/142 (13.38%) showed positivity for IgG antibodies for HEV.

Conclusion: This study showed the importance of screening for HEV, as there is increased rate of positivity among the blood donors in our study population.

Keywords: *Hepatitis E, Parenteral Transmission, Blood Transfusion, Maternal Mortality, HEV IgG*

Introduction

Hepatitis E is an enterically transmitted infectious virus, which causes acute self-limiting hepatitis.[1] Initially it was considered as only water borne outbreak infection, now it has been proven that there are other modes of transmissions like zoonotic transmission, person-to-person transmission and through blood transfusion.[1] Transfusion transmitted infections (TTI) are one among the major risks during blood transfusion.[2] Among them, most commonly screened infections during blood and blood products transfusion in health care centers are as follows: Human immuno deficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), syphilis and malaria. Other least common infections screened are Toxoplasmosis, Leishmaniasis and Hepatitis E virus (HEV).[3] Developed countries have a constant vigilance in the estimation of prevalence of transfusion related HEV infections. It was estimated that 80,000 to 1,00,000 HEV infections had occurred due to transfusion in England in 2013, with seroprevalence rate of 6.8% and annual incidence rate of 0.35%.[4] Studies in developing countries like India are very limited and only little evidence is available till now. In a study conducted among blood donors in Pune, 1.5% of HEV RNA was estimated during the year 2000 and 3.7% during the year 2004.[5,6] Blood transfusions are carried out in critically ill cases that include solid organ transplantation, pregnancy, hematological disorders and neoplasm. Hepatitis E, even though most commonly causes self limiting diseases, in the above said critically ill and immunocompromised cases, it leads to more severe presentations that may end up in chronic hepatitis and cirrhosis. Though acute morbidity due to transmission of HEV infection through blood transfusion is rare and supporting evidences are still lacking, studies need to be explored in all developing countries to know the real burden of transfusion transmitted HEV infections.[7] Hence we aimed at estimating the seroprevalence of HEV IgG antibodies among healthy blood donors in our tertiary hospital, Chennai, South India.

Materials and Methods

A prospective study was conducted in the Department of transfusion medicine in a tertiary care teaching hospital in Chennai, South India. Institutional ethical committee approval was obtained and the serum samples were anonymized. By simple random sampling, 142 samples of the healthy donors were chosen as study population during the study period of January to November 2016. Serum anti HEV IgG antibody was detected by Enzyme Linked ImmunoSorbent Assay (ELISA). Third generation enzyme immune assay for the detection of IgG antibodies to Hepatitis E virus kit (Diagnostic Bioprobes Srl Via G. Carducci nº 27 20099 Sesto San Giovanni - Italy) was used and procedure was done as per manufacturer instructions.

The sensitivity and specificity of the kit is 100%. The positive cases were retested in duplicate. The results were tabulated in MS office Excel 2016 and analyzed using SPSS software.

Results

The study population that included 142 samples showed predominant gender distribution of 141 males and 1 female donor. The range of age is from 18 years to 51 years with a mean age of 25 years. Age distribution was analyzed by stratification of age group into 18-20 years (33.09%), 21-30 years (49.29%), 31-40 years (13.38%), 41-50 years (3.52%) and 51-60 years (0.7%).

Out of 142 samples, 19/142 (13.38%) showed positivity for anti-IgG antibodies for HEV. The distribution of HEV positive cases based on age stratification is depicted in Figure 1.

Table 1: Comparative evidence of seroprevalence of Hepatitis E among blood donors in different countries.

Study Place	Study Year	Methodology	Detection	Sero-prevalence	Ref
Spain	1998	ELISA	HEV IgG	2.8	(13)
Pune,India	2000	PCR	HEV RNA	1.5	(5)
United States	2002	ELISA	HEV IgG	18.3	(14)
Saudi Arabia	2004	ELISA	HEV IgM	8.96	(6)25 of whom were transfused with 107 blood units, while the other 25 did not receive any transfusions.\nRESULTS: In our retrospective study, markers of acute HEV infection (IgM anti-HEV and HEV RNA
Pune,India	2007	PCR	HEV RNA	0	(18)
South west France	2007	ELISA	HEV IgG	16.6	(17)19.1% of rural donors and 14.2% of urban donors had anti-HEV antibodies (P = 0.13
Japan	2007	ELISA	HEV IgG	7.1	(19)serum samples were collected from 6700 voluntary blood donors with an elevated alanine aminotransferase (ALT
Gujarat, India	2012	ELISA	HEV IgM	4.78	(12)
Southeast England	2012	PCR	HEV RNA	0.04	(7)but is probably widespread, and the virus has been detected in pooled plasma products. HEV-infected donors have been retrospectively identified through investigation of reported cases of possible transfusion-transmitted hepatitis E. The frequency of HEV transmission by transfusion and its outcome remains unknown. We report the prevalence of HEV RNA in blood donations, the transmission of the virus through a range of blood components, and describe the resulting morbidity in the recipients.\nMETHODS: From Oct 8, 2012, to Sept 30, 2013, 225,000 blood donations that were collected in southeast England were screened retrospectively for HEV RNA. Donations containing HEV were characterised by use of serology and genomic phylogeny. Recipients, who received any blood components from these donations, were identified and the outcome of exposure was ascertained.\nFINDINGS: 79 donors were viraemic with genotype 3 HEV, giving an RNA prevalence of one in 2848. Most viraemic donors were seronegative at the time of donation. The 79 donations had been used to prepare 129 blood components, 62 of which had been transfused before identification of the infected donation. Follow-up of 43 recipients showed 18 (42%

Study Place	Study Year	Methodology	Detection	Sero-prevalence	Ref
Germany	2013	ELISA	HEV IgG	6.8	(4)incidence, and viremia in blood donors for the assessment of risk of transfusion-transmitted (TT
Central Italy	2014	ELISA	HEV IgG	49	(20)
			HEV IgM	0.63	
			HEV RNA	0.63	
Punjab, India	Not given	ELISA	HEV IgG	10.7	(21)
Chennai, India	2016	ELISA	HEV IgG	13.38	Current study

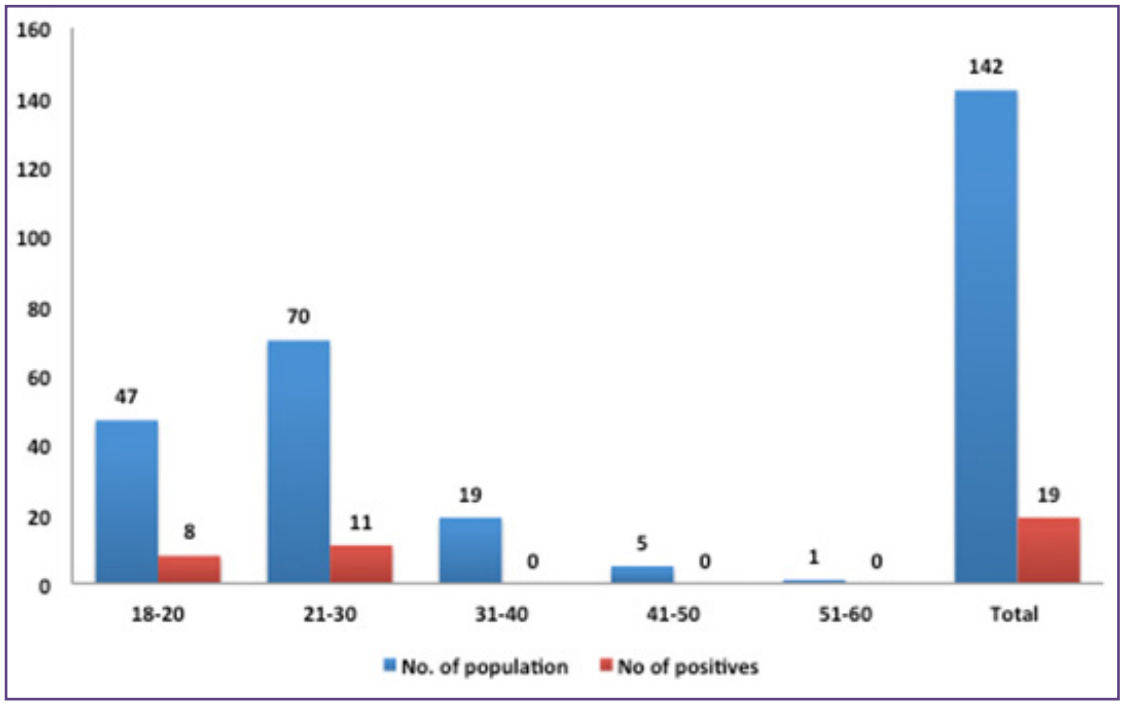

Fig. 1: Age wise distribution of study population and HEV seropositivity.

Discussion

In our study, among a total of 142 voluntary blood donors screened for HEV antibodies, 13.38% showed seropositivity. Gender preponderance for HEV infection cannot be concluded from our study, as there is male dominance in study population. However certain studies conducted at Japan and Iran have shown a male preponderance. In contrary, studies from France and Brazil have shown no difference among gender distribution in HEV seroprevalence.[8,9,10] Age stratification has shown that majority of the positives are amongst younger age group 8/47 (17.02%) among 18-20 years followed by 11/70 (15.71%) among 21-30 years. None of the population above the age group of 31 years showed positivity. This may be contrary to the study conducted in western India, which showed increased positivity among older age groups supported by studies in USA and Denmark.[11,12,13,14] This may be due to decreased number of study population in age group more than 31 years.

Seroprevalence rate in our study is comparable with the higher prevalence as seen in southwest France and England.[15,16,17] Few Indian studies have shown lesser seroprevalence rates of 1.5% and 3.7%, but the methodology used was polymerase chain reaction, which detected HEV RNA.[5,6] Comparison of prevalence rates among different studies are represented in Table 1.

The reasons behind these variations may be due to different sample size, different age groups and due to different methodology used that included serological methods and molecular methods.

Seroprevalence rates are detected based on the presence of anti-HEV IgG in blood. Though the duration of persistence of anti-HEV IgG in blood after exposure is not certain, it mainly depend upon immune status of individual and other factors. Antibody levels are in peak during acute phase of illness and it may persist up to a maximum of 12 years or even more.[22] Clinical risk of transmission of infection can be evidenced only by detection of viremia either in asymptomatic form or in subclinical presentation. Studies estimating seroprevalence helps in estimating the burden of HEV. However only HEV RNA detection aids in identifying viremic cases and transmission related risk could be estimated with increased specificity.[23] Since molecular methods are limitedly used only for epidemiological studies due to cost factor; serological methods using more sensitive and specific kits (anti HEV IgM and anti HEV IgG) is mandatory in all health care centers.

Limitations of our study are small sample size, increased distribution of younger age groups in study population and molecular method couldn't be used due to increased cost. Future plan has been proposed to conduct studies overcoming the above limitations.

Conclusion

The above study throws light about screening for HEV in blood donors in general. Though little knowledge is available about HEV transmission risk during blood transfusion, in endemic countries it is advisory to screen the blood and blood products for HEV for effective prevention. More longitudinal studies need to be conducted in all health care centers to estimate the burden of HEV among Indian population, so that a national policy can be implemented to screen and prevent transmission of HEV during blood transfusion.

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Immunohistochemical Profile of Lung Tumors in Image Guided Biopsies

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ABSTRACT

Background: Lung cancer is the leading cause of cancer related mortality in both men and women worldwide. Establishing the histological type and grade of pulmonary carcinoma is very important especially for the therapy and prognosis. Study design: cross-sectional descriptive study. The study analyses various histomorphological patterns of lung tumors in correlation with immunohistochemical profile.

Methods: All the bronchoscopic and CT guided needle biopsy specimens (50 biopsy specimens) received in the Pathology department of Coimbatore medical college hospital over a period of one year were analysed. Both H&E and immunohistochemical sections were studied with panel of markers- CK7, CK20, TTF-1, chromogranin, synaptophysin, CD45, vimentin, smooth muscle actin.

Result: The most common histological type was squamous cell carcinoma (48%), followed by adenocarcinoma (28%) and small cell lung carcinoma (18%). Large cell neuroendocrine carcinoma and metastatic deposit constituted 2% each. Out of 50 cases, 24 cases were squamous cell carcinoma which showed positivity with HMWCK (20 cases) and P63 (22 cases) ($p<0.001$). Fourteen cases reported as adenocarcinoma showed positivity with CK7 (14 cases) and TTF-1 (13 cases) ($p<0.001$). All the nine cases of small cell carcinomas showed positivity with both TTF-1 and Ki 67. One case of large cell carcinoma with neuroendocrine features showed immunopositivity with neuroendocrine markers.

Conclusion: Integration of conventional histomorphological diagnosis with panel of immunohistochemical markers allows more accurate identification of histological type, which has significant treatment implications.

Keywords: *Lung Tumor, Non-small Cell Lung Carcinoma, Small Cell Lung Carcinoma, Cytokeratin, TTF-1, p63.*

Introduction

Lung cancer is one of the most deadly cancers with increased morbidity and mortality in the world leading to 1.58 million deaths in 2016.[1] Clinically, lung carcinomas are classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), as the therapy and prognosis varies. The most common histological pattern is squamous cell carcinoma, but recently the incidence of adenocarcinoma has increased significantly and now it is the leading malignancy in both sexes.[2]

Despite new diagnostic techniques, the overall 5-year survival rate is only 14% in males and 9% in males.[3] Oncogenes c-myc, k-ras, EGFR, c-kit are involved in lung cancers.[4] Presence of EGFR mutation may predict response to therapy.[5]

Histomorphological assessment on hematoxylin–eosin (H&E) stained sections remains the most important diagnostic tool for classifying lung carcinomas.

Immunohistochemistry (IHC) can be used for the diagnosis and classification of lung tumors as NSCLC or SCLC. IHC can also be used to interpret neuroendocrine differentiation of a neoplasm. Panel of immunohistochemical markers have been developed and used both as diagnostic and prognostic markers.

The purpose of this study was to analyze the histomorphological patterns of lung tumors and to study the immunohistochemical profile using panel of markers- Cytokeratin (CK)7, CK20, thyroid transcription factor (TTF-1), high molecular weight cytokeratin (HMWCK), P63, CD45/ leukocyte common antigen (LCA), chromogranin, synaptophysin, vimentin and smooth muscle actin.

Materials and Methods

This was a cross-sectional study conducted in the Department of Pathology, Coimbatore medical college Hospital, spanning over a period of 1 year. A total of 50 cases of lung biopsy specimens including bronchoscopic and needle biopsies received were studied. Treated patients and inadequate biopsies were excluded from the study. Detailed history regarding age, sex, clinical findings,

history of primary tumor elsewhere in the body and radiological investigations were reviewed in all the cases.

Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

Histomorphological patterns and immunohistochemical profiles of the lung tumors were analysed. Because of the availability of very limited tissue in the lung biopsy specimens, panel of markers limited to the particular histological type diagnosed in H&E were used. Panel included markers of squamous differentiation- p63, HMWCK, cytokeratin specific for primary pulmonary origin- CK7, TTF-1, cytokeratin for primary gastrointestinal tract origin- CK20, markers of neuroendocrine differentiation- chromogranin, synaptophysin, proliferation antigen- Ki67, LCA and vimentin. Tumour cells were scored positive based on the pattern and intensity of staining in the neoplastic cells.

Statistical data analysis of various histomorphological patterns and percentage positivity of various immunohistochemical markers were studied and compared with those in the literature.

Result

In our study, it was observed that the peak incidence of lung malignancies occured in the age group of 51-60 years (46%) with a male preponderance (84%). 43 out of 50 cases could be diagnosed and subtyped precisely using routine Hematoxylin & Eosin stained sections and confirmed by IHC. Remaining seven cases were diagnosed as NSCLC and could not be subtyped as SCC or adenocarcinoma requiring the aid of IHC. With the IHC findings, four cases were concluded as SCC and two cases as adenoarcinomas. Only one case showed inconclusive result with IHC and reported as NSCLC. (Table 1)

In our study, we observed 24 cases were squamous cell carcinoma (48%), 14 cases of adenocarcinoma (28%) and 9 cases of small cell lung carcinoma (18%). Large cell neuroendocrine carcinoma and metastatic deposit constituted 2% each. One case was reported as NSCLC alone. (2%). It was observed that among the 20 cases reported as squamous cell carcinomas and 4 cases as NSCLCs histologically, HMWCK was expressed in 20 cases with a sensitivity of 83.3%. P63 was expressed in 22 out of 24 cases with a sensitivity of 91.7%. (Table 2) P63 was not expressed in any of the adenocarcinoma cases with 100% specificity (Table 3). It was observed that both HMWCK and p63 were equally good immunohistochemical markers for the diagnosis of squamous cell carcinomas.

All the 12 cases reported as adenocarcinomas in H&E stained sections and 2 cases as NSCLCs, showed immunopositivity with CK7, with a sensitivity of 100%. 13 out of 14 cases of adenocarcinomas showed nuclear immunoreactivity with TTF-1. TTF-1 was not expressed in any of the squamous cell carcinomas; thus the sensitivity of TTF-1 in this study was found to be 92.8% and specificity 100%. (Table 3). One case reported as NSCLC alone histologically, gave inconclusive results with IHC markers of both squamous and glandular differentiation.

In the present study, 9 cases reported as small cell lung carcinomas histomorphologically showed positivity with both TTF-1 and Ki 67 immunohistochemically and negative staining with LCA. (Table 4).

Table 1: Interpretation of Results with H&E Sections and Immunohistochemistry.

Total cases (n=50)	RESULTS CONCLUSIVE	RESULTS INCONCLUSIVE
H&E SECTIONS	43	7
IHC	49	1

(H&E- haematoxylin and eosin, IHC- immunohistochemistry)

Table 2: Expression of Ihc Markers in Scc (N=24).

S. NO	IHC MARKERS	POSITIVITY	POSITIVE PERCENTAGE (%)	NEGATIVITY
1.	HMWK	20	83.3%	4
2.	P63	22	91.7%	2
3.	TTF 1	0	0%	24

(HMWK-high molecular weight cytokeratin, TTF-1- thyroid transcription factor)

Table 3: Expression of Ck-7, Ck-20, TTF-1 and P63 in Lung Adenocarcinoma (N=14).

S.NO	IHC MARKERS	POSITIVITY	POSITIVEPERCENTAGE(%)	NEGATIVITY
1.	CK-7	14	100%	0
2.	CK-20	0	0%	15
3.	TTF-1	13	92.8%	1
4.	P63	0	0%	15

(CK-Cytokeratin, TTF-1- thyroid transcription factor)

Table 4: Expression of TTF-1, KI 67 and LCA in Sclc (N=9).

S.NO.	IHC MARKERS	POSITIVITY	POSITIVE PERCENTAGE %
1.	Ki 67	9	100%
2.	TTF-1	9	100%
3.	LCA	0	0%

(SCLC- small cell lung carcinoma, TTF-1- thyroid transcription factor, LCA- leukocyte common antigen)

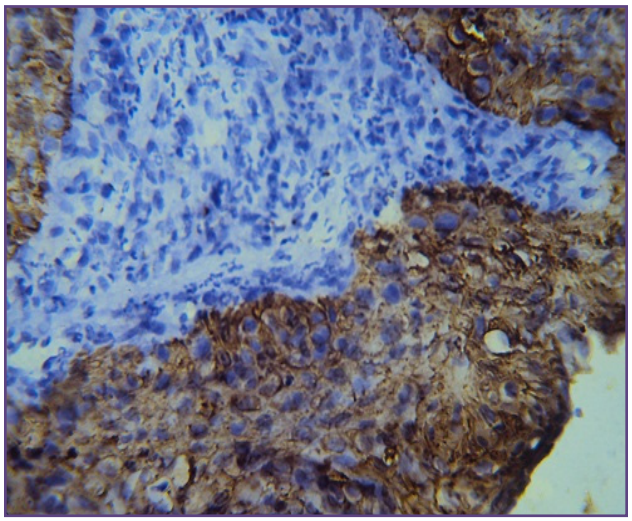

Fig. 1: SCC showing cytoplasmic positivity with HMWCK.

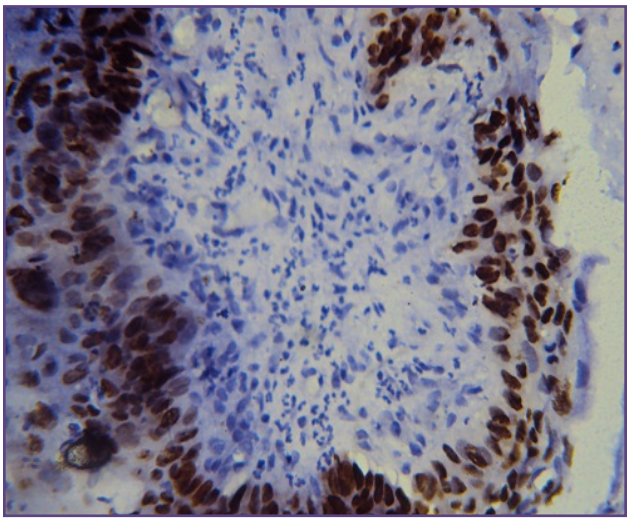

Fig. 2: SCC showing strong nuclear immunostaining with P63.

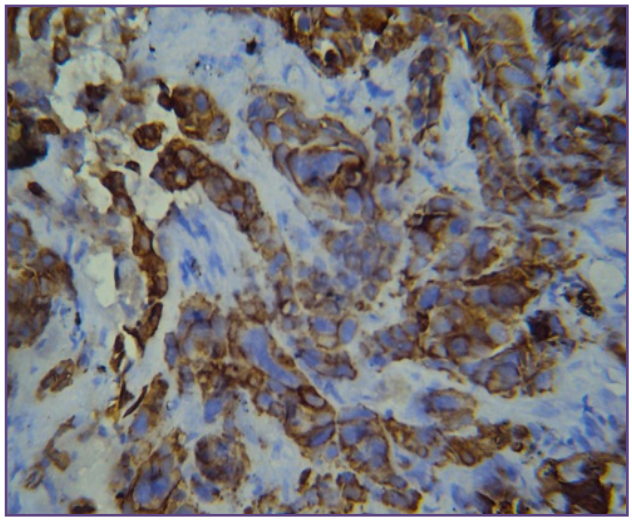

Fig. 3: Adenocarcinoma- CK7 cytoplasmic positivity.

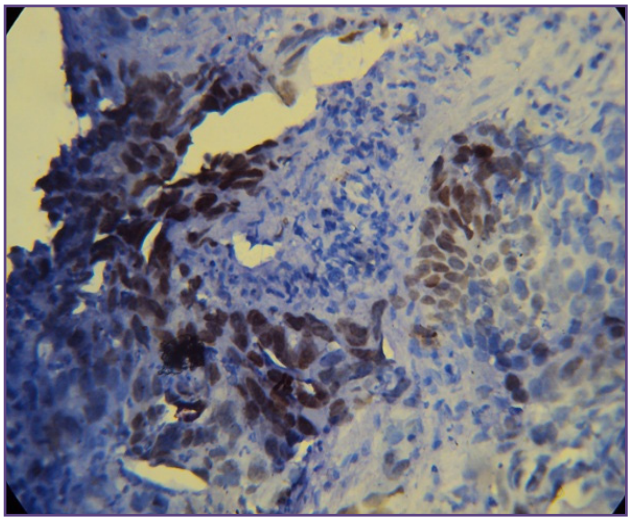

Fig. 4: Immunostaining with TTF-1 showing nuclear positivity.

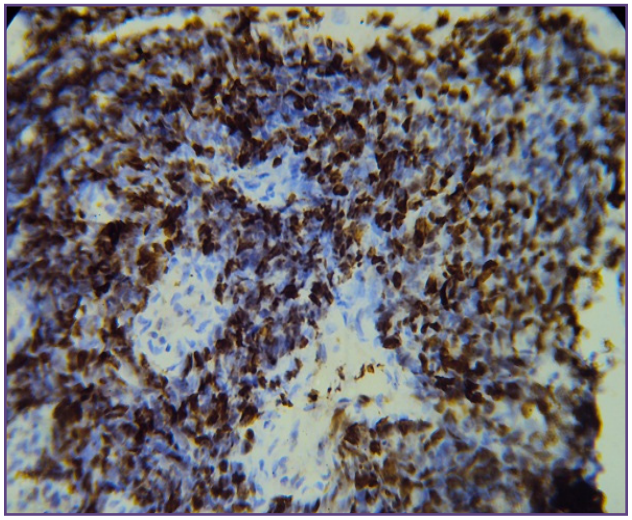

Fig. 5: Tumor cells of SCLC show strong nuclear immunostaining with Ki67.

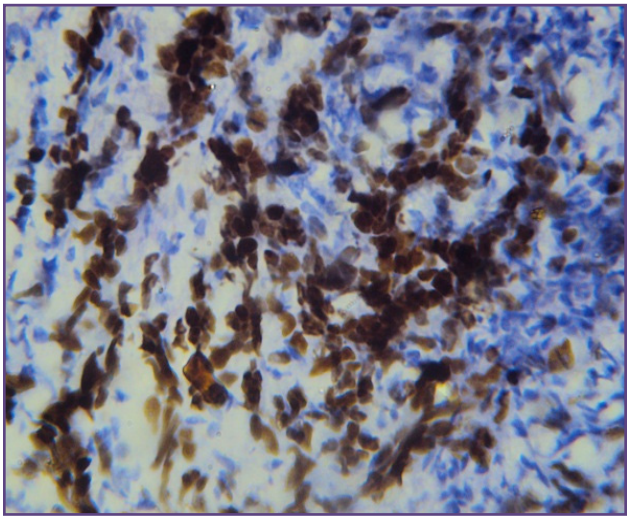

Fig. 6: SCLC showing nuclear immunoreactivity with TTF-1.

Discussion

Lung cancer is the leading cause of cancer related morbidity and mortality. The primary intent of histopathological study is to classify lung tumors as primary pulmonary tumors or metastatic lesions. Primary lung carcinomas are classified as small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). NSCLC accounts for 75-80% of all the lung carcinomas and further subtyped as squamous cell carcinoma and adenocarcinoma.[6]

The objective of this study is to analyze the histomorphological patterns and immunohistochemical profile of lung tumors in bronchoscopic and needle biopsy specimens. Panel of immunohistochemical markers were used to confirm the histopathological diagnosis and correctly classify the lung tumors. It is a global observation that lung cancer has a higher incidence in males than in females with a male to female ratio of 2.7:1. [7] These tumors commonly affect individuals in the 6th to 7th decades of life. In our study also, males are more commonly affected (84%) and the peak incidence occurred in the age group of 51-60 years.

In our study, NSCLC accounted for 78%; Squamous cell carcinoma was the most common histological pattern (48%), followed by adenocarcinoma (28%). Small cell lung carcinoma constituted 18%, large cell neuroendocrine carcinoma and metastatic deposit of sarcoma constituted 2% each. In a study by S. Sheikh, A. Shah et al., (2010), they observed squamous cell carcinoma (71.3%) as the most common histological pattern, followed by small cell carcinoma (20.8%), adenocarcinoma (2.6%), bronchoalveolar carcinoma (1.8%) while other tumors constituted 3.6%.[7] Because of its relative ease of use and specificity, immunohistochemistry has largely replaced mucin histochemistry and electron microscopy in diagnosing pulmonary neoplasms. Many studies have proposed panel of markers for lung tumors.

In our study, panel of markers were restricted according to the histological type of tumor. For NSCLC, panel of markers included- P63, HMWCK for squamous cell differentiation and CK7, CK20 and TTF-1 for glandular differentiation. In case of SCLC, panel included CK, TTF-1, Ki67, neuroendocrine markers and LCA (CD 45), to confirm the diagnosis and to differentiate it from lymphoma.

HMWCK is usually expressed in SCC and does not show reactivity in adenocarcinomas and SCLCs.[8] P63 is also expressed only in SCCs. Both HMWK and P63 are useful to differentiate SCC from adenocarcinoma and poorly differentiated SCC from SCLC and large cell neuroendocrine carcinoma.[9] In our study, out of 20 cases reported as SCC and 4 cases as NSCLC in H&E, HMWCK was expressed in 20 cases (sensitivity 83.3%) (fig.1) and P63 showed immunopositivity in 22 cases (sensitivity 91.7%) (fig.2). P63 was not expressed in any of the adenocarcinoma cases with 100% specificity. (p value<0.001) (Table-2).

This observation was similar to the study by N.Kalhor, D.S.Zander et al., 2006. According to their study, P63 expression was found in all 13 cases of squamous cell carcinomas.[10] In a study by R.Ocque, N.Tochigi et al., 2011, P63 expression was found in all the 30 cases of SCCs with 100% sensitivity.[9]

Recent studies have proposed a panel of markers for pulmonary adenocarcinomas- CK-7, CK-20, TTF-1.

Primary lung adenocarcinoma shows strong and diffuse positivity with CK-7. CK-20 is expressed in metastatic deposits from colonic carcinomas. CK-7 is used in combination with CK-20, in differentiating primary pulmonary carcinoma from metastatic colonic carcinoma. [11] 75% of primary pulmonary adenocarcinomas express TTF-1.[12,13] Napsin A is also a sensitive marker for adenocarcinoma that has a stronger intensity than TTF-1.[14]

Y.Su, Y.Hsu et al., used a panel of markers CK-7, CK-20, TTF-1 to differentiate primary from metastatic lung adenocarcinomas. They observed that 73% of primary pulmonary adenocarcinomas expressed TTF-1, whereas all the metastatic adenocarcinomas lacked TTF-1 staining. They concluded in their study that TTF-1 has high sensitivity and specificity for primary pulmonary adenocarcinomas. CK-7 expression was present in 75% of pulmonary adenocarcinomas and none of the cases expressed CK-20. In their study they found combination of $CK\text{-}7^{+}/CK\text{-}20^{-}$ along with TTF-1 immunoreactivity was highly specific for primary pulmonary adenocarcinoma.[15]

In a study by R.Ocque, N.Tochigi et al., 2011, CK-7 expression was found in all the cases of adenocarcinomas (100%) and 86.2% of cases expressed TTF-1. They also observed in their study the immunoreactivity of TTF-1 in 9 out of 43 cases of SCCs with a sensitivity of 86% and specificity of 73%.[9]

In our study, all the 12 cases reported as adenocarcinomas and 2 cases reported as NSCLC, showed positivity with CK7 immunostaining with a sensitivity of 100% and negative immunoreactivity with CK-20. (fig.3) TTF-1 immunoreactivity was positive in 13 out of 14 cases of adenocarcinomas. (fig4) TTF-1 was not expressed in any of the squamous cell carcinoma cases with a sensitivity of 92.8% and specificity 100%. (p value <0.001) (Table-3). The results were consistent with the prior studies.

In our study, 9 cases were reported as SCLCs in H&E stained sections with crush artefacts. Crush artifact can also occur in carcinoids, lymphomas, poorly differentiated non-small cell carcinomas and lymphocytes of inflammation. [16] Mitotic activity is impossible to assess in small biopsy specimens with crush artifacts. In such cases, Ki67 labelling can be more reliable.

In a study by D. L. Aslan, H. E. Gulbahce et al., 2005 they found all the 34 cases of SCLC showed immunoreactivity with Ki-67 even in the crushed areas, with diffuse staining in more than 80% of all tumor cells.[17] Small cell carcinoma is positive for TTF-1 in 90% of cases.[18]

In our study, all the 9 cases of SCLC showed positivity with both TTF-1 (100%) and Ki67 (100%) immunohistochemically. (fig 5&6) Immunostaining with Ki67 showed reactivity in more than 80% of the tumor cells. (fig5) Immunostaining with LCA (CD 45) showed negative results and hence lymphoma was ruled out.

In our study, one case of large cell carcinoma with neuroendocrine features was reported in H&E. Immunohistochemistry showed strong cytoplasmic positivity with neuroendocrine markers- chromogranin and synaptophysin. HMWCK and p63 expression, largely restricted to NSCLCs showed negative results.[19] CK-7 showed focal cytoplasmic positivity. Thus the possibility of poorly differentiated NSCLC was excluded. With the combined H&E and immunohistochemical profile, it was concluded as 'Large cell neuroendocrine carcinoma.'[20]

Large cell carcinoma with neuroendocrine architecture but without immunoreactivity for neuroendocrine markers should be concluded as 'Large cell carcinoma with neuroendocrine architecture'.[20]

With the clinical history, radiological findings, H&E and IHC reports with vimentin positivity, one case was concluded as metastatic high grade spindle cell sarcomatous deposit.

We observed in our study that 43 out of 50 cases could be diagnosed and subtyped precisely in H&E stained sections histomorphologically and confirmed by IHC. Remaining seven cases were diagnosed as NSCLC alone and proceeded with IHC. With the IHC findings, four cases were concluded as SCC and two cases as adenoarcinomas. Only one case showed inconclusive result with IHC and reported as NSCLC. (Table 1) This negative result may be due to reaction bias like specimen fixation, tissue processing and antigen retrival.

Integration of conventional histomorphological diagnosis with immunohistochemistry increases the refinement of diagnosis , so that a diagnosis of NSCLC can be avoided. Subclassification of NSCLC has significant treatment implications, especially for advanced stage tumors for which chemotherapy is being considered. [18]

Conclusion

From this study it is concluded that immunohistochemistry should be done in all the small lung biopsy specimens to confirm the histomorphological diagnosis as well as in cases where histological subtyping is difficult with H&E sections. Also, panel of markers can be restricted to the histological type because of the limited availability of tissues in bronchoscopic and CT-guided biopsy specimens.

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Case Report

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Melorheostosis: A Case Report in Pediatric Age Group

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ABSTRACT

Melorheostosis is a rare, benign, and disabling condition. It is a rare mesenchymal bone disease classified as a sclerotic bone dysplasia. We present this case because of its rarity and highlight the importance of Fine needle aspiration cytology which is a minimally invasive technique in ruling out the possibilities of osteomyelitis and malignancies. Fine Needle Aspiration Cytology (FNAC) negates the use of unwarranted biopsies. The disease is a benign entity with a generally chronic course and periods of exacerbation and remission. Therapy is symptomatic and is aimed at controlling pain.

Keywords: *Sclerotic Bone Dysplasia, Cytology, Biopsy, FNAC*

Introduction

Melorheostosis is a rare disorder characterized by mesodermal dysplasia of bone first described by Leri and Joanny in 1922.[1] It occurs sporadically with an incidence of 0.9 in 1 million with equal sex distribution. It usually occurs in the limbs and frequently crosses synovial joints. The etiology is unknown.[2] We present the case of a 14-year-old female diagnosed with localized melorheostosis in the middle third of tibia. This case is presented to emphasize upon the awareness of the lesion to avoid unnecessary biopsy done to diagnose this case. It can be simply diagnosed on the basis of clinical and radiological features with a correlation with various laboratory investigations. Fine needle aspiration cytology proves to be an effective tool in ruling out the various infective and malignant differential diagnosis.

Case Report

A 14-year-old female presented with continuous pain in the middle third of tibia. No skin atrophy or erythma of the affected limb was detected. However, there was a diffuse bony prominence below the tibeal tuberosity. There was no restriction of movement of the limb or any soft tissue contracture. There was no other bony involvement. She was advised a simple X-ray, Fine needle aspiration cytology (FNAC) of the lesion and various blood tests. Ultrasound guided FNAC was performed which yielded bloody aspirate. Smears examined showed blood and blood elements. No malignant cell was identified. There was no evidence of inflammation in the form of sheets of polymorphs or lymphocytes or necrosis. No fungus or any other organism was identified. The cytological aspirate was also sent for microbiological culture which was negative for aerobes, anaerobes, typical and atypical mycobacteria and fungi. Based on the above findings possibility of osteogenic sarcoma, osteomyelitis and any fungal or bacterial infection was ruled out. X-Ray of the left leg presented with hyperostosis and thickening of the cortex of the middle third of tibia extending along the bone and showing a dripping candlewax image (Fig 1). Various laboratory investigations included complete blood count, calcium, phosphate, bony alkaline phosphatase, thyroid hormone profile and proteins. All these levels were within normal limits. She had normocytic normochromic anemia with a moderately elevated C-reactive protein of 60mg/L (normal <10mg/L). Based on the radiological and laboratory investigations she was diagnosed as a case of melorheostosis. She was started on treatment with analgesics and NSAIDS to which she responded very well.

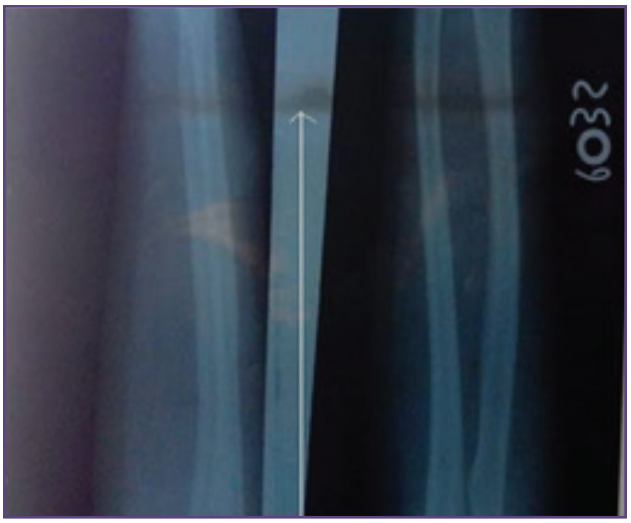

Fig. 1: Radiograph showing the tibia of 14 year old girl who had melorheostosis

Discussion

Melorheostosis is derived from the Greek melos, meaning limb and rheos means flow. It is mixed sclerosing dysplasia with disturbance of both enchondral and intramembranous ossification, in which disordered intramembranous ossification dominates.[3]There is wide range of age distribution from 2-64 years with equal sex predilection. The patients can present with various symptoms including pain, limb swelling, restricted range of motion of extremity due to soft tissue contracture. Other symptoms include stiffness, swelling, numbness, tingling and carpal tunnel syndrome. Lower extremities are more frequently involved. Other sites involved are skull, facial bones, ribs, scapula, pelvic bone and spine. Involvement can be monostotic, polyostotic or monomelic. Other changes include pigmentation, erythma, odema, periarticular fibrosis, weakness and atrophy of muscles, perivascular fibrosis with obliteration of blood vessels. Vascular lesions associated with melorheostosis include hemangiomas, vascular nevi, varices, glomus tumors, arteriovenous malformations and aneurysms. It has also been associated with tumors, including osteogenic sarcoma, malignant fibrous histiocytoma and dermoid tumors. Other associations are neurofibromatosis, tuberous sclerosis, scleroderma, rheumatoid arthritis and hypophosphatemic rickets.[4]

The etiology of this disorder is unknown. It is hypothesized that it may be due to vascular insufficiency or failure in intra-membranous or enchondral ossification. Freyshimidt[5] suggested that it is a form of mosaicism rather than early embryonic infection of the sensory nerve, in which the disorder could be due to the action of a lethal gene that survives only in mosaic state. Other theories propose a vascular disorder, inflammation, a degenerative lesion of the connective tissue, and embryonic damage as etiopathogenic factors. Recent studies have reported loss of function mutations in the LEMD3 gene, encoding an inner nuclear membrane protein that influences Smadsignalling, as a cause of osteopoikilosis, Buschke-Ollendroff syndrome and melorrheostosis.[6]

The classic radiographic features of melorheostosis are regular and wavy hyperostosis referred to as flowing candle wax appearance. Radiologically, five patterns have been described- classical, osteoma-like, myositis ossificans-like, osteopathiastriata-like and mixed type. There is usually a distinct demarcation between the affected bone and normal bone. Most typically the outer bony cortex is affected, but extension into the cancellous bone is also seen. In children, the hyperostosis is endosteal, marked by streakiness of the long bones and spotting of the small bones, whereas in adults it is in an extracortical, subperiosteal location. Four types have been described depending on the clinical situation: monostotic, polyostotic, monomelic and with generalized skeletal involvement. Diaphysis of long bones is more commonly involved. Other sites include the pelvis and bones of hands and feet. The ribs and the craniofacial complex are affected least often. CT and MRI are more useful for assessment of lesions involving the axial skeleton.[5,7]

Laboratory abnormalities reported in association with bone and soft tissue lesions of melorheostosis affect osteoblastic specific factor-2 (osf-2), osteonectin, fibronectin, transforming growth factor-β, and fibroblastic growth factor-23 (FGF-23). Serum calcium, phosphorus, and alkaline phosphatase levels have been reported to be within normal limits in melorheostosis.[8]

Histologic findings include variable degree of cortical thickening consisting of chondroid islands surrounded by woven or non-lamellar dense bone depending on stage of maturation with thickened, sclerotic, and irregular lamellae. An adjacent zone of fibrocartilage may show irregular surface fibrillation. A large quantity of osteoid without mineralization suggests over-production of bone matrix in affected bone. Increased osteoclasts reflect increased bone resorption. This indicates that increased bone formation and bone resorption are combined processes in melorheostosis. Rarely, presence of a cartilage cap over a portion of the sclerotic bone in intra-articular melorheostosis, could lead to misinterpretation of osteochondroma. Cells immunopositive for TGF β and bFGF are densely present in melorheostotic bone. Both of these cytokines are osteogenic and angiogenic, probably these cytokines play a role in increased bone formation and increased angiogenesis. Increased expression of procollagen alpha1 mRNA expression and alpha1 (l) , alpha2 (l),and alpha (lll) collagen secretion has been observed in dermal fibroblasts obtained from a skin biopsy overlying the involved bone. Overall histology is nonspecific, but is used to exclude malignancy. Moreover FNAC can also serve this purpose so minimally invasive techniques should be applied to rule out other causes.[9]

Various differential diagnosis include myositis ossificans, osteopetrosis, osteopoikilosis, chronic osteomyelitis, parosteal and periosteal osteosarcoma, calcium pyrophosphate dihydrate (CPPD), deposition disease, osteoma, and Caffey's disease. Rare differentials include calcified synovial sarcoma, extraskeletal osteosarcoma, or tumoralcalcinosis.[3,4,7]

The treatment is limited and includes surgical interventions in the form of tendon lengthening, excision of soft tissue masses, release of joint contractures and occasionally amputation. Primary aim of treatment is pain relief and

restoration of full range of motion. Conservative measures include analgesia, manipulation, braces, sympathectomy, physiotherapy, nerve blocks and serial casting. In some cases, good results are obtained with infusions of zoledronic acid.[10] The prognosis is variable and depends on the anatomical location, extension into the soft tissues, and soft tissue changes. Melorheostosis does not shorten life span, however morbidity may be considerable. The disease exhibits a slow, chronic course with periods of exacerbation and arrest. Recurrence usually is expected after operative excision.[4]

Conclusion

To conclude melorheostosis is benign in nature with chronic pain in which deformity can be debilitating. FNAC is an effective measure to rule out various differential diagnoses of malignant and infective lesions. Biopsy should be avoided. Findings should be correlated with characteristic radiological features. Surgical intervention is advocated in chronic debilitating symptoms. Early diagnosis and effective treatment can translate these lesions into near complete resolution of the symptoms.

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Case Report

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Leiomyosarcoma of Spermatic Cord: Mimicking Inguinal Hernia

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ABSTRACT

Leiomyosarcomas of the spermatic cord are very rare malignant neoplasms. We report one case, a 64-year-old man presented with the painless, swelling, ball shaped mass in the right inguinal canal. Histopathological evaluation including immunohistochemistrystudiesconfirmed a conventional leiomyosarcoma. Currently there has been no evidence local recurrence or distant metastasis for three months of follow-up. Leiomyosarcoma is a extremely malignant tumor; survival is poor. The long-termprognosis of leiomyosarcoma depends principally on stage and histologic grade. There appears to be a lack of concurrence aboutdiagnostic criteria for malignancy opposite the leiomyosarcoma spectrum, but more studies are needed. This article reviews in the literature and the criteria of these tumors is also discussed.

Keywords: *Hernia, Inguinal, Leiomyosarcoma, Spermatic Cord*

Introduction

Leiomyosarcomas prevalence ranging from approximately 5-10% of all soft tissue sarcomas [1]. Paratesticular leiomyosarcomas which is usually located on the spermatic cord or epididymis [2]. Leiomyosarcoma of the spermatic cord is very rare and nowadays, 110 cases have been reported in the literature [3]. A small number of cases of leiomyosarcoma appear to be primary spermatic cord, are more common uterin corpus and ganstointestinal system. There may be considerable problems with clinical and pathological the differential diagnosis, particularly malignant and benign smooth muscle cell tumor [4]. The major differential diagnosis clinically is between inguinal hernia and inguinal ring (hernial) lipoma. In this case report, leiomyosarcoma of the spermatic cord soft tissue in a 64 year old man is described for its infrequency.

Case Report

A 64 year-old male arrived to the state hospital with compliant of a pain, constipation and burning/hot sensation around in the right groin. Clinical examination identified irreducible/strangulated righ inguinal hernia. Abdominal ultrasonography imaging revealed the presence of a solid mass in the right spermatic cord region, which was clinically considered as a may be tumor and was resected completely. Macroscopically, the tumor measured 5x2x3 cm in diameter. Gross findings nodular, grayish, firm, well-localized and well-demarcated and smooth mass. The cut surface of the tumor revealed ill-defined solid areas that were gray-white and brown, with focally hemorrhagic appearance and central degeneration **(Figure 1).** Macroscopically, tumor perforation and ulceration were not observed and the tumor margins were negative. Microscopically, the tumor appeared to consist predominantly interlacing bundles of smooth muscle cells with elongated and pink/eosinophilic cytoplasm, centrally located nucleus and blunt-ended, cigar-shaped, frequently vesicular nuclei **(Figure 2).** Significant cytological pleomorphism was observed focally areas. Mitotic activity was showed; mitotic rate; 5-9 per 10 high-power fields (HPF)), in addition, focal necrosis were also present. Histologic Grade (French Federation of Cancer Centers Sarcoma Group [FNCLCC]): 2 (tumor differentiation is scored 2, mitosis countscored 1 and tumor necrosis scored 1). The tumor cells were positive for caldesmon, smooth muscle actin and desmin **(Figure 3)** but negative for S100, CD117 and CD34. The Ki-67 (MIB-1, nuclear positivity) expression was approximately 40% positive in tumor cells, which showed a moderately proliferation index for the tumor. It was decided that all of these histopathological and immunohistochemically studies were diagnosis of conventional leiomyosarcoma (LMS).

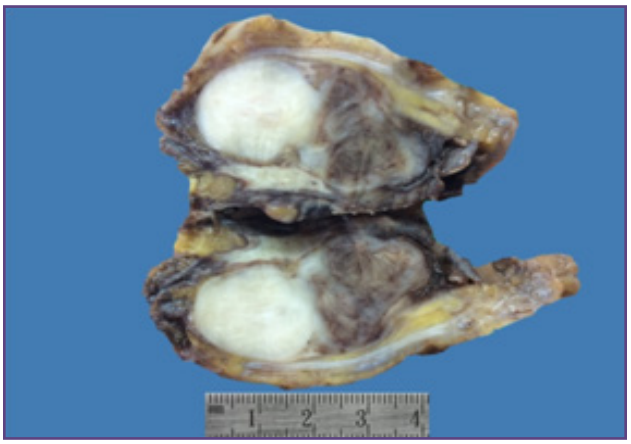

Fig. 1: Gross features: The macroscopic appearance nodular, well-localized and well-demarcated mass, ill-defined solid areas that were gray-white and brown, with focally hemorrhagic appearance and central degeneration.

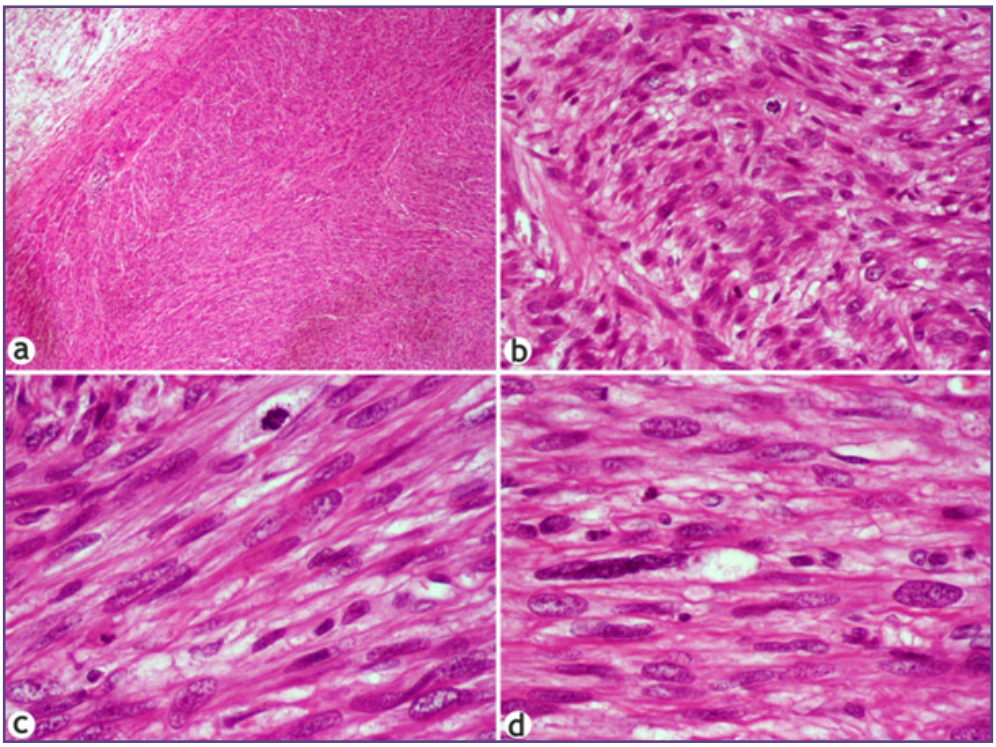

Fig. 2: Microscopically, A,B) The tumor appeared to consist predominantly interlacing bundles of smooth muscle cells with elongated and pink/eosinophilic cytoplasm, centrally located nucleus and blunt-ended, cigar-shaped, frequently vesicular nuclei. C,D) Significant cytological pleomorphismand mitotic activity was showed (H&E, 4x, 10x, 20x objective).

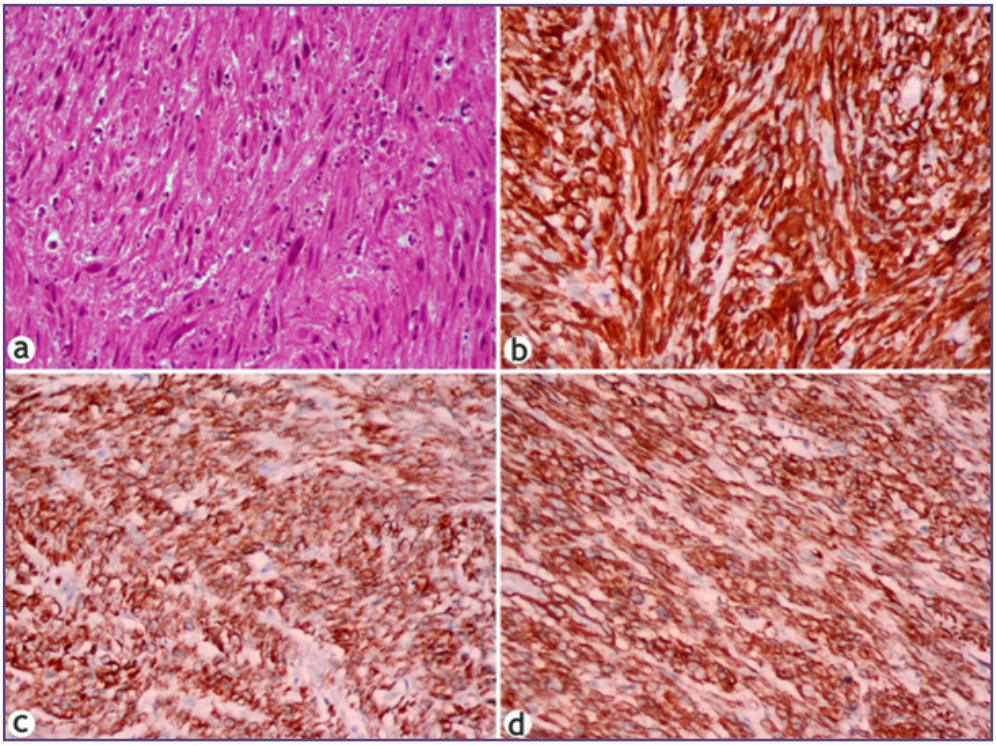

Fig. 3: A)Coagulative tumor necrosis (H&E, 10x objective). Immunohistochemically positive for; B) Caldesmon; C) Desmin; D) Smooth Mucle Actin (SMA) (20x objective).

Discussion

The paratesticular region is composed of the following anatomic structures: Rete testis, efferent ductules, epididymis, vas deferens (ductus deferens), testicular tunics and spermatic cord.Liposarcomas are the most prevalent malignant neoplasms of the paratesticular area [5]. Sarcoma of the spermatic cord first reported by Lesauvage in 1845 [6] . LMS of the spermatic cord are uncommon neoplasia of non-testicular origin and generally arises in the sixth or seventh decade. Originally considered to be malignant smooth muscle tumors or leiomyosarcomas arise from embryologic undifferentiated mesenchymal cells of the cremasteric mucle or vas deferens cells [7]. Most paratesticular LMS are 2 to 9 cm in diameter [5]. LMS is subdivided into different groups on the basis of degreeand distribution of organ or tissue involvement; the three main subcategories are LMS of deep soft tissue, cutaneous/ subcutaneous tissue and vascular origin. Concordant with currentAmerican JointCommitee on Cancer Staging System, paratesticularsarcomas must be to the ‘‘deep’’ subgroup [8].Smooth muscle tumours of the scrotum are are categorized into three major subtypes; leiomyoma, atypical leiomyoma and leiomyosarcoma [9, 10]. The majority of these classifications believe mainly on histopathological four criteria to differentiate between subtypes, and were designed to be used; tumor size ≥5 cm; infiltrating margin; ≥5 mitotic figures 10 high-power field (HPF) and moderate cytological atypia [9]. Unfortunately, the morphologic features approved as predictive variables for uterine smooth muscle neoplasms were not, until recently, applied in extra-uterine smooth muscle tumors in the pelvic, retroperitoneum [11]. Because, it is not clear whether the few reported cases of LMSs of this paratesticular region. On the other hand, at least criteria for malignancy also is smooth muscle neoplasms contains different anatomic region and sex predicletion. In the retroperitoneum in female leiomyoma may exhibit mitotic activity (5-10/50 HPF) this presentation is probably considered leiomyoma [11]. However, Newman and Fletcher reported, the exhibit of along any mitotic activity was supported as a criterion of potential for malignancyin scrotal lesions [10]. Paratesticular leiomyosarcomas with mitotic activity, variable cellularity or atypia sometimes seen necrosis [5]. More recently reported any mitotic activity in a paratesticular region of smooth muscle neoplasia is an indicator to identify a LMS [5]. Similarly, Farshid et al reported mitotic activity seems to be significant in determination a malignant indication [12]. Most cases are usually positive for muscle-specific actin, smooth muscle actin, desmin [5], H-caldesmon and LMSs may also express cytokeratin, CD34, S-100, estrogen and progesterone receptors [1]. Caldesmon seems to be well specific stain for smooth muscle cells and extremely helpful in discrimination between smooth muscle neoplasia and other mesenchymal or stromal tumors [11]. The major prognostic factor predictive of metastasis and death is the histologic grade. Svarvar et al reported none of the grade 1 tumor metastasised while grade 2 and grade 3 tumors %6.1, %25 metastasised, respectively [13]. Quite often there is distant metastasis along hematogenous than the lymphatic line [4]. The most common location of the development of metastatic lesions is the lymph nodes, lungs, or liver [14].Wecase underwent an without any complications postoperativecourse and after approximately three month of follow-up, there was no evidence of recurrent disease or metastasis.Local prophylactic radiotherapy planned postoperative. Considerations in the differential diagnosis of LMSs includes spindle cell neoplasms especially leiomyoma, and gastrointestinal stromal tumor, or other tumors; synovial sarcoma, malignant peripheral nerve sheath tumor, inflammatory myofibroblastic tumor, and pleomorphic sarcomas [1]. The standart treatment for spermatic cord LMSs radical orchidectomy and followed by adjuvant radiotherapy [15].

Conclusion

Leiomyosarcomaof spermatic cord is an unusual neoplasm of soft tissue andshould be differential diagnosis forparticularly spindled cells tumors showing a predilection for the inguinal region.However, it should be kept in mind that these diagnostic criteria for malignancy across the leiomyoma spectrum, but more studies are needed. In addition to that close follow-up is extremely recommended for local recurrence or metastasis risk.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Case Report

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Cutaneous Bronchogenic Cyst: An Unusual Cause of Lump: A Diagnostic Challenge

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ABSTRACT

Bronchogenic cysts are rare developmental anomalies, seen primarily in the pediatric population. Cutaneous manifestations of bronchogenic cysts are still rarer. This lesion poses diagnostic challenge for the clinicians and in almost all, histopathological examination gives the precise diagnosis. We report a case of presternal cystic lesion in 11 year old boy, clinically diagnosed as dermoid cyst, however the lesion was suggestive of cutaneous bronchogenic cyst on histopathology.

Keywords: *Bronchogenic Cyst, Cutaneous, Presternal*

Introduction

Bronchogenic cysts are rare benign congenital developmental anomalies of the ventralforegut.[1] They are endodermal cysts and when they are lined by the respiratory epithelium predominantly, it is termed as a bronchogenic cyst.[2]Bronchogenic cysts have a reported prevalence of 1 in 42,000 to 1 in 68,000. [3] They are usually intrapulmonary and most common extrapulmonary location is the mediastinum,particularly posterior to the carina.[2] In a large series composed of 2,163 cases of mediastinal lesions, 3.3% were foundto be bronchogenic cysts. [3]Unusual sites of presentation include skin, subcutaneous tissue,pericardium and retroperitoneum.[2,4]Under cutaneous location, most common site is the suprasternal notch, followed by presternal area, neck and scapula.[5]

Case report

An 11 year old male child presented to the outpatient department with a swelling over manubrium sterni since birth. The swelling was initially smaller in size and in the past few months has progressed to its present size. On examination, a cystic, fluctuant, nontender, 2x 2 cm mass was noted in the upper presternal region. The overlying skin showed no signs of inflammation or presence of punctum. There were no other associated respiratory complaints. The child was scheduled for an elective surgery. Surgical exploration revealed a cystic mass which wascompletely removed and was sent for histopathological examination. Grossly a single irregular, grey brown toyellow fatty tissue bit was identified which on cut section showed a tiny cyst surrounded by yellow fatty areas.Microscopy revealed a cyst lined by ciliated columnar respiratory epithelium, overlying oedematous fibrocollagenous stroma with occasional mucinous glands, scattered scant lymphocytic infiltrate, muscle fibres (Massonstrichrome positive) and congested blood vessels overlying fibroadipose stroma (Fig 1,2,3,4). A diagnosis of Cutaneous bronchogenic cyst was given.

Discussion

Cutaneous bronchogenic cysts are rare cystic lesions that occur due to congenital developmental aberrations.Ever since its first description in literature in the year 1945, not many cases of cutaneous bronchogenic cysts have been reported.[4]This is possibly due to its rarity and at times unusual presentation. The diagnosis of cutaneous bronchogenic cyst is usually a histological surprise and preoperative diagnosis of this entity is seldom made.

Majority of these lesions have been reported in pediatric population, however, few cases involving the adults have also been mentioned in literature.[3] Sanli et al. [3] reportedbronchogenic cyst in the cervical area in a 48year old woman. Shreya et al. [6] identified a case of cystic cervical mass proved to be bronchogenic cyst on histopathology in an elderly female with history of thyroiditis. Moz et al.[7] also reported bronchogenic cyst in a 39 year old male. In pediatric population, most cases present shortly after birth, however until 14 years of age cases have been reported. [8] Bronchogenic cysts are four times more common among the male population than females. [9]

Bronchogenic cysts can be intrathoracic or extrathoracic in location. Most common extra thoracic location being the mediastinum. Nearly 50% of these cysts are located in the posterior mediastinum, 14% in the superior mediastinum and around 35% are seen in the pericardial area.[1]

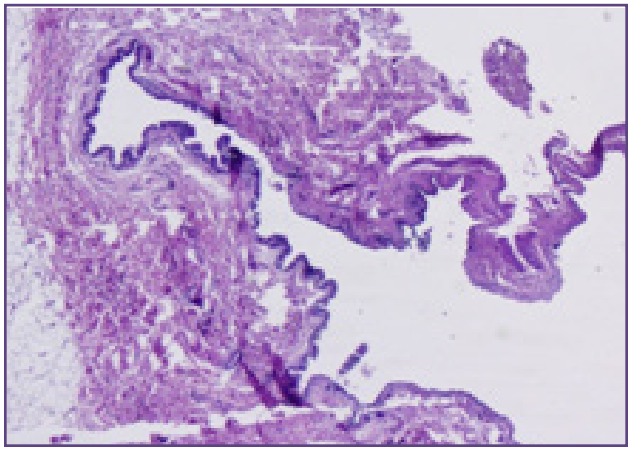

Fig. 1: Cyst overlying fibrocollagenous and fibroadipose stroma H&E X20.

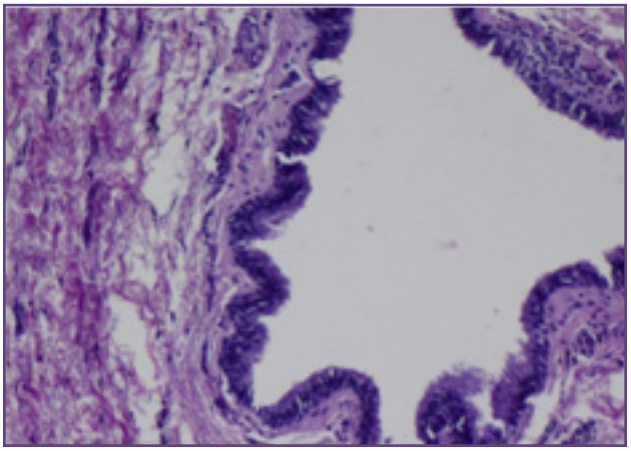

Fig. 2- Respiratory epithelium overlying oedematous fibrocollagenous stroma with mucin glands H&E X100.

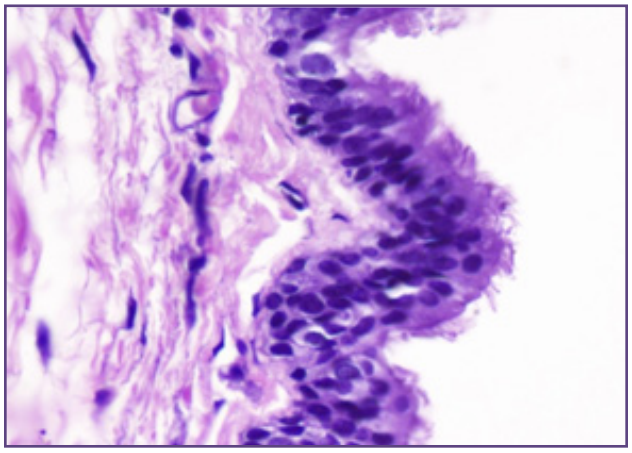

Fig. 3: Cyst lined by pseudostratified ciliated columnar respiratory epithelium H&E X400.

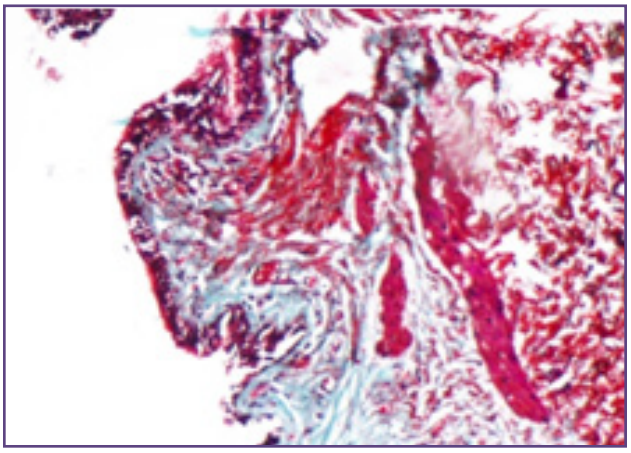

Fig. 4: Smooth muscle fibres in red Masson trichrome X100.

The pathogenesis for the development of bronchogenic cyst lies in embryogenesis. During the beginning of the 5th week of gestation, laryngo-tracheal groove separates the primitive foregut into dorsal and ventral structures.[2,4,5] The dorsal anlage forms the oesophagus, whilethe ventral anlage develops into tracheobronchial tree.Bronchogenic cysts form due to abnormal development occuring in the distal tracheobronchial tree.[8] However, the origin of the cutaneous bronchogenic cysts can also be explained by another hypothesis which states that a part of the anterior portion of the developing lung gets pinched off during the process of fusion of the right and left bars of sternum precursors, leading topresternal localization of the bronchogenic cysts.[4]As far as extrathoracic locations at unusual sites like neck, chin,shoulder are concerned, pathogenesis can be explained by the migration of these sequestered structures in the developing embryo or in situ development of metaplastic respiratory epithelium from pre-existing cutaneous tissue and primary anomalous differentiation in the developing skin.[2]

These lesions usually present as an asymptomatic midline subcutaneous cystic and nontender nodule. [8]If the cysts are large in size and localized in the cervical area, then symptoms like dyspnoea, respiratory distress, cough and dysphagia may be seen. The cases of secondary infection may result in the formation of sinus tract causing external drainage of purulent material or an abscess formation.[1] However, our case did not present with any respiratory symptoms or any associated secondary infection. Due to the lack of any characteristic clinical features it is clinically challenging to distinguish bronchogenic cysts from other cysts occurring in the same region.

Bronchogenic cysts are characteristically lined by pseudostratified ciliated columnar respiratory epithelium with interspersed goblet cells. Smooth muscle fibers are associated in 70%, mucus gland in 53% and cartilage in upto 7% cases.[1,4,5] Our case also had similar clinical presentation and microscopy to warrant the diagnosis of bronchogenic cyst.

A differential diagnosis for bronchogenic cyst includes branchial cleft cyst, thyroglossal duct cyst, mature cystic teratoma, cutaneous ciliated cyst, dermoid cyst, epidermal inclusion cyst, infundibular cyst and trichilemmal cyst. Characteristically, branchial cysts are located either along the sternocleidomastoid muscle, or mandibular region or in the preauricular region. They are predominantly lined by stratified squamous epithelium or rarely by pseudostratified ciliated columnar respiratory epithelium with surrounding lymphoid follicles and lymphocytic infiltrate.Thyroglossal duct cysts are noted as midline cystic neck nodules and characterised by the presence of thyroid follicles and lymphocytic infiltrate.Trichilemmal cysts are derivative of outer root sheath of hair follicle, can occur at any site and are lined by stratified squamous epithelium withcharacteristic absence of granular layer andabrupt keratinization. Cutaneous ciliated cysts usually occur in females, seen in the lower extremities and lined by ciliated columnar epithelium with papillary projections resembling fallopian tubes.Dermoid cysts can be differentiated as they possess epidermal appendages and epidermal inclusion cyst have stratified squamous layer as the liningepithelium.[1,2,4,5]

Bronchogenic cysts are benign lesions, however few cases with malignant transformation have been reported in literature. Tanita et al. [10] have reported development of malignant melanoma from a cutaneous bronchogenic cyst over scapular region. Calzada et al. [11] mentioned about a case of poorly differentiated adenocarcinoma arising from a bronchogenic cyst and Jakapovic et al. [12] reported transformation of bronchogenic cyst into large cell carcinoma.

Treatment of choice for bronchogenic cyst is complete surgical excision. Due to the possibility of development of malignancy in these lesions, histopathological examination of the entire cyst is considered very important. [1] Incomplete excision may lead to recurrence, hence follow up is advocated.Hasegawa et al .[13]reported a case where recurrence of bronchogenic cyst in a 42 year old man occurred nearly 15 years after initial resection and the probable cause stated was incomplete resection.

Conclusion

Bronchogenic cysts are uncommon congenital malformations. They may occur at unusual locations and thus should be included in the differential diagnosis of cystic and nodular skin lesions on the upper chest, neck and upper back. A high index of clinical suspicion is required to diagnose this lesion preoperatively. Histopathological examination is mandatory and plays an integral role for establishing precise diagnosis.

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Case Report

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Severe Low Back Pain as The Presenting Feature of Alkaptonuria in A Young Female

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ABSTRACT

Alkaptonuria is a rare inborn error of metabolism, caused due to the deficiency of the enzyme homogentisic acid oxidase. The manifestations of this disease are initially non specific, hence the condition is often under diagnosed as the patients present at later stages with associated complications. Although the treatment of alkaptonuria is mainly supportive, the diagnosis and reporting of this rare condition is necessary as it avoids subjecting the patients to unnecessary investigations and interventions.

We report a case of a thirty five year old lady, who presented with severe low back pain. X ray showed features of disc prolapse. On table intraoperative findings showed blackish discolored disc material during discectomy. After doing simple ancillary tests, and correlating with other clinical features, the lady was diagnosed as a case of Alkaptonuria.

We report this case as not only is the metabolic condition rare, but also to highlight the importance of simple urinary tests which are of great help in diagnosing such conditions, even in this era of automation considering the cost effectiveness in rural population like ours.

Keywords: *Low Back Pain, Urine Examination, Homogentisic Acid, Oochronosis, Alkaptonuria*

Introduction

Alkaptonuria is a rare, inborn error of metabolism. It is an autosomal recessive condition caused due to the deficiency of the enzyme homogentisic acid oxidase. It was also one of the four inborn errors of metabolism described by Garrod in his croonian lectures of 1908**.** [1] The common clinical manifestations of alkaptonuria are (i) homogentisic aciduria, (ii) ochronosis (deposition of bluish-black pigment in all connective tissues), and (iii) arthritis.

[2]**.** Though no specific treatment is available till date, there is need to diagnose and report this condition as patients' survival can be improved by dietary modifications and other ancillary treatments.

Case Report

A thirty five year old female was admitted with history of severe low back pain and stiffness since the past two months, which aggravated since a week. The backache was of gradual onset initially, reaching to a severity that she was not able to get up from the sitting position without support at the time of presentation. The patient had no other significant complaints. She also gave history of being operated in ankle at the region of the Achilles tendon two years back, but no documents of the same were available. The patient had no significant family history.

General examination showed brown to black pigmentation of the ear cartilage and the anti helix. [FIG 1]The interpalpebral fissures of both eyes showed light brownish pigmentation.

X ray of the spine showed extensive calcifications of the intervertebral disc spaces and osteophytosis. Disc prolapse was noted at the level of L3 – L4 spine. Also noted was

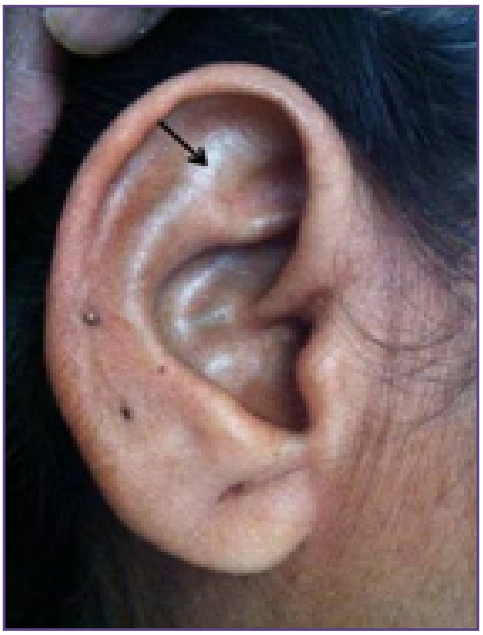

Fig. 1: Ear showing light brownish pigmentation in the anti helix region (Arrow).

mild osteoarthritis of the knee and hip joints on X ray of these joints. In view of disc prolapsed a discectomy was planned. Intra operative findings showed brown to blackish pigmentation of the disc material.The scooped disc material was sent for histopathological examination.**[FIG2A].** Histopathological examination showed fragments of mature cartilage showing mild degenerative changes and brownish pigmentation at places.[FIG 2B]

Laboratory investigations:Simultaneously, other laboratory investigations were done. Routine biochemical parameters including the ECG were normal. However taking into consideration the clinical findings and the disc material discoloration, Alkaptonuria was suspected. The patient's urine sample on standing for twenty four hours turned black in color [FIG3A]. Benedict's test done on the urine sample showed a brick red precipitate .A black precipitate was observed on doing ammoniacal silver nitrate test. Ferric chloride test done on urine showed a transient dark green colour. [FIG3B]

The patient was thus diagnosed as a case of Alkaptunuria based on the clinical and other investigation findings. She was given required doses of vitamin C, and advised dietary modification to avoid phenylalanine and tyrosine rich foods. Symptomatic treatment for joint pains was given with analgesics, and sessions of physiotherapy was advised. The patient, being from a rural background, was eventually lost to follow up.

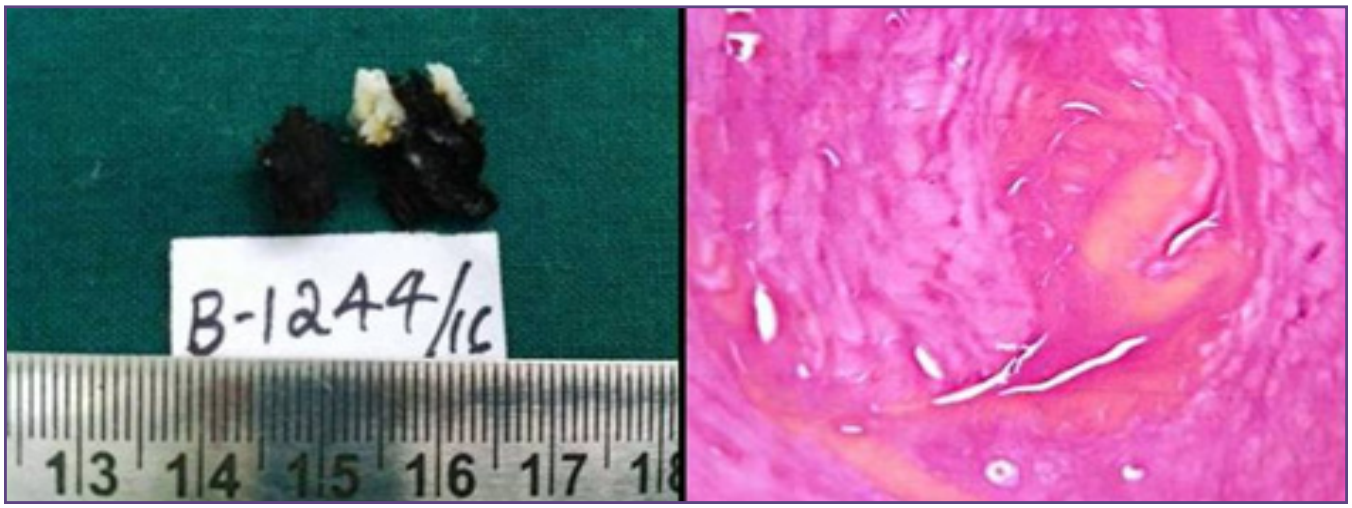

Fig. 2 A: Scooped out disc material sent for histopathological examination .Fig 2B: Photomicrograph showing cartilaginous bits with foci of brownish pigmentation, H&E X100.

Fig. 3A: Urine sample after standing for 24 hours (right) as compared to fresh sample(left).FIG 3B: Urine sample showing control sample, Ferric chloride test , Benedicts test and silver nitrate test (left to right).

Discussion

Alkaptonuria [AKU] is a rare autosomal recessive metabolic disorder, characterized by the deficiency of homogentisic acid oxidase, an enzyme in the metabolic pathway of phenylalanine and tyrosine. It also is credited with being one of the first diseases for which the laws of Mendelian inheritance was proposed.[1] The deficiency of homogentisic acid oxidase leads to the accumulation of a melanin like granular pigment, which has affinity for cartilage, and resembles ochre (yellow), hence the name oochronosis. [3]

Although one of the earliest manifestations of this disease is the darkening of urine, this is seldom the presenting complaint as patients do not observe the same. Our patient also did not notice any change in the colour of urine. However, her urine sample on standing turned dark after twenty four hours during urine examination. Benedicts test, a non specific test for reducing substances is positive for homogentisic acid. Sachadevet al[4] have reported a case of alkaptonuria, misdiagnosed initially as diabetes mellitus on basis of Benedicts test alone. The patient was given hypoglycaemic drugs, after which he went into hypoglycaemic coma. This situation is however uncommon in current practice as dipsticks, which specifically test glucose are used in the clinical laboratories. We would also want to highlight the importance of simple, inexpensive but valuable tests like ferric chloride and the silver nitrate test , which can detect the homogentisic acid in urine quantitatively and are highly cost effective, considering the rural population of our country.[5]

The major weight bearing joints of the body get affected after the third decade of life in Alkaptonuria , most common being the intervertebral discs of the lumbar spine, hip and the knee. [6]Low back pain is one of the commonest presenting symptoms in young patients. The deposition of pigment happens in these joint s as the ochronotic pigment has a very high affinity for collagen fibers of the joints.[7]As most of the patients present with non specific symptoms of low back pain and stiffness of joints, the metabolic defect is often masked, as in our case.[8] Degenerative arthritis and ankylosing sponylitiis [AS] are the close differentials to be considered. Annular sclerosis is a feature of AS, unlike AKU. The sacro – iliac joints in AKU show narrowing and marked sclerosis, which is not seen in AS.[9] Intra – operative findings of blackish discolored disc material as seen in our case, provided us some clue to work up for alkaptonuria. On histopathological examination the ochronotic pigment needs to be differentiated from melanin. Ochronotic pigment does not stain with silver nitrate, and blackening on methylene blue or cresyl violet staining is a useful method to highlight it. [10]

Spontaneous rupture of the Achilles tendon is also an important clinical manifestation of Alkaptonuria. Deposition of the pigmented polymers weakens the structural integrity of the tendons causing their rupture. Most cases of Achilles tendon rupture till date have been reported from India. [9, 11] Interestingly even our patient gave a similar history of having some surgical correction in the region of achilles tendon, but no documents or discharge summary of the same was available for confirmation.

Renal, prostatic , salivary stone formation, cardiovascular manifestations (valvular calcification , coronary artery calcification , aortic stenosis), rupture of ligaments are some of the documented complications of Alkaptonuria. [2, 8, 12].There is no definitive treatment for this condition till date. The management strategies are directed towards early diagnosis of this condition and timely intervention to delay the complications. Ascorbic acid is shown to inhibit the binding of homogentisic acid to the chondrocytes, hence the ideology in giving patients supplementation of the same in doses of 1g/day. Nitisinone an inhibitor of 4 – hydroxyphenylpyruvate, the enzyme that produces homogentisic acid has also been tried in these patients. A recent study by Millucci et al[13]provided experimental evidence that AKU osteoarticular tissue contains AA-amyloid deposits .This opens new perspectives for AKU therapy. The study also showed that methotextrate was able to significantly prevent in vitro HGA-induced A-amyloid aggregates, thereby contributing new perspectives to the clinical picture of this disease.

Conclusion

Alkaptonuria is a rare metabolic disease, which is often underdiagnosed as patients often present at later stages with associated complications. Though no specific treatment has been discovered till date, the importance of recognizing this condition lies in helping the patients delay the onset or tide over the already manifested complications. Also, diagnosis of this metabolic disease with simple urine tests can prevent the patient from being subjected to unnecessary medications and interventions in this era of automation.

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Case Report

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Primary Non Hodgkins Lymphoma of Bilateral Breasts: A Rare Entity

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ABSTRACT

Extranodal Non-Hodgkin's Lymphoma (NHL) of the breast is a rare entity, representing 0.04-1.1% of malignant tumours of the breast. Most breast lymphomas are the non-Hodgkin's B cell type, with DLBCL being the most common. We present the case of a 32 years old female who presented with bilateral hard breast lumps, which were clinicoradiologically thought to be carcinomas. But on histopathology and immunohistochemistry, it was finally diagnosed as a lymphoma. Primary and secondary lymphomas of the breast, though rare, should be considered in the differential diagnosis of breast malignancies.

Keywords: *Breast, Lymphoma, NHL*

Introduction

Extranodal Non-Hodgkin's Lymphoma (NHL) of the breast is a rare entity. It constitutes 0.04-1.1% of malignant tumours of the breast, 1.7-2.2% of extranodal lymphomas and 0.7% of all NHL.[1] However, primary NHL (PNHL) is the most frequent hematopoetic tumour of breast[1]. Mucosa associated lymphoid tissue (MALT) lymphoma is another common type of breast lymphomas.[2]

Non-Hodgkin's typebreast lymphoma represents approximately 70– 90% of breast lymphomas. In patients diagnosed with NHL, primary involvement of the breast is seen in 0.4–0.7% of the cases.[3] Almost all primary breast lymphomas have a B-cell phenotype, while primary breast lymphomas with a T-cell phenotype are extremely rare.[4] 46–71% of primary breast lymphomasare diffuse large B-cell lymphomas (DLBCL).[5,6]Primary breast lymphoma exhibits a poor prognosis.and the therapeutic management is controversial and is not fully established.

Case report

A 32-year-old woman presented with a hard mass in the bilateral breasts for 3 months. Mammography showed a diffuse increase in the density of the breasts. Other investigations were unremarkable. Clinicoradiologically, it was suspected to be carcinoma breast. Biopsy was done from bilateral lesions and histopathological picture was similar showing sheets of dyscohesive tumour cells entirely replacing the normal breast tissue. The tumour cells were large, round with moderate to scanty cytoplasm. Tumour cells were showing severe degree of pleomorphism. High degree of mitosis could be identified.

Differential diagnosis of poorly differentiated carcinoma and lymphoma were considered. Immunohistochemistry panel of ER, PR, Her2neu, Ki-67, LCA, CK and EMA was put up. LCA, EMA and Ki-67 came out to be positive, and rest of the markers were negative. This directed us towards lymphoma.A final diagnosis of NHL was given.

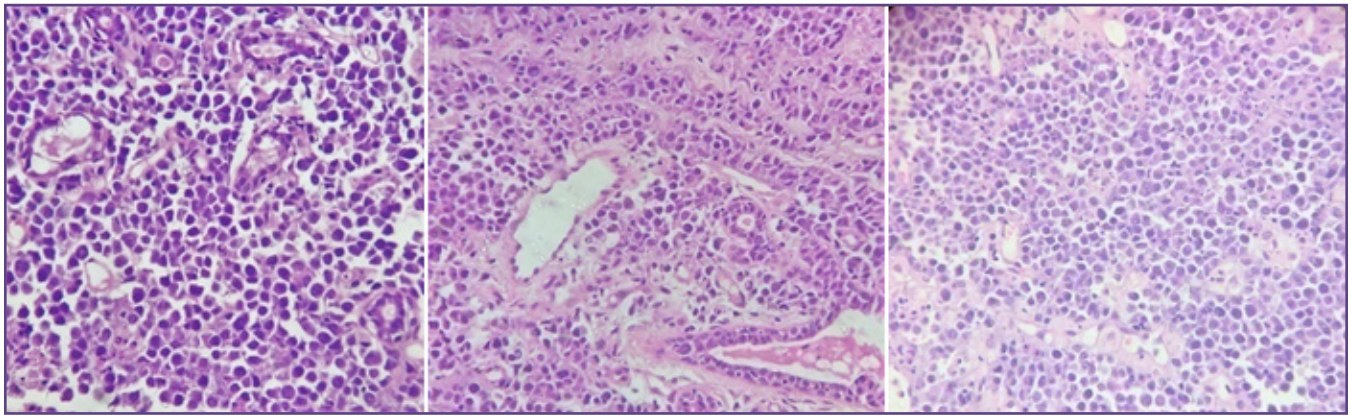

Fig. 1,2,3: H&E showing features of NHL (40X).

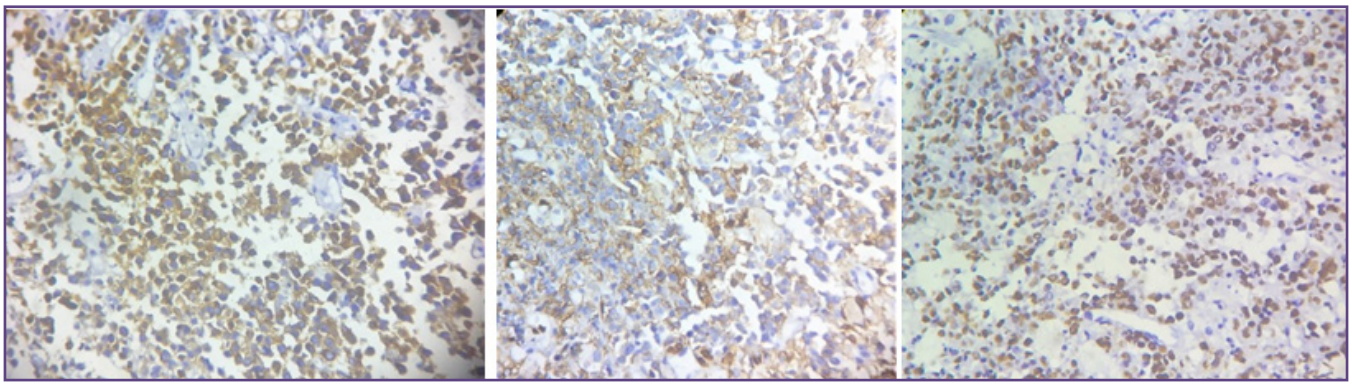

Fig. 4 A: IHC showing positivity of LCA (40X), B: positivity of EMA (40X); C: high positivity index of Ki-67 (40X)

Discussion

NHL involving the breast either as a primary site or as a site of recurrence from lymphoma previously diagnosed elsewhere is rare. Several series have reported varying incidences of primary and secondary cases. Primary NHL of the breast is a rare entity, constituting only 0.04%-0.50% of malignant breast neoplasms,1.7% of all extranodal NHL and 0.7% of all NHL.[1]

Clinically, primary breast lymphoma presents with features similar to that of breast carcinoma. It usually presents with right sided painless lump, sometimes multinodular, and which can be bilateral in 10% of cases.[7] Our patient had bilateral breast involvement. It affects two distinct age groups, one which affects a young woman is frequently bilateral, often associated with pregnancy, and is Burkitt-type lymphoma. The second group affects older women and is usually unilateral.[6,8]

Since there is considerable overlap in clinical and radiological features of breast lymphoma and carcinoma, pathology remains gold standard to differentiate these two malignancies. Although sensitivity of FNAC in diagnosis of lymphoproliferative disorder is 90%, several diagnostic pitfalls exist. Confirmatory core needle biopsy is recommended for a suspected primary lesion.[4]

Histologically, primary breast lymphomas are predominately of B-cell origin and most commonly large cell type.[9]The rate of secondary lymphoma metastatic to breast only slightly exceeds primary breast involvement.[9,10]

The following strict criteria must be met for a neoplasm to be characterized as PNHBL: (1) an adequate pathologic specimen, (2) close association of mammary tissue and lymphomatous infiltrate, (3) no evidence of disseminated lymphoma at the time of diagnosis, and (4) involvement of ipsilateral axillary nodes only if it occurs concomitantly with the primary lesion.[4]In our case, all these criteria were met for the diagnosis of PNHBL.

The histological differential diagnosis of breast lymphomas include lobular carcinoma, medullary carcinoma, amelanotic melanoma and poorly differentiated duct carcinoma. IHC and/or flow cytometry is helpful in differentiating these. In addition to physical examination, radiology of the chest, skull and pelvis is a reliable method for detecting visceral and nodal dissemination, and should always be performed.Contralateral breast involvement is best ruled out by MRI scan. It is also useful in follow up of patients to monitor response to chemotherapy and radiotherapy and to diagnose disease recurrence. The risk of CNS relapse in patients with primary breast lymphoma is greater than that of aggressive nodal NHL, and approximately estimated as 5%.[11]

The treatment of PNHBL is similar to that of other lymphomas and depends on the histological typeand histologic grade. Patients with low grade disease can be managed with local therapy alone. Patients with intermediate or high grade disease have better outcome if chemotherapy is included. Recent studies have shown that aggressive B-cell lymphomas should always be treated with chemotherapy alone or in combination with radiotherapy. The most effective combination reported in literature is radiotherapy and 3 to 10 cycles of CHOP regime.

Overall 5 year survival rate is 43%.[12]Survival rate of primary breast lymphoma is better as compared to systemic lymphoma with secondary breast involvement. [4] Anticancer drugs are the main treatment rather than surgery, so it is very important to accurately diagnose primary lymphoma of breast.

Conclusion:

In summary, we report a case of primary lymphoma of bilateral breasts, which was clinically andradiologically

suspected to be a carcinoma. Histologically, the tumour demonstrated the characteristic histopathological and IHC features of a lymphoma. Primary and secondary lymphomas of the breast, though rare, should be considered in the differential diagnosis of breast malignancies.

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Case Report

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Isolated Tubercular Liver Abscess: An Entity Rarely Thought, Diagnosed on Cytology

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ABSTRACT

Introduction: Tuberculosis is a rare cause of liver abscess in the Indian subcontinent even though the disease itself has high prevalance rates here. Isolated tubercular liver abscess presenting without any other foci of infection is a rare presentation with a documented prevalence of 0.34%. In an adult immune competent patient diagnosis can be challenging as it is likely to be confused with pyogenic and amoebic liver abscess or hepatoma.

Case History: A 30 year old female patient presented with complaints of low grade fever, vague abdominal pain of 4 months duration, breathlessness and loss of appetite for 1 month. Past or family history was non-contributory. Ultrasonography abdomen revealed a small hypoechoic lesion in sub-hepatic region of size 49x20mm suggestive of a liver abscess. Computerized Tomographic scan images confirmed loculated irregular collections in and around left lobe of liver and Reidel's lobe. Fine needle aspiration cytology from the lesion revealed ill formed granulomas on a necrotic background. Zeihl Neelson staining was positive for acid fast bacilli.

Conclusion: Isolated tubercular liver abscess is a rare entity, particularly in immunocompetent individuals. Symptomatology and radiologic findings may not be contributory. Diagnosis of this entity rests on the demonstration of acid fast bacilli in material obtained. FNAC is a simple minimally invasive, cost effective procedure which helps attain the same.

Keywords: *Liver Abscess, Tuberculosis, Rare Entity, Cytology*

Abbreviations

FNAC: fine needle aspiration cytology

USG: ultrasonography

CT: computerized tomography

MGG: May Grunwald Geimsa

ZN: Ziehl Neelson

AFB: acid fast bacilli

HIV: human immunodeficiency virus

Introduction

Liver is a frequently involved intra-abdominal organ susceptible to the development of abscess for a variety of causes chiefly organisms, be it parasitic (amoebic), fungal or bacterial (including mycobacterial).[1] In the Indian sub-continent Tuberculosis continues to be a major public health problem and is known to exist in two forms pulmonary and extra-pulmonary. Hepatic involvement in tuberculosis has been associated with both. Bacilli from respiratory and gastro-intestinal lesions reach via Hepatic vein or Portal vein. Isolated tubercular liver abscess is a rare entity seen in 0.34% of cases with hepatic tuberculosis.[2]

Tuberculosis is a rare cause of liver abscess in the Indian subcontinent even though the disease itself has high prevalance rates here. Isolated tubercular liver abscess presenting without any other foci of infection is a rare presentation with a documented prevalence of 0.34%. In an adult immune competent patient diagnosis can be challenging as it is likely to be confused with pyogenic and amoebic liver abscess or hepatoma.

Case Report

A 30 year old female patient presented to the medicine outpatient department with complaints of low grade fever and vague abdominal pain of 4 months duration. Occasional episodes of vomiting were reported during this period. She had additional complaints of breathlessness and loss of appetite for 1 month. Cough, expectoration and hemoptysis was not present. There was no past history for any chronic illness such as asthma, tuberculosis or diabetes. Prior contact with patients of tuberculosis was not documented. There was negative history of intake of anti-tubercular drugs. Her last child birth was six months prior. No history of blood transfusion or high risk behaviour was found.

General examination was non-contributory. Systemic examination showed slight dullness in right lower lung and costo-phrenic angle. Per abdomen mild tenderness in right upper quadrant was present. Laboratory investigations revealed haemoglobin of 10.7gm/dl, haematocrit 35.3%, total leucocyte count of 13,000/mm^3 with neutrophilia and raised liver enzymes (SGOT= 153.0 IU/L, SGPT=95.0

IU/L, serum Alkaline Phosphate=150.0 IU/L). Patient was seronegative for HIV1 & HIV2. Chest radiograph showed mild right sided pleural effusion.

Ultrasonography (USG) abdomen revealed a small hypoechoic lesion in sub-hepatic region of size 49x20mm suggestive of a liver abscess. Computerized Tomographic (CT) scan images confirmed loculated irregular collections in and around left lobe of liver and Reidel's lobe with small perigastric extention as well as thickening of omentum (Figure 4). Follow up chest radiography showed resolution of right sided pleural effusion by the time patient was investigated thoroughly. Possibility of liver abscess of infectious etiology was most probable diagnosis. Patient underwent an ultrasound guided fine needle aspiration of the hepatic lesion. Smears were stained with May Grunwald Geimsa (MGG) and showed ill formed collection of epithelioid cells, transformed macrophages and lymphocytes (Figure 1) with giant cells (Figure 2) seen on a necrotic background. Ziehl Neelson (ZN) confirmed presence of acid fast bacilli (AFB) (Figure 3) and a final diagnosis of tubercular hepatic abscess was rendered.

Discussion

Hepatic tuberculosis is not a rare disease, however isolated tubercular liver abscess is, even in a country like India

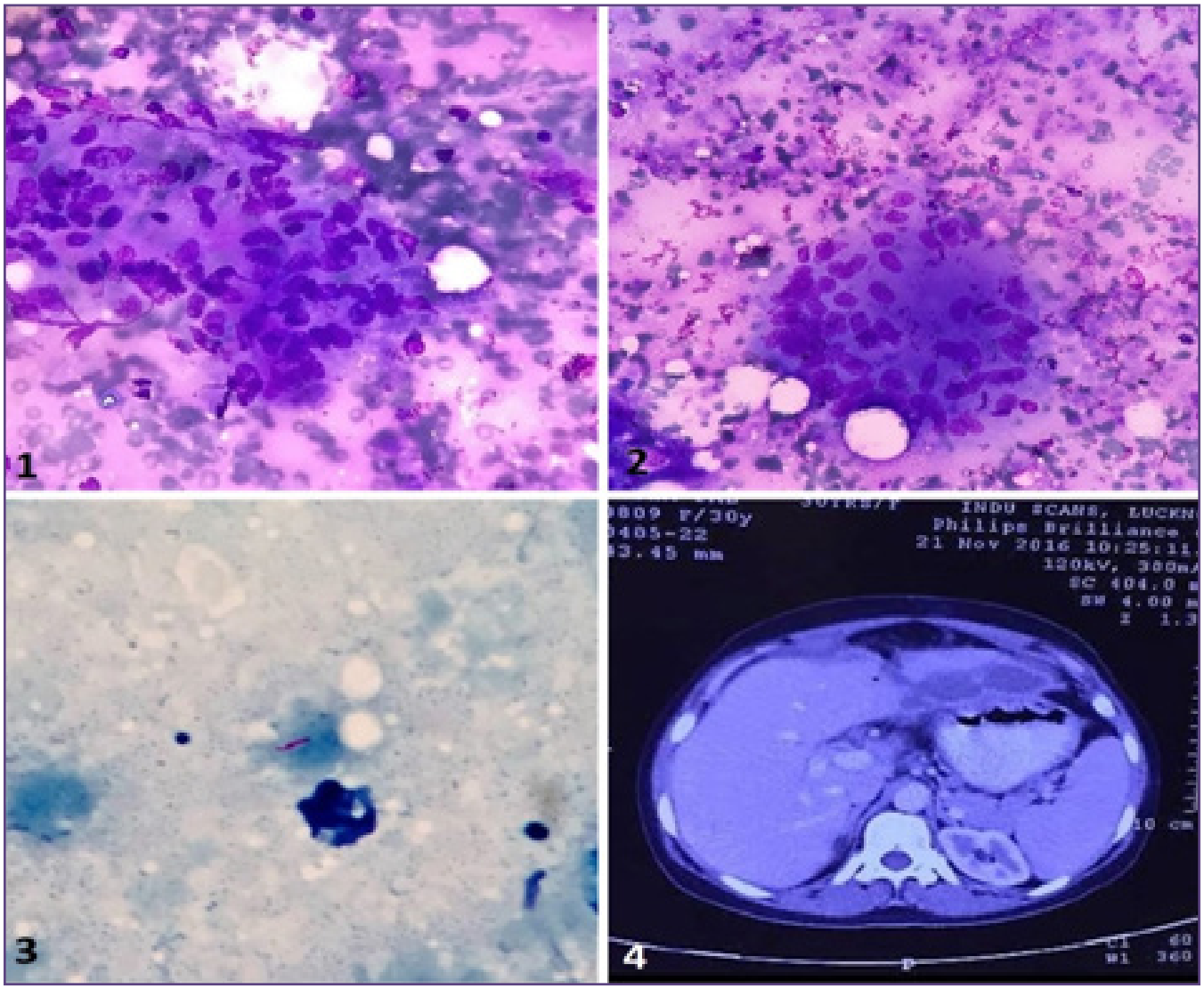

Fig. 1: ill formed granulomas consisting of epithelioid cells, lymphocytes and transformed macrophages (MGG,400X)2:- photomicrograph shows langhans type of giant cell on a necrotic background(400X); 3:- acid fast bacilli seen on Zeihl-Neelson stain(oil immersion 1000X); 4:- CECT axial section shows well defined hypodense loculated collection with enhancement in subcapsular region along postero-inferior margin of left lobe of liver suggestive of abscess.

where tuberculosis is an alarming health problem.[3,4]Low oxygen levels make liver an inhospitable organ for bacterial growth.[5] Approximately a 100 reported cases are known in literature, to the best of our knowledge.[6] It was first described in 1858 by Breastowe.[7] Three forms of hepatic involvement described in tuberculosis are:- i) diffuse hepatic along with pulmonary or miliary tuberculosis ii) diffuse hepatic without pulmonary involvement iii) focal tuberculoma or abscess, the rarest of all.[2]

Involvement of liver is usually secondary to a primary focus in lungs and gastro-intestinal tract. Hematogenous dissemination of mycobacteria to liver takes place via hepatic artery or portal vein. Various authors have reported abnormalities in chest x-ray of patients with tubercular liver abscess ranging from 65% to 78%.[8,9,10] Moreover presence of immunosuppression as a result of concomitant diabetes mellitus, AIDS, steroid therapy or chronic renal failure may further mask presenting features and delay diagnosi s.

Symptoms are usually non-specific and vague including fever, abdominal pain/discomfort and anorexia with weight loss. Jaundice is usually rare though hepatomegaly is commonly documented.[11] Liver function test shows elevation of transaminases to variable levels with prolonged prothrombin time and reversed A:G ratio. Our case had delivered a healthy child six months prior following an uneventful pregnancy. She was immunocompetent and had presented with low grade fever and abdominal discomfort. Some loss of appetite and breathlessness possibly due to reactionary pleural- effusion had been present.

Common differential diagnosis to be considered and excluded are pyogenic and amoebic liver abscess and hepatoma. Fine needle aspiration cytology (FNAC) is an inexpensive and minimally invasive procedure that when used under USG guidance helps localize lesions and improves yield of diagnostic material. ZN stain confirms the presence of AFB on necrotic material aspirated following screening of routinely stained smears by MGG. Identification of AFB by ZN satin ranges from 0% -45%.[12]

Use of alternative diagnostic tests such as culture on LJ media to directly demonstrate Mycobacterium *tuberculosis* is practised however reported sensitivity of culture for tubercular liver abscess is 10%.[12] PCR is another diagnostic tool that enables rapid identification of the infectious organism but is costly. Due to the limitation of resources, following radiographic imaging demonstration of AFB by ZN stain aided with similar results in simultaneously put positive control smears helped us reach definitive diagnosis of tubercular liver abscess, following which appropriate medical therapy was instituted.

Conclusion

Isolated tubercular liver abscess is a rare entity, particularly in immune-competent individuals. In a country like India which has nearly 25% of global incident tuberculosis cases, its possibility should be kept in mind. Global travel trends have necessitated that elsewhere in the world too tubercular liver abscess though rare should be kept as a differential along with amoebic abscess in unknown hepatic mass lesions. Symptomatology and radiologic findings may not be contributory. Diagnosis of this entity rests on the demonstration of acid fast bacilli in material obtained and FNAC is a simple minimally invasive, cost effective procedure which helps achieve this, particularly where resources are limited and modalities like polymerase chain reaction (PCR), though available are expensive.

Isolated tubercular liver abscess is a rare entity, particularly in immunocompetent individuals. Symptomatology and radiologic findings may not be contributory. Diagnosis of this entity rests on the demonstration of acid fast bacilli in material obtained. FNAC is a simple minimally invasive, cost effective procedure which helps attain the same.

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Case Report

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Riedel's Thyroiditis in a 78 Year Old Male: A Rare Experience

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ABSTRACT

We present a rare case of Riedel's thyroiditis in a 78-year-old male, native of hilly region of Nepal who presented with chief complaint of long standing swelling of the thyroid with discharging sinus. Right hemithyroidectomy with excision of sinus was done. Gross examination showed asymmetrically enlarged right lobe of thyroid with adherent fibroadipose and muscular tissue. Microscopy revealed diffuse hyalinised fibrosis of the thyroid parenchyma with presence of variable number of atrophic to few normal thyroid follicles in between. The stroma showed dense lymphoplasmacytic infiltration with foci of calcifications and hemorrhage. There was extension of fibrosis beyond the thyroid capsule, encasing the skeletal muscle bundles at many places. Diagnosis of Riedel's thyroiditis was made.

Riedel's thyroiditis is a rare entity but can occasionally be encountered. Various imaging modalities may not be helpful for the definite diagnosis. Diagnostic thyroidectomy should be performed for the accurate diagnosis and further management.

Keywords: *Thyroiditis, Hashimoto Thyroiditis, Fibrosis, Hürthle Cell, Thyroid.*

Introduction

Riedel's thyroiditis (RT) is a chronic thyroiditis characterized by an inflammatory proliferative fibrosing process that partially destroys the thyroid gland and extends into the surrounding tissues beyond the thyroid capsule.[1] It is an uncommon disease found in about 0.05% of all thyroidectomies.[2] Clinical symptomatology and imagery overlap makes it difficult to differentiate from malignancy. Riedel's thyroiditis usually cannot be diagnosed accurately by preoperative cytology.[3]

Case Report

A 78-year-old male presented in the Department of Otorhinolaryngology and Maxillofacial Surgery of B. P. Koirala Institute of Health Sciences, Dharan, Nepal, with complain of mass at right side of anterior neck and a discharging sinus since 20 years which was not associated with pain or fever. On physical examination, mass moved with deglutition and was non-tender. Thyroid Function Test showed subclinical hypothyroidism. T3 and T4 levels were within the normal range while thyroid stimulating hormone (TSH) was raised (10.7 µIU/mL).

Neck Ultrasound showed enlarged right lobe of thyroid with multiple nodules and coarse calcifications. A large, eccentric, heterogeneous, predominantly hypo echoic area with echogenic foci within a hypo echoic tract with internal echoes extending from the lesion to skin surface was observed, which was suggestive of an infective pathology.

Preoperative diagnostic fine-needle aspiration cytology (FNAC) performed yielded non- diagnostic material. Right hemithyroidectomy with excision of sinus was done.

On gross examination, the gland was found to be asymmetrically enlarged with adherent fibroadipose and muscular tissue on the right. The cut surface was grey white to yellowish, firm to hard, with gritty sensation on sectioning (Figure 1). The attached soft tissue showed presence of a sinus tract which was not reaching up to the thyroid. Normal thyroid parenchyma was not identified grossly.

Microscopic examination revealed diffuse hyalinised fibrosis of the thyroid parenchyma with presence of variable number of atrophic to few normal thyroid follicles in between (Figure 2 & 3). The stroma showed dense lymphoplasmacytic infiltration (Figure 4) with foci of calcifications and hemorrhage. The fibrosis extended beyond the thyroid capsule and were seen encasing the skeletal muscle bundles at many places (Figure 5 & 6). Sections from sinus tract revealed a tract lined by granulation tissue and mixed inflammatory cells. The tract was not extending into the thyroid parenchyma microscopically as well. There was absence of hürthle cells, lymphoid follicles, multinucleated giant cells or granulomas. Based on these findings a diagnosis of Riedel's thyroiditis was considered.

Discussion

Riedel's thyroiditis (RT), also known as Riedel struma, fibrous thyroiditis or invasive thyroiditis is an extremely

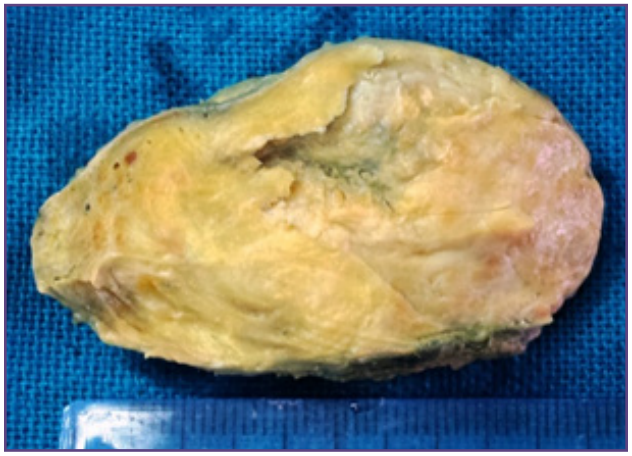

Fig. 1: Gross appearance of thyroid revealing a solid grey white to yellowish cut surface. Normal thyroid parenchyma is not seen.

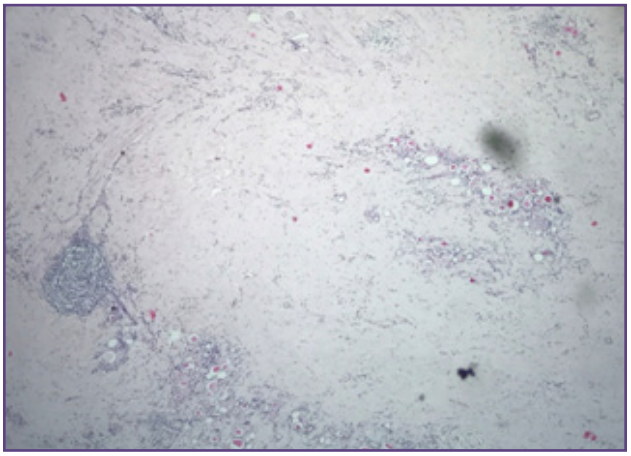

Fig. 2: Extensive fibrosis of parenchyma with residual atrophic and normal thyroid follicles and lymphocytic infiltrates. H & E stain.

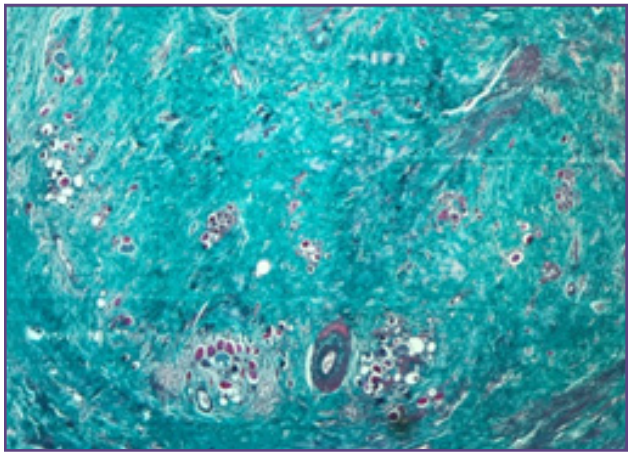

Fig. 3: Masson Trichome stain demonstrating extensive fibrosis of thryroid parenchyma with residual and atrophic thyroid follicles.

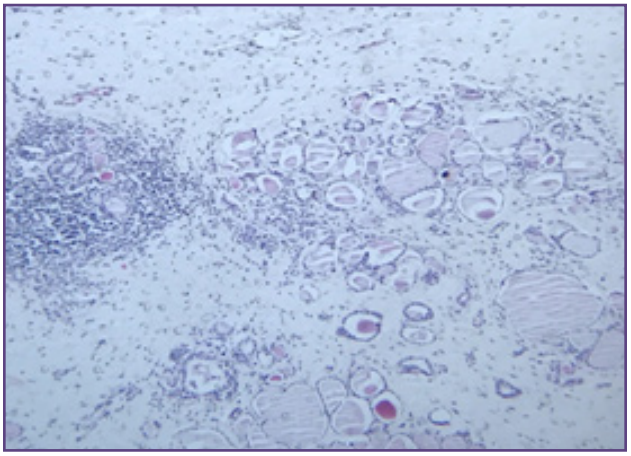

Fig. 4: High power view revealing residual atrophic and few normal thyroid follicles with mononuclear cell infiltrates.

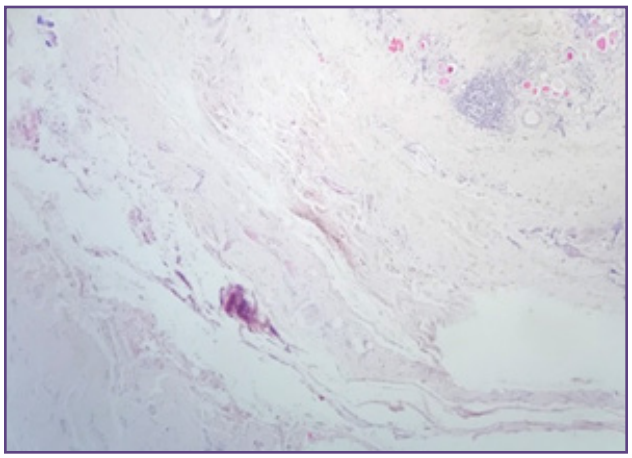

Fig. 5: Extensive fibrosis extending into the capsule and surrounding structures, with entrapment of skeletal muscle bundles at the left lower end. H & E stain.

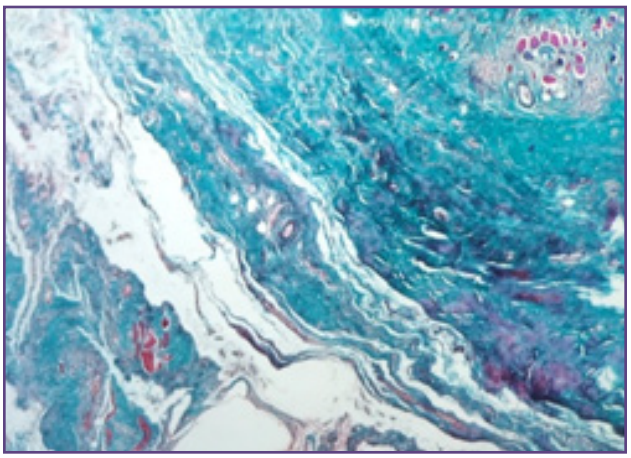

Fig. 6: Masson trichome stain highlighting the extracapsular fibrosis with entrapment of skeletal muscle bundles at the left lower end.

rare form of infiltrative and inflammatory disease of the thyroid and was first described by Bernard Riedel in 1896.[4,5] Reports of Riedel's thyroiditis in the literature are often limited to case reports and small case series. The true incidence is unknown, but in a 1985 review of 56,700 thyroidectomies performed at Mayo Clinic, only 37 cases of Riedel's thyroiditis were identified.[6]Due to extensive fibrosis of the thyroid tissue along with surrounding soft tissue, it generally presents as a firm mass in the thyroid, with compressive symptoms.[5,7] Riedel's thyroiditis presents as a painless mass as is not preceded by acute inflammatory process.[5]

Similar to cases reported by Pi GY et al[3] and Wojciechowska-Durczynska[8] et al our case presented with non-specific cervical discomfort, painless mass which was firm in consistency. In our case thyroid function test showed subclinical hypothyroidism in contrast to the euthyroidism in the case reported by Wojciechowska-Durczynska et al.[8] Hypothyroidism was seen in the case reported by Pi GY et al.[3]In 2 case reports by Zakeri H et al one case presented with hypothyroidism and the other with thyrotoxicosis.[7]

It is difficult for physicians to distinguish Riedel's thyroiditis from malignant neoplasms of the thyroid clinically because both clinical examination and imaging of Riedel's thyroiditis suggests malignancy. Ultrasound of Riedel's thyroiditis shows a hypo-echoic and hypo-vascular mass with extension into adjacent soft tissues as in our case. However, this appearance is nonspecific and can be seen in other disease processes that present with diffuse fibrotic involvement, such as Hashimoto thyroiditis, lymphoma, and thyroid carcinoma.[9,10]Thus, it is hard to distinguish Riedel's thyroiditis from other forms of thyroiditis.

Preoperative diagnostic modalities such as imaging and FNAC are inconclusive, as in our case and in cases reported by Pi GY et al[3] and Wojciechowska-Durczynska et al[8].

Grossly, the gland is asymmetrically enlarged with adherent fibroadipose and muscle tissue and is stony hard in consistency. The tissue is difficult to cut with gritty sensation and cut section reveals a firm to hard, grey white fibrotic tissue with complete obliteration of the normal thyroid gland.[4,5]Our case showed similar finding. The sinus tract that was present in the soft tissue did not reach the thyroid gland.

One of the difficult and important microscopic differential of Riedel's thyroiditis is with fibrosing variant of Hashimoto's thyroiditis. Lack of extension of fibrosis into the adjacent soft tissue, absence of extensive hürthle cell metaplasia and granulomatous inflammation are key features of Riedel's thyroiditis which helps to differentiate from fibrosing variant of Hashimoto's thyroiditis.[4,5,9,11] Our case typically lacked hürthle cell metaplasia and granulomatous inflammation. There was extensive fibrosis of the with replacement of the normal thyroid, however few normal thyroid follicles were seen in between the fibrous tissue along with dense infiltration by lymphocytes and plasma cells. The plasma cells present in Riedel's thyroiditis are polyclonal with numerous IgA and IgG4 producing cells.[5]Studies have been attempted to link Riedel's thyroiditis with IgG4-related systemic disease and have thought to be the underlying condition.[11] However, further studies are required to study the disease at various stages. Occlusive phlebitis is an important diagnostic feature but was not seen in our case.

Conclusion

Riedel's thyroiditis is an extremely rare entity but can occasionally be encountered. Preoperative diagnostic procedures including US, CT, MRI, and FNAC are not helpful for the definite diagnosis of Riedel's thyroiditis and differentiation from thyroid malignancy. Diagnostic thyroidectomy should be performed for the accurate diagnosis of Riedel's thyroiditis with histopathological evaluation for further appropriate management of the patient.

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Case Report

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Myelolipoma: A Rare Incidentally Detected Adrenal Lesion

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ABSTRACT

Adrenal myelolipomas are benign, uncommon neoplams that are being increasingly detected due to frequent use of imaging studies. They are lipomatous tumours, that are usually asymptomatic and sometimes associated with endocrinological dysfunction.

We present a case of middle aged gentleman, with accidentally detected adrenal mass and no hormonal disturbances. The patient was evaluated initially for fever. Laboratory investigations revealed evidence of urinary tract infection, which was conservatively managed. Ultrasound abdomen showed a hyperechoeic mass in suprarenal region with a hypoechoeic component. On further work up, MRI showed a well defined suprarenal mass with hyperintensity, possibly adrenal myelolipoma. The tumour was removed laparoscopically and histopathology revealed features of myelolipoma. The patient was discharged after an uneventful postoperative period.

Adrenal myelolipomas are rare, benign tumours of adrenal gland diagnosed incidentally. Careful evaluation is important including imaging studies and endocrinological testing. Larger or symptomatic tumours can be excised surgically. Laparoscopic resection is a safe procedure in tumours considered for surgery, with favourable patient outcome.

Keywords: *Adrenal Myelolipomas, Lipomatous Tumours, Benign, Rare*

Introduction

Adrenal myelolipomas are unusual, benign tumours of adrenal gland that are usually asymptomatic.There has been an increase in detection of these lesions due to more frequent use of imaging techniques. [1] Majority of these tumours are diagnosed incidentally although some may present with varied symptoms. [2] Histologically, they are composed of an admixture of lipomatous component and haematopoetic component, in varying proportions. We report an unusual case of adrenal myelolipoma, incidentally discovered in a 56 year old gentleman.

Case report

A 56 year old gentleman presented with fever, generalized weakness, and frequency of micturition. He had a past history of hypertension and type 2 diabetes mellitus, controlled on medication. The patient had undergone surgery for inguinal hernia 6 years back. There was no history of any drug allergy. General and systemic examination showed no positive findings. The patient was mildly hypertensive with blood pressure of 140/90 mmHg.

Routine investigations showed presence of pus cells in urine, mild anaemia with borderline leukocytosis on blood counts. ESR was elevated. Malarial parasite antigen test was negative. Blood glucose, serum electrolytes and liver enzymes were within normal range. Tests for viral markers were non reactive. Urea and creatinine levels were mildly elevated (41.8mg/dl, 1.4mg/dl respectively). HbA1c was borderline (6.6 %). ECG did not show any significant abnormality, apart from sinus tachycardia. Urine routine examination revealed pus cells 25-30/hpf. Urine culture showed Escherichia coli with significant colony count, and blood culture showed Escherichia coli.

On USG, Right kidney showed a hyperechoic oval shaped lesion measuring 6.8x5.7cm in upper pole with 2.4x2 cm hypoechoic area within it, most likely adrenal mass lesion. Rest of abdominal organs including urinary tract did not show any significant abnormality. Patient was managed conservatively with medications for urinary tract infection. He improved symptomatically and blood counts came to normal. After six days, the patient was discharged.

Meanwhile further investigations were done to ascertain the nature of suprarenal lesion. Endocrine workup revealed serum aldosterone, serum cortisol and plasma free metanephrines within normal range. (S aldosterone 3.24 ng/dl , Plasma free metanephrine 50 pg/ml (normal <65); serum cortisol 17.1 µg/dl)

MRI whole abdomen showed a large well-defined solid looking altered signal intensity mass lesion measuring 65x52x55mm, appearing slightly hyperintense on T1

images with loss of signal intensity on fat suppression images (Figure 1) in right supra renal gland. The lesion was abutting the inferior surface of liver with slight displacement of IVC. There was no significant abnormality in the urinary tract, no obvious pelvic or abdominal lymphadenopathy. On CECT there was a well circumscribed oval mass lesion, measuring 65x67mm in right renal upper pole in region of adrenal gland with soft tissue and fat; density -40 to +34 HU, with minimal punctate enhancement on contrast administration, most likely to be adrenal myelolipoma.

One month later the patient was taken up for laparoscopic adrenalectomy after routine investigations which did not show any abnormal findings. At that time, the patient was afebrile with general and systemic examinations not revealing any abnormality. There was no complaint of pain in abdomen or fever. His blood counts and urea/creatinine were normal.

Right laparoscopic adrenalectomy was done. Post operative stay was uneventful and the patient was discharged next day with follow up advice. At one month follow up, the patient was asymptomatic.

Pathological Findings: Gross examination revealed grey yellow, capsulated soft tissue measuring 6 x 5 x 5 cm. Cut surface was grey yellow with haemorrhagic areas (Figure 2). On histopathological examination (Figure 3), sections showed a tumor composed of lobules of mature adipocytes, with focal fat necrosis. Focal haemorrhage and scattered foci of hematopoeitic elements including erythroid precursors, few myeloid cells and occasional megakaryocytes were seen. Normal adrenal gland tissue was present in the periphery. There was no capsular breach or any other evidence of malignancy in the sections examined. Based on these findings, a diagnosis of adrenal myelolipoma was given.

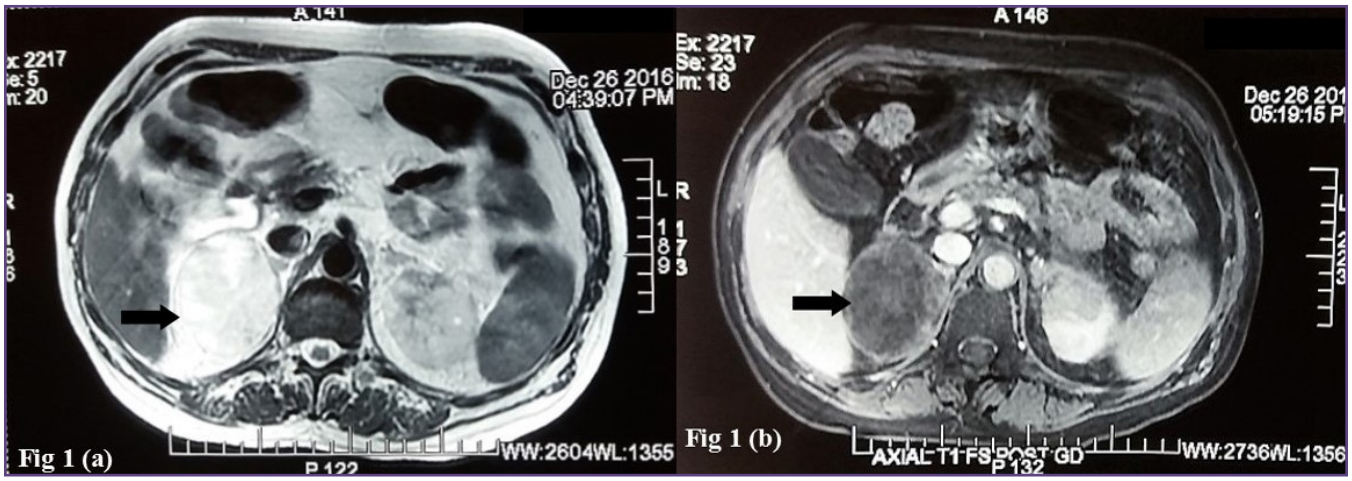

Fig. 1 (a) : MRI features of myelolipoma - hyperintense mass (arrow) in right suprarenal in T1 image.; (b): MRI features of myelolipoma - Loss of signal intensity in the lesion (arrow)with fat suppression on T1 MRI.

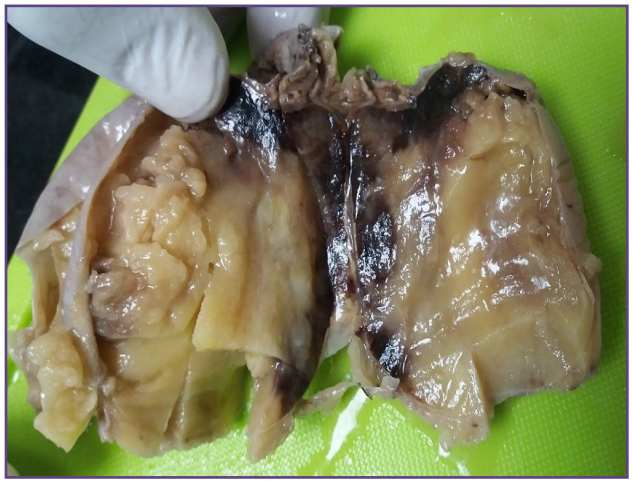

Fig. 2: Gross specimen showing encapsulated fat rich lesion with variegated areas.

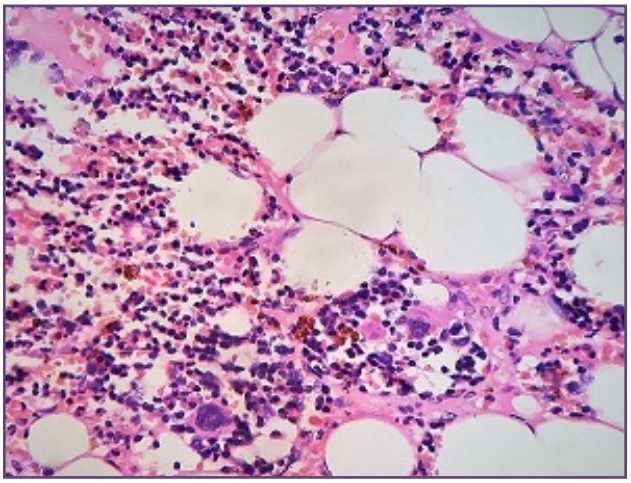

Fig. 3: H&E 40x showing adipose tissue admixed with trilineage haematopoeisis including megakaryocytes.

Discussion

Adrenal myelolipomas are rare tumours, constituting 2.6-6% of primary adrenal tumours.[2-4] They are being reported more frequently due to increase in use of imaging investigations.[1,3] They may be detected as an incidental finding in imaging studies, done for other reasons. [3] Sometimes adrenal myelolipoma may be symptomatic, presenting with pain, palpable abdominal mass, hypertension, endocrine disorders. [3,5,6] Large tumours may rupture, leading to intraabdominal haemorrhage and may present with shock. [6]

Adrenal myelolipoma may be associated sometimes with endocrinological disturbances such as hyperaldosteronism, hypercortisolism, or hyperandrogenemia. There may be associated clinically apparent hormonal imbalance due to adrenocortical dysfunction in some cases, and aldosterone hypersecretion may be induced by some factor released by adrenal myelolipoma or due to pressure effects on the adjacent gland by tumor. However, further observation is required to investigate the association of endocrinological imbalances with adrenal myelolipoma. Compression of the surrounding tissue by the tumour may also lead to adrenocortical abnormalities in some cases. There may be associated hyperplasia of adrenal gland which may result in endocrinological disorder. [6] Endocrinological work up may be helpful in excluding functional myelolipomas. Gershuni et al recommend evalulation of fractionated plasma metanephrines to exclude pheochromocytoma before proceeding for surgery. Cortisol evaluation and further hormonal workup may be considered depending on the clinical scenario. [2] Our case was clinically asymptomatic and the patient did not show any evidence of endocrinological abnormality on investigations.

Imaging techniques may be the first to point towards an incidental adrenal tumour. Adrenal myelipomas can be radiographically detected as well circumscribed lipomatous lesions with heterogenous density.[1-3] Adrenal myelolipoma shows heterogenous echogenicity on ultrasonography. [7] CT imaging indicates the suprarenal location, and shows characteristic appearance with a well circumscribed lesion with heterogeneous enhancement containing a fatty component. [3,7] On Magnetic resonance imaging there is increased signal intensity in fatty areas on T1 weighted images. T2 weighted images show moderate hyperintensity admixed with medium signal intensity due to presence of haematopoetic elements. [1] Fat suppression images allow better characterisation of lesion.[8] Myelolipomas are the most commonly encountered lipomatous tumours of adrenal gland. Rarely, they have been reported in the mediastinum, thorax, kidney and presacral region. [9-12]

Other lipomatous tumours that occur in adrenal include teratoma, lipoma, angiomyolipoma and liposarcoma. [3] Adrenal adenoma and adrenal carcinoma may also enter the differential diagnoses based on imaging findings, and it may not always be possible to make a definitive diagnosis on imaging in cases with lesser amount of fat component. [13]

On pathological examination, adrenal myelolipomas are macroscopically encapsulated with presence of fatty areas and tan brown areas, sometimes with haemorrhagic foci. [14] Size may vary from small subcentimeter tumours to large masses upto 21 cm. [2,3] Microscopically they are composed of adipocytes and haematopoetic elements in varying proportions.[1,13] Adjacent adrenal tissue is usually evident. [1,14] Larger tumours may show haemorrhage, necrosis, cystic change, calcification, ossification. [14]

For large tumours (>=6cm) or symptomatic tumours, surgical excision may be performed. [14] Smaller and asymptomatic tumours may be managed with careful patient follow up. Increase in size and atypical radiological features may be considered when making a clinical decision. [2, 14] Recently, laparoscopic adrenalectomy has been shown to be an effective methodology for excision of adrenal myelolipoma with lesser blood loss, shorter duration of post-operative stay. [13] In our case, succesful laparoscopic excision of the adrenal tumour was performed without any complications and uneventful recovery of the patient. Laparoscopic adrenalectomy is a safe approach with favorable outcome in patients where surgical removal is indicated. [2,13]

Conclusion

Adrenal myelolipomas are unusual, benign adrenal lesions that are may be detected as an incidental finding following imaging studies. Characteristic imaging findings may allow a presumptive diagnosis. Careful work up is recommended including hormonal evaluation before making a clinical decision. Surgical excision may be considered in larger tumours, to prevent development of future complications. Laparoscopic surgery is an effective modality with good patient outcome and minimal complications.

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Case Report

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CyclinD1 Positive High-Grade Endometrial Stromal Sarcoma: A Fascinating Entity!

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ABSTRACT

The 2014 WHO classifies endometrial stromal tumours into endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS), high-grade endometrial stromal sarcoma (HGESS), and undifferentiated uterine sarcoma (UUS). LGESS and HGESS are histomorphologically, immunohistochemically and genetically distinct from each other.

A 51-year-old postmenopausal lady presented to us with vaginal bleeding. Radiological findings revealed a well defined heterogeneous lesion involving the whole of uterus. Hysterectomy revealed a large polypoidal tumour, occupying the entire uterine cavity. Microscopically, the tumor was predominantly composed of epithelioid cells with very few intervening spindle cell areas. Immunohistochemically, epithelioid cells were diffusely positive for cyclinD1, while were negative for CD10, ER, PR. Diagnosis of cyclinD1 positive HGESS was rendered. This case highlights the importance of performing cyclinD1 immunostaining in diagnosing HGESS.

Keywords: *CyclinD1, Endometrial Stromal Sarcoma, High-grade*

Introduction

Endometrial stromal tumours (EST) of the uterus are uncommon tumours, which account for less than 2% of all uterine tumours. The 2014 WHO classifies these tumours into endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LGESS), high-grade endometrial stromal sarcoma (HGESS), and undifferentiated uterine sarcoma (UUS). [1–3] Out of all these entities, HGESS is unique and has gone through several modifications since the earliest study by Norris and Taylor. [4] This entity was removed from WHO 2003 classification, however, with progress in molecular understanding of ESSs, and identification of *YWHAE-NUTM2A/B* (previously known as *YWHAE-FAM22A/B*) gene fusion, HGESS has again been reintroduced in the updated 2014 WHO classification of uterine mesenchymal tumours. LGESS and HGESS are not only histomorphologically and genetically distinct but also express different immunomarkers. While LGESS usually expresses strong CD10, ER, PR; HGESS is typically negative for CD10, ER, PR.[3] Although molecular confirmation (RT-PCR or FISH analysis) currently represents the standard to establish a definitive diagnosis of *YWHAE- NUTM2A/B* ESS, these tests are presently offered only at a few centers. Nevertheless, it is necessary to identify HGESS, as these patients may not respond to anti-estrogenic therapy unlike LGESS and the prognosis of *YWHAE*-rearranged cases is intermediate between LGESS and UUS. [3] Lee et al has demonstrated consistent upregulation cyclinD1 in *YWHAE-NUTM2* ESS. [5] Their study also revealed that *YWHAE*-rearranged cases show diffuse ($\geq$ 70%) moderate to strong immunostaining for cyclinD1 in tumour cells. Thus they deducted that cyclin D1 can be used as a surrogate marker for identification of *YWHAE-NUTM2* ESS in appropriate setting.[5] Here we present a case of HGESS, which was diagnosed based upon diffuse cyclinD1 positivity.

Case Report

A 51-year-old postmenopausal lady presented to us with history of off and on vaginal bleeding of 01 year duration. Per abdominal findings were a 12 weeks size mass which was firm, tender with smooth well defined borders.

Radiological Findings: She underwent transabdominal ultrasonography that revealed a bulky uterus measuring 11.5x10x 3 cm and showing heregenous echotexure. Right ovary was also bulky. She also underwent MRI pelvis which disclosed a well defined heterogeneous lesion involving the whole of uterus and measuring 8.3 x 5.9 x 7.5 cm. The lesion was predominantly soft with cystic areas. The lesion was extending upto uterocervical junction. Fat planes between the mass and urinary bladder were illdefined. The right adnexal mass measured 5.6 x 5.2 cm.

She underwent transabdominal hysterectomy with bilateral salpingoophrectomy. During intraoperative examination, a large uterine mass of the size of 12 weeks was noted, with extra uterine extension to right adnexa. The mass was adherent to the bladder and rectum. The mass could not be removed in toto.

Pathological Findings: Grossly, the hysterectomy specimen revealed an enlarged uterus measuring 11.5 x 10 x 3.5

cm. On cutting open, entire uterine cavity was occupied by a large polypoid mass measuring 9x6 cm. The tumour seemed to be involving entire myometrium and overlying serosa. (Figure 1a) The enometrium varied in thickness from 0.1 to 0.2 cm. Cut surface of the tumour was soft and fleshy with few areas of hemorrahge and necrosis. Few areas showing cyst formation were also noted. Tumour grossly involved right parametrium and right adnexa.

Multiple sections from the tumour revealed predominantely large areas of monomorphic proliferation of epithelioid cells in vague nested pattern seperated by delicate curvilinear vasculature. (Figure 1b) Few areas of spindle cell component with fibromyxoid stroma were also noted. (Figure 1c) The epithelioid cells showed moderate amount of eosinophilic cytoplasm with irregular nuclear contour, fine evenly dispersed chromatin with nuclear clearing and lack of prominent nucleoli. (Figure 1d) Mitotic count was 10-12/10 HPF. Areas of necrosis were seen. Extensive sampling (total 15 sections from tumour) did not reveal any carcinomatous component.

On Immunohistochemistry, epithelioid tumor cells were negative for CD10, broad-spectrum CK, EMA, desmin, smooth muscle actin (SMA), caldesmon, ER, PR, LCA, CD43, inhibin, CD99, WT-1, HMB-45, c-Kit/ CD117, DOG1, and p53. Epithelioid tumor cells exhibited diffuse nuclear staining for cyclinD1, which was negative in areas of spindle cell component (Figures 2a-d). However, spindle cell component exhibited positive staining for CD10.

Based upon histomorphology and immunohistochemistry, diagnosis of cyclinD1 positive high-grade endometrial stromal sarcoma (HGESS) was finally rendered. Post-operatively, the patient underwent 40Gy/28# of radiotherapy. She is presently doing well and is under follow-up.

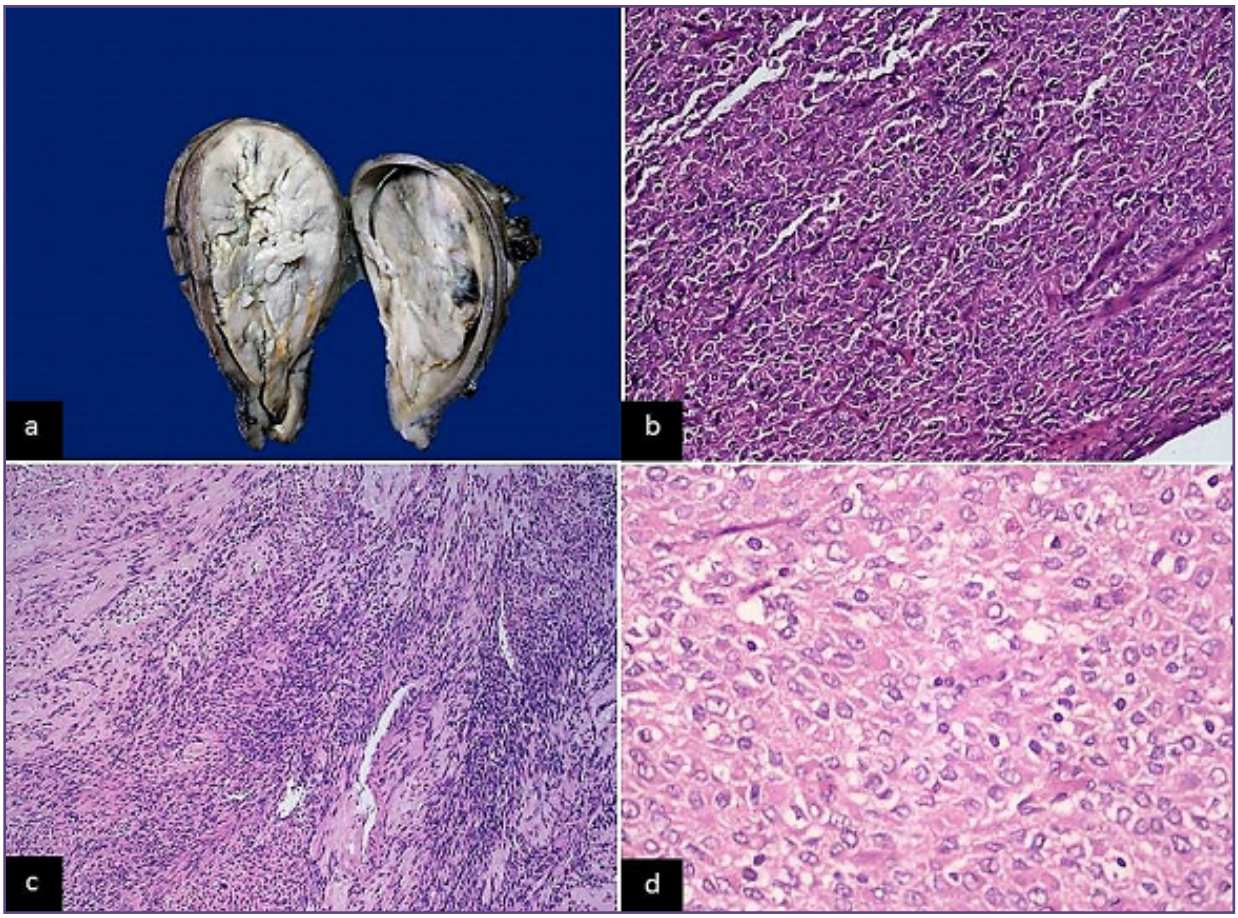

Fig. 1: Endometrial stromal sarcoma, high grade. (a) Gross examination revealed a large polypoidal mass occupying the entire uterine cavity. (b) Infiltrating tumor composed of predominately nested growth of epithelioid cells separated by thin vascular channels. H and E, ×200. (c) Few areas of spindle cell growth with fibromyxoid stroma were also seen. H and E, ×200. (d) The epithelioid cells showed moderate amount of eosinophilic cytoplasm with irregular nuclear contour, fine evenly dispersed chromatin with nuclear clearing and lack of prominent nucleoli. H and E, ×400.

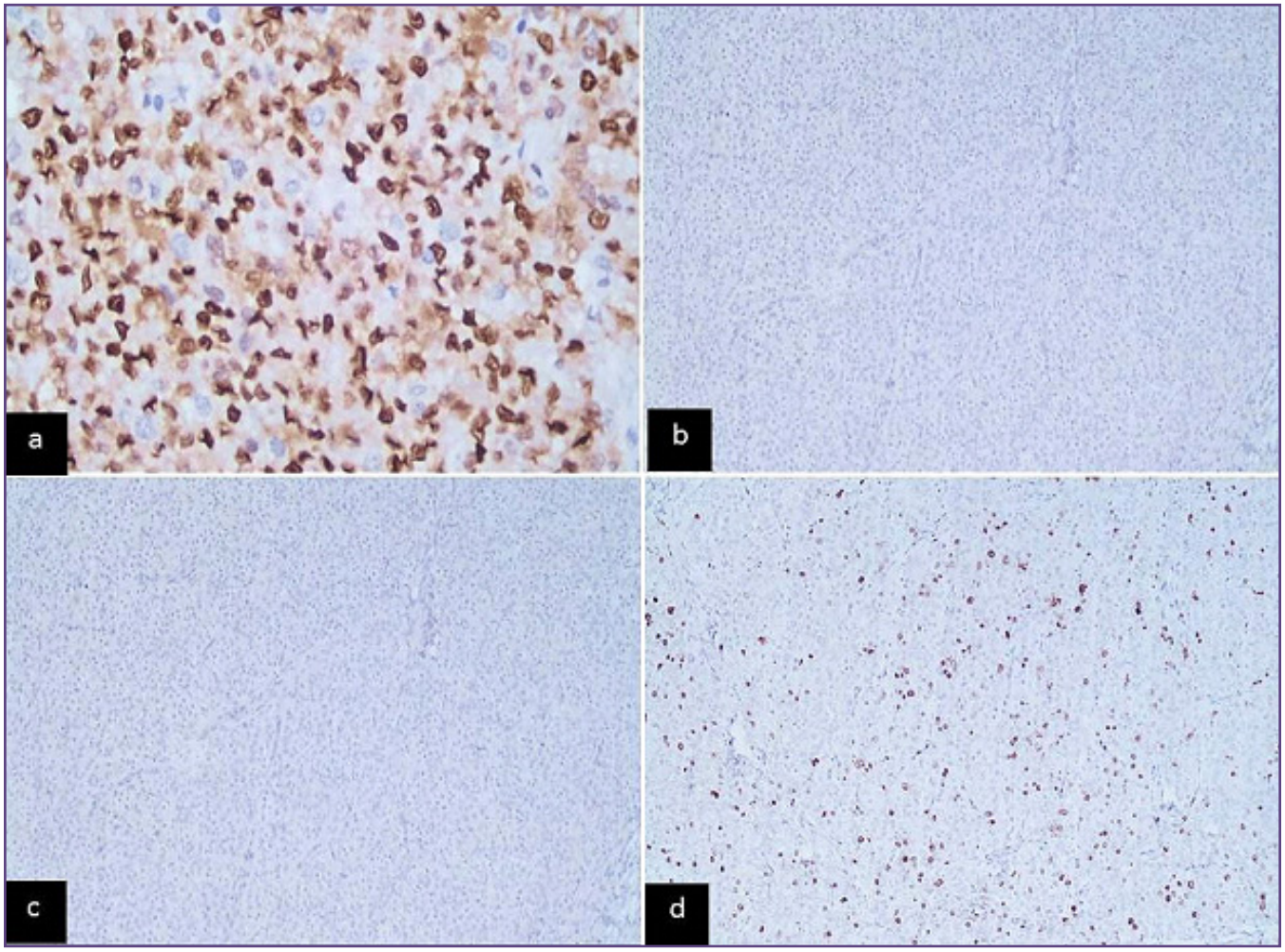

Fig. 2: Immunohistochemistry results of epithelioid component. (a) Epithelioid tumour cells showed strong and diffuse positivity for cyclinD1. Diaminobenzidine (DAB), ×200. (b) CD10 negativity in epithelioid cells. DAB, ×200. (c) ER negativity in epithelioid cells. DAB, ×200. (d) MIb-1 labelling index was 20-25%. Diaminobenzidine (DAB), ×200.

Discussion

Endometrial stromal sarcomas accounts for approximately 0.2% of all malignant uterine tumors and 10–15% of uterine sarcomas. These tumours frequently occur in women between 40 and 55 years of age, as seen in the present case.[6]

Norris and Taylor, in 1966 first classified EST into ESN, LGESS, and HGESS.[4] The subdivision into low-grade (<10 mitosis/10HPF) and high-grade(≥ 10 mitosis/10 HPF) was based on mitotic count. They studied necrosis and cytological atypia but found these to be prognostically not relevant. However, further studies confirmed that even mitotic count was not prognostically significant. Consequent to this, the 2003 WHO classification removed the category of high grade ESS and reclassified these tumours into 'ESS' (low-grade tumours with histological resemblance to proliferative endometrial stroma) and 'undifferentiated endometrial sarcoma (UES)' (pleomorphic tumours with no resemblance to endometrial stroma). Problem with the 2003 WHO classification was that not only UES was a heterogeneous category comprising of tumours with different clinical behavior but it was also silent on categorization of tumours with components of high-grade and low-grade ESS. Further molecular studies showed that ESSs was characterized by translocation involving chromosomes 7 and 17 [t(7; 17)(p15; q21)], leading to fusion of JAZF1/JJAZ1/SUZ12, however, only 50-60% of UES cases demonstrated this translocation. [6,7] Lee et al in 2012 described a novel genetic fusion between *YWHAE* and *FMS22A/B* (now *NUTM2A/B*) in ESS harbouring translocation involving chromosome 10 and 17 [t(10;17)(q22;p13)] and associated clinicopathological features. [8] Of the 11 *YWHAE*-rearranged primary uterine tumors

described by them, 7 contained a mixture of round cell and spindle cell areas, whereas 3 and 1 showed a purely round cell and purely spindle cell appearance, respectively. The round cell component described was highly cellular, and the tumor cells were typically arranged in a vaguely nested growth pattern, with the nests being separated by a delicate stromal capillary network. The round cells were epithelioid in appearance with scant to moderate amount of eosinophilic cytoplasm, had irregular nuclear contours with inconspicuous nucleoli. These tumours showed brisk mitosis and areas of necrosis. Our case also demonstrated high - grade round cell component with characteristic nuclear features, brisk mitosis and areas of necrosis.

Immunohistochemically, the high-grade round cell component of the tumour shows diffuse strong nuclear staining for cyclinD1, lack of CD10, and weak or absent staining for ER and PR. [6] This is in contrast to LGESS which characteristically show diffuse CD10, ER, PR positivity and weak/patchy cyclinD1 staining. Our case also showed strong nuclear staining for cyclinD1 in more than 70% of epithelioid tumour cells and negative staining for ER, PR, and CD10. Spindle cell component showed CD10 positivity and cyclin D1 negativity.

YWHAE-NUTM2A/B (previously *YWHAE- FMS22A/B*) rearrangement needs to be confirmed by molecular tests such as reverse transcription polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH) analysis. However, in limited resource settings, these tests may not be available at all the centers. In this scenario, the distinctive morphological and immunohistochemical features of HGESS are generally good surrogate markers for *YWHAE*- rearrangement. In fact, Lee et al observed diffuse cyclinD1 positivity in YWHAE-rearranged ESS cases with sensitivity of 100% and specificity of 99%. [5] Genetic analysis could not be performed in our case due to non-availability of the test in our laboratory.

Differential diagnosis in our case included epithelioid leiomyosarcoma, malignant perivascular epithelioid cell tumour (PEComa), undifferentiated uterine sarcoma, and undifferentiated uterine carcinoma. No conventional areas of leiomyosarcoma were seen and tumour cells were negative for SMA, desmin, and caldesmon. PEComa was excluded by absence of immunoexpression of muscle and melanocytic markers. Undifferentiated uterine carcinoma (UUC) can occur in pure form or in combination with low-grade endometrioid adenocarcinoma and show diffuse positivity for cyclinD1. However, they usually show focal/ patchy positivity for EMA/ broad-spectrum CK. In our case, no areas of low-grade endometrioid adenocarcinoma were identified despite of extensive sampling (total 15 sections studied of tumour). The tumour cells showed no immunostaining with EMA/ CK. Adequate tumour sampling is of paramount importance in the cases where UUC and HGESS are in differential diagnosis. This fact was highlighted by Shah et al, in their study of cyclinD1 expression in ten cases of UUC.[10] All the tumours showed cyclinD1 expression, with six cases showing diffuse and strong staining and four cases with patchy staining. Thus, adequate sampling and staining for EMA/broad-spectrum CK cannot be overemphasized to rule out any carcinomatous component.

UUS is a high-grade sarcoma that lacks a specific line of differentiation. Histologically, these have been classified into uniform (UUS-u) and pleomorphic (UUS-p) type. [9] Out of these, UUS-u can show morphological and immunohistochemical overlap with HGESS. However, finding of CD10 positivity in UUS-u usually excludes HGESS. [4]

It is important to diagnose HGESS, because these patients usually present with advanced stage disease (stages II–IV) and frequently have recurrences, usually within a few years after initial surgery. Anti-estrogenic therapy is likely ineffective given the lack of ER and PR immunopositivity in the high-grade component. Furthermore, although experience is limited, adjuvant therapy may provide survival benefit. [3]

Conclusion

In high grade uterine mesenchymal tumours showing round cell component in isolation or with spindle cell component, revealing absent CD10 immunoexpression, an extended panel of immunohistochemistry, including cyclinD1 should be added, before labelling the tumour as undifferentiated uterine sarcoma. This has prognostic as well as therapeutic implications.

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Letter to Editor

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Cytokeratin Expressing Oncocytic Variant of Gastrointestinal stromal tumor : A Morphological Mimicker of an Epithelial Malignancy

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Keywords: *cytokeratin, oncocytic, gastrointestinal stromal tumor*

Dear Sir

A 61 year old male presented with abdominal swelling and vague abdominal discomfort since 1 month. His laboratory parameters were within normal limits. CT scan revealed a large, hypervascular, heterogeneously enhancing mass within the abdominal cavity displacing the intestinal loops.

Excision of the mass was performed and grossly it was a large well circumscribed mass measuring 16.5 x 10 x 8 cm, cut section of which was grey white solid with a firm consistency. This mass was attached to the small intestinal loops. Histopathological examination revealed an encapsulated tumor composed of diffuse sheets of polygonal to ovoid cells with well defined cell membranes, abundant pale eosinophilic cytoplasm, centrally placed ovoid nucleus, fine chromatin and small conspicuous nucleoli(figure1). Mitotic activity was 8-10/50hpf. Necrosis was not present. These tumor cells were limited to the submucosa, muscularis propria and serosa of the small intestine. No mucosal involvement was present.

The following differentials were considered: GIST (oncocytic type), metastatic hepatocellular carcinoma, metastatic eosinophilic variant of clear cell RCC, metastatic hurthle cell carcinoma thyroid, metastatic sex cord stromal tumor, melanoma. A wide IHC panel including the markers CD117, CD34, DOG 1, CK, EMA, S100, glypican, PAX 8, inhibin was applied. The tumor cells revealed positivity for, CK (cytoplasmic granular) CD117, DOG1,CD34 (figure 2:A-D), while negativity for S100, EMA, glypican, PAX 8 and TTF1.A diagnosis of oncocytic variant of gastrointestinal stromal tumor, high risk, was rendered considering the histomorphological as well as immunohistochemistry profile.

What was unique in this case was the morphology: oncocytic tumor cells with abundant eosinophilic cytoplasm mimicking an epithelial malignancy; what was even more mystifying was the cytokeratin expression in tumor cells creating a confusing picture. However DOG1, CD117 and CD34 positivity helped in labelling the neoplasm as oncocytic variant of GIST. The tumor cells may mimic any oncocytic tumor metastasising from different sites like liver, kidney, thyroid gland or testis.

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract.[1] Most GISTs exhibit a spindled, epithelioid, or mixed (spindle/epithelioid) cell morphology.[1] Oncocytic variant characterised by the presence of abundant mitochondria has been described in literature.[2] CK expression in GIST is a rare manifestation, that may lead to diagnostic difficulty and errors as they can easily be mistaken for other epithelial or epithelioid mesenchymal tumors.[3] Aberrant expression of CK is identified to be a consequence of aberrant synthesis of CK by tumor cells or cross-reactivity to other intermediate filament proteins.[4] CK expression in GISTs may be a phenomenon related to tumor progression, akin to meningioma where CKs are preferentially expressed in high grade tumors and malignant melanoma where CK positivity is perceived more often in metastatic rather than primary melanoma.[5] In such problematic cases, a diagnosis of GIST can be made when DOG1 immunoreactivity or mutation of KIT or PDGFRA are witnessed.[3]

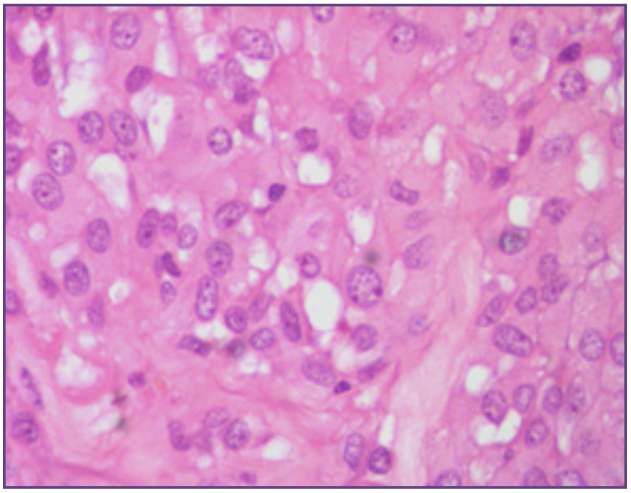

Fig. 1: photomicrograph showing a tumor composed of diffuse sheets ofpolygonal to ovoid cells with well defined cell membranes, abundant pale eosinophilic cytoplasm, centrally placed ovoid nucleus, fine chromatin and small conspicuous nucleoli,H&E,400X.

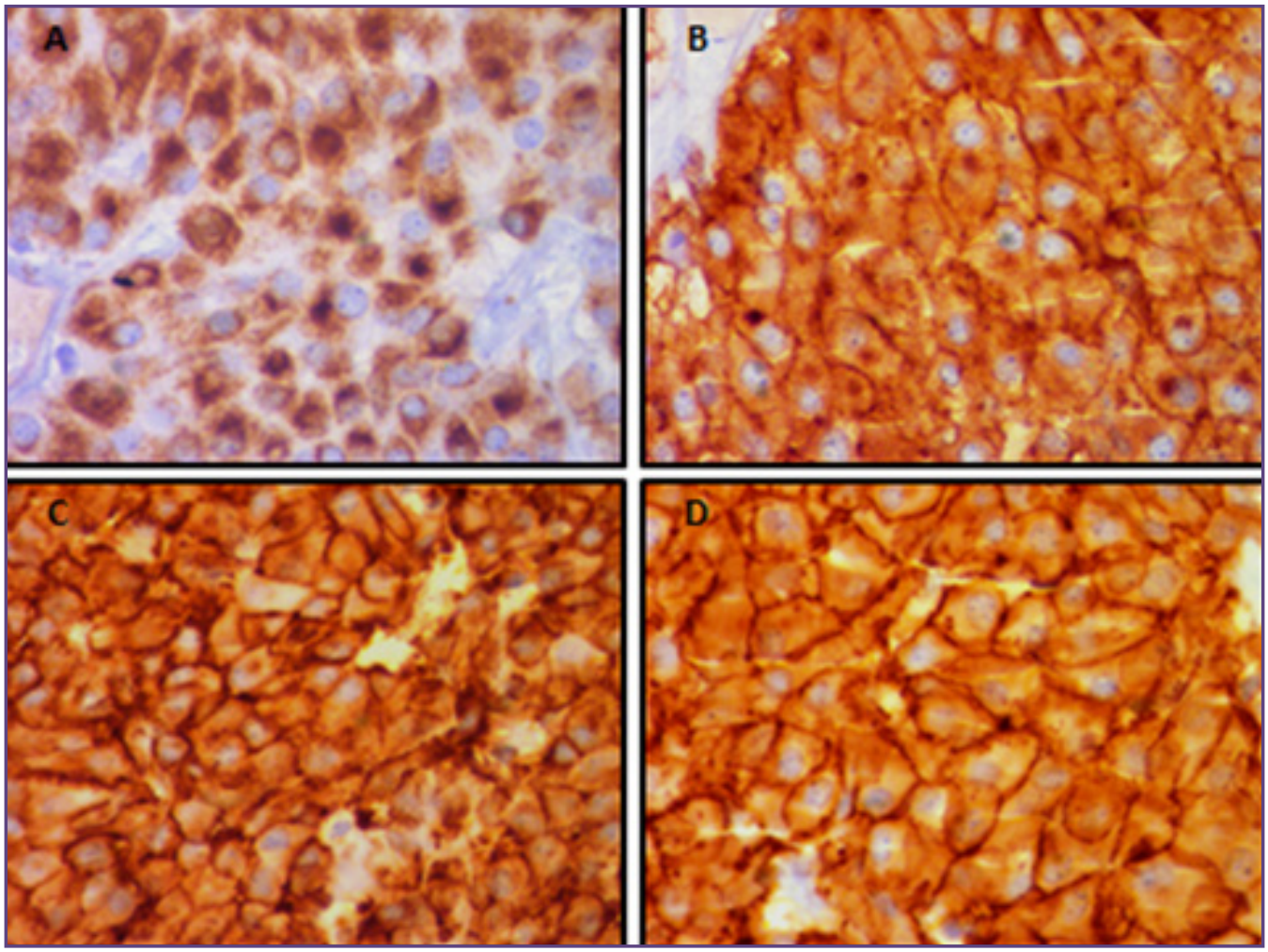

FiG.2:A:photomicrographshowingtumorcellsexpressingcytokeratin(cytoplasmicgranular),CKIHC,400XB:photomicrograph showing tumor cells expressing CD117(cytoplasmic and membranous),CD117 IHC,400X C:photomicrograph showing tumor cells expressing DOG1(cytoplasmic and membranous),DOG1 IHC,400X D:photomicrograph showing tumor cells expressing CD34(cytoplasmic and membranous),CD34 IHC,400X Figure2:A:photomicrograph showing tumor cells expressing cytokeratin(cytoplasmic granular),CK IHC,400X B:photomicrograph showing tumor cells expressing CD117(cytoplasmic and membranous),CD117 IHC,400X C:photomicrograph showing tumor cells expressing DOG1(cytoplasmic and membranous),DOG1 IHC,400X D:photomicrograph showing tumor cells expressing CD34(cytoplasmic and membranous),CD34 IHC,400X.

In conclusion, cytokeratin expression in GISTs more so with an epithelioid/oncocytic morphology is an unwarranted diagnostic pitfall, especially in high grade GISTs with limited biopsy material and from metastatic sites, thereby necessitating the use of molecular analysis or comprehensive immunohistochemistry for difficult cases.

Abbreviations and Symbols

CT: computed tomography GIST: gastrointestinal stromal tumor RCC: renal cell carcinoma IHC: immunohistochemistry CD: cluster of differentiation DOG1: discovered on GIST CK: cytokeratin S100:100% soluble in ammonium sulphate at neutral PH PAX 8: paired box gene 8 TTF1: thyroid transcription factor 1 EMA: epithelial membrane antigen PGDFRA: platelet derived growth factor

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I would like to thank Dr. Anurag Mehta, who is the head of department, pathology, RGCIRC, for his encouragement and support.

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Letter to Editor

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Detection of Microfilaria on fine Needle Aspiration from Breast Lump: An Uncommon Finding

Anchit Goel, Roopak Agarwal, Natasha Singh, Jyoti Mishra* and Geeta Deshmukh

Department of Patholo-gy, School of Medical Sciences and Research, Sharda Hospital, Great-er Noida, Uttar Pra-desh, India

Dear Sir,

We report a case of 18 year old female, who presented with ill defined breast lump and was diagnosed with microfilaria along with fibroadenoma.

An 18 year old female presented to the surgical OPD of Sharda Hospital, Greater Noida, with ill defined lump in lower outer quadrant of right breast measuring 1x1cm. It was tender and firm in consistency and was not freely mobile. The axillary lymph nodes were not palpable. The overlying skin did not show discoloration, any ulceration and there was no nipple discharge. The patient was for FNAC to department of pathology. Fine needle aspiration from breast lump yielded a scant blood mixed aspirate. Microscopically, the smears revealed a microfilaria lying near a small cluster of benign ductal epithelial cells. The microfilaria was ensheathed and the nuclei were arranged within the long axis and spared the tip ends. (Figure 1a, c) The ductal epithelial cells were disposed in small tight clusters. The cells were mildly enlarged and showed moderate amount of cytoplasm. (Figure 1b, c) The background showed fibromyxoid stroma along with mild inflammatory infiltrate.

Microfilaria is a major public health problem in tropical and subtropical countries and is an endemic problem in India. Despite its high incidence it is unusual to find microfilaria on cytological smears. Mammary filariasis is rare. There are only few reported cases of coexisting microfilaria with breast lesions. Sane KC et al. and Khare P et al., reported a microfilaria in association to fibroadenoma in a 20 year old female and in two patients of breast lumps respectively. [1, 2] Nalini et al., have reported a case of filariasis of breast masquerading a fibroadenoma clinically. [3] Mitra et al., in their FNAC study found filariasis in 8 cases of breast. Out of these, eosinophilia and microfilaraemia was present in 8 and 3 cases respectively. [4] Singh NG et al, reported a case of breast lump showing microfilaria and filarial worm along with marked inflammatory infiltrate on cytological smears. [5] In contrast to these studies in our case there was sparse inflammatory cell infiltrate only. The cytological smears had no haemorrhage which ruled out contamination from the blood. No parasite was detected on peripheral blood smears. The possible explanation for this unusual occurrence is the lodgement of the parasite in the vessels of breast, including lymphatics.

The reporting pathologist while performing FNAC from sites such as breast, thyroid, and lymph nodes while screening the slides; should keep the possibility of filariasis in mind even in the absence of dense inflammatory infiltrate so that specific treatment can be administered to avoid complications associated with lymphatic filariasis.

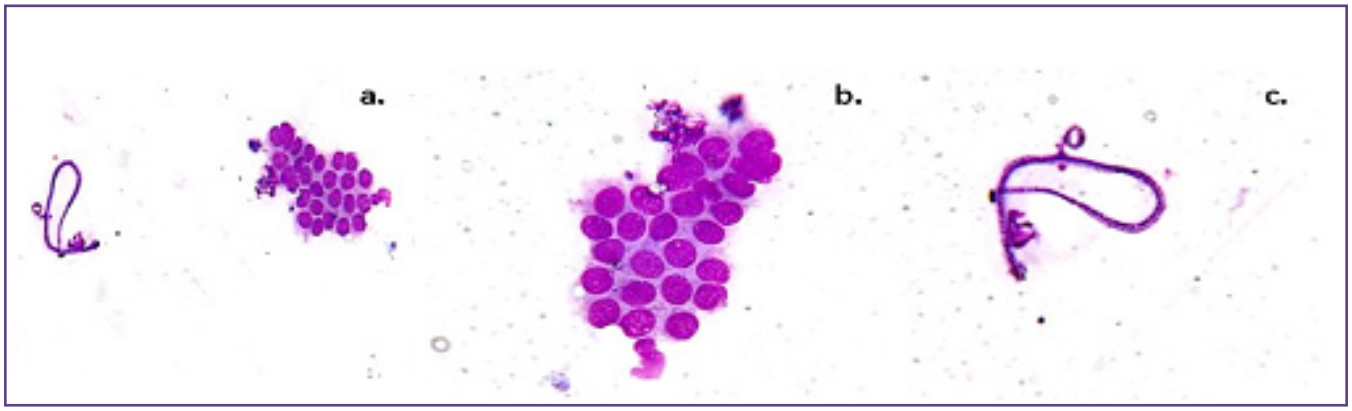

Fig. 1a: FNAC breast showing monolayered cluster of benign ductal epithelial cells with microfilaria, (Wuchereria bancrofti) (Giemsa, 40X). Figure 1b: Smears showing monolayered cluster of benign ductal cells (Giemsa, 100X). Figure 1c: Smear showing sheathed microfi-lariae with nuclei dispersed along the long axis. (Giemsa, 100X).

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We thank our patient for her support.

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