Original Article

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Non-Hematological Malignancies Seeding in Bone Marrow: A 6 Years' Experience at Tertiary Care Hospital

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ABSTRACT

Background: Bone marrow is the site of origin for primary haematological malignancies and is the third common preferred site metastasized by the solid tumors. The malignant infiltration of the hematopoietic tissue alters the clinical course of disease, response of the treatment and influences the overall survival. The aim of this study was to assess pattern of bone marrow involvement by different solid tumors and their correlation with hematological parameters.

Methods: In this retrospective study, 8064 bone marrow examinations from Jan 2011-Aug 2017 at tertiary health and research centre of Northern India were evaluated to access spectrum of different solid tumors infiltrating the bone marrow alongwith their clinical, hematological and histopathological findings.

Result: Total 38 cases of non-hematological malignancies metastasizing to bone marrow were evaluated with main indications of lytic lesions, cytopenia and Pyrexia of Unknown Origin. The most common metastasis were adenocarcinoma of prostate and lung. In 33 cases, the clinical, cytomorphological and immunohistochemical analysis findings were correlated to know primary site, while in remaining five patients, even after complete diagnostic evaluation, the definitive origin could not be ascertained, therefore categorized as Carcinoma of Unknown Primary Site (CUPS).

Conclusion: Our series showed that anemia as commonest parameter, followed by leukopenia, thrombocytopenia. Many cases were misdiagnosed as multiple myeloma due to lytic lesion, anemia and hypercalcemia. We concluded that unexplained cytopenia are strong indicators of bone marrow examination; an easy, convenient, sensitive, effective procedure of staging of tumor, monitoring the course and prognosis of solid tumors.

Keywords: *Bone marrow, Metastasis, Solid tumours, Immunohistochemistry, Adenocarcinoma, Carcinoma of Unknown Primary Site (CUPS)*

Introduction

Bone marrow aspiration (BMA) and bone marrow biopsy (BMB)are well established procedures to diagnose hematological disorders for staging, prognosis and diagnosis of various solid tumors. Bone marrow infiltration can lead to varied cytopenia which can scale down the chemotherapy dose or alter the schedule.

Lung and liver are usual metastatic sites for solid tumours whereas bone marrow stands at third position by the infiltration of prostate, lung, breast kidney, thyroid and gastric carcinomas in adults and neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma and retinoblastoma in children.[1]Metastasis to bone marrow causes consequences of it as well as change in therapeutic decisions.[2-6]It is therefore considered imperative to rule out marrow involvement in any curative malignancy or where autologous transplantation is an option.[7]

At times, demonstration of tumor cells in the marrow might be the first available evidence of malignant solid tumors.[8-10] The recognition of metastasis in random biopsies presents challenges to clinicians and pathologists in cases of CUPS.[4,10] In such scenarios clinical, radiological findings and other laboratory tests plays a major role in evaluating cases with bone marrow metastasis. Clinically these patients may present with bony pain, pathological fracture, breathlessness and radiological lytic or sclerotic lesions present on X-ray. Other frequent laboratory findings are hypercalcemia and elevated serum alkaline phosphatase. However, in day today reporting, it is often seen that metastasis may be present in the bone marrow without above mentioned abnormalities. In those cases, other sensitive technique plays a vital role for the detection of bone marrow metastasis.[6,11] The meticulous use of immunohistochemistry (IHC) with new evolving markers plays an important role

in short listing the primary lesion with high sensitivity. The aim of this study is to observe the clinical presentations of solid malignancies and cryptic lesions metastasizing to the bone marrow and their correlation with haematological findings. This also highlights the importance of this easy and cost-effective method of reporting of metastasis in a very short span of time.

Materials and Methods

We performed a retrospective analysis of bone marrow aspiration and biopsies involved by the metastatic solid tumours that were diagnosed at tertiary health and research centre of Northern India from Jan 2011-Aug 2017. Various parameters like patient's clinical history, physical findings including age, sex, duration of illness and provisional diagnosis were also incorporated for evaluation. Paediatric population were defined as patients with an age group of less than 12 years. While bone marrows involved by the metastatic deposits were included, those which were involved by lymphoma deposits were excluded.

All of the trephine biopsies were performed from the same site i.e. posterior iliac crest using a Jamshidi aspiration and trephine needle. Length of the biopsy cores ranged between 1.0 cm and 2.5 cm. Trephine biopsies were sent in picric acid and then fixed and decalcified overnight to 24 hrs in a in 10% neutral buffered formalin, followed by automated tissue processing, embedding, and cutting of sections to 0.3-0.5 micrometer. Bone marrow aspiration/touch preparations were stained with May-Grunwald-Giemsa stain. Bone marrow biopsies were stained with Hematoxylin-Eosin (H&E) to evaluate cytomorphology and reticulin stain to access the associated fibrosis.

Wherever morphology alone could not able to ascertain the type of metastasis, immunohistochemistry for pancytokeratin, Leukocyte Common Antigen (LCA) and vimentin were done. For an epithelial tumor, cytokeratin (CK-7, CK-20, CK8/18), Carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), Napsin, TTF-1, Hepar1 and CDX2 were used.

Two ml blood sample was collected in EDTA for evaluation of complete blood picture and peripheral smear. Leishman's stained peripheral blood smears were examined under microscope.

Result

Total 38 cases of solid metastatic tumour deposits were detected in 8064 bone marrow examinations, performed for the various indications during the period 2011-Aug 2017. Among these cases, only four were of paediatric age group (10 months, 2 years and 2.5 years and 6 years) and incidentally all were male. Three of these paediatric cases were diagnosed as neuroblastoma and fourth one as small cell neuroendocrine tumour. Other 34 adult cases had median age of 62 years (range 21-86 years) and comprised of 24 males and 10 females. Overall there were 28 males (73.7%) and 10 females (26.3%). Clinical indications of these metastatic cases for bone marrow examination varied from multiple myeloma, pyrexia of unknown origin, lytic lesion with hypercalcemia and anaemia [Table: 1]. Among these cases, 14 were presented with known primary solid tumours, while remaining 24 cases had different clinical diagnosis. The haematological profile showed anaemia (36 cases; 94.7%) as most common clinical presentation followed by leukopenia (13 cases; 34.2%), thrombocytopenia (12 cases; 31.5%), bicytopenia (10 cases; 26.3 %), leukoerythroblastic picture (8 cases; 21.0%) and pancytopenia (7 cases; 18.4%) respectively. Mean haemoglobin level of 8.4 gm/dl (range: 5-16 gm/dl), mean TLC counts of 8066.57/cumm (range 2100-64810/cumm) and mean platelets counts of $162.3x10^3$/cumm (range 80-230 $x10^3$/cumm) were present. Adenocarcinoma (29 cases; 76.3%) constituted as most common type of metastatic deposit and immunohistochemistry (IHC) were done in 23 cases to ascertain the primary origin [Table: 2]. Morphological appearance, IHC and more sensitive advanced radiological tools (Computed Tomography scan/Magnetic Resonance Imaging/Positron Emission Tomography) were used to reveal the primary site in 19 out of 25 cases. Among adenocarcinoma most common subcategories metastasised to bone marrow were adenocarcinoma lung and prostate each comprising 6 followed by 5 cases of CUPS and 4 cases of carcinoma breast, 3 cases of renal cell carcinoma and one case each of gall bladder, rectal carcinoma, carcinoma ovary, carcinoma pancreas and carcinoma colon respectively. [Table: 3].

Table 1: Clinical Indications for Bone Marrow Examinations.

Clinical Indications	Number of cases	Percentage of cases
Multiple myeloma	6	15.8%
Carcinoma lung	4	10.5%
Carcinoma prostate	3	7.9%
Bicytopenia	2	5.3%
Non-Hodgkin's Lymphoma	2	5.3%

Clinical Indications	Number of cases	Percentage of cases
Carcinoma breast	2	5.3%
Carcinoma of Unknown Primary Site (CUPS)	2	5.3%
Pyrexia of Unknown Origin (PUO)	2	5.3%
Lytic lesions	2	5.3%
Suprarenal Mass	2	5.3%
Anemia	1	2.6%
Chest mass	1	2.6%
Myelodysplatic syndrome	1	2.6%
Myelofibrosis	1	2.6%
Neuroblastoma	1	2.6%
Osteosarcoma	1	2.6%
Pancytopenia	1	2.6%
Prostate mass	1	2.6%
Subdural hemorrhage	1	2.6%
Carcinoma Rectum	1	2.6%
Pain abdomen	1	2.6%
Total	**38**	

Table 2: Patterns of Diagnosis of Bone Marrpow Metastasis with Immunohistochemistry.

Bone marrow morphological diagnosis	Number of cases	Known primary (Solid tumor)	Diagnosis By IHC
Adenocarcinoma	29 (76.3%)	11 (28.9%)	18 (47.4%)
Neuroblastoma	3 (7.9%)	1 (2.6%)	2 (5.3%)
Squamous cell carcinoma	2 (5.3%)	0	2 (5.3%)
Small cell neuroendocrine carcinoma	1 (2.6%)	0	1 (2.6%)
Osteosarcoma	1 (2.6%)	1 (2.6%)	0
Hepatic carcinoma	1 (2.6%)	01 (2.6%)	0
Gastrointestinal stromal cell tumor	1(2.6%)	1(2.6%)	0
Total	**38**	**15 (39.5%)**	**23 (60.5%)**

Table 3: Subcategories of Primary Solid Malignancies.

Primary solid malignancy	Number of cases	Percentage of cases
Carcinoma lung	6	15.8%
Carcinoma prostate	6	15.8%
Carcinoma of Unknown Primary Site (CUPS)	5	13.1%
Carcinoma breast	4	10.5%
Renal cell carcinoma	3	7.9%
Neuroblastoma	3	7.9%
Squamous cell carcinoma oropharynx	2	5.3%
Small cell neuroendocrine carcinoma	1	2.6%
Hepatic cell carcinoma	1	2.6%
Carcinoma colon	1	2.6%
Sarcoma	1	2.6%
Gastrointestinal stromal cell tumour (GIST)	1	2.6%
Carcinoma pancreas	1	2.6%
Carcinoma ovary	1	2.6%
Carcinoma Rectum	1	2.6%
Ca Gall Bladder	1	2.6%
Total	**38**	

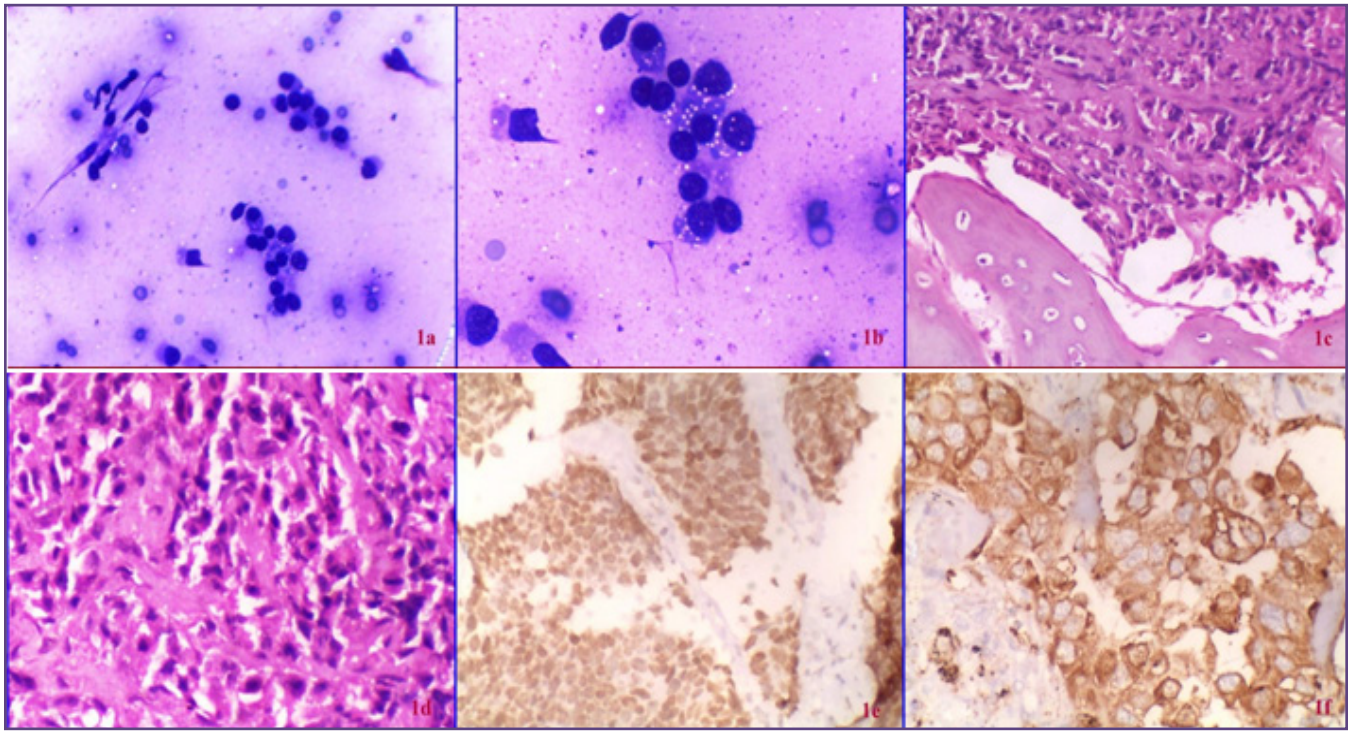

Fig. 1; Microphotographs of May-Grunwald-Giemsa (MGG) stained bone marrow aspirates show few atypical cells in small clusters and vague glandular patterns (1a; 40x, 1b;100x) H & E section of BM biopsy show medium to large sized atypical cells in sheets and glandular patterns (1c;40x, 1d;100x), IHC is positive for TTF1 (1e;40x) and Napsin A (1f;40x).

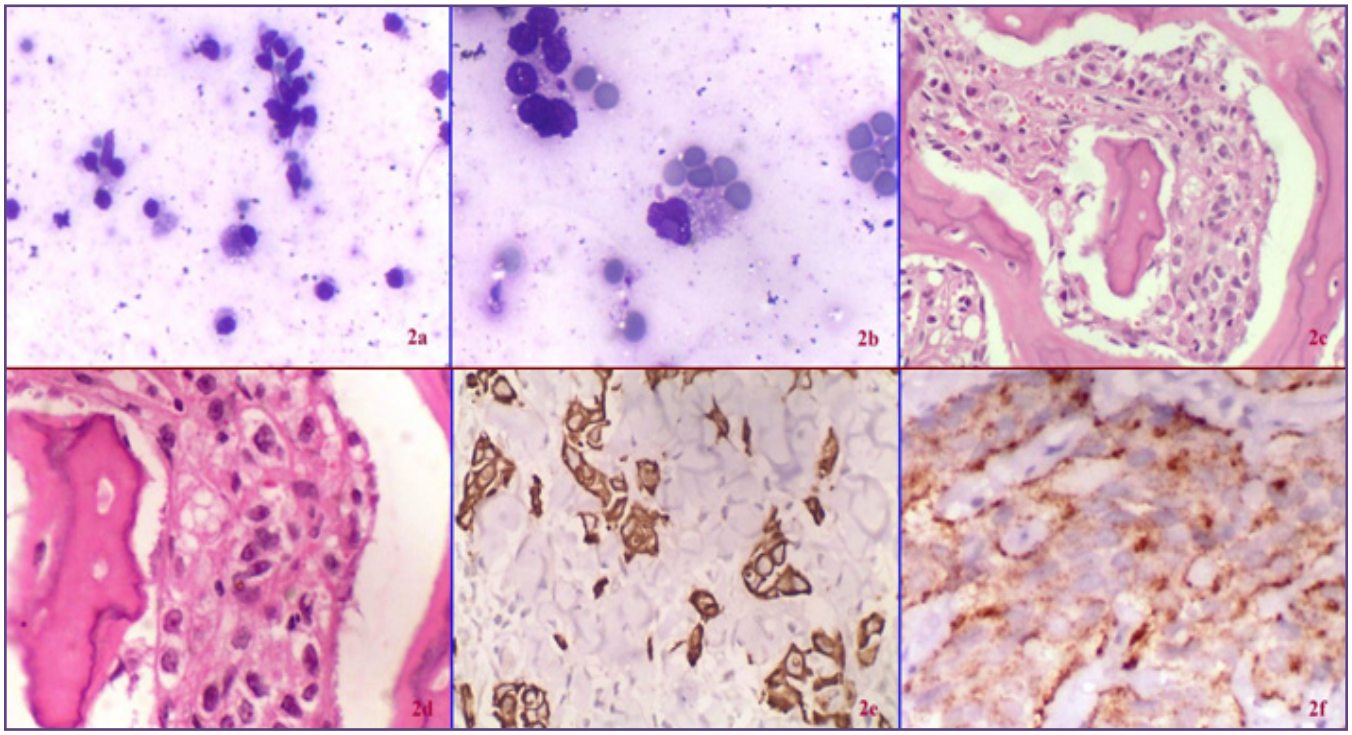

Fig. 2; Microphotographs of MGG stained bone marrow aspirates show few atypical cells in singly scattered and vague glandular patterns (2a; 40x, 2b;100x)H & E section of BM biopsy show medium to large sized atypical cells in sheets and glandular patterns (2c;40x, 2d;100x), IHC is positive for PANCK (2e;40x) and PSA (2f;40x).

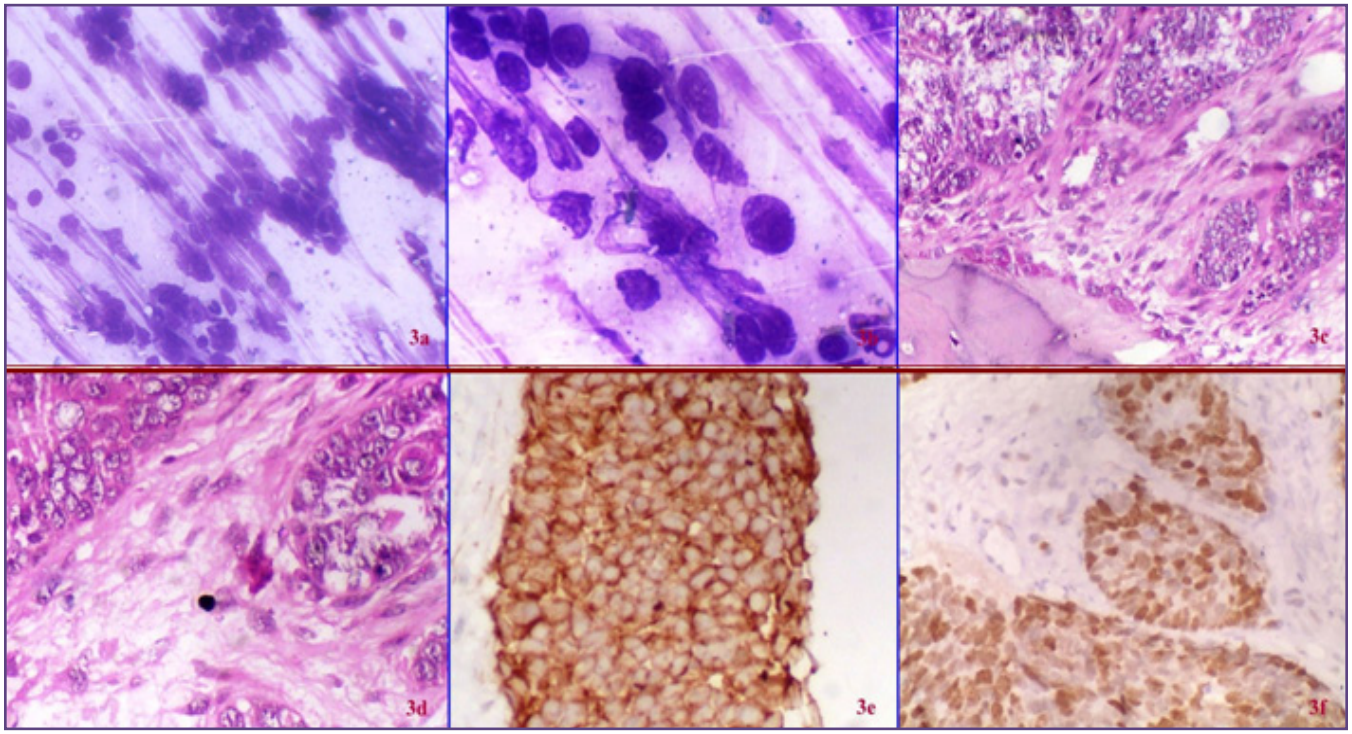

Fig. 3; Microphotographs of MGG stained bone marrow aspirates show few atypical cells having ice bluish cytoplasm arranged in small aggregates and single patterns (3a; 40x, 3b;100x) H & E section of BM biopsy show medium to large sized atypical cells in sheets and nests (3c;40x, 3d;100x), IHC is positive for PANCK (3e;40x) and p63 (3f;40x).

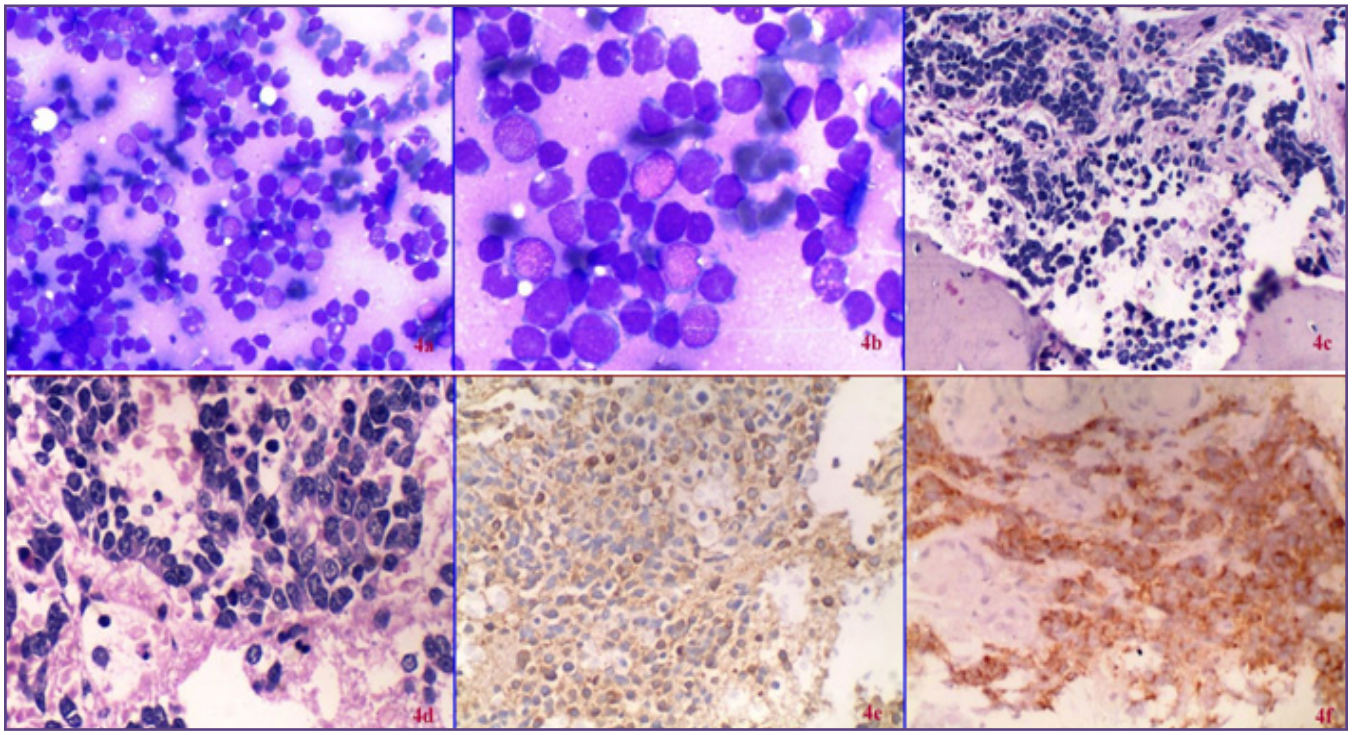

Fig. 4; Microphotographs of MGG stained bone marrow aspirates show numerous atypical cells in small clusters, glandular patterns with prominent nuclear moulding (4a; 40x, 4b;100x) H & E section of BM biopsy show small sized cells with high N:C ratio and crushing artifacts (4c;40x, 4d;100x) IHC is positive for chromogranin (4e; 40x) and synaptophysin (4f;40x).

Discussion

Bone marrow aspirates and trephine biopsies (length > 1cm) are sensitive techniques for detecting bone marrow infiltration.[4]The bone marrow metastasis occurs through the hematogenous spread of tumor cells and affects the normal hematopoiesis leading to myelopthesic anemia and other cytopenia. In advanced stages, this infiltration results in thrombocytopenia and bleeding manifestations. The morphology of metastatic tumor cells should be correlated with the clinical presentation and histopathological features to clinch the final diagnosis. The presence of tumor cells in the bone marrow is considered to be of diagnostic and prognostic importance in the form of metastases and relapse of disease. Mundy GR et al. reported in their study that tumors which commonly metastasized to the bone marrow were from lung, prostate, breast while in paediatric population most common metastasized solid tumour were neuroblastoma. A similar pattern of involvement was observed in our study. Commonly the metastatic cells are manifested in a trephine biopsy, while they were not demonstrated in bone marrow smears. In our analysis of 6 years, we revealed 21 such cases; where in 17 cases bone marrow smears were either dry tap or grossly hemodiluted, while in 4 cases the bone marrow aspirate despite being adequate were negative for malignant deposits. This low sensitivity of bone marrow aspiration can be explained owing to fibrosis, patchy involvement, packed and tight clustering of cells. This also depicts an importance of adequate bone marrow biopsy.

Bone marrow examination plays a pivotal role for prognosis and diagnosis of cryptic metastatic deposits. In our study, the initial diagnoses of solid malignancies were not suspected in 23 cases. After the diagnoses were revealed in the bone marrow examination, work up of the solid malignancies were started. Out of 23 cases, in 18 cases primary site of origin was revealed with the help of IHC and radiological correlation, thus leading to an early appropriate management. While in 5 cases which comprised all of adenocarcinoma cases, primary site couldn't be ascertained. We in our hospital have facility of utilisation of wide panel of IHC antibodies which helped us in evaluation of cryptic lesions. IHC panel antibody if judiciously used can help other ancillary techniques to find out the primary lesion with confidence. We used step by step approach by first using pancytokeratin, leukocyte common antigen, S-100 to find out the cell type and then with the help of CDX2 (colon, small intestine), CK7, TTF-1 and napsin (lung), Hepar1, arginase (liver), CK-7, ER/PR (breast), CK8/18, CA 19.9, (pancreatobiliary), CD56, chromogranin, synaptophysin (Neuroendocrine) further characterization was done. However, the role of IHC was limited in poorly differentiated malignancies.[12,13]

Clinically besides pancytopenia, the most common complications of skeletal metastases are pathologic fractures, pain, spinal cord compression as the result of vertebral compression fracture or extension of the tumor beyond the epidural space, and hypercalcemia.[14]

Osteolytic metastases in comparison to osteoblastic lesions more likely cause these symptoms and therefore manifest themselves earlier, however we in our study we have experienced that they clinically resembled multiple myeloma so close that lot of time was wasted in various unwarranted tests. In our study there were six patients who were under evaluation for multiple myeloma because of anaemia, hypercalcemia and lytic lesions but on bone marrow examination were found to have metastatic deposits. These symptoms were managed by use of osteoclast inhibitors (bisphosphonates/denosumab). Metastatic bone marrow lesions may not always be symptomatic or just presents with fewer symptoms and are diagnosed incidentally during the initial staging evaluation with normocytic normochromic anaemia as commonest associated finding. Hence a clinical suspicion of metastatic deposit should be kept in mind with various cytopenia. In our study among the haematology parameters anaemia was detected in 94.7% and leukopenia in 34.2% and thrombocytopenia were detected in 31.5% cases respectively which were similar to the observations of Kaur G et al.[15] Syed RM et al studied 124 cases and found anaemia in 71.4% and thrombocytopenia in 45.1% in the metastatic deposits to bone marrow.[16]Sar R et al in his study observed marked thrombocytopenia in 83.3% and leukopenia in 50% cases with metastatic bone marrow involvement.[17]Leukoerythroblastic picture was once considered to be the common manifestation of metastatic bone marrow disease. Various studies have reported variable rates of leukoerythroblastic picture and have concluded that its absence cannot exclude marrow involvement.[4,18-20]In our study, 21% patients had leukoerythroblastic blood picture similar to studies of Kaur G et al and Jonsson U et al.[15,21,22] Variations in leukoerythroblastic picture may be explained by diagnosis of disease at different stages. In our cases we had lower rate of leukoerythroblastic picture probably owing to presence of disease in less advanced stage as evident by 23 cases which were firstly diagnosed after bone marrow examination without having any primary malignancy manifestations. Presence of leukoerythroblastic picture in a known case of solid tumour is a strong indication of the bone marrow involvement by the metastatic deposits.

Similarly, the cytopenia in a known case of solid tumour carries a strong possibility of involvement of metastatic deposits.

Marrow infiltration by metastatic tumours may be focal or diffuse. Reticulin and collagen fibrosis are commonly present and are most marked in areas of greater degree of infiltration. Metastatic tumour deposits lead to increased fibrosis. In our study we found 1 case of grade I/III fibrosis, 22 cases of Grade II/III fibrosis while the rest 15 cases had grade III/III fibrosis. We didn't find any correlation of fibrosis with any specific tumours though some studies have suggested to have increased in carcinomas of the breast, stomach, prostate and lung.[23-25] "Dry tap" is used to describe failure to obtain bone marrow aspirate which may be due to extensive marrow fibrosis and hypercellularity and thus is a major cause of poor sensitivity of bone marrow aspirate. [26,27]

Importance of bone marrow examination in evaluation of metastatic disease also lies in identification of the two primary sites because of their sensitivity to hormonal therapy are breast and prostate. Frequently these two malignancies involve the bone marrow in early stages

Extensive and specific immunohistochemistry panel like prostate-specific antigen (PSA), TTF-1, CDX-2, Hepar1 and CA 19-9 are helpful in establishing primary diagnosis of metastatic prostate cancer, lung, intestines, liver and pancreas respectively as done in the 18 cases of index study. Thus, the cytomorphological bone marrow examination with IHC now can be aided in the protocol wherever there is a suspicion of solid tumour.

Conclusion

Bone marrow examination has been a good modality for staging and monitoring the prognosis and treatment in cases of malignant solid tumours.[7]Theimportance for the metastatic disease lies in its lead role for the primary diagnosis with the help of extensive IHC panel. Cryptic lesion can be revealed in cases of pyrexia of unknown origin, lytic lesions and hypercalcemia especially in the setting of leukoerythroblastic picture, anaemia and unexplained cytopenia.

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Competing Interests

There is no competing interests in the present study

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