

Role of Cytology in Detection of Unsuspected Carriers of Microfilariae: A Retrospective Study in National Capital Region (NCR), India

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ABSTRACT

Background: Filariasis, a global problem, is a major public health issue in India. Despite its high incidence, it is unusual to detect microfilaria in cytological smears, though unexpected detection of microfilariae in fine needle aspiration cytology (FNAC), exfoliative and fluid cytology have been reported previously. The study analyzed the role of cytology in detection of asymptomatic carriers of microfilariae.

Methods: This was a retrospective, observational study and included cases of filariasis from cytological records spanning five years. The epidemiological, clinical, hematologic and radiologic details were noted. The slides were retrieved and examined. Descriptive statistical analysis was utilized.

Result: Filariasis was diagnosed in 0.03% of the total cytology cases studied during the period, constituting 0.04% of FNACs (two cases of inguino-scrotal swellings, two of breast lumps, one of thyroid swelling) and 0.02% of cervicovaginal smears (two cases). None of them was clinically suspected to be filariasis. Radiological examination was also misleading. None of the cases demonstrated raised leukocytosis or microfilaremia and eosinophilia was present in 3 cases (42.9%). Apart from microfilariae of *Wuchereria bancrofti*, eggs were present in one case. This was associated with inflammation in all cases, cell adherence in 42.9% and coexistent hyperplastic and neoplastic conditions in 57.1% cases.

Conclusion: The detection of microfilariae in cytological material in the absence of clinical, radiologic or hematologic suspicion, in an area of relatively low prevalence of filaria, points to the need for a high index of suspicion and careful screening of all smears, as cytology may play an important role in the diagnosis of asymptomatic carriers of microfilariae.

Keywords: *Microfilaria, Filariasis, Fine Needle Aspiration Cytology (FNAC), Exfoliative Cytology*

Introduction

Filariasis is a global problem, involving over 120 million people in tropical and subtropical areas of the world, chiefly in the South-East Asia Region (SEAR) and Africa.

^[1] In India, filariasis is a major public health problem, second only to malaria. It is estimated that approximately 60 million people are infected and approximately 45 million people have clinical manifestations of disease, with at least 6 million attacks of acute filarial disease per year. ^[2] Despite efforts by the government, filariasis is endemic in the eastern region and coastal areas, while the north-western region including Delhi/ National Capital Region (NCR), and the north-eastern states are relatively free from indigenously acquired filarial infection. ^[3]

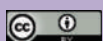
Filariasis is caused by infection with threadlike worms of the family Filarioidea and is transmitted by mosquitoes, generally of the genus *Culex*. Adult worms lodge in the lymphatics of man and damage the lymphatic system, leading to elephantiasis, lymphadenitis, lymphangitis

and hydrocele. They mate and produce larvae known as microfilaria which circulate in the blood, the demonstration of which helps in diagnosis of filariasis. ^[1,3]

Despite its high incidence, it is unusual to detect microfilaria in cytological smears. There have been reports of single or small number of cases of microfilariae in fine needle aspiration cytology (FNAC) various sites such as breast, ^[4-13] lymph nodes, ^[4,6,8-15] scrotum, ^[6,8,9,11] soft tissue swellings, ^[4-6,8,10-13] thyroid, ^[6-8,10-13,15] ovary, ^[16] lung, ^[7,12] liver, ^[4] salivary gland, ^[5,7] gall bladder, ^[7] bone marrow, ^[4,10] spine, ^[7] pancreas ^[12] and eyeball. ^[13] They have also been rarely reported in exfoliative cytology of cervicovaginal smears, ^[13,16] sputum ^[13] or in fluids like ascitic fluid, ^[4,11,13,16] pleural fluid, ^[4,5,13,14,16] pericardial fluid, ^[14,15] bronchial washings, ^[13,15,16] and urine. ^[16]

Materials and Methods

This was a retrospective, observational study spanning five years conducted in the Department of Pathology in a tertiary care centre in the National Capital Region



(NCR), India. Cytological records from July 2012 to June 2017 were retrieved and all diagnosed cases of filariasis were included in the study. In all cases, FNAC had been performed using a 10ml disposable syringe and 22-24 gauge needle. Few slides were air-dried and stained by May-Grünwald Geimsa (MGG) stain and few were fixed in 95% ethanol and stained by Hematoxylin and Eosin stain. Cervicovaginal smears had been stained by Papanicolau (Pap) stain. The epidemiological and clinical findings were noted and the slides retrieved and examined. The results of blood tests performed in these patients were also retrieved from the electronic database of the hospital.

Ethics: Permission was obtained from Institutional Ethics Committee after approval from Medical Superintendent of the hospital to access the records and from the Head of Department, Pathology to retrieve the slides of the enrolled cases.

Statistics: Descriptive statistical analysis was done by using the SPSS software and an Excel worksheet, whenever required.

Result

The total number of cytology cases during the study period was 23254, out of which 7 cases of filariasis (0.03%) were detected. These included five cases diagnosed by fine needle aspiration cytology (FNAC) of various sites out of a total of 12588 FNACs (0.04%) and two cervicovaginal smears out of a total of 9376 Pap smears (0.02%) performed during the study period. Out of 1290 cases of fluid cytology, none were diagnosed as filariasis (0.0%).

None of the patients were clinically suspected to be filariasis (Table 1). Their age varied from 18 to 60 years,

with 42.9% cases in the 20-29 age group. Male to female ratio was 3:4. Most of them presented with firm swellings varying in size from 1-8 cm, of which two (40.0%) were tender (1 scrotal, 1 breast). Radiological examination was also misleading and provided no clue to the real diagnosis. Total leucocyte count (TLC) was within normal limits in all cases. Peripheral smear examination demonstrated eosinophilia in 3 cases (42.9%). None of the cases showed microfilaremia on thick and thin blood smear examination. Nocturnal blood smear examination performed in 2 cases (1 scrotum, 1 cervicovaginal) was uniformly negative.

As shown in Table 2, the nature of the aspirate varied greatly, from dirty/ necrotic (1 scrotal), chylous (1 scrotal), haemorrhagic to colloid-like (1 thyroid). All cases showed viable microfilaria (Figure 1a,b), varying from 1-5 in number. The largest number of parasites were visible in the inguino-scrotal mass (Figure 2a,b), also contained sheathed, coiled microfilaria and numerous eggs, some of which were packed with embryoid bodies (Figure 1c,d and Figure 2b). All cases revealed microfilariae of *Wuchereria bancrofti*, which have sheath longer than the larval body, with graceful curves, discrete nuclei extending from the head to the tail and tail tip free from nuclei.[2] None of the cases studied showed adult worms.

Associated inflammatory infiltrate was found in all cases, with marked mixed inflammation observed in the 2 inguino-scrotal swellings and 2 cervicovaginal smears (Figure 3c,d). Other than inflammation, filariasis was associated with other pathology in 4 cases (57.1%), like benign proliferative breast disease, fibroadenoma breast (Figure 2c,d), colloid goitre (Figure 3a,b) and reactive changes in epithelial cells of Pap smear. However no association with granulomas or malignant pathology was seen.

Table 1: Epidemiological, clinical, radiologic and haematological findings with cytological diagnosis.

Site	Age/ Sex	Presentating Complaints						Clinical Diagnosis	Radiologic Diagnosis	Peripheral smear	Cytologic Diagnosis	Associated conditions
		Swelling (cm)	Consistency	Pain	Fever	Others	Duration					
Right inguinal	27/M	1x0.5	firm	-	+	-	3 days	Inguinal swelling	Not done	Normal	Filariasis	Inflammation
Scrotum	20/M	8x6	Soft tense	+	-	-	6 months	Bilateral hydrocele	Bilateral hydrocele	Eosinophils 15%	Filariasis	Inflammation
Right Breast	18/F	1x1cm	Firm	+	-	-	1 month	Fibroadenoma	Fibroadenoma	Normal	Filariasis	Fibroadenoma
Left Breast	32/F	2x1.2 cm	Firm	-	-	-	3 months	Fibroadenoma	Fibroadenoma	Eosinophils 6%	Filariasis	Benign proliferative breast disease
Thyroid	60/M	2.7x1.3 cm	Soft to firm	-	-	-	3 years	Colloid goitre	Thyroid adenoma	Eosinophils 10%	Filariasis	Colloid goitre
Cervicovaginal	40/F	-	-	-	-	Pain & burning during micturition; Cervix bleeds on touch, discharge+	6 months	Recurrent urinary tract infection	Cystitis with cervicitis	Normal	Filariasis	Reactive changes associated with inflammation
Cervicovaginal	34/F	-	-	-	-	Cervical discharge	1 month	Cervicitis	Cervicitis	Normal	Filariasis	Inflammatory pathology

Table 2: Cytological findings.

Site	Aspirate	Filarial parasite			Cytologic findings						Final diagnosis
		Microfilaria	Adult worms	Eggs	Neutrophils	Eosinophils	Lymphocytes	Macrophages	Adherent cells	Others	
Right inguinal	Dirty viscous	+	-	+	+	+	+	+	+		Inguinoscrotalfilariasis
Scrotum	8 ml chylous	+	-	-	+	+	+	+	+	Necrosis	Scrotal filariasis
Right Breast	Scanty hemorrhagic	+	-	-	-	-	-	+	-	Sheets of ductal & myoepithelial cell + fibromyxoidstroma	Filariasis with Fibroadenoma breast
Left Breast	Hemorrhagic	+	-	-	-	-	-	+	-	Enlarged ductal cells	Filariasis with Benign proliferative breast disease
Thyroid	1 ml colloid	+	-	-	-	-	-	+	-	Follicular cells + colloid	Filariasis with Colloid goite
Cervicovaginal	NA	+	-	-	+	-	-	+	+	Reactive changes associated with inflammation	Cervical filariasis with reactive changes
Cervicovaginal	NA	+	-	-	+	-	-	+	-	-	Cervical filariasis

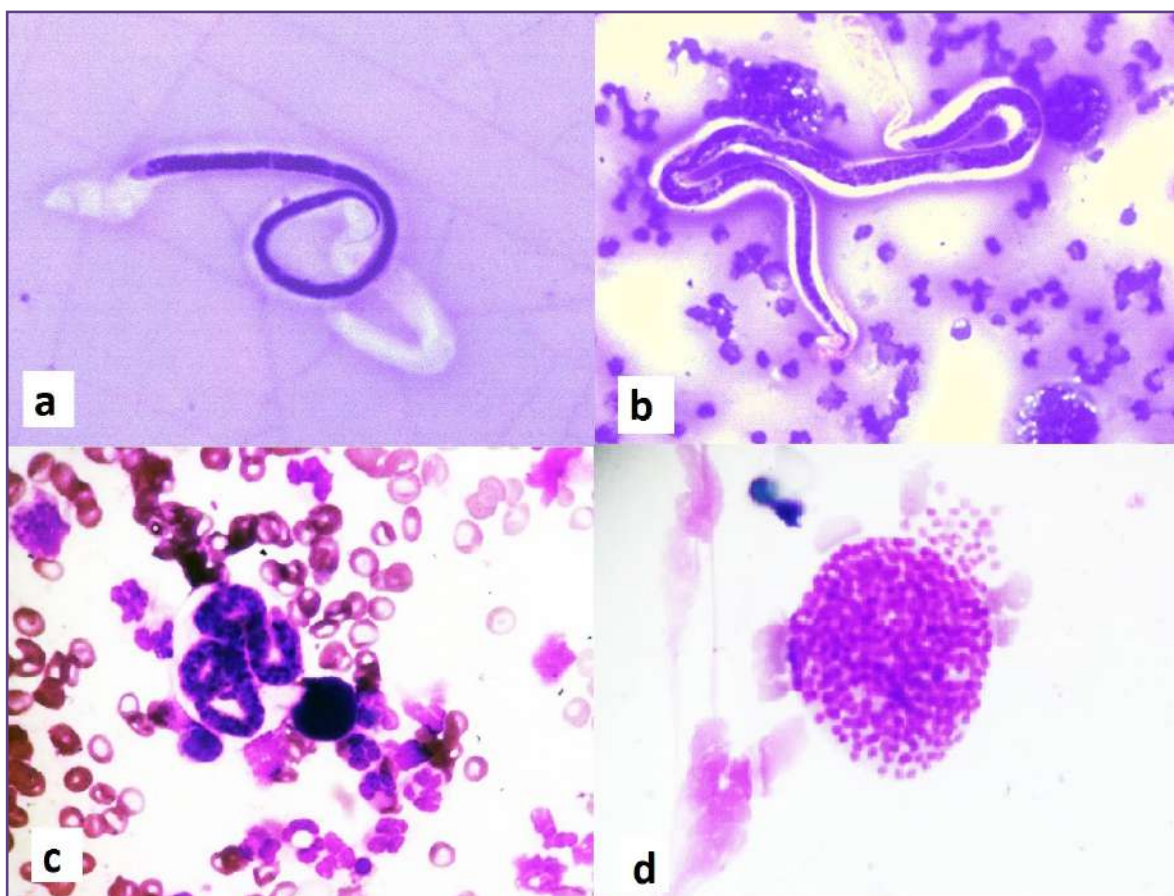


Fig. 1a. FNAC from inguinoscrotal swelling showing microfilaria of *Wuchereria bancrofti* which has sheath longer than the larval body, discrete nuclei extending from the head to the tail and tail tip free from nuclei (MGG, 400X). **Fig 1b.**FNAC from thyroid swelling showing sheathed microfilaria of *W. bancrofti* with numerous nuclei and graceful sweeping curves and macrophages, some adherent, against a background of colloid (MGG, 400X), **Fig 1c.** FNAC from inguinoscrotal swelling showing sheathed, coiled microfilaria of *W. bancrofti* with adherent polymorphs and lymphocyte (MGG, 400X). **Fig 1d.** FNAC from inguinoscrotal swelling showing egg of *W. bancrofti* packed with embryoid bodies (MGG, 400X)

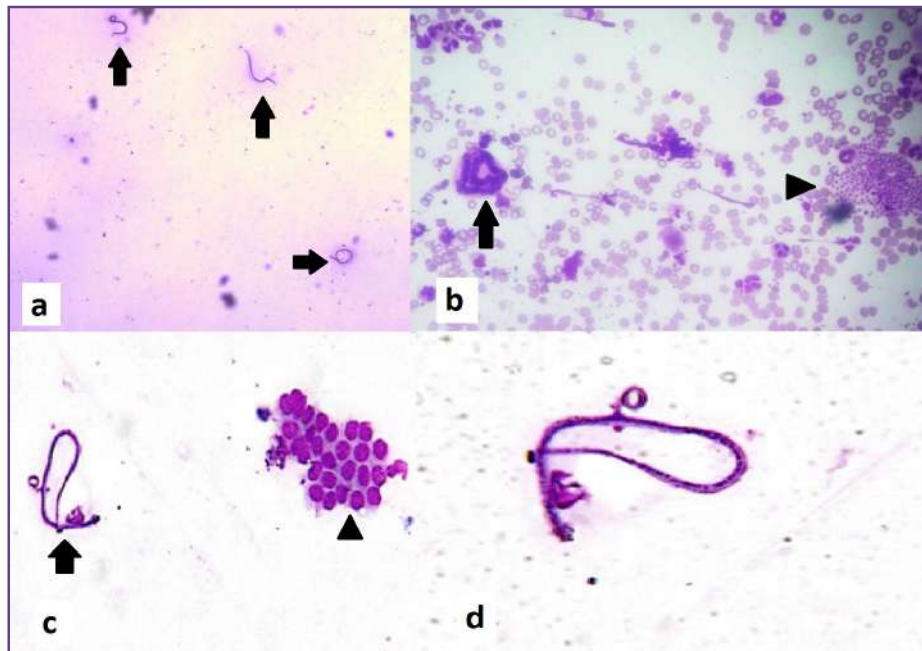


Fig. 2a. FNAC from inguinoscrotal swelling showing 3 microfilariae of *Wuchereria bancrofti* (arrows) with graceful curves (MGG, 40X). Fig 2b. FNAC from inguinoscrotal swelling showing sheathed coiled microfilaria of *W. bancrofti* (arrow) and a partially ruptured egg containing embryoid bodies (arrowhead) with polymorphs in the background (MGG, 100X), Fig 2c. FNAC from breast swelling showing microfilaria of *W. bancrofti* and a monolayered cluster of benign ductal cells (MGG, 100X). Fig 2d. FNAC from breast swelling showing sheathed microfilaria of *W. bancrofti* with nuclei dispersed along long axis (MGG, 400X)

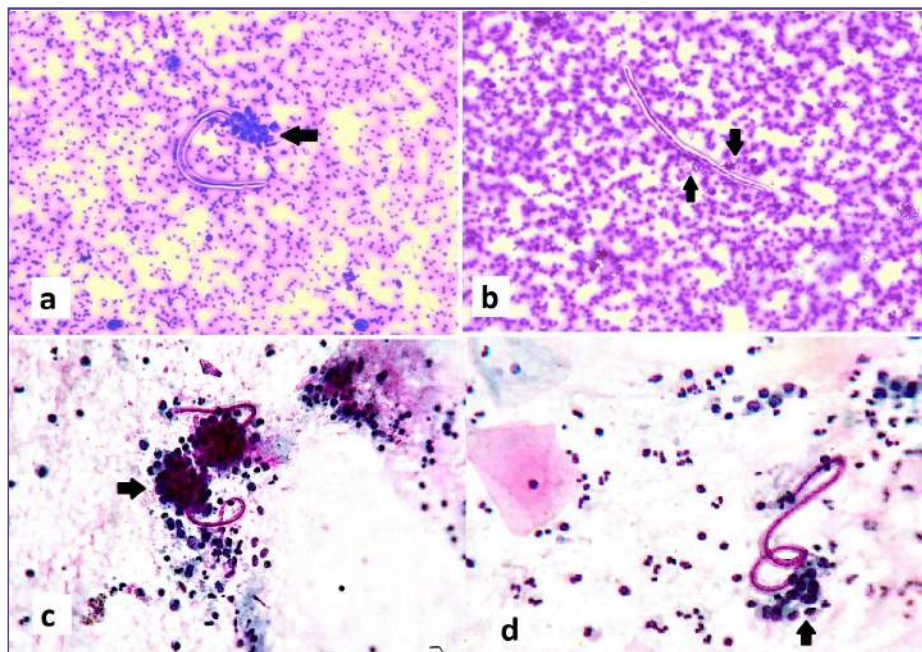


Fig 3a. FNAC from thyroid swelling showing sheathed curved microfilaria of *W. bancrofti* with adherent cluster of follicular cells (arrow) against a background of colloid and macrophages (MGG, 100X). Fig 3b. FNAC from thyroid swelling showing sheathed microfilaria of *W. bancrofti* having discrete nuclei with adherent macrophages (arrows) (MGG, 100X). Fig 3c. Cervicovaginal smear showing microfilaria of *W. bancrofti* with adherent mixed inflammatory cells. Background shows mucus and inflammatory cells (Pap, 100X). Fig 3d. Cervicovaginal smear showing sheathed microfilaria of *W. bancrofti* having discrete nuclei, tapering ends and no nuclei on the tail tip, with adherent histiocytes. Background shows squamous cells and inflammatory cells (Pap, 100X).

Discussion

Filariasis is a major social and economic scourge in India. The National Filaria Control Programme (NFCP) was launched in 1955, leading to a decrease in the national average microfilaria rates from 1.24% to 0.26%. According to the latest data available, heavily infected areas include West Bengal, Bihar, Telangana and Karnataka. On the other hand, north-western states (Jammu & Kashmir, Himachal Pradesh, Punjab, Haryana, Rajasthan, Delhi, and Uttaranchal) and north-eastern states (Sikkim, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura) have a low prevalence. It is worthwhile to note that the prevalence of filariasis is low in the NCR, where this study was conducted. The detection rate of 0.03% detected in this study is similar to the value of 0.078% in other studies conducted in Delhi/NCR,^[6] while it is low as compared to studies in endemic areas.^[4,8-10]

Among the various nematodes which can cause filariasis, *Wuchereria bancrofti* is responsible for 90% cases worldwide and 99.4% cases in India, while *Brugia malayi* accounts for the remainder.^[1,2] This is corroborated by the detection of microfilariae of *W. bancrofti* in all the cases in this study, similar to other Indian studies.^[4-16]

Filariasis causes a spectrum of diseases including asymptomatic amicrofilaremia, asymptomatic microfilaremia, acute lymphangitis, lymphadenitis, lymphedema, chronic lymphadenitis, elephantiasis and tropical pulmonary eosinophilia.^[2] Majority of the infected individuals in filarial endemic communities are asymptomatic carriers.^[3] These are the cases detected in our study, similar to other studies, where detection of microfilariae in cytological material has been incidental.^[4-16] In our study, we have additionally retrieved the radiologic data in all cases, which was of no additional help, and was even misleading in two cases (1 thyroid, 1 breast swelling). Predominance in the third decade has been noted in other studies.^[5,6] A mild preponderance towards females is seen, possibly because of the inclusion of a large number of cervico-vaginal smears, constituting 40.32% of the study group. Only one case in our study, an inguinal swelling of 3 days' duration, was accompanied by acute manifestations like fever or leucocytosis. Pathogenesis of the pain present in 3 cases varied from case to case. The bilateral scrotal swelling was possibly tender because it was tense and cystic; and pain during micturition in a case of filarial cervicitis could be explained by coexistent cystitis. Painful breast fibroadenoma had no direct cause, but was possibly due to blockage of lymphatics by microfilaria.

Mild eosinophilia was noted in 42.9% cases which is similar to that observed in other studies.^[6,11,13] However,

definitive diagnosis of filariasis is through demonstration of microfilaria in peripheral blood. All the cases in this study showed amicrofilaremia, which is consistent with the observation that filariasis can exist without microfilaremia.^[8,16] Thus, not only clinical and radiologic, but haematological parameters were also innocuous.

In the absence of any leads and in an area of low endemicity, the presence of microfilariae of *W. bancrofti* clinched the diagnosis in all the cases. Apart from open and coiled sheathed larvae, numerous eggs were demonstrated in the right inguinal swelling.^[4,6,8,9,12,13] However, unlike some reports,^[4,6,8-10,12] adult worms were not demonstrable in any cytological material in this study.

Study of cytological material further showed the ubiquitous presence of macrophages. Prominent mixed inflammatory infiltrate, which was primarily neutrophilic, was observed in both inguinoscrotal swellings and both cervico-vaginal smears. Cell adherence of inflammatory cells and macrophages to microfilaria, which was observed in 42.9% cases (1inguino-scrotal, 1 thyroid and 1 cervico-vaginal smear) has special significance. It has been described in other studies,^[6,11,13,16] and is believed to represent the immune status of the patient with regard to his or her filarial disease. Pandit *et al* noted that leukocytes adhered to microfilariae only in patients of elephantiasis who were amicrofilaremic, and did not adhere to dead microfilaria. Thus they concluded that cell adherence is due to presence of anti-filarial antibodies in the sera of these patients.^[17] This correlates with the findings in our study, where all the cases were amicrofilaremic and the cases exhibiting cell adherence were also those who had exhibited a brisk inflammatory immune response. However, unlike many studies,^[4,6,9,11,12] associated granulomatous reaction was not seen in this study.

Despite the vast population infected in endemic zones, microfilariae have been detected rarely in cytological smears. A reason for this could be that though they circulate freely in blood and lymph, extravasation into tissues or exfoliated material occurs only when there is either obstruction to the vascular and lymphatic system, caused by scars or tumours, or damage to the walls, which may be caused by inflammation, trauma or stasis.^[16] The presence of coexistent inflammation in all cases and coexistent hyperplastic and neoplastic conditions in 57.1% cases (2 breast, 1 thyroid and 1 cervico-vaginal smear) support this hypothesis. However the possibility that microfilaria are capable of penetrating intact vessels using their cephalic hooks, cannot be ruled out.^[18] Some authors feel that the detection of microfilaria in tissues is accidental; they wander in tissue fluids and get trapped during aspiration.^[19]

Data from Indian sources reveal that the lymphatic vessels of the male genitalia are most commonly affected in bancroftian filariasis, producing episodic funiculitis, epididymitis and orchitis, while hydrocele is the most common sign of chronic bancroftian filariasis.^[3] It is the commonest site for complications in most Indian studies.^[6,8,9,11] We reported two cases involving the inguinoscrotal region, one of which presented with a unilateral inguinal swelling of short duration, and the other presented with a long-standing bilateral hydrocele containing chylous fluid. Both cases showed associated inflammation and one showed cell adherence, with the chylous fluid also containing necrotic material, but associated infertility^[6] was not reported in any of these cases.

Isolation of microfilaria from breast is also rare.^[4-13,20] They have been reported in isolation^[6,8,9,10,11,12] and in association with breast abscess,^[4] granulomas,^[11,12] benign proliferative disease,^[5] fibroadenoma,^[4,19] and adenocarcinoma.^[7] Two such cases were reported in this study. In one, smears showed monolayered sheets of ductal cells, some of which were enlarged, bare nuclei, fragments of fibromyxoid stroma in conjunction with a single microfilaria; a diagnosis of filariasis with fibroadenoma was made. In the other, tight clusters of enlarged ductal cells and bare nuclei were seen with a single microfilaria, rendering a diagnosis of filariasis with benign proliferative disease of breast. Inflammation was minimal in both cases, though the lump was tender in the first case.

Microfilariae have been detected incidentally in the thyroid,^[6-8,10-13,15] sometimes in association with other diseases like colloid goitre,^[6,10,15] lymphocytic thyroiditis,^[6] undifferentiated carcinoma,^[7] and anaplastic carcinoma.^[12] In this study, a single case of non-tender, soft to firm, diffuse, slow-growing mass in a biochemically euthyroid patient led to a clinical diagnosis of colloid goitre. However, ultrasonography showed a heteroechoic mass, with vascularity on colour Doppler, which was suggestive of thyroid adenoma. Thyroid aspirate yielded colloid aspirate and smears revealed microfilarial larvae in the background of blood mixed colloid, along with a few clusters of benign follicular cells and few macrophages, leading to a diagnosis of filariasis with colloid goitre.

Microfilaria have been rarely reported in cervico-vaginal or Pap smears.^[13,16] Vaginal bleeding was seen to be the most common symptom in such patients,^[16] which was seen in one of the cases reported. Both cases had vaginal discharge, radiologic features suggestive of cervicitis and showed a predominance of acute inflammatory infiltrate, with presence of microfilaria. In one smear, cell adherence was marked and associated epithelial cells showed nuclear

enlargement, pleomorphism and mild hyperchromasia, leading to a diagnosis of cervical filariasis with reactive changes associated with inflammation. Follow-up smear after a 21-day course of DEC (diethyl carbamazine) showed mild inflammatory changes only.

Filariasis has been reported in literature in association with neoplasms,^[4,5,7,13-15,21] both benign^[4,21] and malignant.^[4,5,7,13-15,21] There are several theories to explain this. Tumours have a rich blood supply due to increased vasculogenesis, leading to concentration of parasites;^[7,12,21,22] Increased cell proliferation leads to blockage to vascular and lymphatic channels, thereby trapping microfilaria.^[4,7,12,16] Aspiration may lead to rupture of tumour vasculature, which already contains microfilaria, causing their release into the aspirate.^[7,21] It has also been suggested that filarial organisms play a role in tumorigenesis by release of toxic mediators or by chronic mechanical irritation.^[12,22] It has also been associated with other diseases like tuberculosis,^[4,14] leprosy,^[23,24] leishmaniasis,^[25] and HIV,^[26] apart from proliferative non-neoplastic lesions in the associated organ. It is believed to be an opportunistic infection due to this association with debilitating diseases.^[7,14,22] In this study too it has been found to be associated with other pathology in 57.15% cases, but no malignant condition was found associated.

Conclusion

Cytology has a major role in diagnosis of asymptomatic carriers of filariasis. The incidental detection of microfilariae in cytological material, derived by FNAC, exfoliative or fluid cytology, in areas where prevalence is low, points to the need for a high index of suspicion and careful screening of all smears.

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