Original Research Article

Unanticipated cytodiagnosis of filariasis: A Study of 16 cases

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Abstract

Background: Bancroftian Filariasisisatropical and subtropical disease caused by Wuchereriabancrofti and transmitted by the Culexmosquitoes.The diagnosis of it is conventionally made by demonstrating microfilariae in the peripheral blood smear. Microfilariae and adult filarial worm have been incidentally detected in fine need leaspirates of swelling at various sites in amicrofilaremic patients. Currently 1.4 billion people world wide are at risk of being infected. In India 600 million people are at risk of filariasis.Cytology has important role in the diagnosis of occult Filariasis. **Objectives:** To discuss the role of FNAC in diagnosing occult Filariasis. **Material and Methods:** This is a retrospective study carried out in the Department of Pathology at SRMS IMS, Bareilly, Uttar Pradesh. Aspiration was made by 22-23 gauze needle, smears were stained by Papanicolaou and May-Grünwald Giemsa stain. **Result**: 16 cases of cytological specimens incidentally showed microfilaria without any clinical suspicion which included breast lumps (three cases), thyroid swelling (three cases), lymph nodes (two cases), subcutaneous swelling (two cases), ascitic fluid (one case), pleural fluid (one case), lung mass (one case), scrotal swelling (one case), cervical smear (one case) and space occupying lesion in liver (one case). Fragments of adult worm were reported in four cases. Only one patient with scrotal swelling had microfilaremia.**Conclusion:** FNAC can play an important role in diagnosing occult Filariasis in clinically unsuspected cases.

Key words: Filariasis, Fine needle aspiration cytology, Microfilariae

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Introduction

Filariasis affects over 120 million people worldwide and is endemic in 80 countries. India accounts for about 40% of the total global disease burden [1,2]. Filariasis of skin and subcutaneous tissue is caused by Onchocerca volvulus and Loa loa. Lymphatic filariasis is caused by Wuchereriabancrofti, Brugiamalayi and Brugiatimori. W. bancrofti accounts for over 90% of global filariasis and affects 115 million people worldwide, whereas Brugia filariasis is restricted in South and Southeast Asia and affects 13 million people [2,3,4]. Overall, 700 million people live in endemic areas [5]. In India W. bancrofti is the commonest cause of filariasis followed by B. malayi[6]. Other species of filariasis are only rarely reported from the Indian subcontinent [7]. It is distributed chiefly along the sea coast and along the banks of big rivers; it has also been reported from Rajasthan, Punjab, Uttar Pradesh and Delhi[8].

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The life cycle of the filarial worms (bancroftian and brugian filariasis) can be divided into the mosquito phase and the human phase. Infective larvae are transmitted by infected biting mosquitoes during a blood meal. The larvae migrate to lymphatic vessels and lymph nodes, where they develop into adults. The adults dwell in lymphatic vessel and lymph nodes where they can live for several years.

The female worms are ovo-viviparous (laying eggs and well-developed embryos). The embryos or microfilariae circulate in the blood. The microfilaria infects biting mosquitoes. Inside the mosquito, the microfilariae develop in 1 to 2 weeks into infective filariform (third stage) larva which again infect the vertebral host during the next meal and the cycle continues [9].

The clinical manifestation of lymphatic filariasis may range from the asymptomatic filariasis to lymphangitis, lymphadenitis, hydrocele, chyluria, filarial fever and elephantiasis [10,11].

The conventional diagnosis of filariasis relies on finding microfilariae on a peripheral blood smear [12]. Occult filariasis is a condition characterized by absence of microfilaraemia because the microfilarias are destroyed in the tissues [11].

Cytological examination plays an important role in the diagnosis of such unsuspected cases. The finding of microfilaria in fine needle aspirates is uncommon[13].

The literature contains few reports of finding micro-filariae in various locations including thyroid, skin & soft tissue swelling, epididymis, breast, salivary gland, cervicovaginal smear, ovarian cyst, urine, lymph node, endometrial smear, nipple secretion, hydrocele fluid, lung, bronchial washings, bronchial brushings, bone marrow aspirate, brain aspirates and effusion [10,13-16,18-20,21-32]. Appearance of adult worms & eggs have also been described.

Although exact characterization of microfilariae requires DNA analysis, most cytopathologist rely on morphological features Microfilariae of both bancrofti and malayi are sheathed. But microfilariae of W. bancrofti are longer have smooth curves and tail tip is free of nuclei whereas microfilariae of B. malayi are smaller possess secondary kinks and unlike the former tip is not free of the nuclei[11].

It is unusual to find microfilariae in routine cytological smear and their recognition is considered as incidental finding.

Methods

Place of study: The present study was done in Rural and Urban areas, in & around Bareilly region of North India.

Nature of study: This is a retrospective study of clinically unexpected filariasis diagnosed by cytology in Department of Pathology in a tertiary care hospital of this region. All the cytological records between this period was retrieved and cytologically diagnosed cases of filariasis were included in the study. Clinical records and cytology slides were reviewed.

FNAC had been performed with a 23-gauze needle and 10 ml syringe. Aspirates were air dried for MGG stain and also wet fixed in 95% alcohol for Papanicolaou stain in some cases. The fluid specimen centrifuged and the smears were prepared from sediments.

Unit of study: 16 cases of clinically unexpected filariasis diagnosed by cytology were studied.

Study period: 10 years (September 2007 to September 2017).

Study schedule: The smears were evaluated for the presence of microfilaria and their number, eggs & embryoid bodies, adult worm, associated inflammatory response and any other associated pathology.

Microscopic examination of microfilariae and evaluation of morphological features like presence of sheaths and distribution of nuclei helped in species identification. Sometimes microfilariae of *W.bancrofti* appear unsheathed due to staining problems. Arrangement of nuclei and free spaces helped in species identification in these difficult cases.

Blood examination records were retrieved and evaluated for eosinophils and microfilaremia.

Inclusion criteria: Cases in which microfilaria or adult filarial worm was detected on cytological examination.

Exclusion criteria: Poorly prepared smears where morphological features were not distinct.

Statistical analysis: Statistical analysis was carried out wherever necessary.

Result

The study included sixteen cases of filariasis diagnosed on cytological specimens which included 13 cases of FNAC, two cases of effusion cytology (one case each of ascitic and pleural fluid) and one case of exfoliative cytology (cervical smear).

Respective frequencies of detection of organisms in FNAC from different sites are shown in Table 1.

The patients presented predominantly as swelling at various sites with or without pain and fever. The duration of history ranged from weeks to years. In none of these cases was filariasis considered a diagnostic possibility. The number of parasites varied from one to many per slide in various stages of development. Microfilaria, fragments of adult worm, eggs, cuticle and empty sheath were seen.

Table-1: Frequency of Filariasis in FNAC

S. No.	Specimen	No. of cases with Filariasis
1.	Breast lump	3
2.	Thyroid swelling	3
3.	Lymph node	2
4.	subcutaneous swelling	2
5.	Lung mass	1
6.	Liver SOL	1
8.	Scrotal swelling	1

In our series of 16 cases, the age of the patients ranged from 10 to 69 yrs with no specific age preponderanceand the Male (11): Female (5) ratio was 2.2:1.

Smears revealed microfilaria in 15 out of the 16 cases. Fragments of adult worms in 3 cases; and embryoid bodies in two cases, both these cases were from lymph node swelling. The number of microfilariae was much more in lymph node and breast aspirates as compared to other sites. In one case with subcutaneous nodule at angle of mandible only adult worm was identified without any microfilaria. Empty sheaths were seen in only two cases.

In all the aforementioned cases, the morphology of the microfilariae was consistent with *W. bancrofti*. Microfilaria had sheath, smooth curves and pointed curved tail which were free of nuclei. In one case the microfilaria appeared unsheathed and the species identification was done on the basis of cephalic space and nucleus free tail tip. The length of the sheath was variable.

In one smear from from lymph node aspirate we could demonstrate that the gravid female worm was ovo-viviparous where many embryoid bodies and microfilaria were seen comingout from the adult worm.

Additional cytological findings are shown in Table 2.

Table-2: Microscopic findings in cytological smears

S.N.	Microscopic features	No. of cases
1.	Microfilaria	15
2.	Embryoid bodies & Eggs	2
3.	Fragments of Adult worm	3
4.	Granulomas	4
5.	Eosinophils	7
6.	Lymphocytes, polymorphs & histiocytes	11
8.	Adherence of inflammatory cells	2
9.	Giant cells	2
10.	Neoplastic cells	2
11.	Atypical cells	1

Table-3: Summary of clinical data and cytological findings in the 16 case of filariasis

Case no.	Type of specimen	Age/sex	Clinical presentation	Microfilaria	Inflammatory infiltrate	Associated malignancy
1	Aspirate	45y/F	Midline neck Swelling	+	L+,P+,E+	Nil
2	Aspirate	25y/M	Breast lump	+	Nil	Nil
3	Aspirate	10y/M	Left axillary lymph node swelling	+,adult worm + eggs	L+ E +granulomas	Nil
4	Aspirate	22y/M	Epitrochlear lymph node swelling	+, eggs	L+P+ granulomas	Nil
5	Aspirate	60y/M	Liver SOL	+,	P+	Metastatic adenocarcinoma
6	Aspirate	50y/M	Midline neck swelling	+,	Nil	Nil
7	Aspirate	28y/F	Breast swelling	+	L+, E+	Nil
8	Aspirate	52y/F	Breast swelling	+	L+,E+	Nil
9	Aspirate	45y/M	Lung mass	+, adult worm	P+,E+, Granulomas	Nil
10	Aspirate	55y/F	Midline neck swelling	+	Nil	Follicular neoplasm
11	Aspirate	30y/M	Left elbow subcutaneous nodule	+	L+, P+, E+	Nil
12	Aspirate	20y/M	Subcutaneous nodule angle of mandible	-, adult worm	L+,E+, granulomas	Nil
13	Aspirate	54y/M	Scrotal swelling	+	L+	Nil
14	Pleural fluid	69y/M	Pleural effusion	+	L+, P+	Atypical cells
15	Ascitic fluid	71y/M	Ascites	+	L+, P+	Nil
16	Cervical smear	33y/F	Discharge p/v	+	P+	Nil
E = Eosinophils, L= Lymphocytes, P = Polymorphs,						

The smears revealed variable inflammatory infiltrate. Most cases had mixed inflammatory infiltrate composed of lymphocytes, polymorphs and histiocytes. Eosinophils were seen only in 7 cases and their number varied from a few to many. Granulomas were demonstrated in only four cases. Inflammatory cells were seen sticking to microfilariae in four cases. Giant cell reaction was seen in two cases.

Figure 1: FNAC smear reveal microfilaria of Wuchereriabancrofti. Sheathed larva with tail tip free from granules (MGG stain, 40X)

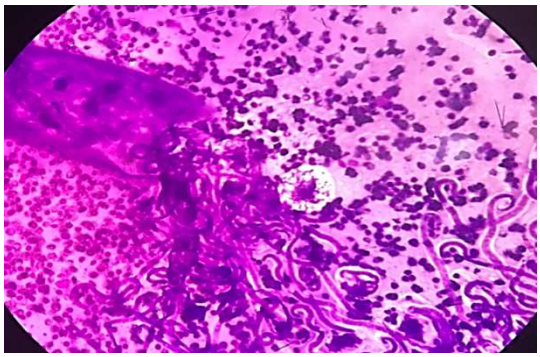

Figure 2: FNAC smear show microfilaria & embryoid bodies coming out from gravid female worm (MGG stain, X400)

Figure 3: FNAC smear show embryoid bodies (MGG stain, X400)

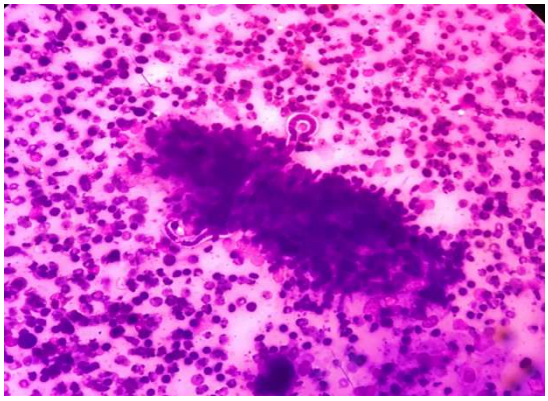

Figure 4: FNAC smear show inflammatory cells adhering to microfilaria. (MGG stain, X400)

The aspirate from the breast lumps revealed variable number of benign ductal epithelial cells, myoepithelial cells, bare nuclei and fat cells in the background. The aspirates from thyroid swellings revealed colloid and benign follicular epithelial cells. In one case, follicular neoplasm was diagnosed co-existing with microfilaria. The aspirates from the lymph nodes revealed reactive lymphoid population in the background. The aspirate from lung revealed pulmonary macrophages and bronchial epithelial cells in the background. The aspirate from liver revealed clusters of malignant cells and benign hepatocytes in the background. Both cases of body fluid cytology revealed reactive mesothelial cells in the background. Some atypical cells were also seen in the case of pleural fluid.

The peripheral blood smear revealed eosinophilia in only 4 cases. The number of eosinophils ranged from 9 to 35%. Nocturnal blood sample examination for presence of microfilaria was done in only ten cases; out of which only one case with scrotal swelling was reported as positive for microfilaria.

Discussion

Filariasis is a major health problem in many countries including India. It is caused by nematodes like *W.* bancrofti, B. malayi, B. timori, Onchocerca volvulus, Mansonellaperstans, M. streptocerca, Dimmitis and loalo[10]. The most common species in India being W. bancrofti

Adult worms live in the lymphatics of definite hosts and microfilariae are released into the peripheral blood. Most frequentlyinvolvedsites are lymphatics of lower limbs, retroperitoneal tissue, spermatic cord, epididymis and mammary glands[10]. It may appear in tissue fluid and exfoliated surface material due to lymphatic and vascular obstruction and subsequent extravasation[39]. In endemic areas people become infected early in life and develop microfilaremia, reaching a peak between 15-20 yrs of age[40]. Most infected people in endemic areas are asymptomatic but are an important source of infection in the community[40,41].

Although the incidence of filariasis is high in the Indian subcontinent, the finding of microfilaria on fine needle aspiration sample is unusual [14,41]. Incidental detection of filarial organism has been reported in cytological smears from almost any part of body. Forms of bancroftianfilaria and background pathology, however, can vary. Microfilaria is the most common form of filarial organism detected in cytological smears; however, eggs of the organism and fragments of adult worms can also be detected rarely [37]. Microfilariae have also been reported in association with various benign and malignant tumors[37] Thus role of cytology in diagnosis of filariasis cannot be underestimated in clinically unanticipated cases. This study is an attempt to prove importance of cytology in diagnosis of filariasis. The diagnosis of filariasis depends on clinical features, history of exposure in endemic areas and on laboratory findings [43]. The common tests include-demonstration of microfilariae in peripheral blood, chylous urine or hydrocele fluid, provocation test with Diethylcar-bamazine drug, detection of filaria in FNA smears, Skin tests with filarial antigens, serological detection of filarial antigen and antibody which include ELISA test, IHA test, Immuno chromate graphic card test. The specificity of these tests is about 99%. PCR test has been developed but is not so much sensitive[11]. X-ray examination shows calcifiedadult worm. USG can detect adult *W. bancrofti* in lymphatics vessels of scrotum in infected male and of breast in infected female[11].

The drug of choice for treatment is Diethylcarbamazine which is filaricidal [11]. In present study none of the patients were clinically suspected of filariasis, other authors also found similar result [10,40,42,43,44]. We did not find any specific age preponderance for the filariasis. However, Andola et aland Mallick et al reported the most common age group afflicted by filariasis to be 21 – 40years [44,45].

We found that male were more commonly affected than females & Male: female ratio was 2.2 : 1 which was similar to study conducted by Mallick et al This is probably due to relatively more exposure of males to the vector during outdoor activities [44].

In the present study, no specific site predilection was seen, with three cases each of breast and thyroid followed by two cases each in lymph nodes and subcutaneous swelling (Table 2). Mitraet al reported breast as the most common site, whereas in studies conducted by Andola et al & Yenkeshwar et al lymph node and soft tissue swelling were found to be the most common sites respectively. This could be attributed to no site preponderance for microfilaria. Thus, it can be seen anywhere [10,40,44].

In the present study, the majority of cases presented with swelling which was similar to that reported by several workers Yenkeshwaret al, Jhaet al, Andola et al&Mallick et al and Mitra et al. However, there have been case reports of unusual presentations like breast filariasis mimicking inflammatory carcinoma, paeud' orange, Paget's disease, abscess, non-healing leg ulcer. [10,40,42,43,44,45,46,47,48,49]

We saw W. bancrofti microfilaria in all the cases. In most studies and in most of the reported cases microfilariae in the cytological smears have been identified to be of W. bancrofti. However, a case of adult worm of B. malayi and its fragments has been reported in FNA smear from epitrochlear lymph node by Arora et al[50].

In our study microfilaria was seen in most of the cases which is similar to various other studies and case reports. We demonstrated fragments of adult worms in 25% of cases which was slightly higher than that demonstrated by Mallicket al [44]. In one case (aspirate from subcutaneous nodule) only adult worm was seen. There are few reports of finding adult filarial worm alone in the smears. Arora et al reported three out of thirty-four cases showing no microfilaria. Arora et al reported adult worms in almost 50% of cases [50].

We found that length of the sheath was variable, from closely fitting to quite loose, which is similar to that reported by Kaushal et al. They also reported empty sheath in two out of 32 cases which is similar to our finding one out of sixteen cases [38]. We observed microfilaria stripped off thin sheath in one case which may be mistaken for unsheathed microfilaria (seen in Onchocerca volvulus, Diptalenemaperstans, D. strepto-cerca and M. ozzardi, none of which are known to occur in India. Similar observations have also been made by Kaushalet al, Hira et al &Sabesan et al [38,39,51].

We found a variable inflammatory response in the smears, ranging from being insignificant to formation of wellformed epithelioid granulomas as described in previous studies Kaushal et al & Jha et al. We found mixed inflammatory infiltrate comprising polymorphs, lymphocytes and histiocytes in most of the cases (11 out of 16) which was 68.75%. Eosinophils were seen in the smears of only 7 cases out of 16 (43.75%) which was almost similar to that reported by Andola et al, Jha et al, Mitraet al & Kaushal et al. All the cases of eosinophilic infiltrate show presence of well-preserved microfilaria which was similar to study conducted by Kaushal et al & Vankateswaran et al [37,38,40,42,43,52].

Significant adherence of inflammatory cells and macro-phages to microfilariae was present in 2 cases out of 15 cases. Cell adherence was first described by Panditet al. Almost similar result was observed byother authors [14,22,38,40,53].

Four cases show granulomas which was similar to study done by, Mitraet al, Andola et al.& Kaushal et al [37,40,43].

The aspirates from the breast swelling revealed benign ductal epithelial cells, myoepithelial cells, bare nuclei, fibrofatty tissue and inflammatory cells which was similar to other studies[14,40,43,44,54,55,].

Three cases of thyroid swellings show microfilariae along with colloid and thyroid follicular cells, similar observation was made by Mitraet al.,Yankeshwar et al, Varghese et al.&Joshi et al.One thyroid aspirate revealed coexisting follicular neoplasm which has also been reported by Kaushal et al.[10,14,25,38,40]

Aspirates from lymph nodes demonstrated microfilariae in a background of reactive lymphoid cells which was similar to that reported by Joshi et al., Mitra et aland Varghese et al[14,25,40].

Interestingly one smear from lymph node aspirate revealed viviparous adult worm which was similar to study done by Mallick et al. which showed the presence of numerous eggs packed within the body cavity of an adult worm and slightly higher number was observed by Kaushal et al. & Jain et al. which may be due to higher number of cases from lymph node in their studies [38,44,56].

In single case in which pleural fluid showed larva, incidental discovery of filariasis was associated with atypical cells, similar observation was observed by Mallicket al. & Kaushal et alin which filariasis was associated with malignant cells in fluid cytology[38,44].

We reported microfilaria in one cervicovaginal smear. Microfilaria in cervicovaginal smears have also been reported by Valandet al, Rohiniet al.&Andola et al [57,58].

We reported microfilaria in one case each of liver and lung. These are extremely rare sites for microfilaria detection. There are few case reports of microfilaria detection in liver by Andola et al., Reddy et al.' Pandey et al. & Arakeri et al.and lung by, Jha et al.& Konar et al [42,43,59,60,61,62].

Association of filarial parasite with malignancy has been described but its role in tumorogenesis is not so far explained and it could be just a chance association. A rich blood supply in the tumors could be a possibility resulting in concentration of parasites at these sites. Although the role in tumorogenesis is controversial [37].

In smears from liver aspirate microfilaria was found together with clusters of malignant cells (Adenocarcinoma) in Thyroid with follicular neoplasm and in pleural fluid with atypical cells. Association of filariasis with neoplasm was seen inmuchhigher number of cases reported by Jha et al. Kaushal et al. Microfilariae have been reported in association with neoplastic lesions such as hemangioma of liver, ewing's sarcoma of bone, squamous cell carcinoma of maxillary antrum, anaplastic astrocytoma of thalamus' low grade astrocytoma of C6-D1 spinal segment, craniphary-ngioma of third ventricle, Nonhodgkin lymphoma, transitional cell carcinoma of bladder,follicular carcinoma of thyroid, seminoma of undescended testis, meningioma, intracranial hemagio blastoma, fibromyxoma, squamous cell and undifferentiated carcinoma of uterine cervix, carcinoma of pharynx, metastatic melanoma to bladder, leukemia, lymphangio-sarcoma, carcinoma of pancreas, dentigerous cyst, carcinoma breast [37,38,42,63-75].

We could demonstrate microfilaria in nocturnal blood sample of only one patient with scrotal swelling (out of the 10 patients in which nocturnal blood sample examination was done). Mitra et Al detected micro-filaremia in 3 out of 32 cases [29,40,68].

The peripheral blood examination revealed eosinophilia in only 4 cases which is similar to that found by Mitraetal, Kaushal et al. Variable numbers of eosinophils have been reported in various studies. Thus, filariasis is not always associated with eosinophilia [38,40].

The present study further proves the role of cytology as a simple and cheap procedure in detection of few occult cases with amicrofilaremia. The study highlights the importance of careful screening of FNAC smears in diagnosing filariasis in clinically unanticipated patients, especially in endemic areas ansd disease may be missed if one is not aware of the possibility.

Conclusions

Despite high incidence of Filariasis, microfilaria in fine needle aspiration cytology is not very common finding. Filariasis can exists without microfilaremia and accompanying eosinophilia. Detection of adult worms, eggs and larva at unusual sites emphasizes that filariasis must be considered as a possibility in differential diagnosis of swellings at various sites, especially in the endemic regions, and careful screening of the cytological smears is mandatory keeping this possibility in mind.

All the cases in the present study were clinically unanticipated. Immunological tests for detection of filarial antigen though sensitive are not widely available and costly for developing countries like India and FNAC can be regarded as a safe, cheap, simple and initial step for diagnosis, which can provide the pathologist as well as clinicians a definite diagnosis within a short period of time.

Undoubtedly the demonstration of parasite, in aspirate, plays a significant role in recognition and its early treatment, thus obviating the severe manifestations of lymphatic Filariasis and reducing health care cost.

Contribution details

Nature of work	Authors		
	1	2	3
Concepts	✓		
Design	✓		
Definition of intellectual content	✓		
Literature search	✓	✓	✓
Data acquisition	✓	✓	✓
Data analysis	✓		✓
Manuscript preparation	✓		✓
Manuscript editing	✓		✓
Manuscript review	✓		

What this study add to existing knowledge?

1. Filariasis can present as swelling at almost any site in the body without accompanying microfilaria & eosinophilia and therefore careful screening of FNAC smears is mandatory, keeping the possibility in mind especially in endemic area.
2. Microfilaria can occasionally be found along with a malignant pathology.
3. Microfilaria can also be detected in body fluids and Pap smear cytology.

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