Unusual Cytological Findings In Breast Aspirates: Case Studies Of Microfilariae With Review Of Literature
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Introduction: Extranodal filariasis is a rare entity and the breast is an uncommon site for filariaisis. Patients commonly present with an underlying lump and may occasionally mimic malignancy. Lymphatic filariasis in humans is commonly caused by Wuchereria bancrofti and Brugia malayi.

Materials and methods: In two cases of breast lump, FNAC was performed by a 24 gauge needle fitted to a 10 cc syringe. Smears were wet fixed immediately in 95% alcohol and stained by hematoxylin and eosin, and Papanicolaou stain. Air dried smears were stained by May-Grünwald Giemsa stain.

Case 1: A 50-year-old female came with history of lump in left breast and a small palpable node in the left axilla since 15 days. The provisional diagnosis was carcinoma breast. The lump was firm, mobile measuring 4 x 3 cm. On Fine needle aspiration (FNA), creamy material was aspirated. Cytological diagnosis was Granulomatous mastitis of parasitic origin – Microfilariae. Case 2: A 30-year-old female came with history of lump in left breast since 15 days. Clinically, it was diagnosed as Fibroadenoma. There was a single well defined, mobile, non tender mass measuring 2 x 1 cm situated in the upper inner quadrant. On FNA, 0.5 cc blood mixed cystic fluid was aspirated. Cytological diagnosis was Mastitis of parasitic origin – Microfilariae (Wuchereria bancrofti).

Results: Two cases of breast lump were reported as Filariasis on FNAC. Case 1 showed granulomas and clinically it was suspicious of malignancy. In case 2 the clinical diagnosis was fibroadenoma. Both the cases were females with mean age being 40 years. Both cases were Filarisis with case 1 showing well-formed granulomas and both the patients presented with lump in the left breast.

Conclusion: In endemic areas filariasis should be considered one of the differential diagnoses of superficial swelling. Careful screening of FNAC smears help in detecting microfilaria even in asymptomatic patients.

INTRODUCTION
Lymphatic filariasis or elephantiasis affects more than 90 million people worldwide and has been identified by WHO as the second leading cause of permanent and long term disability after leprosy. Filariasis is a global problem. It is a major social and economic scourge in tropics and subtropics.

One third of the people infected with the disease live in India, one third in Africa and most of the remainder in South Asia, Western Pacific and parts of America.

The problem is increasing every year due to gross mismanagement of the environment. The disease is endemic all over India especially in Uttar Pradesh, Bihar, Jharkhand, Andhra Pradesh, Orissa, Tamil Nadu, Kerala and Gujarat.

There are at least six million attacks of acute filarial disease per year and 45 million persons are currently having one or more chronic filarial lesions.

A majority of infected individuals in filarial endemic communities are asymptomatic. Conventional mode of diagnosis of filariasis is by demonstration of microfilaria in peripheral blood smear. Despite high incidence, it is infrequent to find microfilariae in FNAC smears and body fluids. Literature contains few reports of microfilariae found in various locations including thyroid nodule, skin and soft tissue swelling, epididymis, spermatic cord, breast, nipple discharge, salivary gland, cervicovaginal smear, ovarian cyst, urine, lymph node, and effusion fluids (pleural, pericardial, peritoneal), laryngeal and pharyngeal brushings, lung, hydrocele fluid, bone marrow, bronchial washing, retroperitoneal tissue, brain aspirates and joint aspirates.

Breast is an unusual site for the occurrence of filarial nodule and very few cases have been documented in the literature.

MATERIALS AND METHODS
In two cases of breast lump FNAC was performed with a 24 gauge needle fitted to a 10 cc syringe. Smears were fixed...
immediately in 95% alcohol and stained by hematoxylin and eosin, and Papanicolaou stain. Air dried smears were stained by May-Grünwald Giemsa stain.

CASE 1
A 50-year-old female presented with a history of lump in the left breast and a small palpable node in the left axilla since 15 days. The provisional diagnosis was carcinoma breast. Ultrasound was done and it was inconclusive. The lump was firm, mobile measuring 4 x 3 cm. Skin over swelling was normal. On FNA, creamy material was aspirated. Smears showed significant number of microfilariae admixed with granulomas consisting of lymphocytes, giant cells, histiocytes, plasma cells, eosinophils and red blood cells. Cytological diagnosis was Granulomatous mastitis of Parasitic origin – Microfilariae

(Figures: 1-5).

Figure 1
Figure 1: Photomicrograph showing coiled microfilaria of Wuchereria bancrofti (10X).

Figure 2
Figure 2: Microfilaria of Wuchereria bancrofti with neutrophils in the background (40X).

Figure 3
Figure 3: Microfilaria of Wuchereria bancrofti (arrow) in the background of granuloma and acute inflammatory infiltrate (10X).

Figure 4
Figure 4: Photomicrograph of breast aspirate showing epithelioid histiocytic cell collection along with acute inflammatory infiltrate predominantly composed of eosinophils (10X).
CASE 2:

A 30-year-old female presented with a lump in the left breast since 15 days. Clinically, it was diagnosed as Fibroadenoma. On examination, there was a single well defined, mobile, cystic, non tender mass measuring 2 x 1 cm situated in the upper inner quadrant. On FNA, 0.5 cc blood mixed cystic fluid was aspirated. Smears showed sheathed microfilaria (nuclei not extending to the tip) along with inflammatory cells, predominantly eosinophils, lymphocytes and occasional neutrophils. Cytological diagnosis was Mastitis of Parasitic origin – Microfilariae (Wuchereria bancrofti) (Figures: 6-8).

RESULTS

Two cases of breast lump were reported as Filarisis on FNAC. Case 1 showed granulomas and clinically it was suspicious of malignancy. In case 2 the clinical diagnosis was fibroadenoma. Both the cases were females with mean age being 40 years. Both cases were of Filarisis with case 1 showing well-formed granulomas and both the patients presented with lump in the left breast.

DISCUSSION

Filariasis is a serious socioeconomic and public health
problem. It is endemic in large areas of India, Srilanka, Africa, and Far East. Wuchereria bancrofti (W. bancrofti) accounts for more than 90% of all filariasis cases in the world followed by Brugia malayi (B. malayi) and Brugia timori (B. timori).\[7\] Brugia malayi is confined to South-east and Eastern Asia.

B. timori is found only in Timor and its adjacent islands.\[5\] In endemic areas like Eastern part of Uttar Pradesh, people become infected early in life with a peak between 15 to 20 years. Majority of infected individuals in filarial endemic areas are asymptomatic.\[3\]

Female breast is an unusual site for the occurrence of filarial nodule and few such cases have been documented in literature.\[6, 9, 10, 11\] It is frequently caused by W. bancrofti. It has not been reported from areas endemic for B. malayi.\[1\]

Filarial granuloma in the breast though not a common occurrence in India is common in some endemic areas in Africa.\[1\] Our case 1 showed granuloma.

Larvae enter the lymphatic vessels causing lymphangitis. When the female breast is involved, the larvae enter the lymphatic vessels causing lymphangitis, fibrosis and disruption of lymphatic drainage.\[12\] Most common site is upper outer quadrant of breast. But central or periareolar nodules occur with notable frequency.\[10\] Hyperemia in the overlying skin with changes of peau d’ orange and enlargement of axillary lymph nodes has also been reported which make it clinically indistinguishable from carcinoma. Most of the lesions involve subcutaneous tissue and present as a hard mass with cutaneous attachment and enlargement of axillary lymph nodes.\[11\]

Ultrasound is a valuable tool in diagnosing cases of lymphatic filariasis and can demonstrate the adult worms. A specific distinctive continuous pattern of movement called the filarial dance has been described by ultrasonologists.\[13\] These worms can later calcify and these calcifications are visualized on breast mammograms. They appear elongated and serpiginous with no evidence of irregularity or pleomorphism and are not oriented or adjacent to the ducts. Due to their location in connective tissue unrelated to the ducts, these can be differentiated from calcifications of intraductal carcinoma. In FNA of breast, epithelioid cell granulomas are more commonly associated with filariasis in the breast but the tissue immune response is variable, with intact worms provoking only minimal reaction. The degenerating parasite is associated with inflammatory cell infiltration particularly eosinophils. Therefore adult worms and microfilaria should be sought in all unexplained granulomas of the breast.

Demonstration and identification of the parasite in the smear plays a significant role in the prompt recognition of disease and institution of specific therapy. Filarial granulomas can be confirmed by histopathology by finding of an eosinophilic granulomatous reaction around the filarial parasites which are in varying stages of degeneration. Filarial antibodies have been demonstrated in these patients and they usually respond to DEC therapy.\[1\]

Filarialis though not fatal disease, is responsible for considerable morbidity. It has been estimated that 905 million people are at risk of filarial infection.\[14\] Most frequently involved lymphatic vessels are those of lower limbs, spermatic cord, epididymis and mammary gland. When infected mosquito bites man, the larvae are deposited at the site of puncture. Later attracted by warmth of the skin, the larvae either enter through the puncture or penetrate through the skin and reach the lymphatic channel, settle down at some spot and begin to grow into adult form. The gravid female gives birth to larvae. Lumps felt may be due to the granuloma formation. Clinically both our patients were asymptomatic as regards filariasis and parasitic infection. FNA was performed to rule out the possibility of malignancy in case 1. Identification of parasite in the cytology was instrumental in the diagnosis of filariasis. The absence of microfilariae in the peripheral blood examined at night is consistent with the observation that filariasis can exist without microfilariaemia.\[8\]

**LIFE CYCLE OF WUCHERERIA BANCROFTI**

Filariasis is transmitted by the Culex mosquito and is caused by 2 closely related nematodes: Wuchereria bancrofti and Brugia malayi. The medical literature documents filariasis back to 600 BC by Sushrutha, recognizing the clinical manifestation of elephantiasis which was referred to as elephantiasis arabicum.\[15\] It is estimated that approximately 600 million people live in areas endemic for lymphatic filariasis in the South East Asian region. It is a major health problem in India particularly along the coastal areas.\[16\] Of the 8 identified species of filarial parasites, only 3 (i.e. W. bancrofti, B. malayi, and Brugia timori) are known to cause lymphatic filariasis.\[17\]

The life cycle of the filarial worms (bancroftian and brugian filariasis) can be divided into the mosquito phase and the human phase. Man is the definitive host, and the
mosquito is the intermediate host. It is transmitted by the Culex mosquito. Culex quinquefasciatus transmit W. bancrofti and Mansonia mosquitoes transmit Brugian filariasis. The mosquito cycle begins when the microfilariae are picked up by the vector mosquito during feeding. The following 4 stages of development have been identified in the vector: (a) ex-sheathing—the larva comes out of the enclosed sheath within 1–2 hours following ingestion inside the stomach of the mosquito; (b) first stage larva—within 6–12 hours, the larva penetrates the stomach wall and migrates to the thoracic muscle growing into a sausage-shaped form; (c) second stage larva—the larva moulds and increases in length with the formation of the alimentary canal, which is still in the inactive form; (d) third stage larva—this is the final stage of moulding making the larva highly active, motile, and infective, in which it migrates to the proboscis of the mosquito making it infective and ready for transmission to a new host. The duration of the mosquito cycle (extrinsic incubation period) is between 10–14 days.

In the human phase, the microfilariae find their way into the lymphatic system by penetrating into the skin following a mosquito bite. The microfilaria of W. bancrofti and B. malayi prevalent in India display nocturnal periodicity as a part of the biological adaptation correlating with the nocturnal biting habits of the mosquito. The infected larvae develop into adult male and female worms. The adult worms are usually found in the lymphatic system of humans. Male worms are 40 mm long while female worms are 50–100 mm long. The females are viviparous; giving birth to as many as 50,000 microfilariae per day. Adult female worms of W. bancrofti and B. malayi cannot be distinguished, though adult male worms show minor differences. Species diagnosis is made on the basis of morphology of the microfilaria. Microfilariae of B. malayi are smaller than those of W. bancrofti, possess secondary kinks instead of a smooth curve, and unlike the latter, the tip is not free of the nuclei. Filarial infection of breast lesions, although unusual, commonly presents with a solitary palpable mass. There have been reports where the filariasis of the breast has either mimicked malignancy or coexists with it.

Table 1 Morphological difference between Microfilariae of W. bancrofti and B. malayi

<table>
<thead>
<tr>
<th>Morphological Features</th>
<th>Microfilaria of W. Bancrofti</th>
<th>Microfilaria of B. Malayi</th>
</tr>
</thead>
<tbody>
<tr>
<td>General appearance</td>
<td>Graceful sweeping curves</td>
<td>Crinkled, secondary curves</td>
</tr>
<tr>
<td>Length</td>
<td>250 ± 46 μ</td>
<td>177 ± 230 μ</td>
</tr>
<tr>
<td>Frequent space</td>
<td>As long as broad</td>
<td>Nearly twice as long as broad</td>
</tr>
<tr>
<td>Caudal end</td>
<td>Uniformly tapering,</td>
<td>Kinked and terminal nuclei</td>
</tr>
</tbody>
</table>

>CLINICAL FEATURES

The majority of infected individuals in endemic areas remain asymptomatic throughout their life. This situation is traditionally classified as ‘endemic normals’. Bancroftian filariasis produce wide spectrum of acute as well as chronic clinical manifestations. Those patients with symptoms may manifest an acute phase of the disease characterized by fever, headache, backache, muscle pain, insomnia, anorexia, urticarial rash, malaise, nausea and fatigue, lymphangitis, lymphadenitis, epididymo-orchitis, and funnunculitis with accompanying eosinophilia and microfilaremia. The chronic stage may be characterized by lymphadenopathy, lymphedema, hydrocele, and elephantiasis. The lymphatics of the lower extremities and genitalia are the most common sites of involvement, followed by the upper extremities. However, the organisms may be found in any organ of the body, provoking an inflammatory reaction and causing a mass lesion in the absence of the classic signs of filariasis.

As the parasite circulate in the lymphatic and vascular systems, appearance of filarial organism in tissue fluids and exfoliated surface material probably occurs due to conditions causing lympho-vascular obstruction resulting into extravasations of blood and release of microfilariae. Such aberrant migration to these dead end sites is probably determined by local factors, such as lymphatic blockage by scars, or tumors, and damage to the vessel wall by inflammation, trauma, or stasis.

Microfilariae in cytological smears have been detected in association with leprosy, tuberculosis, leishmaniasis and esophageal stricture. Association of microfilariae with debilitating conditions suggests that it is an opportunistic infection. Microfilariae have been reported in cytological specimens from pericardial and pleural fluid, urine and in blood smear in HIV positive patient.
DIAGNOSIS

However, in all the cases typical clinical manifestations of filariasis may not be seen. Common methods of diagnosis of filariasis are by demonstration of microfilaria in stained or unstained blood films, circulating filarial antigen detection and demonstration of organism in histopathological sections.\[21\]

In the past, the definitive diagnosis of filariasis has been based on an identification of the microfilariae in blood. The microfilariae of *W. bancrofti* often demonstrate periodicity, and the blood samples must be taken at night, preferably between 10 P.M. and 2 A.M.\[18\]

Parasites can remain in human tissues for varying periods of time without invoking any adverse host inflammatory response. All the factors responsible for initiation of the host inflammatory response are not clear, but it is this host response that brings on the symptoms and signs of the parasites’ presence. Anderson’s Pathology states that the lymphatic vessels of the mammary gland are commonly involved after those of lower extremities, retroperitoneal tissues, and the scrotum.\[19\] The host tissue response to the parasite is extremely variable and ranges from an insignificant response to marked inflammatory cell infiltration with histiocytes and formation of epithelioid cell granulomas as in our case 1. Presence of granulomas in absence of adult filarial worm in the smear should not lead to a mistaken diagnosis of tuberculosis as the necrosis associated with filariasis is never as complete as that of tuberculosis. Moreover the presence of eosinophils should prompt the cytopathologist to search for a parasite.\[20\]

Adult worms are found in the lymphatic vessels and lymph nodes of human beings only i.e. Bancroftian filariasis is not a zoonotic disease. In infection with *Brugia malayi*, domestic animals like cats and dogs may serve as reservoirs of infection. Breast lesions occur due to obstruction of the dermal lymphatics by the filarial worm.\[21\]

The present cases did not have any clinical evidence of filariasis and there was no microfilaraemia i.e. the patient had occult filariasis. In this condition microfilariae are found in affected tissues but not in peripheral blood. This can be seen in endemic areas where filariasis can exist without microfilaraemia or microfilaraemia may be extremely transient and hence overlooked.\[21\]

Patrikar et al.\[22\] reported a rare case of breast filariasis where cytological smears did not yield any granuloma but the excised breast lump showed histopathological features of an adult filarial worm with surrounding granulomatous inflammatory reaction. They opined that although the presence of filarial granuloma is rare in India, the presence of any unexplained granuloma of breast should prompt a search for a filarial etiology.

Satpathi et al.\[23\] reported a rare case of adult filarial worms in the breast aspirate. Thus barring a few reports, the presence of an adult gravid filarial worm in a breast lesion by FNAC is extremely rare finding. In our both the cases there was presence of adult filarial worm. The presence of a host response in the form of foreign body reaction and granuloma formation is also a unique finding. Our case 1 showed a granulomatous lesion along with presence of adult filarial worm.

CONCLUSION

In endemic areas filariasis should be considered one of the differential diagnoses of a superficial swelling. Careful screening of FNAC smears help in detecting microfilaria even in asymptomatic patients. This plays a significant role in recognition of the disease, institution of specific treatment, and to avoid surgical procedure in young patients and thus can obviate the severe manifestations of lymphatic filariasis.

References

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