

Cytological diagnosis of bancroftian filariasis in lesions clinically anticipated as neoplastic

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ABSTRACT

Filariasis is a common disabling parasitic disease in this region and cytological diagnosis is often not required. Cytology has important role in diagnosis of sub-clinical filariasis. Most cases of cytologically diagnosed filariasis are clinically unanticipated. Microfilaria, ova and fragments of adult worm of *Wuchereria bancrofti*, in exfoliative as well as aspiration cytology have been reported and are useful in cytological detection of bancroftian filariasis. Microfilaria is frequently detected in association with neoplasm, although the role in tumorigenesis is controversial. The objective of the study was to investigate importance of cytology in diagnosis of filariasis in lesions clinically anticipated to be of neoplastic and to review the cytomorphology of bancroftian filaria and its association with neoplasm. This is a retrospective study carried out in cytology department of Tribhuvan University Teaching Hospital. 14 cases of cytological specimen out of 4291 (0.3%) showed microfilaria; 12 cases were from FNAC from different sites and 2 cases were from pleural fluid. 2 cases showed ova in addition to microfilaria and one of them in addition showed fragment of adult worm. Microfilaria in 4 cases of FNAC and one case of pleural fluid were associated with malignant cells.

Keywords: Filariasis, incidental cytological diagnosis, cytomorphology, association with malignancy.

INTRODUCTION

Filariasis is disabling parasitic disease prevalent worldwide caused by various species of filarial organism. Bancroftian filariasis is infection by the filarial worm *Wuchereria bancrofti* which causes disease by blocking lymphatic vessels.¹ *W. bancrofti* is responsible for 90.0% of cases of filariasis and is found throughout the tropics and in some sub-tropical areas world-wide. *Brugia malayi* is confined to South-east and Eastern Asia. *B. timori* is found only in Timor and its adjacent islands.² Certain parts of Nepal are endemic for bancroftian filaria; 58 districts of Nepal are potentially endemic for bancroftian filariasis.³

Filariasis may produce acute as well as chronic clinical manifestations or person may remain asymptomatic in endemic areas.¹ However, in all the cases typical clinical manifestations of filariasis may not be seen. Pathological findings associated with filarial lesions are chronic inflammatory cell infiltrate consisting of lymphocytes, histiocytes, plasma cells, and eosinophils. Epithelioid granulomas, necrosis and acute inflammatory cell infiltrate are also seen.¹ Association of filarial parasite with malignancy has been described but its role in tumorigenesis is not so far explained and it could be just a chance association.⁴

Common methods of diagnosis of filariasis in this country are by demonstration of microfilaria in stained

or unstained blood films, circulating filarial antigen detection and demonstration of organism in histopathological sections. Fluid cytology or fine needle aspiration cytology (FNAC) are rarely applied for routine diagnosis of clinically suspected filariasis. But filarial organism can be detected in cytological smears from various sites of body in clinically unsuspected cases of filariasis. Such lesions may be primarily caused by the organism or it may be associated with other pathology such as malignancy. Incidental detection of filarial organism has been reported in cytological smears from almost any part of body. Forms of bancroftian filaria and background pathology, however, can vary. Microfilaria is the most common form of filarial organism detected in cytological smears; however ova of the organism and fragments of adult worms can also be detected rarely.⁴ Microfilariae have also been reported in association with various benign and malignant tumors.⁴ Thus role of cytology in diagnosis of filariasis can not be underestimated in clinically unanticipated cases. This study is an attempt to prove importance of cytology in diagnosis of filariasis.

MATERIALS AND METHOD

Cytological records of the year 2004 to 2005 in department of cytology of Tribhuvan University Teaching Hospital (TUTH) were retrieved for diagnosed cases of filariasis. All the cases were clinically

unsuspected of filariasis. In all the cases cytological smears were stained with Papanicolau and Giemsa stain. Slides with filarial organisms were reviewed and findings are tabulated. Histology was available in only one case.

RESULTS

Total number of cytology during one year was 4291 that included FNAC (2289 cases) from different sites, cervicovaginal smears, sputum cytology, and body fluid cytology including urine cytology (2002 cases). Total numbers of cases with filariasis were only 14 (0.3% of all cytological specimens) that include 12 cases of FNAC (0.5% of 2289 cases of FNAC) from different sites (Table-1) and 2 cases of pleural fluid. Frequency of detection of filariasis in FNAC was 0.5% and in other cytological specimen it was 0.1% (2 cases of 2002 cases). In later only 2 cases of pleural fluid (out of 97 cases) showed the organism. Respective frequencies of detection of the organism in FNAC from different sites are shown in table 1. Out of 4 cases with filariasis in FNAC of lymph nodes, 3 were from axilla and only one from inguinal region. None of the above cases had clinical filariasis. Male and female were in 1:1 ratio (7 each) with age ranging from 11 years to 78 years.

In all the 14 cases microfilaria was identified as microfilaria of *W. bancrofti*, based on its characteristic cytomorphology that is sheathed larvae with tail-tip free from nuclei (Fig. 1). In the smears from breast aspirate in one case showed numerous coiled microfilariae as well (Fig. 2). Ova of the organism in addition to microfilaria were seen in two cases of FNAC, one from the breast lump and other from inguinal lymph node. In

Table-2: Microscopic findings in cytological smears

S. N.	Microscopic features	n. of cases	Sites of cytology
1	Microfilaria	14	All 8 sites. (see table 1)
2	Ova	02	Breast lump and inguinal lymph node
3	Fragments of organism	01	Inguinal lymph node
4	Malignant cells	04	See table 2
5	Necrosis	02	Breast and thyroid
6	Epithelioid granuloma	03	Breast 2 cases and inguinal lymph node
7	Eosinophils	09	Breast 3 cases, inguinal lymph node 4 cases and soft tissue 2 cases
8	Histiocytes, lymphocytes, neutrophils	14	All 14 cases (see table 1)
9	Giant cells	03	Breast 2 cases and inguinal lymph node one case.

latter fragments of adult worm packed with microfilaria and ova of the organism was also seen (Fig. 3 and 4). Aspirate from the breast lump also showed sheets of ova of the organism (Fig. 5). Frequency of additional associated cytological findings such as epithelioid cell granulomas, histiocytes, giant cells, eosinophils, and necrosis is shown in Table-2. All the cases showed mixed inflammatory infiltrate composed of scattered lymphocytes and occasional neutrophils.

Five cases (35.7% of 14 cases) were associated with malignancies (Fig. 6) as shown in Table-3. The second case in the table 3 showed atypical cells clusters in association with microfilaria which was later confirmed as paraganglioma in the tissue biopsy. Microfilaria load was more in the smears from non-neoplastic lesions in comparison to smears associated with malignancy. In later case only occasional microfilaria was seen. Patient profile in these 5 cases is shown in Table-3.

DISCUSSION

Bancroftian filariasis produce wide spectrum of clinical manifestations. The acute phase is characterized by fever, lymphangitis, lymphadenitis, epididymo-orchitis, and funiculitis. Headache, backache, muscle pain, insomnia, anorexia, urticarial rash, malaise, nausea and fatigue are common complaints. Eosinophilia and microfitemia are common in acute phase. Chronic stage of bancroftian filariasis is characterized by lymphadenopathy, lymphadema, hydrocele, and elephantiasis.¹ A significant number of infected individuals in endemic areas remain asymptomatic throughout their life.¹ The later situation is traditionally classified as 'endemic normals'.² In recent years the traditional classification of filarial disease has been challenged. The introduction of assays for

Table-1: Frequency of filariasis in FNAC

S. N.	Specimen	Total n. of cases	n. of cases with filariasis	%
1	Breast lump	549	3	0.5
2	Lymph nodes	320	4	1.2
3	Soft tissue	74	2	2.7
4	Lung	66	1	1.5
5	Thyroid nodule	500	1	0.2
6	Pancreatic tumor	12	1	8.3
7	Other lesions	780	00	00
Total	2289	12	0.524	



Fig. 1. FNAC smear from soft tissue nodule. microfilaria of *Wuchereria bancrofti*. Sheathed larva with tail tip free from granules. (Papanicolaou stain, X100).

circulating filarial antigen, the discovery of occult lymphatic pathology and renal disease in ‘asymptomatic microfilarems’ and the recognition of the role of bacterial infection in the pathogenesis of acute and chronic disease suggests that the old classification based on presence or absence of microfilaremia and/or chronic pathology is outdated. It is no longer wise to think of individuals as having filarial ‘infection’ without ‘filarial disease’ for the same reason – many of the former will have evidence of ‘covert disease’ if the studies are rigorous enough.² Significant numbers of patients never undergo tests for filarial infection because they are never included in epidemiological studies nor they present features typical of filariasis.

In present study none of the patients were clinically suspected of filariasis; clinically they presented with breast lump (3 cases), lymphadenopathy (4 cases), and soft tissue nodule (2 cases). One case of lymph node

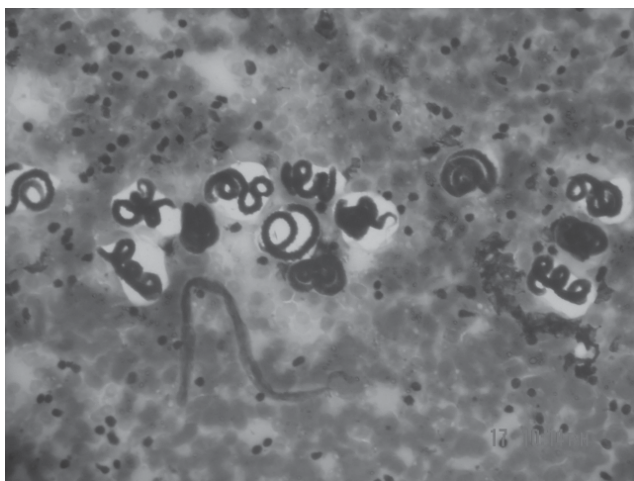


Fig. 2 FNAC smear from breast lump. Numerous coiled microfilariae, a curve straight microfilaria (at the bottom of the field) and numerous ova in the background of the smear. (Giemsa stain, X100).

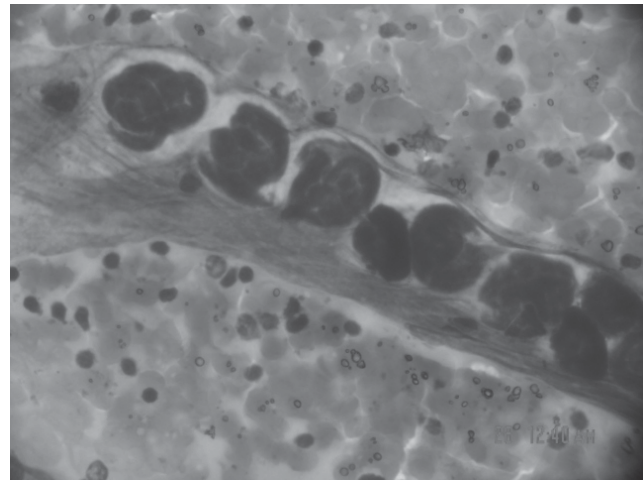


Fig. 3. FNAC smear from inguinal lymph node. Fragment of adult female filarial worm containing numerous coiled microfilaria (Giemsa stain, X200).

enlargement in the axilla was also associated with paraganglioma. Likewise 3 cases were associated malignant tumor in lung, thyroid and pancreas, one in each case. 2 cases presented with features of pleural effusion, one out of that was associated with metastatic adenocarcinoma.

A review of literature reveals detection microfilaria in most of the commonly performed cytological specimens and mostly they are incidental. Microfilaria have been detected in cervicovaginal smears,⁵ endometrial smears,⁶ nipple secretions,⁷ ovarian cyst fluid,⁵ breast aspirates,⁸ hydrocele fluid,⁹ epididymal aspirates,¹⁰ urine samples,¹¹ lung aspirates,¹² pleural fluid,¹³ bronchial washings,⁵ ascitic fluid,¹⁴ intraoperative peritoneal fluid,¹⁵ lymph node aspirates,^{16,17} thyroid aspirates,¹⁸ salivary gland aspirates,¹⁹ bronchial brushings,²⁰ laryngeal and pharyngeal brushings,²¹ gastric brushings,²² pericardial fluid,²³ cutaneous nodule,²⁴ soft tissue nodule,²⁵ oral ulcer,²⁶ bone marrow aspirates,²⁷ brain aspirates²⁸ and joint aspirates.²⁹

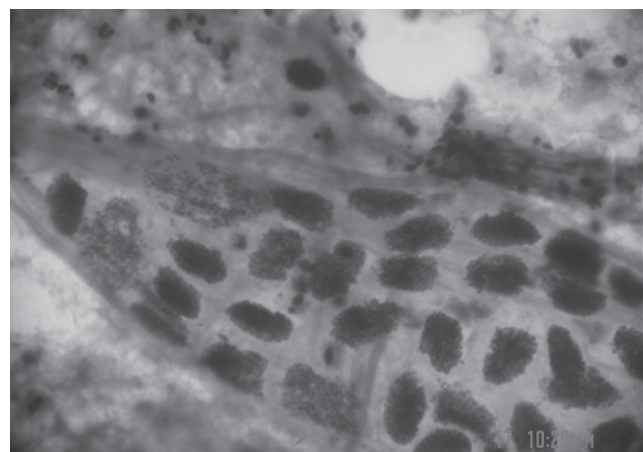


Fig. 4. FNAC smear from inguinal lymph node. Fragment of adult female filarial worm containing numerous ova (Papanicolaou stain, X400).

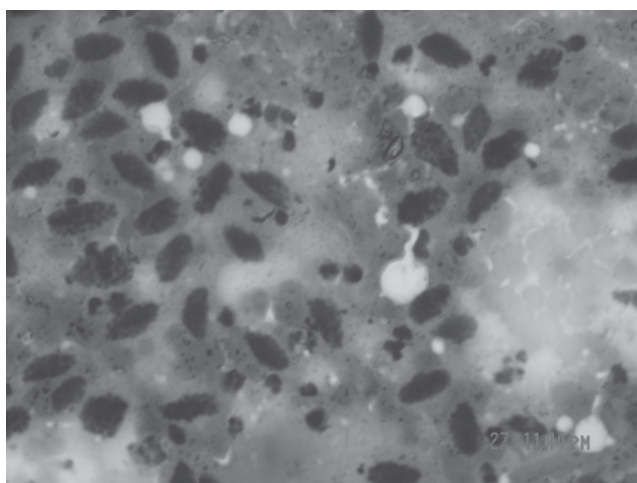


Fig. 5. FNAC smear from breast lump. Sheets of ova of the filarial organism. (Giemsa stain, X100).

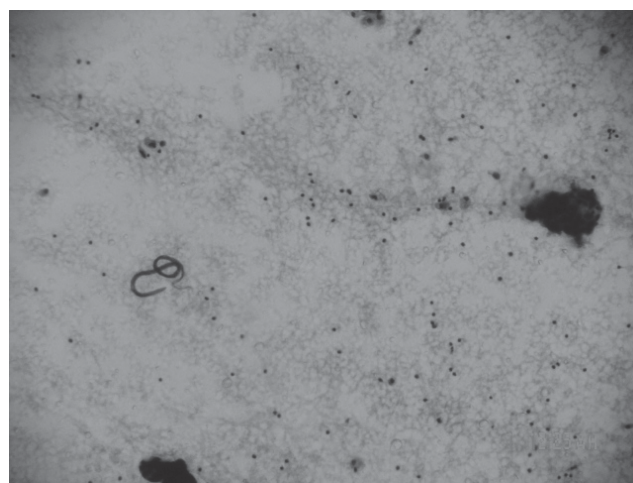


Fig. 6. Pleural fluid smear. A microfilaria (left of the field) and a clusters of malignant cells, diagnosed as metastatic adenocarcinoma (right of the field and left bottom of the field). (Papanicolaou stain, X40).

Microfilariae have been reported in association with neoplastic lesions such as hemangioma of liver,^{4,30} Ewing’s sarcoma of bone,³¹ squamous cell carcinoma of maxillary antrum,³² anaplastic astrocytoma of thalamus,²⁸ low grade astrocytoma of C6-D1 spinal segment,²⁸ cranipharyngioma of third ventricle,²⁸ non-Hodgkin lymphoma,^{4,33} transitional cell carcinoma of bladder,⁴ follicular carcinoma of thyroid,⁴ seminoma of undescended testis,⁴ meningioma,³⁴ intracranial hemagioblastoma,³⁴ Fibromyxoma,³⁴ squamous cell and undifferentiated carcinoma of uterine cervix,³⁴ carcinoma of pharynx,²¹ metastatic melanoma to bladder,³⁵ leukemia,³⁶ lymphangiosarcoma,³⁷ carcinoma of pancreas,³⁸ dentigerous cyst,³⁹ carcinoma breast.⁴⁰

In all 14 cases in the present study, microfilariae of *W. bancrofti* were detected, as suggested by their typical morphologic appearance. *W. bancrofti* microfilariae are sheathed and measure 230-300 X 7-10 microns. The cephalic space at the anterior end is 5-7 microns long and the anterior nuclei are side by side. The caudal space at the pointed posterior end is 5-15 micron long and the terminal nuclei are elongated.¹ *Wuchereria bancrofti* is the most prevalent filarial parasite accounting for 90.0% of filarial infection worldwide and in Southeast Asia region.² In most studies and in most of the reported cases microfilariae in the cytological smears were identified as *W. bancrofti*.¹⁻⁴⁰

Diagnosis of filariasis in cytological smears can also be made by presence of fragments of adult female or male worms and ova of the filarial organism with or without simultaneous presence of microfilaria. Presence of ova and adult worms of filarial organism in cytological smears may or may not be associated with simultaneous presence of microfilaria.^{41,42}

Arora *et al* in a retrospective study reviewed 34 cases of filariasis reported on FNAC for the identification of various parasitic structures and found 3 cases showing no microfilaria but adult male worm (one case), non-gravid female worm (one case) and fertilized eggs (one case). Thirteen cases which showed microfilaria also showed fragments of gravid female worm.⁴¹

Ova and adult worms with or without microfilaria are most often detected in fine needle aspirate smears rather than exfoliative cytology specimen. They have been detected in aspirates from lymph nodes,^{16,17} soft tissue swelling,⁴³ perineal ulcer,⁴⁴ and breast lumps.⁸

Adult female worms were the more common finding than male worm, in most of the studies and in reported cases; in addition most of the female worms detected in smears were gravid containing microfilaria or embryonated ova within their body cavities as well as outside.^{8,16,17,42-44}

Table-3: Profile of patients with filariasis associated with malignancies

S.N.	Age	Sex	Site of specimen	Types of tumor
1	58	M	FNAC lung mass	Adenocarcinoma
2	50	M	FNAC axillary node	Paraganglioma
3	78	F	FNAC thyroid nodule	Anaplastic carcinoma thyroid
4	58	M	Pleural fluid	Adenocarcinoma, metastatic
5	41	M	Pancreatic tumor	Adenocarcinoma

Jain *et al* in a retrospective study carried out over 10 years in clinically unsuspected cases of filariasis diagnosed on cytology, found microfilaria alone in 25 cases and simultaneous gravid female worm of *W. bancrofti* in 4 cases. They also found one case with both adult male and female worms along with microfilariae and eggs.⁴²

Akin to microfilariae in the smears, bancroftian adult worms as well as ova are more common than any other species; however, a case of adult worm of *B. malayi* and its ovarian fragments has been reported in needle aspirate smears from epitrochlear lymph node.⁴⁵

In present study bancroftian microfilariae as well as ova were seen in needle aspirate smears from breast lump (one case) and inguinal lymph node (one case), the later case in addition also revealed fragments of adult female worm packed with microfilaria.

All the three forms of bancroftian filarial organism that is microfilaria, adult worm fragments, and ova have been reported in cytological smears from neoplastic lesions. Their presence along with benign and malignant tumors is a controversial issue and needs to be explored further as this could be just a chance association or the parasite may act as trigger for carcinogenesis.⁴

Their presence in association with tumors of lymph nodes and lymphatic can be explained as they are normal habitation for the filarial organism,^{34,37,46} however, this view does not completely explain their association with tumors from other sites.

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.^{11,47} 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.^{11,47}

A rich blood supply in the tumors could be a possibility resulting in concentration of parasites at these sites.⁴ It has been suggested in a few reports that filarial organism may be involved in tumorogenesis by releasing certain toxic mediators or by chronic mechanical irritation at the sites of infestation.⁴

Not all authors agree with above explanation of tumorogenesis by microfilaria and they explain these phenomena as purely incidental. Microfilariae wander in tissue fluids and may get trapped in needle during aspiration.⁴⁸

It is interesting to notice that there are very few reported cases of filarial organisms associated with tumors in histological sections as compared to cases reported in cytological specimens as is the case in present study.⁴⁸ This finding also cast doubt regarding the role of microfilaria in carcinogenesis. In most of the reported cases microfilariae could not be identified in histological sections despite their presence in cytological smears.^{4,8,49}

In this study one of the case showed microfilaria and atypical cells, subsequent biopsy of the axillary mass revealed paraganglioma but no filarial organisms.

Microfilariae have been detected in association with metastatic malignant cells in pleural fluid,⁵⁰ peritoneal fluid⁵¹ and pericardial fluid.⁵² In present study one case of microfilaria was detected in association with metastatic adenocarcinoma in pleural fluid.

Serous cavity effusion such as pleural effusion can be primarily caused by filarial infection and a search for microfilaria in pleural fluid smears especially in cases of recurrent effusions can be very rewarding if tuberculosis and malignancy are remote possibility.¹³

Microfilariae in cytological smears have been detected in association with leprosy,⁵³ tuberculosis,^{12, 33} leishmaniasis⁴² and esophageal stricture.⁵⁴ In later condition blood smear also revealed microfilaria.⁵⁴

Association of microfilariae with debilitating conditions suggests that it is an opportunistic infection.³³ This statement is further supported by the fact that microfilariae have been reported in cytological specimens from pericardial fluid, pleural fluid, & urine and in blood smear in a HIV positive patient.⁵⁵

In addition to tumor cells in the cases of neoplastic lesions and reactive mesothelial cells in cases of serous effusions, other cytological findings in the cytological smears containing various forms of filarial organism are reactive lymphoid cells, acute inflammatory cells including neutrophils, granular debris, eosinophils, macrophages, epithelioid cells, epithelioid granulomas and necrosis.^{1,5,22,33,52,56} Sometimes smears with microfilaria may not show significant numbers of inflammatory cells.³³

In present study lymphocytes, neutrophils and histiocytes were seen in all the cases. Frequencies of eosinophils, giant cells, epithelioid granuloma and necrosis are shown in Table-2.

As present study was a retrospective study, microfilaria in peripheral blood smear (PBS) was not possible to evaluate. However, absence of microfilariae in PBS does not exclude filarial infection.^{30,49} PBS shows microfilaria only in a handful cases of filariasis diagnosed by cytology.^{12, 34} Microfilariae were even absent in PBS of some cases filariasis in which bone marrow aspirates had revealed the organism.⁵⁷

In present study none of the cases were clinically anticipated of having filarial infection. In most of the reported cases filariasis was diagnosed in cytology in clinical unsuspected cases. Even in the studies involving large number of cases diagnosis was made incidentally.^{5,42}

Maximum number of cases of filariasis in present study was from lymph nodes (4 cases) followed by breast (3 cases), soft tissue nodule and pleural fluid (2 cases each). Rest of three cases and one case from axillary node was associated with adenocarcinoma a lung, anaplastic carcinoma of thyroid, pancreatic adenocarcinoma and paraganglioma respectively. In association with pancreatic carcinoma only few cases have been reported,³⁸ similarly in thyroid microfilaria has been reported in colloid goiter¹⁸ and follicular carcinoma⁴ but not with anaplastic carcinoma. Association paraganglioma has not been reported. Surprisingly in present study no cases detected in common smears like cervicovaginal smears. Similarly no cases detected in urinary sediments, bronchial cytology, gastroesophageal cytology, peritoneal fluid, and synovial fluid. All the cases associated with malignancy were above 50 years of age and male except for one case associated with pancreatic adenocarcinoma, which was 41 years of age and one case associated with anaplastic carcinoma thyroid which was female.

Cytology has important role in diagnosis of sub-clinical filariasis, although cytological diagnosis is often not asked. Most cases of cytologically diagnosed filariasis are incidental and clinically unanticipated. In present study 14 cases of filariasis was diagnosed cytologically in one year period, 12 cases were diagnosed by FNAC and 2 cases were diagnosed on pleural fluid cytology. 6 cases including 2 cases of pleural fluid were associated with malignancy. Microfilaria, ova and fragments of adult worm of *W. bancrofti*, in cytological specimen have been reported and were also seen in present study. They are useful in cytological detection of bancroftian filariasis. Association of filariasis with neoplasm is often seen, although the role in tumorigenesis is controversial.

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