Wuchereria bancrofti and Cytology: A Retrospective Analysis of 110 Cases from an Endemic Area

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Abstract

Background: Wuchereriasis is a significant cause of chronic morbidity. It can affect any organ/tissue in the body. Fine-needle aspiration cytology (FNAC) is an easy method for its detection. A comprehensive analysis of the various facets involved has not been discussed in detail in any publication. Materials and Methods: A twenty-six year (February 1994 to January 2020) retrospective audit of all patients who were cytologically diagnosed with wuchereriasis was performed. Data regarding age, sex, organ/tissue involved, and presence of co-existing disease were noted. Hematoxylin and eosin (H and E) and May-Grünwald-Giemsa (MGG) stained slides were screened for microfilaria, adult worm, larval forms, microfilaria ghosts, epithelioid cell granuloma, and eosinophils. Results: Audit yielded 19,323 cases of which 110 had wuchereriasis giving an incidence of 0.57%. The 11–30 year age group accounted for 41.8% cases. Male: female ratio was 1.04:1. Duration of disease at presentation ranged from 3 days to 24 years. Lymph node was the commonest site involved (40%), followed by soft tissue (23.6%) and female breast (14.5%). Highest parasitic load was encountered in female breast aspirates. Microfilaria bancrofti was seen in 105 (95.4%) cases. In the five cases where microfilaria bancrofti was not encountered, diagnosis was established by the presence of adult gravid female worm (2 cases), coiled larvae (2 cases), and both adult gravid female worm and coiled larvae (1 case). Microfilaria ghosts were seen in 18.2% cases. Coexisting benign and malignant diseases were encountered in 17.3% and 13.6% cases, respectively. Conclusion: FNAC provides a simple and inexpensive means of detecting wuchereriasis and is preferred over histopathology. All stages of development of this nematode in human beings are identified in cytology. Microfilaria ghost is a useful clue in screening. The presence of granuloma and eosinophilic infiltrate indicates tissue reaction only. Patients with asymptomatic microfilaraemia should be reported in cytology as they merit treatment.

Keywords: FNAC, Microfilaria bancrofti, Wuchereriasis

INTRODUCTION

The subject of filariasis does not incite much interest as it is not an immediate health threat having serious consequences. In addition, it has limited geographic distribution in tropics and subtropics and usually runs an insidious course. In India, lymphatic filariasis is prevalent in 18 states and union territories, chiefly found in states of Bihar, Uttar Pradesh, Odisha, Andhra Pradesh, Tamil Nadu, Kerala, and Gujarat. Bancroftian filariasis is responsible for almost 98% of infection. Furthermore, due to increased intercontinental travel and atmospheric warming, it may be encountered by anyone anywhere in the world.

The term “filariasis” is commonly used to denote the morbid changes produced by lymphatic dwelling Wuchereria and Brugia. However, its use can also imply infection with any member of the superfamily Filarioidea, viz., Loa loa, Onchocerca volvulus, Mansonella ozzardi, M. perstans, and M. streptocerca. Thus, infection with Wuchereria bancrofti (W. bancrofti) is more appropriately referred to as “wuchereriasis” or “Bancroft’s filariasis.”

Though microfilaria of W. bancrofti was first described by Demarquay in 1863,[3] not many reports were published before the advent of cytopathology methods. Subsequently, there have been numerous sightings of the parasite with subsequent publication on this subject. This does not imply that there has been an increase in the incidence of this disease. As fine-needle aspiration cytology (FNAC) and effusion
cytology evolved, more and more organs were targeted resulting in more frequent spotting of these parasites. In addition, as the whole of microfilaria [Figure 1], adult worm, and larvae are visible in cytology smears, they are easily identified. This is in contrast to histopathology sections where we see only a cross section of the worm which may be overlooked by an uninitiated pathologist [Figure 2]. Moreover, FNAC is preferred over biopsy for demonstration of the parasite since tissue removal further compromises lymphatic drainage.[4]

There are many subtle clinical presentations which have not been documented but are encountered in endemic areas, viz., decreased motor power in arms and/or legs, decreased power of grip, difficulty/inability to hold a pen, difficulty/inability to write, heaviness of forearm, etc.

Although there are many publications on this subject, mostly as case reports, a comprehensive analysis of the various facets involved has not been discussed in detail. This retrospective study intends to analyze and discuss the various facets involved in diagnosis and interpretation in relation to cytology. As only Wuchereria bancrofti is encountered in our area, the ensuing discussion pertains to this species only.

Materials and Methods

A twenty-six year (February 1994 to January 2020) retrospective audit of all patients who were cytologically diagnosed with wuchereriasis, and those who had not received any prior therapy, was performed. The sample size was determined by the period of the study and the inclusion criteria detailed above. Data regarding age, sex, organ/tissue involved, and presence of co-existing disease were noted. Nonsuction method was mostly used for collecting the sample. Wherever required, aspiration was done using a 22-gauge needle and 10 mL syringe. If fluid was aspirated, the fluid was centrifuged at 3000 rpm for 15 min and the sediment thus obtained was used for making smears after decanting the supernatant. At least two smears were made for each case, one slide was stained with hematoxylin and eosin (H and E) stain and the second slide with May-Grünwald-Giemsa (MGG) stain. Stained smears were screened for microfilaria bancrofti, adult male worm, adult female worm (nongravid and gravid), larval forms, microfilaria ghosts, epithelioid cell granuloma, and eosinophilic infiltrate.

Results

During the twenty-six year study period, 19,323 FNACs were performed, out of which 110 cases were diagnosed with wuchereriasis [Table 1], giving an incidence of 0.57%. The clinical possibility of wuchereriasis was not considered in any of these patients. The age distribution of the patients is shown in Table 2. Most of the cases were seen in 11–30 year age group, which accounted for 41.8% cases (46/110). The male: female ratio was 1.04:1. Duration of disease at presentation ranged from 3 days to 24 years.

Among the tissues affected [Table 1], lymph nodes were most often involved (40%), followed by soft tissue (27.3%) and female breast (14.5%). The highest parasitic load was encountered in female breast aspirates.

Cytologic diagnosis was made by detection of microfilaria bancrofti [Figure 1], adult gravid female worm [Figure 3], and coiled larvae [Figure 4a-e], which were seen in various permutations and combinations as detailed in Table 1. Microfilaria bancrofti was seen in 105 cases (95.4%). In the five cases where microfilaria bancrofti was not encountered, diagnosis was established by the presence of adult gravid female worm (2 cases), coiled larvae (2 cases), and both adult gravid female worm and coiled larvae (1 case). Microfilaria ghosts were seen in 18.2% cases [Figure 5]. Epithelioid cell granuloma [Figure 6] and eosinophilic infiltrate, when present, signified tissue reaction to these nematodes.

Both benign and malignant co-morbid conditions were noted in 19 and 15 cases, respectively, as detailed in Table 3.

![Figure 1: FNA smear from a lymph node showing a sheathed (S) microfilaria of W. bancrofti showing nuclei in central axis of body which do not extend to the tip of tail, anterior cephalic space (CS), nerve ring (NR), excretory pore (EP), excretory cell (EC), and genital cells (GC) (H&E, ×400)](image)

![Figure 2: Histopathology section from a lymph node showing three adult worms (H&E stain, ×20)](image)
Discussion

*W. bancrofti* is nocturnally periodic, i.e., microfilariae forms are scarce in peripheral blood by day and increase at night, mostly between 10 PM and 04 AM. Thus, the timing of sample collection is critical. Though a large number of people are affected by *W. bancrofti*, it is not surprising that microfilariae are not frequently encountered in fine-needle aspirates and other cytologic material as most of the sample collection is done during daytime. Though the town of Munger lies on the southern bank of river Ganges and is an endemic area in the state of Bihar, our study revealed an incidence of 0.57% only among all the patients undergoing FNA of various lesions during the study period.

In cytology, the various stages of development of the nematode in human beings are seen and are enumerated as follows:

(a) **Microfilaria**—It is a highly motile prelarva, about 290 µm in length and 6–7 µm in breadth and has an outer sheath which is much longer than its body and projects slightly beyond its extremities. They have bluntly rounded head and pointed tail. Nuclei, which appear as granules, are seen in the central axis of the body extending from the head to the tail end. However, the nuclei do not extend up to the tip of tail, this being a distinguishing feature of microfilaria bancrofti. At the anterior end is a space, called cephalic space, which is devoid of granules and is as long as broad. The granules are broken at definite places, viz., (1) nerve ring which is an oblique space, (2) excretory pore and excretory cell, (3) genital (G) cells, and (4) anal pore [Figure 1].

(b) **Adult male worm**—Hair like, measures 1.5–2.0 cm in length and 0.8–1.0 mm in thickness with a ventrally curved

Table 1: Site distribution and microscopic findings of 110 cases of wuchereriasis. (* - diagnosis established by presence of adult gravid female worm and/or coiled larva in absence of microfilaria bancrofti; † - single case having both adult gravid female worm and coiled larva in absence of microfilaria bancrofti)

| Site                  | No. of cases | Microfilaria bancrofti | Gravid female worm | Coiled larva | Granuloma | Eosinophilic infiltrate |
|-----------------------|--------------|------------------------|--------------------|--------------|-----------|------------------------|
| Lymph node (n=44)     |              |                        |                    |              |           |                        |
| Cervical              | 18           | 18                     | -                  | -            | 03        | 07                     |
| Axillary              | 10           | 10                     | -                  | 01           | 01        | 05                     |
| Inguinal              | 09           | 07                     | 02*                | -            | 03        | 07                     |
| Femoral               | 04           | 03                     | -                  | 01*          | 01        | 03                     |
| Generalized           | 02           | 02                     | -                  | -            | -         | 01                     |
| Epitrochlear          | 01           | 01                     | -                  | -            | -         | -                      |
| Soft tissue           | 30           | 30                     | 02                 | -            | 01        | 16                     |
| Female breast         | 16           | 15                     | 01†                 | 01†          | 02        | 05                     |
| Salivary gland        | 05           | 05                     | -                  | -            | -         | -                      |
| Thyroid               | 04           | 04                     | -                  | -            | -         | -                      |
| Skin                  | 03           | 03                     | -                  | -            | -         | -                      |
| Liver                 | 02           | 02                     | -                  | -            | -         | 01                     |
| Testis                | 02           | 02                     | -                  | -            | -         | -                      |
| Epididymis            | 01           | -                      | -                  | 01*          | 01        | -                      |
| Scrotum               | 01           | 01                     | -                  | -            | -         | -                      |
| Bone                  | 01           | 01                     | -                  | -            | -         | -                      |
| Buccal mucosa         | 01           | 01                     | -                  | -            | -         | -                      |

Table 2: Age distribution of 110 cases of wuchereriasis

| Age range (in years) | No. of cases |
|----------------------|--------------|
| <=10                 | 11           |
| 11-20                | 20           |
| 21-30                | 26           |
| 31-40                | 15           |
| 41-50                | 14           |
| 51-60                | 12           |
| >60                  | 12           |

Figure 3: FNA smear from a lymph node showing a gravid female worm containing numerous ovoid embryos, some escaping from the uterus (MGG, ×40)
tail. It has a single, long, delicate, thread-like testis. The testis has two distinct zones, the proximal germinal zone, showing solid sex cells, and the distal growth zone, filled with round amoeboid sperm 1–2 µm in diameter. The growth zone merges with the seminal vesicle, which is filled with fusiform sperm 6–8 µm long[4]

(c) **Adult nongravid female worm**—Hair like, measures 8–10 cm in length and 0.2–0.3 mm in thickness with a narrow and abruptly pointed tail. The internal reproductive system shows characteristic long ovaries with numerous nuclei, lipid droplets, and refringent granules. Eggs or microfilariae are not seen in the paired uteri[4]

(d) **Adult gravid female worm**—Distinctive features are prominent, paired uteri filled with eggs and developing microfilariae [Figure 3][4,7,8]
(e) **Embryo**—The young embryos in the inner portion of the uterus are confined within thin, hyaline, ovoid shells, 38 × 25 μm in size. As they progress toward the outer portion of the uterus, the shells become elongated to accommodate the uncoiling embryos and become known as “sheath” of the microfilaria. In this stage, they are discharged from the worm [Figure 4 a-e].

“Microfilaria ghosts” are an additional finding noted in FNA smears [Figure 5]. These microfilaria ghosts have the same size and contours as that of a microfilaria. They are mere impressions of a microfilaria created by the pressure that is exerted while making smears. It is a very useful clue as once these ghost images are identified, a detailed scan of the smear invariably leads to detection of microfilaria. However, if only microfilaria ghost is identified in the absence of microfilaria or any of the other life cycle stage(s) of *W. bancrofti*, a diagnosis of wuchereriasis should not be made. In this study, microfilaria ghosts were identified in 18.2% (20/110) cases. Ours is the first original case series to coin the term “microfilaria ghost” and describe its morphology.

Our study revealed that microfilariae were the most commonly encountered life cycle stage of *W. bancrofti*, being seen in 105 out of 110 cases, which is commensurate with the published literature. In our study in 5 out of 110 cases, microfilaria was not encountered. In these patients, diagnosis was established by presence of adult gravid female worm (2 cases), coiled larvae (2 cases), and both adult gravid female worm and coiled larvae (1 case). While screening smears, one needs to be aware of all these life cycle stages of *W. bancrofti*, as on occasions microfilaria may not be encountered. In such a situation, detection of adult male, adult nongravid female, adult gravid female, or larva, either singly or in combination, will suffice for a diagnosis of wuchereriasis.

Adult worms are largely inaccessible as they live in afferent lymphatics or sinuses of lymph nodes. They have been reported from few sites like epididymal nodule, inguinal swelling and breast lump, lymph node, and soft tissue swelling. In our study, adult gravid female worm was identified in 5 out of 110 cases.

Male worm has been spotted in epitrochlear and inguinal lymph node and adult nongravid female worm has been reported in an epididymal nodule. In our study, we did not encounter any adult male or adult nongravid female worm.

As more and more evaluation is done using FNAC, the frequency of involvement of various sites in wuchereriasis may need to be revised. Epitrochlear, axillary, femoral, and inguinal lymph nodes are the commonest sites of lymph node involvement in wuchereriasis. However, in our study, the lymph node involvement encountered were cervical 16.4% (18/110), axillary 9.1% (10/110), inguinal 8.2% (9/110), femoral 3.6% (4/110), generalized 1.8% (2/110), and epitrochlear 0.9% (1/110). Involvement of genital lymphatics occurs almost exclusively in *W. bancrofti* infection. This can manifest as funiculitis, epididymitis, and scrotal pain and tenderness. The favorite site for the adults of *W. bancrofti* is the globus major of epididymis. Our study revealed the involvement of epididymis in 1 out of 110 cases and scrotum in 1 out of 110 cases only. Testicular involvement was seen in 2 out of 110 cases. In both these cases, azoospermia with maturation arrest at spermatid level was noted. Microfilarial involvement of testis in infertility has not yet been reported. However, it could not be ascertained if this relationship was causal or incidental.

The association of wuchereriasis with comorbid conditions is shown in Table 3. The association with benign and malignant conditions is mostly incidental. As these parasites circulate in the vascular and lymphatic systems throughout the body, their appearance in tissue fluids, exfoliated surface material, and fine-needle aspirates would probably occur only under conditions of lymphatic or vascular obstruction or extravasation. Such aberrant migration to these “dead end” sites is probably determined by local factors such as mechanical blockade of the lumen by dead worms, damage to vessel wall by inflammation or trauma, and lymphatic blockade by scars or tumors. In addition, rupture of fragile vessels created by neo-angiogenesis and lymphangiogenesis and increased blood flow to tumor sites may increase the chance of detection of microfilaria.

Three clinical pictures exist:
(a) Asymptomatic microfilaraemia
(b) Microfilaraemic filariasis
(c) Amicrofilaraemic filariasis (occult filariasis).

Asymptomatic microfilaraemia is one of the most common presentations of lymphatic filariasis. As the microfilaria circulate in the blood and lymphatics, they may be inadvertently picked up in fine-needle aspiration smears, cervical smears, effusion cytology smears, and other cytology specimen. In endemic areas, despite large numbers of circulating microfilariae in the peripheral blood, the overwhelming majority of infected individuals have few overt clinical manifestations. Although they may be clinically asymptomatic, virtually all persons with *W. bancrofti* microfilaraemia have some degree of subclinical disease that includes microscopic hematuria and/or proteinuria, dilated and tortuous lymphatics, and in men scrotal lymphangiectasia. However, in relatively few, the infection does progress to either acute or chronic disease. Nonetheless, early treatment of these patients is recommended to prevent further lymphatic damage and also to eradicate a potential source of infection. Hence, cytologic reporting of microfilaria in such patients is mandatory.

A definite cytologic diagnosis can be made only on the detection of the parasite, either the adult worm or microfilaria. Diagnosis of amicrofilaraemic filariasis, as seen in tropical pulmonary eosinophilia, cannot be made cytologically. It is done either by the demonstration of circulating antigens and/or
by ultrasonographic identification of motile adult worm within dilated lymphatics.\cite{23}

The pathogenic effects seen in wuchereriasis are produced by the adult wuchereria, living or dead. Living microfilariae circulating in blood are not known to produce any pathogenic effect, except in tropical pulmonary eosinophilia. Host reaction to the worms consists of an accumulation of histiocytes, epithelioid cells [Figure 6], eosinophils, lymphocytes, plasma cells, and giant cells in the lumen of the vessel around the worms. Hyperplasia of the endothelium and perilymphatic cellular infiltration occur, not only around the worms but also proximally along the vessel to the regional lymph nodes in an ascending lymphangitis and lymphadenitis. As acute inflammation subsides and fibrosis advances, the worms die and are absorbed or become calcified.\cite{5} In addition, the presence of Charcot Leyden crystals has also been described.\cite{5} The presence of granuloma and/or eosinophilic infiltrate in cytology smears indicates host tissue response to the nematode only. In our study, granulomas were seen in 10.9% (12/110) cases, and eosinophilic infiltrate was observed in 40.9% (45/110) cases. In the absence of microfilaria or adult worm, the presence of granuloma and/or eosinophilic infiltrate does not merit a diagnosis of wuchereriasis.

FNAC is the preferred modality to diagnose Wuchereriasis as compared to histopathology. Following are the demerits of using histopathology for its diagnosis:-

1. Adult *W. bancrofti* resides in the lymphatic vessels and not in the lymph node parenchyma\cite{38}
2. Dead worms appear deformed, fragmented, with ruptured external membranes making their identification difficult on a histopathology section\cite{38}
3. All stages of development of this nematode in human beings are not identified in histopathology sections
4. Surgical tissue injury during biopsy procedure further compromises an already jeopardized lymphatic drainage\cite{46}
5. As the treatment is usually medical, surgery is infrequently indicated for diagnosing such conditions.

To conclude, cytology provides a simple and inexpensive means of detecting wuchereriasis and is preferred over histopathology. In human beings, all stages of development of this nematode are identified cytologically, presence of any of which suffice for diagnosis. Microfilaria ghost is a useful clue in screening and helps in detection of microfilaria. The presence of granuloma and eosinophilic infiltrate indicates tissue response, but when present alone, it is not sufficient to make a diagnosis. Patients with asymptomatic microfilaraemia should be reported in cytology as such patients merit treatment.

To the best of our knowledge, this is the largest case series detailing *Wuchereria bancrofti* in cytology. Ours is the first original case series to coin the term “microfilaria ghost” and describe its morphology.

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**Conflicts of interest**

There are no conflicts of interest.

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