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Review

Filarial hydrocele: a neglected condition of a neglected tropical disease

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Abstract

Filarial hydrocele is the most common chronic manifestation of lymphatic filariasis (LF) and poses a major public health burden to several filarial endemic countries. This review highlights the socio-economic impact of the disease, the role of the immune system in hydrocele development, current diagnostic approaches, and the control and management of filarial hydrocele. In the quest to facilitate the global effort to eliminate filarial hydrocele as a neglected tropical disease, a more comprehensive understanding of the mechanisms underlying the pathogenesis and development of the condition is important. In general, success has been achieved using annual treatment with ivermectin, but much remains to be done, particularly with late-stage infected individuals where surgery remains the only option. Studies have successfully demonstrated that inhibition of embryogenesis in adult female worms occurs after weeks of tetracycline treatment. Even more intriguing was the observation that the *Wolbachia* endosymbionts potently induce proinflammatory cytokines such as tumor necrosis factors (TNFs) and vascular endothelial growth factors (VEGFs), which are crucial for the development of filarial hydrocele. Furthermore, reports from human studies show that doxycycline treatment significantly ameliorates filarial hydrocele and markedly reverses early-stage filarial hydrocele. However, with the enormous challenges that face LF elimination such as global funding, logistics, civil wars, and drug resistance, a more relentless and collective approach from local governments as well as other stakeholders is needed to accelerate the fight against filarial hydrocele if the goal to eliminate it by 2020 is be to achieved.

Key words: hydrocele; filariasis; endemic; proinflammatory; *Wolbachia*.

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Introduction

Lymphatic filariasis (LF) is one of the oldest and most debilitating diseases known to humanity [1]. The disease is caused by the filarial worms *Wuchereria bancrofti*, *Brugia malayi* and *B. timori. W. bancrofti* accounts for 90% of the infections. The worms are transmitted by mosquitoes and are endemic in more than 80 countries in tropical and sub-tropical areas of Africa, Asia, the Pacific, the Middle East, and the Americas [2]. More than 1.4 billion people, or one-fifth of the world's population, most of whom are the world's poorest, are at risk [3]. Infection with *W. bancrofti* is usually acquired in childhood, but the painful and profoundly disfiguring visible manifestations of the disease such as hydrocele and elephantiasis occur later in life [4].

Filarial hydroceles are the most common chronic manifestation of LF [5] and afflict an estimated 27 million men worldwide [6]. Hydroceles result from the gradual accumulation of fluid in the tunica vaginalis of the scrotal sac and may be accompanied by thickening of the spermatic cord and changes in the scrotal skin and subcutaneous tissue [7]. When left unattended, filarial hydroceles may lead to other urogenital complications, including lymph scrotum, a urogenital condition characterized by the presence of lymphatic vesicles on the surface of the scrotal skin that can easily rupture, giving rise to drainage of the whitish secretion typical of the disease. This secretion can then serve as an excellent culturing medium that favors repeated bacterial infections. These repeated bacterial infections can trigger the progression of the condition to acute inflammation of the scrotum and penis, elephantiasis of the scrotum, and inguinal adenitis [8]. Despite the fact that many males in endemic regions are infected, much remains to be established about the factors that drive filarial hydrocele development. Indeed, genetics of infected subjects could be a crucial factor. Other factors, such as co-infection, age, gender, and ethnic background, could also be driving forces that need to be validated further.

The social impact of disease on individuals and productivity

Filarial hydroceles can have significant, and often negative, social impacts [7,9-11]. The degree of social disability varies among cultural settings, but the extent of stigmatization appears to be directly correlated with the severity of visible disease [12,13]. Gyapong *et al.* [14] suggested that the physical and psychological burden borne by men has a negative impact on their marriage and employment prospects. The extent of male sexual disability as a result of LF has not been extensively studied, but investigators believe that there is a significant silent burden [1,15]. Gyapong *et al*. [14] found that hydroceles had a significant impact on young men, particularly at a time when they were struggling to establish their sexual identity and their capacity to be reliable economic providers. However, unwillingness of men to admit to sexual dysfunction may shroud the real extent of this issue [11].

Affected individuals often avoid seeking treatment for fear of drawing attention to their condition [13,16]. Meanwhile, failure to treat the disease results in recurrent acute febrile attacks and progressive damage to the lymphatic system. Without access to simple hygiene practices, infected subjects are unable to prevent further progression of the outwardly visible complications of LF [17]. As the disease progresses, both labor productivity and sexual reproductive potential are increasingly hampered [11].

Immunology of hydrocele development

The events that lead to the development of chronic pathologies in lymphatic filariasis are not fully understood, but the immune responses of the human host to the parasites are believed to play a significant role in determining pathological manifestations such as hydroceles in infected individuals [18-22].

The lymphatic vascular system plays a critical role in immune surveillance, tissue fluid homeostasis, and fat absorption [23,24]. Perturbations in the maintenance and function of the lymphatic system can lead to a variety of pathological disorders, including lymphatic dilation and lymphedema [23,25,26].

Recent studies on the molecular mechanisms controlling the lymphatic vessels have shown that vascular endothelial growth factors C (VEGF-C) and VEGF-D specifically control lymphangiogenesis in humans [27,28]. The expression of VEGF-C has also been shown to be upregulated by proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF), suggesting that proinflammatory cytokines could affect the lymphatic vessels via VEGF-C [22,29].

Taylor *et al*. [30] have shown in animal models that *Wolbachia*-derived molecules from *Brugia* spp. also induced proinflammatory cytokines, including TNF and IL-1B. Soluble extracts of *Brugia* and *Onchocerca volvulus* adult and microfilarial worms were also found to stimulate human peripheral mononuclear cells *in vitro*, resulting in the production of TNF, IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-10 [31,32]. This stimulation was not achieved using extracts from *Acanthocheilonema viteae*, a filarial species naturally devoid of *Wolbachia*, and, importantly, with *O. volvulus* extracts from patients who had been treated with doxycycline to deplete *Wolbachia* from the worms [30].

Thus, it was concluded that in those filarial species that contain these endosymbionts, *Wolbachia* are the major stimulating principle for proinflammatory cytokines such as TNF. From this, it can be further hypothesized that exposure of host cells to *Wolbachia* from worms (either from dying adult worms or incoming L3/4 larvae, or from the proportion of degenerating embryos that are constantly released) may induce the production of lymphangiogenic factors such as VEGF-C by endothelial cells in LF patients [22].

Diagnosis of filarial hydroceles: the challenges

Definite diagnosis of filarial hydroceles requires parasitological techniques to demonstrate the causal organisms. Microfilariae may be found in the blood, hydrocele fluid or, occasionally, in another body fluid. These fluids can be examined microscopically, either directly or, for greater sensitivity, after concentration of the parasites by the passage of fluid through a polycarbonate cylindrical filter (pore size, 5 µm) or by the centrifugation of fluid fixed in 2% formalin (Knott's concentration technique) or 2% formalin/10% Teepol [33]. Indeed, most filarial hydrocele patients, especially at the advanced stages, are amicrofilaremic, and this previously made diagnosis challenging. However, with the development of immunochromotographic test cards, this challenge has been overcome.

DNA-based techniques have been developed to diagnose and differentiate filarial parasites in humans, animal reservoir hosts, and mosquito vectors [34]. The techniques include DNA hybridization, polymerase chain reaction (PCR) amplification using specific

primers (including Ssp I repeat, pWb12 repeat, pWb-35 repeat, and LDR repeat for *W. bancrofti* and Hha I repeat, glutathione peroxidase gene, mitochondrial DNA for *B. malayi*), and universal primers, multiplex-PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), PCR-enzyme linked immunosorbent assay (PCR-ELISA), as well as quantitative PCR. These techniques, however, need at least one microfilaria in the volume of blood used for DNA extraction, and therefore are not more sensitive than microscopic blood examination for microfilariae. They are, however, important tools for xenomonitoring in lymphatic filariasis [34-36].

A major step in the diagnosis of LF was made almost two decades ago, with the observation of what was described by Amaral *et al.* [37] as filarial dance signs (FDS). FDS were described as peculiar, random-appearing movements of objects inside a vessel-like structure. There are varying opinions on whether the echogenic particles represent adult filarial worms [38] or microfilaria, the larval form of filarial worms [39]. The general consensus, however, is that these objects are motile adult worms within dilated lymphatics, which may be visualized in the lymphatics of the spermatic cord in up to 80% of infected men [40-42].

Currently, the etiology of the so-called filarial dance signs is being debated. Adejolu and Sidhu [42] recently demonstrated that the sonographic appearance described as the filarial dance is not characteristic of filariasis but occurs even in non-endemic areas as a manifestation of epididymis obstruction. They suggest that the oscillating particles seen on sonography are not necessarily adult worms or microfilariae, but may be a manifestation of a complex pathophysiologic process common to men with epididymal obstruction and men infected with filariasis.

Their finding is supported by Shyamkumar *et al*. [39], who remarked that the moving particles could not represent adult worms because they are significantly smaller than adult worms. Further, some patients with filarial hydrocele (diagnosed by ultrasonography) do not have detectable microfilaria and circulating filarial antigens (Og4C3) in their blood [42-44]. This apparent discrepancy in ultrasonographic and biochemical/microbiological markers has been explained as resulting from the fact that ultrasonography is more sensitive in the diagnosis of filariasis than detection of either microfilaria or the circulating filarial antigen in the blood, especially when the microfilaria density is low [42,45].

In the face of these conundrums, the World Health Organization (WHO) has made some recommendations to aid in diagnosis of filarial hydroceles. It is recommended that since an estimated 69% of all hydroceles are filarial in origin and the prevalence of hydroceles in non-endemic areas is considerably low, all hydroceles in *W. bancrofti*-endemic areas are to be considered to be caused by filarial worms, unless otherwise proven [4]. Other causes of hydroceles are imbalance in fluid secretion and absorption, lymphatic filariasis, injury, radiation, retroperitoneal fibrosis, other infections and neoplasms [46].

Treatment and management: successes and challenges

The Global Programme for Elimination of Lymphatic Filariasis (GPELF) has two main goals: to interrupt transmission using microfilaricides given in mass drug administration (MDA) programs, and to reduce morbidity associated with chronic pathology including filarial hydrocele [3].

Interrupting transmission of lymphatic filariasis

The first goal of the GPELF has been pursued through the instrumentality of the MDA programs using ivermectin, albendazole, and diethylcarbamazine (DEC) [3]. MDA programs in endemic countries are considered to be more cost effective than properly diagnosing and treating infected individuals [2]. The low-side-effect profile of drugs and the pledge by two pharmaceutical companies to provide them free of charge, as long as necessary, makes the MDA a good elimination strategy [2].

Ivermectin is regarded as a pure microfilaricide, which kills nearly all microfilariae. However, several studies have demonstrated that ivermectin has no macrofilaricidal effect, although there are indications that it reduces fertility of the adult worms [47-49]. Some macrofilaricidal effect might occur though, if ivermectin is combined with the broad-spectrum albendazole [50]. Despite ivermectin's remarkable effect against microfilariae, a suboptimal response has been recently reported in Asubende area of Ghana [51].

In addition to ivermectin, DEC has a potent microfilaricidal effect as well as 50% macrofilaricidal activity [2,52-54]. The combination of DEC and albendazole has macrofilaricidal effects of between 56% and 87% [54]. However, in Africa, DEC cannot be used due to co-endemicity of lymphatic filariasis and onchocerciasis; the rapid killing of *O. volvulus*

worms results in the development of severe adverse events.

Successes of the MDA regimes as reported by several independent investigators include reductions in microfilareaemia, antigenemia, and transmission rates [55-62] after years of administration of the MDA drugs. The results from these studies indicate that it is indeed possible to eliminate lymphatic filariasis and its attendant debilitating chronic pathologies, including filarial hydroceles.

Management of morbidity: hydrocele

Currently, the mainstay of filarial hydrocele management is hydrocelectomy because the current MDA drugs such as DEC or ivermectin, usually given with albendazole, have no effect on the adult worms and subsequently no ameliorative effect on filarial hydroceles [2,4]. This is because the chronic clinical manifestations of lymphatic filariasis such as hydroceles are initiated by activities of adult worms [63].

Adult worms cause mechanical blockage of the lymphatic vessels, eliciting several immune reactions, which culminates in dilation of scrotal lymphatics and attendant drainage of fluids into the scrotal sac. This is unlike the case of onchocerciasis (a closely related disease), where the baby worms are the initiators of pathogenesis. Meanwhile, hydrocelectomies are expensive and invasive, and relapse may occur even after a successful surgery; this is not easily welcomed by many individuals in rural settings where the infection rate is usually high.

A promising development in the management of morbidity is the discovery of the ability of some tetracyclines, especially doxycycline, to kill adult worms. Hoerauf *et al*. [64], in a study using *Litomosoides sigmodontis*, discovered that tetracycline therapy eliminated *Wolbachia*, the bacterial endosymbionts in adult worms and resulted in filarial growth retardation and infertility. Later, a pilot study with doxycycline administered at 200 mg for six weeks depleted *Wolbachia*, sustained amicrofilaremia for a long time, and resulted in 80% disappearance of worm nests from scrotal areas of infected men examined by ultrasonography [65]. Other similar studies have also demonstrated similar activity with doxycycline [66]. Anti-wolbachial chemotherapy with doxycycline appears to have a higher macrofilaricidal effect (80%) than does DEC, which showed 30%–40% worm nest disappearance, a finding that was interpreted as 30%–40% macrofilaricidal effect [50]. Indeed, anti-wolbachia drugs are known to have less severe adverse effects and demonstrable ameliorative outcomes in individuals with clinical pathology [22] compared to all standard anti-filarial treatments.

Going forward: the challenges

Despite 50 years of research into filariasis control, many questions still remain unanswered. Doxycycline is among the registered drugs mostly available and affordable in most endemic regions, which makes it safe to use without much supervision. Nevertheless, the possible risk of resistance among the population must be carefully considered. The use of doxycycline in the treatment of filarial hydrocele is still being investigated as an alternative method of treatment for individuals living with lymphatic pathology in endemic communities [22,41].

Furthermore, there are logistical challenges with regard to the use of doxycycline in filarial hydrocele patients especially on a large scale. These include the currently long regime of drug administration (200 mg of doxycycline daily for six weeks), which may discourage compliance and increase the occurrence of adverse events. Other challenges include the fact that to date, no study has achieved a 100% clearance of adult worms and the contra-indication of doxycycline in pregnant women and in children under nine years of age [66]. In spite of these challenges, individual patients with filarial hydrocele can benefit from doxycycline, which has been shown to ameliorate filarial hydrocele [22,44], especially in the early stages of the pathology.

The exploration of antibiotics for treating filariasis is far from exhausted. The principle of drug synergism can be explored by combining two or more drugs in an effort to reduce treatment time and increase macrofilaricidal effect. Other antibiotics and chemicals should also be tried for their efficacy against the adult worms in lymphatic filariasis. For instance, following these field observational reports, it will be helpful if other alternative drugs, such as rifampicin, could be administered to children who cannot be given tetracyclines.

Conclusions

Although filarial hydrocele is an important pathology of lymphatic filariasis, its burden has largely been underestimated. The disease hampers economic productivity and presents fertility-related issues in most developing countries. Given that most cases of hydrocele go unreported for fear of victimization and stigmatization, there is a need to strengthen the current existing program of elimination

and to campaign for more global support. Although the advent of recent molecular approaches has improved diagnosis approaches, there is still room for improvement. It is clear that the combination of surgery and treatment seems plausible, but the cost of surgery alone poses another layer of challenge. The introduction of anti-wolbachial therapy has provided a promising lead and should be pursued. Besides its macrofilaricidal potentials, anti-wolbachial therapy also leads to the amelioration of chronic filarial pathologies such as hydrocele and lymphedema. Additionally, the incorporation of more educational awareness in endemic community health programs could help infected individuals to report early to health facilities for quick medical attention.

In conclusion, the present review provides a current view of the treatment options, diagnostic approaches, and disease burden of filarial hydrocele, which will be helpful to governments in endemic regions as well as to several workers in the field of filarial biology.

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