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SENSITIVITY AND SPECIFICITY OF ULTRASOUND DETECTION AND RISK FACTORS FOR FILARIAL-ASSOCIATED HYDROCELES

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Abstract. To better understand risk factors for hydrocele as a consequence of *Wuchereria bancrofti* infection, 342 men more than 15 years of age in an endemic area in Papua New Guinea were evaluated. Thirty-four subjects (9.9%) had hydrocele by physical examination. Ultrasound examination detected hydroceles in 57 men (16.7%). Compared with ultrasonography, the sensitivity of physical examination was 44.3%, the specificity was 98.2%, and the positive predictive value was 73.5%. Hydrocele was independently associated with age (odds ratio [OR] = 3.3, $P < 0.01$) and intensity of infection as determined by filarial antigenemia (OR = 2.3, $P = 0.07$). Dilation of spermatic cord lymphatics detectable by ultrasound did not correlate with hydrocele, but was associated with the presence of infection. These observations suggest that filarial pathology of the male genitalia is under-reported when evaluated by physical examination alone and that duration and intensity of infection are risk factors for hydrocele.

INTRODUCTION

Lymphatic filariasis affects more than 120 million people in the world. *Wuchereria bancrofti* infection can produce acute and chronic disfigurement of the extremities, breast, and genitalia.[1] Hydrocele is the most frequent disease manifestation of *W. bancrofti* infection in men,[1] yet its pathogenesis is poorly understood.

Hydroceles develop most commonly during adulthood and are a consequence of the accumulation of excess fluid between the serosal and visceral layers of the tunica vaginalis, which lies between the testis and scrotal sac. Hydroceles may be a consequence of unreduced inguinal hernias or orchitis and epididymitis secondary to infectious agents such as *Mycobacterium tuberculosis*. In *W. bancrofti* infection, it is likely that hydroceles result from inflammation of the tunica vaginalis or impaired lymphatic drainage through the spermatic cord. Since *W. bancrofti* adult worms reside in lymphatic vessels, local host inflammation induced by the parasite or secreted worm products themselves may cause lymphatic dysfunction with formation of hydroceles.[2]

Hydroceles have traditionally been detected by physical examination. Use of this method of detection has led to disparate results for evaluating demographic and other risk factors for this disease manifestation of filariasis.[3–6] Differences in the results of such studies may be due to failure to detect small hydroceles, misdiagnosis because of confusion with other causes of disfigurement of the inguinal area (e.g., inguinal hernia), and inter-observer variation. Ultrasonography, on the other hand, has been shown to be an objective tool to evaluate hydroceles of filarial as well as non-filarial etiology. This non-invasive imaging technique can also detect lymphatic dilation of the spermatic cord and varicoceles.[2] In the present study, we evaluated the sensitivity and specificity of physical examination relative to ultrasonography for detection of hydrocele in a filariasis endemic area of Papua New Guinea. Risk factors for hydroceles are described.

MATERIALS AND METHODS

Study area and population. Prior to the study, 11 villages in the Usino-Bundi District, Madang Province, Papua New Guinea were mapped, demographic information was obtained, and all inhabitants underwent a physical examination and had peripheral venous blood drawn to determine the presence of infection (n = 2,233). Of 516 men less than 15 years old who were initially examined, 342 (66.3%) agreed to participate. Non-participation rates were similar among the 11 villages and did not differ between men with and without hydroceles. Study subjects had not been previously treated for filariasis. Thirty age-matched Papua New Guinean control subjects living in a village where *W. bancrofti* is not endemic (Tubusereia, near Port Moresby) were also examined.

Only males more than 15 years old were included in the current study because previous reports from Papua New Guinea failed to detect hydroceles by physical examination in younger individuals.[7] No hydroceles, both by physical examination and ultrasonography, were identified in 30 circulating antigen-positive individuals 13–15 years of age in two study villages with the highest prevalence.

Informed consent was obtained from all patients according to the guidelines of the authors' institutions. The Institutional Review Board of Case Western Reserve University and the Medical Research Council of the Papua New Guinean Institute for Medical Research reviewed and approved this study.

Physical and ultrasound examination for hydrocele. All participants underwent physical examinations that included evaluation for lymphedema of the extremities, hernias, and palpation of the testicles and scrotum. The presence or absence of a hydrocele was noted and its size was estimated according to guidelines outlined by the World Health Organization.[8] A portable ultrasound machine (Sonosite 180; Sonosite Corp., Bothell, WA) with a variable 5–10 MHz linear array transducer was used to detect fluid accumulated between the layers of the tunica vaginalis. The ultrasound machine was light, compact (about the size of a laptop computer), easy to operate, and battery powered, making it easy to transport and use in remote areas. The cost was approximately $18,000 U.S. dollars, but it could cost less with fewer accessories. The quantity of hydrocele fluid was estimated by measuring the distance between the inner most layer of the scrotal sac (i.e., parietal layer of the tunica vaginalis) and the most superficial, anterior aspect of the testicle (visceral layer of the tunica vaginalis) in millimeters.[9] Ultrasonography was

also used to detect dilated lymphatics of the scrotal contents. Varicoceles (dilated veins of the spermatic cord) were distinguished from dilated lymphatics by the presence of blood flow with pulse doppler observed with the Valsalva maneuver. The ultrasound operator was unaware of the physical examination and infection status results.

Physical examinations for hydrocele were performed by two males nurse with more than five years of experience doing community surveys for hydrocele. They received additional training prior to the current study by one of the investigators (CLK, a physician). The ultrasound operator was a medical student trained for two months with a radiologist specializing in ultrasonography at University Hospitals of Cleveland on adult volunteers. All ultrasounds performed in Papua New Guinea were recorded on digital tape and subsequently reviewed blindly by physicians for presence and size of hydrocele and lymphatic dilation.

Parasitologic measurements. The number of blood-borne microfilariae was quantified in a subset of individuals by filtration of 1 mL of anti-coagulated venous blood and levels of circulating antigen were determined by an enzyme-linked immunosorbent assay based on the Og4C3 monoclonal antibody (TropBio, Pty. Ltd., James Cook University, Townsville, Australia) as described previously.[6] Infection intensity was categorized into four groups based on a log scale: negative (< 32 antigen units), low (32–520), medium (512–5,120), and high (> 5,120).

Statistical analysis. Associations between antigenemia and age for dichotomous variables (e.g., presence or absence of hydrocele, lymphedema, etc.) were calculated using Pearson's chi-square tests. Regression analysis was performed by Wilcoxon rank-sum correlation. Logistic regression analysis was performed using the SAS Institute, Inc. (Cary, NC) statistical software package.

RESULTS

Prevalence of infection in study villages. The overall prevalence of infection in males in the study population increased steadily in the first and second decades of life such that between 70% and 85% of the adult men (n = 1,106) were infected (Figure 1). The prevalence in study subjects closely reflected that observed in the population (Figure 1).

Establishing criteria for hydrocele detectable by ultrasound in the local setting. To establish baseline values for ultrasound diagnosis of hydrocele based on fluid accumulation between the parietal and visceral layers of the tunica vaginalis in a setting where filariasis is endemic, we first examined 30 Papua New Guinean men residing in a non-filariasis endemic area. As shown in Figure 2A, all except two of the subjects had less than 0.30 cm fluid accumulation. Hydroceles detected by physical examination were present in both of these men (fluid accumulation was more than 0.55 cm). One of the subjects had history of trauma to the scrotum. There were no pertinent historical findings to explain the presence of a hydrocele in the other subject. Physical and ultrasound examination failed to reveal any evidence for hydroceles in the remaining 28 men. The mean ± SD fluid width among the 28 individuals with < 0.30 cm accumulation was 0.11 ± 0.14 cm. Since the mean + 2 SD of these subjects was 0.39 cm, any individual in the filariasis endemic area with > 0.40 cm fluid accumulation was considered to have a hydrocele.

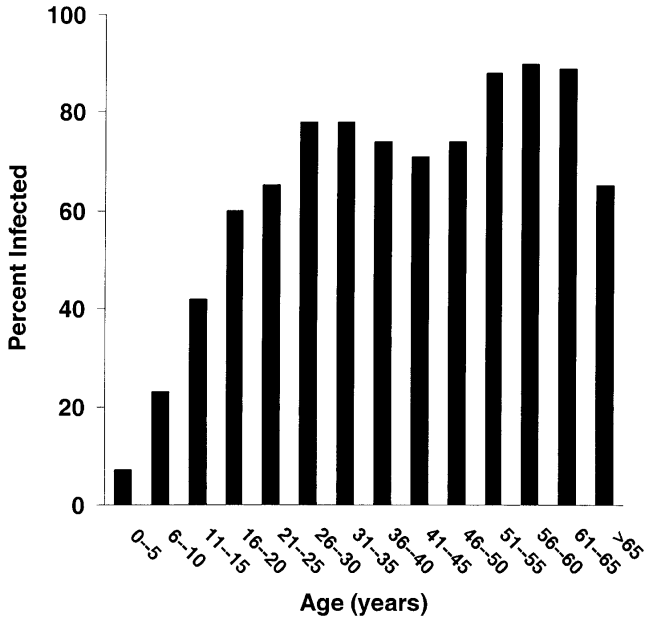

FIGURE 1. Prevalence of infection shown by age among all males in the study community (n = 1,106). Circulating antigen positivity (> 32 units) was used to evaluate whether subjects were infected.

Sensitivity and specificity of physical examination relative to ultrasound. Physical examination resulted in failure to detect 32 of 57 ultrasound-diagnosed hydrocele cases, giving a sensitivity of 43.8% (Table 1). In contrast, physical examination had a specificity of 96.1%, correctly classifying most men as true negatives. The nine subjects that were falsely determined to have hydrocele by physical examination had unreduced inguinal hernias (n = 3) or thickened skin of the scrotal sac (n = 6). The positive predictive value of physical examination was 73.5%. Increasing the cut-off value to the 98% confidence interval (e.g., mean + 3SD, > 0.53 cm) would increase the sensitivity of the physical examine relative to ultrasonography to 55% (10 subjects had a hydrocele width between 0.40 and 0.53 cm). This change in the cut-off level did not affect the positive predictive value of the physical examination.

Relationship of age and intensity of infection with hydrocele. The prevalence of hydrocele increased with age, from 9.6% in 15–24-year-old subjects to 26% for those more than 45 years old (Figure 2B, n = 342). The median fluid accumulation increased from 0.98 cm in the youngest age category to 1.69 cm in the oldest category. The proportion of subjects with bilateral hydroceles also increased with age. No subject more than 35 years old had a bilateral hydrocele, whereas six 35–44-year-old men (prevalence = 9.5%) and seven subjects lees than 45 years old (prevalence = 10.9%) did.

Individuals with hydroceles were more likely to have microfilaremia compared with individuals without hydroceles. Nineteen of 32 persons with hydroceles (59%) were microfilaria-positive (geometric mean = 291 microfilariae/mL of blood). In contrast, 21 of 75 individuals without hydroceles (28%) were microfilaria-positive ($P < 0.01$ versus the group with hydroceles, geometric mean = 97 parasites/mL of blood).

The prevalence of hydrocele increased with infection intensity estimated by circulating antigen level (Figure 2C). The

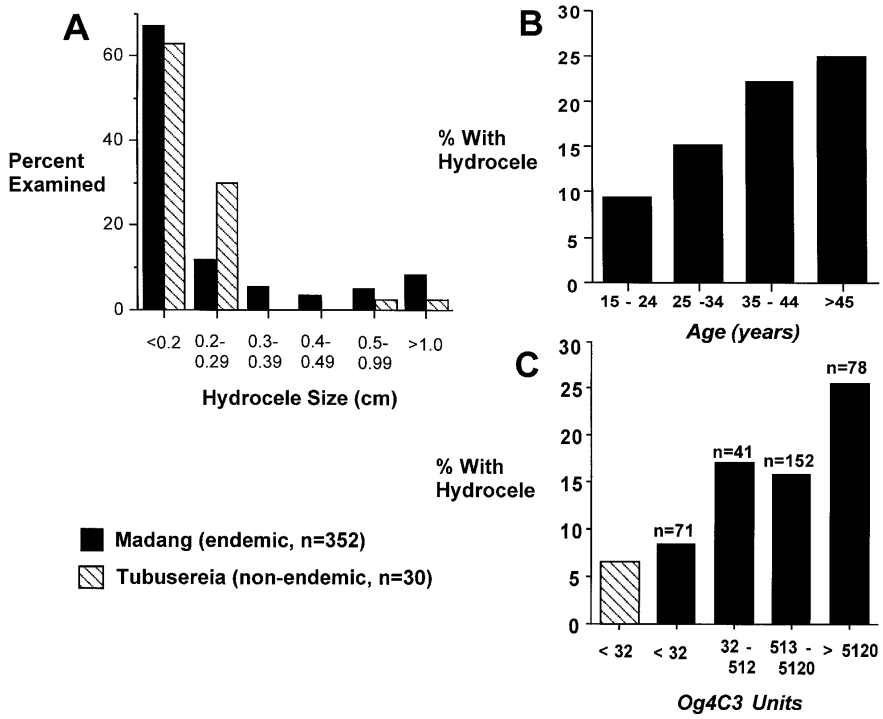

FIGURE 2. **A**, Distribution of hydrocele prevalence among men living in a filarial endemic area of Madang province and in a filarial non-endemic area (Tubusereia) of Papua New Guinea. Hydrocele size was estimated by measuring the distance between the testis and the anterior scrotal wall using a high-resolution 5–10 MHz linear array transducer with a portable SonoSite 180 ultrasound machine. Two individuals in the non-endemic area had > 0.51cm of fluid in the tunica vaginalis. **B**, Relationship of age with hydrocele prevalence. **C**, Relationship between intensity of infection and hydrocele prevalence.

prevalence of ultrasound-confirmed hydroceles among antigen-negative individuals in the filarial endemic population was similar to that of age-matched residents of an area of Papua New Guinea where filariasis is not endemic (6.7% and 8.4%, respectively). The frequency of hydroceles correlated positively with infection intensity estimated by circulating antigen level. Men with low-to-moderate infection levels (32–5,120 Og4C3 antigen units; $P < 0.05$) and heavy infections (> 5,120 antigen units; $P < 0.001$) had 2 to > 3-fold greater frequency of hydrocele compared with antigen-negative individuals in the population ($P < 0.01$ for each comparison). The fluid accumulation of hydrocele subjects also increased with infection intensity. Median hydrocele widths were 0.61 cm, 0.78 cm, 1.61 cm, and 2.46 cm for antigen-negative subjects and those with low (32–512 antigen units), moderate (512–5,120), and high (> 5,120) circulating antigen levels, respectively. Finally, the proportion of persons with bilateral hydroceles also increased according to circulating antigen level. Among men with antigen levels less than 512 units, only one had a bilateral hydrocele (prevalence < 1%). In contrast, six men in the group with 513–5,120 units (prevalence = 3.9%) and > 5,120 units had bilateral hydroceles (prevalence = 3.9% and 7.7%, respectively).

Age and infection intensity assessed by circulating antigen level showed a correlation ($r^2 = 0.21$, $P < 0.01$). A logistic regression model was therefore used to examine these two variables independently. When adjusted for intensity of infection, individuals more than 45 years old had a 3.3-fold greater risk for hydrocele compared with younger men ($P < 0.01$). Heavily infected men, when adjusted for age, had 2.3-fold greater risk for hydrocele compared with moderately to lightly infected men ($P = 0.07$).

Relationship of lymphatic dilation with hydrocele. No lymphatic dilation was observed in healthy adult North American individuals (n = 5), which was consistent with findings of experienced ultrasonographers in the United States (Herbner T, unpublished data) and in Brazil.[13] Although the presence of lymphatic dilation was surprisingly high in the non-endemic population (18 of 30 examined, 60%), residents living in the endemic population had a significantly higher frequency of lymphatic dilation (279 of 342, 81.5%; $P < 0.001$). Lymphatic dilation was higher among infected (89%) compared with uninfected subjects (76%; $P < 0.05$), but did not correlate with the presence of hydroceles. The number and mean ± SE maximum diameter of the lymphatics were also similar for men without (1.4 ± 0.4 mm) and with hydrocele (1.7 ± 0.7 mm) in the filarial endemic area.

TABLE 1

Sensitivity and specificity of physical examination compared with ultrasound for detecting hydrocele*

	PE hydrocele positive	PE hydrocele negative
Ultrasound positive	25	32
Ultrasound negative	9	276

* PE = physical examination.

DISCUSSION

The results of this community-based study demonstrate that the physical examination may be inaccurate and have low

sensitivity relative to portable ultrasonography in identifying hydroceles in areas where *W. bancrofti* is endemic. This could have important implications for the use of hydrocele prevalence as an estimate of filarial endemicity in communities prior to and after introduction of community-based mass chemotherapy.[10]

Age and infection intensity were independent risk factors for hydroceles detected by ultrasonography. Infection intensity, however, did not correlate with hydroceles ascertained by physical examination. We believe this may account for the failure of some studies to find a significant association between hydrocele and infection intensity.[3–6] Hydroceles in some individuals may resolve after treatment of lymphatic filariasis,[11] and smaller ones may resolve without administration of anti-filarial drugs (King C, unpublished data). However, the majority of hydroceles, especially in older men, presumably persist. Therefore, it is likely that repeated and chronic infections with *W. bancrofti* lead to cumulative damage to scrotal lymphatics. Superimposed on other causes for hydroceles, it is possible this results in age-related increased frequency of larger hydroceles.

The increase in prevalence of infection during the first and into the third decades of life in the population that persists at a high level throughout adulthood indicates that the association between age and hydrocele probably reflects duration of infection. These observations parallel those seen in other chronic helminthiases such as urinary schistosomiasis, where the duration and burden of infection contribute to an increased long-term risk of pathology of the bladder.[12]

Adult worms in the lymphatic vessels draining the tunica vaginalis may cause lymphatic dysfunction and/or partial obstruction leading to fluid accumulation and hydrocele. We found no association with lymphatic dilation and the presence of hydrocele, a finding consistent with earlier observations by Noroes and others.[13] Lymphatic dilation was found in more than 81% of men and correlated with the presence of infection. This is consistent with a recent report of a steady progression of lymphatic dilation with age and infection intensity.[14] Surprisingly, 60% of the men who were life-long residents of the filariasis non-endemic area also had evidence for lymphatic dilation in the scrotum. We excluded that the possibility that this finding was mistaken for blood vessels or active lymphatic filarial infection. Similar findings of lymphatic abnormalities in the extremities of Brazilian residents of filarial non-endemic areas[15] highlight the importance of examining local control populations. These data also support studies suggesting that filarial worms release mediators that dilate lymphatics both near and distant from worm nests,[2,14] suggesting that lymphatic dilation in itself does not directly cause hydrocele. On the other hand, dilation of collateral lymphatics may compensate for the loss of lymphatic function and flow from parasitized vessels.

The association of age and infection intensity with hydrocele suggests that death of adult worms, either naturally or by treatment, triggers events that cause or exacerbate disease by mechanisms that still remain poorly understood. It is not clear why the majority of heavily infected men do not develop hydroceles. We speculate this heterogeneity may be related to the strength and type of host innate and acquired immune response to *W. bancrofti*, the precise location of the parasite at the time of its death, or individual differences in lymphatic anatomy and collateralization. Repeated moderate-to-light infections over a prolonged period may modulate host immunity and allow development of adequate lymphatic collaterals to protect many against disease. Alternatively, periods of intense exposure with heavy worm burdens over a short period may increase the risk for disease. A greater understanding of the role of innate and acquired immunity to filarial and *Wolbachia* antigens among men with and without hydrocele will help define these parameters as risk factors for disease.

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