DIAGNOSIS OF GENITAL CERVICAL SCHISTOSOMIASIS: COMPARISON OF CYTOLOGICAL, HISTOPATHOLOGICAL AND PARASITOLOGICAL EXAMINATION

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Abstract. Granulomatous inflammation of the cervix uteri is a common manifestation of infection with Schistosoma haematobium. In women the cervix is the most common site of infection by S. haematobium. Three methods were used to assess the performance of three different ways of detecting schistosome eggs in cervical tissue: cytological examination of a cervical smear, histological examination of a cervical biopsy, and direct examination of cervical tissue obtained by forceps biopsy (quantitative compressed biopsy technique [QCBT]). Of 228 women studied who lived in an S. haematobium endemic area in Tanzania, 112 (49%) had schistosome eggs detected in the cervix using QCBT. Histological examination detected eggs in 40 of 228 (18%). The cytological examination of cervical smears yielded only 6 positive results (3%). The median egg load in the cervical tissue of cases correctly diagnosed by histology was significantly higher than the egg load in the misclassified cases, indicating that the sensitivity of histological sectioning increases with egg density. We conclude that the QCBT is the diagnostic test of choice for schistosomiasis of the genital cervix.

INTRODUCTION

Schistosomiasis is a parasitic disease caused by flukes living in the mesenteric and pelvic veins. In infected women adult worms of all schistosome species can be found in any organ of the upper and lower female genital tract, and eggs are frequently trapped in the various genital organs. In schistosomiasis caused by Schistosoma haematobium the cervix uteri appears to be a common site of infection. Community-based studies have shown that cervical schistosomiasis is very common in women infected with S. haematobium with frequencies ranging from 33% to 75%. Clinically, schistosomiasis of the cervix shows diverse features, all of which result from granulomatous inflammation. Cauliflower-like growth, nodular hypertrophy, ulcerative and polypoid lesions have been documented. Such lesions may resemble malignancy. So-called sandy patches are pathognomic; they may exist on a macroscopically normal cervix, but are often in the vicinity of ulcer and erosions. Ova are predominately located at the ecto-endocervical junction. Symptoms associated with cervical schistosomiasis are dysmenorrhea, menorrhagia, leukorrhrea, lower abdominal pain, post-coital bleeding, intermenstrual bleeding, and dyspareunia.

Genital schistosomiasis responds well to anti-helminthic drugs. A small clinical study in Malawi confirmed that after a single dose of praziquantel (40 mg/kg), lesions usually resolved within nine weeks.

Genital schistosomiasis may occur independent of urinary egg excretion. Therefore, negative results of urine examinations does not rule out the presence of genital lesions, and other diagnostic tests have to be used to detect schistosome eggs in the cervix. Parasitological and histological examination of cervical tissue as well as cytological examination of Papanicolaou-stained cervical smears (PAP smears) have been advocated. However, the diagnostic value of these techniques has never been evaluated. This study had two aims: i) to compare the diagnostic value of biopsy-based examination techniques (histopathological sectioning versus the quantitative compressed biopsy technique [QCBT]); and ii) to assess the diagnostic value of cytological examination of PAP smears against the findings of biopsy-based techniques.

MATERIALS AND METHODS

The study was carried out in Mwanga District in Northern Tanzania. The design of the population-based study has been detailed previously. In brief, a cross-sectional study was carried out in two villages to determine the prevalence of schistosomiasis of the lower reproductive tract. Mass treatment or treatment of school children has never been carried out in the study area. All women aged 15 to 45 years from the study villages were invited to participate. Urine filtration was carried out on three consecutive days. The medical history was taken by a standardized questionnaire covering sociodemographic and health-related topics. A gynecological examination was carried out which included the inspection and palpation of the lower abdominal, genital, and perianal area, colposcopy, and a punch biopsy of cervical tissue at the squamocolumnar junction. Cervical smears were fixed in 95% alcohol and stained by a routine Papanicolaou method.

Each biopsy was divided into two pieces, one for histology and one for QCBT. The former was preserved in 10% formaldehyde solution. Sections were stained by hematoxylin and eosin. Of each biopsy two sections were analyzed. The second half of the biopsy was immediately compressed between two glass slides and examined microscopically for the presence of schistosome eggs using ×100 magnification.

To facilitate the identification of schistosome eggs, a drop of 1% trypan blue in physiological saline was added before compressing the tissue. In order to measure the cervical egg load the surface of the compressed tissue was determined by placing a millimeter paper under the glass slide and counting the number of fields of 1 mm² covered by the biopsy. Results were expressed in eggs/mm² cervical tissue. This QCBT is derived from a similar technique used for rectal biopsies and has been applied to biopsies taken from the vulva, vagina, and the cervix.
Women were defined to have cervical schistosomiasis when at least one egg was detected by one of the biopsy techniques or the PAP smear. Sensitivity and specificity of the PAP smear were calculated using the combined results of the biopsy-based techniques of the cervical tissue as the gold standard. Furthermore, the diagnostic values of histopathological sectioning and the QCBT were compared.

The chi-square test was used to compare groups. Comparisons of the cervical median egg load were made by the Mann-Whitney U-test. Sensitivity and specificity interval (CI). All women provided informed consent. The study was approved by the Research and Ethical Clearing Committee of the Kilimanjaro Christian Medical Center. Women with diagnosed genital or urinary schistosomiasis were treated free of charge with praziquantel (40 mg per kg body weight). Other genital tract and bladder infections were treated appropriately.

## RESULTS

In 119 of 228 study participants, schistosome eggs were detected in the cervical tissue by at least one method used. Schistosome eggs were present in the urine of 65 of 119 (55%) women with genital schistosomiasis and in 40 of 109 (37%) women without genital schistosomiasis. Eighty of 119 (67%) women with diagnosed cervical schistosomiasis showed sandy patches and/or cervical erosion and/or leukoplakia, whereas 47/109 (43%) of women without genital schistosomiasis presented with at least one of these signs (Yates’ corrected $\chi^2 = 12.44$, df = 1, $P = 0.0004$).

Schistosome eggs were found by QCBT in 112 of 228 specimens (49%), by histopathological examination in 40 of 228 samples (18%) and by cytological examination in 6 of 228 cases (3%). The median cervical egg load was 0.3, 2.8, and 0.2 eggs per mm$^2$, respectively, with a maximum of 77 eggs per mm$^2$.

Significantly less cases were detected using histopathological examination compared to the QCBT (Yates’ corrected $\chi^2 = 40.75$, df = 1, $P < 0.0001$). The median cervical egg load of the cases classified as positive by the histopathological examination was significantly higher than the median egg load of those classified as negative (2.8 egg/mm$^2$ versus 0.2 eggs/mm$^2$). Mann-Whitney U = 570, $P < 0.0001$. A higher intensity of infection was associated with a higher likelihood that eggs were detected by histopathological examination. In samples with a cervical egg load of less than 1 egg/mm$^2$, only 27% of the cases were detected, whereas in samples with more than 24 eggs as determined by the QCBT, all cases of cervical schistosomiasis were identified by histological examination. Seven cases were detected by histopathological examination alone, with negative results by the QCBT.

Combining the results of histopathological examination and the QCBT, schistosome eggs were found in 119/228 (52%) of samples. Based on the results for the biopsy-related diagnostic techniques as a gold standard, the cytological examination of PAP smears had a sensitivity of 2.5% (95% CI: 0.7–7.7) and a specificity of 97.2% (95% CI: 91.6–99.3).

## DISCUSSION

Genital schistosomiasis of the cervix has been diagnosed—often accidentally—by histopathological examination of cervical biopsies or routine cytological investigation. Similar to diagnostic approaches used in intestinal schistosomiasis which detects eggs in rectal mucosa, we applied the QCBT to the analysis of cervical tissue. With this method biopsies were easily and rapidly screened for the presence of schistosome eggs. As already demonstrated for rectal biopsies, the QCBT is sensitive enough to detect a single egg in cervical tissue. The QCBT is also specific: artifacts resembling schistosome eggs were not found in this study.

When the three methods were compared, the QCBT was the best method available for the diagnosis of cervical schistosomiasis. We did not determine the negative predictive value (disease absent if test is negative) in this study. In cervical tissue schistosome eggs are not evenly distributed and tend to lie in clusters. In fact, dividing a cervical biopsy of approximately 3 mm diameter for histological examination and QCBT yielded in seven cases negative results by the QCBT and positive results by the histological sectioning. If the cervix appears macroscopically normal, to diagnose genital schistosomiasis, it may be necessary to take a biopsy at a randomly selected site, which may diminish the chance of detecting eggs. To definitively rule out cervical schistosomiasis it may be necessary to perform several biopsies.

The low sensitivity of the histopathological sectioning—in our study only one-third of the genital schistosomiasis cases have been categorized correctly—might also be explained by the formation of egg clusters within the tissue. Thus, not surprisingly the sensitivity of the histological examination depends on the number of eggs in the tissue. The higher the egg burden in the cervix, the more likely genital schistosomiasis will be diagnosed by histopathological examination. In this case, the examination of serial sections of the biopsy would increase the sensitivity.

Biopsy-based methods may not be feasible in primary health care settings in developing countries. If patients could be diagnosed by an examination of a cervical smear, a method routinely used in gynecology, this would be a considerable achievement. Therefore several authors have advocated such an approach. Berry has described the morphology of the schistosome eggs and the cytological changes in routinely taken PAP smears in South African women. In 110 of 139 slides from patients with evidence of genital schis-
tosomiasis S. haematobium ova were seen. Youssef and oth-
ers reported that larger amounts of schistosome ova are
found if the cervical smears were taken from sites of ulcer-
ation.19 The number of eggs found in smears determined by
these authors varied from one to more than 300 eggs per
smear. In a prospective study in Zimbabwe, 1,901 smears
were examined for the presence of schistosome eggs. Forty-
four (2.3%) were found to contain schistosoma ova with an
average of 19 eggs per smear. However, in our study the
 cytological examination proved not to be an appropriate di-
agnostic approach to diagnose cervical schistosomiasis. Only
3% of the positive cases were classified correctly. The lo-
calization of the eggs might be a reason for the poor per-
formance of this diagnostic approach. Ova are usually situ-
ated in the subepithelial connective tissue, the majority being
 present in the squamocolumnar junction.21 If ova are just
beneath the epithelial layer, especially if they happen to be
found under a single layer of columnar epithelium, ova can be
found coincidentally in the cervical smear.14

In conclusion, if genital schistosomiasis of the cervix is
considered in a differential diagnosis of cervical genital le-
sions, the QCBT is the diagnostic method of choice. This
technique can be complemented by histopathology and cy-
tology if other clinical conditions such as pre-malignant or
malignant alterations of the cervix have to be excluded.

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