Sexually transmitted infections other than HIV/AIDS among women of low socio-economic class attending antenatal clinics in Khartoum, Sudan

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Abstract
Sexually transmitted infections (STIs) are major health threats affecting people globally; however, the burden of STIs is greatest in low-income countries. Since they are physiologically more vulnerable, women are mostly affected. The risk is increased dramatically during pregnancy leading to serious health complications that may affect the newborn. Underprivileged pregnant women attending antenatal clinics for routine checkups in displaced camps, a women’s prison and several peripheral health centres were clinically and laboratory screened for trichomoniasis, chlamydial infections, gonorrhea and syphilis. A total of 426 women with an age range of 14-45 years were included. Clinical data, blood, cervical and vaginal swabs were collected. Conventional bacteriological and serological methods were applied. All attendees were HIV1/2-negative. The prevalence of *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Treponema pallidum* infections was found to be 7.8%, 4.9%, 0% and 5%, respectively. Although vaginal discharge, among other symptoms, is known to be the most significant indicator for STIs, our identified positive predictive value was only 14.1%. We conclude that use of syndromic approach for diagnosing and treating attendees of antenatal settings is of low clinical value and many easily curable STIs will be overlooked. Consequently, trichomoniasis, chlamydial infection and syphilis prevailed widely among this population.

Keywords
Prevalence, STIs other than HIV, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Treponema pallidum*, underprivileged pregnant women, antenatal care clinics, Sudan

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Introduction
Globally, more than a million people acquire a sexually transmitted infection (STI) every day. The burden is greatest in low-income countries.¹ In Sudan, available epidemiological studies suggested increase in STIs prevalence, especially among females.²,³ Curable non-viral STIs include gonorrhea, chlamydia, syphilis and trichomoniasis.¹ The estimated average prevalence of these conditions among antenatal attendees in selected African countries is 5.1%, 11.3%, 4.4% and 15.2%, respectively.⁴ According to the World Health Organization (WHO), an estimated 499 million new cases of curable non-viral STIs occur every year globally.¹ Their consequences are foetal and neonatal deaths, pelvic inflammatory disease (PID), ectopic pregnancy, infertility and HIV risk.¹,⁴,²⁴ Syphilis in pregnancy...

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leads to 305,000 foetal and neonatal deaths and leaves 215,000 infants at increased risk of dying from prematurity, low birth weight or congenital disease each year.\textsuperscript{1} Gonorrhoea and chlamydia are an important cause of infertility; in sub-Saharan Africa, untreated genital infection may be the cause of up to 85% of infertility among women seeking reproductive health care.\textsuperscript{1,5} Having an STI such as syphilis increases the chances of acquiring HIV infection by threefold or more.\textsuperscript{1,6,7} In addition, the direct physical, psychological and social consequences of STIs have a major impact on quality of life and are a prime indicator of the quality of global sexual and reproductive health care.\textsuperscript{7} STIs are usually acquired through sexual behavior which is considered unacceptable outside of marriage, socially and morally, in Sudan. This leads to under diagnosis of STIs that in turn imposes obstacles to any effort to curb the mounting number of cases.\textsuperscript{8-10} In the present study, we aimed at exploring the spectrum of curable non-viral STIs among Sudanese pregnant women attending antenatal care clinics.

Materials and methods

Study design and area

This is a multi-centre descriptive study; it was conducted during a one-year period in Khartoum, Sudan. We recruited pregnant women of low socio-economic status attending antenatal care clinics for routine checkups at the following locations: the Academy and Bangaded charity teaching hospitals located at Al-Sahafa and Al-Bangadeed areas, Bashaeer Charity Teaching Hospital at Mayo district, Red Crescent and Med Air health centres at Wad-Elbashir and Dar-Elsalam displaced camps and Omdurman Women Prison in Omdurman city.

Study population

Out of 1400 estimated total attendees at all hospitals and health centres in 40 visits, a total of 426 pregnant women agreed initially to participate in the study. Women with contraindication to pelvic examination or those who received antibiotic treatment within two weeks were excluded from the study. Free and appropriate antibiotic treatment was provided for all infected women and their partners once laboratory evidence was identified. Syndromic management and treatment, however, was applied by health care workers in hospitals and health centres that women were attending.

Data collection and management

Standardized structured questionnaire was used to collect demographic and behavioural information. Inquiries were made on the presence of symptoms related to STIs. Physical, gynaecological and laboratory examinations were also performed. Data were analysed using SPSS software version 13.

Ethical considerations

The ethical clearance for conducting this study was obtained from the Ethical Committee Board of the University of Medical Sciences and Technology. Ethical acceptances and approvals to proceed were collected from administrative bodies of all locations that we visited. Oral and written informed consents were obtained from all women prior to participation in the study and initiation of specimen and data collection.

Specimen collection

Bivalve sterile disposable speculums were used to visualize vaginal walls and the cervix. Vaginal secretions were collected from the wall of the posterior fornix, using sterile cotton swabs, for the detection of Trichomonas vaginalis and measurement of vaginal pH. After cleaning the excess cervical secretions, material from the walls of the endocervical canal were collect in two swabs. The specimens were collected without coming in contact with the vaginal wall. One of the specimens was used for detecting Chlamydia trachomatis antigens and the other for conventional isolation of Neisseria gonorrhoeae. Around 4 ml of venous blood was collected, sera separated and used for detecting treponemal and HIV1/2 antibodies.

Pathogen identification

T. vaginalis. Direct microscopy was used to detect motile flagellates in wet preparations. The presence of single or more motile trichomonads was diagnostic.

N. gonorrhoeae. Cervical swabs were immediately cultured on chocolate blood agar and then on modified Thayer Martin medium. Smears were also prepared, fixed and stained by Gram’s technique. The inoculated plates were incubated at 37°C in an atmosphere enriched with 5% CO2 and moist for 48 h. Gram-stained smears were examined for the presence of small Gram-negative intra- and extracellular diplococci. After incubation, the plates were examined macroscopically for the characteristic colonial morphology of neisseria species. Suspected colonies were Gram stained, examined for oxidase activity, sub-cultured on rich media and inoculated into a set of sugar fermentation test. The observation of a Gram-negative small diplococcic that are strongly oxidase positive and ferments glucose but not maltose,
lactose or sucrose indicates the identity of the aetiologic agent.\textsuperscript{11}

\textit{C. trachomatis}. The second cervical specimens, which were collected from the walls of the endocervix, were used for the detection of Chlamydia antigen using qualitative lateral flow immunoassay. Swabs were tested immediately upon collection using ACON Chlamydia Test Device\textsuperscript{®} and following the manufacturer’s recommendations.

\textit{T. pallidum}. Serum specimens measuring 10 μl were used to detect treponenal IgG and IgM antibodies using qualitative membrane based immunoassay. Sera were examined using ACON Syphilis Ultra Test Device\textsuperscript{®} following the manufacturer’s recommendations.

\textit{Human immunodeficiency virus 1/2}. Serum specimens measuring 10 μl were used to detect IgG, IgM and IgA antibodies specific to HIV-1 (including subtype O) and HIV-2 using a third-generation (direct sandwich) differential and qualitative immunochromatographic assay. Sera were examined using Standard Diagnostics HIV1/2 3.0\textsuperscript{®} kit and confirmed by Colloidal\textsuperscript{®} and Unigold HIV\textsuperscript{®} kits. In case of positive specimens, confirmation by other tests was intended, however, not needed in this study.

\textbf{Results}

A total of 426 pregnant women attending antenatal clinics in Khartoum state agreed initially to participate in the study. The monthly household income is roughly estimated to be less than 500SDG ($50) and, therefore, were classified as low socio-economic class. Their age ranged between 14 and 45 years with an average of 25 ± 6 SD. Of the 426 women, 20 declined to proceed further and hence were withdrawn from the study. General physical examination aiming at identifying abdominal tenderness, lymphadenopathy, skin rash and oral leukoplakia was refused by 44 (10.8\%) participants out of the 406. Gynaecological examination to identify genital ulcers, vaginal and/or cervical discharge was refused by 58/406 (14.3\%) participants. Regarding laboratory investigations, 29/406 (7.1\%) refused blood testing for HIV and syphilis, 56/406 (13.8\%) refused cervical testing for gonorrhoea and chlamydia infections and 47 (11.6\%) refused vaginal testing for trichomonas infection.

Statistical analysis revealed normally distributed data evident by skewness of +0.702 and kurtosis of −0.121. Chi-square tests between related variables were significant under the 0.05 level. Outcomes of cross-tabulations are mentioned hereunder. Average gravidity was 4 ± 2.6, average total number of children was 3.2 ± 2, median number of miscarriages was 1 (range 1 to 8) and median number of stillbirths was 1 (range 1 to 7).

Of the 406 women, 62 (17.7\%) were found to harbour one of the mentioned infections and 7 (1.97\%) had two infections. Among those having mixed infections, 2 (0.61\%) suffered from trichomoniasis and chlamydial infection and 5 (1.36\%) suffered from trichomoniasis and syphilis.

Despite the high number of positive findings on history and symptoms generally indicating to trichomoniasis, chlamydial infection and gonorrhoea, clinical and laboratory examinations revealed diverse findings (Table 1). Our estimated prevalence of the identified

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
Signs and symptoms & No. of positive cases out of total respondents & \% \\
\hline
Increase in vaginal discharge & 149/404 & 36.9 \\
Discharge colour & & \\
Yellow & 22/141 & 15.6 \\
White & 119/141 & 84.4 \\
Smell & & \\
Foul & 40/148 & 27 \\
Not foul & 108/148 & 73 \\
Pelvic pain & 150/405 & 37 \\
Pain during sexual intercourse & 64/404 & 15.8 \\
Episode of painful joint swelling & 140/405 & 34.6 \\
Baby with purulent discharge during the first month & 15/336 & 4.5 \\
Burning sensation when urinate & 152/406 & 37.4 \\
Itching or irritation on the genital area & 93/406 & 22.9 \\
Swelling in the groin & 11/356 & 3.1 \\
Is it painful & 5/11 & 45.5 \\
\hline
Examination findings & No. of positive cases out of total no examined & \\
\hline
Abdominal tenderness & 35/362 & 9.7 \\
Cervical discharge & 176/321 & 54.8 \\
Vaginal discharge & 290/336 & 86.3 \\
of which is clear & 19/282 & 6.7 \\
of which is white & 142/282 & 50.4 \\
of which is curdy & 87/282 & 30.9 \\
of which is yellow & 34/282 & 12.1 \\
vaginal pH ≤ 4.5 & 168/264 & 63.6 \\
vaginal pH > 5 & 96/264 & 36.4 \\
vaginal odour foul smelling & 50/289 & 17.3 \\
vaginal odour no foul smelling & 239/289 & 82.7 \\
Inguinal bubo & 2/317 & 0.6 \\
\hline
\end{tabular}
\caption{Clinical history and examinations.}
\label{tab:clinicalhistoryandexaminations}
\end{table}
STIs is shown in Table 2. Moreover, vaginal pH ≥ 5 was the highest calculated positive predictive value (PPV) (21.9%) followed by foul vaginal odour (18%) and vaginal discharge (14.1%). The remaining PPVs for some signs and symptoms of STIs are demonstrated in Table 3.

T. vaginalis flagellates were detected in 28/359 (7.8%) participants; all presented with vaginal discharge. The discharge was clear in 3 (10.7%) women, white in 8 (28.6%), curdy in 7 (25%) and yellow in 10 (35.7%). Vaginal pH for the majority of positive cases (21, 91.3%) was ≥5 and 9 (33.3%) with foul smelling. Among the positives, 9 (32.1%) cases were found to suffer from increased vaginal discharge with varying colour, foul odour and pH ≥5. Whereas 6/28 (21.4%) cases acknowledged dysuria with itching and/or irritation on their external genitalia. 8/28 (28.6%) responded positively for increased vaginal secretions with varying colour and foul smelling.

Testing for C. trachomatis antigens was performed on 308 participants, of which 15 (4.9%) were positive. Among the positive cases, no mutuality was detected on cross-tabulations with the symptoms; swelling in the groin and purulent discharge during the first months. However, 2/15 (13.3%) cases suffered from dyspareunia, 7/15 (46.7%) from pelvic pain and 4/15 (26.7%) cases had vaginal discharge. On clinical examination, none of the positive cases for chlamydia antigens had inguinal bubo, 4/15 (26.7%) suffered from abdominal tenderness and 13/15 (86.7%) presented with both cervical and vaginal discharge. One (6.7%) of the positive cases presented only with vaginal discharge and one (6.7%) did not have any discharge. Vaginal discharge was clear (1, 6.7%), white (9, 60%), curdy (3, 20%) and yellow (1, 6.7%).

N. gonorrhoeae was not detected in any of the 350 examined participants, although clinical findings suggesting gonorrhea were significantly high (Table 1).

Specific treponemal antibodies were detected in 19/377 (5.0%) cases, of which, one case has been identified as early secondary syphilis, whereas the remaining 18 cases were diagnosed as early primary syphilis, and no latent or late syphilis was detected. For syphilis staging, we used the 2008 European Guideline on the Management of Syphilis.12

Serological testing for HIV1/2 was negative in all tested participants (377/406). However, inquiry on past history and clinical examination suggested the opposite. A total of 39/403 (9.7%) women had an episode of fever which lasted for more than one month, 25/405 (6.2%) mentioned positive history of diarrhoeal episode lasting for more than two weeks and 62/399 (15.5%) suffered from marked weight loss. Oral leukoplakia was seen in 6/362 (1.7%) women and generalized lymphadenopathy in 2/362 (0.6%).

**Table 2. Estimated prevalence of STIs in the present study.**

<table>
<thead>
<tr>
<th>Sexually transmitted infection</th>
<th>Number of positive cases</th>
<th>Percentage among study group</th>
<th>Prevalence rate (%)^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV/AIDS</td>
<td>0 out of 377</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Syphilis</td>
<td>19 out of 377</td>
<td>5</td>
<td>1.4</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>0 out of 350</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>28 out of 359</td>
<td>7.8</td>
<td>2</td>
</tr>
<tr>
<td>Chlamydial infection</td>
<td>15 out of 308</td>
<td>4.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

^4The estimated total attendees of all hospitals and health centres is 1400 in 40 visits (μn = 354, 25%).

**Table 3. Positive predictive value for some signs and symptoms of STIs.**

<table>
<thead>
<tr>
<th>Sign or symptom</th>
<th>No. of positive cases</th>
<th>Positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital ulcer</td>
<td>58</td>
<td>1.70%</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>290</td>
<td>14.10%</td>
</tr>
<tr>
<td>Vaginal pH ≥ 5</td>
<td>96</td>
<td>21.90%</td>
</tr>
<tr>
<td>Foul vaginal odour</td>
<td>50</td>
<td>18%</td>
</tr>
<tr>
<td>Cervical discharge</td>
<td>176</td>
<td>7.40%</td>
</tr>
<tr>
<td>Pelvic pain</td>
<td>150</td>
<td>4.70%</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>64</td>
<td>3.10%</td>
</tr>
<tr>
<td>Dysuria</td>
<td>152</td>
<td>7.20%</td>
</tr>
<tr>
<td>Itching or irritation on the external genitalia</td>
<td>93</td>
<td>7.50%</td>
</tr>
</tbody>
</table>
cost-effective for the syndrome of urethral discharge. However, it performs less well for other syndromes. In most patients, clinical manifestations of STIs are demonstrated at severe disease stage and may facilitate the development of several serious complications, as mentioned above.

STIs other than HIV have been overshadowed in recent years by the heightened public health focus on HIV treatment, despite the strong association between STIs and HIV acquisition. In the present study, our patients were first screened for HIV status to rule out positive cases; however, this was not necessary because all 377 investigated women were negative. It was rather anomalous finding considering a prevalence of 0.95% among Sudanese pregnant women reported by the Federal Ministry of Health/Sudanese National AIDS Program. This may reflect the variation of HIV infection among different population in various geographical areas of Sudan.

Despite the availability of several simple, cheap and cost-effective interventions to combat STIs, little progress has been made, evident by the 2013 WHO reported annual increase of incidence. In this study, several silent STIs other than HIV were detected (Table 2) when attendees for routine pregnancy follow-up were screened. T. vaginalis was the most frequently isolated organism reaching a prevalence of 7.8%. Ortashi et al. and Kafi et al. reported similar findings of 7.3 and 7.7%, respectively, among pregnant and non-pregnant Sudanese women. Though earlier studies during the 80s demonstrated much higher prevalence ranging between 20 and 40%. Although infection with T. vaginalis does not interfere with fertility, frequently it leads to premature rupture of membranes, miscarriage, premature delivery, neonatal low birth weight and perinatal infection in 5% of infected individuals.

Infection with Chlamydia revealed relatively low prevalence (4.9%) compared with the prevalence (19.9%) obtained by Ortashi et al. among pregnant women in Sudan. To the best of our knowledge, in clinical practice in Sudan, chlamydia is ignored when an STI is suspected even though tests of high sensitivity and specificity are now available. Furthermore, we did not detect gonorrhea although Ortashi et al. reported a prevalence of 2.2%. Considering the fact that symptoms indicating PID prevailed, we were still unable to identify significant cases of chlamydia and none of gonorrhea in the present study. It is known that both chlamydia and gonorrhea are notorious for causing PID. When it pursues a chronic course, PID is usually associated with dyspareunia, tubal blockage and hostility of the endometrium to the implanting embryo; all these factors agglomerating to infertility.

The prevalence of syphilis (5%) was relatively high in the present study even though treatment is simple. In the era of antimicrobial therapy, some studies consider syphilis to be virtually non-existent and forgotten in much of the developed world. More recent data, however, suggest increased estimates for the disease in countries with high and upper-middle income. In developing countries, it imposes obstacles to the fight against HIV. Despite global initiatives to eliminate syphilis, implementation remains poor; only about 30% of pregnant women with syphilis in sub-Saharan Africa receive testing and treatment. In the current study, none of the infected women had genital ulceration. However, skin rash resembling secondary stage of syphilis was noted in 13 women (3.6%); with the exception of one, all other patients were negative for anti-treponemal antibodies.

The PPV of most signs and symptoms (Table 3) was poor. For instance, PPV of vaginal discharge was 14.1% even though 290 cases were positive, this may be explained, according to Hill and Embib, by the fact that endogenous vaginitis is the main cause of vaginal discharge rather than STIs. Furthermore, the WHO stated in their last 2013 STIs report that vaginal discharge is a very poor predictor of STIs. Some conclusions have been reached by Ortashi et al. Poor STIs predictors can lead to either under diagnosis and lack of treatment or false diagnosis and overtreatment, with all its social implications. Accordingly, the value of syndromic approach in management of STIs needs to be re-addressed specially in developing countries. Most important, syndromic management misses asymptomatic infections, which are by far the greatest burden of disease.

What is more, in the impoverished and neglected regions, good antenatal and sexual health care is denied so the prospects of STIs detection during pregnancy and treatment are dim. In the industrialized countries, antenatal care coverage reaches up to 98% of pregnant women, whereas only 68% or less women get access to antenatal care in developing countries.

This study was overshadowed by high refusal rates. For instance, 14.3% of the participants declined gynaecological examinations; probably because the majority (13.8%) perceived speculum examinations as painful procedure. In addition, around 11.6% of the patients were embarrassed by their vaginal discharge and thus refused to be examined. Others (7.1%) were in status of doubt and feared to retrieve a positive result especially for HIV. In general, reason for rejecting any of the examinations is because most of the women thought being tested was an endeavour too great for diseases they know they do not have! Another limitation to this study, other than the high refusal rate, was the...
need to rely solely on conventional non DNA-based-molecular assays. In a low-income developing country, it is an inevitable approach when having small budgets and/or lacking access to current technologies and their well-standardized protocols.

Amongst the five screened STIs, we conclude that trichomoniasis, chlamydial infection and syphilis prevailed among this population. Moreover, use of syndromic approach for diagnosing and treating attendees of antenatal settings is of low clinical value and many easily curable STIs will be overlooked.

Sexual health education is a priority in this population. We therefore recommend that it should be adopted by all women’s health-related governmental and non-governmental organizations at both community and antenatal-clinic levels. Furthermore, screening for STIs and STDs should be performed on regular basis on antenatal settings. In addition, attempts to provide appropriate tools and protocols to use the more advanced DNA-based molecular techniques in STIs screening should be sought.

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