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Sero-Detection of Syphilis among HIV Patients attending Al Obeid Teaching Hospital (Sudan)

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Abstract

Background: The burden of syphilis diagnosis had been mainly among patients many of whom are co-infected with HIV. Regular serological screening for syphilis is required to detect patients who may be at risk of asymptomatic HIV infection.

Objective: To sero-detect the frequency rate of syphilis among HIV patients at Al Obied Teaching Hospital (Sudan).

Materials and methods: The study was conducted during the period from June to August, 2013 at Al Obied Teaching Hospital. HIV patients were prospectively investigated for syphilis using the serological rapid plasma reagin (RPR) test. Positive results were confirmed by the *Treponema pallidum* Hemagglutination (TPHA) test.

Results: A total number of 100 HIV patients were investigated. 12 samples (12%) were found positive by the RPR test. From these only seven samples (7%) were confirmed positive by TPHA. The age range of HIV patients investigated was 15-54 years (mean 34.5 years). The gender incidence of patients investigated was 68 (68%) females and 32 (32%) males. The highest frequency rate of confirmed syphilitic infection (8.8%) was among HIV patients aged 25-34 years. On the other hand, the frequency rate of syphilis was highest among females (7.3%) than among males (6.2%). There were 34 HIV patients with a past history of blood transfusion; three of them (8.8%) turned to be positive for syphilis. Also there were nine HIV patients with a past history of sexually transmitted infections; one (11.1%) of them was found to be positive for syphilis.

Conclusion: Routine RPR and TPHA screening tests are effective in detecting the increasing numbers of syphilis cases among HIV patients.

Key words: Syphilis infection, HIV infection, RPR test, TPHA test.

Introduction

Human immunodeficiency virus (HIV) and *Treponema pallidum* (the causative agent of syphilis) can co-infect the same host because their risk factors are similar and they are transmitted sexually. By the end 2007, 33 million people were infected by HIV and meanwhile, syphilis was still one of the major causes of death in some developing countries where HIV infection was also prevalent¹.

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Syphilitic ulcers can facilitate the transmission of HIV, decrease CD4 levels and increase viral load among HIV infected patients. On the other hand, HIV infection increases the risk of neurosyphilis. It may alter syphilis's clinical features and its treatment outcome. It might be related to syphilis treatment failure, especially if neurosyphilis had a delayed diagnosis. In addition, syphilis can mimic various clinical expressions and cause severe cardiovascular and neurological problems among the HIV infected patients².

Sexually transmitted infections (STIs) are the major public health problems in most parts of the world. Based on the WHO estimate, STIs and their complications are among the top five disease categories for which adults seek health care in developing countries¹.

Syphilis is one of the most important STIs, caused by the bacterium *Treponema pallidum*. It has been estimated that about 12 million new syphilis infections occur annually worldwide; of which, almost two-thirds are in sub-Saharan Africa and south/southeast Asia. Unprotected sex, blood transfusion, needle sharing and vertical transmission from mother to the child are major modes of syphilis transmission².

Syphilis, as a cause of ulcerative genital lesions, presents a site for HIV entry and shading. Moreover, by activating immune cells and raising viral load, syphilis could facilitate HIV transmissibility. On the contrary, concurrent HIV infection may adversely affect the natural history, clinical manifestations, and treatment response of syphilis³.

An emerging epidemic of human immunodeficiency virus (HIV) infection in India had made sexually transmitted infection (STI) control as one of the strategies imperative and probably the most important one to decrease HIV transmission⁴.

HIV and syphilis affect similar patient groups and co-infection is common. All patients presenting with syphilis should be investigated for HIV and vice versa. Syphilis can enhance the transmission of HIV. Detection and treatment of syphilis can probably help to reduce HIV transmission⁴.

Syphilis may present with atypical features in HIV patients. There is a higher rate of asymptomatic primary syphilis, and proportionately more HIV patients present with secondary disease. Secondary infection may be more aggressive and there is an increased rate of early neurologic and ophthalmic involvement. Diagnosis is generally made with serology, but the clinician should be aware of the potential for false negative serology tests in both primary and secondary syphilis. All HIV patients should be treated with penicillin-based regimen, and alternative therapies should be used with caution. All HIV patients should be considered when evaluating neurosyphilis. Relapse is a real concern; and careful follow up is required⁵.

Hence this study was performed to sero-detect the frequency rate of syphilis among HIV patients.

Materials and methods

This was a prospective, analytical, cross-sectional, facility-based study. It was conducted at Al Obied Teaching Hospital (Sudan). The population investigated was HIV positive patients. The study was conducted during the period from June to August, 2013. An ethical clearance was obtained from the Ethical Clearance Committee at University of Medical Sciences and Technology, Khartoum (Sudan). Complete information regarding risk factors involved in the study was explained to all participants. Confidentiality of information collected from

participants studied was maintained. Valid verbal consent of the participants investigated was obtained. Results of specimens collected were given to all patients included in the study and some specimens results were dispatched to sponsored physicians. Permission to collect the specimens was granted by the Administration of Al Obied Teaching Hospital. The study data was entered, cleaned, sanity-checked, and analyzed using the Statistical Package of Social Science (SPSS) program.

The selection of HIV patients was made through a stratified simple random sampling technique. Sample size was 100 blood specimens collected from HIV patients. The blood specimens were collected by authorized personnel at the HIV Centre at Al Obied Teaching Hospital, and then transported to the laboratory for syphilis testing. A pretest standard structure questionnaire was used to collect data from the study participants.

Venous blood was collected first by disinfecting the forearm (the site of collection) using alcohol. The vein was located using a tourniquet. Using sterile syringe, 3 ml of blood were withdrawn, transferred into a sterile plain container, and allowed to clot naturally. The clotted samples were centrifuged at 3000 rpm for 5 minutes. The serum was removed to another sterile plain container that was labeled, and transported in an ice bag, from the site of collection to the laboratory where the rapid plasma reagin (RPR) test was performed and positive RPR tests were confirmed by the *Treponema pallidum* hemagglutination (TPHA) test.

Rapid plasma reagin (RPR) test:

This test was processed first by bringing the RPR antigen suspension, positive control, negative control and patients' serum samples to room temperature (20-30°C) before use. Reagents were gently mixed to avoid foaming. Then an RPR card was labeled with patient and control information. Using disposable serum droppers, one free-falling drop (0.05 ml) of serum sample was dispensed onto a circle on the test card. Adding one free-falling drop was repeated for positive control and negative control. The sample was spreaded smoothly across the circle area using the paddle side of the dispenser without scratching the test area. After mixing the antigen solution by swirling, one drop of the antigen suspension was added to each sample/control testing area without stirring or spreading the antigen. Then the card was placed on a rotator and covered to maintain humidity. Rotation was made at 5 rpm for 8 minutes. Following rotation, a brief hand rotation and tilting of the card (3-4 times) were performed to aid in differentiating non-reactive from minimally reactive results. Immediately results were read macroscopically in the "wet" state under a high intensity light source.

Interpretation of RPR test: Non-reactive-smooth suspension showed no clumping or slight roughness. Reactive showed clear clumping.

Treponema pallidum hemagglutination (TPHA) test:

Before performing the test, the sample, diluent, control and test cells were brought to room temperature (25-30° C). A test card with three wells was used. To dilute the patient's, 10µL of patient's serum and 190µL of diluent were added in one well. This was mixed well using a micropipette. Then 75µL of "control cells" was added to another well and 75µL of "test cells" was added to a third well. 25µL of the diluted serum was added on each of "control cells" and "test cells" wells. Then the plate was shaken gently to mix the contents thoroughly. The plate was covered and incubated for 45-60 minutes at room temperature. Results were read and interpreted as follows: Reactive showed full cell pattern covering the bottom of the well. Non-reactive showed cells settling to a compact button with a small clear center.

Results

A total of 100 HIV-positive patients were investigated. 12 samples were found positive by RPR test. From these only seven samples were confirmed positive by TPHA. The age range of HIV patients investigated was 15-54 years (mean 34.5 years). Most of HIV patients studied (34/34%) were in the age group (25-34 years). While the fewest number of HIV patients investigated (16/16%) were aged 45-54 years (Table I).

Table (I): Distribution of HIV patients investigated according to age

Age range (years)	No. investigated	Per cent
15 - 24	18	18
25 - 34	34	34
35 - 44	32	32
45 - 54	16	16
Total	100	100

The gender incidence of patients investigated was 68 (68%) females and 32 (32%) were males. The highest frequency rate of confirmed syphilitic infection (8.8%) was among HIV patients aged 25-34 years; followed by those in the age group 35-44 years (6.4%). On the other hand, the frequency rate of syphilis infection was highest among females (7.3%) than among males (6.2%).

There were 34 HIV patients with a past history of blood transfusion; three of them (8.8%) turned to be positive for syphilis. While 66 HIV patients had no past history of blood transfusion, yet four (6.1%) of them were found to be positive for syphilis.

Also there were nine HIV patients with a past history of sexually transmitted infections; one (11.1%) of them was found to be positive for syphilis. Furthermore, 91 HIV patients had no past history of sexually transmitted infections, six of them (6.5%) were found to be positive for syphilis tests (6.5%).

All HIV patients investigated had previously received antibiotic treatment. Most HIV patients (50%) had carried the disease for 2-4 years; and only one patient (1%) was suffering from the infection for 9-12 years (Fig. 1).

Discussion

Studies showed the spread of sexually transmitted infections in Sudan due to promiscuous sexual practices among some high risk groups in the population. This had resulted from the increasing availability of more diverse illegal social and sexual networks. This behavior was the reason for the storm of HIV and syphilis in some parts of Sudan. This problem requires rapid screening, diagnosis, and treatment of sexually transmitted infections.

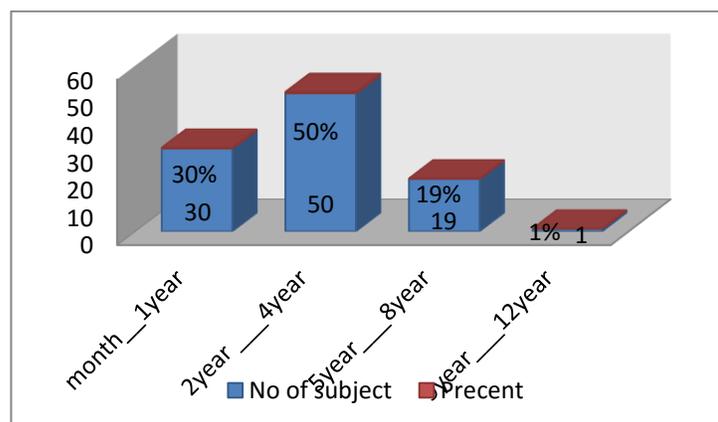


Fig. (1): Distribution of HIV patients according to duration of infection

There is an association between syphilis and HIV transmission. Enhanced laboratory surveillance of syphilis and HIV may help in the prevention and control of these infections. In this study the frequency rate of syphilis among HIV patients was 7% as confirmed by the specific TPHA test. This rate was higher than that reported by Vahdani and his colleagues (0.45%) in Tahrán, after investigating 450 HIV patients at Imam Khomeini Hospital in 2008 using FTA-Abs test⁶.

Also in this study, the highest frequency rate of confirmed syphilitic infection (8.8%) was found among HIV patients aged 25-34 years; followed by those in the age group 35-44 years (6.4%). Also there were nine HIV patients with a past history of sexually transmitted infections; one (11.1%) of them was found to be positive for syphilis. Furthermore, 91 HIV patients had no past history of sexually transmitted infections, six of them (6.5%) were found to be positive for syphilis tests (6.5%). Eticha and his co-workers in Addis Ababa (Ethiopia) reported that the sero-prevalence of syphilis among HIV-infected patients was 9.8% compared with 1.3% among HIV-uninfected individuals. They also reported that the frequency rate of syphilis was found among men (11%) and women (8.9%) with HIV infection; and syphilis prevalence was not significantly increased with age, with highest rate in 40-49 years of age (16.9%). They also stated that except for a history of sexually transmitted infection, which was associated with syphilis (frequency rate 2.25%) other risk factors did not raise the odds of infection⁷.

On the other hand, the frequency rate of syphilis infection in our study was highest among females (7.3%) than among males (6.2%). In Nigeria, Nnoruka and his co-authors reported that the prevalence rate of syphilis among HIV infected patients was 2.0-14.0%. Among the HIV-positive individuals, the prevalence rate of *T. pallidum* infection was higher in females (15.0%) than in males (12.7%). Individuals of the 21-30 and 31-40 years age groups had the highest prevalence rate of 20.5% and 20.0% respectively⁸. In UK, Cohen and his colleagues investigated 2389 HIV patients and 13 patients were found infected by *T. pallidum* confirmed with FTA test⁹.

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In this context, there were 34 HIV patients with a past history of blood transfusion; three of them (8.8%) turned to be positive for syphilis. While 66 HIV patients had no past history of blood transfusion, yet four (6.1%) of them were found to be positive for syphilis. Omer and his co-workers (1982) evaluated the diagnosis of syphilis among Sudanese subjects at Khartoum Teaching Hospital. They investigated 2201 blood donors and 199 patients with sexually transmitted infections using the Venereal Disease Research Laboratory (VDRL) and the fluorescent treponemal antibody absorption (FTA-ABS) tests. The VDRL test was found positive in 30 (1.36%) of the blood donors and 11 (5.5%) of the patients investigated. Syphilis was confirmed by FTA-ABS test in one patient (0.5%) and none in blood donors. The biological false positive (BFP) reactions were 1.36% in blood donors and 5.0% in control patients¹⁰.

The high prevalence of syphilis among people with HIV infection highlights the need to target this population to prevent the transmission of both infections. Screening all HIV-infected people for syphilis and managing those infected would have clinical and epidemiological importance. It is the responsibility of both the clinician and the bacteriologist to make sure that syphilis screening programs are properly applied.

Conclusion: Routine RPR and TPHA screening tests are effective in detecting the increasing number of syphilis among HIV positive patients.

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