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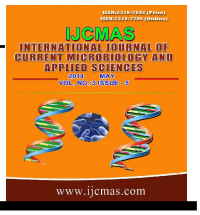

Original Research Article

VDRL v/s TPHA for diagnosis of syphilis among HIV sero-reactive patients in a tertiary care hospital

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Keywords

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A B S T R A C T

HIV and syphilis co-infection is complex and diagnosis of syphilis is more difficult among HIV positive patients. HIV can alter the clinical picture of syphilis and can cause more serious complications. Unusual serological responses like high titre as well as false negative reactions have been reported in VDRL tests in HIV reactive patients, therefore specific tests such as TPHA or FTA-ABS should always be done in all HIV reactive patients. We conducted this study to know the prevalence of syphilis among HIV reactive patients in a tertiary care hospital and to test the adequacy of VDRL as a screening test for the diagnosis of syphilis in HIV patients. Our study showed 11.4% of HIV serum samples reactive with TPHA, while out of TPHA reactive samples only 37.5% were VDRL reactive. 0.3% samples gave non-treponemal false positive reactions, i.e. VDRL reactive but TPHA non-reactive. CD4 count was correlated in TPHA reactive patients and most of the patients (62.2%) were having CD4 count <400 cells/cu.mm. In light of the findings in our study, there is a need to formulate a strict policy to diagnose syphilis especially in HIV sero-reactive patients. Diagnosis on the basis of single non-specific test cannot rule out syphilis, therefore combination of non-specific test along with specific test is required for proper diagnosis of syphilis. Prompt diagnosis and treatment of syphilis is essential not only to lower transmission rates, but also to avoid the complications seen in the later stages of the disease.

Introduction

Treponema Pallidum, causative agent of syphilis and HIV (Human immuno-deficiency virus) co-infection is not uncommon now as both are sexually transmitted and risk factors are the same. Syphilitic ulcers facilitate the transmission of HIV and increase the viral load among HIV positive patients.

Studies demonstrated that individuals with sexually transmitted infections (STI) are 3-5 times more likely to acquire HIV infection, if exposed to the virus through sexual contact (Rolfs RT *et al,* 1997*)* On the other hand HIV alters the natural course of syphilis & response to treatment. Incidence of neuro-syphilis is high among

HIV infected persons (Gordon SM *et al*, 1994).

HIV and syphilis co-infection is complex and diagnosis of syphilis is more difficult among HIV positive patients. HIV can alter the clinical picture of syphilis and can cause more serious complications. Unusual serological responses like high titre as well as false negative reactions have been reported in VDRL test (Venereal Disease Research Laboratory) in HIV reactive patients, therefore specific tests such as TPHA (*Treponema Pallidum* Haemagglutination assay) or FTA-ABS (Fluorescent *Treponema Pallidum* Antibody- Absorption test) should always be done in all HIV reactive patients (Lowhagen GB, 1990).

Timely diagnosis of syphilis in HIV patients would help in better management of these patients and will reduce the transmission of HIV. Considering the increasing prevalence of HIV syphilis co-infection, we conducted the study to know the prevalence of syphilis among HIV reactive patients in a tertiary care hospital and to test the adequacy of VDRL as a screening test for the diagnosis of syphilis in HIV patients.

Materials and Methods

A prospective study was conducted in the Department of Microbiology, Dr. Ram Manohar Lohia hospital and PGIMER over a period of one year from July 2012 to June 2013. All the known HIV reactive serum samples received in the laboratory from Anti-retroviral therapy clinic and wards for VDRL test were included in the study. Both VDRL and TPHA were done in the received serum samples. Also CD4 count was done in all HIV sero-reactive patients. The VDRL test was performed using kit received from Institute of serology, Kolkata and TPHA test was performed by using Immunotrep from Omega Diagnostics. CD4 count was done by using BD FACS Calibur flow cytometer. Positive and negative controls were included with all the tests performed. Comparison of CD4 counts in TPHA reactive/ VDRL reactive, TPHA reactive/ VDRL non-reactive and both non-reactive were done in all HIV patients.

Results and Discussion

During the study period of one year, we received 2670 HIV sero -reactive serum samples for VDRL testing. Out of 2670 samples, 1768 were males and 902 were females (1.96:1). Out of total samples received, 304 (11.4%) samples were found reactive with TPHA. TPHA was found reactive most commonly in males (76.3%) and between age group of 20-40 years (53.6%) followed by 40-60 years (40.8%) as shown in table 1. Among TPHA reactive samples, only 114 (37.5%) samples were VDRL reactive while 190 (62.5%) samples were VDRL non-reactive. Among VDRL reactive samples, 97 patients were having low titre (1:8 or less) and 20 patients having high titre (>1:8). Also 9 (0.3%) samples gave non-treponemal false positive reactions, i.e. VDRL reactive but TPHA non-reactive. Of these 9 biological false positive cases, 6 were having low VDRL titre (1:8 or less) while in two titre of 1:16 and in one sample a titre of 1:32 was obtained. CD4 count was correlated in TPHA reactive patients and most of the patients (62.2%) were having CD4 count <400 cells/cu.mm (Table 3). As shown in table 4, no statistically significant difference was found in CD4 counts in TPHA reactive and non-reactive HIV patients.

Table.1 Age-wise and sex-wise distribution of TPHA reactive patients

Age (years)	Male	Female	Total
0-20	Nil	Nil	Nil
20-40	114	49	163 (53.6%)
40-60	106	18	124 (40.8%)
60-80	11	4	15 (4.9%)
>80	1	1	2 (0.6%)
Total	232 (76.3%)	72 (23.7%)	304

Table.2 Correlation of VDRL and TPHA among HIV-positive patients

VDRL	TPHA	Patients reactive
Reactive	Reactive	114
Non-reactive	Reactive	190
Reactive	Non-reactive	9

Table.3 Correlation of CD4 count in TPHA reactive samples

CD4 count (cells/ cu.mm)	TPHA reactive (304)	Percentage
<200	75	24.7%
200-400	114	37.5%
400-600	66	21.7%
>600	49	16.1%

Table.4 Comparison of CD4 count in TPHA reactive and non-reactive samples

CD4 count (cells/ cu.mm)	TPHA reactive	Both non-reactive
<200	75 (24.7%)	481 (20.4%)
200-400	114 (37.5%)	838 (35.5%)
400-600	66 (21.7%)	659 (27.9%)
>600	49 (16.1%)	379 (16.1%)
Total	304	2357

Syphilis can facilitate HIV transmission, and HIV can influence the clinical features and treatment outcome of syphilis. Since the immunodeficiency is associated with a high risk of treatment failure of syphilis, hence prompt diagnosis in patients with syphilis is extremely important (Kofoed *et al*, 2006 and Gitai *et al*, 2009). In HIV patients there are altered clinical manifestations and serological response in syphilis. Hence serological tests for syphilis may be difficult to interpret in HIV seropositive patients because of atypical responses such as delayed responses to both treponemal and non-treponemal test. VDRL test is less likely to identify syphilis except in primary stage of disease where TPHA may appear non-reactive. Moreover, it produces more false-positive results at all stages and more false-negative results in late disease. Hence a TPHA-positive/VDRL-negative result implies that patient has treponemal infection (Rachel *et al*, 1996). Our study

showed 11.4% of HIV serum samples reactive with TPHA, while out of TPHA reactive samples only 37.5% were VDRL reactive which is comparable to other studies (Paul Diggory, 1996 and Turbadkar *et al*, 2007). Also in 9 samples VDRL +/ TPHA- but the titres were low in most of the samples i.e.< 1/8, which is in accordance with other studies. These nine cases were lost for the follow up, hence, the seroconversion pattern or relevant clinical findings could not be studied. False positive is seen because non-treponemal test detects antibodies against non-specific antigen i.e. reagin, cardiolipin and lecithin. In HIV infection anticardiolipin, antilecithin antibodies and polyclonal gammopathy can occur. Hence it suggests biologically false positive and not syphilis infection (Rachel *et al*, 1996).

TPHA positive/ VDRL negative may result in tertiary or treated syphilis or it can be due to other pathogenic treponemes. The VDRL test provides an excellent, inexpensive method of assessing disease activity and monitoring treatment. It gives low titres or becomes negative with inactive or treated disease and gives high titres in active syphilis. However, syphilis is such a serious, but treatable disease hence initial testing alone by VDRL test is not justified, as a proportion of latent and tertiary disease would be missed (Paul Diggory, 1996). Although cost effectiveness of VDRL test makes it a common screening test for syphilis. We have seen from this study that to accurately diagnose and confirm syphilis it would be better to do TPHA along with VDRL in HIV patients. In our study, TPHA was found reactive most commonly in males (76.3%) and between age group of 20-40 years (53.6%) which is in accordance with some studies (Turbadkar *et al*, 2007), while discordant result was found in other study from Ethiopia (Eticha *et al*, 2013) where females were predominantly affected and most common age group was 40-49yrs . Age group of 20-40 yrs were most commonly affected in this study followed by 40-60 yrs, because it is the most sexually active age group. TPHA is found to be superior for diagnosis of syphilis over VDRL as seen in our studies and is consistent with other study (H. Young *et al*, 1974)

In our study correlation of syphilis sero-reactive patient with CD4 count was done. 62.2% patients were having CD4 count < 400 cells/cu.mm. In a study conducted by Farhi *et al*, 2009 and Sadiq *et al*, 2005 : mean CD4 count in active syphilis in HIV sero-reactive patients were 450 and 410cells/cu.mm respectively. Lower CD4 cell count that is associated with more severe degree of immunosuppression may increase the risk of treatment failure in HIV-infected patients who receive penicillin treatment according to the guidelines. CD4 count ≤ 350 cells/ml and RPR titre≥ 1:32 are associated with increased risk of neurosyphilis in HIV-infected patients (Marra *et al*, 2004 and Libois *et al*, 2007). Hence monitoring of CD4 count in HIV- syphilis co-infected patients is essential (Nicola *et al*, 2007). CD4 count was correlated in TPHA reactive and non-reactive patients and no significant difference was found between the two groups. Since HIV patients are immunosuppressed and are prone to various opportunistic infections hence low CD4 count may be due to other opportunistic infections present in the patient which were not included in this study. However the limitation of this study is that TPHA reactive patients were not followed up and also the CD4 count was not correlated with the treatment of these patients.

In light of the findings in our study, there is a need to formulate a strict policy to diagnose syphilis especially in HIV sero-reactive patients. Diagnosis on the basis of single non-specific test cannot rule out syphilis, therefore combination of non-specific test along with specific test is required for proper diagnosis of syphilis. Prompt diagnosis and treatment of syphilis is essential not only to lower transmission rates, but also to avoid the complications seen in the later stages of the disease.

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