



Research Paper

SEROPREVALENCE OF SYPHILIS IN HIV PATIENTS

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It is known that history of any sexually transmitted disease (STD), including syphilis, is associated with an increased risk of Human immunodeficiency Virus (HIV) infection among both homosexuals and heterosexuals. Serological surveys continue to be the best source of information on the prevalence of syphilis. Venereal Disease Research Laboratory (VDRL) test is a useful screening test to determine seroprevalence of syphilis in a community. MATERIAL AND METHODS- This retrospective study was conducted in the department of Microbiology of Pt. B.D. Sharma, PGIMS, Rohtak. A total of 61,140 serum samples received in the laboratory, from July 2008 to July 2012. RESULTS- Out of 61,140 serum samples, 1735 (2.8%) were VDRL reactive. VDRL reactivity in HIV non reactive serum samples was 2.96% in 2008, 3.0% in 2009, 2.9% in 2010, 2.8% in 2011 and 2.8% in 2012 respectively. VDRL reactivity was found to be maximum among HIV reactive males in which a rising titre was observed. CONCLUSION- Prevalence of syphilis is more in HIV infected population as compared to HIV non infected population.

Keywords: HIV, Seroprevalence, STD, Syphilis, VDRL

INTRODUCTION

The relation of syphilis and Human immunodeficiency Virus (HIV) is complicated and domain of interest for further research and study (Hook, 1992; Tramont, 1995; Douglas *et al.*, 2005; Golden *et al.*, 2003; Hall *et al.*, 2004; Zellen *et al.*, 2004). Many epidemiological studies have demonstrated that sexually transmitted diseases (STDs) including herpes infection, syphilis and specifically genital lesions are associated with increased risk of HIV acquisition (Hall, 2006).

Serological screening investigations e.g. Venereal Diseases Research Laboratory test (VDRL) and Reactive Plasma Reagin (RPR) test are mainstay of diagnosis for syphilis. The VDRL test is one of the most widely used simple and rapid test to determine the seroprevalence of syphilis in a community (Bala *et al.*, 2009). In HIV infected population initial serological responses to syphilis are shown to be equivalent in HIV negative as well as positive individuals. In many other studies it has been demonstrated that history of any STD, including syphilis, is associated with

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an increased risk of HIV disease among both homosexuals and heterosexuals. Sexual behaviors that increase the risk of acquiring STDs further increase the risk of acquiring HIV. Genital lesions and inflammation in syphilis are considered as catalyst for acquiring or transmitting HIV infection (Hall, 2006).

Atypical clinical presentations of syphilis are more common in HIV patients which makes diagnosis of *Treponema pallidum* (*T. pallidum*) infection more complicated and difficult. However, serological tests appear to be accurate and reliable for diagnosis of *T. pallidum* and the evaluation of treatment response in HIV patients. The clinician should seek confirmatory investigation for syphilis in HIV patients (Hall, 2006).

Even though syphilis continues to be a major problem in India, the true incidence will never be known, because of inadequate reporting. Serological surveys continue to be the best source of information on the prevalence of syphilis (Bala et al., 2009). Hence, the present study was designed to determine the trend and seroprevalence of syphilis in HIV patients receiving anti retroviral therapy (ART).

MATERIALS AND METHODS

This retrospective study was conducted in the Department of Microbiology of Pt. B.D.Sharma, PGIMS, Rohtak on total no. of 61,140 serum samples received in the laboratory from July 2008 to July 2012.

The inclusion criteria for blood samples received were-

1. Properly labeled samples
2. Samples with complete requisition slip

The exclusion criteria were:

1. Open vacutainers with contaminated blood
2. Lipolysed blood
3. Samples not processed on the same day
4. Turbid serum

The samples were subjected to VDRL test after proper processing as per Centre for Diseases Control (CDC) guidelines. A quantitative VDRL test was performed for seropositive samples (Young H, 1989). The VDRL antigen was obtained from Laboratories of Serologist, Calcutta, India.

Test Procedure

Serum Preparation

The VDRL test is a simple flocculation test with high sensitivity. It is performed as a, microslide test. 5 ml of clotted blood was taken and serum was separated out and heat inactivated in a water bath at 56 °C for 30 min. The serum was kept at room temperature before testing (Bala et al., 2009).

Antigen Preparation

The VDRL antigen and buffered saline diluent was provided with the antigen kit. The antigen was prepared according to the manufacturers' instructions. Buffered saline of 0.4 ml was pipetted out in 30 ml round bottom bottle. Antigen (0.5 ml) was added drop by drop to the buffered saline while continuously rotating the bottle on a flat surface over a period of approximately 6 seconds. After the last drop was blown out, the bottle was rotated for 10 more seconds. Then 4.1 ml of buffered saline was added to the bottle, and the bottle was shaken for another 10 seconds (Bala et al., 2009).

Qualitative VDRL Test

The glass slide (2-3 inches) with 12 paraffin rings of approximately 14 mm inside diameter were taken. Serum (0.05 ml) was added into one ring and a drop (1 of 60 ml) of antigen was added to the serum. Serum and antigen were mixed with a wooden stick and the slide was rotated for 4 min on a mechanical rotator, set at 180 rpm (Bala *et al.*, 2009).

Reading and reporting of the results – Under microscope with 10x low power objective with 100 times magnification, the antigen was seen as small needles which remain more or less evenly dispersed in a non reactive serum and aggregate into clumps in reactive sera.

Reading	Report	Group
No clumps or very slight roughness	Non reactive	1
Small clumps	Weakly reactive	2
Medium to large clumps	Reactive	3

The quantitative tests were performed on the reactive serum samples in which medium to large clumps were seen. Successive two fold dilution of the serum was made in 0.9% saline and each dilution was tested as described under qualitative serum tests. The results were reported in the

terms of highest dilution which gave a reactive result (Harries *et al.*, 1946; Harries *et al.*, 1948; Harries *et al.*, 1948; Rosenberg *et al.*, 1948; Manual of tests for syphilis, 1969).

HIV reactivity was confirmed as per National AIDS Control (NACO) guidelines.

RESULTS

In the present study, a decrease in the sero-prevalence of syphilis from 2.96% to 2.8% was observed among HIV non-reactive population while increasing trend of seroprevalence was observed among HIV reactive population, i.e., from 4.3% to 4.5% (Table 1).

In HIV reactive population, VDRL reactivity was maximum in adult males (4.87%) and minimum in male children (2.38%).

90 male adults (42.85%) showed a significant titre of >1:8 while each of 60 male adults (28.57%) showed undiluted (1:1) and a weakly reactive titre (\leq 1:8) respectively.

75 female adults (36.6%) showed a significant titre of >1:8 while 55 female adults (27%) and seventy-five female adults (36.6%) showed undiluted (1:1) and a weakly reactive titre (\leq 1:8) respectively. None of the children (both male and female) showed a significant titre (>1:8) while

Table 1: Comparison of Seroprevalence of Syphilis in HIV Reactive and HIV Non-Reactive Population

Year	Total Samples (HIV Non-Reactive)	VDRL Reactive	HIV Reactive	VDRL Reactive in HIV Reactive Population
July 2008-June2009	15,444	457 (2.96%)	2060	89 (4.3%)
July 2009-June2010	15,380	461 (3.00%)	2180	94 (4.3%)
July 2010-June 2011	18,088	522 (2.90%)	3384	149 (4.4%)
July 2011-July 2012	12,228	295 (2.8%)	1906	103 (5.4%)
Total	61,140	1735 (2.8%)	9530	435 (4.5%)

weakly reactive titre ($\leq 1:8$) was observed in 5 male children (100%) and 15 female children (100%) (Table 2).

DISCUSSION

The rate of syphilis has decreased throughout the 1990s, and in 2000 reached an all-time low. (Centres for Disease Control and Prevention, 2005). A sharp decline in trend of syphilis was reported in a retrospective data analysis was carried out to find trends in frequency and distribution of different STDs in North Eastern India during 1995-1999 (Jaiswal *et al.*, 2002)

After steady decrease for > 1 decade, rates of syphilis in the United states reached their lowest point during 2000, when the rate of primary and secondary syphilis was 2.1 cases per 100000 persons. In California, there was a >700% increase in primary and secondary syphilis cases reported between 1999 and 2005 (Centres for Disease Control and Prevention, 2006; Centres for Disease Control and Prevention, 2002).

All the above studies are comparable with the present study showing a decreasing trend of syphilis in general population.

The number of patients with STDs attending hospital(s) is declining. It is not clear whether this is due to an actual decrease in incidence of STDs or due to other factors. The increased availability of facilities for treatment of STDs at peripheral

centres might be a factor leading to decline in the number of patients with STDs approaching higher centres like the teaching hospital (Thappa *et al.*, 2007) where the present study was undertaken.

The syphilis rate among men is now nearly six times the rate among women. Additionally, prior CDC research has estimated that more than half of syphilis cases in recent years have occurred among homosexual males (Thappa *et al.*, 2007) which is in line with the present study.

A rising trend of syphilis was observed in HIV seroreactive population during different periods in a study conducted at New Delhi. The association of HIV seropositivity was consistently more in patients presenting with genital ulcers especially syphilis and increased significantly from 0.6% to 8.8% (Ray *et al.*, 2006).

There was estimated 2 to 5 fold increased risk for acquiring HIV when syphilis is present (New Mexico Department of Health, quarterly report, 2005). Significant association was observed between seropositivity for HIV and syphilis in a study by Shrivastava *et al.*, (2012). The results of above studies correlate well with the present study.

Out of 88 HIV seroreactive patients, 8 (9.1%) patients were reactive for VDRL in various titres. Out of these eight patients, males (75%) were more commonly affected than females (25%) as seen in present study. Males were commonly

Table 2: Seroreactivity of Syphilis in HIV Reactive Population in Both Sex

TITRE	Undiluted (1:1)	Weakly Reactive	TITER >1:8
Male adults	60 (28.57%)	60 (28.57%)	90 (42.85%)
Female adults	55 (27%)	75 (36.6%)	75 (36.6%)
Male child	0 (0%)	5 (100%)	0 (0%)
Female child	0 (0%)	15 (100%)	0 (0%)

affected group reactive for VDRL in various titres as seen in the present study (Turbadkar *et al.*, 2008).

The specificity of the VDRL test may be compromised in HIV infected patients (Augenbraun *et al.*, 1994; Rompalo *et al.*, 1992; Rusnak *et al.*, 1994; Drabick *et al.*, 1990). Many patients with HIV infection have both anticardiolipin- lecithin antibodies and polyclonal gammopathy, which could result in biological false positive results (Bala *et al.*, 2009). Therefore, in this study the VDRL titers ranging from of 1:1 to 1:8 were considered as insignificant and the titers of >1:8 were significant (Bala *et al.*, 2009)

Persons with HIV infection acquired through sexual contact should be tested for syphilis, because syphilis is a disease with broad range of manifestations and unusual clinical presentations, so specific laboratory diagnosis of syphilis is of great aid to clinical management of these cases. All sexually active persons with syphilis should be tested for HIV (with informed consent of the patient) for better management of the patients.

It was recommended that all the patients with newly diagnosed syphilis should be counselled for HIV testing. Similarly serological testing for syphilis in all patients with newly diagnosed HIV infection should be carried out (Turbadkar *et al.*, 2007).

VDRL, a slide flocculation non-treponemal test, provides a simple, rapid, convenient and economical procedure for serologic testing of syphilis. The nontreponemal tests have a sensitivity of 70 to 99%, depending on the stage of disease. The sensitivity of the test approaches 100 percent during the secondary phase of the disease. The specificity of the non-treponemal

tests can be used for a rapid and exact quantitative titration of reactive serum samples (Pope, 2007)

Recent changes in the epidemiology of patients who have concordant syphilis and HIV infection will require innovative public health strategies to control these new and resurgent epidemic (Zetola *et al.*, 2007).

CONCLUSION

In this study we conclude that the prevalence of syphilis was more (about 1.6 times) in HIV infected population as compared to non reactive population. The trend of syphilis infection is increasing from the July 2008 to July 2012 in HIV infected population. Males were predominantly affected with syphilis in HIV reactive population at variable VDRL titres. Whereas, in HIV non reactive population, syphilis is showing a decreasing trend.

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