

# Seroprevalence of Syphilis in Apparently Healthy Population: A Five-Year Study

**Kiran Bala, M.D.,  
Uma Chaudhary, M.D., FIAMS,  
Ritu Aggarwal, M.D.,  
Nidhi Goel, M.D.,  
Narinder Kaur, M.D.**

## ABSTRACT

The Venereal Disease Research Laboratory (VDRL) test is one of the most widely used simple and rapid tests to determine the seroprevalence of syphilis in a community. Early diagnosis and treatment of syphilis is extremely important as it decreases the overall burden and long-term morbidity. In this retrospective study, a total of 80,271 serum samples received from Department of Gynecology and Obstetrics, clinics of skin and venereal diseases, and others, from January 2001 to December 2005 were screened for syphilis by VDRL testing. A total of 1,502 (1.9%) serum samples were found to be reactive, with the decreasing trend over five years from 2.1-percent rate in 2001 to 1.6-percent rate in 2005. Of the total 300 couples with reactive titers, 74 (24.7%) of the wives had significant titers of  $\geq 1:16$ , and 123 (41.0%) of the wives had the titers ranging from 1:1 to 1:8. Of these 123 wives, 25 (8.3%) of the husbands had significant titers of  $\geq 1:16$ . Therefore, testing couples with the VDRL test can play an important role in the detection of apparently healthy cases of syphilis in the community. Early detection and treatment of such cases could further reduce perinatal mortality and morbidity. (*J Infect Dis Antimicrob Agents* 2009;26:55-9.)

## INTRODUCTION

The emerging epidemic of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) globally has made sexually-transmitted disease (STD) control as one of the imperative strategies to decrease the HIV transmission.<sup>1</sup> Syphilis is still a common STD in many areas of the world, despite the availability of effective

therapy. In 1999, the World Health Organization (WHO) estimated that the worldwide annual incidence of sexually-acquired syphilis was 12 million cases. The target for the year 2010 is 0.2 cases of primary and secondary syphilis per 100,000 populations, whereas in 2003 there were 2.5 cases per 100,000 population.<sup>2</sup>

Syphilitic disease is an important cause of perinatal morbidity and mortality. The incidence of congenital

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Department of Microbiology, PGIMS, Rohtak-124001 (Haryana), India.

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Reprint request: Dr. Kiran Bala, 4/6-J, Medical Enclave, Rohtak-124001 (Haryana), India.

E-mail: kiran\_griwan@yahoo.co.in

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syphilis roughly parallels to that of syphilis in females. Some studies have shown that in antenatal syphilis seropositivity rate can be as high as 30 percent, and congenital syphilis has been reported to account for up to 50 percent of still births. Prevention, timely detection, and treatment should reduce this perinatal morbidity and mortality.<sup>3</sup>

Even though syphilis continues to be a major health problem in India, the true incidence will probably never be known not only because of inadequate reporting but also due to the secrecy that surrounds them. Serological surveys continue to be the best source of information on the prevalence of syphilis. Hence, we designed the present study to determine the seropositivity rates of routine Venereal Disease Research Laboratory (VDRL) test of pregnant females and their husbands during antenatal care (ANC) screening and of high risk patients attending clinics of skin and venereal diseases (VDs), and others.

## MATERIALS AND METHODS

### Materials

The present study was conducted in Department of Microbiology, on 80,271 serum samples received from out-patients from January 2001 to December 2005. All the serum samples were subjected to VDRL testing, using the standard methods. A quantitative VDRL test was performed for positive samples.<sup>4</sup> 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 and is performed as a microslide test. Five mL of clotted blood was taken, and serum was separated out and heat inactivated in a water bath at 56°C for 30 minutes. Then, the serum was kept at

room temperature before testing.

#### *Antigen preparation*

The VDRL antigen and buffered saline diluent was provided with the VDRL antigen kit. The antigen was prepared according to the manufacturers' instructions. Buffered saline of 0.4 mL was pipetted out in 30 mL round 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.

#### *Qualitative VDRL Test*

The glass slides (2x3 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 minutes on a mechanical rotator set at 180 rounds per minute (rpm). The tests were read immediately after rotation under a microscope with the low power objective (100 x magnification). The results were read as non-reactive when there were no clumps or very slight roughness, weakly reactive when small clumps were observed, and reactive when medium to large clumps were observed.

#### *Quantitative VDRL test*

A quantitative test was performed on all reactive and weakly reactive serum samples. Successive two-fold dilutions of the serum were made in 0.9 percent saline. Each dilution was treated as an individual serum and tested as described under a qualitative VDRL test. The results were reported in terms of highest dilutions which gave a frank reactive reaction.

## RESULTS

A total of 80,271 sera were screened for syphilis by the VDRL testing over the period of five years, and 1,502 (1.9%) serum samples were found to be reactive. A decreasing trend of syphilis seropositivity was observed in our institute. VDRL reactivity was 2.1 percent in 2001, and it has decreased to 1.6 percent 2005. The seropositivity rate among samples was 1.6 percent, 3.07 percent, and 3.41 percent obtained from the patients attending clinics of ANC, skin and VD, and others departments, respectively (Table 1). Of a total 41,215 samples received from patients attending ANC clinic, the seroprevalence of syphilis was 1.4 percent. Of these ANC samples, 8,146 couples (16,292 samples) were screened; of which 300 (1.84%) couples were reactive. The titers of husbands and wives were also compared.

In Table 2, the titers of the husband's VDRL are shown against the titers of the wife's VDRL, when the wives either tested non-reactive or reactive with non-significant titers ranging from 1:1 to 1:8 or the significant titers of  $\geq 1:16$ . Of a total 300 couples with reactive titers, 74 (24.7%) wives had the titers of  $\geq 1:6$ . One hundred twenty-three (41.0%) wives had the titers ranging from 1:1 to 1:8. Of these 123 wives, 25 (8.3%) husbands had the titers of  $\geq 1:16$ . In addition, 103 (34.3%) wives with non-reactive VDRL had 25 (8.3%) husbands with the titers of  $\geq 1:16$ .

## DISCUSSION

VDRL, a slide flocculation non-treponemal test, provides a simple, rapid, convenient and economical procedure for serologic testing of syphilis. The non-treponemal tests have a sensitivity of 70 percent to 99 percent, 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.<sup>2</sup> It is well suited for mass serologic surveys, and our institution has had extensive experience with this test. Current recommendations in our institute are to screen women at the first ANC visit at third week of gestation and at delivery.<sup>5</sup> The guidelines from the Centers for Disease Control (CDC) of the United States included a non-treponemal test for the probable diagnosis of syphilis.<sup>2</sup>

The present study revealed that the five-year seroprevalence of syphilis in our institute was 1.9 percent. VDRL reactivity the Department of Obstetrics and Gynaecology was found to be 1.6 percent, and the seroprevalence among ANC cases was found to be 1.4 percent. A high rate of seropositivity of 3.1 percent and 3.4 percent was observed among patients attending clinics of skin and VD, as well as other departments, respectively. These higher prevalence rates are probably more high risk patients attending the STD clinic.

A diagnosis of syphilis in clinical settings is usually facilitated conjointly by symptoms and signs as well as non-treponemal serological tests, VDRL and rapid protein reagin (RPR) tests. Atypical presentations of clinical cases almost always necessitate the confirmatory treponemal antibody tests including the *Treponema pallidum* hemagglutination (TPHA).<sup>1</sup> One major problem with performing VDRL test is the interpretation of results when initial titers are  $< 1:16$ , as these may represent biological false positives.<sup>5</sup> Therefore, in the present study, the VDRL titers ranging from of 1:1 to 1:8 were considered as significant, and the titers of  $\geq 1:16$  were significant. The present study revealed 103 reactive husbands whose wives' VDRL were non-reactive. Of these 103 husbands, 75.7 percent

Table 1. The five-year trend of syphilis seropositivity from 2001 to 2005.

Department	Year					Number (percentage) of seropositivity
	2001	2002	2003	2004	2005	
Obstetrics and Gynaecology (N=65,987)	238	224	224	193	181	1,060 (1.6)
Skin and VD (N=13,523)	103	93	67	83	70	416 (3.10)
Others	3	6	4	6	7	26 (3.4)
Number (percentage) of seropositivity	344 (2.1)	323 (1.9)	25 (1.9)	282 (1.7)	258 (1.6)	1,502 (1.9)
VD: venereal disease						

Table 2. The Venereal Disease Research Laboratory (VDRL) titers of 300 couples (when either of the VDRL were reactive) attending antenatal care clinic from 2001 to 2005.

Wife	Husband	Non-reactive					Insignificant (1:1 to 1:8)					Significant (>1:16)				
		2001	2002	2003	2004	2005	2001	2002	2003	2004	2005	2001	2002	2003	2004	2005
Non-reactive (N=103)	*	*	*	*	*	17	11	14	20	16	4	6	3	8	4	
Insignificant (N=123)	9	7	12	8	6	13	10	14	8	11	7	5	2	6	5	
Significant (N=44)	1	2	4	3	1	6	8	5	10	7	3	7	8	5	4	

\*Both husband and wife had non-reactive titers.

and 24.3 percent had the titers ranging from 1:1 to 1:8 and of  $\geq 1:16$ , respectively. Similarly, when 123 wives showed the titers ranging from 1:1 to 1:8, 42 of their husbands were non-reactive, while 56 and 25 husbands showed the titers ranging from 1:1 to 1:8 and  $\geq 1:16$ , respectively. Of 74 wives with the significant titers, only 27, 36, and 11 husbands had significant, insignificant, titers and non-reactive titers, respectively.

Therefore, if only females attending ANC clinic are examined and if their husbands are syphilitic, then women can acquire syphilis late in pregnancy after an initially negative serologic screening. Also a woman who contracted infection once, may be at increased risk of reinfection, especially if her sexual partner has not received treatment. The VDRL testing of both husband and wife is of special value during ANC screening.<sup>6</sup>

The present study is limited by the fact that it is a retrospective study of laboratory-based data, and clinical details have not been included. Moreover, the sexual behavior of patients including the number of partners and the HIV status of patients were not studied. However, our data is useful in providing significant information about the trend of syphilis in our institute.

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