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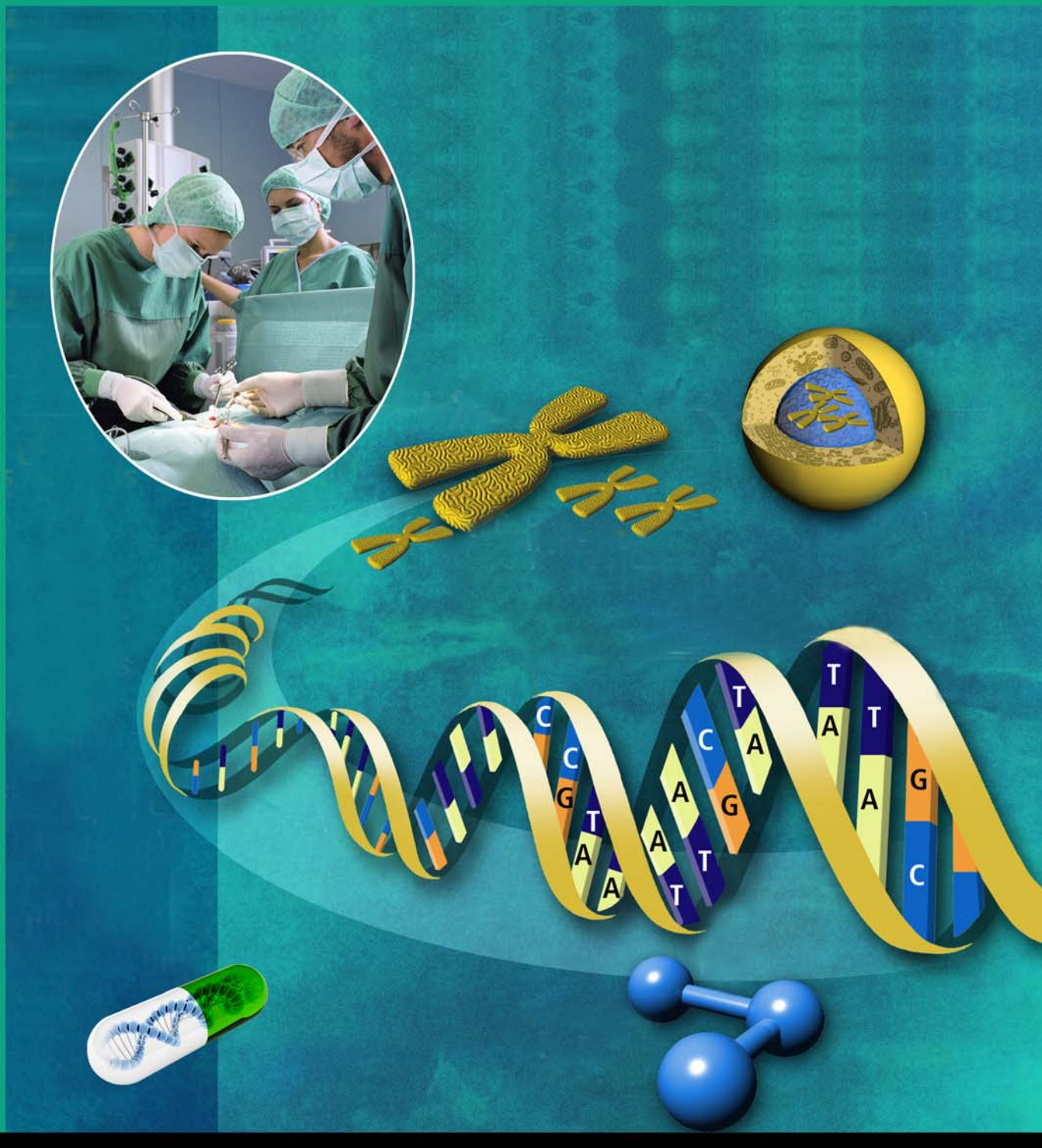

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Research Paper

RECENT TRENDS OF SEROPREVALENCE OF SYPHILIS IN APPARENTLY HEALTHY POPULATION IN A TERTIARY CARE CENTRE

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Syphilis is a sexually transmitted disease (STD), caused by Spirochete *Treponema pallidum*. It is an obligatory parasite of human beings .There are no known animal or environmental reservoirs. It is an old re-emerging disease in most part of world is currently a big challenge to clinicians. Early diagnosis and treatment of syphilis is extremely important to decrease further transmission and long term morbidity associated with syphilis. The Venereal Disease Research Laboratory (VDRL) test is one of the most widely used simple and rapid test to determine the seroprevalence of syphilis in community. We are working in a tertiary care hospital having a heavy load of serum samples of patients from different clinical departments. This study was done from 1st January 2006 to 31st December 2010. We tested 86,126 serum samples received from different clinical departments for VDRL testing. Out of these, 1,559 (1.83%) serum samples were VDRL reactive.

Keywords: Syphilis, *Treponema pallidum*, VDRL, Seroprevalence

INTRODUCTION

Syphilis is a sexually transmitted disease (STD), caused by Spirochete *Treponema pallidum*. It is an obligatory parasite of human beings. There are no known animal or environmental reservoirs. Major route of transmission is sexual intercourse, including genital, oral, and /or anal contact with or without penetration (Pope *et al*., 2007). Syphilis is an important cause of perinatal morbidity and mortality. Despite the availability of effective therapy Syphilis is a common STD in many areas of the world (Lukehart *et al.,* 2008).

The prevalence of syphilis is still high in the developing countries, resulting in a major public health problem (Eddy Van Dyck and Luc Van De Velden 1993; Yinnon AM *et al.,*1996). World Health Organization (WHO) estimated that the worldwide annual incidence of sexually-acquired syphilis was 12 million cases (WHO 1999). The target for the year 2010 was 0.2 cases of primary and secondary syphilis per 100,000 populations, whereas in 2003 there were 2.5 cases per 100,000 population (Pope *et al.,* 2007; and WHO, 1998).

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Congenital syphilis is associated with high healthcare costs and significant morbidity and mortality (stillbirths), but it is a preventable disease. The rate of congenital syphilis parallels that of primary and secondary syphilis in women of childbearing age. In the early 1990s, rates in both infants and women began to decline and continued to decline through 2004. Now the CDC reports that rates of congenital syphilis started to rise again in 2005. The rate increased 23% in 2005–2008, from 8.2 to 10.1 cases per 100,000 live births. Rates increased primarily in the South and among infants of black mothers. The overall syphilis rate decreased for the first time in a decade and is down 1.6% since 2009. However, the rate in young black men increased dramatically over the past years (134%). Other CDC data also show a significant increase in syphilis in young black men who have sex with men, suggesting that new infections among MSM are driving the increase in young black men. WHO estimated that 1 million pregnancies are affected by syphilis worldwide in 2004 (Walker and Walker, 2004)

Syphilis can be transmitted *in vitro* by blood transfusion (Nagi *et al*., 2008). It can also be transmitted by intravenous drug use (Olokoba *et al*., 2009). Most of the studies have shown that, in antenatal syphilis seropositivity rate can be as high as 30%, and congenital syphilis has been reported to account for up to 50 % of still births. Prevention, timely detection and treatment of these patients can reduce this perinatal morbidity and mortality (Lukehart *et al*., 2008). 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 (Young, 2007). 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 Venereal Disease Research Laboratory (VDRL) test of pregnant female and their husbands during antenatal care (ANC) screening and of high risk patients attending clinics of skin and venereal diseases (VDs), and other clinical departments.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, (PGIMS), Rohtak Haryana (India). A total of 86,126 serum samples obtained from patients belonging to Departments of Obstetrics and Gynaecology, Skin and VD, Medicine, Surgery and other clinical departments of PGIMS from January 2006 to December 2010. All the serum samples were subjected to VDRL testing, using the standard methods and quantitative VDRL test was done for positive samples. The VDRL antigen was obtained from Laboratories of Serologist, Kolkata, India. The samples having titre of more than 1:16 were further subjected to *Treponema pallidum* Haemagglutination (TPHA) test to confirm the results.

Test Procedure

Serum Preparation: The VDRL test is a simple flocculation test with high sensitivity and 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 minutes. Then, the serum kept at room temperature before testing.

Antigen Preparation: The VDRL antigen and buffered saline diluents were 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 6 seconds. After the last drop was blown out, the bottle was shaken for another 10 seconds (Winn *et al*., 2005).

Qualitative VDRL Test: The glass slides (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 minutes on a mechanical rotator set at 180 rounds per minute. The tests were read immediately after rotation under a microscope with the low power objective (100× magnification). The results were read as non-reactive when there were no clumps or every slight roughness, weekly reactive when small clumps were observed and reactive when medium to large clumps were observed (Winn *et al*., 2005).

Quantitative VDRL Test: A quantitative test was performed on all 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 (Winn *et al*., 2008)

RESULTS

Table 1 shows, a total of 86,126 serum samples were obtained during five years (January 2006 to

Table 1: Trends of VDRL Reactivity from 2006 to 2010 in PGIMS, Rohtak

Departments	Number of VDRL Reactive Cases					Total No. of Reactive Samples Total No. of Tested Samples Percentage
	2006	2007	2008	2009	2010	
Obstetrics & Gynaecology	243	206	226	176	227	107866,682(1.61%)
Skin & VD	123	109	87	82	71	47218,622(2.53%)
Medicine, Surgery & etc.	12	9	5	6	6	38822(4.62%)
No. of Reactive Samples	378	324	318	264	304	1559
No. of tested samples	16437	18441	17683	17650	16915	86126
Percentage	(2.30%)	(1.76%)	(1.80%)	(1.50%)	(1.80%)	(1.83%)

Table 2: Seroprevalence of Syphilis Among Different Age Groups

Age groups	No. of Samples	No. of Reactive samples (Male)	No. of Reactive samples (Female)	Percentage of Reactive samples
19-23	7,050	42	48	1.27
24-28	34,370	418	315	2.13
29-33	24,675	256	180	1.76
34-38	20,031	198	102	1.49
Total	**86,126**	**914**	**645**	**1.83**

December 2010) and screened for syphilis by VDRL testing. The samples having titre of more than 1:16 were further subjected to Treponema pallidum Haemagglutination (TPHA) test to confirm the results. Out of these, 1559 (1.83%) serum samples were VDRL reactive. VDRL reactivity in samples obtained from Obstetrics & Gynaecology, Skin & VD, Medicine, Surgery and other clinical departments were 2.30% in 2006, 1.76% in 2007, 1.80% in 2008, 1.50% in 2009 and 1.80% in 2010 respectively.

Table 2 shows the seroprevalence of syphilis among different age groups. The highest seroprevalence (2.13%) was observed in the group aged 24-28 years followed by the groups aged 29-33 years (1.76%) and 34-38 years (1.49%) respectively. The lowest prevalence (1.27%) was observed in the group aged 19-23 years. The difference in seroprevalence between the groups aged 24-28 and 29-33 years was statistically significant ($p<0.05$), as was the difference between the groups aged 34-38 and 24-28 ($p<0.05$).

Table 3 shows the comparison of titres of husband and wife when the wives either tested non-reactive , or reactive with insignificant titres ranging from 1:1 to 1:8, or reactive with significant titres ≥1:16. Out of total 313 couples, 111 (35.4%) wives had non reactive titre, 126 (40.2%) wives had the insignificant titre ranging from 1:1 to 1:8., 76 (24.2%) wives had significant titres of ≥1:16.

Of the one hundred and eleven wives with non-reactive VDRL had, 32 (28.8%) husbands with significant titre of ≥1:16. Out of the 126 wives who had insignificant titre, 28 (22.2%) husbands had significant titre of ≥1:16. Out of seventy six wives who had significant titres , 34 (44.7%) husbands had significant titre.

DISCUSSION

Sexually transmitted infections (STI) are one of the major causes of morbidity and mortality among men, women and children. They cause infertility, ectopic pregnancy, stillbirth, cancer and congenital infections. Globally around 340 million cases of curable new STI occur every year. Syphilis is relatively better controlled in the developed countries, but the situation is in the developing countries is not satisfactory (Pramod *et al*., 2004; Prabhu *et al*., 1992).

Laboratory tests are available for the diagnosis of syphilis which are not expensive. Syphilis can be treated with penicillin, which is also used for the prevention of congenital syphilis. Penicillin is

Table 3: The Titre of VDRL of 313 Couples Attending ANC Clinic from 2006 to 2010

Wife	Husband (Non Reactive VDRL)					Husband (Insignificant titre) (1:1 to 1:8)					Husband (Significanttitre) (>1:16)				
	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010
Non-Reactive VDRL(n=111)	0	0	0	0	0	19	13	13	18	16	5	7	6	5	9
Insignificant titre (n=126)	8	6	12	9	7	13	9	12	10	12	7	5	4	3	9
Significant titre (n=76)	2	2	3	3	2	5	7	6	5	7	6	8	7	6	7

cheap and is included in WHO list of essential drugs (Watson-Jones *et al.*, 2002)

Antibody detection tests supplement the direct organism detection methods used for the diagnosis of primary and secondary syphilis and are the only practical methods of diagnosis during latent and late syphilis (Rusell *et al*., 1996)

Serological tests for syphilis are divided into non-treponemal and treponemal types. Non-treponemal tests detect so-called reaginic antibodies that react with lipoidal particles containing the phospholipids cardiolipin (Rusell and Carrie, 1996).

We used VDRL, a slide flocculation non-treponemal tests, which provides a simple, rapid, convenient and economical procedure. The non-treponemal tests have a sensitivity of 70% to 99%, depending on the stage of disease. The sensitivity of the test approaches 100% 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 (Rusell and Carrie, xxxx). This is well suited for mass serologic surveys. In PGIMS, Rohtak we screen women at the first ANC visit at third week of gestation and at delivery (Cunningham *et al*., 2001).

The guidelines from the Centres for Disease Control (CDC) of the US included a non-treponemal test for the probable diagnosis of syphilis (Pope *et al*., 2007; and WHO, 1998).

The present study revealed 1.83% seroprevalence of syphilis over a five years period in apparently healthy population. VDRL reactivity in samples from the department of Obstetrics and Gynaecology was found to be 1.61%. A high rate of seropositivity of 2.53% was observed among patients attending clinics of Skin and VD. Seroprevalence in Medicine, Surgery and other departments was 4.62%.

Out of total 313 couples attending antenatal clinic, one hundred and eleven(35.4%) wives had non reactive titre, One hundred twenty six (40.2%) wives had the insignificant titre ranging from 1:1 to 1:8., seventy six (24.2%) wives had significant titres of $\geq$1:16. Of the one hundred and eleven wives with non-reactive VDRL had 32 (28.8%) husbands with significant titre of $\geq$1:16. Out of the 126 wives who had insignificant titre, 28 (22.2%) husbands had significant titre of $\geq$1:16. Out of seventy six wives who had significant titres 34 (44.7%) husbands had significant titre.

Diagnosis of syphilis in clinical settings is usually facilitated conjointly by symptoms and serological tests of the non-treponemal antibody like VDRL and Rapid Plasma Reagin (RPR) tests (Rajendran *et al*., 2003)

A typical presentations of clinical cases necessitate almost always confirmatory treponemal antibody tests like TPHA (Rajendran *et al*., 2003). Reports from several countries worldwide (Hussain,1986; Lowhagen,1990; Thaker *et al*., 1996) confirm such practice as routine in hospitals receiving STD patients (Wiis and Sheller, 1995). The situation is much more complicated while documenting the prevalence rate of syphilis in a community to draw baseline for instituting preventive measures. The reasons are (a) Population studies on 'apparently healthy adults' are limited. The term apparently healthy adults used herein is taken to mean those without any previous diagnosed STD. (b) The non-treponemal antibody tests like RPR and VDRL which detect only the reaginic antibodies do not conclusively prove the active stage of the disease and the actual need for anti-syphilitic treatment

in the absence of suggestive clinical symptoms. (c) The occurrence of biological false positivity due to physiological conditions and certain infections (Smikle MF *et al*., 1990) (d) Biological false negative results in late and latent syphilis (Smikle *et al*., 1990), (e) Even, TPHA as a treponemal antibody test does not satisfy the requirements since it is insufficient for excluding syphilis in the elderly because of immunological impairment seen in aged persons (Kanda *et al*., 1992). It lacks the sensitivity in sera from patients with primary syphilis (Larsen *et al*., 1981)

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 (Cunningham FG *et al*.2001). Therefore, in the present study, the VDRL titers ranging from 1:1 to 1:8 were considered as insignificant, and the titers of ≥1:16 were significant.

It is also important to note that, the male and female ratio of case increased from 1.6 in 1999 to 5.3 in 2003 Cunningham FG It increases in the rate of primary and secondary cases among men relative to women in the US (Ciesieski, 2003). In the present study, seroprevalence of syphilis was observed highest in group aged 24-28 (2.13%) while lowest prevalence rate was observed in group aged 19-23 (1.27%). Therefore, only antenatal females are examined and if their husbands are syphilitic then women can acquire syphilis late in pregnancy after an initially negative serologic screening (Klass *et al*.,1994). Also a woman who contracted infection once may be at increased risk of re-infection, especially if her sexual partner has not received treatment (Klass *et al*.,1994). In conclusion, the findings of this study further emphasizes that, VDRL testing of both husband and wife during antenatal screening is of special value in the diagnosis, treatment and prevention of syphilis in the newborn and in our institute a decreasing trend of syphilis was observed in the last five years.

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