Research Article

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Screening of syphilis using rapid plasma reagin test in apparently healthy population in Madhya Pradesh: a 6 years study

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ABSTRACT

Background: To analyze the prevalence of syphilis in apparently healthy population in Madhya Pradesh during last 6 years. **Methods:** Serum samples were collected from Departments of Obstetrics and Gynecology, Skin and Venereal diseases, Medicine, Surgery, etc., of the period of 6 years. All the serum samples were screened for syphilis by rapid plasma reagin (RPR) test using standard methods.

Results: Out of 21,742 samples, 133 (0.62%) serum samples were found to be positive. The highest seroprevalence was observed in the group aged 25-31 years (0.89%) and lowest in 18-24 years (0.31%).

Conclusion: RPR testing in this populations group plays a special value in the diagnosis, treatment and prevention of syphilis.

Keywords: Syphilis, Rapid plasma reagin, Syphilis in apparently healthy population

INTRODUCTION

Syphilis is sexually transmitted disease, which is caused by *Treponema pallidum*. It is a spiral, motile, chemo heterotrophic, microaerophilic, Gram-negative bacillus belonging to the Spirochaetaceae. They are mainly transmitted by sexual intercourse (venereal syphilis) and less commonly via the placenta (congenital syphilis) or by accidental inoculation from infectious materials.^{1,2}

However, syphilis can also be transmitted *in-vitro* by blood transfusion and intravenous drug use.^{3,4} Syphilitic disease is an important cause of perinatal morbidity and mortality.⁵ The incidence of syphilis increased dramatically in the past decade.⁶ The reason for this is unclear, but must be sought in sexual behavior, increased travel, immigration, and prostitution, as well as provision, use, and effectiveness of diagnostic, treatment, and contact tracing services.⁷

The prevalence of syphilis is still high in many populations in the developing world, resulting in a major public health problem.⁸ In 1999, the World Health Organization estimated that the worldwide annual incidence of sexually-acquired syphilis was 12 million cases.⁹ Serological surveys continue to be the best source of information on the prevalence of syphilis. That is why, we designed the present study to determine the seropositivity rates in apparently healthy population in Madhya Pradesh.

METHODS

The retrospective study was conducted in the Department of Microbiology, Gajra Raja Medical College, Gwalior. A total of 21,742 serum samples were obtained from patients belonging to Departments of Obstetrics and Gynecology, Skin and venereal disease (VD), Medicine, Surgery, etc. of J.A. Group of Hospitals, Gwalior from January 2008 to December 2013. All the serum samples were subjected to rapid plasma reagin (RPR) testing, using the standard methods, and quantitative RPR test was done for positive samples.¹⁰

Serodiagnosis of syphilis using RPR

RPR card test is a non-treponemal flocculation test for the qualitative, and semi quantitative determination of reagin antibodies in serum or plasma.

Principle

RPR is a modified form of Venereal Disease Research Laboratory antigen which contains carbon particles to improve the visual reading of the results. The binding of cholesterol or cardiolipin or lecithin in the reagent to reagin antibodies in the sample results in large black clumps or aggregates can be seen macroscopically.¹¹

Test procedure

Slide method

One drop (50 μ l) of patients sample was dispensed on to a glass slide or RPR test card and was spread to cover the entire area of the test circle using the flat end of the pipette/stirrer. Using the dispensing bottle or needle assembly, one free falling drop (16 μ l) of antigen was allowed to drop on to the test specimen. The test card was rotated at 100 rpm for 8 min only on an automatic rotator. Immediately, after 8 min the results were observed visually, under good light. Medium and large aggregates indicated reactive, dispersed aggregates weakly reactive, non-aggregates visible smooth gray appearance were considered non-reactive.

Semi quantitative method

Serial dilutions (50 μ l) of the patient serum (1/2, 1/4, 1/8, 1/16, so on) were prepared with isotonic saline. Each serum dilution was then added to the test circle on the slide. After that one drop (16 μ l) of shaken antigen was added to each dilution. The slide/card was then rotated for 100 rpm for 8 min. Immediately, after 8 min the results were observed visually in good light. The last dilution that produces the reactive result was taken as the titer. 12

RESULTS

A total of 21,742 serum samples was obtained during 6 years (January 2008-December 2013) and screened for syphilis by RPR testing. Out of these, 133 (0.62%) serum samples were RPR reactive. RPR reactivity in samples obtained from Departments of Obstetrics and Gynecology, Skin and VD, Medicine, Surgery etc. were 0.45% in 2008, 0.88% in 2009, 1.00% in 2010, 1.06% in 2011, 0.39% in 2012 and 0.23% in 2013 respectively (Table 1).

Table 2 shows the seroprevalence of syphilis among different age groups. The highest seroprevalence (0.89%) was observed in the group aged 25-31 years followed by the groups aged 32-38 (0.59%) years and 39-45 years (0.43%) respectively. The lowest prevalence (0.31%) was observed in the group aged 19-23 years.

Table 1: Trends of RPR reactivity from 2008 to 2013.

| Year | RPR total | RPR non-reactive | RPR reactive |
|-------|--------------|---------------------|-----------------|
| 2008 | 1094 | 1089 | 05 |
| 2009 | 2136 | 2147 | 19 |
| 2010 | 3172 | 3140 | 32 |
| 2011 | 3933 | 3861 | 42 |
| 2012 | 5331 | 5310 | 21 |
| 2013 | 6076 | 6062 | 14 |
| Total | 21,742 | 21,609 | 133 |

RPR: Rapid plasma reagin

Table 2: Seroprevalence of syphilis among different age groups in male and female.

| Age groups | Number of | Number of reactive samples | | Percentage of reactive |
|---------------|--------------|----------------------------|--------|------------------------|
| | samples | Male | Female | samples |
| 18-24 | 2826 | 6 | 3 | 0.31 |
| 25-31 | 6280 | 34 | 22 | 0.89 |
| 32-38 | 8069 | 33 | 15 | 0.59 |
| 39-45 | 4567 | 12 | 8 | 0.43 |
| Total | 21,742 | 85 | 48 | 0.62 |

DISCUSSION

Syphilis, an old re-emerging disease in most parts of the world is currently a big challenge to health practitioners. Although, it is relatively better controlled in the developed countries, the situation is aggravating in the developing countries. 13 Here the study was done on apparently healthy population. The term apparently healthy adult used herein is taken to mean those without any previous diagnosed sexually transmitted diseases. The humoral antibodies produced in response to T. pallidum infection become detectable in the primary stage, increase in concentration during the secondary stage and decline during latent infection. Diagnosis of syphilis is usually facilitated conjointly by symptoms and serological tests. The test like non-treponemal antibody tests like RPR detects only reaginic antibodies, hence do not prove the active stage of the diseases, even T. pallidum hemagglutination as a treponemal test does not satisfy the requirement since it is insufficient for excluding syphilis in elderly, because of immunological impairment seen in aged persons and it lacks the sensitivity in sera from patients with primary syphilis.14

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.¹⁵ RPR test is a simple, rapid, convenient and economical procedure. The present study also revealed the 6-year seroprevalence of syphilis in male-female among different age groups. We found that the seroprevalence rate was 0.62% among

patients attending clinics of obstetrics and gynecology, skin and VD, medicine, surgery, etc. in last 6 years. One major problem with performing RPR test is the interpretation of results when initial titers are < 1:16, as these may represent biological false positive. ¹⁶

Therefore, in the present study the RPR titers ranging from of 1:1 to 1:8 were considered as insignificant, and the titer of \geq 1:16 were considered significant and seroprevalence of syphilis was observed the highest in group aged 25-31 (0.89%), while lowest prevalence rate was observed in group aged 18-24 (0.31%).

CONCLUSION

In conclusion, the findings of this study further emphasizes that, RPR testing in this populations group plays a special value in the diagnosis, treatment and prevention of syphilis.

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