Research Article

APPLICATION OF A RAPID SEROLOGICAL VDRL TEST FOR DETECTION OF ANTI *TREPONEMA PALLIDUM* ANTIBODIES IN SERUM OF SUSPECTED SYPHILIS PATIENTS

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ABSTRACT

Syphilis is a sexually transmitted disease caused by spirochetal bacterium *Treponema pallidum*. VDRL are rapid slide microflocculation tests for syphilis that use an antigen containing cardiolipin, lecithin, and cholesterol. The VDRL tests measure IgM and IgG antibodies bound to the lipoidal material found in infected cells of diseased person. This lipoprotein-like material, cardiolipin released from the treponemes. In the present study 28 blood samples from suspected patients of different age groups and sex were collected from IGMC Shimla and tested for the infection of *Treponema pallidum.* Out of 28 samples only 3 were found positive and other were negative. In 3 positive samples 2 were females and only one patient was male. Study reveals that the cases of syphilis were low in the region. All the positive patients were of between 23 -37 year age group.

Keywords: VDRL, Serological, syphilis.

INTRODUCTION

Syphilis, a chronic and systemic sexually transmitted infection, is caused by the spirochaete *Treponema pallidum* subspecies *pallidum*. *Treponema pallidum* cannot be cultured *in vitro*[1]. Although, it can only be demonstrated by specific laboratory stains or dark background microscopy[2]. Since the organism is not easily accessible in early stage of infection, serologic tests are still the main and essential tools in making the diagnosis of syphilis. Microscopy is useful in diagnosing syphilis in its primary stage, whereas serological tests are used for diagnosing primary, secondary, or latent stages of syphilis. The most widely used screening test for syphilis is Venereal Disease Research Laboratory (VDRL). Syphilis continues to be a major global health threat causing an estimated 12 million infections each year[3] despite the known adverse effects on pregnancy[4] and the synergistic relation with HIV infection,[5] about 90% of these syphilis cases are in low-income countries[6] where traditional syphilis tests are often unreliable[7].

MATERIALS AND METHODS

Collection of clinical samples

Patient blood was collected by vein puncture (from inside of the elbow). Blood was collected by placing an elastic band around the upper arm with slight pressure with the help of 2-4ml sterile syringe. Puncture site was cleaned with suitable antiseptic before taking the sample. Four to five ml of blood sample was taken. Collected blood was centrifuged and serum was separated and tested further[8].

Rapid VDRL test

VDRL test was performed by using Plasmatec VDRL Antigen test kit (Plasmatec laboratory products ltd. UK) following manufacturers protocols. The Plasmatec VDRL Antigen test kit is for the serodiagnosis of syphilis. It is suitable for both the VDRL tube and slide flocculation tests[9-12].

Preparation of Reagents

VDRL Antigen Emulsion for Slide Test

VDRL Antigen emulsion was prepared by Pipetting 0.4ml of the buffered saline diluent into a flat bottomed, stoppered bottle. To this 0.5ml of the VDRL antigen was added drop wise to the diluent whilst rotating the bottle on a flat surface. To this mixture 4.1ml of saline diluent was added and bottle was shaked with vigorous shaking for at least 10 seconds. The prepared reagent was kept at 4°C and used within 24 hrs.

Test procedure

Rapid VDRL test was performed for screening syphilis patients by taking 4-5ml of patient whole blood in to a sterile test tube. Test tubes containing specimens were centrifuged and serum was separated. Half ml of serum was taken in test tubes and kept in a water bath for 30 minutes at 56°C for inactivation. Tubes were cooled at room temperature (25°C ±5°C). In a cavity slide 0.05 ml of patient serum was added and to this one drop of antigen was added. Slide was rotated for 4 minutes by using rotator. Slides were observed for clump formation under low power objective. The results were recorded as non reactive (negative), if no clumps with needle like particles were seen, weakly reactive if small clump with free particle were seen and reactive (positive) if large clumps on a clear background were seen.

Table 1: Detail of patients report for VDRL testing

S. No	Patient Name	Age (years)	Sex (M/F)	Result
1	Radhika	22	F	Non reactive
2	Vandana	25	F	**Reactive**
3	Narpat Ram	25	M	Non reactive
4	Balwant Singh	40	M	Non reactive
5	Kesav	34	M	Non reactive
6	Devita	25	F	Non reactive
7	Gurudyal	40	M	Non reactive
8	Rajkumari	38	F	Non reactive
9	Sangita	28	F	Non reactive
10	Joginder	45	M	Non reactive
11	Bimla	55	F	Non reactive
12	Reena Devi	35	F	Non reactive
13	Vidya Devi	30	F	Non reactive
14	Seema	23	F	**Reactive**
15	Asha	27	F	Non reactive
16	Krishna	33	F	Non reactive
17	Meena	35	F	Non reactive
18	Lata Devi	37	F	Non reactive
19	Lmesh	18	F	Non reactive
20	Kamla	54	F	Non reactive
21	Ram Bhagli	30	F	Non reactive
22	Mohan Singh	44	M	Non reactive
23	Sanjeev Kumar	37	M	**Reactive**
24	Heena	26	F	Non reactive
25	Aman	22	M	Non reactive
26	Seeta Devi	27	F	Non reactive
27	Sonu Kumar	29	M	Non reactive
28	Leela Devi	48	F	Non reactive

Table 2: Details of positive samples after VDRL test

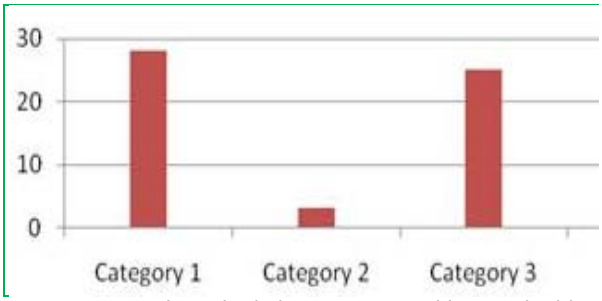
S. No	Name & Age of patient (Years)	Sex	Result
1	Seema, 23	F	Reactive
2	Vandana, 25	F	Reactive
3	Sanjeev Kumar, 37	M	Reactive

RESULTS AND DISCUSSION

Despite the advancement of molecular technique and the whole genome of *Treponema pallidum*, the causative agent of syphilis, has been sequenced, the diagnosis and treatment response of syphilis still rely on serologic tests[13]. Evaluation of syphilis treatment trials is complicated by three factors: first, many studies lack the essentials of good study design: randomisation, blinding, an adequate number of patients (i.e., statistical power), and inclusion of a control group; second, because *T.pallidum* cannot be routinely cultured, diagnosis of active disease often relies on serology tests; third, the natural history of syphilis necessitates long-term follow up to assess treatment efficacy. Because of the last two factors, studies should have clearly defined entry and outcome criteria, but rarely do. As a result, treatment trials are difficult to evaluate, and optimal treatment regimens are difficult to determine[14]. VDRL test is a rapid test to diagnose the syphilis. Pregnant women are also screened by VDRL test in order to avoid the risk of congenital syphilis. In the present study, a total of 28 blood samples were taken (Table 1) from suspected patients at IGMC Shimla (H.P.), India. The blood samples were centrifuged to separate the serum (Figure 1). The serum was tested for syphilis infection on VDRL slide (Figure 2). Out of 28 samples, 3 were reactive and 25 were non reactive (Figure 3).

Figure 1: Patient serum after blood centrifugation

Figure 2: VDRL slide containing patient serum



Category 1= Total samples (28), Category 2= Positive samples (3), Category 3= Negative samples (25)

Figure 3: Distribution of positive and negative samples.

Three positive cases were observed in age group of 23-37 Years (Table 2). This test play important role to reduced mortality and morbidity rate. *Treponema pallidum* penetrates a broad variety of tissues, including central nervous system, eye and placenta, so called "immune privileged, where there is less surveillance by the host's innate immune system and causes great damage to the body tissues. Therefore, it is necessary to diagnose the disease in the early stages.

CONCLUSION

Rapid serological tests for syphilis are an acceptable alternative to conventional laboratory tests. Since, they do not require equipment or electricity; they could increase coverage of syphilis screening, and enable treatment to be given at the first clinic visit. The VDRL tests are fast, easy to perform, and excellent for screening of samples.

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