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Evaluation of Various Techniques for Sero-Diagnosis of Syphilis in Blood Donors

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Abstract:-

➢ ***Introduction:***

Syphilis, the first transfusion-transmitted disease described, was reported commonly before 1950. Screening of blood donors have been performed with serological test from the early of stages of blood transfusion practices.. Although the risk the transmission of syphilis through blood is negligible, in India, it is mandatory that blood banks screen every donation for syphilis[3]. The study was performed to evaluate the true seroprevalence of syphilis in blood donors as well as to evaluate the suitability of Immunochromatographic test, PaGIA, TPHA and ELISA as replacement for RPR either for screening or confirmation of syphilis in blood donors.

➢ ***Material & Methods:***

The total number of donors who were screened for syphilis were 28,544. All the donors were screened for HIV-1, 2, Hepatitis B, Hepatitis C, syphilis and malaria. Rapid plasma reagin (RPR) method was done as a primary screening method of syphilis using carbogen particles (Tulip laboratories). A total of 132 RPR positive and 132 RPR negative control sera were included in the study to compare the additional test performed.

➢ ***Results:***

Out of the total samples tested , 132 donors were found to be RPR reactive with 0.46% seropositivity. The seroprevalence was 0.46% (n= 127) among male donors and 0.43% (n =05) in female donors which was not statistically significantly different (p>0.05). The sensitivity, specificity, PPV, and NPV of treponemal tests was done and it was seen that PaGIA, ICT, and ELISA had sensitivity of 100%, 98.8% and 100%, respectively and the specificity of PaGIA, ICT, and ELISA was found to be 78.7%, 97.8%, and 93.5%. An overall agreement of 91.6 % was found in all four treponemal tests.

➢ ***Conclusion:***

On comparing the RPR titer with other treponemal tests performed, it was found that titer >16 gave the good prediction of positive treponemal tests. Other studies have also shown correlation of RPR or VDRL titre with treponemal tests. In our study, the sensitivity of ELISA, TPHA and PaGIA were similar and comparable. The positive predictive value and negative predictive values for PaGIA were 89.4% and 100%, respectively. In our study, treponemal assay had higher specificity than cardiolipin assay. The number of false positive samples can be reduced by using a method for screening which has higher specificity.

I. INTRODUCTION

Syphilis, the first transfusion-transmitted disease described, was reported commonly before 1950[1]. Screening of blood donors have been performed with serological test from the early of stages of blood transfusion practices [2]. Although the risk the transmission of syphilis through blood is negligible, in India, it is mandatory that blood banks screen every donation for syphilis[3]. The most commonly used serological test are the Venereal Disease Research Lab (VDRL) test which is considered the classical method for testing for syphilis and Rapid Plasma Reagin (RPR) are based on the development of high titers of antibodies to cardiolipin and other phospholipids in patients with active syphilis. There is a need for a confirmatory treponemal rest as most of the non-treponemal test in later stages of infection lacks sensitivity[4] and they may also yield higher false positive reactions during screening of samples [4]. These false positive results may be seen in aute and chromic conditions in absence of syphilis (biological false positive reactions)[5]. Recently there

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are various developments in serological tests for syphilis with availability of a large number of commercial recombinant antigen based treponemal tests [4]. Detection of specific TP antibodies can also effectively be achieved using the sensitive enzyme immunoassay technique. Particle gel immunoassay (PaGIA) has been introduced for detection of infectious disease in addition to red cell serology. Recently in the US, treponemal tests have been introduced for screening in blood banks[6].

This study was performed to evaluate the true seroprevalence of syphilis in whole blood donors in our population as well as to evaluate the suitability of Immunochromatographic test, PaGIA, TPHA and ELISA as replacement for RPR either for screening or confirmation of syphilis in blood donors.

II. MATERIAL AND METHODS

The study was conducted in the department of transfusion medicine, a tertiary care teaching hospital in North India. The total number of donors who were screened for syphilis were 28,544. All the donors were screened for HIV-1, 2, Hepatitis B, Hepatitis C, syphilis and malaria. Rapid plasma reagin (RPR) method was done as a primary screening method of syphilis using carbogen particles (Tulip laboratories). The RPR test was performed quantitatively by double dilution method in all RPR positive sera. The serum samples of RPR positive donors were preserved at -20ºC. A total of 132 RPR positive and 132 RPR negative control sera were included in the study to compare the additional test performed. The additional test performed were as follows.

- *TPHA:*

TPHA was performed using Immunotrep TPHA (Omega Diagnostics, UK). It comprises T. pallidum sensitized formolized tanned fowl erythrocytes. When diluted positive samples were mixed with sensitized erythrocytes, antibody to the sensitizing antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of the microtitration plate well. In the absence of antibody, they form a compact button in the well. Samples giving positive reaction with unsensitized formolized tanned erythrocytes were labelled as Indeterminate.

- *Particle gel immunoassay (PaGIA):*

The PaGIA (DiaMed, Cressiersur Morat, Switzerland) which uses recombinant antigens to detect antibodies to *T. Pallidum.* It is a particle immunoassay in which a gel matrix in contained in microtubes. These microtubes also consist of red polymer particle which are sensitized with ready to use suspension of recombinant antigens primarily, TpN15, TpN17, and TpN47. The assay was done as per kits instructions. A positive test in observed as a red line on the top of gel matrix indicating the presence of antibodies to T. Pallidum. The reaction occurs due to agglutination of particles. . A lower strength reaction when antibodies concentration were low the agglutinated beads were seen in the entire matrix. Formation of pellet at the bottom of microtubes by colored particles represented negative results .

- *Immunochromatic assay (ICT):*

A rapid solid phase immunochromatic test was performed using SD BIOLINE Fast 3.0 (SD. Biostandard diagnostics, India). It is a membrane strip, which is pre-coated with recombinant TP antigens on test band region. The recombinant TP antigens –colloid gold conjugate, patient sample, and sample diluent moves along the membrane chromatographically to the test region and forms a visible line as the antigen antibody- antigen gold complex. It is used for qualitative detection of all isotypes (IgG, IgM, IgA) against treponemal pallidum.

- *ELISA:*

ELISA was performed using Treponostika TP recombinant (Biomeriux) based on one step “sandwich” principle. The test makes use of recombinant T. Pallidum antigens coupled with HRP conjugate, and TMB and peroxide as substrate. If the absorbance of sample had absorbance value which was less than the cut off (mean of three negative control + 0.2) were labelled as negative, while samples with an absorbance more than or equal to cut off were marked positive reaction.

Descriptive parameters and binary function test such as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for validation of various test methods. Kappa indices and agreement rates were used to evaluate the comparability of each test.

III. RESULTS

During the study period, a total of 28,544 donors including 27,381 males and 1163 females were screened for syphilis by Rapid Plasma Reagin (RPR) method. Out of the total samples tested , 132 donors were found to be RPR reactive with 0.46% seropositivity. There was an increasing trend in prevalence with age (0.14-0.66) (Table 1). Maximum donors belonged to the age group 26-35 years with median age of 33 years. The seroprevalence was 0.46% (n= 127) among male donors and 0.43% (n =05) in female donors which was not statistically significantly different ($p>0.05$). The results of comparison of the RPR test versus the TPHA, PAGIA, ICT, ELISA results are shown in Table 2 & 3. It was observed that one sample that was RPR positive and TPHA negative was positive by PaGIA, ICT, and ELISA. Similarly, one nonspecific result by TPHA which had agglutination in both sensitized and unsensitized cells was positive by PaGIA, ELISA and ICT. Out of the 46 RPR positive and TPHA negative samples (biological false positives), eight samples were positive by PaGIA, and 3 samples were ELISA positive. In our study, 36 (27.2 %) samples were found to be false positive by RPR, while all other treponemal tests done were negative. In 132 RPR positive samples, ELISA was found to

be positive in 89 (67.4%) samples. The sensitivity, specificity, PPV, and NPV of treponemal tests was done and it was seen that PaGIA, ICT, and ELISA had sensitivity of 100%, 98.8% and 100%, respectively and the specificity of PaGIA, ICT, and ELISA was found to be 78.7%, 97.8%, and 93.5% . The RPR titre was more than 8 in 35.6% (n=47) RPR reactive donor whereas the titre was less than 4 in 66 reactive donors. It was also observed that TPHA and ELISA reactivity was certain in samples which had RPR titer of 1:16 or more and 1: 8 or greater respectively. In contrast, in 40 % cases ELISA/TPHA were negative with RPR titers of 1 in 2 and 75% in cases with RPR titres of 1:1. Agreement and kappa coefficient of the syphilis screening tests performed in blood donors (Table 5). An overall agreement of 91.6 % was found in all four treponemal tests. When any of the two treponemal tests were compared, the agreement ranged from 93.1 to 98.5 %.

IV. DISCUSSION

The RPR seroprevalence in blood donors was 0.46% with an increasing trend with age (0.14-0.66) shown in Table no 1. Various studies from India have shown similar prevalence in blood donors [7-9.] The true seropositivity of syphilis in blood donors was 0.3% (TPHA) & 36 (27%) sera were found to be BFP by RPR. The RPR test has a reported sensitivity and specificity of 86% and 98% respectively[10]. The RPR quantitative results showed titre ranging from 1 to maximum 128. On comparing the RPR titer with other treponemal tests performed, it was found that titer >16 gave the good prediction of positive treponemal tests. Other studies have also shown correlation of RPR or VDRL titre with treponemal tests[11]

Availability of recombinant antigens commercially for detection of syphilis antibodies has made it appropriate to review the suitability of various methods in various types of blood bank settings In western countries, blood centres have adopted treponemal test over nontreponemal tests (ie, RPR) for donor screening because of reproducibility, automation and subjective variation in interpretation of test results.

When compared with TPHA as a gold standard, the four treponemal tests showed sensitivity, specificity and PPV performance superior to that obtained using RPR method, which is the technique most used in the routine screening of syphilis in India.

In our study, the sensitivity of ELISA, TPHA and PaGIA were similar and comparable. The positive predictive value and negative predictive values for PaGIA were 89.4% and 100%., respectively. Our results are in concurrence with study published by Schmidt who reported a sensitivity and specificity of 91.9% and 99.8% respectively for PaGIA.[12]. Similarly, our study results were in agreement with another published study, where PaGIA was reported to have high sensitivity and specificity for screening of blood donors.[13]. The advantage of the test is that it is easy to perform and requires no complex infrastructure.

ICT, based on recombinant antigens serves as a rapid point of care test with and advantage of decreased false positives. Another advantage of these tests is that they don't require any complex infrastructure such as any equipment or refrigeration. The test can be performed to a high standard at donor site with blood obtained from finger prick without any specialized laboratory training by staff and without any need for electricity. The greatest value is in developing countries like India. ICT which is a rapid treponemal test has shown high sensitivity and specificity and a good agreement with EIA as well as TPHA. Benzaken A.S. showed in his study that immunochromatic test (SD bioline syphilis) had sensitivity and specificity of 90.2% and 99.4% respectively which were similar to our results[14].

There is currently a trend to use automation to reduce personnel costs .The automated test are usually in ELISA formats. For TTI testing in out institute, we perform the entire test on (DA VINCI Quatro, Biomeriux, France). For detection of larger number of specimens EIAs are ideally suited but has a limitations in terms of time and cost whenever small number of samples are to be processed. But in blood bank settings like ours where EIAs are being performed for HIV, HCV and HBV by automated ELISA machine. ELISA because of it sensitivity, specificity and its suitability made it as an ideal screening method for syphilis.. Another advantage are that it has capacity to automated process large number of samples and an objective reading of results.

ELISA can be used for screening of syphilis with advantage of batch testing and objective automated printed results . With the introduction of automation in treponemal antibody enzyme immunoassay, it can provide few benefits in the terms of rapid results and objectivity of the test. The batch testing in high sample load laboratories with low positivity rates can extract advantages as cost effective method. The capacity to process large number of samples and automated results were evaluated subjectively for TPHA and PaGIA. Together these benefits would provide savings in health care expenses and contribute to the prevention of the spread of the disease. These long-term benefits would help to reduce the health care expenses and in turn contribute as a control on transmission of disease. The benefits would certainly outweigh the additional costs incurred.

In our study, treponemal assay had higher specificity than cardiolipin assay. The number of false positive samples can be reduced by using a method for screening which has higher specificity.

A The 36 false-positive RPR results focus our attention on a problem with the RPR. As our results show, there were 36 false positive RPR samples, a the treponemal test which have better specificity than RPR method in our study would have been more beneficial.

Age groups	Total donors	RPR positive donors (n)	Sero-positivity %
<20	2216 (7.8%)	3(2.3%)	0.14
21-30	13369 (46.8%)	50 (37.8%)	0.37
31-40	8980 (31.5%)	55 (41.7%)	0.61
41-50	3525 (12.3%)	22 (16.7%)	0.62
51-60	454 (1.6%)	02(1.5%)	0.44
Total	**28,544**	**132**	**0.46%**

Table 1:- Age wise sero-positivity in RPR positive donors

Result	No of samples with the following results			
	TPHA	ELISA	PAGIA	ICT
Reactive	85	89	95	86
Non-Reactive	46	43	37	46

Table 2:- Comparison of Rapid plasma regain (RPR) test results with the TPHA, PaGIA, ICE, ELISA test results for 132 samples.

RPR Results	No of samples with the following results			
	TPHA R PaGIA R ICT R ELISA R	TPHA NR PaGIA R ICT R ELISA R	TPHA NR PaGIA R ICT NR ELISA NR	TPHA NR PaGIA NR ICT NR ELISA NR
RPR Positive n=132	84	1	8	36
RPR Negative n=132	0	0	1	131

Table 3:- Comparison of RPR test results with the TPHA, PaGIA, ICT and ELISA results for 132 RPR positive and 132 RPR negative results

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
PaGIA	100	78.7	89.4	100
ICT	98.8	97.8	98.8	97.8
ELISA	100	93.5	96.5	100
TPHA	100	100	100	100

Table 4:- Performance of the serological tests for syphilis in comparison with TPHA.

Test/Agreement	Concordance (%)	Kappa Index
TPHA vs ELISA	97.7	0.94
TPHA vs PaGIA	93.1	0.84
TPHA vs ICT	98.5	0.98

Table 5:- Agreement and kappa coefficient of the syphilis screening tests performed in blood donors:

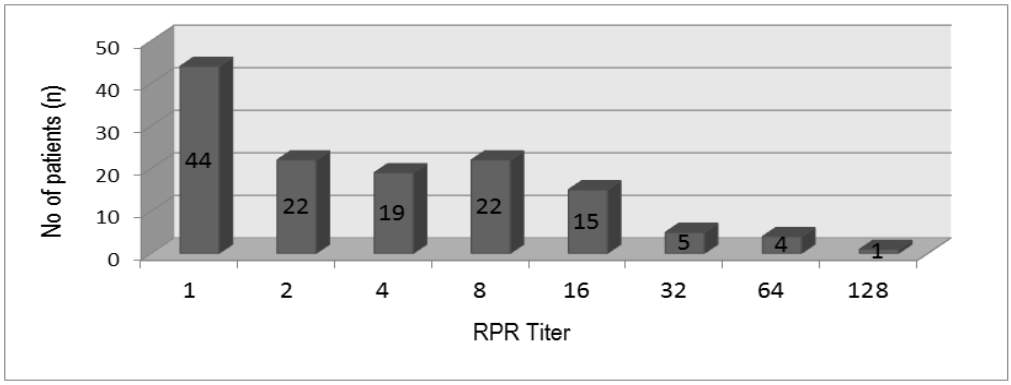

Fig 1:- Quantitative RPR test results

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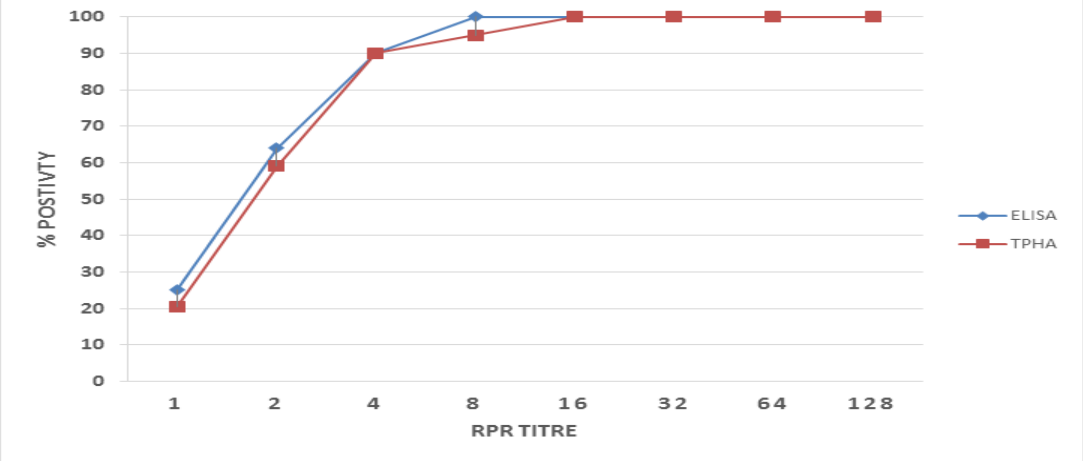

Fig 2:- Comparison of RPR titre with % TPHA / ELISA positivity

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