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Screening of Syphilis Using the Reverse Algorithm and its Trends Among Healthy/Asymptomatic Blood Donors: A Regional Transfusion Centre Study from Northern Pakistan

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ABSTRACT

Objective: To determine the syphilis trends by using the reverse algorithm in healthy/asymptomatic blood donors from Northern Pakistan.

Study Design: Perspective longitudinal study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Transfusion (AFIT), Rawalpindi Pakistan, from Jan to Sep 2022.

***Methodology*:** After taking a complete history from potential donors, an appointed blood bank doctor performed a general physical examination. Potential donors' blood samples were tested for syphilis by the Chemiluminescent Microparticle Immunoassay (CMIA) method followed by reflexively testing of CMIA reactive blood samples with Rapid Plasma Reagin (RPR), and results were interpreted as positive or negative.

***Results*:** A total of 56122 donors donated blood over nine months, including 55742(99.3%) males and 380(0.7%) females. Blood donors ranging from 18-65 years with a mean age of 28.82±7.1 were included in the study. Out of 56122 blood donor samples submitted, 487(0.87%) turned out to be syphilis positive by CMIA method, comprising 485(99.59%) males and 02(0.41%) females. Of 487 CMIA-positive serum samples, 216(44.3%) were RPR-positive, all male donors.

***Conclusion*:** A higher latent syphilis trend was observed in replacement non-voluntary young male donors between 18 and 40 years of age. The majority of the syphilis positives donors were male, while females made up a negligible percentage.

Keywords: Blood donor, Blood screening, Chemiluminescent Microparticle Immunoassay (CMIA), Rapid plasma retin (RPR), Syphilis, Transfusion-transmitted infections (TTIs).

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INTRODUCTION

In Pakistan, the blood transfusion system is demand-driven and fragmented; its reliance on weakly regulated transfusion practices fuels the fire. Despite the presence of legislation, transfusion practices are weakly regulated in Pakistan.[1,2] The safety of millions worldwide depends on safe therapeutic blood transfusion. Owing to good health-seeking and compassionate behaviour, the voluntary donor group is considered the safest group compared to the replacement blood donors. Since the last many decades, data regarding latent syphilis in blood donors has not been very sparse.[3] Transfusion-transmitted infections (TTIs) not only hamper blood safety but also pose a matter of great concern for patients and healthcare authorities. Approximately 3.5 million blood donations are collected each year in Pakistan.[3,4] Indications for blood transfusions include anaemia due to pitiable health conditions, thalassemia patients, emergency/planned surgical procedures and complications related to pregnancy.[4] Transfusion-dependent thalassemia patients utilize one-fourth of the national annual blood collections.[5] Latent syphilis is a stage of syphilis during which syphilis remains in the body even though there are no visible signs or symptoms of syphilis.[6] If infection occurs within the past 12 months, it is called early latent syphilis. As there are variations in diagnostic and therapeutic approaches to syphilis, the use of only one type of serologic test is insufficient for diagnosis, which necessitates multiple diagnostic modalities to be cost-effective and reliable for syphilis diagnosis. Currently, there is limited comprehensive data to be seen in our region about trends of syphilis in healthy blood donors for age groups, gender, and voluntary or replacement transfusions.[7,8]

Guidelines by different international health authorities like WHO, CDC and the Association of Public Health Laboratories (APHL) suggest an

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updated algorithm released which guides that samples may be screened using a treponemal-specific assay followed by positive samples analyzed with a non-treponemal test to assess disease and treatment status in areas having a low disease prevalence.[9] So, the present study was planned as per WHO guidelines by using the CMIA method for syphilis screening followed by CMIA-positive samples processing by the RPR method, and both CMIA and RPR positive, CMIA positive but RPR negative blood bags were rejected and incinerated as per institutional policy. In Pakistan, national strategies like the use of behavioural screening questionnaires to defer the donors who are at high risk of infection, along with testing the blood with highly sensitive and specific laboratory techniques, are executed to minimize the risk of syphilis transmission.[10]

This study aimed to determine not only the syphilis trends in healthy/asymptomatic blood donors by using a reverse algorithm but also its association with age, gender, and type of transfusion among blood donors. This aspect of demographic data will help to assess behavioural patterns and provide a scientific basis to formulate effective blood screening and improve syphilis control strategies.

METHODOLOGY

The prospective longitudinal study was conducted at Department of Microbiology, Armed Forces Institute of Transfusion (AFIT), Rawalpindi Pakistan, from January 2022 to September 2022 after the Institutional Ethical Committee approval (Certificate No. AFIT-ERC-23-01).

Inclusion Criteria: Blood donors of either gender, aged 18 to 60, reported to this transfusion institute and with no past history of transfusion transmissible diseases were included.

Exclusion Criteria: Pregnant females and repeated samples from the same blood donors showing similar syphilis results were excluded.

All healthy/asymptomatic blood donors who qualified the Institute's blood donation criteria as per the standard donor questionnaire, which included age, gender, type of donation (voluntary/replacement) and several past donations, were subjected to an initial general physical examination and pre-donation blood test after taking informed consent. All blood donors were subjected to blood sample collection to detect haemoglobin levels and signs of inflammation using a Sysmex automated blood analyzer, followed by syphilis screening using two screening/diagnostic techniques. In the first step, syphilis screening was done by CMIA technique by using Abbott Architect i2000SR along with Internal positive, negative and cut-off controls run with each batch. In the second step, all CMIA-positive syphilis blood samples were subjected to the Rapid Plasma Reagin (Omega Diagnostics Ltd. UK) test, as per the manufacturer's instructions. Quality control was ensured by running positive and negative controls provided with the kit, with each batch as per manufacturer instructions. Syphilis-reactive blood donor samples and blood components were sorted and subjected to incineration for safe disposal. Blood donors were subjected to five age groups ranging from 18 to 65 years. Physically fit blood donors who donated blood at AFIT were included in the study. In contrast, repeated samples from the same blood donors showing reactive syphilis results were excluded from the study.

The data analysis was conducted using MS Excel 2016 software. The Mean ± standard deviation (SD) was calculated for continuous variables. Categorical variables were analyzed by determining their frequency and percentages. To examine the association of latent syphilis, either Fisher's exact test or chi-square test was employed. A significance level of $p \leq 0.05$ was considered statistically significant.

RESULTS

Fifty-six thousand one hundred twenty-two (n=56122) blood donations were given over nine months, including 55897(99.6%) males and 225(0.4%) females. For nine months, 487(0.87%) blood donors turned out positive for syphilis on screening by the CMIA method. Out of 55742 male and 380 female blood donors, 485(0.87%) male and 02(0.53%) females turned out to be syphilis reactive by the CMIA method. Of 487 CMIA-positive serum samples, 216(44.3%) were RPR-positive, all male donors (Figure).

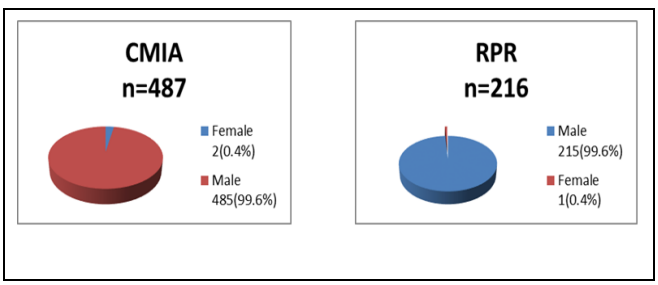

Figure: Gender Wise Syphilis Positive Blood Donor Distribution (n = 487)

The age of donors ranged from 18 to 65, with a mean age of 28.82±7.1. Out of 487 CMIA-positive blood samples, 216(44.4%) were RPR positive/reactive, including one female donor, while 271(55.6%) were non-reactive (Table).

Table: Syphilis positivity in Blood Donors with Reference to Multiple Parameters (n=487)

Parameters	Age of blood donors (in years)				
	18-25	**26-35**	**36-45**	**46-50**	**> 50**
CMIA (487)	89(18.3%)	216(44.3%)	128(26.3%)	35(7.2%)	17(3.5%)
RPR (216)	35(16.2%)	98(45.4%)	60(27.8%)	14(6.5%0	09(4.1%)
Replacement non voluntary donors 444(91%)	72(16.2%)	205(46.2%)	119(26.8%)	35(7.9%)	13(2.9%)
Voluntary non replacement donors 38(7.8%)	14(36.8%)	18(47.4%)	4(10.5%)	2(5.3%)	
Autologous donors 5(1%)		3(60%)	2(40%)		
First time donors 425(87.3%)	78(18.35%)	183(43%)	114(26.8%)	33(7.8%)	17(4%)
2nd time donors 52(10.7%)	10(19.2%)	30(57.7%)	10(19.2%)	1(1.9%)	1(1.9%)
3rd time donors 7(1.4%)		3(42.8%)	4(57.2%)		
4th time donors 3(0.6%)	1(33.3%)	1(33.3%)		1(33.3%)	

DISCUSSION

Blood transfusion is essential to all surgical procedures and most medical emergencies/diseases. Laborious blood screening before issuance for transfusion helps us know about TTIs in healthy populations and safeguards the supply of blood and blood products. Donor selection is the core point for safe blood availability, which depends on the donor's education and the correct and honest disclosure of risk behaviour.[10] Syphilis diagnosis involves clinical evaluation, causative organism detection, and disease confirmation involving serological and nuclear laboratory tests. CMIA assay/TPHA constitute treponemal serological tests, and RPR/VDRL constitute non-treponemal tests. The alone treponemal or non-treponemal test is not sufficient for syphilis diagnosis. RPR guides not only in monitoring syphilis treatment responses but also indicates new or recent infections. Compared to RPR/VDRL, the CMIA test had the advantages of objective interpretation, automation and higher testing quantity.

As per WHO, there occur 6.3 million per annum of new cases of syphilis, 90% of which is constituted by low-income countries.[11] The present study focused on healthy blood donors from twin cities as well as north of Pakistan, intending to monitor the latent syphilis prevalence in this area for evidence-based awareness among society about this silent killer and to make further improvements to control this disease. This study showed that the major contribution to the donor pool belonged to the 26-45 age group, which may be because, at this young age group, the selection criteria of donations are fulfilled more frequently compared to older age groups. Male blood donors, being more than 98% of total donations, constituted the bulk of syphilis-reactive cases. For donor age groups, the majority belonged to younger age groups, which is consistent with a study by Ehsan *et al*.[12] A very low number of female donors was seen, which emphasizes the need to encourage the number of female blood donors. The reason for male predominance might be that most males have larger blood volumes and iron stores, making them more suitable for blood donors. Furthermore, because of anaemia, pregnancy, menstrual cycle and breastfeeding, females are mostly exempted from blood donation. In our study, syphilis prevalence among blood donors is 0.87%, which is consistent with a local study (0.89%) by Waheed *et al*.[13] and also close to another Pakistani study, which showed 0.72% of blood donors reactive for syphilis.[14] However, another Pakistani study by Arshad *et al.* reported 2.1% syphilis reactive cases among blood donors.[15]

On comparing our study results with those from developed countries, our study findings were on the higher side; Syphilis prevalence turned out to be 0.044% in blood donors of Saudi Arabia, 0.16% in the USA, 0.031% in Italy and 0.047% in Israel.[16-19] On the other hand, in contrast to our study results, the latent syphilis trend was high in Nigeria, Angola, and Burkina African continent blood donors, reported as 3.1%, 20% and 1.5%, respectively.[20-22]

Our study results showed that 45% of CMIA reactive blood donor samples also turned out to be RPR positive/reactive, and this reflects the number of donors either suffering from latent or primary syphilis or having previous exposure to syphilis. All syphilis-reactive blood donors were interviewed not only to help and guide them but also to find more details

regarding their lifestyles, which might help update the syphilis prevention and management guidelines.

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LIMITATION OF STUDY

This study covered only Rawalpindi/Islamabad and surrounding rural and urban areas. Blood samples could not be segregated based on early or late latent syphilis.

CONCLUSION

Syphilis, being considered one of the TTIs, causes morbidity in patients of almost all age groups and genders. Syphilis prevalence was higher in replacement non-voluntary and first-time blood donors, so there is a need to increase public awareness regarding voluntary donation and its benefits of early diagnosis of infections in a latent stage that will benefit public health and blood safety improvement.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

TG & NS: Data acquisition, data analysis, critical review, approval of the final version to be published.

AM & RL: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MAR & SF: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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