RESEARCH ARTICLE

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Syphilis Seroprevalence Among Blood Donors in Punjab, Pakistan: A Cross-Sectional Epidemiological Study

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ABSTRACT: *Treponema pallidum*, the causative agent of syphilis, remains an important sexually transmitted and transfusion-transmissible pathogen worldwide. Despite national blood-screening initiatives in Pakistan, the true burden of syphilis among blood donors remains unclear. This study aimed to determine the seroprevalence of *T. pallidum* antibodies among blood donors in District Faisalabad and District Toba Tek Singh, and to evaluate demographic and behavioral risk factors associated with infection. A cross-sectional analytical study was conducted among 400 volunteer blood donors (n=200 from each district). Serum samples were screened for *T. pallidum* antibodies using a rapid immunochromatographic assay (Inst-Answer, USA). Demographic variables and potential risk factors were assessed using a structured questionnaire. Statistical analyses were performed using chi-square tests and odds ratios, with significance set at $P < 0.05$. The overall seroprevalence of syphilis was 3.75% (15/400). Prevalence was higher in Faisalabad (5%) than Toba Tek Singh (2.5%), though not statistically significant. Age was significantly associated with infection ($P = 0.008$), with the highest prevalence in individuals >40 years (10.34%). Males showed a slightly higher prevalence (3.83%) than females (2.85%), but the difference was not significant. Lower educational status was associated with higher seropositivity, although not significantly. Marital status showed significant association, with married individuals exhibiting higher prevalence (5.97%) than single individuals (1.85%) ($P = 0.033$). A history of previous blood transfusion was strongly associated with syphilis (6.66% vs. 2.26%, $P = 0.008$; OR = 4.62). Donors with previous blood-donation history showed a higher prevalence (4.65%) than first-time donors (3.50%), although this was not statistically significant. The findings indicate a moderate burden of *T. pallidum* among blood donors in the study region, with age, marital status, and prior transfusion identified as significant risk factors. Gaps in awareness, sexual health education, and blood-screening practices may contribute to ongoing transmission. Strengthened screening protocols, targeted public health education, and comprehensive risk-reduction strategies are essential to minimize syphilis transmission through sexual contact and blood transfusion.

Keywords: *Treponema pallidum*, syphilis, immunochromatographic assay, potential risk.

INTRODUCTION

Treponema pallidum is a helically coiled microorganism usually 6–15 µm long and 0.1–0.2 µm wide. Treponema are too thin to be Gram stained and only visible under dark field microscope. Syphilis is a condition caused by infection with T. pallidum and commonly known as sexually transmitted disease (Forrestel et al., 2020). It is also a systemic disease with the estimated incidence of 12 million per year according to World Health Organization reports (Alabdulmonem et al., 2020). The infection has three stages: initial genital tract lesion (primary stage) followed by dispersed lesions (secondary stage) and in approximately one-third of untreated individuals, cardiovascular and neurologic problems (tertiary stage). Infection during pregnancy (congenital syphilis) may result in fetal death or birth defects (Forrestel et al., 2020).

In 2022, in a study syphilis were reported on prior to screening of blood donors in US (Drews et al., 2023). Globally, approximately 118.5 million blood donations were collected annually and in Pakistan, 1.5 million units of blood are transfused annually, but a substantial portion of this is not screened properly (Javed et al., 2022). Each transfused unit has 1% chance of transfusion transmitted infections. Due to asymptomatic nature of disease, lack of knowledge, high cost or inappropriate use of screening tests, limited healthcare facilities and absence of monitoring system, the exact burden of disease is still unknown in Pakistan (Saba et al., 2021). The most common mode of transmission is person to person by direct contact with lesions known as chancre. This type of transmission may happen during sexual activity (vaginal, anogenital, urogenital) or childbirth (less common). Several studies showed the higher prevalence of syphilis among people with unsafe sexual behavior such as prostitutes, addictive to drugs and homosexual men (Wang et al., 2022). Some studies show the infection among the female sex workers in Sudan (Elhadi at al 2013).

Syphilis affecting pregnancies results in low birth weight, prematurity and intrauterine death (Salomè et al., 2023). The data of WHO stated that around one million pregnancies were affected by syphilis in 2004 and nearly half (n=460,000) resulted in abortion or perinatal death, 270,000 was born with low birth weight and/or prematurity, and 270,000 was born with congenital syphilis (Mullick et al., 2004). In addition, transference of syphilis can take place through blood and blood products during

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transfusion. This type of transmission has occurred in past and is believed to be rare (Fong, I. W. 2020). Occupational exposures are considered unavoidable and accidental injuries has always been a source of transmission of pathogens such as a case was reported in regards for transmission of syphilis via accidental injury with a scalpel that is another potential mode of transmission via blood (Song et al., 2025).

Around the globe, approximately 4 million blood units are not tested for HIV, and few are tested for hepatitis B and C while none of donation is screened for Treponema pallidum which cause syphilis. Transfusion transmitted infections were considered as unavoidable by patients and physicians in the past (Fong, I. W. 2020) but the blood banks have aggressively pursued strategies to reduce the risks of transfusion transmitted infections. Blood screening systems were initiated to control the blood borne diseases in Pakistan in 2008. These programs were started with combined efforts of national AIDS control program, German agency for international cooperation (GIZ) and financial cooperation funded by KFW, the German development bank.

Diagnostic tests having good sensitivity and specificity is very important to devise proper control measures against different diseases. There is various laboratory methods used to identify syphilis infection at different stages of disease. Mainly two types of tests are available, nontreponemal and treponemal tests. Both types are considered mandatory for the confirmation of active syphilis infection (Park et al., 2020). Nontreponemal tests including Venereal Disease Research Laboratory (VDRL) and rapid plasma regain (RPR) tests can produce false-positive results, hence, are not sufficient for diagnosis and are not specified for syphilis. Therefore, to confirm a syphilis diagnosis, an individual with reactive nontreponemal test should always receive a treponemal test (Satyaputra et al., 2021). Exudates or fluid from lesions is observed under dark field microscope for detection of Treponema pallidum. It is a simple and reliable method however it has limited sensitivity and sometimes fails to detect microorganism due to different factors like quantity of fluid on slide, illegitimate thickness of slide or cover slip, untrained staff and ongoing treatment with antibiotics (Satyaputra et al., 2021). Due to lack of awareness and being Sexually Transmitted Disease (STD), syphilis is considered as a taboo and Pakistani population is unaware regarding the effects and losses of syphilis. The status of syphilis is still unknown across Pakistan. So, to obtain the actual picture of syphilis, the goals of present study was to check the seroprevalence of Treponema pallidum antibodies among blood donor Treponema pallidum antibodies among blood donors. To access the role to demographic factor associated with syphilis as well as to evaluate the role of risk factor associated with Treponema pallidum infection.

MATERIALS AND METHODS

Study Design

It was a cross sectional analytical epidemiological study conducted in district Faisalabad and Toba Tek Singh with a goal to analyze the seroprevalence of syphilis and its associated risk factors in blood donors. A total (n=400) blood samples were collected from volunteer blood donors of both districts (200 from each district). Information regarding the demographic characters and risk factors was recorded on a predesigned questionnaire.

Sample Collection and Processing

A total of 3-5 ml blood was collected in a vacutainer tube. The samples were then shifted to postgraduate laboratory, Department of Microbiology, Government College University Faisalabad for further analysis. These samples were subjected to centrifugation at 1000-2000 rpm for 5 minutes to separate the serum and stored at -20ºC till further processing. Persons falling out of required age criteria were excluded as well as persons having transmissible diseases like HIV, HBV and AIDS etc. were excluded to participate from the current study

A total 400 samples were collected from volunteer blood donors and based on age, the samples were divided into three groups, I (18-25 years), II (25-40 years) and III (Above 40 years). A total of (n=208) samples were collected from group I, (n=163) from group II and (n=29) from group III. Among the (n=400) 365 samples belongs to males while 35 to female blood donors.

Detection of Treponema pallidum Antibodies

The antibodies against *Treponema pallidum* infection were detected with the help of rapid Immunochromatographic kit method (rapid test Inst-answer, USA). Immunochromatographic assay (ICA), namely lateral flow test, is a qualitative immunoassay which is simply proposed to detect the presence or absence of the target component present in the serum or plasma. The kit contains the package inserts, test cassettes and disposable droppers for specimen. A capture antibody against target component is immobilized over test line. A secondary antibody against labeled antibody is immobilized at control zone, so when the serum sample is added to the kit, the antigens if present react with the bound antibodies. A colored line will appear if the antigen- antibody complex or no color will appear otherwise. The intensity of results can be determined by the brightness or strength of color i.e. strongly positive, moderately positive, mildly positive.

Procedure

The sample were removed from freezer and thawed at room temperature for 20 minutes before performing the test. Before using the kit, the expiry date of kit was checked, and it was made sure that the seal was intact and kit was not damaged. The pouch was removed, and the kit was placed on a clean, flat surface which was disinfected earlier. Carefully labeled the kit with permanent marker and 10 µl serum was picked and transferred to the test well with the help of dropper provided with the kit. The result was observed for a positive indicator red line after 15 minutes. The appearance of red line dark, moderate or lighter in color was the indication of positive result. If no line appeared, the result was considered as negative.

Statistical Analysis

Data obtained through questionnaires was arranged in Microsoft Excel sheet and analyzed through statistical software (Minitab, Inc). The role of each risk factor was analyzed by the chi square test analysis and odds ratio. The value ($P<0.05$) was considered as significant

RESULTS

The result of present study concluded that overall prevalence of syphilis in the study area was found 3.75% (15/400). A total of 400 samples were collected from district Faisalabad and Toba Tek Singh, and prevalence of *Treponema pallidum* antibodies were found 5% (10/200) in district Faisalabad, while 2.5% (5/200) in district Toba Tek Singh. The difference in the prevalence of *Treponema pallidum* among both districts was found statistically non- significant ($P> 0.05$).

Sero-Prevalence of Treponema Pallidum Based on Age

In the present study, the collected samples were divided into three groups based on age. The individuals sampled from Group I were 208, II were 163 and III were 29, respectively. The prevalence of syphilis was found highest 10.34% (3/29) in individuals of Group III and 6.16% (10/163) in Group II, while two of the individuals from Group I was detected positive for *Treponema pallidum* antibodies (0.96%) as shown in (Figure 1). The difference was found statistically significant ($P=0.008$).

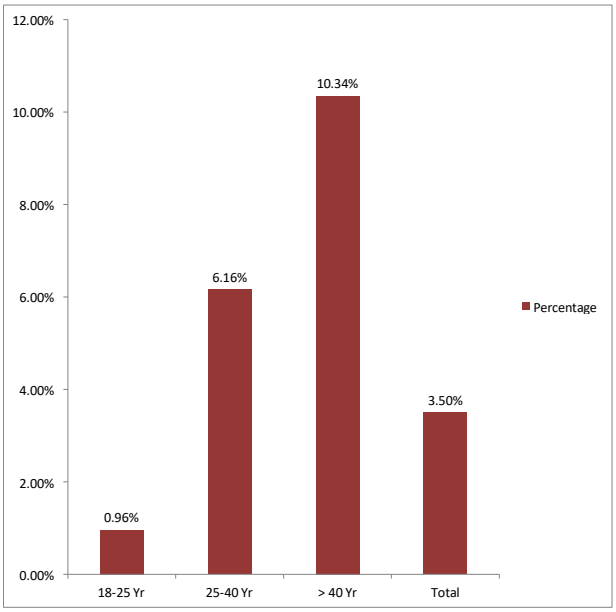

Figure 1. *Sero-prevalence of Treponema pallidum based on age groups.*

Sero-Prevalence of Treponema Pallidum Based on Gender

The prevalence of syphilis based on gender was also detected in the current study, and it was found that among the 365 male donors, 14 were found positive with 3.83% prevalence and only 01 female among 35 donors were found positive. These results were also found statistically non-significant ($P> 0.05$) as shown in (Figure 2).

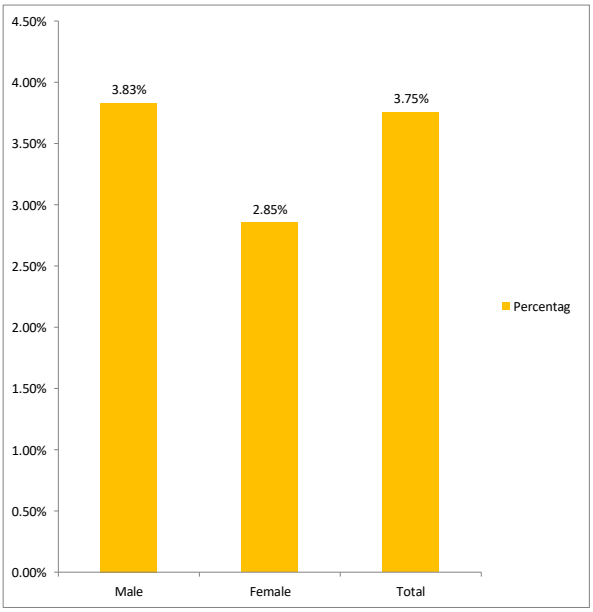

Figure 2. *Sero-prevalence of Treponema pallidum based on gender*

Sero-Prevalence of Treponema Pallidum Based on Education Status

Based on education status the blood donors were divided into three groups including (None/Primary), (Secondary) and (Graduates). The samples collected from these groups were 215, 105 and 80, respectively. It was observed that as the education status increases the percentage of *Treponema pallidum* antibodies decreases. It was found highest among group having up to primary education 4.65% followed by secondary level 2.85% and graduate level 2.50%. This difference was statistically non-significant (P> 0.05) as shown in (Figure 3).

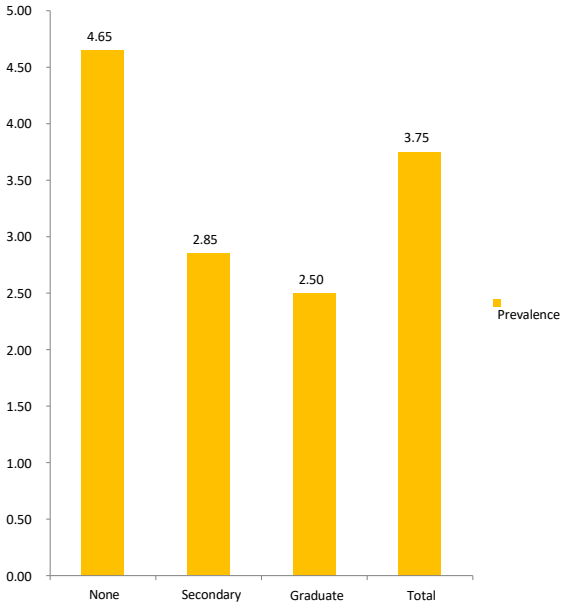

Figure 3. *Sero-prevalence of Treponema pallidum based on education status*

Sero-Prevalence of Treponema Pallidum Based on Marital Status

Among the total 400 blood donors, 216 were single while 184 were married individuals. The sero prevalence of *Treponema pallidum* was detected 1.85% in singles and 5.97% in married individuals. These results were found statistically significant (P= 0.033) as shown in (Figure 4).

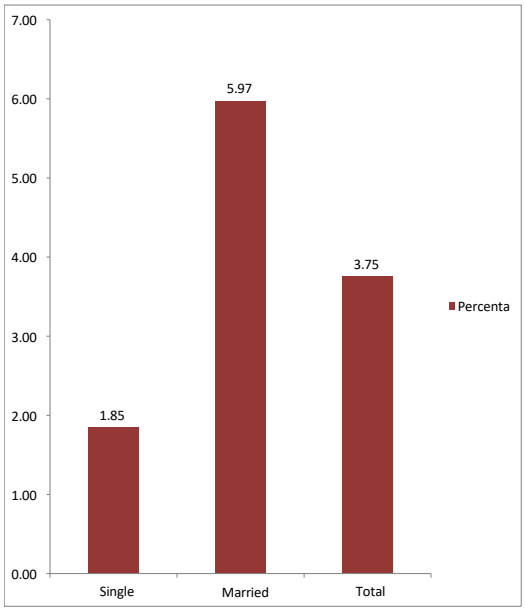

Figure 4. *Sero-prevalence of Treponema pallidum based on marital status*

Sero-Prevalence of Treponema Pallidum Based on Number of Spouses

Number of spouses is one of the important risk factors considered to be associated with syphilis. Among the 184 married individuals, 35 have more than one spouse while the remaining 149 have single spouse. The prevalence of syphilis in individuals having more than one spouse was 8.57% (3/35) and having one spouse was 5.36%. This result was found statistically non-significant (P> 0.05) as shown in (Figure 5).

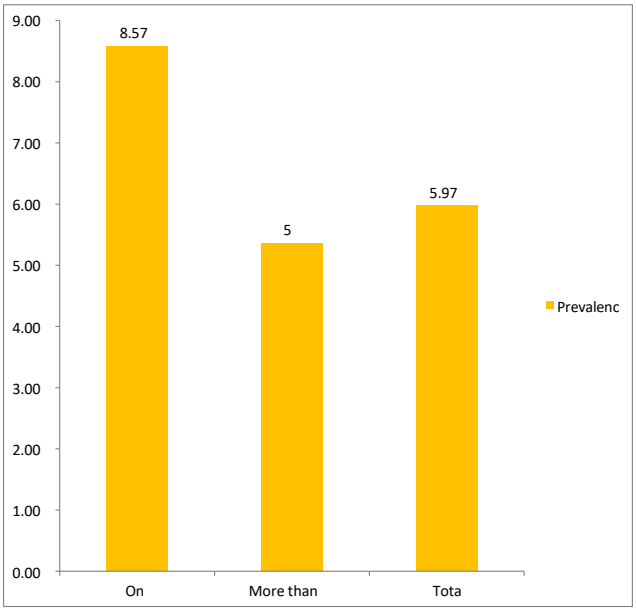

Figure 5. *Sero-prevalence of Treponema pallidum based on number of spouses*

Sero-Prevalence of Treponema Pallidum Based on Number of Blood Transfusions

Out of 400 blood donors, 265 samples were collected from individuals who didn't get any transfusion in their life, while 135 of them had a previous history of transfusion. The results obtained by the research showed that syphilis was detected in 5.92% (9/135) in individuals with previous history of blood transfusion. The prevalence was about half 2.64% (6/265) in those who never got transfused blood and blood products in their life. These finding were statistically significant (P= 0.008) as shown in (Figure 6).

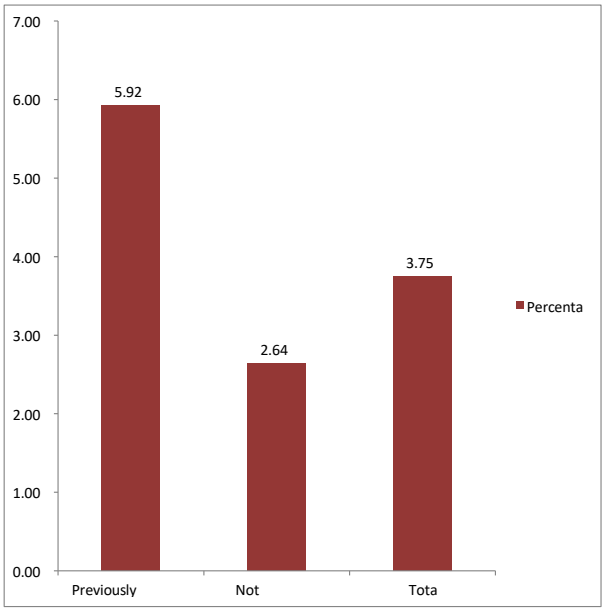

Figure 6. *Sero-prevalence of Treponema pallidum based on blood transfusion*

Sero-Prevalence of Treponema Pallidum Based on Number of Blood Donation

In the present study, among the total blood donors (n=86) had a previous history of blood donation while the remaining (n= 314) didn't donate blood in their life. The prevalence of syphilis was found 4.65% (4/86) in previously blood donated individuals and 3.50% (11/314) in non-donors. Statistically this result was non-significant (P> 0.05) as shown in (Figure 7).

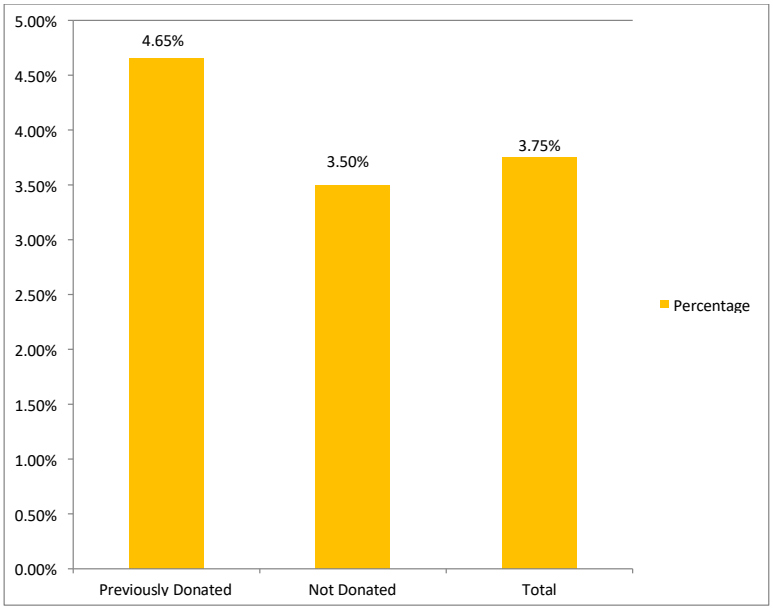

Figure 7. *Sero-prevalence of Treponema pallidum based on blood donation*

DISCUSSION

Treponema pallidum is commonly known as sexually transmitted bacteria, a causative agent of syphilis. It causes small painless sores at genital area or mouth of the host which is known as primary syphilis. The secondary syphilis appears as a rash and lesions at mucus membranes. It is commonly known as contagious at this stage and can be transferred to fetus during childbirth or direct contact with syphilitic sores known as chancre. Both primary and secondary syphilis may resolve without treatment or may enter to a latent phase (Forrestel et al., 2020). Most of patients of secondary syphilis remain asymptomatic and progress to tertiary stage where the bacteria can invade in multiple organs. Another mode of transmission of *Treponema pallidum* is blood transfusion (Jasim et al., 2024).

The present cross sectional epidemiological study was conducted in District Faisalabad and Toba Tek Singh to find out the Sero prevalence of *Treponema pallidum* and its associated risk factors. To achieve this aim, a total of 400 blood samples were collected from blood donors of DHQ hospitals of both districts and subjected to screening by immunochromatographic test. The results of current study showed that among the 400 donors, 15 were found positive making the overall Sero-prevalence 3.75%. These results are much lower than the reported studies of Pakistan with 9.25% by Memon et al. (2017) in Karachi (Memon et al., 2017). In contrast the results of our study are much elevated than the findings of Arshad et al. (2016) with (2.1%) (Arshad et al., 2017), Sultan et al. (2016) with (0.91%) (Sultan et al., 2016) and (0.90%) by (Batool et al., 2017) (Batool et al., 2017). On the other hand, much higher level of syphilis prevalence was detected among the male sex workers in Nigeria (Kokogho et al., 2021). The prevalence of syphilis was found 2.5% in district Toba Tek Singh and 5% in district Faisalabad. The variation in results might be due to deviation in population density, marital status, prescreening of blood before transfusion and transfer of patients at DHQ hospital Faisalabad from other hospitals and cities.

The age group around or more than 40 years of age is more prone to *Treponema pallidum* infections is also endorsed by the findings of Cardona-Arias et al. (2024) in Colombia (Cardona-Arias et al., 2024). The prevalence of syphilis was high in this age group due to sexually active age group and as the age increases the chances of exposure to causative agent also increases.

Gender was also considered a contributing factor to the prevalence of *Treponema pallidum*. Sahi et al (2020) shown the prevalence of *T. pallidum* infection was 1.2%. Risk factors varied by gender (Shi et al., 2020). The results of (Mahmud et al 2023) also stated that males were more prone to this infection. The reason behind was more than one wife or sexual partners is more attached to male members rather than females of study area. Other factors include avoiding the use of condoms and neglected the screening of sexual partners (Mahmud et al., 2023).

Education level also influences the seroprevalence of *Treponema pallidum* among population. The samples were divided into three groups; none/primary, secondary and graduation and the prevalence detected in these groups were 4.65%, 2.85% and 2.50%, respectively. The findings of Lamidi et al, (2020) also indicates that in primary level education group, the prevalence of syphilis is 2%. (Lamidi et al., 2020) The main reason behind this pattern is lack of awareness about STDs. The population having no or primary education had no idea about syphilis and other STDs. This lack of awareness leads towards unprotected sexual activities.

Marital status is another important factor in prevalence of *Treponema pallidum*. In the present study 216 samples were collected from single while 184 from married blood donors. Single blood donors showed a lower prevalence rate 1.85% than married blood donors with 5.97%. These results resemble with recorded findings of Nawaz et al. (2021) in Pakistan. (Nawaz et al., 2021) The main reason behind such results was lack of screening of partner before marriage and sexual behavior of married individuals. Similarly, number of spouses is also considered an important factor in linked with the prevalence of *Treponema pallidum*. Among 184 married donors 149 had single while 35 more than one spouse. The individuals with more than one spouse showed higher prevalence (8.57%) than with single spouse (5.36%). A similar study based on marital status and sexual behaviors of general community was observed in China by (Zhang et al., 2022).

The syphilis is transmitted primarily by sexual contact, and the other mean of transmission is blood transfusion. Out of 400 individuals, 135 who were transfused previously had the syphilis prevalence 6.66%, while the remaining 265 have no history of transfusions and among them the prevalence was 2.26%. Similarly in the sampled population 314 were first time donors and showed lower seropositivity 3.5% of syphilis in comparison to those who already donated blood in their life 4.65%. The high rate of syphilis prevalence in previously transfused persons and regular blood donors was also due to lack of screening facilities in the blood banks, neglected behavior of staff towards sexually transmitted and blood borne disease and limited information of the donor and recipient about the disease.

It is therefore concluded that the frequency of sexually transmitted diseases particularly syphilis is high in the study area and there is a need of national level studies to control and combat this problem. The main reasons behind high burden of disease were lack of information about disease, poor screening measures in the blood banks, unawareness regarding use of condoms and having multiple sexual partners. So, a massive awareness program must be initiated at national level to decrease the prevalence and protect the population from this dangerous disease.

CONCLUSION

Treponema pallidum is a helically coiled microorganism with three subspecies *T. pallidum pallidum, T. pallidum endemicum* and *T. pallidum pertenue*, which causes syphilis, endemic syphilis and yaws, respectively. The infection has three stages: initial genital tract lesion also known as primary stage. Syphilis is commonly known as contagious at secondary stage and can be transmitted to fetus at this stage. About 70% of untreated patients of secondary stage remain asymptomatic and 30% of untreated individuals progress to tertiary stage. It is also a systemic disease with the estimated incidence of 12 million per year according to World Health Organization reports. It was the first transfusion transmitted infection to be screened. According to WHO, globally 81 million blood units are donated annually, and 18 million blood units of total are not screened properly in this regard each transfused unit has 1% chance of transfusion transmitted infections.

This study highlights that syphilis remains a notable public health concern among blood donors in District Faisalabad and District Toba Tek Singh, with an overall seroprevalence of 3.75%. Although the prevalence varied between the two districts, demographic factors such as age and marital status demonstrated significant associations with *Treponema pallidum* infection. Individuals above 40 years of age, married donors, and those with a history of previous blood transfusion were at significantly higher risk. These findings underscore the role of inadequate awareness regarding sexually transmitted infections, insufficient screening measures in blood banks, and limited understanding of safe sexual practices in sustaining disease transmission.

The study emphasizes the need for comprehensive interventions, including strengthened and standardized blood-screening protocols, improved surveillance systems, and widespread awareness campaigns addressing sexually transmitted infections. Enhancing public education particularly among less-educated communities alongside routine screening of blood donors can substantially reduce the risk of transfusion-transmitted syphilis. Overall, proactive and coordinated public health efforts are essential to reduce the burden of syphilis and safeguard both donors and recipients within the healthcare system.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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