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Original Article

Seroprevalence of specific antibodies to *Treponema pallidum* in blood donors with DNA confirmation of seropositivity

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Abstract

The rising global incidence of syphilis underscores the risk of transmission through blood transfusions. *Treponema pallidum*, the pathogen responsible for syphilis, represents a major public health challenge. Accurate detection is essential for controlling the disease, particularly in asymptomatic blood donors. This study aimed to evaluate the seroprevalence of specific antibodies against *T. pallidum* in blood donors, confirmed by DNA testing for seropositivity. The goal was to enhance our understanding of syphilis exposure and improve the safety of blood donations. A total of 1,260 HIV, HCV, and HBsAg-negative blood donors were screened for *T. pallidum*-specific antibodies using enzyme-linked immunosorbent assay (ELISA). Initially, reactive samples were re-evaluated, and those repeatedly reactive were classified as seropositive for syphilis. ELISA-positive samples were further tested for *T. pallidum* DNA using real-time polymerase chain reaction (RT-PCR). Data analysis was done using SPSS with a level of significance $p< 0.05$ Of 1,260 blood donors, the seroprevalence of anti-*T. pallidum* antibodies was 0.158%, with both positive cases confirmed by PCR. The prevalence was 0.2% in males and 0.00% in females, with no significant gender differences ($P > 0.05$). The highest prevalence was in the 31-40 age group (0.5%), but this was not statistically significant ($P > 0.05$). There were no significant differences by donation type or marital status. Significant associations were observed with educational level ($P < 0.05$), with higher prevalence among high school graduates Our results confirm syphilis in Iraqi blood donors, highlighting the need for routine *T. pallidum* ELISA screening at transfusion centers. Positive cases should be discarded and affected donors treated. ELISA is an effective primary screening method, consistent with WHO guidelines for low-prevalence settings, and is essential for preventing transfusion transmission.

Keywords: Seroprevalence, Specific Treponemal pallidum antibodies, DNA, Blood donors

1. Introduction

Blood transfusion is a crucial and life-saving procedure in modern medicine. However, it also carries the risk of transmitting transfusion-transmissible infections (TTIs). These include HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis, among others. Ensuring the safety of blood transfusions is essential to prevent the spread of these infections [1, 2].

Syphilis, caused by the bacterium *Treponema pallidum*, is a chronic sexually transmitted disease (STD) that progresses through multiple stages. It can also be transmitted from mother to baby during pregnancy or childbirth, leading to congenital syphilis, which may cause severe damage to various body systems, including the nervous, skeletal, and mucous membranes, and can result in abortion or stillbirth [3]. Additionally, syphilis can be transmitted through blood transfusion and accidental direct inoculation [4]. Syphilis was the first transfusion-transmitted infection systematically investigated following the introduction of serological screening in blood banks in 1938 [5]. Prior to this screening, over 100 cases of transfusion-related syphilis were reported. Since the implementation of these tests, the incidence of transfusion-transmitted syphilis has significantly decreased, with only three cases reported in the last 40 years [6, 7].

Syphilis remains a major global public health concern, with substantial variations in reported cases over the years. Earlier estimates indicated approximately 5.6 million new cases annually [8]. More recent data from the World Health Organization (WHO) suggests that the number of new cases has risen to 12 million per year, with the highest incidence in South and Southeast Asia [9]. The global prevalence of syphilis is currently estimated at 56.1 million cases [10]. In the United States, the Centers for Disease Control and Prevention (CDC) reported 133,495 new cases in 2020, marking a 6.8% increase in primary and secondary stages compared to the early 2000s [11]. Despite these figures, the prevalence of syphilis antibo-

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dies among blood donors remains relatively low, at 1.0% in India and 1.2% in Dar es Salaam, Tanzania [12, 13]. These statistics emphasize the continued need for rigorous screening protocols to address the risk of transfusion-related syphilis [14, 15].

T. pallidum, the spirochete causing syphilis, cannot be visualized on a Gram stain or cultured using standard methods [16]. Diagnosis primarily relies on detecting specific antibodies in the patient's serum or cerebrospinal fluid (CSF), which become detectable 3-4 weeks after exposure and remain elevated for extended periods post-treatment [17]. Serologic tests for syphilis include non-treponemal tests, such as Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL), which detect immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against lipid antigens like cardiolipin and lecithins, released due to cell damage from *T. pallidum*. These tests are sensitive for detecting active infections and monitoring treatment response, with a fourfold decrease in titers indicating successful treatment [18, 19]. However, they have lower specificity and may yield false negatives (due to prozone phenomena) or false positives [20, 21]. The use of Automated treponemal tests, such as Enzyme Immunoassay (EIA) and Chemiluminescence Immunoassay (CLIA), is crucial for primary syphilis screening, as highlighted by recommendations from the WHO (2003) and ECDC (2010) [22, 23]. These tests detect antibodies against *T. pallidum* and are essential for initial diagnosis. Various diagnostic algorithms—classical, reverse, and ECDC—offer distinct benefits, but there is no universally accepted standard for their application.

Analysis of syphilis outbreaks has demonstrated that *T. pallidum* DNA is detectable in the blood of untreated, infected individuals throughout all stages of the infection. In blood donors, some individuals remain infectious even after antibodies develop during acute infection, with *T. pallidum* DNA persisting for up to three weeks post-antibody development [5]. PCR, with its high specificity, can accurately identify *T. pallidum* and distinguish it from other treponemal infections, especially in early stages when serological tests may not yet be reactive. This high precision reduces false positives and provides a more accurate infection prevalence estimate [24, 25]. Real-time PCR of blood samples from donors with reactive serological tests (VDRL, EIA, FTA-ABS) detected *T. pallidum* DNA in 1.02% of cases. Conversely, no *T. pallidum* DNA was found in VDRL-negative but EIA and FTA-ABS-positive donors. This indicates that donors with all three serological tests positive are more likely to have active syphilis and pose a risk of transmission, underscoring the importance of STS in preventing syphilis transmission through blood transfusions [26].

Recent advancements in transfusion medicine aim to enhance safety protocols for donors, yet current serological screening methods face challenges, particularly in interpreting patterns in asymptomatic individuals. This study aimed to address these limitations by determining the prevalence of antibodies to *T. pallidum* among blood donors, evaluating the effectiveness of the standard ELISA test, and characterizing individuals who test positive.

2. Materials and Methods

2.1. Study Design and Setting

This descriptive cross-sectional study was conducted at the Iraqi National Blood Bank in Erbil City from February 2023 to March 2024.

2.2. Sample collection

A total of 1,260 blood donors, aged 18-56, participated in the study. Blood samples were collected from the cubital vein using sterile 3-5 ml syringes by qualified phlebotomists. The samples were transferred to gel and clot activator tubes, labeled appropriately, and then centrifuged at 3,000 rpm for 10 minutes to separate the serum. The serum was stored at -20°C until further analysis. Routine screening for HIV-1 and HIV-2, hepatitis B (HBsAg and anti-HBc), and hepatitis C (anti-HCV) was performed at the Virology Unit of the National Blood Bank in Erbil as part of standard donor screening procedures.

Donation types were categorized as follows: (i) first-time donation (from individuals who had never donated at our center), (ii) repeat donation (from individuals who had donated at least twice in the past 12 months), and (iii) sporadic donation (from individuals who had donated at least twice, but with intervals longer than 12 months). As part of routine procedures, any donor who tests positive on serologic screening is recalled for a follow-up sample to confirm the initial results. Additionally, these donors undergo an interview to assess risk factors for syphilis and other transfusion-transmitted infection.

2.3. Serological detection of *T. pallidum* antibodies

Serological screening was performed using the DRG Instruments GmbH *T. pallidum* Screen ELISA kit (Cat. No. EIA-4697), following the manufacturer's instructions. Results were interpreted based on optical density (OD) values relative to a cut-off value of 10 Detection Units (DU): samples with OD values >12 DU were classified as positive, <8 DU as negative, and OD values between 8 and 12 DU as inconclusive, necessitating retesting. Initial reactive results were confirmed through repeat testing, with persistent reactivity indicating seropositivity. All ELISA-positive samples were subsequently analyzed using real-time PCR for the detection of *T. pallidum* DNA.

2.4. DNA Extraction and PCR Analysis

DNA was extracted from 500 µl of serum using the MagNA Pure Compact Nucleic Acid Isolation—Large Volume kit (Roche, Germany) with an automated MagNA Pure Compact system (Roche, Germany), according to the manufacturer's protocol. The presence of *T. pallidum* DNA was detected using TaqMan real-time PCR on a StepOne Plus™ Real-Time PCR System (Life Technologies, Foster City, CA, USA). Primers and probes targeting the polA gene of *T. pallidum* were designed using the assay design program (Applied Biosystems, Carlsbad, CA, USA). Detailed protocols and guidelines provided by the manufacturers were strictly adhered to throughout the procedures.

2.5. Ethical Consideration

The study was approved by the Ethical Committee of Hawler Medical University/College of Dentistry. Both written and verbal informed consent were obtained from all participants prior to sample collection.

2.6. Statistical Analysis

Data were analyzed using SPSS version 25 to deter-

mine the prevalence of *T. pallidum* antibodies in blood donors and to confirm the presence of DNA. Sociodemographic variables—gender, age group, donor status (first-time, repeat, or sporadic), marital status, and educational levels, were compared with serological results using the Pearson Chi-square (χ^2) test. Statistical significance was set at $p < 0.05$.

3. Results

The study included 1,260 blood donors, comprising 1,232 males (97.78%) and 28 females (2.22%), aged 18 to 56 years. The donors were categorized into five age groups: ≤20 years (n=7), 21-30 years (n=691), 31-40 years (n=418), 41-50 years (n=115), and 51-60 years (n=29) The values of the parameters are listed in Table 1. The overall seroprevalence of anti-*T. pallidum* antibodies was 0.158% (2/1,260).

Both positive samples were confirmed for Treponema DNA via real-time PCR as indicated in Table 2.

Seroprevalence was 0.2% among male donors and 0.00% among female donors, with no significant gender-based differences ($P > 0.05$). Age-specific prevalence showed a 0.5% rate among donors aged 31-40 years, with other age groups showing no positive cases. This variation was statistically insignificant ($P > 0.05$).

The prevalence among first-time donors was 0.2%, while repeat and sporadic donors tested negative. Although the rate was slightly higher in first-time donors, differences were not statistically significant ($P > 0.05$). The prevalence of syphilis did not vary significantly based on marital status but was higher in married donors (0.31%) compared to singles and divorced individuals ($P > 0.05$).

Significant differences were observed based on educational level ($P < 0.05$), with higher prevalence found

Table 1. Seroprevalence of syphilis in blood donors according to demographic characteristics.

Parameters	Seroprevalence	Seroprevalence		
	Blood Donors N (%)	Negative N (%)	Positive N (%)	P-value
Blood donors	1260 (100%)	1258 (99.8%)	2 (0.158)	0.812
Gender(N=1260)				
Male	1232	1230 (99.8%)	2(0.2%)	0.831
Female	28	28 (100%)	0 (0.0%)	
Age (Years)= (n=1260)				
≤ 20	7	7(100%)	0 (0.0%)	
21-30	691	691(100%)	0 (0.0%)	
31-40	418	416(99.5%)	2(0.5%)	0.401
41-50	115	115(100%)	0 (0.0%)	
51-60	29	29(100%)	0 (0.0%)	
Donation Type (n=1260)				
First time	930	928 (99.8%)	2 (0.2%)	
Repeat	319	319 (100%)	0 (0.0%)	0.701
Sporadic	11	11 (100%)	0 (0.0%)	
Marital Status(n=1260)				
Single	493	493(100%)	0 (0.0%)	
Married	745	743(99.7%)	2 (0.3 1%)	0.500
Divorced	22	22(100%)	0 (0.0%)	
Educational Level(n=1260)				
Uneducated	43	43(100%)	0 (0.0%)	
Elementary School	155	153 (98.7%)	2 (1.3%)	0.003
High School	630	630 (100%)	0 (0.0%)	
College and above	432	432 (100%)	0 (0.0%)	

Table 2. Comparative Analysis of Seropositivity of specific antibodies to *T. pallidum* via ELISA and DNA Detection with PCR.

Seroprevalence via ELISA Seropositive N (%)	Treponema DNA with CPR Positive N (%)	Treponema DNA with CPR Negative N (%)
2 (0.158)	2 (100%)	0 (0.0%)

in high school graduates compared to other educational groups (uneducated, elementary, and college or above).

4. Discussion

The detection of specific antibodies in blood donors is crucial for ensuring blood safety and preventing transfusion-transmitted infections. Accurate identification of syphilis is essential, as undetected infections can pose significant risks to recipients [27]. Current screening methods primarily use serological tests to detect specific antibodies to *T. pallidum*. However, these methods may not always identify the pathogen's DNA, which signals active infection [28]. This study aimed to address this limitation by evaluating the prevalence of *T. pallidum* DNA among blood donors with confirmed seropositivity for syphilis antibodies. Our research seeks to improve the effectiveness of current screening methods and enhance blood safety

This study revealed that the seroprevalence of specific antibodies to *T. pallidum* among blood donors in Erbil was 0.158%, as determined by the ELISA method. This result is similar to those reported by other studies using ELISA, such as 0.18% in Turkey [11], in 2021, the seroprevalence of Syphilis in Basar, Iraq was 0.36% [29], 0.42% in Nepal [30], 0.57% in China [31], 0.69% in Baghdad, Iraq [32], 0.72% in India [33], and 0.84% in Nigeria [34]. In contrast, it is significantly higher than the previously observed rate of 0.01% in Bosnia [35] and the 0.01% and 0.07% found in Brazil in 2017 and 2018, respectively [36]. Additionally, our result is notably lower than the 4.9% in Tchad [37], 3.22% in Burkina Faso [38], and 3.2% in Gabon [39].

Differences in seroprevalence rates among studies can be attributed to variations in sample sizes, study periods, local syphilis prevalence, and the quality of diagnostic kits used can impact results. Geographic location, socio-cultural factors (e.g., sexual behavior, marriage practices), and disparities in healthcare access and laboratory resources also contribute to these discrepancies. These factors highlight the need for context-specific interpretations of seroprevalence data.

Our study found that 0.158% (2/1,260) of blood donors with confirmed syphilis seropositivity had detectable *T. pallidum* DNA. This finding is consistent with Attie et al. (2020) [36], who reported that 2.2% of EIA-IgM-positive donors had *T. pallidum* DNA, and aligns with earlier findings by Dow et al., suggesting that *T. pallidum* may persist in some individuals despite an antibody response. Importantly, the detection of *T. pallidum* DNA does not distinguish between viable and non-viable organisms [5, 40].

Similar results were observed in a previous study conducted in 2014 [26], which detected *T. pallidum* DNA in 1.02% of blood donors positive for syphilis. Although nucleic acid amplification tests (NAATs) can detect *T. pallidum* DNA, their routine use in blood screening is limited due to high costs, the need for specialized personnel, and their inability to determine organism viability. Consequently, NAATs are not recommended for all blood donors [5, 26].

In contrast, a study conducted in 2024 [41] reported a significantly higher rate of *T. pallidum* DNA detection. Out of 32,812 blood samples, 272 (0.83%) were reactive to the chemiluminescent microparticle immunoassay (CMIA), with 46 testing positive via PCR. This study found a much higher DNA detection rate of 16.91%, revealing notable discrepancies compared to other findings. This variation underscores the need for further investigation into the factors affecting *T. pallidum* DNA detection rates.

The study observed a higher seroprevalence of *T. pallidum* among male blood donors (0.2%) compared to female donors (0.00%), though this difference was not statistically significant ($P > 0.05$). This finding is consistent with other studies that have reported higher seroprevalence rates in males compared to females [11, 32, 41, 42]. This difference may be attributed to the fact that a larger proportion of blood donors in this study, as well as in other studies, are male. In our society, blood donation is more prevalent among men than women, which could contribute to the higher seroprevalence observed in males. Additionally, societal and cultural factors influencing blood donation practices may explain this gender disparity

In this study, age-specific prevalence revealed a 0.5% seroprevalence of *T. pallidum* among donors aged 31-40 years, with no positive cases in other age groups. This variation was statistically insignificant ($P > 0.05$). These findings are consistent with studies that also observed higher seroprevalence in older age groups [30, 31, 36, 42]. Increased seroprevalence in this age group may be due to higher cumulative exposure to risk factors for syphilis over time and a potential decline in immune system efficacy with age, which could contribute to an increased susceptibility to infections.

Our study observed a prevalence of *T. pallidum* DNA among blood donors with confirmed syphilis seropositivity of 0.2% in first-time donors. Notably, repeat and sporadic donors tested negative for *T. pallidum* DNA, though this difference was not statistically significant ($P > 0.05$). This finding is consistent with previous studies, suggesting a generally low prevalence of syphilis among blood donors, with some variability based on donation frequency.

For instance, a study by Kane et al. (2015) [43] reported a higher seroprevalence of syphilis among first-time donors in the U.S. blood donor population. This higher prevalence was attributed to the lower likelihood of first-time donors engaging in regular health screenings or disclosing high-risk behaviors compared to repeat donors, who are generally more consistent in their health checks and risk reduction efforts [43]. Similarly, Jones et al. (2020) and Attie et al., (2021) [36, 44] observed a marginally higher prevalence of syphilis among first-time donors, suggesting that initial screenings may be more likely to identify individuals who have not previously been tested and may be

at higher risk for syphilis. The study indicates that, despite any marginal differences, the prevalence of *T. pallidum* antibodies among blood donors does not significantly vary based on donation type. This supports the broader understanding that while first-time donors may show a slightly higher prevalence, the comprehensive screening processes in place effectively mitigate the risk.

Regarding the association between marital status and syphilis prevalence, our results indicated a higher prevalence among married donors (0.31%) compared to single and divorced individuals, though this difference was not statistically significant ($P > 0.05$). Previous research presents mixed insights. This finding is consistent with previous studies [45–47], showing higher syphilis prevalence among married individuals. These studies suggest that marital status might be associated with different sexual behavior patterns or access to healthcare access. Moreover, Nawaz et al. (2021) reported a statistically significant correlation between marital status and syphilis prevalence, suggesting that factors like socioeconomic status or geographic location might play a more critical role [42]. Conversely, Attie et al., (2020) [36] observed higher seroprevalence among unmarried individuals, highlighting that demographic factors influencing syphilis prevalence can vary and that marital status alone may not fully account for these trends. Overall, while our study did not find significant differences based on marital status, the broader literature indicates that marital status can impact syphilis risk, emphasizing the need for targeted screening and prevention strategies that consider these diverse factors.

Our study found a significant association between education level and the prevalence of *T. pallidum* antibodies, with higher prevalence observed among high school graduates ($P < 0.05$). This finding aligns with Kane et al., et al. (2015) and Attie et al. (2020) [36, 43], who reported elevated syphilis prevalence among individuals with high school education. Similarly, Mutagoma et al. (2016), Gomes et al. (2017), and Keleta et al. (2019) [48–50] reported a higher prevalence of syphilis among high school graduates, attributing this trend to gaps in health literacy and access to preventive services. This contrasts with the study by Nawaz et al. (2021), which found a strong association between lower educational levels and higher syphilis prevalence among blood donors in Punjab, Pakistan, particularly highlighting significant rates among individuals with secondary education [42]. These findings collectively emphasize the need for targeted health interventions addressing educational disparities to effectively reduce syphilis rates.

5. Conclusion

In conclusion, this study found that seroprevalence of anti-*T. pallidum* antibodies among blood donors was low, with *T. pallidum* DNA confirmed in a small subset of cases. Gender, age, and marital status did not significantly influence seroprevalence, though a slightly higher rate was noted among first-time donors and married individuals. Educational level emerged as a significant factor, with higher prevalence observed among high school graduates compared to other educational groups. Despite the detection of *T. pallidum* DNA, the low seroprevalence emphasizes the effectiveness of current screening measures and the need for ongoing monitoring to address any emerging trends.

Conflict of Interest

The authors declare that there is no conflict of interest.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Hawler Medical University/College of Dentistry. Both written and verbal informed consent were obtained from all participants prior to sample collection.

Authors' contributions

All authors contributed in this research equally.

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