Original Article

Treponema pallidum hemagglutination assay seroreactivity among healthy Indian donors and its association with other transfusion transmitted diseases

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Abstract:

Background: The aim of the present study was to determine the prevalence of syphilis infection by *Treponema pallidum* hemagglutination assay (TPHA) among blood donors in Delhi and to study their correlation with other markers of transfusion transmitted infections such as hepatitis C virus (HCV), human immunodeficiency virus (HIV) and hepatitis B surface antigen (HBsAg) so as to establish the utility of TPHA over and above venereal diseases research laboratory test (VDRL), not only as a marker for testing *T. pallidum* infection, but also as a marker of high risk behavior. **Materials and Methods:** This prospective study was carried out in the Regional Blood Transfusion Centre, Lady Hardinge Medical College and associated Sucheta Kriplani Hospital, New Delhi for a period of 2 years. Donated blood was screened for TPHA seroreactivity along with screening for anti HIV I and II, anti-HCV, HBsAg by third generation enzyme-linked immunosorbent assay test. A total of 8082 serum samples of blood donors were collected from healthy blood donors in our blood bank. They were classified into two groups- test group and control group based on TPHA positivity. The co-occurrence of HBsAg, HIV and HCV infection were determined in TPHA positive blood donors (test group) in comparison with TPHA negative blood donors (control group). **Results:** We found the TPHA seroreactivity to be 4.4% in Delhi's blood donors. Nearly 8.2% (663/8082) of the donated blood had serological evidence of infection by at least one pathogen (syphilis/HIV/hepatitis B virus/HCV) and 6.63% (44/663) donors with positive serology had multiple infections (two or more). Quadruple infection was seen in one donor, triple infection was seen in three donors and double infection was seen in 40 donors. Prevalence of HIV seroreactivity was found to be statistically significant and HCV seroreactivity statistically insignificant in TPHA positive group in comparison to TPHA negative group. **Discussion:** In our study, the TPHA seropositivity correlated with higher HIV and HCV seropositivity and the same correlation has been observed by several other studies also. In view of these observations, we propose that testing for syphilis by more sensitive and specific treponemal markers like TPHA rather than VDRL, rapid plasma reagin tests; as TPHA also has the added advantage of picking up all the high risk donors, whereas, VDRL picks up only currently infected donors. Moreover, TPHA should be continued as a marker of high risk behavior especially in high prevalence areas like India where we don't have universal access to markers like nucleic acid amplification technique.

Key words:

Syphilis, *Treponema pallidum* hemagglutination assay, transfusion transmitted infections, Co-infection

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Introduction

Blood transfusion has become much safer today than it was about a decade ago. However, we are way behind achieving a "zero risk" of transfusion transmitted infections (TTIs). New pace at which medicine is progressing to control the evils of TTIs has reduced the risk of these TTI's to a major extent with the advent of nucleic acid amplification technique (NAT testing).

In India, drug controller authorities have made screening of five markers of TTIs mandatory. Testing of donated blood for syphilis started as early as 1958[1] in western countries, but in India, test for syphilis was made mandatory in 1999.[2] There is an ongoing controversy in Food and Drug Association (FDA) regarding inclusion of testing for syphilis in donated blood as a mandatory marker and its various testing modalities. Those in favor of testing donated blood for syphilis consider it as a good marker for predicting high risk behavior; while those against it argue that the causative organism doesn't survive beyond 48-72 h in refrigerated blood.[3] Drug controller of India[2] and National Aids Control Organization[4] have left the choice of testing modalities (treponemal vs. non-treponemal) at the blood bank's discretion. Keeping this in mind, we undertook the study to determine the prevalence of syphilis infection by *Treponema pallidum* hemagglutination assay (TPHA) among blood donors in Delhi and to study their correlation with other markers of TTIs.

We hereby discuss the seroprevalence and association of syphilis with other TTIs like hepatitis C virus (HCV), human immunodeficiency virus (HIV) and hepatitis B virus (HBV) so as to establish the utility of TPHA, not only as a marker

for testing *T. pallidum* infection, but also as a marker of high risk behavior.

Materials and Methods

Study design

This prospective study was carried out in the Regional Blood Transfusion Centre (RBTC), Lady Hardinge Medical College and associated Sucheta Kriplani Hospital, New Delhi from June 2008 to May 2010 for a period of 2 years. Blood donors were screened using strict screening criteria as per the standard operating procedures of RBTC. All the donors having a history of jaundice, drug abuse, promiscuous sexual behavior and history of major and minor surgeries were deferred from donating blood. All these donors were 1st time donors. Donated blood was screened for TPHA seroreactivity along with screening for anti HIV I and II, anti-HCV, HBsAg by third generation enzyme-linked immunosorbent assay (ELISA) test.

A total of 8082 serum samples of blood donors were collected from healthy blood donors in our blood bank. Of these, 8076 blood donors were males and only 6were females. They were divided into two groups- test group and control group based on TPHA positivity. We further determined the co-occurrence of HBsAg, HIV and HCV infection in TPHA positive blood donors (test group) in comparison with TPHA negative blood donors (control group).

HBsAg antigen was tested by using third generation ELISA (Eliscan ELISA kit, replication factor C large) with reported sensitivity and specificity of 99.8% and 99.9% respectively (as per manufacturer's manual). HCV was screened using third generation ELISA kits (HCV Bioelisa; Biokits S.A.) with reported sensitivity and specificity of 100% and 99.8% respectively. HIV was screened by third generation ELISA kit (Bioelisa HIV 1 + 2; Biokit S.A) with reported sensitivity and specificity of 100% and 99.98%.

Syphilis was screened by Syphagen TPHA Rec Plus. This is based on the principle of indirect hemagglutination test for the detection of specific antibodies to *T. pallidum* in human serum or plasma. The reagent is a suspension of stabilized chicken erythrocytes coated with P15, P17 and P47 *T. pallidum* antigen obtained from cellular culture of recombinant *Escherichia coli* and subsequent purification. Test was performed and interpreted as per manufacturer's instructions.

Statistical analysis

The statistical analysis was performed by using Statistical Package for the Social Sciences version 13 software (Chicago, Illinois, USA).

Results

All the units of donated blood were tested for anti HIV 1 and 2 antibody, anti HCV antibody, HBsAg, TPHA and malaria. Our samples of blood donors comprised largely of male subjects - 99.92% (8076/8082) and only 0.074% (06/8082) female donors. The donors were divided into two groups, TPHA positive group (test group) and TPHA negative group (control group). The age distribution of donors was from 18 to 62 years (median age 36 years).

Out of 8082, 356 units were tested positive with TPHA, making the overall prevalence to be 4.4%. In TPHA positive group; a total of 355 donors were males and only one donor was female. Nearly 8.2% (663/8082) of the donated blood had serological evidence of infection by at least one pathogen (syphilis/HIV/hepatitis B virus/HCV) and 6.63% (44/663) donors with positive serology had multiple infections (two or more). Quadruple infection was seen in one donor, triple infection was seen in three donors and double infection was seen in 40 donors. Correlation of TPHA results with other TTIs is depicted in Table 1. We looked for association between TPHA and other markers of TTIs. Prevalence of HIV seroreactivity in TPHA positive and TPHA negative group was 2.24% (08/356) and 0.69% (54/7726) respectively. Correlation was found to statistically significant with $P = 0.003$, odd's ratio (OR) = 3.27 (95% confidence interval [CI] 1.43-7.18). However, no such statistically significant correlation was observed between HBsAg and TPHA (1.68% in TPHA positive vs. 2.52% in TPHA negative group).

Prevalence of HCV seroreactivity in TPHA positive and TPHA negative group was 1.68% and 0.75% respectively. However, this correlation was statistically insignificant ($P = 0.101$, OR = 2.22 [95% CI, 0.88-5.51]).

HIV seroprevalence in TPHA positive group (2.24%) was significantly more than the prevalence reported in the general population. On the other hand, HIV seroprevalence in TPHA negative group was only 0.69% (similar to the prevalence reported in the general population).

Discussion

For any seroprevalence study, sample from the general population is ideal. However, prevalence amongst healthy blood donors is often used as representative of the general population. Seroprevalence of syphilis varies world-wide.[5-14] We found the TPHA seroreactivity to be 4.4% in Delhi's blood donors. This is in accordance with Matee *et al*.[7] (4.7%; Tanzania) while Rahlenbeck *et al*.[6] (Ethiopia) and Adjei *et al*.[11] (Ghana) found much higher seroprevalence of syphilis, that is, 12.8% and 7.5% respectively. On the contrary, Mathai *et al*.[9] (Kerala, India) and Kocak *et al*.[12](Turkey) found a very low seroprevalence of 0.2%. Review of world literature on seroprevalence of syphilis is shown in Table 2. There is variability in test results reported by various studies due to differences in testing modalities (treponemal [TPHA] vs. non-treponemal markers e.g., venereal diseases research laboratory [VDRL] and rapid plasma reagin [RPR]), study designs, geographical, cultural and ethnic differences in various states.

In our study, we found significantly higher prevalence of HIV infection in TPHA positive group in comparison with TPHA negative. This is in agreement with other studies by Rahlenbeck *et al*.[6] and Adjei *et al*.[11] However, no such correlations were seen between TPHA and HCV and TPHA and HBsAg in our study.

Correlation of TPHA and HCV has also been extensively studied and reviewed. Barusrux *et al*.[17] showed statistically significant association of anti HCV ($P = 0.0008$) and *T. pallidum* particle agglutination ($P = 0.045$) in 21-30 years old HIV group. However, no such correlation was seen between TPHA and HCV in the studies by Matee *et al*.[7]

The present study was conducted in response to the proposal rule published on August 19, 1999 (FDA report), which raised

Table 1: Correlation of TPHA results with other transfusion transmitted infections

Study Group	TPHA positive (*n* = 356) (%)	TPHA negative (*n* = 7726) (%)	Statistical Values
HIV +ve	8 (2.24)	54 (0.69)	*P*=0.003, OR=3.27 (95% CI=1.43-7.18)
HIV –ve	348 (97.76)	7672 (99.31)	
HBsAG +ve	6 (1.68)	195 (2.52)	
HBsAG –ve	350 (98.32)	7531 (97.48)	
HCV +ve	6 (1.68)	58 (0.75)	*P*=0.101, OR=2.22 (95% CI=0.88-5.51)
HCV –ve	350 (98.32)	7668 (99.25)	

TPHA: *Treponema pallidum* hemagglutination assay; HIV: Human immunodeficiency virus; HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; OR: Odds ratio; CI: Confidence interval

Table 2: Review of world literature on prevalence of syphilis and its association with other markers of TTIs

Studies	Year	Prevalence of syphilis	HIV	HCV	HBsAg	Comments
Sebastian *et al*.[5] Brunei Darussalam	1989	0.64% (VDRL)	—	—	4.71%	—
Mundee *et al*.[15] Northern Thailand	1995	—	3.4%			
Rahlenbeck *et al*.[6] Ethiopia	1997	12.8%	16.7%			HIV positive group had increased risk of syphilis
Matee *et al*.[7] Tanzania	2006	4.7%	3.8	1.5	8.8	Syphilis more fq among HIV+gp
Aydin *et al*.[8] Trabzon Farabi	2002	0.47% (RPR)	—	0.47%	3.94%	—
Mathai *et al*.[9] Kerala (India)	2002	0.2% (RPR)	0.2%	1.4%	1.3%	
Adjei *et al*.[11] Ghana	2003	7.5% (VDRL and TPPA test)				
Nantachit *et al*.[16] Thailand	2003		2.24% in male, 0.64% in female			
Kocak *et al*.[12] Istanbul, Turkey	2004	0.2% (RPR)	0.001%	0.5%	2.07%	
Chinkhumba.[13] Malawi	2006	1.4	9	—	3.2	Co-infection-1.4%
Bhatti *et al*.[14] Pakistan	2007	0.75% (TPHA)	0.004%	4.16%	2.16%	

TTIs: Transfusion transmitted infections; HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; HBsAg: Hepatitis B surface antigen; VDRL: Venereal diseases research laboratory; RPR: Rapid plasma regain; TPPA: *Treponema pallidum* particle agglutination; TPHA: *Treponema pallidum* hemagglutination assay

questions regarding the utility of testing donors for syphilis. FDA has received conflicting views regarding this issue, more views were in favor of eliminating, while few who were in support for testing donors for syphilis regarded this as a marker of high risk behavior as well as for eliminating the chance of transmitting syphilis through blood transfusion.[3]

It has been documented that *T. pallidum* is viable for approximately 96 h in stored blood. Turner and Diseker injected rabbits with human or rabbit's blood contaminated with *T. pallidum* and found no evidence of syphilis with blood stored at 4-5°C for longer than 48 h.[18] Kolmer's studies confirmed the findings in which transmission was prevented by 72-96 h of cold storage.[19] This is the main argument against testing of donated blood for syphilis.

However, a history of syphilis results in 1 year deferral because of concerns that active syphilis might correlate with increased risk for HIV. In our study, nearly 8.2% of the donated blood had serological evidence of infection at least one pathogen and 6.63% donors with positive serology had multiple infection.

Discretion has been left with the testing laboratories both by FDA[3] and drug controller[2] regarding testing modalities for syphilis. Treponemal based antibody tests (e.g., TPHA) remain reactive regardless of whether the individual is currently infected or the individual has been infected in the past and is no longer infected. Sustained reactivity is not a characteristic of non-treponemal based tests (e.g., VDRL test, RPR assays) therefore their results generally reflect an individual's current status and after successful treatment these tests becomes non-reactive.[3] Hence, utility of TPHA test for screening of blood donors is not only for preventing the transmission of syphilis, but it also has an additional advantage of serving as a marker of high risk behavior that can be further proved by concomitant infection of TPHA with other markers such as anti-HIV, HBsAg and anti-HCV as found by Matee *et al*.[7]

In our study, prevalence of syphilis among healthy blood donors by TPHA was 4.40% and positivity of anti-HIV was significantly higher in TPHA positive group in comparison with TPHA negative group (OR = 3.2) but not with anti-HCV and HBsAg (OR = 2.22 and 0.662, respectively). The variability in test results can be explained by different testing modalities used by different laboratories and the fact that seroprevalence of syphilis has been reflected by various studies using either treponemal test (e.g., TPHA) or non-treponemal tests (e.g., VDRL, RPR etc.). Rahlenbeck *et al*.[6] found that HIV positive donors had an increased risk for being positive for syphilis antibodies (OR = 3.69, 95% CI = 2.69-4.96) but not for HBsAg (OR = 0.79, 95% CI = 0.36-1.67) since syphilis and HIV infection are associated with each other at a higher rate than expected by chance.[9] Barusrux *et al*.[17] showed statistically significant association of HIV with syphilis in young adults and with HCV in mature adults.

There is an overall decline in the incidence of syphilis in the United States during recent decades. No case of transfusion transmitted syphilis has been referred since 1968. Therefore, in a proposal rule published in 1999, FDA report raised questions regarding the utility of testing donors for syphilis. Important difference is that non-treponemal tests become non-reactive after successful treatment, whereas treponemal tests continue to show reactivity even after successful treatment. However, in developing countries like India, we don't have universal access to markers like NAT to eliminate window period residual risk of TTIs and

have high prevalence of HIV, HCV, HBsAg. Therefore, we propose that testing for syphilis by more sensitive and specific treponemal markers (TPHA) as opposed to VDRL should be continued as a marker of high risk behavior.

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