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Research Article

SERO- PREVALENCE OF SYPHILIS AMONG VOLUNTARY BLOOD DONORS: AN INSTITUTIONAL STUDY

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ABSTRACT
The appropriate diagnosis of syphilis is of particular importance because of its considerable importance. Moreover, syphilis being an epidemiological synergy and cofactor in transmission of HIV and other sexually transmitted infections. 10,000 donor serum samples were tested for syphilis using enzyme immuno assay and immunochromatographic test. 35 samples were detected by the enzyme immuno assay and the reaming 5 samples by the immunochromatographic test. No discrepancies were noted between these two methods. Respective positive controls and negative controls were used. Of the total of 10,000 samples 35 (0.35%) samples were found to be reactive for syphilis. 33 males (94.29%) and 2 females (5.71%). Transfusion syphilis, although now rate, does still occur, and although routine screening of blood donors for syphilis has been called into question, it is still a legal requirement. Furthermore, there is an increasing tendency to use blood product which have been held at 22^{o} C, at which temperature *Treponema pallidum* can survive for longer periods than at the normal refrigeration temperature of 4^{o}C. There is thus a continuing need for an effective, practicable, and economical serological test.

Keywords: *Syphilis, Enzyme Immuno Assay, Trepolisa, Treponema pallidum, Hemagglutination Assay*

INTRODUCTION
Every year, millions of people are exposed to avoidable, life-threatening risks through the transfusion of unsafe blood. As per a global database, 6 million of 81 million units of blood collected annually in 178 countries are not screened for transfusion-transmissible infections (Willcox *et al.*, 1966). Blood transfusion is a life-saving intervention that has an essential role in patient management within health care systems. The World Health Organization (WHO) estimates that 12 million new cases of syphilis occur each year and the greatest number of cases was estimated to have occurred in South and Southeast Asia.

Syphilis is a sexually transmitted infection (STI) caused by the *Treponema Pallidum* spirochete. The route of transmission of syphilis is almost always by sexual contact, although there may be congenital syphilis via transmission from mother to child in-utero. Syphilis may also be transmitted via blood and blood products, and intravenous drug use. STIs are widespread in the developing countries and constitute a major public health problem and have now acquired a new potential for morbidity and mortality through association with increase risk for HIV infection.

Information regarding the sero-prevalence of syphilis among the blood donors of the rural Karnataka is not available. The study is expected to be useful in making plans and policies for management of safe blood supply in rural Karnataka and would generate the valuable data regarding the same.

MATERIALS AND METHODS
Ours is the licenciated 900 bedded, tertiary cares, teaching hospital based blood bank situated in Kolar, southeastern Karnataka, with the facilities for blood and blood components collection, preparation, storage & distribution. The population of Kolar district is 2,523,406, population density is 307 sq. km and females are 970 per 1000 males In addition to the routine hospital demand, our blood bank caters to the demand of the neighboring districts of Chikkaballapura, Chittor district of Andhra Pradesh and also Hosur and Krishnagiri district of Tamil Nadu.

The study includes 10,000 voluntary donors over a period of 1 year. Each donor was requested to fill the blood donors form and the donors were assured that the confidentiality would be strictly maintained

during testing and recording and recording of the mandatory screening test as per the by Director General Health Service (DHGS) and Drugs' Controller of India. Ethical clearance was obtained for our study and our findings were compared with other similar studies.

Table 1: Total no. of donors and Annual incidence of Syphilis among the voluntary blood donors (n =45)

Sl.No.	Categories	no.
01.	Male Voluntary donors	8,500
02.	Female Voluntary donors	1,500
03.	Total Donors	10,000
04.	Total no of VDRL reactive donors	45
05.	Incidence of reactive donors	0.45%

An initial reactive test result was reconfirmed by repeat testing and repeatedly reactive samples were considered sero-positive.

About 5ml of blood sample was aseptically collected from each bag into sterile anticoagulant free blood samples tubes. The blood was centrifuged for about 10 minutes at 200 rpm to separate the plasma from the packed cells. The plasma was then screened for antibodies specific for *T. Pallidum*.

Blood donors were tested for detection of specific antibodies against Treponemal pallidum by Enzyme Linked Immunosorbent Assay (ELISA) Trepolisa (Qualpro Diagnostic India) in an automated ELISA processor. An initial reactive test result was reconfirmed by repeat testing and repeatedly reactive samples were considered seropositive.

Microwell strips are coated with recombinant 47Kd and 17Kd antigens. The same antigens are conjugated to HRP. Samples along with positive and negative controls are added in the coated wells and incubated simultaneously with antigen HRP conjugate. The wells are washed to remove unbound components. Captured antibodies are detected by adding substrate. The reaction is stopped after specified time with acid and absorbance is determined for each well at 450 nm with an ELISA reader. The cutoff value is calculated by the given formula and absorbances of all the wells are compared with the cutoff value. Any sample having absorbance more than the cutoff value is considered reactive.

In times of emergency, strip method was also used. The Syphilis Ultra Rapid Test Strip (Whole Blood /Serum/Plasma) is a qualitative membrane strip based immunoassay for the detection of *T. Pallidum* antibodies (IgG and IgM) in whole blood, serum or plasma. In this test procedure, recombinant Syphilis antigen is immobilized in the test line region of the strip. After a specimen is added to the specimen pad it reacts with the Syphilis antigen coated particles that have been applied to the specimen pad. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized Syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains *T. Pallidum* antibodies, a red line will appearing in the test line region, indicating a positive result there by indicating the presence of anti- *T. Pallidum* antibodies in the serum samples.

The result were read immediately after 10 minutes and reported as positive, negative or invalid against the appropriate donor's identification number. Manufactures instructions with regard to the test kits were followed Chi Square study were done. The results obtained by the strip method were reconfirmed by EIA. No discrepancies were noted between the same.

RESULTS AND DSCUSSION

Of the 10,000 donor sample, 45 samples were found to be reactive for syphilis. Of these, 40 samples were found to be reactive by Enzyme immuno assay (EIA) and the remaining 5 samples were deducted by immunochromatographic test (ICT). Subsequently the 5 samples were also reconfirmed by EIA and no discrepancies were noted. Of the 45 samples 41 (91.11%) were males and 4 (8.9%) were females donors.

Demographic details of the reactive donors are described in subsequent table nos. 2, 3, 4, 5 and 6.

Table 2: Gender profile of the reactive donors (n =45)

Sl.No.	Categories of Syphilitic donors	No	%
01.	Male Voluntary donors	41	91.11
02.	Female Voluntary donors	04	8.9
03.	Total	45	100

Table 3: Age profile of reactive donors (n =45)

Sl.No.	Age	**No.**	**%**
01.	18-22	16	35.5
02.	23-27	13	28.8
03.	28-32	13	28.8
04.	33-37	03	6.6

Table 4: History of exposure to Risk Factors among the VDRL reactive donors (n =45)

Sl.No.	Name	**No.**	**%**
01.	Promiscuous sexual behaviour	31	68.8
02.	History of previous surgical intervention	14	31.1

Table 5: Occupational profile of reactive donors (n =45)

Sl.No.	Name	**No.**	**%**
01.	Agriculture	23	51.1
02.	Student	13	28.8
03.	Transport	09	20.0

Table 6: Marital profile of reactive donors (n =45)

Sl.No.	Name	**No.**	**%**
01.	Un married	33	73.3
02.	Married	12	26.6

Discussion

The risk of transmission through blood is negligible due to improved donor selection, uniform serologic testing of all blood donors and increased trend of transfusion of refrigerated blood components. Transmission via blood products is nonetheless theoretically possible since organisms may survive for upto 5 days in blood stored at 4^{o} C (Willcox *et al.*, 1966).

The first case of transfusion-transmitted syphilis was reported in the year 1915 (Fordyce *et al.*, 1998) More than 100 cases have subsequently been reported in different countries including USA and Great Britain. However, the numbers of transfusion-transmitted syphilis cases has decreased all over the world. In the past 35 years, only three cases of transfusion-transmitted syphilis have been reported in the English Literature and the last one was reported since more than forty years ago in USA. The absence of transfusion-transmitted syphilis in many developed countries leads to question the rationale for continuing syphilis testing of blood donors (Lafond *et al.*, 2006).

Serological tests for syphilis contributed greatly to the detection of T. pallidum infection in blood donors and especially in those who were not identified during the medical selection (Avelleria *et al.*, 2006). Wasserman detected the first test of syphilis in 1906 (Avelleira *et al.*, 2006). Although it had some false positive results, it was a major advancement in the prevention of syphilis because it helped to diagnose the disease before the clinical manifestation and thus prevent its spread (Garnett *et al.*, 1997). In the 1930s the Hinton test, developed by William Augustus Hinton, and based on flocculation, was shown to be more specific than the Wassermann test (Larsen *et al.*, 1995).

Syphilis was the first infectious agent shown to be transmitted by blood transfusion and, in the past there was a reasonably significant number of transmissions. Occasional cases still occur even today in some countries with a high incidence of syphilis. However, it is very unlikely that transfusion has ever been a major factor in the spread of the disease.

Neither there is a specific type of method absolutely indicated, nor is there any confirmatory algorithm for testing based on the different assays available. In fact, the laboratory assessment of syphilis is generally based on the detection of antibodies against T.pallidum antigens in blood by the use of the either specific or nonspecific reagents. The detection of genomic particle are more specific but not affordable for most of laboratories (Avelleira *et al.,* 2006).

The detection of specific Treponema antigens is possible using methods as passive agglutination, as T. pallidum hemagglutination assay or the T.pallidum particle agglutination assay (TPHA), indirect immunofluorescence as the fluorescent treponemal antibody absorbed assay (FTA- Abs) or enzyme immunoassay (EIA) for the detection of specific IgG and IgM or total Ig. Non-treponemal methods are based on the flocculation technique (Larsen *et al.,* 1995). Of these, the Venereal Disease Research Laboratory (VDRL) and rapid plasma regain (RPR) tests are the most commonly used. These tests are cheap, fast and more sensitive. They are able to identify the contaminated blood donors few days before the treponemal test and thus useful for acute infection. However, VDRL and RPR cannot be automated and are time-consuming if used for large scale testing. Moreover, they produce more false positive results in hepatitis, tuberculosis, malaria, or varicella etc. According to the guidelines published by the U.S. Centers for Disease Control and Prevention (CDC), the diagnosis of syphilis should be based on the results of at least two tests: one treponemal and the other non treponemal (Willcox *et al.,* 1966). According to WHO, blood banks may choose VDRL & RPR or EIA. VDRL and RPR are sensitive for recent syphilis infection, but not for past infection. Recent good laboratory practices suggest that screening should be performed using a highly sensitive and specific test for treponemal antibodies: either TPHA or (EIA) (Garnett *et al.,* 1997).

The global incidence of syphilis among the blood donors is variable. In a study by Adjei *et al.,* (2003) the 7.5% incidence rate of syphilis were noted among the Ghanian donors where as an incidence of 12.7% was noted amongst the Tanzanian donors by Matee *et al.,* (2006). Gupta *et al.,* (2004) observed an incidence of 0.85% among the Indian donors and Bhatti *et al.,* (2007) found an incidence of 0.75% among the Pakistani donors.

Table: 7 Comparative studies regarding the incidence of syphilis among the blood donors

Sl. NO	Name	%
01.	Incidence of syphilis among Kenyan donors	**1.2%**
02.	Incidence of syphilis among Indian donors	**0.85%**
03.	Incidence of syphilis among Pakistani donors	**0.75%**
04.	Incidence of syphilis among Nigerian donors	**0.12%**
05.	Incidence of syphilis in the present study	**0.45%**

The global variation in the incidence of the syphilis among the blood donors may be due to the differences in geographical locations, age range of blood donors, sample sizes, the period of time the studies were carried out, and the different socio-cultural practices such as sexual behavior, marriage practices etc which take place across the world (Garnett *et al.,* 1997).

Access to healthcare and the laboratory test reagents used may also be contributory factors (Bhatti *et al.,* 2007). The implication of syphilis in voluntary blood donors is the risk of transmission of this infection to recipients of blood and blood products. This can contribute to the ever-widening pool of infection in the wider population. Syphilis has also acquired a new potential for morbidity and mortality through association with increase risk for HIV infection thus making safe blood more difficult to get (Garnett GP *et al.,* 1997).

In developed countries, transfusion- transmitted infections are now extremely rare because of improved donor selection processes, and universal serologic screening of donors for blood-borne pathogens coupled with the shift from transfusion of fresh blood components to transfusion of refrigerated products (Garnett *et al.*, 1997). Less developed countries are not able to fully implement the above procedure to ensure the safety of transfused donor blood. Furthermore, resource-poor settings tend to have relatively high demands for blood transfusion on account of high incidence of anemia, malnutrition, and surgical/obstetric emergencies that are associated with blood loss. The consequent high volume of transfusion has the potential for the transmission of unscreened pathogens such as syphilis, and other blood-borne viruses.

The testing strategy employed varies-either a non treponemal test alone, a treponemal test alone, or both in combinations may be used, depending on several factors, including whether the aim is to detect all stages of syphilis or only infectious syphilis. In the United States (US) and certain European countries, including France and Belgium, non-treponemal tests are used for screening. One advantage of this approach is that it is relatively simple procedure but has several disadvantages that can heal false negative results in the presence of high titres of antibody (the prozone phenomenon), in early infections and with concomitant HIV infections.

In large diagnostic laboratories screening with both VDRL and TPHA has been common practice for many years as it provides sensitive and specific screening for all stages of syphilis but it is more labour intensive than a single screening test, requires subjective interpretation, and cannot readily be automated.

With these practical disadvantages, and with the recent commercial availability of EIAs, the VDRL and TPHA combination for screening is being widely replaced by the use of EIA tests that detect trepnomal IgG or IgM. The advantages of the EIA format include the production of objective results, minimize subjective interpretations and facilitate automation.

Published data show both that screening with a treponemal IgG EIA gives comparable results to the VDRL and TPHA combination and that it may be a useful method for detecting treponemal antibody in patients who are infected with HIV. Furthermore, a recent report suggested that a new recombinant antigen-based treponemal IgG and IgM and EIA is the most sensitive treponemal test, and that it is also highly specific and thus suitable for screening.

Conclusion

The provision of safe and efficacious blood and blood components for transfusion or manufacturing use involves a number of processes, from the selection of blood donors and the collection, processing and testing of blood donations to the testing of patient samples, the issue of compatible blood and its administration to the patient. There is a risk of error in each process in this transfusion chain and a failure at any of these stages can have serious implications for the recipients of blood and blood products. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of blood-borne infections (Avelleria *et al.*, 2006).

During syphilis infection two groups of antibodies are formed viz. one reactive with the non-Treponemal antigen (RPR & VDRL), the other reacting with specific antigens of *Treponema pallidum*. The non-Treponemal tests react to cardiolipin and lecithin released from the damaged host cells, as well as lipoprotein-like material released from the spirochete. The non-Treponemal tests are found to be sensitive in early syphilis, but they have several disadvantages (Garnett *et al.*, 1997). Recently several EIA based on specific Treponemal pallidum recombinant antigen are being increasingly used.

The recent introduction of rapid immunochromatographic strip (ICS) to screen for treponemal infection would allow syphilis to be both diagnosed and treatment in a single visit. Unlike RPR reagents, the ICS can be stored at room temperature and does not require special procedures.

Scarcity of laboratory services, staff, and training as well as late diagnosis and treatment have hampered efforts to prevent among the blood donors. Furthermore, doubts have also been raised about the accuracy of the currently used syphilis screening tests, such as the RPR, especially in population with a high prevalence of HIV and malaria (Adjei *et al.*, 2003).

The challenge and perspectives of syphilis during transfusion is related to improvement of clinical selection of blood donor (identifying the precise risk factor) and to development of tools for the treatment of red blood cell concentrates.

Eliminating high risk sexual behaviors is very effective in helping prevent syphilis. Moreover, donors sexually exposed to a person with primary, secondary, or early latent syphilis within 90 days preceding the diagnosis should be assumed to be infected. They should be treated and educated, even if they are sero-negative at the time of donation (Adjei *et al.*, 2003).

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