PREVALENCE AND DETECTION OF LEPTOSPIROSIS AMONG VOLUNTARY BLOOD DONORS

ABSTRACT

Introduction: Blood transfusion is a life-saving measure in emergencies and is important for the medical treatment of every patient. Among all adverse effects of transfusion, transfusion transmitted infections (TTI) are very important. Although the risk of transfusion-transmitted infections today is lower than ever, the supply of safe blood products remains subject to contamination with known and yet to be identified human pathogens. Our aim was to study the sero prevalence of leptospirosis among voluntary blood donors in Chennai.

Materials and Methods: A total of 100 voluntary healthy blood donors selected from different regions of Chennai were included in the study. Five ml of blood was collected directly from the donor in a sterile plain test tube. Serum was stored at 4°C and -200°C in two separate aliquots for MAT and PCR studies respectively. Another 5 ml of blood was collected from donor's blood bag immediately after collection and used within 4 hours for DFM study.

Results: Prevalence of leptospirosis among voluntary blood donors in our study was found to be 21%. Of the 100 samples studied, 31 were positive by DFM, 40 by MAT, 21 by both DFM and MAT; however, none of the samples were found positive by PCR.

Conclusion: Leptospira survived for 48 hrs in stored plasma at 4-6°C and 24 hrs in fresh frozen plasma stored at -18°C. DFM technique can be used as a simple, rapid and inexpensive means of leptospira detection. Leptospiral screening can be made mandatory in endemic regions like Chennai after undertaking a larger study.

KEYWORDS

Infection, MAT, DFM, seroprevalence

INTRODUCTION

Blood transfusion carries the major risk of transmission of infections because of large volume of human source material is infused directly into the body and blood collected from a single infectious donor may be transfused to a large number of recipients. Transmission transmissible agents are present in an infectious or potentially infectious form in the blood, and are stable during storage and transmitted via the parenteral route. Most importantly, they can cause asymptomatic infections in the donor and an apparently healthy prospective donor could potentially be harboring infections.

Leptospirosis has been recognized as an emerging global public health problem because of its increasing incidence in both developing and developed countries. In India leptospirosis has been documented from all over southern India, Maharashtra, Gujarat, Bengal and the Andamans. The worldwide prevalence of leptospirosis among voluntary blood donors is 1.01%. In India, the prevalence is 2.3% and in Chennai, 2.7% prevalence has been reported.

Leptospirosis has been recognized as an important emerging global public health problem because of its epidemic properties and increasing incidence in both developing and developed countries. It is an active bacterial infection caused by spirochetes, with different pathogenic species of the genus Leptospira.

Leptospirosis has wide geographic distribution and occurs in tropical, subtropical and temperate zones. In the developed world, the incidence of the disease has come down substantially and most cases that occur now are associated with recreational exposure to the contaminating water. Contrastingly, the incidence appears to be increasing in developing countries.

Leptospirosis is of interest in transfusion medicine because it is possible to transmit and acquire leptospirosis through blood transfusion. With this background, we did a study to know the prevalence of leptospirosis among voluntary blood donors in Chennai. Our objectives were to detect leptospiro by the three methods- Dark Field Microscopy (DFM), Microscopic Agglutination Test (MAT), Polymerase Chain Reaction (PCR) and to find out the feasible screening method for routine use in blood banks if needed; to detect the optimum survival time of leptospiro in plasma stored at -30°C and to assess whether leptospiro screening should be included as a screening for voluntary blood donors.

MATERIALS & METHODS

This is a prospective study conducted amongst voluntary healthy blood donors in the Department of Transfusion Medicine at The Tamilnadu Dr.G.R. Medical University, Guindy, Chennai. A total of 100 voluntary healthy blood donors selected from different regions of Chennai were included in the study. Five ml of blood was collected directly from the donor in a sterile plain test tube and allowed to clot; serum was separated and stored at 4°C and -20°C in two separate aliquots for MAT and PCR (Medox Biotec, India) studies respectively. Another 5 ml of blood was collected from donor's blood bag immediately after collection and used within 4 hours for DFM study. All these test were done as per our standard operating procedure and manufacturer's instructions. Statistical analysis was done with “Chi-square Test” using statistical software packages (Microsoft Excel, SPSS22). Groups were assumed to differ significantly when the probability (p value) was less than 0.05.

RESULTS

In the present study, the most common age group of voluntary blood donors was between 21 to 30 yrs (45%), followed by 18% (31-40 yrs), 17% (41-50 yrs), 10% (18-20 yrs) and 10% (51-60 yrs). Sex distributions of the donors were 75% males and 25% females.

In our study, the percentage distribution of voluntary blood donors on the basis of their occupations were 43% professionals, 26% clerical job workers, 16% students and 10% Coolies. The remaining 5% were unemployed.

Of the 100 plasma samples 31 were positive by DFM study at the time intervals of 4 hours after collection at room temperature, and 24 hrs & 48 hrs after storage at 4°C. Subsequent examination at 96 hrs the samples were negative by DFM. DFM was negative after 24 hrs of storage.
storage at -18°C.

MAT test was performed on all the samples irrespective of the DFM results. Of the 100 serum samples 40 were positive by MAT. The serovars were Patoc (37%), autumnalis (32%), australis (21%) and Canicola (11%) icterohaemorrhagiae (5%). Of the total, 21% of the donors were positive by both DFM and MAT. The serovars in these samples were autumnalis (38%), patoc (14%), australis (29%), canicola (10%) and Icterohaemorrhagiae (9%) and 10% of the donors were positive only by DFM.

Of the 40 samples positive for MAT, 19% were negative by DFM. The distribution of serovars were autumnalis (32%), patoc (36%), australis (21%), canicola (10%) (Figure 1). In 50% of donors, both DFM and MAT were negative.

**Figure 1. Prevalence of serovars in DFM Negative & MAT Positive Donors**

The distribution of the samples positive for DFM alone were 29% between 21-50 yrs, 6.5% each in 18-20 and 51-60 yrs.

The distribution of the samples positive for MAT alone were 32.5% between 21-30 yrs, 30% between 31-50 yrs, 5% between 51-60 yrs and 2.5% among 15-20 yrs.

Two donors who showed DFM and MAT positive were also positive for HBsAg. All samples which were examined by PCR were found to be negative.

**DISCUSSION**

In the present study, the most common age group of voluntary blood donors were between 21-30 years (45%) with sex distribution of 75% males and 25% females.

As our blood bank adheres strictly to 100% voluntary blood donation, all the donors included in the study were Voluntary blood donors. Hence, the prevalence of Transfusion Transmitted Infections among our donors was low. Ekdashi, et al., from New Delhi, reported that HIV seropositivity and syphilis seroreactivity were more among replacement donors than voluntary donors.

In our study, by DFM 31% of voluntary blood donors' samples showed organisms with characteristic pattern of motility suggestive of leptospires. Similar study by Saranya narayan among 14760 units of apparently healthy individuals tested positive for anti-leptospira antibodies by MAT in Cordillena province of Bolivia.  

In a study by Bovet et al on random sample of 1067 persons in Seychelles showed a sero-prevalence rate of 37% in the year 1999, whereas 54% sero-prevalence rate was observed among healthy population from North Andaman, Andaman and Nicobar archipelago by Murhekar et al.in the year 1998. Consequent to an outbreak of bovine leptospirosis in Chennai, serological evidence of leptospirosis was more evident among human. Also there was a high prevalence of leptospiral antibodies in humans was reported from Somalia in 1982. The results of high seroprevalence rate of leptospirosis in these studies among healthy population is similar to our study.

In our study all the 100 samples from voluntary blood donors were subjected to PCR, none of the samples showed a positive reaction. The possible reasons could be due to the fact that most of the sera used in the study were stored for considerable time at 4°C. Sera on repeated freezing and thawing might have had led to DNA degradation.

Gravekamp et al, showed PCR's failure to detect leptospiral DNA is leptoprosis patients. PCR may fail when there are inhibitory factors present in the sample that impede the amplification process, as well as when leptospires are present in very low numbers below the detection level of 1-10 leptospires / ml. The low sensitivity of PCR may be attributed to the lower number of leptospires in the blood of patients with less severe infections.

The diagnostic accuracy of DFM in leptospirosis evaluated by Vijayachari et al showed a sensitivity of 40.2%, specificity of 61.5%, a positive predictive value of 55.2%. In our study, of the 31 samples positive for DFM, 21 showed MAT positivity.

In 10% of the donors, in our study, DFM alone was positive, while MAT was negative. This might be because, of recent acute infection where the antibody level would not have started to rise. DFM negativity in 19 of the MAT positive samples might be attributed either to the absence of disease in the donor, or seroconversion has occurred with the clearance of leptospira from blood and production of antibodies that remain detectable for months or years, at a low titre. However, DFM is a cost-effective and rapid technique which can help in the early diagnosis and management of patients.

The observation of a significant titre of MAT in a single sample does not necessarily indicates current disease as it may be attributed to persisting antibodies of a past infection. In endemic areas, persisting antibodies may be found in a large proportion of population. In our study 40% of the samples were positive for MAT. A negative MAT does not exclude current leptoprosis if the considered serogroup is not available. MAT has some disadvantages such as technical complexity, high cost and the need to maintain live strains of different serovars with an associated risk of infection to the professionals performing the test. However, MAT remains very useful for epidemiologic studies, identification of strains, assessment of the probable infecting serogroup, and confirmation of illness for public health surveillance. It was an incidental finding in which two of the samples positive for DFM and MAT, also showed HBsAg positivity. The significance is not known.

**CONCLUSION:**

Prevalence of leptospirosis among voluntary blood donors in our study was found to be 21%. Of the 100 samples studied, 31 were positive by DFM, 40 by MAT, 21 by both DFM and MAT; however, none of the samples were found positive by PCR. Leptospirosis survived for 48 hrs in stored plasma at 4-6°C and 24 hrs in fresh frozen plasma stored at -18°C. DFM technique can be used as a simple, rapid and inexpensive means of leptospira detection. Also, the survival of leptospirosis in the recipient blood and its disease causing virulence in the recipient remains a question. Hence, the Pandora box of leptospirosis in transfusion medicine can be opened up in future with more extensive studies regarding the survival time of leptospires in whole blood, packed cells, FFP, platelets individually, and also about the effects of storage on the organism's survival. Leptospiral screening can be made mandatory in endemic regions like Chennai after undertaking a larger study.
REFERENCES:


