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Original Research Article

Effect of grey zone sample testing of transfusion transmissible infectious diseases on safety of blood-experience of a tertiary care referral teaching hospital blood bank from South India

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ABSTRACT

Background: Grey zone samples with optical density (OD) lying between cut-off OD and 10% below the cut-off OD (cut-off OD × 0.9) were identified during routine transfusion transmissible infectious disease (TTIs) screening. Enzyme-linked immunosorbent assay (ELISA) used for this purpose can sometimes fail to detect blood donors who are recently infected or possessing the low viremia. Estimation of a grey zone in ELISA testing and repeat testing of grey zone samples can further help in reducing the risks of TTI in countries where nucleic acid amplification testing for TTIs is not feasible.
Methods: On performing repeat ELISA testing on grey zone samples in duplicate, the samples showing both OD values below grey zone were marked nonreactive, and samples showing one or both OD value in the grey zone were marked indeterminate. The samples on repeat testing showing one or both OD above cut-off value were labelled reactive.
Results: Of the 21,908 blood donors screened during the study period, a total of 144 blood donors were found to be in grey zone. On repeat testing of these grey zone samples, 35 (24.30%) were found to be reactive for TTIs.
Conclusions: Estimation of grey zone samples with repeat testing can further enhance the safety of blood transfusion in resource poor developing nations where more sophisticated and sensitive methods such as nucleic acid amplification test (NAT) is not available in all the blood banks.

Keywords: Enzyme linked immunosorbent assay, Grey zone, Transfusion transmissible viral infections

INTRODUCTION

Blood transfusion is a life-saving measure in emergencies and is important for the medical / surgical management of most of the patients. Among all adverse effects of transfusion, transfusion transmitted infections (TTI) are very important. These include human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Malaria parasite (MP) and Syphilis. Despite advanced screening test technologies, recipients still have an increased risk of becoming infected with these TTIs.[1] The situation becomes graver in developing countries, as improvised testing technologies for screening of TTIs leads to increased cost of blood components for the patients. Hence, transmission of HIV and other viral infections continues to be a serious threat to safe blood transfusion in these areas. The fact that the developing countries account for more than 90% of all new HIV cases worldwide makes the task of screening blood donors more challenging.[2] The risk of TTIs is estimated

to be 1 in 6,77,000 units for HIV, 1 in 1,03,000 for HCV, and 1 in 63,000 for HBV and the risk of transmission of these infectious agents through infected blood products exceeds that of any other exposures.[3] In multiply transfused hemophiliac patients, the prevalence of HCV was found to be as high as 23.9%.[4] In India, mandatory screening for HCV was implemented quiet late in 2002.[5] Several tools have been implemented for preventing TTIs ranging from donor selection to donor testing.

Screening of blood donors for infectious markers is done by immunoassay in the form of antigen/antibody detection methods such as latex agglutination, Immunochromatography, enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA) or by genetic tests like nucleic acid amplification technology (NAT) assay.

As per Drugs and Cosmetics Act,1940 and Rules, 1945, it is mandatory to test all blood donations for HIV, HBV, HCV, syphilis, and malaria in blood banks.[6] In India, screening of all blood donors is done complying with the strategy I as laid down by world health organization (WHO). Strategy I of World Health Organization mentions subjecting all blood donors sample to one-time ELISA for screening purposes and marking samples with optical density (OD) above or equal to cut-off OD as reactive and samples below cut-off OD as nonreactive.[7] There is scarcity of literature regarding detection of grey zone sample and its application in TTI screening procedures in blood bank set up.[7]

Therefore, it becomes very prudent to assess the utility of grey zone calculation and its role in improvising the current screening methodologies. Hence, this study was undertaken to analyze the importance of grey zone testing of TTI of apparently healthy blood donors and its role in enhancing the sensitivity of current ELISA technology at our blood bank.

METHODS

This prospective, cross-sectional, analytical study was conducted during the period from March 2015 to April 2017 in the department of Transfusion Medicine attached to a tertiary care referral teaching hospital, Andhra Pradesh, India.

Written informed consent was obtained from all the donors. Blood donors, fulfilling the criteria for donor selection as per the selection criteria laid down by Drugs and Cosmetics Act,1940 and Rules,1945 were considered for the present study. A total of 21,908 blood donors were screened during the study period. The donors were either voluntary or replacement donors. Voluntary donors are those who voluntarily donated their blood either at the blood bank or at voluntary blood donation camps, while the replacement donors were either relatives or friends of patients. Five milliliter of whole blood samples were collected from the subjects into plain sterile tubes which were centrifuged and the sera were separated and analyzed for different TTIs; HIV, HBV and HCV as per the standard operating procedures followed in the blood bank.

Samples were analyzed for antibodies to HIV1, 2 and p24 antigen (Microlisa HIV Ag and Ab, J. Mitra and CO. PVT. Ltd, New Delhi, India), HBsAg (Hepalisa, J. Mitra and Co. Pvt. Ltd, New Delhi, India), and HCV (Microlisa HIV Ag and Ab, J. Mitra and Co. Pvt. Ltd, New Delhi, India), by ELISA as per the manufacturer's instructions. The validity of the test was assured as per the given criterion and the results were computed.

All the samples with optical density (OD) more than the cut-off were considered reactive and blood units were discarded and donors were notified as per departmental standard operating procedure. Grey zone was calculated as 10% below the cut-off OD. All the samples with OD between cut-off value and 0.9 × cut-off value was marked as grey zone and were quarantined. All the grey zone samples were retested in duplicate for their respective viral marker using the same or different manufacturer's ELISA kits the next day. On repeat testing, the grey zone samples showing both OD values below 0.9 × cut-off value were marked as nonreactive and the blood units were included in the inventory. If on repeat testing the grey zone sample showed one or both OD value above the cut-off value it was marked as reactive and blood units were discarded and donor notified. The grey zone sample showing one or both OD value again as grey zone on repeat testing was marked as indeterminate and blood unit was discarded, but the donor was documented as nonreactive and notified for repeat testing after 6 months.

RESULTS

Of the 21,908 healthy donors screened for mandatory infectious markers during the study period, HIV reactivity was found in 42 (0.19%) donors with HBV and HCV in 317 (1.44%) and 123 (0.56%) donors, respectively. Cumulative overall reactivity for all infectious markers was found to be 2.20%. Excluding all reactive samples, about 144 (0.65%) more samples were found to lie in a grey zone with 20 belonging to HBV, 54 belonging to HIV, and 70 belonging to HCV. On repeat testing of these grey zone samples, 35 were found to be reactive with 03 for HBV, 16 for HIV, and 16 for HCV. Eighteen grey zone samples showed indeterminate results on repeat testing with 02 for HBV, 05 for HIV, and 11 for HCV (Table 1).

The inclusion of criteria of grey zone calculation in routine ELISA screening test at our blood bank increased the reactivity of infectious markers from 2.0% to 2.20% (Table 2).

Table 1: Repeat testing of grey zone TTI samples.

TTI marker	Grey zone sample n (%)	Repeat reactive n (n)	Repeat nonreactive (n)	Repeat grey zone (indeterminate) n (%)
HBV	20 (13.9)	03 (8.6)	15 (16.5)	02 (11.1)
HCV	70 (48.6)	16 (45.7)	43 (47.2)	11 (61.1)
HIV	54 (37.5)	16 (45.7)	33 (36.3)	05 (27.8)
Total	144	35	91	18

TTI= transfusion transmissible infections; HBV= hepatitis b virus; HCV=hepatitis c virus; HIV= human immuno-deficiency virus.

Table 2: Seroreactivity after grey zone sample assessment.

TTI marker	First time seroreactivity of 21,908 donors (%)	Repeat reactive of 144 grey zone donors (%)	Total yield on grey zone testing of 21,908 donors (%)
HBV	314 (1.43%)	03 (2%)	317 (1.44%)
HIV	26 (0.11%)	16 (11.2%)	42 (0.19%)
HCV	107 (0.49%)	16 (11.2%)	123 (0.56%)
Total	447 (2%)	35(24.4%)	482 (2.2%)

TTI= transfusion transmissible infections; HBV= hepatitis b virus; HCV=hepatitis c virus; HIV= human immuno-deficiency virus.

DISCUSSION

Blood is life. Transfusion of blood and blood components, as a specialized modality of patient management saves millions of lives worldwide each year and reduce morbidity. It is well known that blood transfusion is associated with many complications, some are only trivial and others are potentially life threatening, demanding for meticulous pretransfusion testing and screening particularly for transfusion transmissible infections (TTI). These TTI are a threat to blood safety. In developing nations like India, blood safety continues to be a major problem due to the high prevalence of infectious markers among blood donors compounded with the problem of limited resources that preclude the use of sophisticated, sensitive but expensive technologies for screening of blood products.

The prevalence of TTI varies from country to country depending on the population from where blood units are collected. HBV, HCV, and HIV are the most important agents responsible for TTIs and thus their testing on blood donors is mandatory worldwide due to potential serious clinical complications associated with these agents.[8,9]

With advances in screening techniques in the form of NAT, the risk of TTI's has decreased considerably.[10] Still TTI's remains a threat to blood safety due to several factors such as genetic variations of infectious agents, presence of immunologically silent carriage, laboratory errors, and variations in the window period of the infectious agent, as well as limitations in screening testing methodology.[8]

In developing countries where NAT test is not routinely practiced for screening due to non-affordability, immunological assays like ELISA serves as a main screening tool in blood bank setup. Several methods have been devised to improve the sensitivity of ELISA such as the inclusion of borderline reactive control samples in each run to minimize batch to batch, as well as day to day variation in testing. These borderline reactive samples are also able to detect minor variation in the assay procedure.[7] Another method to enhance the sensitivity of ELISA as screening assay is an estimation of the sample lying in a grey zone and its repeat testing. It has been very well illustrated by Pereira et al, that ELISA-based screening test for TTI in blood banks does involve a certain amount of uncertainty especially around the cut-off zone used for calculating the reactive samples.[11]

Hence, they have emphasized on the measurement of this uncertainty around the cut-off zone in the form of grey zone sample testing. Presently, there are no such existing guidelines for grey zone sample testing in any regulatory authority in India and most of the blood banks in India follow the strategy I of one-time ELISA testing as screening procedure as per WHO guidelines.[7]

Grey zone sample testing might not have gained much relevance due to the issues of false positivity on repeat testing wherein a study conducted in Turkey have reported 70% false positivity on testing grey zone samples.[12] It has also been estimated that on applying the confirmatory test to grey zone samples resulted up to only 2% of true positivity.[13]

The study found a total of 144 (0.58%) samples in grey zone area for all three viral markers as compared to 0.14-0.29% found by other authors.[13,14] This may be due the differences in the type of ELISA kits used for performing the test. We detected 24.30% of 144 grey zone samples showing repeat reactivity, for either of the viral markers,

on repeat testing as that of 4.6-75% in relevant studies.[13-15]

One of limitations of this study is we could not be able to subject the grey zone samples for confirmatory assay. Hence, we are not able to comment on the overall effectiveness of repeat grey zone sample testing in improving the transfusion safety. However, repeat reactivity in grey zone sample testing is an alarming indication for mandatory implementation of more sensitive testing technologies like NAT in developing countries. Since at our blood bank we separate majority (more than 85%) of collected blood units into at least three components, we effectively discarded 92 infectious components considering 35 grey zone samples showing reactivity on repeat testing. On the inclusion of grey zone sample testing, we observed the prevalence of HBV, HIV, and HCV to be 1.29%, 0.23%, and 0.56%, respectively.

Recently Acar et al, have also reported similar findings of 1.76%, 0.17%, and 0.50% for HBV, HIV, and HCV in Turkey.[13] The risk of TTI remains despite of serological test because of donors' window period, viral variants, immunosilent or delayed seroconverting carriers and laboratory errors. Necessary precautions should be undertaken to prevent transmission through transfusion such as a careful selection of potential blood donors through a health history questionnaire and create opportunities for self-deferral. Programmes to prevent TTI infected donors should be aimed primarily at reducing elevated risk behaviors. It is hoped that the continued education of public about the methods of transfusion and increased availability of TTI testing will further reduce the seroreactivity and spread of the diseases. If a case of TTI is suspected, clinicians should report the case to the blood bank personnel.

The public health surveillance system may perhaps collaborate with blood banks and health departments to investigate and look back into various other procedures although the risk of TTIs today is lower than ever, but to achieve an era of zero risk transfusion remains a challenge as the supply of safe blood remains subjected to contamination known and yet to be identified pathogens. Continuous improvement in the form of diligent donor screening and implementation of sensitive screening assay and effective pathogen inactivation procedures can ensure the elimination or at least reduction of the risk of acquiring TTIs.[9]

Implementation of cost-effective measures to improve the sensitivity of screening assays can be practiced especially in areas where TTI are highly prevalent. Higher discard rate of reactive blood units and minor increase in cost due to new testing methodologies can be justified by the impact it would have in reducing the mortality and morbidity of patients due to TTIs in an already resource burden nation.

CONCLUSION

NAT technology for TTI screening being far from reality in developing nations, hence several alternative methods, in the form of assessment of grey zone samples to improve the sensitivity of the current screening procedure holds special importance.

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