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Utility of grey zone sample testing strategy for transfusion transmittable infections to improve blood safety of a tertiary care center

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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 were identified during routine transfusion transmissible infectious disease screening by Enzyme-linked immunosorbent assay and its application in blood transfusion services.
Materials and Methods: The present is an observational study where blood donors samples have been screened by performing repeat ELISA testing on Grey zone samples in duplicate at the A.D Gorwala Blood Centre, Karamsad between January 2018 to December 2020.
Results: A total of 20560 blood donors were screened during the study period, out of which 12 blood donors were found to be in grey zone. On repeat testing of these, 02 were found to be reactive and 01 was found to be in grey zone again.
Conclusion: Appropriate quality control measures can improvise the current screening methodologies in TTIs of blood bank centres, especially in resource constrained settings, where the NAT technology is not financially feasible.

Keywords: grey zone, transfusion transmissible infectious, immunosorbent, ELISA, blood donors

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. In India these include human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Malaria parasite (MP) and Syphilis. 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 centers [1]. Integrated strategy for the provision of safe blood and blood products includes recruitment and retention of blood donors who are at low risk of transmitting infection, stringent donor screening consisting of meticulous donor history and examination, quality-assured screening of all donated blood units for transfusion transmissible infections (TTI), rational use of blood to reduce unnecessary transfusions, and the use of alternatives to transfusion, wherever possible [2].

The risk of transfusion transmitted infections 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 [3]. Blood transfusion is an effective mode of transmission of TTIs as it allows entry of large quantity of infective virions into the recipients. 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.

Screening of blood donors for infectious markers such as human immunodeficiency virus (anti-HIV), hepatitis B virus (HBsAg), and hepatitis C virus (anti-HCV) is commonly done by immunoassay in the form of antigen/antibody detection methods, such as enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immuno-assay (CLIA) [5]. Nucleic acid testing (NAT) is a newer molecular technique for screening blood donations to reduce the risk of transfusion transmitted infections (TTIs) in the recipients, based on amplification of targeted regions of viral ribonucleic acid or deoxyribonucleic acid (DNA), thus, narrowing the window period and providing an additional layer of blood safety. However, it is highly technically demanding, involving issues of high costs, dedicated infrastructure facility,

equipment, consumables and technical expertise. 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.

In India, screening of all blood donors is done complying with the strategy I as laid down by world health organization (WHO) [6]. Strategy I of World Health Organization mentions subjecting all blood donors' sample to one-time ELISA for screening purposes and marking samples with samples with optical density (OD) above or equal to cut-off OD are defined as reactive and samples below cut-off OD as nonreactive. Some centres additionally use Grey Zone (GZ) phenomenon (defined as samples with OD within 10% below the cut off) in a bid to further enhance blood safety. However, there is paucity of data regarding usefulness of GZ samples and its application in TTI screening procedures in blood transfusion services. 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 centre.

Aim and Objectives

The present study aims to improve the blood safety for patients, with respect to transfusion transmitted infections. The objectives of the study were to:

- To analysed GZ sample results, and confirmatory test results to verify if it adds to blood safety in our setting and its role in improvising the current screening methodologies.
- To validate the algorithm prepared for the re-testing of grey zone samples and their appropriate use/ disposal.

Materials and Methodology

Study setting

This is an observational cross-sectional study done at A.D. Gorwala Blood Centre of Shree Krishna Hospital, Karamsad, a rural tertiary care centre located in Central Gujarat. Samples of blood donors were utilized as per departmental standard operating procedures (SOP) over the period of January 2018 to December, 2020, for the present study.

Methodology

An informed consent was obtained from the donors stating that their blood samples would be tested for transfusion transmitted infections such as HIV, HBV, HCV, Syphilis, and Malaria. Two milliliter of whole blood samples were collected from the donated units into plain sterile tubes which were centrifuged and the sera separated and analyzed for HIV, HBV and HCV as per the standard operating procedures followed in the blood center.

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 blood center 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.

The algorithm used for processing the grey zone sample is illustrated in Figure 1:

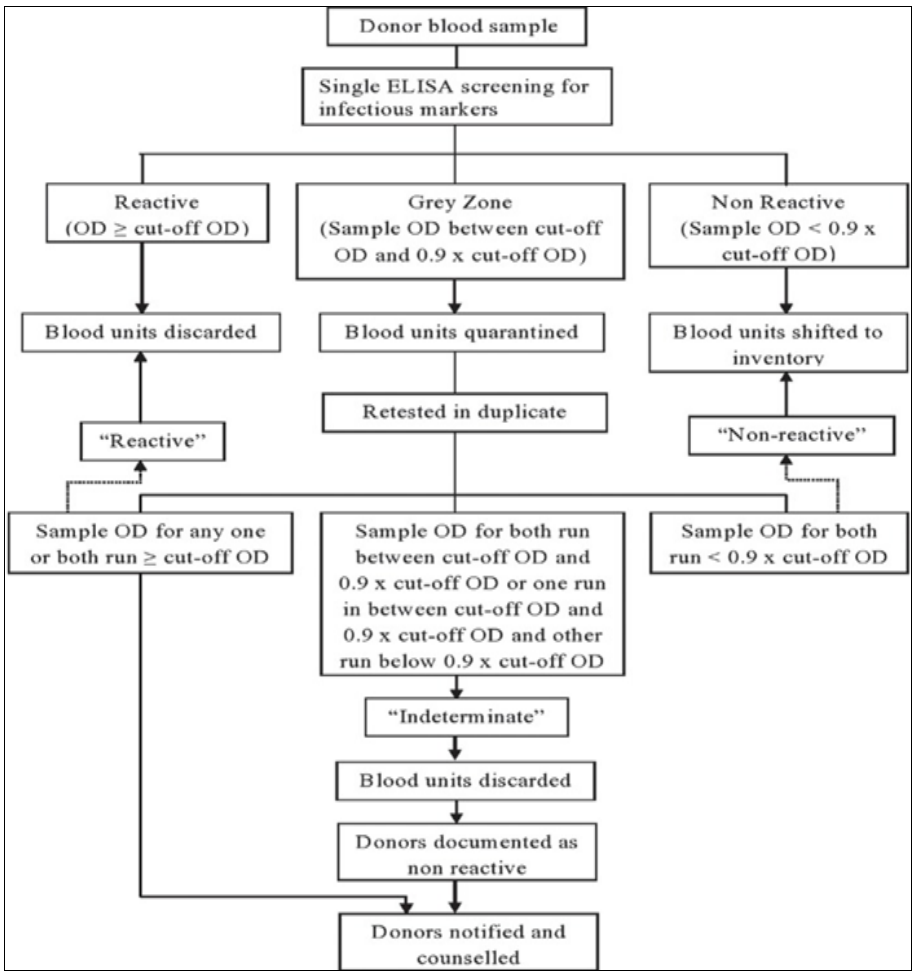

Fig 1: Algorithm for evaluating grey zone samples

Data Collection: The data for grey zone sample testing and re-testing results shall be available from the daily TTI run sheets and the online (Laboratory Information System) TTI Register.

Data Analysis: Frequency distribution and descriptive analysis is primarily done for comparison using Microsoft Excel 2019.

Results and Observations

20, 560 healthy donors were screened for mandatory infectious markers during the study period. HIV reactivity was found in 104 (0.50%) donors with HbsAg and HCV in 118 (0.57%) and 27 (0.13%) donors, respectively. Cumulative overall reactivity for all infectious markers was found to be 0.4%. Excluding all reactive samples, about 12 (0.05%) more samples were found to lie in a grey zone the interpretation, and results obtained on repeat testing of GZ samples are shown in Table 1.

Table 1: Frequency distribution of the various grey zone samples

TTI Parameter	Grey zone sample	Repeat nonreactive	Repeat sero-reactive	Repeat Grey Zone (Indeterminate)
HIV	04	03	01	00
HbsAg	07	05	01	01
HCV	01	01	00	00
Total	12	09	02	01

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

Discussion

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.

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 [6, 7]. With advances in screening techniques in the form of NAT, the risk of TTI's has decreased considerably.

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 center setup. ELISA-based screening test for TTI in blood centers does involve a certain amount of uncertainty especially around the cut-off zone used for

calculating the reactive samples [9].

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 centers in India follow the strategy I of one-time ELISA testing as screening procedure as per WHO guidelines [8].

Acar *et al*. estimated that on applying the confirmatory test to GZ samples resulted in only 2% confirmed positivity which is higher than our study. GZ results may be of limited relevance due to reported false positivity on repeat testing. A study conducted in Turkey reported 70% false positivity on re-testing GZ results [13].

A study Anitha M *et al.* Stated that a total of 144 (0.58%) samples in grey zone area for all three viral markers as compared to 0.14-0.29%. While our study found a total 12 (0.05%) sample in grey zone. It is suggested that which is lower than other study done by different authors. This may be due the differences in the type of ELISA kits used for performing the test.

Bhardwaj, *et al.* Found that among the 50,064 blood donors a total of 47 (0.1%) blood donors' samples were GZ positive of which HBsAg was 05 (0.01%), HCV was 29 (0.06%), and HIV was 13 (0.03%). However, this may be continued for sake of "erring on the side of caution" and since this only results in negligible wastage (0.1%) of blood units.

One of limitations of 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.

Conclusion

Though NAT technology is an advanced screening method for screening of all sero-positive donors in the context of reducing the window period still in developing countries like India, installation of this technique in all blood centre centres is not feasible due to cost factor. So, ELISA test still remains a prominent screening tool in most blood centre centres in India. Proper donor screening, sensitive screening assays and effective pathogenic inactivation procedures can minimize the risk of TTI to a great extent. Repeat testing of grey zone samples will help in improving the safety of blood transfusion by improvising the current screening methodologies.

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