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ORIGINAL ARTICLE

Performance of Enzyme Linked Immunosorbent Assay and Rapid Screening Techniques for detection of Transfusion Transmitted Infections among blood donors

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Abstract

Introduction: Blood transfusion is associated with many risks, especially exposure to a transfusion-transmitted infections (TTIs). Lack of effective diagnostic techniques that can identifying the infection at window period contributes to the major health problem in most developing countries. The aim of this study was to compare diagnostic performance of enzyme linked immunosorbent assay (ELISA) and rapid screening techniques for detection of transfusion-transmitted infections (TTIs) among blood donors

Methodology: This study was conducted in the Department of Haematology and Blood Transfusion Services Federal Teaching Hospital, Ido-Ekiti, Nigeria from February 2021 to July 2023. A total of 3150 eligible blood donors that were screened fit for blood donation using rapid kits were subjected to ELISA screening technique. Questionnaire was used to inquire about risk factors for transfusion transmissible infection among blood donors and to sort the consent of all the blood donors before collecting their blood sample for study

Result: A total of 3150 blood donors that were screened negative for TTIs (HbsAg, HCV, HIV and syphilis) by rapid test kits were recruited for the study. Out of 3150 blood donors that were re-screened for TTIs by ELISA techniques, 34 (1.08%), 50 (1.59%), 07 (0.22%) and 13 (0.41%) were sero-positive for HbsAg, HCV, HIV and syphilis respectively. In general, the percentage seroprevalence of TTIs in our study was 104/3150 (3.3%), HCV had the highest prevalence of TTIs and Blood group O Rh D positive has the highest frequency among the study population

Conclusion: This study concluded that rapid test did not show any promising results when compared to ELISA. This study suggested that rapid test kits should not be recommended in transfusion center solely for screening blood donors for TTIs. There is need for combination of rapid test kits along with ELISA technique in

the diagnosis of TTIs for blood donors. We advocate for legislation to make screening with ELISA mandatory for TTIs in all blood transfusion center and other health facilities in the country

Key words: Blood transfusion, Transfusion transmissible infection, Blood donors, Rapid kits, ELISA technique.

Introduction

Blood transfusion services is a vital part of modern health care system which is an essential life-saving treatment, it's part of the World Health Organization's list of essential medicines. Safe blood transfusion being the cornerstone of any blood transfusion services requires meticulous testing for transfusion transmitted infections (TTIs) markers in donated blood (1). It is essential that blood and blood products should be available and accessible to the generality of Nigerians irrespective of gender, geopolitical setting, socioeconomic standing, cultural background and other societal variables. Such blood should be sourced from low-risk voluntary non-remunerated blood donors. Screening for transfusion-transmissible infections to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible (2). Effective screening for the presence of most common and dangerous TTIs can reduce the risk of transmission to very low levels. Worldwide, transfusion-transmitted infections (TTIs) remain a major health problem in most developing countries due to facilities with scarce resources and a shortage of staff members (3). Various assay systems with differing sensitivities and specificities are available for blood screening. Decrease in the sensitivity and specificity of rapid diagnostic testing proves that this method is not suitable for quality testing of infectious markers for blood donors. Window period is the interval between the donor's exposure to a virus until antibodies against the virus are produced. It is during this period that the risk of infection in donated blood can be missed by the rapid testing. ELISA is more reliable than the rapid test because it is proved to be more sensitive, specific and has the capacity to reduce the window period of the infections significantly compared to the rapid screening tests due to inability of rapid technique to detect recently infected subjects. Serological, window period detection of infections may be up to 12 weeks, this makes rapid screening test for blood donors a source of residual risk for transmission of TTIs. As a result of this, the risk of transfusion transmissible infections is still a big challenge in most developing countries including Nigeria (4). However, the efficacy of screening depends on their correct use in laboratories that are appropriately resourced, qualified staff and well-maintained quality systems (5). Majorly, blood transfusions are carried out globally to save countless lives, but unsafe practices risk the recipient of transfusion transmittable infections (TTIs). Screening of blood donors is a critical issue as the outcome of the test if not properly performed can result in serious consequences for either the blood service or the blood donor. False positive result can lead to a larger number of blood donors being deferred, while a false negative testing may jeopardize blood safety, also improperly trained laboratory personnel may also produce a false screening result (6). Transfusion transmittable infections can be reduced by improving donor selection criteria, donor awareness regarding TTIs and implementing sensitive screening tests. Screening of donated blood for TTIs represents one element of strategies for blood safety and availability (7). A lower prevalence of TTIs in the blood donor population also reduces the

discard of donated blood and hence results in improved efficiency and use of resources. Screening donated blood for transfusion-transmissible infections is to support countries in establishing effective national blood screening programs to protect the recipients of blood transfusion from TTIs. Transfusion of infected blood to patients in need is a crime, it is an offence to issue or transfuse unscreened blood or blood components (8). Therefore, blood donors are screened for viral markers such as hepatitis B surface antigen (HbsAg) and hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Treponema pallidum (syphilis) before transfusion to prevent transmission of infectious diseases. An important issue regarding blood safety is identifying infectious donors and preventing transmission in order to protect the recipients (9). There are two important strategies for the success of blood transfusion, the first is the adaptation of the national blood transfusion policy to select donors, which aims to exclude donors with a high risk of infections such as HBV, HCV, Treponema pallidum (syphilis) and HIV. The second strategy is the application of methods with high specificity and sensitivity for identifying true positive and true negative individuals or blood units (1). On every unit of blood, there is 1% chance of transfusion associated problems which includes transfusion transmitted infectious diseases. It is mandatory to test each and every unit of donated blood for antibodies to HIV-1 and 2, Syphilis, Hepatitis C and Hepatitis B surface antigen (3). ELISA is recommended and preferred screening technique for blood banks due to its effectiveness and it is ability to detects the presence of specific protein (antigen or antibody) in a given sample. However, many blood banks still lack this technology and prefer rapid test kits because it is an easy-to-use, inexpensive method and does not require advanced equipment and detailed training (10). Rapid tests are a rapid screening method used for the qualitative detection of infection in whole blood samples, serum, or plasma. Rapid tests use monoclonal and polyclonal antibodies to detect elevated levels of infection in samples. ELISA is a type of "sandwich" enzyme immunoassay for detecting infections in plasma or serum (3). This test uses monoclonal antibodies due to its ability to bind to different subtypes of viral infections recognized by the World Health Organization (WHO). Presently, both rapid screening test kits and ELISA technique are employed for the diagnosis of TTIs among blood donors, this therefore suggested the need to compared the performance of the two kits in the diagnosis of TTIs among blood donors (10). Hence, the aim of this study is to compare diagnostic performance of ELISA and rapid screening techniques for detection of TTIs among blood donors.

Materials and Methods

This study was conducted in the Department of Haematology and Blood Transfusion Services Federal Teaching Hospital, Ido-Ekiti, Nigeria from February 2021 to July 2023. Majority of the recruited blood donors for this study were regular blood donors and family replacement donors. Five (5mls) of blood was aseptically collected from the donors into an EDTA tube. Packed cell volume and wet preparation of blood microfilaria of each donor were determined to ascertain the adequate blood level of the donors and that donors were free from microfilaria. Plasma from donor sample was tested with the rapid diagnostic kit for HIV I and II antibodies, hepatitis B surface antigen, hepatitis C antibody and antibody to treponema palladium according to the manufacturer's specifications as initial screening. The donors that tested negative to all the TTIs were allowed to donate a unit of blood. Subsequently, plasma of donor samples was separated into plain bottles, stored at -400C for TTIs screening using the ELISA technique. During this period, a total number of 3150 blood donors that were screened fit

for TTIs (HBsAg, HCV, Treponema pallidum and HIV) by rapid test kits which are ready for blood donation were recruited for this study, blood group of the recruited blood donors were determined by both tile and conventional tube techniques. Plasma samples from 3150 eligible blood donors that were screened fit for blood donation using rapid test kits for TTIs were re-screened for TTIs using ELISA screening technique following manufacture instruction. TTIs results from both techniques used in this study were compared. Questionnaire was used in accordance with Hospital blood bank guidelines policy, to inquire about risk factors for transfusion transmissible infection among blood donors according to blood donor selection criteria and guideline. Consent of all the recruited blood donors were sought before collecting their blood sample for study.

Results

This study compared ELISA with rapid techniques results for detecting TTIs. During this study, a total of 3150 recruited blood donors were screened negative for TTIs (HbsAg, HCV, HIV and syphilis) by rapid test kits. The same group of blood donors were re-tested using ELISA technique. Out of 3150 blood donors that were re-screened for TTIs by ELISA techniques, 34 (1.08%), 50 (1.59%), 07 (0.22%) and 13 (0.41%) were sero-positive for HbsAg, HCV, HIV and syphilis respectively. In general, the percentage seroprevalence of TTIs in our study was 104/3150 (3.3%), this study showed the degree of false negative with the rapid test kit for TTIs among study population, comparison of rapid and ELISA techniques results is showed in table 1. Blood group O Rh D positive has the highest frequency and highest sero-positive of HCV among the study population as showed in figure 1. HCV had the highest prevalence of TTIs, the prevalence of TTIs by ELISA technique according to blood group in this study population were presented in table 2 and 3.

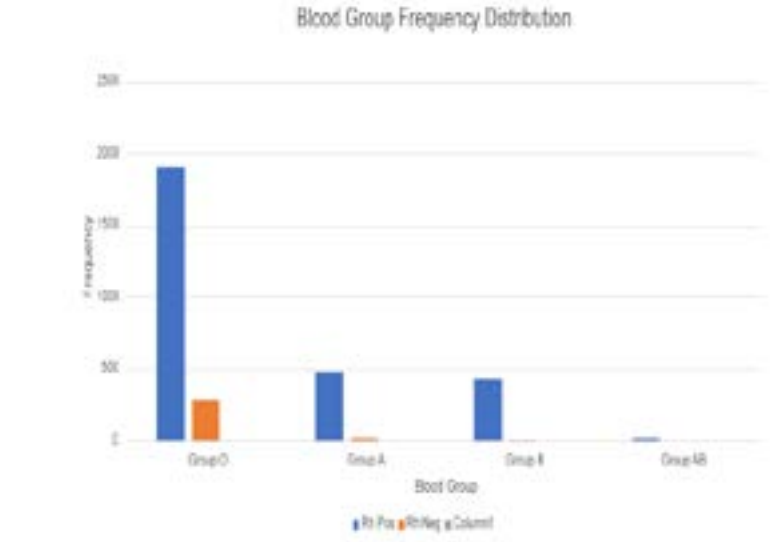

Figure 1: Blood Group Frequency Distribution

Table 1: Comparison of TTIs results using rapid and ELISA Techniques

Techniques	**Rapid Kit Result**		**ELISA Result**	
TTIs	Positive	Negative	Positive	Negative
HbsAg	--------	3150 (100%)	34 (1.08%)	3116 (98.92%)
HCV	--------	3150 (100%)	50 (1.59%)	3100 (98.41%)
HIV	--------	3150 (100%)	07 (0.22%)	3143 (99.78%)
Syphilis	--------	3150 (100%)	13 (0.41%)	3137 (99.59%)
Total	--------	3150 (100%)	104 (3.30%)	3046 (96.70%)

Table 2: Sero-prevalence of TTIs according to the blood group by ELISA Techniques

TTIs	O Pos	O Neg	A Pos	A Neg	B Pos	B Neg	AB Pos	AB Neg	Total
HbsAg	20	04	06	-----	04	-----	-----	-----	34
HCV	34	----	06	-----	10	------	-----	-----	50
HIV	05	01	01	-----	-----	-------	-----	-----	07
Syphilis	09	----	02	-----	01	------	01	-----	13
Total	68	05	15	-----	15	-----	01	-----	104

Table 3: Distribution of TTIs results according to blood group by ELISA Techniques

TTIs	O Pos	O Neg	A Pos	A Neg	B Pos	B Neg	AB Pos	AB Neg	Total
HbsAg Pos	20	04	06	----	04	----	----	-----	34
HbsAg Neg	1885	278	474	20	431	11	17	-----	3116
HCV Pos	34	----	06	-----	10	----	-----	----	50
HCV Neg	1871	282	474	20	425	11	17	----	3100
HIV Pos	05	01	01	-----	-----	----	-----	----	07
HIV Neg	1900	281	478	20	435	11	17	----	3143
Syphilis Pos	09	----	02	-----	01	----	01	----	13
Syphilis Neg	1896	282	478	20	434	11	16	----	3137

Discussion

Blood transfusion has been established as a route of transmission of the transfusion-transmissible infectious agents. Hence, hepatitis B virus (HBV), human immunodeficiency virus (HIV), hepatitis C virus (HCV) and syphilis are among the greatest threats to blood safety for blood transfusion recipients. TTIs pose a serious public health problem, although transfusion of infected blood to patients in need is a crime. An effective blood donor sensitive screening tests can reduce the risk of acquiring TTI's. The prevalence of TTIs using the ELISA technique among blood donors in this study were 34 (1.08%), 50 (1.59%), 07 (0.22%) and 13 (0.41%) for HBV, HCV, HIV and syphilis respectively. In this study, sero-prevalence of HCV by ELISA technique was higher compared with other TTIs among study population. In general, the percentage seroprevalence of TTIs by ELISA technique in our study was 104/3150 (3.3%) which is comparable to other studies where 2.35% and 3% were reported (1,8). The implication of this study is that using the rapid kits technique alone, 104/3150 (3.3%) of infected blood which was initially tested negative with the rapid kits to TTIs would have make patients to be transfused with one or more TTIs. It was established that, in places where effective screening programs exist, the risk of transmission of transfusion-transmissible infections (TTIs) has progressively and significantly decreased (4). The seroprevalence of HBV (1.08%) by ELISA technique in this study is similar to that of other previous studies where 1.8%, 1.6%, 1.4% and 1.3% were reported (11-13). Low prevalence of HIV and HbsAg by ELISA technique in this study may be due to awareness of HIV infection and availability of effective vaccine to hepatitis B virus. Although, this finding is contrary to other previous study which reported that HBV was the most prevalent life threatening TTI (5). The risk of transfusion-transmissible infections (TTIs) through blood is determined by its prevalence in the blood donor population, the problem of sero-conversion window (where the donor is infectious but seronegative), the availability and affordability of sensitive diagnostic capabilities (2). This study showed that ELISA test is more sensitive, reliable, specific and superior for the testing of blood donors for TTIs than rapid test kits. In this study, we observed high false negative results with the rapid diagnostic kit, this may be due to relative effectiveness of ELISA technique to detect infectious at window period (the time between development of infectious viraemia and reactivity by routine serological). Finding in this study is consistent with previous report which indicated that ELISA technique is superior to rapid kits in the diagnosis of transfusion transmissible infections among blood donors. According to Salawu's findings, he reported that there are high false negative results with the rapid diagnostic kit as compared to ELISA testing which is similar to the findings in this present study. This indicated that there is a possible risk of donor blood containing TTIs being transfused to patients due to suboptimal or insufficient testing using rapid kits only (4).

This study observed that the rate of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), in addition to determine the diagnostic accuracy rate and error rate for rapid diagnostic kits in detecting TTIs are less accurate and associated with more false negatives compared to the ELISA technique. It was reported that, failure of the rapid kits to detect the presence of markers of infectious viral diseases may be due to; inadequate coating of the antigen, nature of the antigen used and genetic heterogeneity of the virus, low antibody titres especially in recent infections where the levels may be below the detection limit of rapid test kits but are picked up by the more sensitive

enzyme immunoassay (ELISA technique) (10). However, Improvement of ELISA technique for the detection of TTIs has always been predicted to be due to emergence of mutant isolates and the need for early diagnosis of this TTIs. The results of ELISA technique in this study showed a better performance (higher Sensitivity, higher Specificity, higher positive predictive value, higher negative predictive value and fewer cases of false negatives value).

Sensitivity is the ability of a test to correctly identify patients with a disease and give a positive finding, expressed as a percentage, Specificity is the ability of a test to correctly identify people without the disease and give a negative finding, expressed as a percentage. Positive Predictive Value (PPV) is the ability of an assay to identify actual infected individuals among all persons giving a positive result with the kit being used. Negative predictive value is the ability of an assay to identify correctly the real non-infected individuals among persons giving a negative result with the kit being used (1). A good assay for an infectious agent like TTIs from a diagnostic point of view is one with a high positive predictive value and less cases of false negatives. Our finding is consistent with a previous report which indicated that rapid test has not shown any promising results compared to ELISA. Discordant results between the two assays for the diagnosis of an infectious disease can cause a huge challenge and have serious consequences among subjects which can cause undue mental stress and tension.

Recommendation

Rapid test kits should not be recommended in transfusion center solely for screening blood donors for TTIs, there is need for combination of rapid test kits along with ELISA technique in the diagnosis of TTIs for blood donors. Although, cost effectiveness has erroneously been the reason why ELISA technique has not been fully incorporated in the detection/ screening of TTIs among blood donors in many facilities. It must be noted that the cost of long-term effect of a patient who contracts any of the TTIs from blood transfusion is far outweigh the cost of testing donor units for TTIs using ELISA. An unsafe blood transfusion is very costly from both human and economic points of view. Morbidity and mortality resulting from the transfusion of infected blood have far-reaching consequences, not only for the recipients themselves, but also for their families, their communities and the wider society. Proper screening of the donor using standard techniques would ensure safe blood transfusions. Kit evaluation is vital in determining the diagnostic kit of better performance, there is need for the Nigerian Government to develop a safe blood donor screening strategy for TTIs by combining the use of less sensitive rapid screening techniques with more sensitive and sophisticated evidenced- based ELISA screening to ensure the safety of blood donation in the country. We therefore encourage the government and funding agencies to procure ELISA testing equipment for all healthcare providers involved in blood transfusion. We also advocate for legislation to make screening with ELISA mandatory for all health facilities in the country.

Conclusion

This study concluded that rapid test did not show any promising results when compared to ELISA, because of false negative result observed with rapid test kits.

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