COMPARISON OF IMMUNOCHROMATOGRAPHIC TEST NEGATIVE HEPATITIS B VIRUS AND HEPATITIS C VIRUS BLOOD DONATIONS WITH ELISA IN PUBLIC AND PRIVATE BLOOD BANKS OF LAHORE - PAKISTAN

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ABSTRACT

Objectives: To determine the frequency of immuno- chromatographic false negative HBV and HCV testing among healthy blood donors in city Lahore.

Methodology: This was 18 months cross sectional pilot study, conducted in private and public blood banks of city Lahore. After taking formal consent from head of selected Hospital and in charge blood bank/ donors, blood bags screened as HBs Ag and Anti HCV negative by immune-chromatographic (ICT) method, 3-5 ml blood was transferred to coded tube and later transferred to ex-PHRC research Centre NHRC Lahore, where plasma was separated through centrifugation and stored at -40/-20C. Collected samples from this Centre sent to ex-PHRC Research Centre Khyber Medical College Peshawar in cold chain for ELISA testing.

Results: Study found that among 385 HBs Ag and anti HCV ICT negative Labeled blood bags 0.8 % was positive for HBs Ag and 2.1% blood bags was positive for anti HCV antibody on ELISA testing. Frequency of AB –ive and A-ive blood bags availability were very rare, 1 % for both types. False negative testing rate of blood bags was comparatively high in public sectors hospitals (1 % for HBs Ag and 3.2% for anti HCV) than private sector hospitals (0.5% for HBs Ag and 1% for anti HCV. False negative ICT testing rate was observed high among blood group B +ive (0.8% for HBV and 4.1% for HCV) than all other blood groups.

Conclusion: Study found that 0.8% blood bags were screened falsely negative by immuno-chromatographic method (ICT) for HBs Ag and 2.1% for anti HCV.

Keywords: Immunochromatographic Test; Enzyme-Linked Immunosorbent Assay; Hepatitis B Virus; Hepatitis C Virus.

INTRODUCTION

A number of bacteria, viruses, and parasites can be transmitted through blood or blood products. Amongst these, the hepatitis C virus (HCV) and hepatitis B virus (HBV) testing in blood and blood products are mandatory worldwide because its transmission cause potential serious chronic clinical complication.[1] A number of testing methods are used to screen these viral agents in blood products, despite the introduction of sensitive screening assays for specific viral antigens and antibodies, the transmission of viral infection by transfusion is estimated to occur at low but significant levels.[2-4] In an Attempt to understand the sources of residual virus transmission it has been determined for theoretical sources of transmission risk. The predominant source of risk is blood collected from donors who are in the pre-sero-conversion window phase of infection. Other source of transmission risk is false negative testing of blood products with low sensitive rapid testing methods.[2]

One of the key considerations when employing rapid screening tests is achieving an optimal balance between sensitivity and specificity. This is crucial to limit instances of both false positives and negatives. If a test generates a substantial quantity of false positive results, we may inadvertently defer a significant number of willing donors. Conversely, false negative outcomes could potentially compromise blood safety. Even with advanced, cutting-edge testing methods, there's a risk of obtaining false negatives if the donor is within the so-called 'window period' and has not yet undergone seroconversion. Therefore, strict hemo-vigilance and quality control systems are need to ensure safety and quality.[5] Infection transmission continues to pose a minor threat owing to a variety of factors. These include genetic diversity among pathogens, the existence of

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symptomless carriers, inaccuracies in laboratory processes, and fluctuations in the window period of the infectious agent. The shortcomings inherent in current screening and testing methodologies also contribute to this ongoing risk.[6-8]

Numerous investigations have been carried out to determine the prevalence of HCV and HBV across various regions of Pakistan.[9-11] Despite these efforts, there is a noticeable lack of population-based studies that accurately measure the incidence of hepatitis in different locales and demographic groups. Volunteers donating blood are a high-risk group for hepatitis transmission, due in part to potential testing errors.

Typically, volunteer blood donors are considered a healthier subsection of any population, as blood banks generally implement stringent selection criteria. This effectively ensures only fit and healthy individuals donate blood.[9]

However, the blood transfusion service in Pakistan has encountered significant difficulties in choosing appropriate testing kits for screening blood donors, a problem compounded by the need to extend these services into rural regions.[5] Consequently, the aim of this study is to ascertain the rate of false negative results for HBV and HCV tests in blood donors from Lahore city. Moreover, the study also seeks to understand the role of Information and Communication Technology (ICT) based testing in the transmission of HBV and HCV.

METHODOLOGY

This cross-sectional study spanned over 18 months, from June 2015 to December 2016, and was conducted in both private and public blood banks within the city of Lahore. After obtaining formal consent from the heads of the selected hospitals and the responsible individuals overseeing the blood banks and donors, the study protocol received approval from the local ethical committee. Blood bags screened as HBs Ag and Anti HCV negative by only immune-chromatographic (ICT) method and stored for less than one year will be selected for sampling. A 3 to 5 ml blood from those blood bags was transferred into coded tubes (Serial No/ Centre name/ Hospital name/ blood sample number) and same code both in study proforma. Blood samples were transported to participating ex-PHRC (Pakistan Health Research council) research Centre NHRC (National health Research complex) Lahore plasma was separated through centrifugation and stored in coded tubes at -40/-20 C. Collected samples were sent to ex-PHRC Research Centre Khyber Medical College Peshawar in cold chain for ELISA testing.

ELISA testing for anti HCV and HBs Ag was done using kits Dia Sorin, Murex Anti HCV version 4and Murex HBs Ag version 3. Random 12 tested samples were retested externally for anti HCV and HBs Ag as external quality for the confirmation of test results. Accuracy between lab ELISA tests and external quality was 100%. Data entered and analyzed using SPSS version 16.

RESULTS

Study found that among 385 HBs Ag and anti HCV ICT negative marked blood bags 0.8 % were positive for HBs Ag and 2.1% blood bags were positive for anti HCV antibody on ELISA testing. (Table # 3, 6). Blood bags with B +ive (31.4%) and O +ive (30.9%) blood were available frequently in blood banks of both private and public hospitals. Frequency of AB –ive and A-ive blood bags availability were very rare, 1% for both types. Results showed that blood bags availability of B+ive blood group was high in private sector hospitals (39.1%) than private hospitals (23.8%) while A+ive , O+ive blood groups were high in public sector hospitals (23.3%, 30.9% respectively) as compared to private sector hospitals (13.3%, 22.4% respectively). (Table 2)

False negative testing rate of blood bags was comparatively high in public sectors hospitals (1 % for HBs Ag and 3.2% for anti HCV) than private sector hospitals (0.5% for HBs Ag and 1% for anti HCV) (Table 4, 7). False negative ICT testing rate was observed high among blood group B +ive (0.8% for HBV and 4.1% for HCV) than all other blood groups.

DISCUSSION

This research study demonstrated the comparison of immunochromatographic (ICT) and ELISA testing of donated blood for transfusion in different private and public blood banks of city Lahore. Blood donation transmit HCV and HBV virus and old age blood donors normally transmit HBV and HCV virus with high rate.[12] This study provided information that with which frequency we are donating false negative ICT tested blood in private and public hospitals. The limitation of the study was that false negative ICT tested HCV samples were not confirmed through PCR, because many time false anti HCV antibodies may develop against any other antigens.

The principal finding of our study was that among 385 HBs Ag and anti HCV ICT negative labeled blood bags 0.8 % were positive for HBs Ag and 2.1% blood bags were positive for anti HCV antibody on ELISA testing. Although the false negative testing rate for HBV was low, however special attention must be given on predisposing factors in order to control transfusion related HBV and HCV transmission. This study provided similar consistence results of an Indian study conducted among blood donors, where out of 2000 blood donors samples tested negative for HCV and HBV through ICT method, 6.55% blood samples were found positive for anti HBc antibody and DNA was detected in 0.8% samples.[13]

Table 1: Availability frequency of ABO & RH Blood bags in blood banks of city Lahore

S#	Type of Blood group	Frequency	Percentage (%)
1	A +ive	70	18.2
2	A -ive	4	1.0
3	B +ive	121	31.4
4	B -ive	11	2.9
5	AB +ive	38	9.9
6	AB -ive	4	1.0
7	O +ive	119	30.9
8	O -ive	18	4.7
Total		385	100.0

Table 2: Frequency of different ABO & RH blood bags in public and private hospitals blood banks of city Lahore

S#	Type of Blood bags	Type of Blood Bank n(%)		Total n(%)
		Public sector hospital	Private sector hospital	
1	A +ive	45 (23.3)	25 (13)	70 (18.2)
2	A -ive	3 (1.6)	1 (0.5)	4 (1)
3	B +ive	46 (23.8)	75 (39.1)	121(31.4)
4	B -ive	4 (2.1)	7 (3.6)	11 (2.9)
5	AB +ive	11 (5.7)	27 (14.1)	38 (9.9)
6	AB -ive	0 (0.0)	4 (2.1)	4 (1.0)
7	O +ive	76 (39.4)	43 (22.4)	119 (30.9)
8	O -ive	8 (4.1)	10 (5.2)	18 (4.7)
Total		193 (100)	192 (100)	385 (100)

Table 3: HBsAg ELISA test Result of ICT negative screen blood bags samples

S#	Hbs Ag ELISA Results	Frequency	Percent (%)
1	Negative	382	99.2
2	Positive	3	0.8
Total	385	100.0	

Table 4: Hbs Ag ELISA results of ICT negative blood bags of public and private blood banks of city Lahore

S#	HBs Ag ELISA results	Type of Blood Bank n(%)		Total n(%)
		Public sector Hospital	Private sector Hospital	
1	Negative	191(99.0)	191(99.5)	382(99.2)
2	Positive	2(1.0)	1(0.5)	3(0.8)
Total		193(100)	192 (100)	385(100)

False negative ICT testing for anti HCV was 2.1 % in our study. It should be of high concern and if false negative ICT testing let the transfusion of infected blood with this rate, than HCV transmission cannot be controlled. Our emphasis must be the confirmation of HCV infection through more sensitive tests. In Pakistan no study has been done on false negative HBV and HCV testing among blood donors, however a study was carried out in … on false positive testing among blood donors (0.15% false positivity rate for HCV, HBV and HIV) to highlight the donor deferral rate.[14]

The efficacy of anti-HBc approach has been evaluated in low prevalence areas where a few seropositive samples contained HBV DNA. Data from 10 studies in seven Asian countries revealed that the prevalence of anti-HBc is from 7% to 43%, and about 5% (range: 0 -18%) of anti-HBc samples contained HBV DNA.[15, 16, 17]

More importantly, false negative HBsAg blood samples are observed at a mean of 298 (5-95 percentile: 14-893) blood units per million in the model at producer-claimed sensitivity If more realistic test properties are taken into consideration, the mean rate of false negative HBsAg is found to be 1,087 per million blood units (5-95 percentile: 762-3220) as lower test sensitivity at local laboratories was reported in Cambodia and Vietnam.[18] This shows the importance of test sensitivity and demonstrates that low HBsAg test sensitivity is the main cause of transfusion-transmitted HBV infections in Vietnam

This study provided that among ABO groups blood bags included in the study B +ive (31.4%) and O +ive (30.9%) blood was available frequently in blood banks. When a similar kind of study was conducted in DHQ Hospital Mardan in 2012 it was found similar pattern of results like 29.9% blood donors were B +ive and 27.9% were O +ive.[19]

Availability of Rh negative blood was very low in our study especially in public sector hospitals). All Rh negative people should be encouraged towards blood donation to meet emergency situations.

It was also found that false negative ICT testing rate was relatively bit high in public sector hospitals (1% for HBV, 3.2% for HCV) than private hospitals (0.5% for HBV and 1 % for HCV),. Based on this result, role of testing errors and negligence in public sector hospital could not be nullified. It has been observed that in many public sector hospitals the external & internal quality, strict vigilance and monitoring have not been maintained

Table 5: HBs Ag ELISA test results of different ABO & RH blood bags

S#	Blood group	HBs Ag ELISA Results		Total
		Negative	Positive	
1	A +ive	69(98.6)	1(1.4)	70(100)
2	A-ive	4(100)	0(0.0)	4(100)
3	B +ive	120(99.2)	1(0.8)	121(100)
4	B -ive	11(100)	0(0.0)	11(100)
5	AB +ive	8(100)	0(0.0)	38(100)
6	AB -ive	4(100)	0(0.0)	4(100)
7	O +ive	119(100)	0(0.0)	119(100).0%
8	O -ive	17(94.4)	1(5.6)	18(100)
Total		382(99.2)	3(0.8)	385(100)

Table 6: Anti HCV ELISA Result of ICT negative screen blood bags samples

S#	Anti HCV ELISA results	Frequency	Percent (%)
1	Negative	377	97.9
2	Positive	8	2.1
Total		385	100.0

Table 7: Anti HCV ELISA results of ICT negative blood bags of public and private blood banks of city Lahore

S#	Type of Blood Banks	Anti HCV ELISA Results		Total
		Negative	Positive	
1	Public Sector Hospital	181(96.8)	6(3.2)	187(100)
2	Private Sector Hospital	196(99)	2(1.0)	198(100)
Total		377(97.9)	8(2.1)	385(100)

Table 8: Anti HCV ELISA tests result of ABO & RH blood bags

S#	Blood Group	Anti HCV ELISA Results		Total
		Negative	Positive	
1	A +ive	70(100)	0(0)	70(100)
2	A -ive	4(100)	0(0.0)	4(100)
3	B +ive	116(95.9)	5(4.1)	121(100)
4	B -ive	10(90)	1(9.1)	11(100)
5	AB +ive	38(100)	0(0.0)	38(100)
6	AB -ive	4(100)	0(0.0)	4(100)
7	O +ive	117(98.3)	2(1.7)	119(100)
8	O -ive	18(100)	0(0.0)	18(100)
Total		377(97.9)	8(2.1)	385(100)

properly, therefore possibilities of testing error are high in public sectors hospitals. The contribution of testing errors to the risk of virus transmission by transfusion depends on the rate of false-negative testing errors and the prevalence of infected seropositive donations. Although, the false negative testing error rate has been estimated at 0.1 to 1% on the basis of proficiency studies. Retro virus epidemiology donor study from the duration of 1991 to 1995 depicted about false positive and false negative blood donation for HCV,HIV, and among 1500000 blood donations 0.5% false negative and false positive testing error were determined. [20]

False ICT negative testing for anti HCV and HBs Ag was found high in B+ ive group blood bags (4.1% for anti HCV and 0.8% for HBs Ag) than other ABO groups. No other studies have been found to justify the results. However, another study is needed to confirm the relation of high false negative testing among people with B blood group.

CONCLUSION

The present study revealed the occurrence of false negative ICT testing of blood donation for HCV and HBV virus in public and private blood banks. False negative testing rate was high in public hospitals blood banks. Prompt measurements regarding blood transfusion are needed for the prevention of transfusion related HBV and HCV transmission. Public sector hospitals should strengthen their potential for provision of quality lab services and proper lab management. False negative testing have many predisposing factors, including type of ICT kits, experimental errors, equipment working efficiency, technical expertise and lab quality supervision. It is recommended that public and private blood bank frame their proper transfusion policy fulfilling all quality services. It would be much better if all type of blood banks use ELISA testing as first line of screening for HCV and HBV virus in stored blood bags.

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Author's Contribution

AK conceived the idea and designed the study and checked the manuscript for technical errors. FG helped in data collection and data analysis and drafted the manuscript. OU helped in data collection and writing of the manuscript. IA performed the statistical analysis and reviewed the manuscript for critical issues. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.