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Detection and prevalence of hepatitis B, C and HIV viral infections among hemophilia patients in Peshawar, Pakistan

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Abstract

This multidimensional study was conducted on three hundred and ninety-six (396) hemophilia patients (331 males and 65 females). The hemophilia patients were screened for HBsAg, anti-HCV antibodies, and anti-HIV antibodies by using automated advanced examination diagnostic technique that is Chemiluminescent Microparticle Immunoassay (CMIA) to detect HBV, HCV, and HIV infections. The blood samples were collected by venipuncture and the serum was separated by centrifugation. After that, all the samples were run on ARCHITECT i1000SR automated immunoassay analyzer Chemiluminescent Microparticle base technology to get results. Out of 396 blood samples, we have investigated in this study, 72 (18%) were positive for HCV infection and 12 (3%) were confirmed to be positive for HBV infection. HIV infection was not detected in any patient. Our study demonstrates that the prevalence of HCV amongst Pakistani donors is highest while HBV is on a decline.

Keywords: Prevalence, CMIA, transfusion transmitted infections, hemophilia, Peshawar, HBV, HCV, HIV

1. Introduction

The three main pillars of blood borne diseases are Human Immunodeficiency Virus, Hepatitis B Virus and Hepatitis C Virus [1]. According to independent statistics people suffering from HIV are 34 million in number, 2 billion are suffering from HBV while total of 130 million are suffering from HCV [2-4]. Blood transfusion system in Pakistan is unfortunately poor and conventional. In Pakistan health care institutions are working on blood dealing in their own blood banks having substandard system that is resulting in poor and inadequate transfusions and severe consequences [5]. Viral proliferation through blood transfusion is decreased due to several initiatives. Reason for the decrease in viral proliferation is standard raise in manufacturing blood products and inactivating viruses. Secondly, most of the countries are screening HBV and HCV vaccinations. Recombinant coagulation factors making are also more updated than before now [6-7].

Hemophilia is a disease indicated by significant bleeding indication due to a lack of factor VIII for hemophilia A (Classic hemophilia) and factor IX for hemophilia B (Christmas disease) [8]. The disease treatment method differs across the globe and there is a vast variability not merely in the kinds of products used (plasma-derived factor concentrates, recombinant factor concentrates, plasma, and cryoprecipitate) but also in the dosages managed (minimal to very high) for identical symptom [9]. Medication with fractionally screened blood products has been interrelated with viral infections such as hepatitis B, hepatitis C, and HIV. Transfusion-transmitted hepatitis was acknowledged in 1943, and it is now an important difficulty of hemophilia patient's treatment in different countries [10].

Different studies conducted on blood donors in Pakistan have shown a prevalence of different disease-causing pathogens approximate ranges as hepatitis B 1.46% to 2.99%, hepatitis C 3.01% to 4.99%, and HIV 0% to 0.06%, respectively [11-12]. In a report from the United States, the key reason of hepatitis transmitted by blood products was HCV and in another report, anti-HCV was positive in 60-90% among hemophiliacs who are receiving commercial clotting factor concentrates [13-14]. The screening of blood donors for antibodies to hepatitis B surface antigen and hepatitis C is useful in preventing post-transfusion hepatitis [15]. In contrast to neighbor India, hepatitis B, and C happens at the same rate, however HIV is seen less

common. Screening of blood products for HCV started around 10-12 years ago in Pakistan. A method for screening has been insignificant because of the high fee of these tests. There is a high prevalence of HBV and HCV in blood donors and in a general population [16]. Infection with HBV, HCV, and HIV leads to different complications which contribute to the morbidity and mortality in these patients. Developing nations are prone to transfusion transmitted infections due to lack of standardized pre-transfusion blood screening protocol [17].

TTIs remain to be the main challenge for Blood transfusion centers in the world and are directly proportional to the prevalence of the infections in the blood donor public [18]. Different techniques are enforced for the identification of HBV, HCV, and HIV infections which is Immune Chromatographic Technique (ICT), Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR), but due to the wrong positivity rate of ICT, ELISA is advised to be more reliable than ICT based Hepatitis B, C, and HIV identification [19]. The recognition of HBV, HCV, and HIV by PCR is more efficient and accurate but it is not cost effective and it needs skillful manpower for the process and explanation. A poor socioeconomic indicator of the society depicts that locals can not to afford such costly diagnosing. Detection of HBV, HCV and HIV infections in hemophiliacs through Chemiluminescent Microparticle Immuno Assay (CMIA), which provides a profitable, fast and trustworthy way to detect these infections. In current study, we aimed to determine the prevalence of HBV, HCV and HIV infections and do an analysis of HBV, HCV and HIV infections in hemophilia patients.

2. Material and Methods

2.1 Sample Collection

The sample collection and study was conducted at Fatmid Foundation Peshawar, Pakistan from July 2015 to June 2016. Fatimid Foundation is an engaged transfusion service helping not only a largish portion of the local hemophilic population of Peshawar Pakistan but also from the neighboring country Afghanistan. Fatimid provides more than 100 units of Packed RBCs, platelet, plasma and whole blood per day to different patients and carries out 100 blood transfusions. All blood donors were registered with a name, gender, age etc. About (3 milliliters) of venous blood was collected from each patient in anticoagulant EDTA tube. The significant data of each person was saved in separate forms. All blood donors ranging from 03–34 years was involved in this study.

Table 1: Age group distribution of hemophilic patients

Age Groups	Gender	
	Male	Female
Below 10 years	118	26
11-20 years	171	30
21-28 years	34	07
29 & Above	08	02
TOTAL	331	65

2.2 Sample processing

The samples were centrifuged at a high speed about 5000 revolutions per minute (rpm) for 15 minutes at room 4°C to get clear supernatant serum and then the serum was kept at (-20°C). When all samples were collected then they were run on ARCHITECT i1000SR automated immunoassay analyzer of Chemiluminescent Microparticle Abbott diagnostic services.

2.3 Chemiluminescent Microparticle Immuno Assay (CMIA)

CMIA is an improved form of ELISA method. The screening was done using Architect machine which is an immunochemical automated analyzer. Architect machine is aimed to detect antibodies to purported non-structural and structural protein of HBsAg, HCV and HIV genome. In the final reaction of HBsAg, anti-HCV, and anti-HIV observation bound achrindinylated conjugates were utilized to produce chemiluminescent signals. The samples collection and testing were performed on the same day. According to the manufacturer's literature, the relative susceptibility and particularity of this method is 99.6% and 99.7% respectively. Test results were kept confidential and were only communicated to the participants. Then the results were automatically gained by the software package by evaluating the chemiluminescent signals gained from the response solution of the sample together with the signal of the cutoff worth formerly gained by HBsAg, anti-HCV and anti-HIV standardization. Those with a positive test result were advice to do further testing and treatment and were referred to the nearer health facility centers. The severity of hemophilia was based on level or amount of clotting factor in the blood. The persons having 6-49% factor defined as mild hemophilia, 1-5% as moderate and <1% as severe.

2.4 Statistical analysis

Descriptive statistics were performed using the GraphPad Prism 5.0 software. Chi-square test was used to examine the subjective information. The differences were considered important if $P < 0.05$. Odds ratio (OR) with 95% confidence intermission was used to assess the association of diverse age groups with respect to HBV, HCV and HIV status of a female and male population.

3. Results

The mean age of candidates was 16.44 years (± 8.82 years) ranges 3 to 34 years. Mostly (84%) of the patients were males ($N = 331$) and (16%) of the patients were females ($N=65$). The hemophilia A was reported in 241 (61%) patients, Von Willebrand disease was reported in 82 (21%) patients, Platelet function defect was reported in 31 (8%) and the severity of disease was mostly (79%) mild ($N = 315$).

Table 2: Illustrative disease statistics of hemophilic patients ($N = 396$)

Variables	Frequency	Percentage
Age (mean \pm SD)	16.44years \pm 8.82	Range: 3-34 years
Gender		
Male	331	84%
Female	65	16%
Marital status		
Single	372	94%
Married	24	6%
Type of disease		
Hemophilia A	241	61%
Hemophilia B	5	1%
Von Willebrand disease	82	21%
Platelet function defect	31	8%
Aplastic anemia	15	4%
Other	22	5%
Severity of disease		
Mild	315	79%
Moderate	36	9%
Severe	45	12%

The most common blood groups were, in descending order, group B-positive (34%), AB-positive (29%), A-positive (16%), and O-positive (8%).

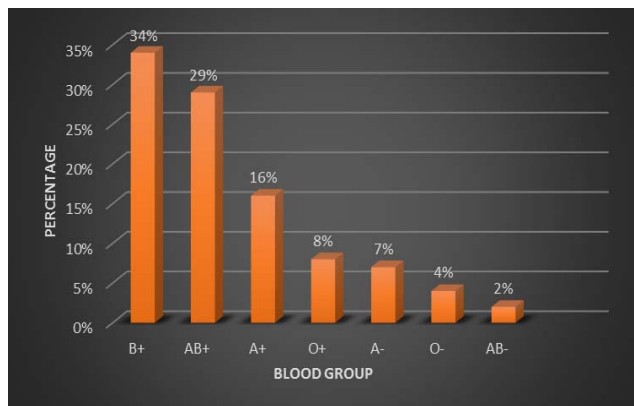


Fig 1: Illustrative blood group percentage of hemophilic patients (N =396)

A total of 396 patients were tested for HBV, HCV, and HIV. The prevalence of anti-HCV antibody was 18% (72 of the 396 patients), of which 63 were males and 9 were females. The prevalence of HBsAg was 3% (12 out of 396 patients) and all were male. All patients were negative for an anti-HIV antibody.

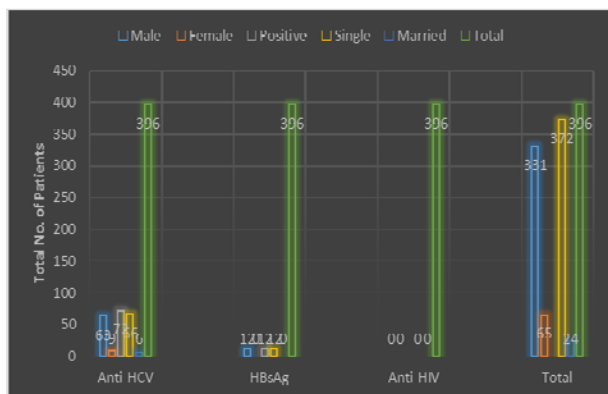


Fig 2: Results of HBV, HCV, and HIV test among hemophilic patients

4. Discussion

Transfusion-transmitted infections is an emergent public health problem in various parts of the globe, mostly in areas where blood screening practices are poor and the prevalence of parenterally transmitted infections between blood donors is high [20]. Eighty percent of the world’s hemophilia population exists in nations with imperfect economic or limited medicinal resources. They cannot obtain virally inactivated clotting products easily. Numerous patients are cured with locally supplied blood and its components only. Other blood products or plasma they receive are not all the time screened for these transmissible disease causing agents. Therefore, transfusion transmitted infections stay to have an important effect on patient administration mainly in developed nations like Pakistan [21].

Most of these patients are not capable of paying for expensively virally inactivated clotting factors and are reliant on wet blood products i.e. Fresh Frozen plasma or cryoprecipitate for the administration and inhibition of bleeding periods. However, these products are commonly not

treated to eradicate blood-borne infections. Therefore, Transfusion-transmitted infections such as Hepatitis B virus, Hepatitis C virus and Human Immunodeficiency virus remain to bear problem to healthcare solution association with administration of hemophilia patients [22].

In this study, we have found the overall prevalence of HCV as 18% which is greater than 8% previously reported amongst the general community of Islamabad, Pakistan [23]. This main change in the whole prevalence might be due to using changed investigative methods, the immunological status of the individuals and insufficiency of understanding about HBV and HCV mainly In Peshawar Pakistan. Another study arranged in Baluchistan Pakistan told 21% prevalence of anti-HCV which was slightly higher than our present study [24]. This is due to the shortage of education, the unhygienic health status of the persons and primary poverty in the area. Thus, countries with a greater HCV prevalence in the general population had a greater prevalence level in multiple-transfused patients. Prevalence of HCV in blood donors from diverse nations of the established world has been stated to be between 0.3 and 1.5% and in Iran, the prevalence was 0.14% [25]. Whereas from other nations such as India 0.25% to 0.9% [26]. The prevalence rate of HCV is much higher in Pakistani donors. The essential anti-HCV screening was announced to blood transfusion centers in Pakistan nearby the year 2000. But still, standard screening approaches are accessible only in few blood transfusion centers of large cities.

In our study, 3% hemophilic patients were positive for HBsAg. According to World Health Organization (WHO), Pakistan drops in the low common area of HBV infection with a prevalence of 3% infected population. Diverse studies present a quite variable image of the disease dependent on the factors focused like sample size, objectives of the study, population under study, associated risk factors, socio-economic status, diagnostic assays practiced, origin and general population behavior. The low prevalence of HBV infection in our members might be due to the introduction of hepatitis B vaccination, strict blood donor selection policy and mass screening programs. The rate of positivity among blood donors is also lower in other nations like Iran to 0.61% in 2005/2006 [27]. In South East Asia and China, it ranges from 1% to 15% [28].

Our study had no positive HIV infection. International literature has stated high frequencies (15-45%) of HIV infection in hemophilia patients formerly the almost simultaneous introduction of the inactivation processes and screening of anti-HIV between blood donors [29-30]. The rate of HIV infection has dropped further, due to deaths and an increasing number of hemophilia patients born after plasma products became safe [31]. However, Pakistani study showed that the HIV prevalence of. 02% [32] while another study reported no positive case of HIV [33]. In one center the positivity rate for HIV was 0.003% [34]. HIV appears to be lowest among the various transfusion-transmitted viral infections. The percentage of HIV infection in multi-transfused patients differs from country to country. This looks to be even lower than HIV seroprevalence in blood donors reported from neighboring countries, which is 0.249% HIV prevalence for India [35] 0.004% HIV prevalence for the Islamic Republic of Iran [36] and 0.1% HIV prevalence for Nepal [37]. Generally, the prevalence looks low, but the restricted number of studies reclaimed from the published literature may mean that the calculated figure does not represent the true prevalence. Blood screening method is effective in minimizing the risk of HBV, HCV, and HIV

infections but still there is dire need of using more accurate viral detection technique, in order to treat hemophilia patients with HBV, HCV and HIV infections more cautiously. The best way to minimize these transfusion transmitted infections is to educate peoples and create awareness among the public. Health care facility makers should put in serious effort to improve transfusion services in Pakistan. The facilities for screening tests for Hepatitis B virus, Hepatitis C virus, and Human immunodeficiency virus would be available in all health care and transfusion centers. The healthy donor selection criteria should be implemented strictly. Good quality blood bags, disposable syringes, and needles should be used. Staff working at health care and transfusion centers should be properly trained and educated.

Further studies are needed to investigate HBV, HCV and HIV prevalence in Pakistan at a molecular level. Large multicenter studies are needed to get the broader outlook of problems faced by transfusion dependent children of our region.

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6. Declaration of interest

None of the authors of this paper had any personal or financial conflicts of interest.

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