

FREQUENCY OF HEPATITIS B AND HEPATITIS C IN HEALTHY BLOOD DONORS OF NWFP: A SINGLE CENTER EXPERIENCE

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ABSTRACT

Objective: To determine the frequency of hepatitis B and hepatitis C in asymptomatic healthy blood donors.

Material and Methods: A prospective study was conducted in the department of Pathology, Rehman Medical Institute, Peshawar from April 2002 to March 2003. A total of 4000 healthy blood donors were selected. Blood samples were collected for screening of HBs Ag and anti HCV antibodies using IMX or Axsym, MEIA technology (Abbott, USA).

Results: Even after strict selection of healthy donors 1.9% were found to be reactive for hepatitis B and 2.2% were found to be reactive for hepatitis C. The rate of incidence of hepatitis C was higher in blood group B negative (6.81%) while the rate of incidence of hepatitis B was higher in donors with blood group B positive (2.47%). Screened blood bags received from other transfusion centers showed higher rates (4.27%) of hepatitis viral infections.

Conclusion: These results indicate that most of the patients suffering from hepatitis B and hepatitis C do not realize that they have got such deadly viruses in their body and can transmit it to non-infected healthy individuals via different parenteral routes including blood transfusion. The rate of infection of hepatitis B and C found in this study is low compared to other regional studies. One possible explanation could be the introduction of pre-donation filtration by questionnaire method which has been found to be an effective tool in addition to routine virological screening using high quality standard methods.

Key words: Hepatitis B, hepatitis C, transfusion, blood screening, ELISA.

INTRODUCTION

Hepatitis B virus infection (HBV) is responsible for an estimated 1-2 million deaths worldwide every year and around 300 millions are carriers for HBV out of which around 80% reside in Asia¹. In southern parts of Saudi Arabia, the prevalence of hepatitis B is about 10%, with delta virus (HDV) occurring both as co- and superinfection² while the prevalence of HCV is 1.4% among healthy blood donors³. Acute hepatitis B has a fatality rate of 1% while 10% of this infection progresses to chronic liver disease leading to cirrhosis and might end up with hepatocellular carcinoma (HCC)⁴. In a study from Karachi, 67% of patients infected with HBV and 33% with hepatitis C (HCV) developed HCC⁵. In Pakistan the rate of HBV and HCV infection ranges from 8-15% in general population with variations in the frequency and characteristics in various parts of the country^{6,7}. Varying prevalence rates for vertical and familial transmission have been reported for hepatitis C but major studies have failed to provide convincing evidence, ranging from 0.07% in England to 25% in Egypt. In general, significantly higher prevalence rate has been reported from Africa, the Middle East and Southeast Asia as compared to those from Europe, the United States or Australia⁸.

It is estimated that 150 million people are infected worldwide with HCV⁹ and approximately 0.5% of the blood donors¹⁰. HCV can also produce acute and chronic hepatitis progressing to cirrhosis and hepatocellular carcinoma¹¹. The most common route of transmission of hepatitis B and C is blood transfusion but it cannot be considered the only source of spread of hepatitis viruses as at maximum only 1% of general population receives blood transfusion in a year¹². Some of the patients are dependent on blood transfusions such as patients suffering from haematological disorders requiring replace-

ment therapy, patients undergoing major surgical procedures i.e. cardiac surgery and those with chronic renal failure.

Four percent prevalence of hepatitis C infection among first time illiterate family blood donors, vs. 0.7% among higher secondary school literate blood donors and 5% prevalence of hepatitis C infection among first time family blood donors residing in low socio-economics localities of Karachi, comparing to 1% among first time family blood donors staying in more urban part indicate some correlation of HCV infection with low socio-economic conditions¹³. The presence of HCV-RNA in semen is a strong argument in favour of HCV sexual transmission from men to women but HCV viral loads detected in semen were low, which suggests that the risk of HCV sexual transmission is probably also low¹⁴. Other routes of transmission such as sharing of the needles, razors, tooth brushes, injections and blood transfusions, accidental needle stick injuries in health-care workers, and organs for transplant from infected donors have all been implicated as routes of transmission for hepatitis B and C^{15,16}.

HCV accounts for 80-90% of post transfusion hepatitis although other hepatitis viruses such as HGV has also been isolated from 4% blood donors of Queensland¹⁷. Therefore screening of blood for antibodies to HCV is very important to control the spread of this rapidly spreading hepatitis. Testing for HCV RNA is also helpful in patients in whom Enzyme linked Immunoassay (EIA) tests for anti-HCV are unreliable. For instance, immunocompromised patients may test negative for anti-HCV despite having HCV infection because they may not produce enough antibodies for detection with EIA. Likewise, patients with acute hepatitis may test negative for anti-HCV when the physician first tests. Antibody is present in almost all patients by one

month after onset of acute illness; thus, patients with acute hepatitis who initially test negative may need follow up testing. In these situations, HCV RNA is usually present and confirms the diagnosis.

The purpose of this study was to improve the transfusion services in NWFP and prevent the deadly complications associated with the outcome of hepatitis B and hepatitis C. A prospective study was undertaken in Rehman Medical Institute (RMI), Peshawar, NWFP to investigate the frequency of hepatitis C and hepatitis B in healthy blood donors of NWFP. RMI is a 200 beds general hospital. Majority of the patients were admitted for cardiac surgery during the one year period of this study. Cardiac surgery is an expensive procedure and individuals from high socioeconomic conditions can afford it. Majority of the donors tested in this study were friends or relatives of these patients, therefore this population sample is representing educated and affluent class of the society. Donors were selected after a thorough interview and filling up of a performa.

MATERIAL AND METHODS

A total of four thousand donors were screened for hepatitis B and hepatitis C, using IMX or AxSYM, MEIA technology (Abbott, USA).

Selection of donors

Healthy individuals aged between 17-55 years of age without any previous history of jaundice, tuberculosis, heart, lung or kidney disease, prolonged fever, convulsions etc., and no history of major surgery, dental procedures, transfusion in the past 6 months, or blood donation during last three months and not receiving any drugs or current medication. Donors with Hb < 12.5 gm/dl or blood pressure > 145/95 mm Hg were also rejected.

Specimen collection and preparation

Fresh blood specimens were collected in vacuette (Greiner Bio-One Austria) from the donors for processing. Specimens were handled gently to avoid haemolysis of blood. After clotting of blood, serum was separated by centrifugation for 20 minutes initially at 3500 rpm (Labofuge, Heraeus, Germany). Serum was transferred into a second tube and centrifuged again at 10,000 rpm (Heraeus Germany) for 20 minutes to remove most of the medium weight interfering substances.

How does the Microparticles Enzyme Immunoassay (MEIA) Technology works?

IMX / AxSYM (Abbott Diagnostics, Abbott Park, USA) was used for the determination of HBs Ag or Anti HCV Antibodies according to the instructions of the manufacturer. The IMX / AxSYM uses MEIA technology where microparticles are coated with a capture molecule specific for the analyte being measured. During the reaction the analytes are bound to the microparticles creating an immune complex. The immune complex irreversibly binds to the glass fiber matrix. Alkaline phosphatase labeled conjugate is added to the matrix which binds to the immune complex to complete the antibody-analyte-conjugate "sandwich". This conjugate catalyzes the hydrolysis of the fluorogenic substrate 4-Methylumbelliferyl phosphate (MUP) to the 4-Methylumbelliferone (MU). The MEIA optics measure the rate at which MU, the fluorescent product, is generated on the glass fiber matrix. The rate at which the MU is generated on the matrix is proportional to the concentration of analyte in the test sample.

Proper quality control procedures were followed in the lab. as recommended by manufacturer (Abbott laboratories, USA). Calibration of each kit was carried out according to manufacturer's instructions.

Daily, weekly, and monthly checks of equipment were carried out. Proper handling and storage of the samples was done. Positive and negative controls were used daily in every assay.

Interpretation of the results

Specimens with S/CO values <1.00 were considered non-reactive for HCV (S/CO = sample rate/cutoff rate). Cutoff rate = Index calibrator mean \times 0.12 on AxSYM for HCV. Specimens with S/CO between 0.8-1.2 were considered borderline greyzone. In the blood bank of RMI, specimens with S/CO > 0.7 were also discarded, though those donors were issued a negative result according to the recommendations of manufacturer. Greyzone borderline cases were retested after a second centrifugation at 1000 rpm for 10 minutes.

Specimens with S/N >2.00 were considered reactive for HBs Ag on AxSYM (S/N = Sample rate/Index calibrator mean rate).

RESULTS

In every transfusion services setup whether Government or private, cost effectiveness is the primary objective of the service provider. An effort was made to extensively scrutinize healthy blood donors before donating their blood using a standard method keeping in mind quality first and cost later. Other methods of screening i.e. Latex agglutination and immunochromatographic techniques are less specific and less sensitive compared to ELISA and give higher false positive results¹⁸. That was the reason MEIA technology was used for screening because it was found to be more sensitive and specific for the detection of hepatitis B and hepatitis C. Specificity in random blood donors on AxSYM HCV version 3.0 is reported to be 99.84% and on IMX / AxSYM HCV version 3.0 is 99.98%¹⁹. Cut off rate and greyzone borderline cases were calculated as

per manufacturer's recommendations. Patients with repeated borderline greyzone results were requested to repeat the test after 4-6 weeks interval or confirm the results by Western blot for the presence of antibodies.

A total of 4000 healthy, adult donors (between the age of 17 years and 55 years) were included in this study. Out of 4000 donors, 47 were female and 3953 were male. Only 65 donors were volunteers, the rest were relatives or friends of the patients. Out of 4000 donors 123 (120 male and 03 female) were Afghans and the rest were Pakistanis belonging to various districts and provinces (Fig. 1). Out of 123 Afghans, 10 were found to be reactive for HBs Ag (8.13%) and 3 were found to be reactive for HCV antibodies (2.43%). Statistically significant difference was found between the prevalence rate of hepatitis B among Afghani and Pakistani donors when Chi squared test was applied (χ^2_{cal} 0.003) but no statistically significant difference was found between the prevalence rate of hepatitis C (χ^2_{cal} 0.28).

Frequency of hepatitis B and hepatitis C in different blood groups is given in Table 1. According to the results obtained, the frequency of hepatitis B infection is 1.9% and the frequency of HCV antibodies is 2.2%. The frequency of HBs Ag among healthy female donors is high (6.38%) compared to male (1.92%). However this difference was statistically not significant (χ^2_{cal} 0.011). The frequency of HCV antibodies is more common in male (2.2% vs. 0.0%) compared to female (Table 1). Hepatitis B infection is more common in blood group "B" positive (2.47%) followed by "A" negative (2.27%) and "B" negative (2.27%). Hepatitis C infection is more common in blood group "B" negative (6.81%) followed by "B" positive (2.79%) and O positive (2.03%). Overall the rate of infection of these viruses is higher in blood group "B" compared to other blood groups (Table 1).

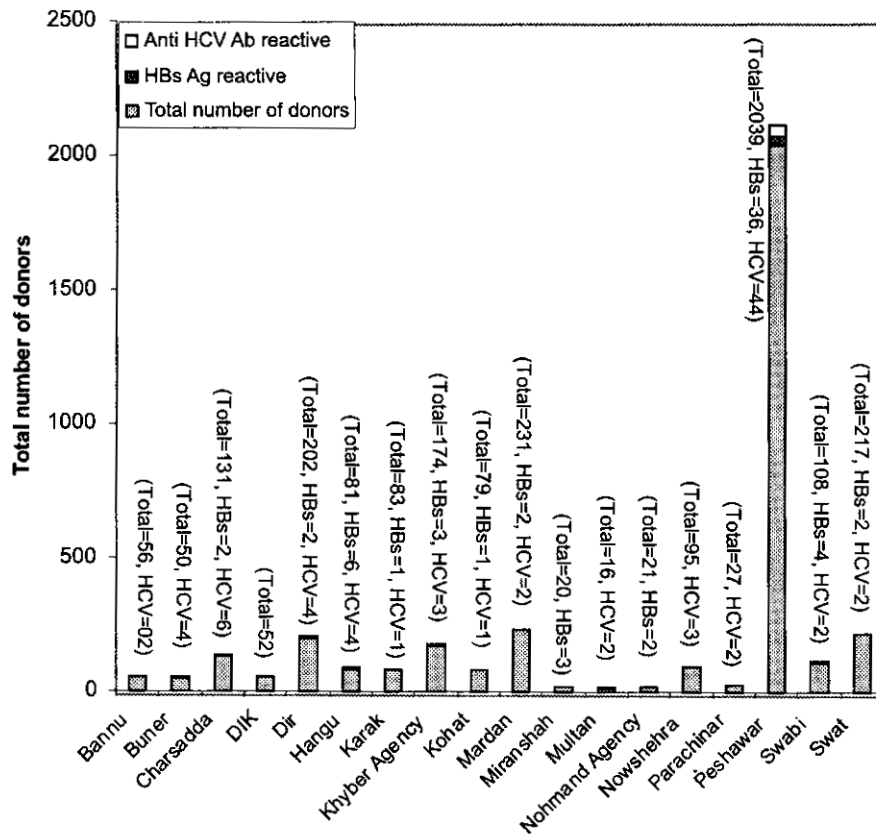


Fig. 1: Prevalence of HBsAg/Anti HCV Ab in healthy blood donors of NWFP – District wise distribution.

DISTRIBUTION OF HBS AG AND ANTI HCV AB IN 4000 HEALTHY BLOOD DONORS OF NWFP WITH RESPECT TO DIFFERENT BLOOD GROUPS

Blood Group	Total number	HBs Ag Reactive				Anti-HCV Ab Reactive			
		Male	Female	Total	% age	Male	Female	Total	% age
A Positive	991	16	00	16	1.61	17	00	17	1.71
B Positive	1251	30	01	31	2.47	35	00	35	2.79
AB Positive	333	05	00	05	1.50	06	00	06	1.80
O Positive	1079	17	01	18	1.66	22	00	22	2.03
A Negative	88	01	01	02	2.27	00	00	00	0.00
B Negative	88	02	00	02	2.27	06	00	06	6.81
AB Negative	58	00	00	00	0.00	01	00	01	1.72
O Negative	112	02	00	02	1.78	01	00	01	0.89
Total	4000	73	03	76	1.9	88	00	88	2.2

TABLE – 1

Interestingly, when blood bags were received in emergency patients from local transfusion centers in the Government or private sector, 4.27% of these already screened blood bags came out to be positive for anti HCV Ab (2.57%) or HBs Ag (1.7%). All these blood bags were screened by these blood banks using low quality screening methods and were labeled as non-reactive for anti HCV Ab and HBs Ag. Total number of bags received in the blood bank of RMI were 117, out of which two came out to be reactive for HBs Ag and three came out to be reactive for anti HCV Ab.

Geographical distribution of blood donors is given in Fig. 1. Majority of donors belong to Peshawar, and its suburban districts of Mardan, Nowshehra, Swabi, Charsadda, Kohat, Bunnu, DI, Khan Dir, Hangu and Karak etc. (Fig. 1)

DISCUSSION

National progress cannot be achieved without a healthy nation. Our nation is confronted with a large number of health related problems, including deadly viral infections of the liver, known as HAV, HBV & HCV etc. Some of these conditions lead to chronic state and leave the victim physically inactive and non-productive. Two of these conditions (HBV, HCV infections) are highly prevalent in the age group of 30-55 years, the real productive lifetime. One patient suffering from hepatitis in a family drains out financial resources of the whole family. A patient, in our set-up, if hospitalized, keeps at least two healthy family members, off their work. If we calculate total number of infected individuals in Pakistani population, using our results (combined 4.1% infection rate for both HBV & HCV) then around 5.658 million infected individuals are suffering either from hepatitis B (2.622 million) or hepatitis C (3.036 million).

The cost of pre treatment investigations of HBV or HCV investigation, amounts to @ Rs. 10,000/-, while full treatment with alpha Interferon is @ Rs. 100,000/-. An ordinary middle class citizen of a poor nation like Pakistan is in no way in a state to afford such a huge financial burden with prevailing economic conditions²⁰. If we calculate the cost of treatment for only 10% of those infected (keeping in view results of this current study, 4.1% total rate of infection both for hepatitis B and C) it turns out to be Rs. 56.58 billion. If the problem of deadly viral hepatic diseases is not attacked from all fronts in an integrated manner, we are going to have a sick nation in the next 15-20 years. This will also drain out our major chunk of the national financial resources.

If we succeed in establishing an integrated network of quality Biochemistry, Hematology, Histopathology, Cytology, Microbiology and finally link them all into one biotechnological sphere, we will help a lot not only in correct diagnosis but also proper management. If the cost of the treatment of a hepatitis patient is out of the reach of an ordinary citizen of Pakistan then the best strategy is to control its spread. There is no way to prevent transmission of hepatitis (particularly Hepatitis C) in a recipient of blood if contaminated blood or blood products are transfused. This was the reason why this study was initiated to introduce awareness towards quality transfusion services to help control the spread of these deadly viruses in the community.

In patients with chronic renal failure undergoing hemodialysis, blood transfusions, dialyzer reuse, all play a major role in the higher prevalence rate HBV and HCV infections, in hemodialysis patients²¹. Many methods for screening of hepatitis B and hepatitis C are now a days available in the market with different specificity and sensitivity. Even with the introduction of this strict criterion for the selection of donors who did

not have any history of jaundice or any signs/symptoms of hepatitis, 4.1% turned out to be positive for hepatitis B and hepatitis C virus (Table 1). It means that in our society many more healthy individuals are unaware that they have got a deadly virus in their body and that they can transmit it to their spouse and other individuals very easily via parenteral routes such as blood transfusion.

It is the policy of RMI that no blood or blood product will be accepted for transfusion brought from outside to RMI except in acute emergency cases when the required blood group is not available immediately. In the Government run hospitals where other methods are used for screening patients and donors, when blood bags were received in acute emergency for patients in RMI and were screened 1.7% of these bags were found to be reactive for hepatitis B and 2.57% for hepatitis C. These bags were already screened in the hospitals and were labeled non-reactive for hepatitis B and hepatitis C. If such infected blood is accepted safe and transfused to patients then transmission of these viruses will be promoted rather than prevented. Therefore it is strongly suggested to either improve or stop these presently applied methods of screening in these institutions/transfusion outlets.

The rate of infection of hepatitis B and C found in this study (1.9% and 2.2% respectively) is much less compared to other data from transfusion centers in this area. The prevalence rate of HBV (2.21%) and HCV (4.1%) in blood donors in Punjab, Pakistan is much higher to that seen in other countries. The prevalence amongst blood donors is 0.15% in Zimbabwe, in Malaysia 0.4% and in Argentina 0.9%²². According to others²³ 4% donors were found to be reactive for hepatitis B and 3.5% were found to be reactive for hepatitis C. Some investigators have reported much higher rates of anti-

HCV Ab in blood donors (20.89%)²⁴. This higher prevalence could be due to the use of low specific and low sensitive methods resulting in higher false positive and ultimately reflecting exaggerated prevalence rates¹⁸. This difference clearly indicates that pre-screening filtration of donors help in improving transfusion services and must be implemented in those centers where standard screening facilities are not available. False positive results due to difference in methodology and difference in the sample populations are two other explanations for higher prevalence rate in other centers.

Those patients who were found reactive either for hepatitis B and hepatitis C were referred to hepatologists for further management. In our study, we have found that individuals with blood group "B" are more prone to develop hepatitis B or C compared to individuals with other blood groups (Table 1). Further studies are required to investigate the higher prevalence rate of hepatitis in individuals with blood group B. In our study, we have also found that Afghans have got higher rates hepatitis B and C compared to Pakistanis (Table 1). The reasons may be less education, low socio-economic conditions leading to overcrowding and poor hygienic conditions of Afghans.

DNA based techniques such as polymerase chain reaction (PCR) offer improved diagnosis by differentiating between current and resolved infection. Due to its speed, sensitivity, specificity and simplicity, PCR has become the method of choice for the application in most diagnostic laboratories. The development of specific diagnostic assay on molecular level using PCR are essential to answer many questions on the epidemiology and pathogenesis of HCV, HBV and HGV virus. Although these tests are technically demanding and expensive but can answer many questions which are difficult to answer using other methods.

Prevention strategies to control the spread

Majority of the infectious diseases are curable and the treatment is inexpensive, but successful treatment requires timely diagnosis, proper and uninterrupted medication. As the signs/symptoms are vague and unreliable for the diagnosis of hepatitis B and hepatitis C, therefore proper screening is required for the whole population of this region. Only after proper screening, the actual frequency of these diseases will come to the surface. The incidence of post-transfusion hepatitis has decreased markedly since the implementation of donor screening for HBV, HCV, there still remains an irreducible minimum due to limitations of various techniques applied and ever increasing list of hepatitis viruses.

- Vaccine for hepatitis C is not available but hepatitis B could be controlled by proper vaccination. The Government has recently included hepatitis B vaccination in the national immunization programme for children under one year but must also run a mass vaccination programme for adults. Those particularly at high risk i.e. healthcare workers should be provided vaccination on priority basis.
- All those individuals vaccinated for hepatitis B need screening for antibodies titer, which if not develop with vaccine, needs either a booster dose of vaccine or full course of re-vaccination. Some cohort studies are required for investigating effectiveness of vaccine which has been introduced in the national immunization programme and which has been provided in the public sector organizations.
- All the blood transfusion services must follow internationally acceptable standards for screening blood and blood products. Less specific and less sensitive methods for screening will misguide everyone and will promote transmission of these viruses in population. Proper performance should be introduced for selection of donors in every setup of blood transfusion services. Screening results of donors in the blood bank must be carefully monitored by the supervisors. All the greyzone borderline cases (doubtful results with cut off rate close to reactive) must be rejected for blood donation. Selected donors must have very clear negative results. They must not possess even slight potential threat of transmission these viral diseases.
- The devices based screening should be discouraged and banned particularly in the blood transfusion services where the post-transfusion hepatitis has been reported as 80-90%²⁵. Cheap and unreliable devices for screening should be banned from the market and standard protocols should be followed for screening infectious viruses.
- Those patients who are found positive for hepatitis B and hepatitis C should be given proper counseling. If they are left as such without proper guidance they will ultimately infect more and more individuals and therefore the gravity of the existing problem will increase. Volunteers from Non-Governmental Organizations (NGOs) should be provided proper training and involved in this campaign.
- All those diagnostic outlets/transfusion centers in the private sector should be closed down where

proper pathologists/trained personnel are not available for carrying out such vital diagnostic tests. Because it will promote transmission of these infections instead of controlling them.

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