Screening Blood Donors for Antibodies to Hepatitis C Virus and Surrogate Marker

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The present study was designed to find out the association of surrogate marker (ALT) and HCV-antibody in blood donor population of Lahore. A sera of 186 blood donors were tested for anti–HCV by ELISA(ABBOTT). Out of 186 sera, 8(4.3%) were reactive for HCV. Risk factors like, H/O blood donation in last six months, showed 5.26% positivity in donors who had donated blood on previous occassions, than in index donation, 37.5% HCV–positive cases gave a past history of hepatitis; 12.5% positive cases gave H/O exposure (contact) to other hepatitis patients, while 2.8% of HCV–negative donor also gave H/O contact, but the number was too small to calculate any significance. In HCV positive donors, Liver function tests, in relation to ALT levels were raised (5/8) about 1 to 2 times above the normal range; and were normal in (3/8); while AST, Alkaine phosphatase and bilirubin levels were within normal range (BOEHRINGER MANNHIEM).

Key words: HCV, ALT

Hepatitis C virus has been identified as the major causative agent of transfusion–associated non–A, non–B hepatitis, and circulating antibodies of HCV reflects persistent infection rather than immunity (Esteban,1991). The incidence of post–transfusion hepatitis has decreased progressively during the last 25 years, since the exclusion of commercial donors and hepatitis B surface antigen(HBsAg)–positive blood donors. Most recently screening of potential donors for antibodies against the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) has also decreased transfusional acquired hepatitis. Despite these measures, post–transfusion hepatitis, usually of the non-A, non–B type, develops in 7% to 12% of recipients of blood and blood products.(Dienstag, 1983).

The Transfusion Transmitted Virus Study (TTVS), has recently reported a significant association between donor ALT and recipient non-A, non-B hepatitis. Data from the transfusion transmitted virus study(TTVS) and from prospective studies at NIH, predicted an efficacy of 29 to 30% in reduction of NANBH by excluding donors with elevated ALT at the loss of 1.3 to 1.6% of the donor population (Aach,etal,1981andAlter,etal,1981). Hence use of surrogate markers such as, antibodies to hepatitis B core antigen (anti–HBc) and elevated alanine aminotransferase levels(ALT), was recommended to screen for non–A, non–B hepatitis and thus to reduce further the incidence of post–transfusion hepatitis (Stevens et al, 1984 and Koizol et al, 1986).

Keeping this in mind the project was planned to find out the incidence of hepatitis C virus in our blood donor population and its association with ALT levels.

Patients and Method

The study was conducted on 186 blood donors, at random attending the blood banks of Mayo Hospital, Services Hospital and Sir Ganga Ram Hospital, Lahore. Brief H/O each donor was taken regarding :number of blood donations given within last six months, contact with hepatitis patients in last six months, any previous history of hepatitis and blood transfusion or administration of blood products.

Sample Collection: Peripheral venous blood samples were collected aseptically. The skin over the selected area was first cleaned with 2% iodine and then rubbed with 70%alcohol. Then about 10 ml of blood was withdrawn in a sterile disposable syringe. The blood was allowed to clot, then centrifuged at 300 rpm for 10 minutes. Serum was separated by using Pasteur pipette, and divided into two halves, one half was tested for liver function tests (Bilirubin, ALT, AST and AP levels) on the same day(According to the instruction of manufacturer–Boehringer Mannhiem). Other half of serum was stored in serum storage tubes, labelled and stored at 20°C, till testing for antibody to hepatitis C virus by Elisa kit of Abbott Laboratories.

Results

The sera of 186 blood donors was tested for anti–HCV by ELISA, the kit provided by Abbott HCV(r–DNA). The overall positivity for anti–HCV was 4.3% (8/186) Table 1.

In our study, donors who were HCV positive (8/186), showed that their Liver Function Tests in relation to ALT (5/8) were raised; while AST, alkaline phosphatase and bilirubin levels were within normal range. Table 2.

In our study regarding risk factor like H/O blood donations given in last six months, the sero–positivity was slightly higher in blood donors, 3(5.26%) were reactive out of 57 donors, who gave the positive H/O donating blood, than those who had not donated blood prior to the index donation. About 97% of donors who gave no H/O hepatitis (174/178) were also negative for HCV, but only 37.5%(3/8) positive HCV cases gave a past H/O hepatitis. About 12.5% (1/8) gave history of contact with hepatitis, while 5(2.8%) of HCV–negative donors also gave history of contact; but the number was too small to calculate any

significance. Only one donor gave H/O blood transfusion or blood product administration, but was sero–negative for HCV. Table 3.

Discussion

NANBH is the most common type of transfusion-related hepatitis in the world (Dienstag,1983). Since the development of a specific antibody test for HCV (Choo et al 1989 and Kuo,etal,1989), and the initiation of routine screening of blood donors in the West, has led to marked decrease in the incidence of post–transfusion hepatitis (Donahue,etal,1992 and Shakil,etal,1995). The initiation of routine screening of blood donors has also led to the identification of many persons with anti–HCV, who are asymptomatic, have no history of liver disease, and deny any risk factors for exposure to viral hepatitis. The clinical significacnce of finding anti–HCV in these other wise healthy persons is unclear. (Gronbaek,1994). Previous studies have shown that 60% to 80% of blood donors with anti–HCV have elevated amino transferase(ALT) levels ; in most cases these elevations are persistent, indicating the prescence of chronic hepatitis. Furthermore, most anti–HCV-positive blood donors can be shown by molecular techniques to harbor HCV RNA, in serum and can transmit HCV regardless of whether serum aminotransferases are elevated or not (Weiner,etal1990;Alberti,etal,1991, Esteban et al, 1991 and Alberti,etal,1992).

The present study was under taken to evaluate the frequency of anti–HCV in our setup of blood donor population and its association with ALT, in the anticipation that HCV carriers might be found with an increase frequency as compared with western countries. If we assume that the prevalence of anti–HCV in blood donors is indicative of the frequency of donors who may transmit HCV, our figures presented falls within the wide range quoted worldwide. Figures from Europe ranges 0.2 % in Finland, 0.68% in North Italy and 1.37% in South Italy(Sirchia,etal,1990). In North America 0.4 % to 1.0 %, 2% in Taiwan. 1.01% in Saudia Arabia (Bernvil,etal,1991). In Indonesia 2.3% in healthy blood donors, while in Egyptian paid donors 27% and 17% in unpaid blood donors. (Soetjipto,etal,1996 and Attia,etal,1996).

In our study, the surrogate marker, ALT, identified a relatively large group of donors, only a small sub-group of these were reactive in the HCV antibody screen test. While AST alkaline phosphatase and bilirubin levels were within normal range. This was comparable to study of Katayama (1990), who also noted that prevalence of HCV antibody was higher in blood donors with high ALT levels. In the same study, it was noted that, within normal ALT levels (less than 34 Karmen units), as ALT levels increased so did the likelihood of HCV antibody positivity; which was confirmed in blood with ALT values over 34 units. They also showed that the majority of antibody positive, normal blood donors were healthy but infectious carriers of HCV, and their blood should not be used for transfusion (Katayama ,et al, 1990).

In our study ALT levels showed wide range of fluctuation between normal to raised levels. Similarly in Taiwan, Lee (1991), noted no co-relation between fluctuations in serum alanine aminotransferase levels and anti – HCV titres. In Saudi Arabia, Bernvil (1991), showed among multi-nationals blood donors 5.5% units of blood were found to have raised levels of ALT, out of which 4.4 % were reactive to HCV. Similarly in Indonesia, Soetjipto (1996), showed that among healthy blood donors(2,234), 260 donors with raised ALT levels(8.8%) were reactive to HCV, while donors with normal ALT levels, randomly picked samples 1.4 % were HCV positive.

Table 1.

Total No.	HCV+Ve	HCV-ve	%age
186	8	178	4.3

Table 2. Liver function tests in HCV positive donors

Total Bilirubin (mg/dl)	ALT (IU/L)	AST (IU/L)	A) (IU/L)
0.50	80	47	55
0.50	17	29	55
0.50	13	22	63
1.20	40	35	71
1.60	103	38	68
0.90	120	39	70
1.40	95	31	40
0.80	64	29	50

Normal value:
ALT: 41IU/L
AST: 37IU/L
AP: upto 90 IU/L
Bilirubin: upto 1.0mg/dl
Kit Bochinger

Table 3. Risk factors

n=	HCV Status	H/O Bllod donations in last six months		H/O hepatitis		Contact with hepatitis patients	
		Multiple	First	Present	Absent	Present	Absent
8	+ve	3	5	3	5	1	7
		5.26%		37.5%		12.5%	87.5%
178	-ve	54	124	4	174	5	173
					97%	2.8%	97.2%
186		57	129	7	179	6	180
						3.2%	96.8%

Conclusion:

Transmission of Hepatitis B and C is parenteral, suggesting an equal risk of HCV infection in our community. Thus need of most effective measures to reduce its transmission, as no effective vaccine is available. This agent should be included among the list of high risk agents along with HBVand HIV etc., for the purpose of screening of blood in the blood banks. HCV is known for its chronicity leading to chronic liver disease, cirrhosis and even hepatocellular carcinoma in the recipients of anti–HCV positive blood in due course of

time. ALT levels showed variation between normal to raised levels, as noted by other workers world wide. This could be used as a marker for the elimination of HCV from blood donations and blood products, before being transfused to the recipients, till such time that our underdeveloped country, could afford a cheaper, cost-effective kit for its routine screening of blood donors. Despite its limitations, the ELISA is recommended to screen blood donors for anti–HCV.Sero-positive blood should be discarded. This measure will result in reduction in number of cases of post-transfusion NANBH, with minimal exclusion of blood donors. We hope, incorporation of additional HCV antigens into new HCV assays will improve the accuracy of the current ELISA test.

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