Prevalence & significance of hepatitis C virus (HCV) seropositivity in blood donors


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Background & objectives: The clinical significance of anti HCV antibodies in healthy blood donors remains uncertain. These donors are usually asymptomatic and it is difficult to elicit risk factors of acquiring HCV infection during pre-donation questioning. Limited information on donor recall and follow up studies on anti HCV positive blood donors have been reported from India. Paucity of data which is likely to have an impact on safe blood transfusion programme has prompted us to undertake this study to assess the significance of HCV seropositivity in blood donors with respect to their clinical, biochemical and virological profile.

Methods: A total of 16,250 blood units were screened for the mandatory tests using third generation ELISA (anti HIV 1&2, anti HCV, HBsAg), VDRL and peripheral smear for malaria. Donors reactive for anti HCV were informed. Repeat anti HCV reactive donors were subjected to detailed clinical history focusing on risk factors for HCV transmission. The blood tests included liver function tests (LFT), coagulation and autoimmune profile, qualitative serum cryoglobulins and HCV RNA detection. These donors were followed at 2-3 monthly intervals for a minimum period of six months by LFT.

Results: An overall seropositivity of 0.44 per cent (72/16,250) was observed in our donors which was significantly lower in first time, young voluntary donors as compared to replacement donors (0.27 vs. 0.60%). In contrast to drug abuse (6.4%) we found minor percutaneous routes like sharing of shaving kits or visit to a road side barber (32%) as the major risk factor for HCV transmission. There was no prior history of blood transfusion in any of these donors; however history of some surgical procedures was present in 25.8 per cent. Raised transaminases and HCV viraemia were observed in 87 and 71 per cent donors respectively. An association was observed between HCV RNA when the ELISA ratio was >5.

Interpretation & conclusion: Voluntary donors form a safe source of blood supply and efforts should be made to increase this precious source to 100 per cent. Abbreviated behavioural donor screening questionnaire for repeat donors is not advisable. Awareness and education of donors is required regarding modes of HCV transmission. HCV positive donors should be informed about their disease, counselled and referred to hepatologist, and permanently deferred for future donations.

Key words Anti HCV - autoantibodies - HCV viraemia - LFT - risk factors
Hepatitis C virus (HCV) is the major cause of post transfusion non-A, non-B hepatitis (NANB). It is estimated that 3 per cent of the world’s population or almost 200 million individuals have chronic HCV infection

The global seroprevalence of HCV among blood donors varies from 0.4-19.2 per cent. The seroprevalence of HCV in voluntary blood donors in India is between 0.12-4 per cent. The geographical variability of HCV seroprevalence can be explained by the extent to which different risk factors contribute to the transmission of HCV infection.

HCV infection can be determined by detecting HCV antibodies using enzyme linked immunosorbent assay (ELISA) which has sensitivity ranging from 90-97 per cent depending upon the kit. The use of third generation ELISA shortens the pre-seroconversion window period to 7-8 wk but does not distinguish among acute, chronic or resolved infection. HCV infection can be confirmed by using highly specific reverse transcriptase polymerase chain reaction (RT-PCR) for HCV RNA detection. This test can detect HCV RNA in serum within 1-2 wk following exposure. The clinical significance of the presence of anti HCV antibodies in the healthy blood donors remains uncertain. Various western studies suggest that 60-80 per cent of blood donors with anti HCV have elevated serum aminotransferases levels. The elevations in most cases are persistent indicating the presence of chronic hepatitis. Furthermore, most anti HCV positive blood donors have HCV RNA in serum and can transmit hepatitis C regardless of whether serum aminotransferases are elevated or not. The anti HCV positive blood donors are usually asymptomatic and have no symptoms that obviously relate to liver disease and deny any risk factors from exposure to viral hepatitis during pre-donation questioning. No study on donor recall and follow up of anti HCV were informed telephonically. Those who agreed to enter the study signed an informed consent and were subjected to further detailed clinical history, physical examination and blood tests.

(i) Risk factors for acquiring HCV infection: A detailed clinical history focused on various risk factors for acquiring HCV infection and for the presence of severity of liver disease.

(ii) The blood tests included repeat anti HCV testing, liver function tests (LFT), coagulation profile [prothrombin time (PT), activated partial thromboplastin time (APTT), prothrombin time ratio (PTR)], qualitative serum cryoglobulins by precipitation method at 4°C for 72 h and HCV RNA detection. Twenty ml of blood was collected at the initial visit and 5 ml at follow up. After the separation of the serum, a part of it was stored at -80°C for RNA processing and the remaining part was used for other tests.

(iii) Repeat anti HCV ELISA: Cut-off values in ELISA tests were calculated according to the manufacturer’s instructions. The ELISA ratio was calculated by dividing the test optical density to the
cut-off value. The criterion for inclusion in the study was samples which showed E-ratio >1 on two occasions. The initial ELISA reactive sera if found non-reactive during repeat testing were further tested both by recombinant immunoblot assay-2 (RIBA-2; LG Life Sciences Ltd, Korea) and HCV RNA. RIBA-2 though its sensitivity was less than that of RIBA-3, was still used because of inclusion of a more specific HCV RNA test along with it for confirmation. If results were negative by both the assays, the initial ELISA was considered false positive and the individual donor was counselled and thus not included in the study analysis.

(iv) Follow up by liver function tests (LFT): The repeat anti HCV positive donors were followed at 2-3 monthly intervals for a minimum period of six months by LFT.

(v) Detection of HCV RNA by RT-PCR: Qualitative HCV RNA was detected using in-house standardized nested RT-PCR (reverse transcriptase-polymerase chain reaction). Positive samples showed a single major band of size 270 base pairs when the PCR products were run on 2 per cent agarose gel containing ethidium bromide. The following primer sequences were designed from the conserved 5' - UTR region of the HCV genome:

(a) Outer sense: 5' - CTGTGAGGAAGCTACTGTCTT - 3' (20 mer)
(b) Outer antisense: 5' - ATACTCGAGGTGCACGGTCTACGAGACCT - 3' (29 mer)
(c) Inner sense: 5' - TTCACGCAGAAAGCGTCTAG - 3' (20 mer)
(d) Inner antisense: 5' - CACTCTCGAGCACCCTATCAGGAGCAGT - 3' (26 mer)

(vi) Autoimmune profile: The autoimmune profile included anti-nuclear antibodies (ANA), anti smooth muscle antibodies (SMA), anti mitochondrial antibodies (AMA), liver-kidney microsomal antibodies (LKM-1) by indirect immunofluorescence test. Serum rheumatoid factor was detected using commercial kit (Tulip Diagnostics, India) based on the principle of latex agglutination.

Statistical analysis: The data were statistically evaluated using Chi square test wherever appropriate.

Results

Of the 16,250 blood units screened, 72 were found reactive for anti HCV antibodies, giving an overall seropositivity of 0.44 per cent in our donor population. No anti HCV reactive donor was found co-infected with HIV or HBV infection. Of the 72 reactive donors, 38 responded back to the department after being informed, thus making an overall response rate of 52.7 per cent. A significantly lower HCV seropositivity (P<0.05) and a higher response rate (P<0.01) was observed in voluntary donors as compared to replacement donors (Table I).

Depending upon the number of previous donations, the voluntary and replacement donor groups were divided into first time and repeat donors. The HCV seropositivity in first time voluntary donors was 0.27 per cent (10/3617) as compared to 0.60 per cent (37/6109) in the first time replacement donors and this difference was statistically significant (P<0.05). Anti HCV reactivity of repeat voluntary donors and repeat replacement donors [0.39% (22/5645) vs. 0.34% (3/879)] was comparable.

<table>
<thead>
<tr>
<th>Table I. Anti HCV seropositivity and response rate according to donor characteristics</th>
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<tr>
<td>Type of donor (n)</td>
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<td>-------------------</td>
</tr>
<tr>
<td>Voluntary donors (9262)</td>
</tr>
<tr>
<td>Replacement donors (6988)</td>
</tr>
<tr>
<td>Total (n=16250)</td>
</tr>
</tbody>
</table>

P */<0.05; **<0.01 compared to replacement donors
HCV seropositivity increased with age in voluntary donors (Table II) while no such trend was seen in replacement donors. Though the overall seropositivity was significantly higher in replacement donors, the difference was most apparent in the age group 18-30 yr. Out of 38 HCV positive responders, seven were anti HCV, RIBA-2 and HCV RNA negative on repeat testing. These donors were thus not included in the analysis and appropriately counselled.

The various probable risk factors for acquiring infection were found in 25 out of 31 (81%) anti HCV positive blood donors as is depicted in Fig. 1. The most common risk factors studied in our donors for acquiring HCV infection was sharing of shaving kits or visit to a roadside barber (32%). This history was elicited more in rural than urban blood donors. There was no prior history of blood transfusion in any of these donors; however history of some surgical procedures was present in 25.8 per cent. Six donors gave no risk factor for acquiring the infection.

The 31 anti HCV reactive donors were divided into 3 groups based on mean ALT levels during three monthly follow up for at least 6 months to determine chronicity. (i) Group I (n=4): anti HCV reactive with normal ALT levels (2-40 IU/l); (ii) Group II (n=13): anti HCV reactive with ALT levels <2 times the upper limit of normal (41-80 IU/l); and (iii) Group III (n=14): anti HCV reactive with ALT levels > 2 times the upper limit of normal (81 IU/l).

Thus ALT levels were found to be elevated in 27 (87%) blood donors, thus indicating chronic hepatitis in majority of the blood donors biochemically. On serial follow up of these anti HCV positive blood donors, majority of them showed fluctuations in their ALT levels ranging from 2-10 folds.

HCV RNA was detected in 22 out of 31 anti HCV positive blood donors thus showing a detection rate of 71 per cent. HCV RNA was positive in acquiring HCV infection.

Table II. HCV seropositivity according to age distribution among blood donors

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Voluntary donors</th>
<th>Replacement donors</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No. screened</td>
<td>Anti HCV reactive (%)</td>
</tr>
<tr>
<td>18 - 30</td>
<td>5260</td>
<td>15 (0.28)</td>
</tr>
<tr>
<td>31 - 40</td>
<td>2548</td>
<td>10 (0.39)</td>
</tr>
<tr>
<td>41 - 50</td>
<td>1259</td>
<td>6 (0.47)</td>
</tr>
<tr>
<td>51 - 60</td>
<td>195</td>
<td>1 (0.51)</td>
</tr>
<tr>
<td>Total</td>
<td>9262</td>
<td>32 (0.43)</td>
</tr>
</tbody>
</table>

*P<0.05 compared to voluntary donors

Fig. 1. Risk factors in anti HCV reactive donors. Number in parentheses indicate donors eliciting risk factors. *donors (n=10) who gave history of more than one risk factors.
Of the 31 anti HCV positive blood donors, autoantibodies were detected in two. One donor had both rheumatoid factor (RF) and anti smooth muscle antibody (1:40) and in the other only RF was detected. Both of them had ALT levels more than two times the upper limit of normal with HCV RNA in the serum. No donor showed ANA, LKM 1, AMA or cryoglobulins in their serum.

Discussion

The efficiency of transmission of HCV through transfusion of a reactive unit is about 90 per cent. The risk of post transfusion HCV infection per unit transfused varies from 1:288,000 to 1:28,000 with an average of 1:103,000 using third generation ELISA. Thus, to ensure transfusion of safe blood to the recipient, not only mandatory screening of blood for such infections markers is necessary, it is also important to study the prevalence, age distribution and risk factors for causing HCV infection among the donor population. This will help to target and increase the low risk voluntary donor base and incorporate necessary changes in the donor questionnaire at the time of donor selection.

In the present study, anti HCV seropositivity was 0.44 per cent among healthy blood donors. Studies form northern India found HCV seroprevalence ranging from 0.53 to 5.1 per cent in their blood donors. HCV seropositivity from western India has been reported between 0.34 to 2.5 per cent. The variation in HCV seropositivity has also been seen globally due to differences in the donor base, testing methodology and its stringent regulation, the degree to which the risk factors are prevalent in the donor population, literacy rate and self exclusion by high risk donors.

HCV seropositivity was found to be lower in our voluntary donors as compared to replacement donors which is similar to another Indian study. Contrary to the above studies a Spanish study did not find replacement donors to represent an increased risk of window period donation. There was no significant

An association was observed between ELISA ratio and HCV RNA whereas this was lacking between ELISA ratio and ALT levels or between ALT levels and HCV RNA detection. When the ELISA ratio was < 2, HCV RNA detection was seen in 40 per cent of donors as compared to 85.7 per cent when the ratio exceeded 2. HCV RNA was seen in 100 per cent of donors when ELISA ratio was more than 5. When the ELISA ratio was <2, raised transaminases occurred in 70 per cent of donors but as ratio increased to >2, the corresponding transaminase derangement was seen in 95 per cent. As the ALT levels increased from normal to more than twice the upper limit of normal, HCV RNA detection increased from 50 to 71.4 per cent (Fig. 2).

Fig. 2. Association between ELISA ratio, ALT groups and HCV RNA detection.
difference in the frequency to conceal deferrable risk factors between the voluntary and replacement donors. However, in India voluntary donors are safer than replacement donors because they invariably are more educated and can better understand the implication of donor questionnaire. Replacement donors on the other hand, give blood under compulsion and thus may conceal answers relating to their health and sexual habits.

First time voluntary donors were found to be safer than first time replacement donors with regard to HCV infection. No difference in seropositivity was observed between first time and repeat donors because repeat donors were screened for the first time for HCV antibodies because of its mandatory introduction in India since June 2001\(^1\), and also there may be lack of awareness among both the donor groups regarding minor modes of HCV transmission like tattooing, ear/nose piercing or sharing of shaving kits. In another study from North India\(^1\), no difference in HCV seropositivity was observed between first time and repeat blood donors. Our results were in accordance with study by Retrovirus Epidemiology Donor Study (REDS) group\(^1\) who also found no difference in donor behavioural risk factors and incidence of HCV infection among frequent repeat whole blood donors than infrequent repeat whole blood donors (rate of donation <2 per year or donation count <2 in the preceding 2 yr). Thus, abbreviated behavioural screening questionnaire for repeat donors may not be advisable.

In the voluntary donor group minimum seropositivity was observed between 18-30 yr. Similar results have been reported in another study\(^1\). Thus, efforts should be made to increase and retain the young motivated voluntary donors to maintain safe blood supply. In our donor population, the seropositivity followed an increasing trend with age as most infections were seen among older age group. This is similar to the pattern reported in studies from Japan\(^2\) showing that the infection would have been acquired in the last few years due to the use of unsafe needles and contaminated equipment used in healthcare related procedures. In a recent study from western India, the HCV seropositivity in the general population was 0.87 per cent which also showed increased prevalence with age (0.31% in <10 yr to 1.85% in >60 yr)\(^3\). In contrast to the above studies, a study from north India showed a trend of decreasing HCV seropositivity with increasing age with a maximum prevalence rate of 1.8 per cent in the age group of 20-29 yr\(^4\). This difference implies that the risk factors and route of acquiring HCV infection may vary from one region to another and needs to be studied for incorporation in donor screening questionnaire.

Our study highlighted an important fact that percutaneous exposure through minor routes of transmission like sharing of shaving kits or visit to a roadside barber, surgery and multiple unsafe intravenous injections have played an important role in HCV transmission in our blood donors. The percentage of our donors reporting high risk behaviour like intravenous drug abuse and having multiple sexual partners was lower than that reported from Western literature\(^5,22\). The possibility that such high risk behaviour might have been concealed by some donors, there might have been difficulties in understanding the health questionnaire or recalling remote events and denial because of a desire to be tested cannot be excluded.

ALT levels were found to be elevated in 87 per cent and HCV RNA was found in 71 per cent of our anti HCV positive blood donors. In a community based study from India, HCV RNA was detected in 81 per cent of the anti HCV positive subjects, however only 31 per cent showed ALT elevation\(^2\). This apparent difference in HCV causing more severe liver injury in our donors needs future studies on HCV genotype infecting our population. The sample size of seroreactive donors who responded for confirmation of the diagnosis and follow up was small and thus future studies on larger sample size would help in drawing valid and better conclusions.
Treatment is generally recommended for chronic hepatitis C individuals who are at increased risk of disease progression such as those with elevated amino transferases, hepatitis C viraemia and at least portal fibrosis or moderate inflammation on liver biopsy. Only two donors underwent treatment for HCV infection who currently are non viraemic with normal transaminases one year post treatment. Some donors did not agree for treatment due to fear of liver biopsy, concomitant alcohol abuse and financial constraints whereas others did not fit into the above profile.

The percentage of HCV RNA detection increased as the ELISA ratio increased. Low ELISA ratios probably represent a false positive reaction. Since we had just qualitatively identified RNA, further studies are required to define the relationship between anti HCV titres and HCV RNA levels. Dufour et al. found a positive correlation between ELISA ratio and HCV RNA positivity when the former was more than 3.8.

Presence of autoantibodies in our donors with hepatitis C infection was lower than that reported in various studies. In an Indian study autoimmune markers were detected in 72 per cent of patients with chronic HCV infection as opposed to 6.5 per cent seen in our blood donors with anti HCV positivity. The lower detection rate of autoimmune markers in our study could possibly be due to the fact that majority of our donors were asymptomatic as compared to 70 per cent of the patients in the above study who had rheumatological symptoms. A study by Homerg et al. found LKM-1 to be present in only 0.07 per cent of their HCV patients.

In conclusion, it is essential to follow up the anti HCV reactive blood donors on two accounts - firstly for permanent deferral for blood donation and secondly for early management of the HCV infection. The initiative taken by Government of India (Action Plan of Blood Safety, 2003) to know the status of transfusion-transmissible infections to the donor is a step in the right direction.

References


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