

Prevalence of Markers of Hepatitis C virus among the Blood Donors

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ABSTRACT

Introduction: Hepatitis C infections continue to be a threat to safe transfusion practices. We analyzed the prevalence and trends of the Hepatitis C infection among voluntary and replacement donors in a 6.5 years retrospective study from January 2005 till June 2011 at the blood bank at Sri Guru Ram Das Institute of Medical Sciences And Research, Vallah, Amritsar, India. The donors were evaluated for the sero prevalence of the Hepatitis- C virus (HCV).

Methods: A total of 35793 healthy blood donations which were collected from January 2005 to July 2011 were screened for anti-HCV antibodies by using third and fourth generation (for confirmation) ELISA kits (HCV Microlisa; J. Mitra/ Eliscan HCV; RFCL Qualisa HCV; Qualpro) with a reported sensitivity and a specificity of 100% each (for the fourth generation kits).

Results: Out of these, 7089(19.8%) were voluntary donors and 28704 (80.1%) were replacement donors. 493 blood donors tested positive for HCV. The average prevalence in percentage was found to be 1.38%. The prevalence rate showed a decline from 2.03% in 2005 to 0.87% by June 2011. The statistical analysis which was done by using the Chi-square test (51.193), demonstrated that the decreasing trend in the prevalence of HCV was statistically significant.

Conclusion: The screening of blood products is the only way to prevent the transfusion associated complications and this should be rigorously implemented. There is a need to stress more stringent donor selection criteria to ensure a safer blood supply. The health authorities need to include hepatitis C on their radar as a disease which can result in significant morbidity and mortality in the years to come.

Key Words: Hepatitis C, Prevalence, Donors, ELISA(Enzyme Linked Immunosorbent Assay), TTI (Transfusion Transmitted Infection)

INTRODUCTION

The use of blood transfusion in clinical practice has brought untold benefits to countless individuals, but simultaneously, unsafe transfusion practices carry the risk of transmitting life-threatening Transfusion – Transmissible Infections [1]. The strategies which were used to reduce Transfusion Transmitted Infections (TTI) have been extremely effective. Some of the strategies include improving the donor selection, testing the donated blood for antibodies for infectious agents, reducing the exposure to allogenic blood by the use of autologous transfusions and changing the transfusion guidelines to use blood more conservatively [2]. TTI is a major concern to the patients, physicians and the policy makers who wish to see a risk free blood supply.

Hepatitis C is the major cause of acute hepatitis after a blood transfusion, that is neither related to Hepatitis A nor to Hepatitis B. It was first detected in 1989 by the extensive testing of serum from experimentally infected animals by using molecular biology techniques [3]. Later, it was characterized to be a small, enveloped, RNA virus which belonged to the flavin family. An important feature of the HCV genome is its high degree of genetic variability and the different mutants of the parent strains which co exist as quasi species in a single infected individual. The genomic instability and the antigenic variability have seriously hampered

the efforts which were made for developing an HCV vaccine. It has been estimated that 3% of the world's population or that almost 200 million individuals have chronic HCV infection [4]. Several studies on voluntary and mixed blood donors have noted a prevalence of hepatitis of below 2% in India [5,6].

Punjab has been found to have the maximum number of HCV carriers due to the use of unsterilized syringes and recycled needles by drug abusers and lack of awareness about the prevention and the treatment of this disease [7]. More recently, blood transfusion has emerged as one of the most common transmission pathways of HCV, which is the major cause of acute hepatitis after a blood transfusion, that is neither related to Hepatitis A nor to Hepatitis B. Serosurveys one of the primary methods to determine the prevalence of the HCV seropositivity. The assessment of the infection thereby helps in determining the safety of the blood products.

The present study was a six and a half year retrospective study. It presented the data on the seroprevalence of the Hepatitis C virus among the voluntary and the replacement donors in the Amritsar district of Punjab, in a teaching tertiary care hospital. This study gave an overview of the epidemiology/prevalence of Hepatitis C in this part of the country. The aim of this study was to lay stress on enhancing the standards of the blood banking system and to

make long term strategies to decrease the transmission of this deadly virus.

MATERIALS AND METHODS

This study was conducted in the blood bank, a unit of the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab, India. A total of 35,793 healthy blood donors (both voluntary and replacement) were reviewed for Hepatitis C among other mandatory tests, over a period of 6.5 years, from the records of the blood bank of the institute. The blood donations which were collected from January 2005 to July 2011 were screened for the presence of anti HCV antibodies by using third and fourth generation (for confirmation) ELISA kits (HCV Microlisa; J. Mitra/ Eliscan HCV; RFCL Qualisa HCV; Qualpro) with a reported sensitivity and a specificity of 100% each (for the fourth generation kits).

The third generation anti-HCV ELISA tests kits utilize a combination of antigens with the sequences of both the HCV structural and non structural antigens i.e. Core, E1, E2, NS3, NS4 and NS5, with increased sensitivity and specificity. The ELISA was validated by the acceptance criteria which were laid down by the manufacturer for the absorbance of the reagent blank as well as for the mean absorbance of the positive and negative controls which were provided with the test kits. The cut off value was calculated as per manufacturer's directions for reporting the positive and the negative results. Known positive and negative samples were used randomly as the external controls in each screening.

The donors were selected and screened thoroughly, as per the guidelines of the Gazette of India [8]. Professional blood donors and those with a previous history of jaundice were excluded.

All the blood donors were pre-counseled and they were asked to fill a form as a part of the routine blood donation screening procedure. In this form, the donors gave their consent and they authorized the blood bank to test their blood as per the law and to use it as it was deemed fit. The information regarding the risk factors like a history of surgery, hospitalization, blood transfusion, occupation, high risk behaviour and tattoo marks was specifically taken. All the reactive samples were repeat tested before they were labelled as seropositive.

The data retrieved was tabulated annually. The statistical analysis was done. Chi-squared (χ^2) statistics was used to estimate whether a significant difference existed between the prevalence of Hepatitis- C over the years or not.

RESULTS

A total of 35793 units of blood were collected over a period of 6.5 years. Among these, 19.8% were from voluntary donors and 80.2% were from replacement donors [Table/Fig-1]. The voluntary donations increased in number year over year, though the replacement donors still continued to comprise the major chunk of the donations. Even the number of donations gradually increased during the past 6 years from 4720 in 2005 to 6743 in 2010.

The %age of the HCV seropositivity showed a gradual downward trend from 2.03% in 2005 to 0.87% in July 2011, with an overall seropositivity of 1.38%. Chi-squared test = 51.193; Degree of Freedom (df) = 6; p-value = <0.001, which was significant. The year wise change in the Hepatitis C prevalence had declined over the years and it was statistically significant.

Year	Total	Voluntary	Replacement	HCV + ve cases	Prevalence in percentage
2005	4720	708 (15%)	4012 (85%)	96	2.03
2006	4570	754 (16.4%)	3816 (83.6%)	86	1.88
2007	4379	876 (20%)	3503 (80%)	80	1.82
2008	4900	931 (19%)	3969 (81%)	65	1.32
2009	6215	1429 (23%)	4786 (77%)	59	0.94
2010	6843	1471 (21.5%)	5372 (78.5%)	66	0.96
2011 till 30th June	4166	920 (22.1%)	3246 (77.9%)	42	1.008
Total	35793	7089 (19.8%)	28704	494	1.38

[Table/Fig-1]: Seroprevalence of voluntary and replacement donors

Place	Study period	No. of donors	Average Prevalence of HCV
Thakral et al., 2006 [5], Pgi, Chandigarh	June 2001 -Jan 2002	16250	0.44%
Garg et al., 2001 [10] Rajasthan,	June 2004 - May 1999	46957	0.29%
Adhikari et al., 2010 [11], Sikkim	2001- 2008	3735	0.27%
Bhattacharya et al., 2007 [12], West Bengal	2004- 2005	106695	0.35%
Gupta et al., 2006 [13], Armed Forces	2000 - 2004	39646	0.51%
Bhawani et al., 2010 [14] Andhra Pradesh	2004-2009	8067	0.84%
Gupta et al., 2009 [15] East Delhi	1997- 2002	157466	0.65%
Arora et al., 2010 [16] Southern Haryana	Oct 2002 - April 2006	10374	0.91%
Gupta et al., 2004 [17] Ludhiana, Punjab	Jan 2001 - Oct 2003	44064	1.09%
Pahuja et al., 2007 [18] New Delhi	2002- 2005	28,956	1.01%
Present Study	Jan 2005 - June 2011	35793	1.38%

[Table/Fig-2]: Comparison of various studies

DISCUSSION

In the present retrospective study, we evaluated the seroprevalence of the Hepatitis C virus among the blood donors in and around the Amritsar district. This study gave an overview of the epidemiology of the disease in the community. The figures from the epidemiology studies in different regions of India were compared [Table/Fig-2]. The data will help in evaluating the seroprevalence of the Hepatitis C infection in India.

The Hepatitis C infection is spreading fast in India. Over one lakh people get infected by the deadly HCV virus every year. According to the Indian National Association for the Study of the Liver, nearly 12.5 million Indians are suffering from the Hepatitis C disease, with the death rate exceeding over one lakh. Hepatitis C has increasingly been found to be a significant aetiological agent

which causes liver disease in India. The clinical manifestations include acute hepatitis, chronic hepatitis, cirrhosis and hepatocellular carcinoma [9]. The Hepatitis C infection is one of the transfusion transmissible infections and hence, it is mandatory to test all the blood donors for its presence. To ensure the transfusion of safe blood to the recipient, not only a mandatory screening of such infection markers is necessary, but it is also important to study the prevalence and the risk factors of the HCV infection among the donor population.

In the present study, the number of HCV positive cases dropped from 96 in 2005 to 66 in 2010. The anti-HCV seropositivity also came down from 2.06% to 0.96% in 2010 and till mid 2011, it was found to be 1.008%. The average seroprevalence of HCV over a period of 6.5 years was found to be 1.38%. A similar study in Chandigarh observed an overall seropositivity of 0.44% (72/16,250) among the blood donors. There was no prior history of blood transfusion in any of these donors; however, a history of some surgical procedures was present in 25.8% of the donors. Another study in Rajasthan evaluated the seroprevalence of the blood donors to be 0.29%. However, the data in this study was collected in 5 years from 3870 donors [10].

Studies from eastern India, one from Sikkim, detected the seroprevalence of HCV to be 0.27% among 3735 blood donors [11]. The other from West Bengal detected an increased prevalence of HCV from 314 in 2004 to 372 in 2005. The percentage increase was from 0.28% in 2004 to 0.35% in 2005, which was statistically significant [12].

A similar study which was carried out among the Armed Forces showed that the seropositivity for anti-HCV had decreased amongst the voluntary donors from 0.46% to 0.20% and that in the replacement donors, it had increased from 0.43% to 0.65% in a span of 4 years from 2000 till 2004 [13]. The study emphasized the implementation of strict donor criteria and the use of sensitive laboratory screening tests to reduce the incidence of TTI in Indian scenarios.

A study from Andhra Pradesh showed a higher seroprevalence of HCV in the blood donors. The study estimated the prevalence to be 0.84% from a total of 8067 donors over a six year period from 2004 to 2009. In this study, the prevalence of HCV among the donors showed a downward trend over a period of six years [14]. Our present study showed similar results, in which the prevalence of HCV among the donors also showed a decreasing trend over a period of more than 6 years from 2.03% in 2005 to 1.008% by mid 2011. A study from northern India, from east Delhi, showed that the prevalence of the antibodies for HCV ranged from 0.65% [15]. The anti HCV positivity was found to vary from 0.91% to 1.09% (483/44064) in two other studies [16,17].

The prevalence of the seropositivity for anti-HCV in a 4 year study which was done by Pahuja et al., was found to 0.66%. The study showed a decline of the seroprevalence of HCV from 1.01% in the year 2002 to 0.29% in 2005 [18].

The seroprevalence in the present study has also reduced over the years. The number of new infections have dropped, probably due to the identification of the virus and due to the increased awareness of the blood borne pathogens. This has reduced the spread of the virus through transfusion, injection drug use, unprotected sexual activity and workplace exposure incidents.

A variation in the seropositivity was also seen due to the difference in the donor base, the testing methodology and its stringent regulations, the degree to which the risk factors are prevalent in the donor population, the literacy rate and the self exclusion by the high risk donors.

There are studies which raise serious concerns regarding the Hepatitis-B virus (HBV) and the HCV prevalence in our country. Although the HBV transmission is showing a decreasing trend, it cannot be relied upon, because the donors were screened only for the HBsAg. In fact, the inclusion of the antibody to the Hepatitis B core antigen and other sensitive markers to the screening protocol, and better donor recruitment is the need of the hour. HCV is clearly on the rise. An increasing trend in the seroprevalence of Hepatitis C shows that it may replace Hepatitis B as the most common cause of liver failure [19].

Currently, the blood donations are screened only for anti-HCV. The screening for the HCV RNA is at present, not practical, and probably not appropriate in most of the cases. A major problem that was being faced by the transfusion services and the laboratories at the start of the anti-HCV screening was the generally poor specificity of the anti-HCV screening assays. A second problem was that true confirmatory assays were not yet available.

The prevalence of HCV, as was apparent from this study, has gradually declined. But still, the seropositivity of HCV in the blood donors of Amritsar is high as compared to that which was found in the studies from other parts of India. The data in [Table/Fig- 2] presents the picture of the prevalence of the Hepatitis C infection in various regions of India.

The reasons for the increasing trend in most of the studies are widespread drug abuse and lack of awareness about the prevention and treatment of Hepatitis C, especially in rural areas. Besides the difficulty in developing a vaccine against HCV, there is an added handicap that the elevated titres of anti-HCV IgG which occur after the infection do not confer active immunity. Moreover, HCV is able to actively evade the Interferon mediated cellular antiviral response. And to add to the problem, the window period for HCV has a long range (6-12 weeks), during which anti HCV cannot be detected in the blood, thus making the blood donation very dangerous and life threatening for the recipient.

The clinical significance of the presence of the anti-HCV antibodies in healthy blood donors remains uncertain. The anti-HCV positive blood donors are usually asymptomatic and they have no symptoms that obviously relate to liver disease and deny any risk factors from the exposure to viral hepatitis during the pre-donation questioning. Various western studies have suggested that 60-80 per cent of the blood donors with anti-HCV have elevated serum aminotransferase levels [20,21]. The elevations in most of the cases are persistent, indicating the presence of chronic hepatitis. Furthermore, most of the anti-HCV positive blood donors have HCV RNA in the serum and they can transmit Hepatitis C, regardless of whether the serum aminotransferases are elevated or not [22]. The HCV positive donors should be informed about their disease, counseled and referred to a hepatologist. They should also be permanently deferred for future donations [5].

CONCLUSION

The given data raises serious concerns regarding the HCV prevalence in Punjab. Although HCV showed decreasing trends, there

is an immediate need of developing locally relevant guidelines for the counseling and the further management of the HCV seropositive donors. Prevention is the only effective cure which is available at present for the HCV infection and so in such scenarios, propaganda and education of the masses regarding the prevention and the consequences of acquiring the HCV infection becomes extremely important. This increasing trend should be considered seriously and alarming bells should be raised by the health authorities. The screening and the treatment of blood products is the only way for preventing the transfusion associated cases and these should be rigorously implemented. The introduction of donor counseling, screening for different pathogens and donor self exclusion and ensuring a 100% voluntary blood donation will be effective in decreasing the hepatitis rate. The results of these studies will help in improving the standards of the blood banking systems in India and long term strategies can be made to improve the public health and to decrease the transmission of this deadly virus.

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