Section: Transfusion Medicine

Research

SEROPREVALENCE OF HEPATITIS B AND C AMONG BLOOD DONORS - A RETROSPECTIVE CROSS-SECTIONAL STUDY FROM A TERTIARY CARE CENTRE IN SOUTH INDIA

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Abstract

Background: Chronic liver disease associated with viral Hepatitis B and C is a major cause or mortality and morbidity worldwide. In developing countries like India, delay in diagnosis and treatment can further aggravate the burden of the disease. As screening for both types of Hepatitis is a mandatory part of blood donor screening and blood donors reflect the general population of a region, estimating the seroprevalence in blood donors helps understand the disease load of the region. This five year retrospective study, aimed at estimating the prevalence of Hepatitis Band C among Blood donors and detecting any regional trends in disease pattern was conducted at the Department of Transfusion Medicine, Government Medical College, Kozhikode, Kerala, India. **Materials and Methods:** This was a five-year retrospective cross-sectional study from 1st January 2015 to 31st December 2019 on all accepted donors who bled at the Department of Transfusion Medicine, Government Medical College, Kozhikode, Kerala, India. Donor Selection was based on the latest Drug Controller and National Blood Transfusion Council guidelines. Both Hepatitis B and C were screened by the ELISA method using standardized kits approved and supplied by the Government of Kerala for hospital use. All seroreactive cases were subjected to a second confirmatory test as per the Standard Operating Procedure to confirm true positives. Statistical analysis, Chi square test with *p* value<0.05 as significant was carried out using computer assisted software SPSS 17. **Result:** Over the five year study period seroreactivity of Hepatitis B was 0.13% and for Hepatitis C it was 0.03%. None of the donors were positive for both types of Hepatitis. Apart from a dip in seropositivity for Hepatitis B in 2018 the positivity rate for both types were almost consistent over 5 years. Of all donor factors analyzed voluntary donation was the only donor characteristic found statistically significant. **Conclusion:** Low prevalence of both types of Hepatitis, with Hepatitis B being more prevalent than Hepatitis C was noted in this study. Voluntary donation being statistically significant stresses the need for voluntary donors over replacement.

INTRODUCTION

Viral Hepatitis B and C are a major cause of morbidity and mortality worldwide due to association with chronic liver disease, both as Cirrhosis and Hepatocellular carcinoma. Prevalence varies in different geographic regions with Eastern Mediterranean and African regions being maximally affected as per WHO Global Hepatitis report 2021.[1] As per the report, with data available at the end of 2019, 296 million people were living with chronic Hepatitis B virus (HBV) infection and 58 million people with chronic Hepatitis C virus (HCV) infection worldwide. Newly acquired infections per annum stand at around 1.5 million people- both for Hepatitis B & C and 96% of deaths from viral hepatitis were also caused by these viruses.[2]

A major challenge faced in developing countries with high incidence is timely diagnosis together with access to affordable and uninterrupted treatment. One aim of the WHO global report 2021 is 90% of chronic viral Hepatitis B and C diagnosed by 2030.[1]

Targeting vulnerable groups with screening tests is a strategy forward; together with Immunization against Hepatitis B.[1] Target groups can be focused through the major modes of transmission in both these types

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of Hepatitis, such as unprotected sex, transfusion of infected blood/needles and vertical transmission from mother to child. Several countries including India have included Hepatitis control as part of their National Health Program.

As far as a Transfusion centre is concerned, even an asymptomatic carrier of these viral infections is capable of transmitting the disease and herein lies the importance of screening all the donated blood in a blood bank for these viral markers.[3-11] Screening offers a double advantage in that it not only can reduce the risk of transmitting Hepatitis through blood transfusion, but it also gives information about the prevalence of the disease in the community.

The Blood Centre at Govt. Medical College Kozhikode, caters to around 28,000 blood donors annually. All donated Blood is screened for Hepatitis B and C by ELISA (Enzyme Linked ImmunoSorbent Assay) method. A five-year retrospective study of the donors who have bled here is aimed at for identifying the prevalence of Hepatitis B and C in the region and to identify any trend in the disease in the last five years.

MATERIALS AND METHODS

This was a retrospective cross-sectional five-year study from January 1st2015 to December 31st2019 on all donated blood, both Whole blood as well as Apheresis at the Department of Transfusion Medicine, Govt. Medical College Kozhikode, Kerala, India.

The study had two objectives; to assess the prevalence of Hepatitis B and Hepatitis C among blood donors screened by ELISA method over a five-year period and to identify any trend in the disease pattern among the seroreactive cases identified.

The Standard Operating Protocol (SOP) for Donor screening in the Blood Centre was as per the latest Drug Controller/ National Blood Transfusion Centre/National AIDS Control Society (NBTC/NACO) guidelines,[12,13] amended from time to time. All Prospective donors were also counseled by trained Blood bank counsellors about high risk behavior and provided the option of self-deferral.

Only donors who met the standard criteria were accepted for blood donation. In addition to medical history, this included weight above 45kg, Hemoglobin level of ≥12.5g/dL, and normal vitals like blood pressure, pulse rate and temperature. All donated blood was screened for the recommended five transfusion transmissible infections as per the National policy. This included both Hepatitis B and C, for which the Drug Controller rules states that blood be non-reactive for Hepatitis B surface Antigen (HbsAg) and Hepatitis C Virus Antibody (HCVAb).

Screening for HbsAg and HCV Ab was done by the Enzyme Linked Immunosorbent Assay Method (ELISA). The test uses the direct 'sandwich' principle to immobilize antigen-antibody complexes onto a solid phase, which are polystyrene microtitre plates. The unbound material is removed by washing. An antibody labeled with an enzyme/ co-enzyme usually horseradish peroxidase (HRP) with a tetramethylbenzidine substrate is added. Binding results in antibody-antigen-antibody/enzyme conjugate complex and is followed by a wash step. A light-blue color is developed which becomes yellow when the reaction is stopped with sulfuric acid. Color change is proportional to the amount of bound enzyme and the activity is detected by measuring the signal emitted by the complexes. The HbsAg ELISA kits use Anti-HBs antibody as capture antibody and HCV ELISA kits use a mixture of core, NS3, NS4 and NS5 antigen as capture antigens and enzyme-labeled anti-human IgG as conjugate.

Standardized kits which were approved and supplied by the Government of Kerala for hospital use were used. All seroreactive cases were subjected to a second confirmatory test as per the Standard Operating Procedure to confirm true positives. The true positive (seropositive twice) cases identified were recalled, counseled and referred to the gastroenterology department of the hospital for further management. All Blood bags from these donors were pulled out from the quarantine/ unscreened storage, autoclaved and discarded.

For this study, the blood donor screening records of all the donors during this period were retrospectively reviewed for obtaining relevant data. Standard Abstraction form (Performa) was used for data collection [**Table1**].

The main donor characteristics studied included- Age, Sex, Type of donation (Voluntary OR Replacement/Directed), First time OR Repeat Donor, State of residence, District if Keralite (importance given to parent district of Blood centre, Kozhikode and three immediate neighboring districts of Malappuram, Wynad and Kannur.

Seroreactivity of Hepatitis B and C, association with other transfusion transmitted diseases tested (HIV 1&2, Syphilis and Malaria) and call back pattern to Department of transfusion Medicine was also analyzed.

Data entry was done and statistical analysis carried out using computer assisted software SPSS 17. Tests of significance used - Chi square test and a p value of less than 0.05 was considered significant.

RESULTS

During the study period of 5 years from January 1st 2015 to December 31st 2019 there was a total of one lakh forty-three thousand five hundred and sixteen (1,43,516) blood donations which included both whole blood and apheresis donations. Blood donations at the blood centre as well as from the outreach voluntary camps conducted during this period were included. The analysis of general blood donor data is given in [**Table 2**].

All donated blood was tested for five transfusion transmitted diseases - HIV 1&2, Hepatitis B,

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Hepatitis C, Syphilis and Malaria. Since this study was on Hepatitis among blood donors, these were reviewed and donor records showed 182 (0.13%) cases of HbsAg and 40 (0.03%) cases of HCVAb reactivity during the 5 year study period. None of the donors tested positive for both types of Hepatitis. 143294 (99.84%) of donors were non reactive for both types of Hepatitis.

Various donor characteristics were studied in relation to the Hepatitis seroreactivity pattern and [**Table 3**] shows the summary.

With regard to Age of donor and seroreactivity, the chi-square statistic for Hepatitis B was 0.2654, with p value 0.8757; the result is not significant with relation to age. For Hepatitis C the chi-square statistic was 2.5432, with p value 0.2804, again showing no significance with age at $p<0.05$.

All the 182 (0.13%) seroreactive cases of Hepatitis B and 40(0.03%) of Hepatitis C were males; 99.84% were Non-reactive for any Hepatitis. 100% of females donations were non-reactive for both types of Hepatitis due to which chi-square statistical analysis was not possible with regard to sex.

The next characteristic analyzed was the type of donation. To know the impact of voluntary donation, voluntary donors were segregated and compared with replacement and directed donors. Among our Voluntary donors 23 (0.05%) tested positive for Hepatitis B and 6 for Hepatitis C (0.01%) and 44447 (99.94%) were non-reactive. Hepatitis B seroreactivity in Directed / Replacement was159 (0.16%); for Hepatitis C it was 34 donors (0.03%). 98847 (99.8%) of our directed/ replacement donors were non-reactive. Statistical analysis for Hepatitis B the chi-square statistic was 28.7127, p value <0.00001, the result is significant. In Hepatitis C, the chi-square statistic was 4.7956; p value 0.029, result is again significant at $p< 0.05$.

Analysis of seroreactivity among first time donors to repeat donors - The chi-square statistic for Hepatitis B was 1.0156, p value 0.313561, the result is not significant. For Hepatitis C also the results were not significant with chi-square statistic at 0.8704 and p value 0.35085.

At 98.1% of total donation, majority of the donors were residents of home state Kerala. All seroreactive cases of Hepatitis B and C were among these groups of donors. However classifying these donors into districts to see if there was any geographical distribution was not possible as addresses in records were incomplete.

Whether the Hepatitis seroreactive cases had tested positive for the three other transfusion transmitted diseases screened which included HIV 1&2, Syphilis and Malaria were also reviewed. However, none of the donors were positive for any other diseases.

Donor callback was done on all 222 seroreactive cases of Hepatitis. 213 cases were called back and referred to Gastroenterology Department of Govt. Medical College, Kozhikode for further evaluation. Of the 9 cases where call back was unsuccessful, the reasons were- Call answered but not reported- 0, Wrong contact details; unable to connect- 5, Refusal to test here (personal inconvenience)- 4 cases

The trend in Hepatitis seroreactivity over the 5 year study period was analyzed. **Table 4** compares the seroreactivity of both types of Hepatitis in comparison with the total donation in each of the five years. **Figure 5** represents the prevalence. As is evident from the two, Hepatitis B prevalence was lowest at 0.06% in 2018 and highest in 2015 at 0.17%. For Hepatitis C lowest was in 2016 at 0.01% and highest in 2015 at 0.05%. For Hepatitis B after the drastic dip in cases in 2018, seroreactivity increased in 2019 though not as much as the first three years. In Hepatitis C however, the pattern is almost the same over three years.

Table 1: Performa (Tick Appropriate column, Q.No's: 9-12 : Answer only if Q.No: 8 is reactive)

1.Blood Centre Donor Number									
2. Age	18-30yrs	31-40yrs		41-50yrs		51-60yrs			
3. Sex	Male			Female					
4. Type of Donation	Voluntary			Replacement / Directed					
5. First : Repeat	First Time Donor			Repeat Donor					
6. State	Kerala			Others					
7. If Keralite, District	Kozhikode	Malappuram		Wynad	Kannur	Others/Incomplete			
8. Hepatitis ELISA Test	Hepatitis B			Hepatitis C					
	Reactive	Non-Reactive		Reactive	Non-Reactive				
9. Others Transfusion Transmitted disease status	HIV	Syphilis	Malaria						
10.Call Back answered	Yes	No							
11. If Yes, Reported to Blood Centre and referred	Yes	No							
12. If No, reason	a. Answered, but did not report to blood centre b. Wrong contact details c. Difficult to test in our blood centre d. Others (specify) ……								

Table 2: Blood Donor Data

Age	**18-30 yrs** 58841 (41%)	**31-40yrs** 53101 (37%)	**41-50yrs** 25832 (18%)	**51-60yrs** 5742 (4%)
Sex	**Male** 139129 (96.94%)		**Female** 4387 (3.06%)	

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Type of Donation	**Voluntary** 44476 (31%)		**Replacement & Directed** 99040 (69%)		
First : Repeat Donor	**First time** 90251 (63%)		**Repeat** 53265 (37%)		
State	**Kerala** 140789 (98.1%)		**Others** 2727 (1.9%)		
District **(if Keralite)**	**Kozhikode** 45712 (32.5%)	**Malappuram** 43147 (30.6%)	**Wyanad** 9263 (6.6%)	**Kannur** 2816 (2%)	**Others/ Incomplete** 39851 (28.3%)

Table 3: Prevalence of Hepatitis in relation to various Donor Characteristics

Age group	**Age Group**	**No: of donors**	**Hepatitis B**	**Hepatitis C**
	18-30yrs	58841 (41%)	79 (0.13%)	21 (0.04%)
	31-40yrs	53101 (37%)	67 (0.12%)	15 (0.03%)
	41-50yrs	25832 (18%)	36 (0.14%)	4 (0.02%)
	51-60yrs	5742 (4%)	None	None
Sex	Male	139129 (96.94%)	182 (0.13%)	40 (0.03%)
	Female	4387 (3.06%)	None	None
First time: repeat donor	First time Donor	90251 (63%)	121(0.13%)	28(0.03%)
	Repeat Donation	53265 (37%)	61(0.11%)	12(0.02%)
Type of donation	Voluntary	44476 (31%)	23 (0.05%)	6 (0.01%)
	Replacement / Directed	99040 (69%)	159 (0.16%)	34 (0.03%)

Table 4: Hepatitis B & C: Prevalence over 5 year study period

	2015	**2016**	**2017**	**2018**	**2019**
Total Donors	27487	29118	28332	27800	30779
Voluntary Donors	7941(28.9%)	10617(36.5%)	6808(24%)	9221(33.2%)	9889(32.1%)
Hepatitis B (prevalence)	47(0.17%)	40(0.14%)	43(0.15%)	18(0.06%)	34(0.11%)
Hepatitis C (prevalence)	14(0.05%)	4(0.01%)	7(0.02%)	5(0.02%)	10(0.03%)

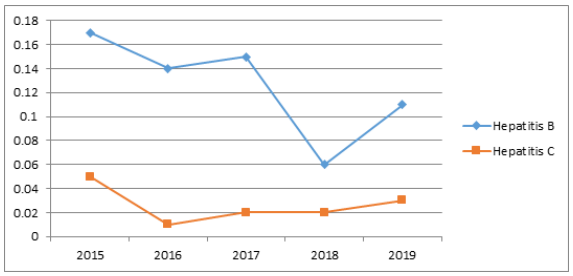

Figure 1: Trend in Prevalence of Hepatitis B & C over 5 years

DISCUSSION

Donor screening for Hepatitis B and C over the study period of five years at Transfusion Medicine, Govt. Medical College Kozhikode showed a prevalence of 0.13% for Hepatitis B and 0.03% Hepatitis C. None of the donors were positive for both Hepatitis B and C or for the other transfusion transmitted diseases screened, namely HIV 1&2, syphilis or malaria.

The prevalence of both types of Hepatitis was lower than similar studies conducted in India and around the world. It was 1.43% for HBV and 0.57% for HCV in the study by Meena M et al which also retrospectively reviewed hospital records (AIIMS, New Delhi) over five years and had a total of 94,716 donations.[7] In the study by Singh B et al,[9] among 1, 28,589 donors the prevalence ranged between 1.7 - 2.2% and 0.25 - 0.9% for HBV and HCV respectively.

Prevalence of HbsAg ranged between 1.55% to 0.99% and Hepatitis C virus antibodies showed a prevalence of 0.4% in the study by Sharma RR et al.[11] In the metaanalysis by M Babanejad et al, prevalence in Eastern Mediterranean countries for Hepatitis B was 1.99% and 1.62% in the Middle Eastern countries, with pooled prevalence 2.03%.[14]

The other significant finding in our study with *p* value <0.05 was the one that reinforced the importance of voluntary donation. Both Hepatitis B and C had a lower prevalence in the voluntary compared to replacement/directed donors. Studies in Indian population by Meena M et al,[7] Singh B et al,[9] Sharma RR et al,[11] all showed a higher seropositivity rate in the replacement compared to the voluntary donors.

However the attempt to analyze any other association between donor characteristics and positivity rate for Hepatitis did not yield any significant findings. The points that were analyzed were donor age, sex and first timers/ repeat donors.

The present study did not show any significant trends in disease positivity over the 5 year study period, apart from a dip in Hepatitis B positivity in 1 year (2018), followed by a rise the next year. In the study by Meena M et al,[7] the annual rates showed decreasing trends in case of HBsAg and a linear increase in HCV. Decreasing trends in HBsAg was also seen in the study by Sharma RR et al.[11]

Studying any geographical pattern or increased prevalence in districts of North Kerala which provided the bulk of the donors was not possible due to several incomplete addresses written in records.

The link between occupation or socioeconomic status with prevalence was also not possible due to incomplete filling in forms.

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Donor callback was an efficient part, as 217 of the 222 seroreactive cases were contacted. 213 cases were referred to the Department of Gasteroenterology of Govt. Medical College, Kozhikode. Four cases had personal inconveniences for getting tested at this centre. Five cases that could not be called back were due to incorrect contact number provided.

CONCLUSION

This 5 year retrospective study showed a very low prevalence of transfusion transmissible Hepatitis B (0.13%) and C (0.03%) in the donor population comprising of 98.1% Keralites. This is also is a reflection of the efficient donor screening and pre-donation counseling given to the blood donors. The importance of voluntary donation has to stressed, being the only donor characteristic studied to have a statistically significant association.

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