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Experience of hepatitis C virus seroprevalence and its genomic diversity among transfusion-dependent thalassemia patients in a transfusion center

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Abstract:

INTRODUCTION: One of the most common blood-borne transfusion-transmitted diseases is hepatitis C. Patients with a history of multiple blood transfusions are significantly at a greater risk of infection by contaminated blood and blood products. Beta thalassemia major is one such condition where repeated blood transfusions are required for patient management.

MATERIALS AND METHODS: The present study was conducted to investigate the serological prevalence of hepatitis C virus (HCV), its viremia, and genotype distribution with clinical parameters among multitransfused thalassemic individuals. In this study, a total of 300 patients were screened to detect anti-HCV antibody in serum, along with liver function parameters and genotyping.

RESULTS: Seventy-five (25%) patients were found to be HCV positive by enzyme-linked immunosorbent assay (ELISA). Among them, 49 (65%) were HCV RNA positive having a significant viral load in their blood and rest 26 (35%) were below detection level, which signify auto clearance of the virus in those patients. According to our study, HCV genotype 3 was the major circulating strain (92.59%) followed by genotype 1. Liver enzymes, such as alanine aminotransferase, aspartate aminotransferase, and total bilirubin, were significantly elevated among HCV seroreactive individuals.

CONCLUSIONS: This study clearly indicates that the incidence of transfusion-transmitted hepatitis C is high in thalassemia patients, but actual scenario of HCV viremia can only be found by HCV RNA qualitative and quantitative detection method and not by ELISA, is a major concern for this high-risk group of population.

Keywords:

Beta thalassemia, enzyme-linked immunosorbent assay, hepatitis C, hepatitis C virus genotype, real-time polymerase chain reaction method

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Introduction

Hereditary disorders of hemoglobin (Hb) result either from qualitative defect (structural alteration of a globin polypeptide chain such as HbS, HbD, and HbE) or quantitative defect (reduced synthesis or complete absence of one globin polypeptide chain like alpha and beta thalassemia). The World Health Organization (WHO) estimates that at least 7.0% of the world populations are carriers of different forms of inherited disorders, related to Hb.[1,2] Parents with various heterozygous states can lead to offspring with homozygous (like beta thalassemia major) or compound heterozygous (such as HbE-beta thalassemia)

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defects. Beta thalassemia major patients show severe hemolytic anemia that can be treated with multiple blood transfusions.[3] HbE-beta thalassemia (also very common in eastern India) shows great variety in clinical expressions of the disease, as it ranges from nearly asymptomatic to severe transfusion-dependent thalassemia. These two are certainly the most common genetic noncommunicable disorders and one of the major public health-related problems in the Hooghly district as well as the entire state of West Bengal.

However, transfusion therapy does have some limitations. The children, who are on regular transfusion schedule like in thalassemia, develop secondary iron overload as a common problem, as human body has no active mechanism for the excretion of iron.[4] Furthermore, there remains an increased risk of exposure to some blood-borne infections such as hepatitis. The agents responsible share the following characteristics such as persistence in donor's bloodstream, the ability to cause asymptomatic infections, giving rise to carrier or latent states, and their stability in stored blood. Ideally, blood for transfusion should either be tested for all pathogens that are prevalent in a given population and cause serious disease or treated to inactivate all such pathogens. However, in practice neither is possible.[5] In India, the blood donor screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection (along with HIV and malarial parasite) became mandatory since 2002. Since then, the risk has been limited to the blood units collected during the "window period" (the period between the time of viral entry to an individual and the time when antibodies against the virus are detectable in the serum). HBV and HCV are transmissible by the parenteral route and may be found not only in blood but also in other body fluids. From the bloodstream, the viruses travel to the liver where they replicate in hepatocytes, resulting in an acute or chronic liver infection.[6] In many developing countries like India, HCV is now the major cause of chronic liver disease and hepatocellular carcinoma (HCC). HCV is a hepatotropic, enveloped, ssRNA virus belonging to the *Flaviviridae* family, which leads to cirrhosis of the liver and finally HCC.[7] Near about 200 million people are estimated to be infected with HCV, all over the world.[8] Chronicity occurs in almost 80% of infected patients. Its importance is particularly vital when effective HCV vaccine is not available.[9] Long-term complication of iron deposition is HCC and presence of superadded HCV and or HBV increases the risk further.[10]

Materials and Methods

A group of 300 patients suffering from thalassemia (beta and E-beta) and taking treatment at Imambara Sadar Hospital, Chinsurah, Hooghly, West Bengal, India, were included in the study. These patients had been receiving blood transfusions and other treatment regularly at Imambara Sadar Hospital from 2011 till the end of 2016. Patients who had received at least 5 previous blood transfusions were included for serological follow-up. Transfusion and clinical records of all patients were maintained. About 5 ml blood sample was collected from each patient and samples were preserved. Investigation was done in two stages. Enzyme-linked immunosorbent assay (ELISA) tests to identify HCV antibodies (anti-HCV) used to detect HCV infection. Detection of HCV RNA by qualitative, quantitative method and genotype tests were performed at ICMR Virus Unit, Kolkata.

Serological study

Samples were tested for anti-HCV antibody in the same laboratory by one person using the same brand of reagents and kits. Tests were carried out with the commercially available, third generation, ELISA for the following transfusion-transmitted infection (TTI) markers: antibodies to HCV [Kit ErbLisa HCV Gen 3 (V2)].

Virological testing and genotyping

HCV viral RNA was extracted from HCV seroreactive serum samples according to the manufacturer's protocol (Qiagen, Hilden, Germany) and eluted with 50 µl elution buffer. Detection of HCV viral RNA was done by nested real-time polymerase chain reaction (RT-PCR) based on 5'-noncoding region (5' NCR) of HCV genome described elsewhere.[11] Quantitative HCV RNA was estimated using in-house Qiagen real-time qRT-PCR kit (QuantiFast Pathogen RT-PCR + IC Kit). The HCV primers and probe sequences were directed against the 5' NCR of the HCV genome.[12] The 4th WHO International Standard for HCV, NIBSC code 06/102, was used as standard.

Nested RT-PCR amplified amplicons of partial HCV core genome (405 bp) were gel purified and directly used for DNA sequencing analysis in an automated DNA sequencer (model 3130XL [ABI, USA]) using BigDye terminator 3.1 kit (Applied Biosystems, USA). The genotypes of the sequences obtained were determined using the NCBI genotyping tool.

Determination of clinical parameters

Liver function parameters such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed by kinetic rate methods and total bilirubin by diazo method (Beckman Coulter Synchron CX5Pro, USA). Hb and complete hemogram were estimated by Automated Cell Counter (Medonic CA530-16 Open, Merck, Germany) and serum ferritin levels by chemiluminescence enzyme immunoassay methods (Beckman Coulter Access 2, USA), respectively.

Written informed consent was obtained from adult participants in our survey and in case of children, from their parents (as per guidelines of the Institutional Ethics Committee).

Results

The present study was conducted to observe the incidence of viral infection in thalassemia patients (n = 300), who received multiple blood transfusion in Imambara Sadar Hospital, of which 156 patients (52%) were male and 144 (48%) patients were female. Their ages ranged from 3 to 38 years with average age being 12.25 ± 6.38 years. Their body weight ranged from 7.0 to 49.0 kg and height ranged from 68 to 168 cm. All the patients were diagnosed as thalassemia major within 6 months to 2 years of age. Most of the individuals undergo transfusion of 1–3 units of blood per month depending on their need [Table 1].

According to our study, out of 300 multitransfused thalassemia patients, 75 (25%) patients were positive for anti-HCV antibody by third-generation ELISA technique. Samples were also tested for HIV (1 and 2) and HBV screening. Among 300 patients, 6 (2%) patients were found HIV (1 and 2) positive and 4 (1.33%) were found positive for HBV [Figure 1]. Out of 75 HCV-seropositive patients, 49 (65%) were detected RNA positive by nested RT-PCR by quantitative RT-PCR and remaining 26 (35%) patients were below detection level (<50 IU/ml). HCV viral load was ranged from 173 to 84.76 × 10^5 IU/ml and having a significant viral load in their blood.

We could successfully amplify 27 HCV RNA-positive samples by nested RT-PCR for core gene-based genotyping. It showed that two major genotypes with only four subtypes were circulated within our study population. HCV genotype 3 (n = 25, 92.59%) was the major prevalence strain, followed by Gen 1 (n = 2, 7.41%) and the subtype characterization showed 1a (n = 1, 3.70%), 1b (n = 1, 3.70%), 3a (n = 23, 85.19%), and 3b (n = 2, 7.41%) [Figure 2].

It is clearly evident that seropositivity of anti-HCV antibody is a marker of hepatocyte damage, but liver function parameters (total bilirubin, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase) and serum ferritin level are also important to know about damages of the hepatocytes. We have found a significant correlation between HCV seroreactive and sero-nonreactive thalassemia patients [Table 2]. Mean Hb and complete hemogram were calculated among HCV seroreactive and sero-nonreactive individuals, but no correlations were found [Table 3].

Discussion

HCV, first identified in 1989, was a major cause of non-A and non-B hepatitis. HCV has been identified as an important etiological agent responsible for transfusion-associated hepatitis and accounts for about 50% of the sporadic cases of non-A and non-B hepatitis.[13] The risk is significantly high in patients who receive multiple blood transfusions. Risk of infective complications due to blood transfusion is a major public health concern nowadays, particularly in countries like

Table 1: Demographic parameters of multitransfused thalassemic individuals in this study (n=300)

Test	n (%)
Sex (%)	
Male	156 (52)
Female	144 (48)
Weight	7.0-49 kg
Height	68-168 cm
Blood transfusion/month	1-3 units
Total transfusion till date (%)	
>100 transfusions	51 (17)
<100 transfusions	249 (83)
History of splenectomy (%)	
Yes	36 (12)
No	264 (88)
Residence (%)	
Rural	252 (84)
Urban	48 (16)

n = Total number of individuals

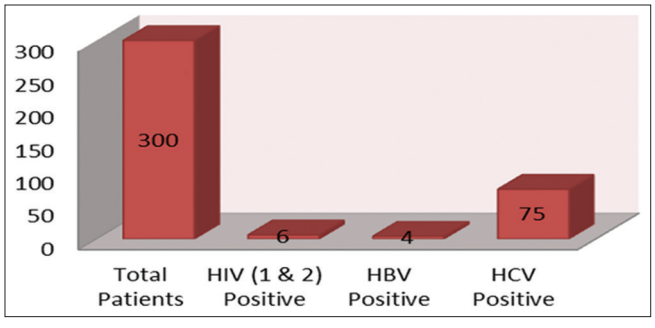

Figure 1: Seroprevalence of HIV, hepatitis B virus, and hepatitis C virus in thalassemic patients

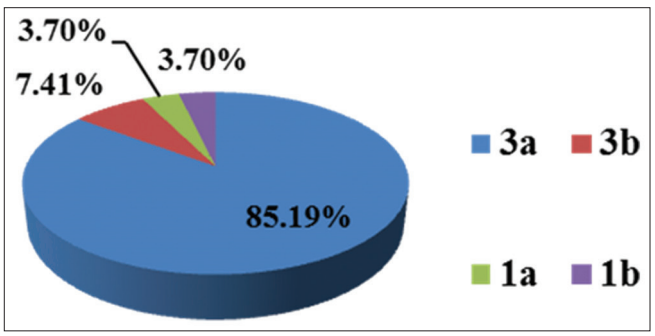

Figure 2: Hepatitis C virus genotypic distribution (n = 27)

Table 2: Serum ferritin, liver function profile level of the thalassemic patients

Parameters	ELISA negative (*n*=225)	ELISA positive (*n*=75)	ELISA positive and RT-PCR positive (*n*=49)	ELISA positive. and RT-PCR negative (*n*=26)
Serum ferritin (mean±SD)	2087.56±648.16	2428.89±861.47	2471.93±884.47	2347.76±827.19
P	0.0003		0.5560	
SGOT (mean±SD)	78.72±32.80	100.04±40.85	103.77±45.23	89.15±25.24
P	<0.0001		0.1408	
SGPT (mean±SD)	94.64±45.36	116.92±49.47	123.12±54.60	105.36±30.12
P	0.0004		0.1289	
Total bilirubin (mean±SD)	2.91±1.14	2.60±1.01	2.86±0.98	2.98±1.01
P	0.0369		0.3628	

n = Total number of individuals, SD = Standard deviation, SGOT = Serum glutamic oxaloacetic transaminase, SGPT = Serum glutamic pyruvic transaminase, RT-PCR = Real-time polymerase chain reaction, ELISA = Enzyme-linked immunosorbent assay

Table 3: Hematological parameters of the patients (*n*=300)

Parameters	ELISA negative (*n*=225) (mean±SD)	ELISA positive (*n*=75) (mean±SD)	*P*
Hemoglobin (g/dl)	6.8±2.42	6.5±1.33	0.3073
PCV	23.12±5.54	21.65±5.85	0.0507
MCV	71.36±9.48	69.2±11.0	0.1021
MCH	23.76±4.68	22.85±3.53	0.1238

n = Total number of individuals, ELISA = Enzyme linked immune sorbent assay, SD = Standard deviation, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin

India where medical facilities are not up to the best of the standards. Consequence of HCV infection may turn into a chronic one with evidence of infection for more than 6 months with ongoing hepatic damage and a threat of developing cirrhosis and HCC in future or spontaneous resolution with two consequent negative tests for HCV RNA at least 6 months apart. Our result showed that 75 (25%) patients were positive for anti-HCV antibody by ELISA. Our data are also corroborated with the previous study conducted by different groups which showed that HCV seroprevalence in thalassemia patients varies from 11% to 30% in India.[14-16] HCV seroprevalence rate is high among multitransfused individuals due to unavailability of vaccine till date and poor blood-screening procedure in blood bank. According to our study, 49 individuals (65%) were HCV RNA positive out of 75 HCV seroreactive individuals. Twenty-six individuals (35%) with different age groups presented lower viral load (within 5000 IU/ml). When the antibody test is positive, but the HCV RNA test is negative, this may be an indicator of resolution of HCV infection. Interestingly, 26 individuals had spontaneously cleared the virus. This may be due to low age in most of the thalassemia patients and iron chelation therapy which may indirectly or partially prevent the replication of the HCV virus. There may be cases of cross-reactive infections (other than HCV) causing false-positive reactions. Furthermore, it can be considered that HCV qualitative and quantitative methods are a better diagnostic tool than ELISA. However, this should be investigated further in a large cohort of the population before coming to any conclusion.

According to our study, liver function enzyme (total bilirubin, ALT, and AST) levels were significantly ($P \leq 0.05$) elevated in all HCV seroreactive individuals than HCV sero-nonreactive individuals [Table 2]. This result suggested that liver function parameters are important clinical indicators for liver health and its function. This finding also corroborates with one of the previous studies conducted on HCV genotypes and viral titer distribution in thalassemia patients.[17] Multitransfused thalassemic individuals have an increased tendency of deposition of iron in the liver. In our study, most of the individuals were found high serum ferritin load; hence, significant correlation was drawn between HCV-infected and HCV-noninfected individuals [Table 2]. High level of serum ferritin was found among HCV seroreactive/RNA-positive individuals than sero-nonreactive/RNA-negative individuals though no significant correlation was found [Table 2]. However, an interesting point to note that serum ferritin level was the important factor affecting the natural history of chronic hepatitis. This should be investigated further in a large cohort of population before coming to any conclusion.

Seven distinct genotypes (1–7) and more than 67 subtypes of HCV have been described till date.[18] Each genotype differs in its amino acid sequence by 31%–34%. Among these genotypes 1–3 have a worldwide distribution, genotypes 4 and 5 are found mainly in Africa, and Genotype 6 is primarily found in Asia.[19] In our observation, we have found that HCV genotype 3 was major circulating genotype (92.59%) followed by genotype 1 (7.41%). This finding also corroborates with previous researches from India showed a preponderance of genotypes 3 and 1.[20,21] Among HCV subtype, 3a was the common strain followed by 3b, 1a, and 1c [Figure 2]. This is also supported by one previous research finding that genotype 3a was predominant in thalassemia patients.[17] Genotypic information is important because it provides information about strain variation and is potentially associated with treatment strategy and its duration. Furthermore, different studies have reported a close relation of subtype 1b with

advanced liver disease, higher viral load, severe iron overloads and needs splenectomy.[22,23] However, as our study was based on cross-sectional data, further studies are needed to clarify that.

Therefore, we suggest the future researchers to study with a large sample size in this domain. On the other hand, it is recommended to introduce the nucleic acid testing (NAT), which is a molecular technique for screening blood donations to reduce the risk of TTIs in the recipients, which also resolve false reactive cases and narrows the window period (for HCV to 1.34 days),[24,25] thus will be very useful in highly populated countries like India.

Conclusions

The result of this study clearly indicates that though incidence of transfusion-transmitted hepatitis C is high in thalassemia patients, the actual scenario can be found only by HCV qualitative and quantitative method, not by ELISA. This should be a matter of concern and further research interest in coming days. In conclusion, proper screening of blood or blood products with latest NAT assays should be given highest priority before administrating it to individuals requiring transfusion.

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Conflicts of interest
There are no conflicts of interest.

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