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Seroprevalence Anti HCV Antibodies, HCV- RNA and its Genotypes among Patients of Hemophilia, at Hemophilia Treatment Centre Pakistan Institute of Medical Sciences, Islamabad

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Objective: To evaluate the frequency of Hepatitis C antibody, viral load and genotypes of HCV in Hemophiliacs at Hemophilia Centre Pakistan Institute of Medical Sciences, Islamabad

Study Design and Duration of Study: A prospective cross-sectional study, done at Hemophilia Centre (PIMS) during a year from January to December 2006.

Material & Methods: Among a total of 215 cases registered for different bleeding disorders at Hemophilia centre PIMS, 161 were Hemophiliacs (both factor VIII and factor IX deficiencies). Out of these 103 were tested for Hepatitis C antibodies by ELIZA method. Viral load was detected using HCV Real Time Amplification Method. Serum was also submitted for genotyping by identifying the genospecific cDNA band.

Results: Among 103 patients of hemophilia A and B tested for Hepatitis C antibody positivity, 36% were found positive. All of these cases were tested for viral load by PCR and 57% cases had detectable viral load. On genotyping most of the cases (38%) were 3a type and a few patients had mixed infection. ALT was elevated in most of the patients. Alterations in hematological parameters have also been described.

Conclusion: We found 36% positivity for HCV antibodies. Detectable viral load was observed in 57% cases. On genotyping most of the cases (among the typable ones) were 3a type. Since the HCV endemicity in general population is already a major public health problem and this is even worse in Hemophiliacs we need to take strict measures to provide safe blood and its products to everyone.

Keyword: Genotype, HCV, Heomophilia,

Introduction

Hepatitis C virus (HCV) infection is a global public health problem particularly in multiple transfused patients as hemophiliacs and thalasemics. Among these patients, blood and blood products are the major sources of infection. The prevalence of HCV antibody in thalassemia subjects has dropped after implementation of anti-HCV screening. However higher prevalence of HCV antibody is still found in hemophiliacs (43.4%) compared with thalassemia patients (5.1%) correlating with the numbers of clotting factor concentrates transfused.[1] Advances in the treatment of hemophilia have resulted in a dramatic increase in both longevity of patients with hemophilia and their quality of life. However, the use of coagulation factor concentrates derived from pooled donations of thousands of donors introduced the risk of transmission of viruses.

In our setup, because of cost of factor VIII and IX concentrates, most hemophiliacs depend upon plasma and cryoprecipitate transfusions for the management of their symptoms. In the early 1990s, methods were developed to adequately inactivate HCV and subsequently donor screening for HCV was introduced, resulting in HCV safe clotting products. Recombinant factor concentrates now in widespread clinical trials should eliminate totally the burden of human transfusion- transmitted viruses.[2] Once infected, about 10–20% of the patients are able to clear the virus spontaneously, while the others develop a chronic carrier state. Liver disease caused by HCV is now recognized as an important cause of morbidity in hemophilia patients.[3] Hepatitis C infection is by far the commonest cause of chronic liver disease among hemophiliacs in the developed countries. In the case of HCV also, there are immunized subjects without viral

replication which is detected as a result of a healed infection, either spontaneously or after treatment. However, this is a quantitatively less important population and it does not remain out of the transmission chain since reinfection is possible**.** The seropositivity of hepatitis C is believed to be significantly associated with duration of transfusion and severity of disease.

The hepatitis C virus is a single-stranded RNA flavivirus which has been further classified into a series of six major genotypes on the basis of variations in the non-structural NS-5 region of the viral genome. Genotypes 1, 2 and 3 predominate in Northern Europe and North America, whereas type 4 is encountered in the Middle East and North Africa, type 5 in South Africa and type 6 in parts of the Far East. The majority of hemophiliacs infected through blood products are infected with types 1 or 3, reflecting the origin of the blood donors used in the manufacture of pooled products. Mixed infection with several genotypes was also documented in a small minority of patients in these studies. It has been suggested that genotype may influence progression of chronic liver disease and response to interferon.[4]

Since the prevalence of Hepatitis C is a major health problem particularly in countries like Pakistan (due to lack of strict regimens for screening of blood and its products before transfusion) we conducted this study to determine seroprevalence of this infection and its genotypes in patients of hemophilia.

Material and Methods

A single centre based prospective study was conducted at Pakistan Institute of Medical Sciences during the year January 2006 to December 2006. Among a total of 215 cases registered for different bleeding disorders at Hemophilia centre PIMS, 161 were Hemophiliacs (both factor VIII and factor IX deficiencies).Out of these 103 were tested for Hepatitis C. Patients' serum samples were used for:

1. Detection of anti-HCV antibodies by ELIZA method at department of Pathology, PIMS
2. Viral load detection using HCV Real Time Amplification Method at CEMB (Centre for Applied Molecular Biology). In this method HCV RNA was extracted from sample, amplified using Real Time Amplification and detected using fluorescent reporter dye probes specific for HCV patients.
3. Genotyping of HCV RNA at CEMB (Centre for Applied Molecular Biology, Lahore). The RNA was isolated from150 ml serum of patient and was reverse transcribed into core DNA. The HCV genotype for the sample was determined by identifying the genospecific cDNA band. The results were labeled untypeable If, the titer of HCV was below the sensitivity limit of genotype assay then either the HCV strain had mutation in the core region or the HCV genotype was other than genotypes 1a, 1b, 1c, 2a, 2b, 3a, 3b, 3c, 3d, 4, 5a and 6a.

Results

Among 161 patients of hemophiliacs studied, 144 (89.4 %) had factor VIII and 17 (10.6 %) had factor IX deficiency. All these patients had received factor concentrates and fresh frozen plasma (FFP) from different sources depending upon availability.

Table No 1: Distribution of age Groups in all Cases .Total Number of Cases: 103

Age Groups (years)	Number	Percentage (%)
0 - 10	59	57.45
11 - 20	30	29.78
21 - 40	13	12.35
> 40	1	0.42

Table No 2: Distribution of age Groups in HCV positive Cases
Total Number of Cases: 37

Age Groups	Number	Percentage (%)
0 - 10	12	32.5
11 - 20	18	48.6
21 - 40	6	16.2
More than 40	1	2.7

Table No 3: Genotype (PCR Positive cases) Total Number = 21

Type	Frequency	Percentage (%)
3a	08	38.0
3a+3b	01	4.8
3a+1a	1	4.8

Untypeable	11	52.4

Age of the patients ranged from 3 to 73 years. As shown in table 1, among all cases more than half of the patients were between 0-10 and about a quarter between 11 and 20 years of age. On the other hand, amongst HCV positive cases about half of the patients were between 10 and 20 and about one third of cases were < 10 years of age (Table 2). However in both groups the lowest percentage was of patients above 40 years.

Seroprevalence of Anti HCV Antibody in Different Regions

Place	Percentage
Islamabad (PIMS)	36%
Peshawar	25%
Lahore	56%
West India	23%
Israel	75%
Brazil	44%
Holland	54%
Iran	15%

ALT was elevated in most of the patients with mean value of 75 iu/l and a range of 12-287 iu/l).

One hundred and three patients were tested for Hepatitis C positivity by ELIZA method. The percentage of patients positive for antibodies to HCV was 36%. All of these cases were further tested for viral load by PCR and 57% cases had detectable viral load. Genotyping of 21 PCR positive patients showed that most of the cases were 3a type (38%), 9.6% patients had mixed infection and in 52.4% cases virus could not be typed.

Discussion

Transfusion-transmitted diseases are amongst the most important complications observed in hemophilic patients and among them chronic hepatitis C is a major cause of morbidity and mortality[5]. Different studies have shown that mortality among hemophiliacs from liver disease was 16·7 times higher than that of the general population, and the mortality from liver cancer 5-6 times higher[4]. In the present study incorporating 103 Hemophiliacs, we found anti-HCV antibodies positive in 36% of the patients which is quite low as compared to other studies. Mayor et al at Israel National Hospital observed 75% positivity for anti HCV antibodies [6], Dutch Hemophiliacs showed 68% positivity in 2001 and 54% positivity in the year 2002. Tarar et al have reported anti-HCV antibody positivity amongst 100 hemophiliacs registered with Haemophilia Patient Welfare Society (HPWS), Lahore Zone as 56%, as compared to 23% anti-HCV seropositivity in Western India, 33% in Sri Lanka and 15% of those in Iran, 44% in Brazilian group and 41% in Jamaican hemophiliacs. They proposed that probably practice of unscreened blood/blood-products transfusions is responsible for high figures of HCV in our population[7]. Another cross sectional descriptive study was conducted at the Department of Child Health Khyber Teaching Hospital & Fatimid Transfusions Center Peshawar, from August 2000 to September 2001, on forty hemophiliac children below 14 years who had received at least two or more transfusions and were tested for HBsAg & anti-HCV antibodies. They found that ten (25%) children showed seropositivity for anti-HCV. There also observed a linear correlation of anti-HCV positivity with the number of transfusions [8]. A study done by Ghosh et al reported a figure of 23% positivity in western India. HCV transmission by blood products is prevented by viral inactivation steps now used in the production of plasma-derived factor concentrates, but transmission remains a problem in many areas of the world especially in developing countries where hemophilia is treated with fresh frozen plasma or cryoprecipitate as 80% of individuals using these products are estimated to be HCV infected [9]. Patients with hemophilia thus constitute a high-risk group for acquisition of HCV infection. During last decade, several cross-sectional studies have been published in the world concerning the prevalence of hepatitis C among hemophilic patients, indicating a wide range from 25% to 100%.The older hemophiliacs are exposed to HCV infection for a longer time compared with the younger ones, and it seems that the prevalence of HCV should be higher in this group. Most of the infected hemophilic patients are asymptomatic. Screening of these patients has a critical importance as infected patients should be identified, followed, and treated.[10]

Age distribution showed that most of our patients were between 3 to 20 years and this is quite comparable to a study done at Lahore in which also showed that most of HCV positive cases were in young age group (between 11 to 30 years).However In another study, done in Netherland, the risk of HCV transmission was found to be lower among younger patients probably due to lower number of exposures.. In our study ALT was raised in 55% cases and this finding is quite comparable with other studies. Studies

of multiple cohorts of hemophilic patients have shown that up to 90% of these patients also have elevated amino transferase (ALT). Genotyping of 21 PCR positive patients showed that most of the cases were 3a type (38%), 9.6% patients had mixed infection. It is proposed that patients who have received clotting factors from geographically different sources may harbor two or more than two HCV strains.[11] Similar observations were made in a study carried out in India on HCV positive cases in general population which showed that 80% of patients had genotype 2 and 3 and 20% had genotype 1 and 4,

This is in contrast to studies done in Iran the USA, Europe, Japan and Brazil which showed that predominant genotypes was type1 and most studies from have reported a prevalence rate of 60%-70% of genotype 1b and 1a.[12,13.] An interesting study done in Italy showed that among the 65 cases of community-acquired hepatitis, HCV genotype II was dominant (60%), followed by genotypes IV (15%), whereas in 45 hemophilia-associated cases, the distribution of the four HCV genotypes was markedly different: genotype I was the most prevalent (61%), followed by genotypes II (25%). According to them genotype II was associated with more severe liver damage than the other types.[14]

It has been suggested that genotype may influence progression of chronic liver disease and response to interferon. Several studies in non-hemophiliac as well as hemophiliac subjects have suggested that HCV genotype 1 is associated with more aggressive liver disease. The genotype determination is also a relevant predictive parameter of response to antiviral treatment since genotype 1 is associated with a lower sustained virologic response (40 - 45%) compared to genotypes 2 and 3, whose sustained virologic response is 70-80%.[15] Lower viral loads also seem to be indicative of a better reaction to treatment [12]

Genotype analysis and viral load quantitation are therefore useful tests in the evaluation and treatment of hemophiliacs exposed to hepatitis C virus. Several other risk factors in hemophiliacs are also associated with hepatitis infection, particularly hepatitis C, for example, age at diagnosis, disease severity, and the use of factor concentrate

Conclusion and Recommendations

Since the HCV epidemics in general population is already a major public health problem and this is even worse in Hemophiliacs, we need to take strict measures to provide safe blood and its products to everyone.

Factor concentrates having minimum risk of transmission of transfusion related viruses, should be preferred in the management of hemophiliacs instead of plasma transfusions.

The screening tests to detect viruses during window period should also be included in the screening process by blood banks to minimise window period. Spot tests which are less reliable than ELISA tests should not be used for screening purpose.

Genotype analysis and viral load quantification are useful tests in the evaluation and treatment of hemophiliacs exposed to hepatitis C virus infection.

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