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Hepatitis C Virus Antibody Positive Cases in Multitransfused Thalassemic Patients in South of Iran

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Background and Aims: **Hepatitis C virus (HCV) infection is the common cause of liver disease among thalassemic patients who receive recurrent blood transfusion. Recent studies in Iran has shown a high prevalance of HCV among Iranian thalassemic patients, inspite of low HCV seroprevalence in general population. In this study we investigated the prevalence of new cases of hepatitis C and its risk factor in the group of thalassemia major patients.**

Methods: **This study was carried out in thalassemic ward of Shiraz University of Medical Sciences, southern Iran in year 2003. Cases were interviewed using a standard questionnaire including demographic, clinical history and HCV related risk behaviors. Then 3 blood samples were investigated by serum marker of anti-HCV antiboby (anti-HCV Ab) via Eliza-3th method in times zero, 45 days and after 6 months follow-up.**

Results: **The mean age of patients were 15.2 ± 6.3 (1-36 years old) and the proportion of male to female 1 to 1. The rate of HCV antibody positivity in the first sample was 25%. All of the second and third blood samples were negative. There was significant correlation between age and being positive for anti-HCV Ab ($P < 0.001$) and also between being positive anti-HCV Ab and the time they began to receive blood transfusion. The patients who started blood transfusion before 1996 (the year we started screening program for blood donors in Blood Bank in Iran) had 12.5 times more risk for being positive for anti-HCV Ab (odds ratio = 12.5).**

Conclusions: **Our results approved the effect of screening program for blood donors in blood transfusion centers.**

Keywords: **HCV, Thalassemia, Blood Transfusion, Blood Bank, Iran**

Introduction

Hepatitis C infection is common in patients receiving life-long blood transfusion therapy [(1)]. The World Health Organization estimates that there are at least 21.3 million hepatitis C virus (HCV) carriers in the Eastern Mediterranean countries, which is close to the number of carriers estimated in the Americas and Europe combined [(2)]. HCV is a blood-borne pathogen affecting an estimated 4 million Americans and 170 million people worldwide. An estimated two-thirds to three-fourths of those infected with HCV have not been diagnosed, and in the next 10 to 20 years, the number of patients diagnosed with HCV is likely to increase [(3)]. In Mirmomem *et al.* survey, the prevalence of HCV infection is much higher (more than 20%) among Iranian beta-thalassemic patients as compared with HBV and HIV infections. Routine screening of donated blood for HCV is

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highly recommended. Also they found a significant relation between positive anti-HCVAb and age, duration and interval of blood transfusion; but in their patients who received blood transfusion before donor screening program, the prevalence of anti-HCVAb was more significant [4].

Chronic liver disease involves great amount of thalassemic patients. The leading cause of liver damage in this group is HCV infection and liver cirrhosis which both of them are secondary to recurrent blood transfusion. The global prevalence of HCV is estimated to be about 3%. This prevalence is lower than 2.5% in American, European, east of asia and most region of Africa. In east of Australia, the prevalence is between 2.5-4.9%. In middle East, the prevalence is between 1% to 12% [5]. After begining of donor screening program, the risk of acquired post transfusion HCV infection has been particulary reduced. Although the rate of HCV infection because of donor screening program and program of changing neddles has been particulary reduced from 1980 to1990 years, the mortality of liver disease and liver cancer from 1990, because of high prevalence of hepatitis has been increased [6].

In Cappellini study on 117 patients with thalassemia major, 66% of patients were positive for HCV RNA. In this study, HCV infection has been reported to be the only risk factor of liver necrotic inflammation [6]. In a multicenter prospective study of Prati *et al.* on 481 patients of 31 therapeutic center, from 219 (14.8%) patients with negative anti-HCV Ab, 181 of them has terminated the period of following up for 3 years, from these patients 10 have been positive for anti-HCVAb at end of study [7]. In Franchini *et al.* study on 102 patients who have been negative for hepatitis B virus and HIV, all of these patients have been infected by HCV before second half of 1980, 14 patients (13.7%) became negative HCV-RNA and 88 (86.3%) positive for HCV-RNA [5].

Recent studies in Iran has shown the prevalance of HCV infection among Iranian thalassemic patient to be high, in spite of low HCV seroprevalence in general population. As there was insufficient data on efficacy of donor screening program on the prevalence of HCV among multitransfused thalasemic patients and related risk factors, we conducted a case-control study on the epidimiology of HCV infection in this population in Shiraz, south of Iran. In our study we are up to investigate the prevalence of new cases of hepatitis C and its risky factor in group of thalassemia major patients. Furthermore, the analysis of associated risk factors allowed for the development of more effectivness donor screening program to reduce the prevalence and incidence of HCV infection among thalassemic patients.

Materials and Methods

A case-control study was carried out in thalassemia ward of Shiraz University of Medical Sciences, south of Iran from 2003 to 2004. Two-hundred multitransfused thalassemic patients were randomly selected by means of a questionnaire, and asked about their demographic data, past medical history including surgical, dental and other possible high risk procedures, transfusion onset and intervals and family history for hepatitis B and C, and also the results of blood samples. All participants were assessed for HCV infection by testing for anti-HCVAb using an enzyme linked immunoassay (ELISA), 3rd generation, randox-UK, which were then confirmed by recombinant immunoblot assay (RIBA), and polymerase chain reaction (PCR). Three blood samples were taken which first one was at the beginning of the study for evidence of HCV. According to our results, the patients were divided into two groups of positive anti-HCVAb who considered as cases and negative anti-HCV Ab as the control group. Second sample was taken 45 days after passing incubation period, again for finding evidences of HCV. Third sample was taken after 6 months follow up for risk factors of HCV transmission. All patients lived within this area for at least 10 years.

Statistical analysis

Collected data were coded and computed by Microsoft Epi Info 2000 and the statistical analysis was done using SPSS 10.0 (SPSS Inc, Chicago, Illinois, USA). Simple descriptive statistics such as frequency, standard deviation, percentage, and ratios were used. Analytic statistics such as chi-square, fisher exact test, student t-test and Pearson Constant were used for comparison. We conducted a multivariate logistic regression analysis to identify factors associated with HCV infection. Confidence interval in all calculation was 95% and P value<0.05 was considered significant.

Results

A total of 200 thalassemic patients (100 females and 100 males) participated in our study including 143 (89%) thalassemia major and 22 (11%) thalassemia intermedia, of which 50 (25%) were

positive anti-HCVAb and 143 (71.5%) were negative for anti-HCVAb compromising the control group. 70 (35%) of patients was splenectomised. All patients have no evidence of hepatitis B virus. 120 (60%) patients used red blood cell via leukofilter and 80 (40%) used washed red blood cell. The mean age onset of transfusion was 21.3 months old and means age onset of desferal injection was 63 months old. Only 11 (5.5%) patients had past medical history of surgery (except splenectomy). Mean serum ferritin level of last 3 years was 2287 ± 932 ng/dL. Mean transfusion interval was 19.2 ± 4.2 days. Mean annual transfusion was 10755 ± 9289 mL. Results of first sampling in 50 (25%) were positive for anti-HCVAb and in 143 (71.5%) was negative. In this period 6 (3%) cases were expired due to thalassemia complications and excluded from study and also one patient voluntarily excluded from the study.

According to results of second and third samples on negative group for anti-HCVAb, all patients were negative. The onset of transfusion in 163 patients was before the end of year 1996 and in 30 was after year 1996 (the year began blood donor screening program in Iranian Blood Bank Organization. In Table 1, comparison of quantitative factors of HCV positive and negative groups and in Tables 2 and 3, comparisons of other factors are shown. According to Table 3, positive and negative groups had significant difference in year of transfusion onset. Patients who started blood transfusion before 1996 had 12.5 times risk of being Anti-HCVAb positivity.

Table 1. Comparison of quantitative factors between positive and negative anti-HCV Ab patients

Factor	Positive anti-HCV Ab (n=50)	Negative anti-HCV Ab (n=143)	P value
Age (yr)	17.8 ± 5.4	14.4 ± 6.3	0.001
Transfusion onset (month)	20.6 ± 28.3	21.8 ± 3.7	0.8
Desferal onset (month)	72.9 ± 66.9	60.5 ± 48.9	0.2
Mean ferritin (ng/dL)	2454 ± 985.2	2214 ± 932.2	0.1
Transfusion interval (day)	18.8 ± 3.6	19.5 ± 4.5	0.3

Table 2. Comparison of qualitative factors between positive and negative anti-HCVAb patients

Factor		Positive anti-HCV	Negative anti-HCV	P value
Thalassemia type	Major	43 (24.3%)	128 (72.3%)	0.5
	Intermedia	7 (21.8%)	15 (68.2%)	
Sex	Male	27 (27%)	69 (69%)	0.5
	Female	23 (23.2%)	74 (74.7%)	
Splenectomy	Yes	23 (32.9%)	43 (62.4%)	0.3
	No	27 (20.9%)	100 (74.7%)	
Hx of surgery	Yes	1 (9.1%)	10 (90.9%)	0.3
	No	49 (26.1%)	133 (70.7%)	
Dentistery referral	Yes	32 (26.2%)	90 (73.8%)	0.9
	No	18 (25.4%)	53 (74.6%)	

Table 3. Comparison of transfusion onset between positive and negative anti-HCVAb patients

Transfusion onset	Positive anti-HCV	Negative anti-HCV	P value	Odds ratio (95% CI)
Before 1996	49 (30.1%)	114 (69.9%)	0.001	12.4 (1.6-99.1)
After 1996	1 (3.3%)	29 (96.7%)		

Discussion

Liver diseases are considered as the second cause of death in adolesencee and adult thalasemic patients [8]. The incidance of hepatitis decreased significantly after hepatitis B vaccination and donor screening program but these patients untill now are prone to liver infection due to known or idiopathic risk factors [9]. The most common cause of postransfusion hepatitis is HCV and also HCV infection associted with iron overload is main cause of liver disease in thalassemic patients [5]. Cross-sectional studies in 10 years ago in the prevalence of HCV infection in thalassemic patients were in ranged from 11 to 88 percent. The reason of this wide range can be due to difference in type and sensivity of testes and also difference in the total prevalence of selected population [4].

In this case-control study, we evaluated evidence of HCV in multitransfused thalassemic patients in south of Iran. In our study, the prevalence of anti-HCVAb was 25%, that is near to other HCV seroprevalence studies in our country. In other studies HCV seropositivity ranged from 13% to 34% in different regions of Iran [4]. Results of studies in other countries on seroprevalence of HCV mainly before year 1980 showed the higher prevalences than our study [6, 10, 11]. Result of our

study showed the mean age in positive anti-HCVAb were significantly higher than negative anti-HCVAb. This result has been expected due to more exposure to HCV infection due to recurrent blood transfusion.

The patients who started blood transfusion before 1996 (The year started screening program for blood donors in blood bank in Iran) have 12.5 time risk of being Anti-HCVAb positivity. Through patients started blood transfusion after 1996, only a 4-years old child was positive for anti-HCVAb. This patient started blood transfusion since 3 month old and has did not have history of splenectomy, dentistry referal or other surgeries. In our study like other studies which have not been significant for relation between thalassemia type (major and intermedia), family history of hepatitis, type of blood transfusion, dentistary referral and surgeries in positive and negative anti-HCVAb groupes [4].

As results of 2nd and 3th sample testes in our study showed all samples were negative. This is a hopefull result and showed effectivness of donor screening program in our blood bank organization. Also Niyh *et al.* reported the 42% prevalence of HCV which decreased to zero percent after Taiwan donor screening program in 4 years period [6]. We suggested conduction of future studies on HCV seroprevalence in mutitransfued thalassemic patients that only received transfusion after donor screening program. Comparison of these results with results of this study can show effectivness of donor screening program in deletion of HCV infected blood packs.

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