The Prevalence and the Quantification of Hepatitis C Virus among Thalassemia Patients using ELISA and PCR in Diyala Province

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Abstract

Background: Hepatitis C virus is the most common cause of post-transfusion hepatitis and end-stage liver disease in many countries. Regular blood transfusion in patients with hereditary hemolytic anemia, particularly thalassemia, has improved their overall survival, but carries a definite risk of acquisition of blood borne virus infections, especially viral hepatitis.

Objective: To determine the prevalence and the quantification of HCV infection among thalassemia patients in Diyala province by using enzyme- linked immunosorbent assay and polymerase chain reaction techniques.

Patients and Methods: This study was conducted on 215 thalassemia patients in the thalassemia unit in Al-Batool Teaching Hospital of Maternity and Pediatrics in Baqubah city-Diyala province during the period from September 2013 to December 2015. Demographic data were obtained from the patients records. Sera were tested with anti-HCV assays by use enzyme linked immunosornbant assay and positive result were tested by a Real time PCR technique to assess HCV infection.

Results: A total of 215 thalassemic patients, 26 (12.1%) patients were HCV positive by enzyme linked immunosornbant assay and only 3 of these 26 patients were positive by RT-PCR. The rate of HCV infection in thalassemic patients is associated directly with the number of blood transfusions and the age of the patients and It is more in the period before 1996.

Conclusion: The prevalence of HCV infection in thalassemic patients is relatively low in Diyala province in comparison with other areas and PCR technique is essential to detect and treat active infection.

Key words: Prevalence, thalassemia, hepatitis C virus.

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Introduction

Hepatitis C virus accounts for over 85% of transfusion-associated hepatitis cases. The chance of the infection progressing to a chronic state is more than 50%, leading to cirrhosis or hepatocellular carcinoma in 25%. Almost all world countries had established their own data base regarding HCV. It is estimated that more than 170 million persons are infected worldwide. In industrialized nations, HCV prevalence is typically 1%-2% in the general population and < 0.5% in blood donors [1]. Hepatitis C virus is the most common cause of post-transfusion hepatitis (PTH) and end-stage liver disease in many countries. Regular blood transfusion in patients with hereditary hemolytic anemia, particularly thalassemia, has improved their overall survival, but carries a definite risk of acquisition of blood borne virus infections,

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especially viral hepatitis [2, 3]. Moreover, with respect to marked liver iron overload, which is often inevitable in patients on regular blood transfusion, HCV infection have been shown to have a potentiating effect on hepatic fibrogenesis in thalassemia patients [4].

Hepatits C virus was identified to the first time in 1989 by Choo *et al* [5] and kuo *et al* [6].

Thalassemia is an important health problem throughout Iraq, particularly in Diyala province. The present study design to identify the prevalence of HCV infection in thalassemia patients in Diyala province.

Patients and Methods

Sample Collection

A total of 215 thalassemia patients, 130 (60.4%) males and 85 (39.6%) females, their age range from 1 year to 36 years. They were attendant's of thalassemia unit in Al-Batool Teaching Hospital of Maternity and Pediatrics in Diyala Province. Sampling lasted from September 2013 to December 2015. The clinical diagnosis was obtained from patient records and interview and ethical approval for use of all specimens was obtained. A written questionnaire including age, gender, address, duration of diagnosis of thalassemia, total number of blood transfusion, family history of hepatitis, previous medical and surgical history was done.

Sample processing

Ten milliliter of blood was obtained by vein puncture using disposable latex gloves and syringes from all patients. Sera were separated from whole blood under optimal conditions for RNA extraction. For this purpose, The blood samples were allowed to clot in the room temperature for 20 minutes and then centrifuged at 2,000 rpm for 10 minutes at room temperature at the laboratory of Al-Batool Teaching Hospital of Maternity and Pediatrics. All samples were

divided into three aliquots then immediately frozen and stored at (-20ºC) at Medical Research Laboratory of Diyala College of Medicine and (-80ºC) at the laboratory of Baqubah Teaching Hospital, for serological and molecular assays respectively to minimize degradation of viral nucleic acid, prevent cross contamination and unnecessary thawing and freezing.

Methods

Four anti-HCV antibodies detection commercial kits were utilized in this part of study. The initial screening for anti-HCV Immunoglobulin G (IgG) antibody was determined by fourth generation ELISA (Bioelisa HCV 4.0 ELISA, Biokit, Spain) at Central Health Laboratory in Baqubah city. The results were interpreted according to manufacturers' instructions. All seropositive samples were tested individually for the presence of HCV RNA by quanitative real time-PCR at Medical Research Laboratory of Diyala College of Medicine.

Real time PCR based on four major processes: isolation of HCV RNA from specimens, reverse transcription of the RNA, nucleic acid amplification and detection of the amplified products on realplex 2 eppendorf mastercycler. To avoid possible contamination with exogenous sequences during extraction or amplification, all nucleic acid extraction, amplification, and detection steps were performed in separate laboratories. Negative and positive controls were extracted, reverse transcribed, and amplified in each batch of samples tested by PCR. Once the program will be finished one can see the graphics. The negative control should run along with the bottom and positive control must give a curve in the software graphics.

Statistical analysis

The statistical analysis was done using the Statistical Package for Social Sciences (SSPS), version 22. The data were analyzed as frequencies and proportions and chi-square with probability P-value $\leq$ 0.05 regarded as significant value.

Results

The mean age of the study group was 11.7±7.8 years. (1 year-36 years), the prevalence of HCV infection detected by ELISA technique in the study group was 12.1% (26 patients from a total of 215 patients). Only 3 of theses 26 patients were positive by PCR.

The mean age of thalassemic patients with ELISA positive HCV infection was 18.4±4.2 years while the mean age of patient without HCV infection was 10.7±7.7 years. There was a statistically significant relation between the age group and HCV infection detected by ELISA with P<0.0005 as shown in table (1).

Table (1): Distribution of HCV infection detected by ELISA in relation to age group of thalassemic patients.

Age group (yr)	ELISA				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
0-2	0	0	27	100	27	100
2-5	0	0	18	100	18	100
5-8	0	0	26	100	26	100
8-11	2	4.7	41	95.3	43	100
>11	24	23.8	77	76.2	101	100
Total	26	12.1	189	87.9	215	100

The mean duration of thalassemia for patients with ELISA positive HCV infection was 16.5±5.1 years while that for ELISA negative was 9.5±7.5 years. There was a statistically significant difference between the duration of thalassemia and HCV infection detected by ELISA with P<0.0005 as shown in table (2).

Table (2): Distribution of HCV infection detected by ELISA in relation to the duration of thalassemia

Duration (yr)	ELISA				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
0-2	0	0	30	100	30	100
2-5	0	0	33	100	33	100
5-8	1	2.9	33	97.1	34	100
8-11	5	13.2	33	86.8	38	100
>11	20	25	60	75	80	100
Total	26	12.1	189	87.9	215	100

P- value <0.0005

The mean number of blood transfusion for thalassemic patients with ELISA positive HCV infection was 190±74 while that for ELISA negative was 42±32 years. There was a statistically significant difference between the number of blood transfusion for thalassemic patients and HCV infection detected by ELISA with P<0.0005 as shown in table (3).

Table (3): Distribution of HCV infection detected by ELISA in relation to number of blood transfusion of thalassemic patients.

| Number of blood transfusions | ELISA | | | | Total | |
| | Positive | | Negative | | | |
	No.	%	No.	%	No.	%
0-50	1	8	124	99.2	125	100
51-100	3	4.6	62	95.4	65	100
101-150	4	100	0	0	4	100
101-150	4	100	0	0	4	100
>200	14	82.4	3	17.6	17	100
Total	26	12.1	189	87.9	215	100

P- value<0.0005

The percent of thalassemic patients with HCV infection detected by ELISA before 1996 was 29.8% while that after 1996 was 7.1 which reveals a statistically significant difference (P<0.0005), table (4).

Table (4): The distribution of thalassemic patients with HCV infection detected by ELISA before and after 1996

| Date of diagnosis | ELISA | | | | Total | |
| | Positive | | Negative | | | |
	No.	%	No.	%	No.	%
After 1996	12	7.1	156	92.9	168	100
Before 1996	14	29.8	33	70.2	47	100
Total	26	12.1	189	87.9	215	100

P-value <0.0005

The total number of thalassemic males was 130 (60.4%) and that for females was 85 (39.6%). The number of thalassemic males with HCV infection detected by ELISA was 19 (14.6%) while that for females was 7 (8.2%). There is no statistically significant relation between gender and thalassemic patients with HCV infection detected by ELISA, p>0.05, figure (1).
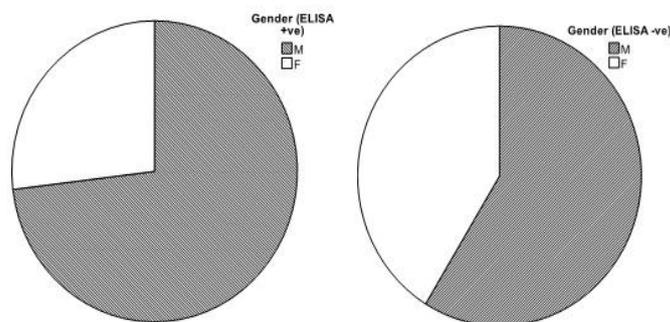

Figure (1): The relation of HCV infection detected by ELISA to gender.

The study found no significant relation between thalassemic patients with HCV infection detected by ELISA and the residency of patient (P>0.05), as shown in table (5).

Table (5): The distribution of HCV infection detected by ELISA to the address of patient.

Residency	ELISA				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Baquba	15	13	100	87	115	100
Baladrooz	1	5	19	95	20	100
Khalis	4	11.4	31	88.6	35	100
Muqdadiya	6	14.3	36	85.7	42	100
Khanaqin	0	0	3	100		100
Total	26	12.1	189	87.9	215	100

p- value > 0.05

Discussion

Thalassemia is common chronic diseases which require frequent blood transfusion as a standard treatment. Thalassemia patients are susceptible to various infectious agents due to their exposure to frequent blood transfusion.

Hepatitis C virus is the most infectious virus in poly transfused children. This especially evident before the period of 1996, the time of starting screening programme of blood for HCV in blood banks in Iraq. This study proved this finding by the presence of a significant difference in HCV infection in thalassemic patients before and after 1996. This result goes with other studies in the world [7][8].

In our study, I observed that there were statically significant difference in number of blood transfusion between patients with positive and negative HCV infections respectfully. The more exposure to blood transfusion can simply lead to more probability of HCV infection. This finding is consistent with other studies in Iran [7][8] and India [9].

Diyala is a province lying in the northeast of Iraq. The pupils of this province were complaining (as several other areas in Iraq) from unstable general circumstances due to violence and terrorism. The great efforts of health authorities to overcome these difficulties were relatively successful toward patients attending thalassemia department in Baquba city , the center of Diyala. Some other patients cannot regularly attend this department which may affect the quality of medical services.

The mean age of the study group was about 12 years (11.7±7.8 years). The place of the study is a pediatrics hospital and although the thalassemia unit at of Maternity and Pediatrics is the only place in the province, the main patients lies in pediatrics age group due to several factors like attending other major centers in Iraq and death of patients due to complications in adulthood period.

The prevalence of HCV infection detected by ELISA technique was 12.1% (26 patients). There was only 3 of these 26 patients had positive results by PCR technique. This difference can be explained

by decreased infectivity of HCV throughout time and the difference between present and past infections. HCV pathogenesis is regulated by many factors like host immunity and metabolic changes like oxidative stress, insulin resistance and hepatic steatosis [10]. When we compare the prevalence of HCV infections in thalassemic patients in our study (12.1%) with other studies in the world, we can observe that it is lower than most of studies in the Middle East and other area of the world. Al-Kubaisy *et al* (2006) found a prevalnce of 61.5% in large study for Iraqi thalassemic patients in Baghdad [11], while Muhsin *et al* (2013) found a prevalence of 25% in Bbylon [12]. Several other studies in Iran show in most instances a higher prevalence than that of this study [13-16]. But one study showed lower figure (10.6%)[8]. Al-Gohary *et al* (1995) found a prevalence of 75% of hepatitis C in thalassemic patients in Egypt [17]. Other studies revealed variable higher figures like that in India [18] and other Arab counteries [19][20]. This relatively low prevalence level in Diyala province may give us a good marker of the quality of the medical services for thalassemic patients in spite of difficult known circumstances, although difference in laboratory technique can play a role in this variability also.

In this study, there was a statically significant difference between the mean age of thalassemic patients with positive HCV infections (18.4±4.2 years) and that with negative HCV infections (10.7±7.7 years). This important difference can be due to the increasing blood transfusion times in the first group as well as to the absence of HCV screening for blood products in older patients. Tow studies in Iran show similar findings [7][8]. This finding was supported by finding a significant difference in the duration of thalassemia between HCV positive and HCV negative patients.

Regarding sex distribution, the study revealed insignificant difference between males and females with HCV infection (19 from 130 males and 7 from 85 females respectifully). This finding is consistent with the results of tow studies in Babylon – Iraq (1997 and 2013) [12][21]. As well as with other studies in middle east [7][8]. The study also found insignificant difference in HCV infection regarding the residency of the patients in several province sectors. This can be explained by the similarity in life conditions and relatively small size of the province.

The study concluded that the prevalence of HCV infection is relatively low in Diyala provinc in comparison with other areas. Although it seems blood donor screening project after 1996 reduced HCV infection, using more accurate technique like PCR is necessary in order to find viral infection and treat thalassemia patients with HCV infection more carefully.

Acknowledgement

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