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Prevalence of hepatitis C in patients with thalassemia major in Qir and Karzin County

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ABSTRACT

The severe form of thalassemia (major) is a beta-globin chain synthesis genetic disorder, in this kind; life is possible only with regular blood transfusions. In time blood transfusions, most clinical problems will appear after the first decade due to excessive accumulation of iron leading to liver disease. In patients with thalassemia, there is also the possibility of infection with hepatitis C that can cause liver damage as well as weakened the immune system. Therefore, the main objective of the study is to investigate the relationship between changes in iron content, the amount of hepatitis C infection and changes of liver enzymes in patients with thalassemia major. Methods: The study conducted on all thalassemic patients admitted to Imam Muhammad Baqir (AS) hospital in Qir and Karzin County (24 patients) for treatment,(The population is all thalassemia patients requiring treatment in Qir and Karzin County). Demographic information is obtained through the questionnaire and patient records; a 5 ml sample blood is obtained from each of samples for HCV-Ab tested. In addition, after sampling, samples analyzed by ELISA for measurement of antibodies against hepatitis C. RT-PCR analysis have been performed to approval the conducted tests. Then last ferritin by levels obtained from patient records. In addition, ANTI-HCV prevalence studied Results: The results show that 7 patients (29%) .age, gender and frequency of injection were ANTI-HCV positive, with 5 patients (4/71%) HCV-RNA positive, and out of seven patients, five are females and two are males. The mean age of thalassemic major patients is 18/67; the mean age of ANTI-HCV positive patients are 22/1; and the mean age of negative thalassemia subjects is 17/5, respectively. The age of patients with thalassemia major in Qir and Karzin County ranges between 31-6 years with male to female ratio of 0.6. The results indicate that there is no significant correlation between Anti-HCV positive and high serum ferritin level Conclusions: The prevalence of hepatitis C in patients with thalassemia major with poor immune function is high and still there is no effective vaccine to prevent hepatitis C, for the prevention given that of new infections through blood products, the use of more sensitive methods for determining pollution and increase accuracy in screening donated blood is the only solution available.

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INTRODUCTION

Thalassemia is an inherited disease, which is transmitted from carrying parent to offspring. A large percentage of children in different parts of the world, especially in areas known as thalassemia belt are infected with. Thalassemia disease is divided into two main categories; Alpha Thalassemia in which the synthesis of alpha globin chain is reduced or completely stopped and Beta Thalassemia with disorders in beta chain [1].

Thalassemia Disease is an inherited autosomal recessive. In order to be infected with beta thalassemia disease defects in both beta - chain genes is necessary. A gene defect causes limited negative features in its agent but does not make a person sick and the defected gene shows no phenotypic effect on the individual with thalassemia gene therefore, recessive gene is being kept in the population [2].

Thalassemia syndromes based on severity of clinical and hematologic findings are divided into four categories:

1- Major: Clinical and severe hematologic symptoms dependent on regular blood transfusions
2- Minor: No clinical symptoms, diagnosed by hematologic test and is carrier

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3- Intermedia: Clinical severity is less than thalassemia and is not dependent on irregular blood transfusions.

4- Alpha thalassemia: No clinically and hematologic significant symptoms and diagnosed by globin or genetic tests [2]

The only way to adjust body iron content is to balance between excretion and absorption of it. There is no physiological mechanism to regulate iron excretion, so a factor affecting on iron absorption is the erythropoiesis. In patients with thalassemia the number of blood red cells is ten times the normal; therefore, circulating plasma and iron absorption from the gastrointestinal tract increases. Patients with thalassemia major often followed by ineffective blood production have a high iron absorption from the gastrointestinal tract. Iron, in the presence of superoxide and hydrogen peroxide generated in cells, produces cells damaging radicals, such as hydroxyl that this radicals lead to cell membrane lipid peroxidation, the protein degradation and DNA damage [3].

A blood transfusion is a basic therapy in patients with thalassemia major. In this genetic disorder, due to an imbalance in the structure of hemoglobin, the red blood cells made coup-life in blood circulation are quickly destroyed. Therefore, severe hemolysis and anemia are complications of this disorder. Thus, blood transfusion treatment is lifetime and blood transfusions intervals should be adjusted so that hemoglobin level before transfusion keep at 9.5 -10 ng/dl. The second treatment in these patients is related to iron overload; since the iron that enters the body through blood transfusion has no way to dispose of body and deposits in parenchyma tissues and causes damage to vital organs such as the heart, liver, and lymph [4]. The most common test to diagnose iron overload is the measurement of serum ferritin. Excess iron chelates often starts from when ferritin level is above 1000 ng/dl and only therapeutic medication commonly used is desferal which is a siderophore , iron carrier, obtained by culturing Streptomyces pilosus[3].

The only way to treat thalassemia is through receiving blood and therefore the patients are at exposures to blood-borne viral disease including hepatitis. Long time increasing use of hepatitis B vaccine has caused a sharp decline in the rate of transmission of infection through blood transfusion, but because there is no vaccine to prevent hepatitis C, the disease in thalassemic patients remains a serious problem. before the introduction of blood tests for HCV infection detection thalassemic patients received blood transfusions with very high probability of contamination so that in 60-80% of patients, according to various reports were ANTI-HCV positive. A study titled “to investigate factors associated with the incidence of complications in patients with thalassemia major visited Shahid Dastgheib Shiraz in, 2005-2006) was conducted. The study shows the prevalence of 14.4 % hepatitis C [5]. According to the above and ferritin sedimentation rate, and increased hepatitis C infection in patients with thalassemia this study is necessary to prevent the spread of disease.

Methods:

The study conducted on all thalassemic patients admitted to Imam Muhammad Baqir (AS) hospital in Qir and Karzin County (24 patients) for treatment (The population is all thalassemia patients requiring treatment in Qir and Karzin County). Demographic information is obtained through the questionnaire and patient records; a 5 ml sample blood is obtained from each of samples for HCV-Ab tested. Then the patients were interviewed and made aware of research process and their consent was taken (all tests will be performed free of charge, and the results will be sent to them). Demographic and biographical questionnaires data including age, sex, age at diagnosis, and number of blood transfusions etc were filled. Notably, the sample preparation for antibodies against hepatitis C counts needs no prior special notice of preparation. After blood sampling, the tubes are marked and serum was prepared from blood samples. Then anti-hcv test was performed using ELISA method. The test is requested for screening and diagnosis of hepatitis C virus infection as well as monitoring the treatment of the infection in thalassemic patients exposed to hepatitis C viruses due to repeated blood transfusions. Moreover, NESTED PCR method was used NS5 in virus after complementary DNA was made.

Sequences of the primers are as follows:

F1: caggcagaaacgtctagccatg

F2: ccc ctg tga ggaact actgtc

R1: tcg caa gca ccc tat caggcag

R2: tgc acg gtc tac gag acc tc

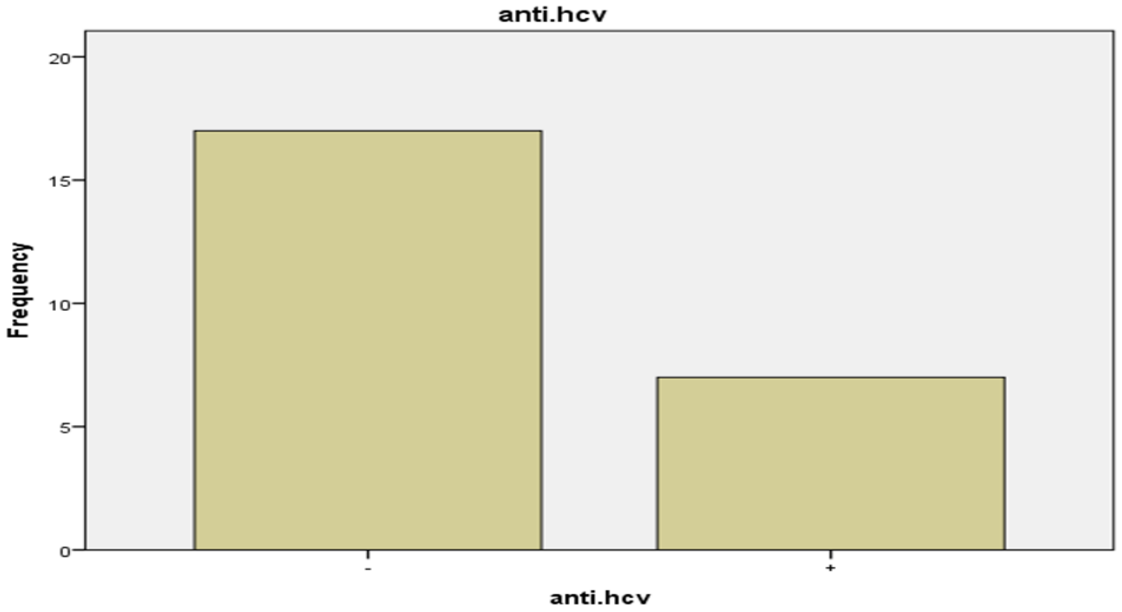
Then to extract HCV virus genome from blood samples RNA-PLUS method was used. After electrophoresis, the gel was transferred to Duke Gel and u/v ray was radiated to the gel. A wavelength emitted from the sample on the gel recorded and analyzed by computer and then compared with the control (Fig. 1). Then last ferritin levels were extracted from the records.

Results:

Seven out of 24 studied patients, i.e. 29.2% were ANTI-HCV positive (Table 1) and (chart 1).

Table 1: Distribution of ANTI-HCV in 24 patients with thalassemia Qir and Karzin County

Percentage	NO.	ANTI-HCV
29.2	7	Positive
70.8	17	Negative



Distribution of HCV patients tested with RT-PCR

Chart 1: Distribution of ANTI-HCV in 24 patients with thalassemia Qir and Karzin County

Currently the sensitivity of serological tests for the diagnosis of HCV is high; however, due to false-positive results and or previous infection with hepatitis C ,the tests cannot accurately identify a definite and active HCV in the body. Therefore, molecular tests are necessary. In this study, five subjects out of seven with ANTI-HCV positive according to ELISA method, show HCV-RNA according to molecular RT-PCR. This indicates that molecular analysis is essential for the diagnosis and therapy of these patients (Tables 2 and 3).

Table 2: Distribution of HCV patients further confirmed by RT-PCR

Percentage	No.	
20.8	5	Positive
79.2	19	Negative
100	24	Total

Table 3: Distribution of Anti-HCV p atients further confirmed by RT-PCR

Percentage	No.	Results RT-PCR
71/4	5	Positive
28/6	2	Negative
%100	7	Total

ANTI-HCV frequency distribution by age:

The average age in patients with thalassemia is 18/6 that the number in ANTI-HCV negative patients is 17/5 and ANTI-HCV positive patients is 22/1. The difference is statistically significant, i.e., in thalassemia patient's subjects, the likelihood of a positive result for HCV testing increases with increasing age.

Table 4: Comparison of age factor in studied thalassemia patients according to Anti-HCV test result

maximum	minimum	Standard deviation	median	mean	No.	Anti-HCV
26	6	4/97	18	17/35	17	Positive
31	16	5/01	22	21/85	7	Negative

Frequency distribution according to gender:

Regarding the sex, there were 15 female (63.5%) patients and 9 males (37.5%) out 24 patients with thalassemia major who were prospectively studied.

ANTI-HCV testing in males is 22% positive and 78% negative and in females is 33% positive and 67% negative, that shows the higher prevalence of Hepatitis C in females is in this study.

Table 5: Comparison of sex factor in the two groups ANTI-HCV positive and negative tests in patients with thalassemia major

Percentage	Positive	Percentage	Negative		
%71/4	5	%58/8	10	F	sex
%28/6	2	%41/2	7	M	

Frequency Distribution based on the number of blood transfusions:

Another related to hepatitis C patients evaluated variable was the frequency of blood transfusion. The maximum blood transfusion annually was 24 times with the minimum of 12 times per year, showing the mean of 20 and standard deviation of 3.31. 60% or more thalassemia patients with ANTI-HCV positive had 20 or more blood transfusion annually. The mean blood transfusion frequency in these subjects was 392 times with the median of 440 times up to the date of sampling , but in ANTI-HCV negative patients the mean annual blood transfusion was 319.5 with the median of 369, that indicates that increased number of blood transfusions increases the incidence of hepatitis C.

Table 6: comparison of the frequency of blood transfusions factor according to Anti-HCV test results in patients with thalassemia major

maximum	minimum	Standard deviation	median	mean	No.	Anti-HCV
440	90	101/74	360	319/5	17	Negative
460	294	75/5	440	392	7	Positive

According to test results, Gel Doc analyzed CDNA amplified by RT PCR and the result is shown in figure (1).

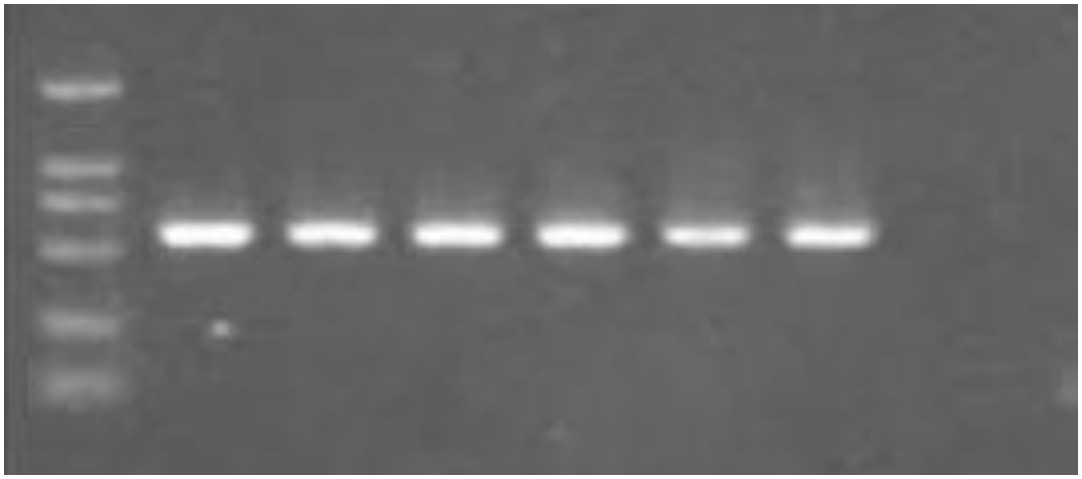


Fig. 1: CDNA amplification by RT PCR results

Discussion and Conclusions:

Today, molecular methods to detect and differentiate between microorganisms are much more efficient and effective than the classical methods. Meanwhile, the RT-PCR method because of its features such as speed and low cost, and high sensitivity without high technical skills required is considered. On the other hand, expressed in reviews Hepatitis C is known as the most common hepatitis after blood transfusion so that in some countries such as Japan, the U.S. and West Europe 90% of the diseases are due to transfused bloods [6]. The research stated that HCV is associated with iron overload [7]. Due to the constant need for blood transfusion in patients with thalassemia major and given the drastic reduction of risk of hepatitis B transmission from a blood transfusion, hepatitis C virus is transmissible agent in patients with thalassemia major. The total number of thalassemic patients in this hospital is 24 of which 15 are female and 9 others are male. Educational level of the patients is 4/2% illiterate, 16.7% elementary, 25% high school 54.2% diplomas and more, respectively. The patients with thalassemia major in Qir and Karzin County are ranged between 6-31 years and male to female ratio is equal to 0.6.

The prevalence of 24.2% have been reported in a study in our country conducted by doctor Alavi et al (2002) in Qazvin province on 95 thalassemia major patients[8]. In addition, according to another study by doctor Mir Momin (2007) on 410 patients with thalassemia HCV prevalence of 27% was reported [9].

In our study, the prevalence of hepatitis C in patients with thalassemia major Qir and Karzin County is 29%, which in comparison with other studies, the prevalence of HCV in patients with thalassemia is the same or relatively high.

According to the study, the mean age of patients with ANTI-HCV thalassemia positive is 22/1 and in ANTI-HCV negative patients are 17.5, respectively. This finding is expected because the higher the patients' age, the more time of exposure to infected with HCV, as a result the higher prevalence of HCV.

In similar studies, there is a statistical relationship between increasing age and increasing number of ANTI-HCV positive patients, respectively .Also in this study, a comparison between the number of blood transfusions in patients with ANTI-HCV thalassemia major positive and negative show that thalassemia major positive subjects had higher or equal to 15 years of blood transfusions than thalassemia major negatives. This result was expected, as well.

In our study, ANTI-HCV test result was positive in 22% of males and 33% of females.

In a study by Malek Sahi et al conducted in Kerman province on 181 patients with thalassemia the HCV test results of males was half positive and half negative, while in females it was 59% negative and 41% positive. However, generally it does not seem that a relationship between gender and HCV infection in patients with thalassemia exists. According to other studies with different statistics on males and females infections, there is a need for greater accuracy.

In this study, 71% of patients with serum ferritin levels between 1000- 2500 ng/ml showed ANTI-HCV positive without any significant relationship between the ANTI-HCV infection and serum ferritin. In the current study, normal serum ferritin in patients with thalassemia was considered more than 700 ng/ml. In this study, seven patients showed HCVAb positive with five patients HCV-RNA positive, or in another word RT-PCR positive. In a study in our country by Ismail Doki et al in Babel, as case-control, 2 patients (2%) were ANTI-HCV-positive with one of them PCR positive.

Conclusions:

In this study, the prevalence of hepatitis C in patients with thalassemia major is very high; and given that an effective vaccine to prevent hepatitis C is still not available, to prevent new infections through blood products, the use of more sensitive methods to detect contamination and increase accuracy in screening donated blood is the only solution available. serologic screening for hepatitis C infection in patients with thalassemia is used; but to determine the absolute infection with preventive and treatment goals in situ RT-PCR tests should be considered to the prevent the risk of transmission of infection to other critical medical equipment and laboratory and clinical staff, as well. Unfortunately, the molecular tests are not supported by insurance, as a result due to the high cost of these tests it is expected the insurers to endeavor to provide services to the test.

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