PREVALENCE OF HEPATITIS C VIRUS INFECTION AMONG CHILDREN WITH BETA THALASSEMIA MAJOR IN SULAIMANI

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ABSTRACT

Background

Hepatitis C infection is a worldwide problem, especially in multi transfused patients including those with Thalassemia. Thalassemia is one of the inherited diseases, in which there is partial or complete failure of globin chain synthesis.

Objectives

To evaluate the prevalence of hepatitis C virus infection in multiple blood transfused children with beta-thalassemia major and related risk factors.

Pateints and Methods

A cross-sectional study was conducted on 459 patients (229 males and 230 females) with β-thalassemia major at Sulaimani thalassemia centre from January 2015 to June 2015. Data regarding age, sex, number of blood transfusions, chelating agents and history of splenectomy were obtained. Serum was used for detection of antibodies against hepatitis C virus, hepatitis B surface antibody, hepatitis B surface antigen and, human immunodeficiency virus, also in liver enzymes (Aspartate aminotransferase, Alanine aminotransferase) were checked.

Results

Sixty-four (13.9%) patients were found to be seropositive for anti-Hepatitis C virus antibodies; this was correlated with age, sex, history of splenectomy, number of blood transfusions and chelating agents. Liver enzymes were significantly higher in infected patients. The risk of exposure to hepatitis C virus was higher than the hepatitis B virus surface antigen and human immunodeficiency virus among the same patients.

Conclusion

Multi transfused thalassemic patients are at high risk for HCV infection, so more accurate techniques for screening of blood products is suggested.

Keywords: *Hepatitis C virus, Thalassemia major, Prevalence.*

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INTRODUCTION

Thalassemia refers to a group of genetic disorders of globin chain production in which there is an imbalance between the α-globin and β-globin chain production [1]. The type of thalassemia usually carries the name of the underproduced chain or chains. The reduction varies from a slight decrease to a complete absence of production. The consequences of impaired production of globin chains ultimately result in the deposition of less haemoglobin into each red blood cell, leading to hyperchromasia. The haemoglobin deficiency causes red blood cells to be smaller, leading to the classic hypochromic and microcytic picture of thalassemia [2]. The most common type is beta-thalassemia [3].

Two related features contribute to the sequelae of β-thalassemia major: inadequate β-globin gene production leading to decreased levels of normal haemoglobin (HbA) and unbalanced α- and β-globin chain production. In β-thalassemia major, α-globin chains are in excess to non–α-globin chains and α-globin tetramers (α4) are formed and appear as red cell inclusions. The free α-globin chains and inclusions are very unstable, precipitate in red cell precursors, damage the red cell membrane, and shorten red cell survival leading to anaemia and increased erythroid production. This results in a marked increase in erythropoiesis with early erythroid precursor death in the bone marrow. Clinically, this is characterized by a lack of maturation of erythrocytes and an inappropriately low reticulocyte count. This ineffective erythropoiesis and the compensatory massive marrow expansion with erythroid hyperactivity characterize β-thalassemia [1] .

Because the β-thalassemia patient cannot make HbA, the α-chains combine with γ-chains, resulting in HbF (α2γ2) being the dominant haemoglobin. δ-Chain synthesis is not usually affected in β-thalassemia or β-thalassemia trait, and, therefore, patients have a relative or absolute increase in HbA2 production (α2δ2). Infants with β-thalassemia major become symptomatic only after birth when HbA predominates and insufficient β-globin production manifests in clinical symptoms [1]. Infants with severe beta-thalassemia major (BTM) are well at birth because the production of beta-globin is not essential during fetal life or the immediate perinatalperiod [4]. Symptoms emerge during the second six months of life when gamma globin chain production decreases and is normally replaced with the production of beta-globin to form adult haemoglobin (Hb A,alpha2/beta2) [5].

If not treated, children with homozygous β-thalassemia usually become symptomatic from progressive hemolytic anaemia, with profound weakness and cardiac decompensation during the 2nd 6 month of life. Depending on the mutation and degree of fetal haemoglobin production, transfusions in β-thalassemia major are necessary beginning in the 2nd mo to 2nd yr of life, but rarely later. The decision to transfuse is multifactorial but is not determined solely by the degree of anaemia. The developing signs of ineffective erythropoiesis such as growth failure, bone deformities secondary to marrow expansion, hepatosplenomegaly are important variables in determining transfusion initiation [1].

The classic presentation of children with severe disease includes thalassemic facies (maxilla hyperplasia, flat nasal bridge, frontal bossing), pathologic bone fractures, marked hepatosplenomegaly, and cachexia and is now primarily seen in countries without access to chronic transfusion therapy. Occasionally, patients with moderate anaemia develop these features because of severe compensatory ineffective erythropoiesis. In nontransfused patients with severe ineffective erythropoiesis, marked splenomegaly can develop with hypersplenism and abdominal symptoms. The chronic anaemia without transfusion exposure produces an increase in iron absorption from the gastrointestinal tract and secondary hemosiderosis-induced organ injury [1].

Chronic transfusion therapy dramatically improves the quality of life and reduces the complications of severe thalassemia. Transfusion induced hemosiderosis becomes the major clinical complication of transfusion-dependent thalassemia. Each mL of packed red cells contains 1 mg of iron. Physiologically, there is no mechanism to eliminate excess body iron. Iron is initially deposited in the liver. Liver hemosiderosis develops after 1 year of chronic transfusion therapy and is followed by iron deposition in the endocrine system. This leads to a high rate of hypothyroidism, hypogonadotropic hypogonadism, growth hormone deficiency, hypoparathyroidism, and diabetes mellitus. After 10 years of transfusion, cardiac dysfunction secondary to hemosiderosis begins. Eventually, most patients not receiving adequate iron chelation therapy die from cardiac failure and/or cardiac arrhythmias secondary to hemosiderosis [1].

Eighty per cent of untreated children will die within the first five years of life, due directly to the consequences of severe anaemia, high output heart failure, inanition, and unusual susceptibility to infection [6].

Risk factors for HCV transmission in US included blood transfusion before 1922 as the most common route of infection, but with current screening practice, the risk of HCV transmission is approximately 0.001% per unit transfused [7].

The aims of the study were to evaluate the prevalence of hepatitis C virus(HCV) antibodies in patients with thalassemia major on multi transfused regimens, who were regularly attending Sulaimani thalassemia centre, and to determine the correlation of anti-HCV seropositivity with certain variables including age, sex, number of blood transfusions, type of chelating agent and history of splenectomy.

PATIENTS AND METHODS

A cross-sectional study was conducted on 459 patients (age range, 1-18 years) with β-thalassemia major at Sulaimani thalassemia centre from January 2015 to June 2015. The diagnosis of thalassemia was confirmed by haemoglobin electrophoresis. Data regarding age, sex, number of blood transfusions, use of chelating agents and history of splenectomy were obtained. The patient's blood samples were taken and the following serological tests were performed using standard methods:

Anti-HCV antibodies were screened for using third generations ELISA assay by ELx50 microplate strip washer supplied from BioTek Company. HBsAg and anti-HIV antibodies were screened by using a quantitative third-generation microparticle enzyme immunoassay by ELx50 microplate strip washer supplied from BioTek Company.

Anti-HCV antibody-positive cases were confirmed by polymerase chain reaction (PCR) by using Exicycler96 quantitative Real-Time PCR system from Bioneer Company. (This study was retrospective only these results were available.)

Serum liver enzymes [serum Alanine Aminotransferase (ALT) and serum Aspartate Aminotransferase (AST)] levels were measured and the tests performed according to the international federation of clinical chemistry standards by using automatic biochemistry analyzer Flexor EL200.

Data were analyzed using the computer facility-IBM SPSS Statistics 21. The seroprevalence for infection was calculated. Chi-square test was used to assess the correlation of age, sex, number of blood transfusions, receiving chelating agents and splenectomy to acquiring positive anti-HCV antibodies. Results are considered as highly significant at P0.05.

RESULTS

The demographic data of all 459 patients included in this study are shown in Table 1. There were 229 male and 230 female patients. 213 patients were between 1-9 years of age and 246 patients were above 9 years of age.

Two hundred eighty seven patients had received blood less than 15 times/year after the initial diagnosis while 172 patients received blood more than 15 times/year. Eighty-seven patients were splenectomised while 372 patients were not.

The seropositive cases for HCV, HBV and HIV among this group of 459 are shown in table 2. Sixty-four out of 459 patients (13.9%) had positive anti-HCV antibodies, 5 were positive for HBsAg and none was positive for HIV.

From the 64 seropositive patients, two patients were between 1-9 years of age and 62 patients were above 9 years of age. There was a significant relation between age >9 years. P-value <0.01 which was highly significant.

From the 64 seropositive patients, 42 were males and 22 females. There was a significant relationship between male gender.P-value <0.01 which was highly significant.

From the 64 seropositive patients, 15 patients received blood less than 15 times/year after the initial diagnosis while 49 patients received blood more than 15 times/year. There was a significant relation of the number of blood transfusions/year (≥ 15 times).P-value <0.01 which was highly significant.

From 64 seropositive patients, 32 patients had splenectomy. There was a significant relation of the history of splenectomy (splenectomised). P-value <0.01 which was highly significant.

From 64 seropositive patients, 22 patients were on desferal as a chelating agent. There was a significant relation to the type of chelating agent (desferal).P-value <0.05 which was significant. From 64 seropositive patients, 37 patients had normal liver function tests and 27 had high liver function tests. There was a significant relation to the high liver enzymes P-value <0.01 which was highly significant.

Table 1. Sociodemographic characteristics of multi transfused thalassemic patients.

Characteristics	Number of patients (n=459)	Percentage of patients(%)
Sex		
Male	229	49.8
Female	230	50.2
Age group(years)		
1-9 years	213	46.4
> 9 years	246	53.6
Number of blood transfusion/year		
< 15 times	287	62.5
≥ 15 times	172	37.5
Splenectomy		
Yes	87	18.9
No	372	81.1

Table 2.Prevalence of viral infection in multi transfused thalassemic patients (n=459).

Serology	Number of positive Results	Percentage (%)
Anti-HCV	64	13.9
HBsAg	5	1.09
Anti-HIV	0	0

Table 3. Prevalence of HCV positive patients according to age.

Age(years)	Seropositive patients (%)	Seronegative patients (%)	Total number of patients
1-9 years	2(1%)	211(99%)	213
> 9 years	62(25%)	184(75%)	246

Table 4. Prevalence of HCV positive patients according to sex.

Sex	Sero(+) patients (%)	Sero(-) patients (%)	Total number of patients
Male	42(18%)	187(82%)	229
Female	22(10%)	208(90%)	230

Table 5. Prevalence of HCV positive patients according to the number of blood transfusions/year.

Number of blood transfusions/year	Sero(+) patients (%)	Sero(-) patients (%)	Total number of patients
< 15 times	15(5%)	272(95%)	287
≥ 15 times	49(28%)	123(72%)	172

Table 6. Prevalence of HCV positive patients in relation with splenectomy.

Splenectomy	Sero(+) patients (%)	Sero(-) patients (%)	Total number of patients
Yes	32(37%)	55(63%)	87
No	32(9%)	340(91%)	372

Table 7. Prevalence of HCV positive patients according to the type of chelating agent.

Chelating agent	Sero(+) patients (%)	Sero(-) patients (%)	Total number of patients
Desferal	22(20%)	87(80%)	109
Exjade	42(12%)	308(88%)	350

Table 8. Liver function tests (ALT, AST) in seropositive and seronegative thalassemic patients.

Liver function test	Sero(+) patients (%)	Sero(-) patients (%)	Total number of patients
Normal	37(9%)	385(91%)	422
High	27(73%)	10(27%)	37

DISCUSSION

Before the introduction of routine testing for HCV antibody in blood donation, the risk of exposure to HCV directly related to the number of transfused blood units and on the prevalence of HCV in blood donor population [8]. In consequence, patients affected with haemoglobinopathies such as thalassemia major, being those most transfused with packed red blood cells were frequently contaminated by HCV, with prevalence varying geographically from 23% to 72% [9].

In this study, we found a prevalence of 13.9% for HCV infection. Only 5 patients were positive for HBsAg, which give a prevalence rate of 1.09%, the low prevalence of HBV may be due to the use of hepatitis B vaccine in the immunization schedule, in addition to using third-generation ELISA technique for screening of donated blood, and the strict precautionary measures applied at hospital against spread of HBV infection. None of the patients tested was positive for HIV. HCV sero positivities reported in some Arabian countries like Egypt, Bahrain, and Saudi Arabia, in addition to other countries like China was higher than our result. The prevalence in these countries were 44% [10], 40% [11], 70% [12], 34% [13], respectively. However, in the USA and since the introduction of screening of blood products, transfusion now accounts for less than 5% of new hepatitis C- cases [14]. The difference in the results among different countries could be explained by different methods of screening (including First & second-generation ELISA) with variable sensitivity and specificity which give high false-positive results [12], in addition to high frequency of blood units used in the treatment.

In this study, we use the third generation ELISA with very high sensitivity & specificity [14]. This difference also could be explained by two other factors, i.e., the prevalence of HCV in the relevant population (and therefore also in the blood donors), and the practice of HCV antibody screening before the transfusion is instituted [15]. The countries with a higher HCV prevalence in the general population had a higher prevalence rate among thalassemia patients, too. For Instance, a study in Egypt reported 75% of HCV prevalence among thalassemia patients, because the prevalence in their blood donor population was 14.5% [16]. However, in India with a low HCV prevalence among

blood donors (1.78%), the prevalence in thalassemic was reported relatively low (25.5%) [17].

Regarding the practice of HCV antibody screening before transfusion, it shows that Prevalence of blood transfusion-associated HCV infection is lowered in developed countries after implementing mandatory screening in 1990s [18], in Japan prevalence, dropped from 4.9% to 1.9% after mandatory screening was introduced in1990 [18] . In the US the prevalence dropped from 3.84% to 0.57% after 1990 [19]. Still, high rates encountered in many countries. In India, mandatory screening for HCV was introduced as late as 2002 [20], the prevalence in thalassemic was 25.5% [17].

Prevalence of hepatitis C (HC) positivity among thalassemic patients in Iraq ranges from 10% to 67.3% according to different studies [21]. HCV-specific antibodies were detected in 67.3% among thalassemic patients in Ibn Al Balady hospital, Baghdad [22]. In another study done at the same hospital, the prevalence is 37.6% [21]. The prevalence is 30% in Mosil, and 10% Thiqar [21].

In our study the prevalence rate of HCV was directly related to the number of transfused units of blood, 28% of patients who had yearly received blood for more than 15 times were seropositive, compared to 5% of patients who had received less than 15 times/ year, other studies have revealed similar results [12, 23- 27] & these results can be explained by the increased risk of transmission of infection with increasing number of blood units transfused. We found that there is a significant difference of Anti HCV sero-positivities prevalence rate among males and females, other studies have revealed similar results [24, 26].

This study showed that the prevalence of HCV infection increases with increasing age of the patients, ranging from 1% in patients less than 9 years to 25% in those more than 9. This rising figure has been documented in other studies [28, 29, 30]. This could be explained by an increased chance of exposure to infected blood, by the increased number of blood units transfused as children getting older or by increased frequency of admission to hospital with increased possibility of exposure to infected device or materials.

The prevalence of HCV infection is significantly increased in thalassemic children with splenectomy 37%, while it was 9% in non-splenectomised patients. This could be explained by that splenectomised patients have had a more severe form of thalassemia and receive more frequent blood transfusions than non-splenectomised patients. In contrast to our study, other studies recorded that there was no significant difference in the prevalence of HCV infection between splenectomised and non-splenectomised children [28,29,31].

In developing countries acquisition of HCV infection is mainly nosocomial [32,33]. As a sequel of low health care standards. While the major route of HCV infection in developed countries is through the use of intravenous drugs [34] .

In our study, there was a significant difference in the prevalence of HCV infection in patients receiving desferal as a chelating agent (20%) from those receiving oral exjade(12%). This could be explained by that the patients on desferal receive more blood units than those on oral exjade. This is in contrast to the result of other studies [35].

In the comparison of our results to other study done in Sulaimani- Iraqi Kurdistan at 2005, there was a decrease in the prevalence of HCV antibodies from 24% to 13.9% and this could be due to improvement in the methods practised to prevent the infection withHCV [27].

In conclusions; the study revealed that the prevalence of HCV antibodies among thalassemic children was 13.9% and this rate is lower than that reported in many countries. The prevalence of anti HCV seropositivity is higher than that for other viral infection transmitted mainly through the blood like HBV and HIV infection. Anti HCV sero positivities were directly related to the frequency blood transfusion, age of patients, sex of patients, type of chelating agent and splenectomized patients.

Recommendations

1- All thalassemic patients should be screened for HCV infection periodically.

2- Availability of ELISA that detects antibody rather than antigen after 14-16 weeks from onset of infection (20-22 weeks from blood transfusion) may result in false-negative results. Therefore, it is best to do PCR which detect viral antigens as early as 1-2 weeks from onset of infection.

3- Encouragement of bone marrow transplantation in the treatment of thalassemic patients as an advanced method. (This is nearly radical treatment for thalassemic patients which is available in our city now.)

4-Education of the population about the disease and the importance of screening tests.

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