Frequency of Viral Hepatitis in Thalassemic Patients Receiving Multiple Blood Transfusions

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الخلاصـــة:

الهدف: أجريت هذه الدراسة أثناء الفترةِ مِنْ الأول مِنْ تشرين الأول **2007** إلى الأول من مايس **2008**. كان هدفَ هذه الدراسةِ هو تقدير إنتشارَ إلتهابِ الكبد الوبائي في مجموعة من المرضى العراقيين المصابين بالثلاسيميا الخاضعين لنقل الدم المتكرر مقارنة بمجموعة المرضى قبل نقل الدم والمجموعة الضابطة للاصحاء.

المنهجية: عينات الدمّ جُمِعتْ مِنْ مائة شخص؛ 50 عينة مِنْ المرضى المحالين إلى مستشفى العباس في مدينةِ الكوت و50 عينة أخرى مِنْ المتبرّعين بالدم الأصحاء في مصرف الدم . بالإضافة إلى ذلك، تمّ فحص 25 عينة دم من المرضى قبل إجراء نقل الدم . تمّ في هذه الدراسة البحث عن وجود المستضد السطحي لفايروس إلتهاب الكبد (B) والأجسام المضادة لكل من فايروس التهاب الكبد (A) و(C) في عينات المصل باستخدام تقنية الجيل الثاني من المقايسة المناعية الإنزيمية (ELISA) .

النتائج: ظهر من خلال النتائج أنّ نسبة المستضد السطحي لفايروس (B) والأجسام المضادة لكل من فايروس (A) و (C) كانت على التوالي : أربعة من 50 (8 %، 95% فترة ثقةِ =0.17-1.3)، اثنان من 50 (4 %؛ 95 % فترة ثقة =12.6-19.2)، ستة عشر من 50 (32 %؛ 95 % فترة ثقةِ =0.12-2.3). النتائج الموجبة للأجسام المضادة لفايروس (A) في مجموعة المرضى قبل نقل الدم كانت 2 من 25 (8%)، والنتائج الموجبة للأجسام المضادة لفايروس (C) في المجموعة الضابطة للأصحاء كانت اثنين من 50 (4%) . معدل الأجسام المضادة لفايروس (C) في مجموعة المرضى كان أكثر بشكل مهم من مجموعة المرضى قبل نقل الدم والمجموعة الضابطة .

الاستنتاجات: يستنتج من هذه الدراسة أنّ فايروس التهاب الكبد نوع (C) هو المشكلة الرئيسية في الوقت الحاضر في مرضى الثلاسيميا الخاضعين لعمليات نقل الدم المتكررة.

التوصيات: أوصت الدراسة بإيلاء أهمية قصوى لفحص الدم والكشف عن وجود الأجسام المضادة لفايروس التهاب الكبد نوع (C) قبل نقله إلى مرضى الثلاسيميا. تقترح هذه الدراسة أجراء المزيد من الأبحاث على عدد كبير من المرضى باستخدام التقنيات الجزيئية المتقدمة للبحث الدقيق عن الفايروس.

Abstract:

Objective: The present study was conducted during the period from October 1st 2007 to May 1st 2008. The aim of this study was to estimate the prevalence of viral hepatitis in a group of Iraqi thalassemic patients receiving multiple transfusions compared with pre-transfused thalassemic patients and apparently healthy controls.

Methodology: Blood samples were collected from one hundred patients, 50 samples from patients with beta thalassemia major referred to Al-Abbas Hospital in Al-Kut City and 50 samples from apparently healthy blood donors in blood bank, in addition to 25 blood samples from pre-transfused thalassemic patients. Hepatitis B surface antigen (HBsAg), anti-hepatitis A virus (HAV), and anti-hepatitis C virus (HCV) antibodies were checked by using a second-generation enzyme-linked immunosorbent assay (ELISA).

Results: Hepatitis B surface antigen, anti-HAV, and anti-HCV antibodies were positive in four of 50 (8%, 95% confidence interval (CI)=0.17-1.3), two of 50 (2%; 95% CI=12.6-19.2), and 16 of 50 (32%; 95% confidence interval (CI)=0.12-2.3), respectively. Positive sera for anti-HAV Abs were found in 2 of 25 (8%) pre-transfused patients. Positive sera for anti-HCV Abs were found in 2 of 50 (4%) healthy control. The rate of anti-HCV Ab was significantly higher in multitransfused patients than in the pretransfused and control groups ($P < 0.01$).

Conclusions: It was concluded that HCV is the current major problem in multitransfused patients with thalassemia major.

Recommendations: More careful pretransfusion screening of blood for anti-HCV must be introduced in our blood banks. More cohort studies are needed by applying advanced molecular techniques for accurate viral detection.

Key words: Viral Hepatitis, Beta-Thalassemia Major, Multiple transfusions

Introduction:

Thalassemia is a group of genetic blood disorders characterized by the absence or reduction in the production of hemoglobin. Severity is variable from less severe anemia, through thalassemia intermedia, to profound severe anemia (thalassemia major) [(1)]. Management of patients with beta-thalassemia is based on adequate, safe blood transfusions (free of transfusion-transmitted diseases) and prevention of iron overload [(2)]. Among people who have thalassemias, infections are a key cause of illness and the second most common cause of death.

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The major complications of blood transfusions are those related to or the development of iron overload and transmission of infectious agent.

As recently as a few years ago, 25% of transfused patients were exposed to hepatitis B virus. At present, both immunization and strict screening of potential donors have significantly decreased the incidence. Hepatitis C virus (HCV) is the most common cause of hepatitis in adolescents older than 15 years with thalassemia (risk of exposure was 6%). Because both liver failure and hepatocellular carcinoma have been attributed to HCV in patients with thalassemia, aggressive treatment with interferon alpha is warranted in patients who contract Hepatitis C virus [3]. The incidence of transfusion-transmitted HCV is expected to drop significantly because of stricter blood screening now mandated; patients with thalassemia are surviving their disease longer and reaching old age. With this longer survival come new issues related to complications that need to be addressed. HCV has emerged as the paramount risk in patients who have been receiving blood transfusions all their lives. HCV screening was initiated in 1990. Since then, according to the Registry of the Thalassemia Clinical Research Network, the incidence rate of HCV has dropped significantly.

Unfortunately, a high incidence rate of HCV continues in developing countries, leading to an increased incidence of fibrosis, cirrhosis, and hepatocellular carcinoma, especially in the presence of a second risk factor such as iron overload [4]. It was recorded that beta-Thalassemia is an important health problem in Iraq because of its high carrier rate and the frequency of consanguineous marriages [5]. This study was designed to evaluate the rate of seropositivity to hepatitis A, B and C infections among patients with beta-thalassemia major who are receiving multiple transfusions in a group of Iraqi patients, compared with pre-transfused patients and apparently healthy controls.

Methodology:

A total number of 75 blood samples were collected from 50 patients (24 males, 26 females) with beta-thalassemia major receiving multiple blood transfusions, and an additional 25 patients (10 males, 15 females) with recently diagnosed thalassemia before starting a regular multi-transfusions program. In addition, 50 blood samples were collected from apparently healthy blood donors (38 males, 12 females) attending blood bank as a control group. It was unknown if blood that obtained from the control group might be used for transfusion in the studied thalassemic patients or not. The healthy controls had no history or clinical evidence of thalassemia and any other hematological disease. The study was conducted during the period from (October 1st 2007 to May 1st 2008). All blood specimens were examined at the Department of Virology in Public Health Laboratory in Wassit Province and the Thalassemia Unit in Al-Abbas Hospital in Al-Kut City. The selection of patients was achieved according to clinical and hematological criteria suspecting the possibility of beta-thalassemia major documented by the hospital records. A blood sample of 5–10 ml was obtained from each subject. Sera were separated immediately (within 1 hour) and each serum sample was then dispensed into a screw-capped vial stored at –20 °C and –70 °C. All specimens were utilized for detection of HBs-Ag, anti-HAV, and anti-HCV antibodies. The ELISA technique was performed for the quantitative determination of antibodies to hepatitis A and C viruses, (UBI, HAV, EIA, United Biomedical, USA). The determination of HBs-Ag was performed by (biolcit, bioelisa HBs-Ag color Kit according to manufacture instructions. Each specimen of all pre-transfused patients was tested repeatedly at least three times to confirm the same results obtained.

Statistical analysis: Descriptive statistics were used for creating statistical tables (observed frequencies, percentages) and creating contingency tables. Inferential statistics were used in order to accept or reject the statistical hypotheses they include: Binomial test for testing the difference between two ratios related to binary nominal responding with pointed their *P*-values, and Chi-square test for testing independency between the two categories factors in the

contingency table with pointed their *P*-values. For all analysis, statistical significance was considered at highly significant level *P*-values of < 0.01, significant level *P*-values of < 0.05, and insignificant level *P*-values of > 0.05. All the statistical analysis was done by using SPSS computer program version 10 and Excel application.

Results:

Table 1. Demographic characteristics of study patients with beta thalassemia major receiving multiple blood transfusions

Characteristic	Thalassemic patients	
	Frequency	Percentage
1- Gender		
-Male	24	48%
- Female.....	26	52%
2- Age		
- <1year	3	6
- 1-10 years.....	11	22
- 11-20 years.....	14	28
- 21-40 years.....	20	40
- 41-60 years	2	4

Table (1) shows demographic characteristics of the study patients. The table revealed that 24 (48 %) were male patients and 26 (52%) were female patients of different age groups ranged between less than one year to about 60 year. The age group between 21-40 years constitutes the higher number and percentage, 20 (40%).

Table 2. Clinical presentation and chelation therapy for study patients with beta thalassemia major receiving multiple blood transfusions

Items	Thalassemic Patients	
	Frequency	Percentage
1- No. of blood transfusions		
0-10 units.....	3	6
11-50 units.....	7	14
51-150 units.....	7	14
151-250 units.....	4	8
251-500 units.....	15	30
501-750 units.....	10	20
751-1020 units.....	4	8
2-Complications		
- Cardiomyopathy.....	15	30
- Delayed puberty.....	9	18
- Diabetes mellitus.....	9	18
- Splenomegaly	17	34
3-Chelation therapy		
- Iron (desferal)	21	42
- Folic acid +vitamins.....	19	38
- Hydrocortisone.....	3	6
- Lasix.....	7	14

Table (2) shows that the majority of patients received more than 250 blood units. Delayed puberty represents the most frequent complications of thalassemia that was recorded

in 19 (38%) patients. In case of complications, clinical examination revealed that 15 (30%) of study patients have cardiomyopathy, (9) 18% of study patients suffering from delayed puberty (9) 18% of study patients have diabetes mellitus and (17) 34% of study patients have splenomegaly. Most of patients received iron chelating therapy.

Table 3. Frequency of viral hepatitis in thalassemic patients and controls according to number of transfused blood units

Studied groups	Positive Anti-HAV Abs		Positive HBs-Ag		Positive anti-HCV Abs	
	F	%	F	%	F	%
1- Pretransfused patient group (n=25)	2	8 %	-	-	-	-
2- Multitransfused patient group (n=50)	-	-	-	-	-	-
-Receiving 0-10 units	-	-	-	-	-	-
-Receiving 11-50 units	-	-	-	-	2	4 %
-Receiving 51-150 units	1	2 %	2	4 %	2	4 %
-Receiving 151-250 units	-	-	1	2 %	3	6 %
-Receiving 251-500 units	-	-	1	2%	5	10 %
-Receiving 501-750 units	2	4 %	-	-	4	8 %
-Receiving 751-1020 units	-	-	4	8 %	16	32 %
2- Healthy donors control (n=50)	-	-	-	-	2	4 %

F= frequency; %= percentage

Table (3) shows the examination of 75 serum samples of thalassemic patients for the detection of viral hepatitis, pre- and post-transfusions. Only two (8%) patients were detected with positive anti-HAV Abs in pretransfusion group. Out of the total, it was shown that two (4%, 95% CI=12.6-19.2) patients were with hepatitis type A, four (8%, 95% confidence interval (CI)=0.17-1.3) patients were with hepatitis type B, and 16 (32%, 95% confidence interval (CI)=0.12-2.3) patients were with hepatitis type C. The highest number and percentage of patients with positive anti-HCV Abs were shown in the group receiving 501-750 blood units, 5 (10%). The results of studied patients are presented in comparison with the results of apparently healthy controls. There was a highly significant difference between the group of patients receiving multiple blood transfusion and apparently healthy control group, (*P*-value=<0.01). The same statistical highly significance was observed when comparing with patients before blood transfusion. Serum samples from healthy donors control revealed only two (4%) positive anti-HCV Abs.

Discussion:

The results of the present study revealed that male and female incidence of thalassemia was 48% and 52%, respectively which is equal to what showed in a study conducted on thalassemic patients in India [6]. The incidence of hepatitis B infection in our study was 8%, compared with 20% that revealed in the Indian study, whereas hepatitis C infection was 32% while in the Indian study was 30%. The incidence of hepatitis B and C in our study is higher than what recorded by Karimi in an Iranian study conducted on thalassemic patients, 0.53% for hepatitis B and 15.7% for hepatitis C [7].

The high incidence of hepatitis B and C infection among patients with thalassemia receiving frequent blood transfusion in spite of history of vaccination might raise the question on the efficacy of the vaccines which were given according to immunization schedule in Iraq and the reliability of the history taken from the parents. Analysis of data collected as part of the Comparative Risk Assessment component of the Global Burden of Disease study suggests that the region which includes Iraq faces substantial challenges in terms of unsafe injection

practices and transmission of blood-borne pathogens through injections. In this region, the proportions of new infections with Hepatitis B, Hepatitis C, and HIV that are attributable to unsafe injections practices are 58.3%, 81.7% and 7.1% respectively [8].

In a study conducted in Baghdad on 1998 by W.A. Al-Kubaisy *et al,* it was recorded that the seroprevalence of HCV-antibody among multi-transfused children with thalassemia was (84.5%) [9]. Surveys on thalassemic patients worldwide have found variable rates of anti-HCV-antibody seroprevalence. Moreover, Iraqi children with thalassaemia have demonstrated a higher rate than that reported in other countries, such as 40.7% in Jordan [10], 40% in Saudi Arabia [11], and 14% in Turkey [12]. It was concluded from Al-Kubaisy's study that there were two important reasons could be suggested as an explanation for such a high rate reported in that study. First, screening of blood for HCV in blood banks in Iraq started in 1996, and secondly, shortage of blood and other supplies in the health services as a result of the international sanctions against Iraq in the 1990s resulted in lower standards of cleaning and sterilization of medical instruments and a shortage of disposable syringes and needles.

It was clearly demonstrated from the present study that the frequency of viral hepatitis was increased proportionally with the number of blood transfusion units received by thalassemic patients. The impact of increment blood transfusions on hepatitis infection might give an idea of inadequate hepatitis screen in the national blood bank center, but doesn't explain why the children got infection while they have recent vaccines in the hospital. Laboratory errors, whether personal errors or quality of the kit's material might occur, but it wasn't evident in this study as the entire patients got nearly normal hepatitis screen at time of first evaluation during first admission to the hospital or during routine follow-up. The incidence of hepatitis C infection has no explanation and again might raise the accuracy of hepatitis C screening which depends on the method and quality of kits in the laboratory department or might reflect better screening of hepatitis C in the national blood bank center. There might be some patients missed screening for hepatitis C in the initial evaluation due to shortage of lab material and the concentration in the records was mainly on the results of hepatitis B screen. There should be a better assessment of the number of admissions to hospital that might give a hint of the unsafe procedures that made by paramedics during invasive procedures or drug injections. There should be an observation to the invasive procedures done in the unit by doctors or paramedical staff like cannula insertion, drug injections, etc [13].

In the present study, serum samples from healthy donors control revealed only two (4%) positive anti-HCV Abs. Nevertheless, as was mentioned above, it was unknown if that blood obtained from the control group might be used for transfusion in the studied thalassemic patients or not. Negative screen on blood specimens for anti-HCV before transfusion doesn't eliminate the risk of infection by HCV, because the donor may be in the window period which gives negative result for anti-HCV antibodies. So, it is unlikely that any test or combination of tests will be 100% effective in detection window period infection [14,15].

However, It was shown that both HCV and iron overload are the main causes of abnormal liver function in Thai patients with thalassemia. The treatment of both problems, if coexisting in patients with thalassemia, is required to prevent progression to chronic liver disease [16].

Recommendations:

In conclusion, HCV is the current major problem in multitransfused patients with thalassemia major and more careful pretransfusion screening of blood for viral hepatitis antibodies must be introduced in our blood banks. More large-scale studies are needed by applying advanced molecular techniques to avoid false negative and positive results.

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