Reliability of Clinical Aspect and Transaminases Level as a Guidance for Screening for Sero-positive Hepatitis C in Multi-transfused β-Thalassaemia Patients

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Abstract

Background: Although regular blood transfusion enhances the overall survival of thalassaemia patients, it have a significant threat of infection with transfusion-related infections, including hepatitis C; therefore, screening is mandatory. Routine screening with anti-hepatitis C Ab was recommended many years ago; sometimes they depend on level of liver enzymes.

Objective: To evaluate the clinical and biochemical features of seropositive hepatitis C virus infection in multi-transfused β-thalassaemia patients to verify if they can be used as a guidance for screening.

Patients and Methods: A retrospective- study done from June to September of 2014 at Thalassaemia Center in Baqubah city - Diyala province - Iraq. All registered β- thalassaemia major and intermedia patients were included, involving adults. Enzyme linked immunosorbant assay was used to screen for hepatitis C virus. Symptoms, signs, and biochemical features, including liver transaminases level, of sero-positive patients were gathered and analyzed. Descriptive statistical analysis and Pearson chi- square test was appliedto analyze data by using Statistical Package for Social Sciences software, version 16.

Results: The total enrolled subjects were 215, male gender comprises 54.9% (n=118); most of the included patients were under 12 years old. The results indicate that 11.2%(n= 24) of multi-transfused β- thalassaemia patients showed serological evidence of hepatitis C. The highest anti-HCV prevalence was observed at ≥ 18 years old patients (p value=.000). More than two third of cases were male gender (p value=.041), but the gender was not associated with positive serology for hepatitis C in the whole sample of the study (p value=.096). All anti-HCV positive patients were clinically asymptomatic; biochemically, 75% (n=18) of them had normal liver transaminases levels (p value=. 014).

Conclusion: Asymptomatic clinical status and unreliable transaminases level elevation cannot be helpful as directory for screening, we encourage the continuation of the usual routine screening test by enzyme linked immunosorbant assay for hepatitis C in β-thalassemia patients; it is more crucial for an older patient.

Key words: β-thalassaemia, hepatitis C virus, serology, screening test.

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Introduction

Thalassaemia is a diverse group of inherited disorders resulted from a reduced proportion of α or β globin chain synthesis [1]. The imbalanced globin chain synthesis will result in red blood cell destruction in the bone marrow (ineffective erythropoiesis) and peripheral circulation (hemolysis) [2].

Repeated blood transfusions of thalassaemia patients have improved the overall survival, though these transfusions may lead to a significant consequence of transmission of particular viruses, including hepatitis B, C and HIV [3]. Hepatitis C virus is now accounted for the majority of post-transfusion non-A non-B hepatitis cases in β- thalassaemia major patients world widely [4]. the rate of infection, in many states, is ranging between 12% and 85% [5].

The acute hepatitis C viral infection is usually mild and have insidious onset. Acute hepatic failure rarely develops. Hepatitis C virus is the most probable hepatotropic virus causing chronic hepatitis. Less than 15% of the affected adults heal the virus; the remaining develop chronic hepatitis. In children, 6-19% of attained spontaneous sustained viral clearance during follow-up of 6 years. Chronic Hepatitis C viral infection is also clinically silent till development of complications. Hepatic fibrosis usually evolutes slowly along several years, unless comorbid causes are present, which may hasten the progression of fibrosis. About 25% of hepatitis patients eventually develop cirrhosis, hepatic failure, and, rarely, primary hepatocellular carcinoma within 20-30 years of the cute infection. Chronic Hepatitis C infection may be associated with small vessel vasculitis and is a frequent cause of essential mixed cryoglobulinemia. Other extra-hepatic manifestations include peripheral neuropathy, cutaneous vasculitis, membranoproliferative glomerulonephritis, nephrotic syndrome, andcerebritis [6,7].

Routine screening of thalassemia patients for hepatitis C with anti-hepatitis C Ab was recommended many years ago. In Canada, it was monitored annually and in cases having two-fold rising of liver enzymes [4-8]. whereas others advocated screening of thalassemia patients when the aminotransferase levels remain persistently elevated for more than 6 months [9].

Liver function tests include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gammaglutamyltransferase, serum bilirubin, prothrombin time, and serum albumin. They reflect different functions of the liver that is, to excrete anions (bilirubin), hepatocellular integrity (transaminases), formation and the subsequent free flow of bile (bilirubin and ALP), and protein synthesis (albumin). Aminotransferase, AST and ALT, are an excellent marker of hepatocellular injury. Hepatocellular injury and not necessarily cell death is the trigger for release of these enzymes into the circulation [10].

This study was designed to re-evaluate the clinical and liver transaminases of seropositive hepatitis C in β-thalassaemia patients to verify their usefulness to be reliably used as a guide for screening for hepatitis C.

Patients and Methods

A retrospective- study carried out within 4 months (June to September of 2014) at Center of Thalassaemia patients at Al- Batool teaching hospital for maternity and children in Baqubah city- Diyala province - Iraq. All registered patients were enrolled in the study, these consisting of patient from different age groups, including adults. None of these

patients had a previous history of anti-viral treatment and intravenous drug abuse.

Routine screening

All β-thalassaemia patients, regardless clinical state and liver enzyme levels, had been scheduled for screening for hepatitis viral infection in the center every 6 months using anti-HCV assays with enzyme linked immunosorbant assay ELISA (BioTeck, USA) according to the manufacturer instructions. This test had been used to detect antibodies against the hepatitis C antigen; these include both IgM and IgG. The positive result was confirmed by recombinant immunoblot assay.

Clinical and biochemical, and other criteria

Positive sero-prevalent hepatitis C patients' files were reviewed. The symptoms and signs had been recorded monthly on routine visits, together with many laboratory investigations, including liver function tests (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), renal function tests, C- reactive protein, and serum ferritin.

The relationship of sero-positive hepatitis C with the type of thalassaemia was assessed. Non-splenectomized patients were also compared with splenectomized patients. For evaluation of the significance of iron overload in hepatitis C cases, the patients were classified into two categories: first one with serum ferritin levels< 2500 µg/L and the second with serum ferritin levels ≥ 2500 µg/L.

Statistical analysis

Data were analyzed through the application of descriptive statistical analysis that include frequency and percentage by using electronic calculator. The pearson chi-square test was also used to assess the associations, the level of significance was set at 0.05 level; the data were analyzed by Statistical Package for Social Sciences (SPSS)software (version16).

Results

The total enrolled patients were 215, most of them were under 12 years old (p value .000); male gender comprises slightly more than half (pvalue0.152), table(1).

Table (1): Distribution of the studied group, according to the demographic characters.

Age/ Gender	Male number (%)	Female number (%)	Total number (%)
1year - < 12yr	71(33)	55(25.6)	126(58.6)*
12yr-< 18yr	22(10.2)	20(9.3)	42(19.5)
≥ 18yr	25(11.7)	22(10.2)	47(21.9)
Total	118(54.9)**	97(45.1)	215(100)

* p value (.000), ** p value (0.152)

The positive serologically tested patients for hepatitis C were 24 patients (mean ± standard deviation age = 18.3 ± 6.6 years), representing about 11.2 % of the enrolled subjects. The highest anti-HCV prevalence was observed at ≥ 18 years old patients (peaked at 18- 25 years old), (p value=.000).

More than two third of cases were male gender (p value=.041), but the gender was not associated with positive serology for hepatitis C when taking in consideration the anti-HCV negative cases in the whole sample of the study (p value=.096). Most of the anti-HCV positive cases were thalassaemia major

(n=17,71%) and the remaining were thalassaemiaintermedia, table (2), (3), and(4). Most of positive cases were having regular visits to the center for one time blood transfusion per month (p value =.000), table (3).

All anti-HCV detected patients were clinically asymptomatic, whereas, biochemically, 75% (n=18) of them had normal liver transaminases levels (p value = .014), table (3). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased in 6 out of 24 sero-positive HCV patients (25%), their elevation was 0.3 to 4 fold upper normal limit, whereas alkaline phosphatase (ALP) was normal in all seropositive patients. Splenectomy was exist in 13 out of 24 Anti-HCV (+) patients (54.2%) and they were significantly associated when compared with sero-negative patients regardless the timing of splenectomy, table (3) and (4).

Renal function tests and C-reactive protein level were normal in most seropositive cases. Serum ferritin level variations were not associated with serological results for hepatitis. One half (n=3) of seropositive patients and elevated transaminases had serum levels of ferritin ≥ 2500 µg/l, table (3) and (4).

Table (2): Relationship between demographic characters and Anti-hepatitis C antibody positivity of the studied group.

Character		Anti- Hepatitis C Results		P-Value
		Positive number (%)	Negative number (%)	
Age	1year - < 12 yr	3 (2.4)	123 (97.6)	0.000***
	12 yr-< 18 yr	6 (14.3)	36 (85.7)	
	≥ 18 yr	15 (31.9)	32 (68.1)	
	Total	24 (11.2)	191 (88.8)	
Gender	Male	17 (14.4)	101 (85.6)	.096
	Female	7 (7.2)	90 (92.8)	
	Total	24 (11.2)	191 (88.8)	

*** highly significant association.

Table (3): Characteristics of Anti-hepatitis C positive patients.

Character	Groups	Number (%)	P value
Age	- 1year - < 12 yr - 12 yr-< 18 yr - ≥ 18 yr	3 (12.5) 6 (25) 15 (62.5)	.008**
Gender	- Male - Female	17 (70.8) 7 (29.2)	.041*
Routine visits to thalassaemia center	- Regular - Irregular	21 (87.5) 3 (12.5)	.000***
Number of blood transfusions per month	- 1 - 2	24 (100) 0	-
Symptoms and signs of hepatitis	- Positive - Negative	0 24 (100)	-
Splenectomy	- Positive - Negative	13 (54.2) 11 (45.8	.683
Liver transaminases	- Normal - Elevated	18 (75) 6 (25)	.014*
Renal function tests	- Normal - Abnormal	24 (100) 0	-
C- reactive protein	- Positive - Negative	6 (25) 18 (75)	.014*
Serum ferritin levels	- < 2500 µg/L. - ≥ 2500 µg/L.	13 (54.2) 11 (45.8)	.683

* significant association, ** very significant association, *** highly significant association.

Table (4): Show the relationship of Anti- hepatitis C antibody positivity with type of thalassaemia, splenectomy, and levels of serum ferritin.

Factor	Anti- hepatitis C Antibody Results		P-Value
	Positive number (%)	**Negative number (%)**	
Type of thalassaemia **- Major** **- Intermedia** **Total**	 17(11.6) 7 (10.1) 24 (11.2)	 129 (88.4) 62 (89.9) 191 (88.8)	.745
Splenectomy **- Positive** **- Negative** **Total**	 13 (23.2) 11 (6.9) 24 (11.2)	 43 (76.8) 148 (93.1) 191 (88.8)	.001**
Serum ferritin level **- < 2500 µg/L.** -≥2500**µg/L.** **- Missed** **Total**	 13 (12) 11 (12.4) - 24 (11.2)	 95(88) 78 (87.6) 18 191 (88.8)	.945

** very significant association.

Discussion

Hepatitis C virus is now accounted for the majority of post-transfusion non-A non-B hepatitis cases in β- thalassaemia major patients world widely[4]. In the present study, hepatitis C virus sero-conversion rate was 24 (11.2 %) of 215 patients, previously, Tareef *et al.* reported that its prevalence was 26.4% by analyzing data collected at 1999-2000 at the same center, this difference appeared most likely due to the extended effect of routine blood screening for viral hepatitis, which had been introduced in Iraq in 1996 [11]. The decline of the HCV prevalence in this study looks to be similar to that reported in other states after introducing ELISA screening test, e.g.in Iran, it decreased from 34.6% to 15.7% [12]. And in the United States it dropped from 3.84% to 0.57% [13].

The current study demonstrated that there is a highly significant increase in rate of HCV infections with increasing age; the highest affected age was 18-25 years old. This result is compatible with other studies[14][15]. Cacopardo *et al.*, 1992 declared that the highest anti-HCV prevalence was noticed between 16 and 20 years[16]. This association may reveal the effect of increasing exposure to hepatitis C virus by the increased frequency of blood transfusion within life. This was in contrast to Tareef *et al*, 2011 when they found a high prevalence of AHC positivity in the younger age group advocated them to think about other modes of viral transmission than blood transfusions [11].

The present study demonstrates that male gender had more anti-HCV positive cases than females (p value=.041), this was in agreement with other studies [11,14]. whereas females have a higher prevalence in Ahmad study [17]. Males and females are similarly affected according to Ansar MM's study [18]. However, this gender incidence dissimilarity was statistically not significant when compared to sero-negative patients (p value .096), because male gender was already more recruited in the study.

Type of thalassaemia (major and intermedia) had no significant association with AHC positivity in this study, we thought that the frequency of blood transfusions is the most effective part of viral transmission; the difference between thalassaemia major and intermedia regarding rate of transfusion was not evaluated in the current study.

The present study showed all anti-hepatitis C positive patients were asymptomatic, it seems that these patients might be either acute hepatitis, chronic hepatitis, or resolved hepatitis [19]. All these possibilities were compatible with previous studies. It is known that acute hepatitis C is usually silent or insidious in onset, chronic hepatitis is asymptomatic during the first few decades until happening of complications or the patient may experience non specific symptoms that are difficult to explained by chronic hepatitis C, thus they are most frequently detected by elevated hepatic enzymes or during a routine screening of high-risk individuals [6,7].

The positive serological tests also might be caused by resolved hepatitis, although all enrolled patients didn't received antiviral treatment previously; in pediatric studies, there is a opportunity of 6-19% of cases achieved natural sustained clearance of the virus within a 6 yr follow-up and in adults less than 15% clear the virus; whereas the rest develop chronic hepatitis. False positive results were unlikely in this study because the test was repeated for all patients routinely every 6 month period and all positive results were confirmed by recombinant immunoblot assay.

A limited non significant number (n=6, 25%) had elevated serum levels of alanine aminotransferase and aspartate aminotransferase with normal alkaline

phosphatase levels in all patients. A previous study reported that ALT screening is not useful in detecting HCV positive subjects [20]. In chronic hepatitis, serum aminotransferase levels is usually fluctuated and may be normal, but histological inflammation is universal [7]. Regarding ALP, our results were compatible with other findings, reported by [14]. Due to ALP is expected to be increased in obstructive jaundice rather than hepatocellular jaundice, which is developed in hepatitis C virus infection.

Splenectomy was done for more than half of anti- hepatitis C positive patients and their relationship were statistically significant, this may be explained by the high prevalence of anti- HCV antibody among older age groups while splenectomy is more possible to be indicated. This association was supported by Sammimi- Rad *et al* (2007) study in Iran;[21] whereas other study showed no significant association [14]. Anyhow, another isolated study is needed to define the exact prevalence of hepatitis C before and after splenectomy.

Limitation of the study include the deficit of confirmatory diagnostic test (e.g. polymerase chain reaction "PCR" or histological examination) for hepatitis C to identify its association with the mentioned criteria after exclusion of resolving infections.

In conclusion, the results of the study revealed that clinical aspect and variable transaminases levels were unreliable to guide for detection of hepatitis C; therefore the study encourages the continuation of routine ELISA screening test for hepatitis C in β-thalassemia patients; a critical look for an older patients (those transfused before implementing routine screening of blood).

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