

## Original paper

### Hepatitis C Infection Among Children with Beta- Thalassemia Major in Babylon Center of Hereditary Blood Disorders

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#### Abstract

**Background:** Hepatitis C infection is the most common cause of post transfusion hepatitis and end stage liver disease in many countries. B-Thalassemia major is one of the most prevalent hereditary blood disease world wide. Polymerase chain reaction is a highly sensitive technique for the detection hepatitis C virus-RNA in serum

**Aim of study:** To determine the percentage of hepatitis C infection (positive polymerase chain reaction) and possible risk factors in children with B-Thalassemia major in Babylon Center of Hereditary blood disorders.

**Patient and Methods:** A prospective study was done on two hundred twenty six child with B-Thalassemia major (aged from 2 years to 18 years) that have been transfused with blood , as part of their management , at least 10 units of blood irrespective of their age , sex were included in this study from a period 1 of march 2013 to 1 of July 2013 in Babylon center of hereditary blood disorder in Babylon Gynecology and Children Teaching Hospital .Serum was stored at -20 then tested for hepatitis C (HCV antibodies) by ELISA, HCV antibodies positive cases were confirmed by polymerase chain reaction test.

**Results & Discussion:** Total number of patient was 226, male 129(57%), female 97(43%) with minimal age 2 years and maximal 18 years with mean age 8.4 with st. deviation 4.3 year. HCV Ab was positive in 17 (7.5%), only 12 (70%) were +ve PCR for hepatitis C and 5 (30%) were -ve PCR . There are significant statistical relation regarding the increase number of blood transfusion units (more than 150 units) P value 0.045, Huge splenomegaly (more than 8 cm) P value 0.002 , Abnormal liver enzyme with P value 0.032 , and male gender (P value 0.029) with +ve PCR HCV patient. There are no significant statistical relation regarding huge hepatomegaly (p value 0.107) and type of chelating agent (p value 0.107) with +ve PCR HCV patient.

**Conclusion:** Children with B- Thalassemia major are more prone to get Hepatitis C infection especially if get blood transfusion more than 150 units, huge splenomegaly, abnormal liver enzymes and male gender.

**Key words:** hepatitis C Antibodies, B-thalassemia major, blood transfusion, polymerase chain reaction

#### Introduction

Hepatitis C virus (HCV) is the common cause of post-transfusion hepatitis (PTH) and end -stage liver disease <sup>(1)</sup>. HCV is the most common cause of chronic viral hepatitis in the many developed countries and a significant cause of cirrhosis, hepatic failure and hepatocellular carcinoma <sup>(2,3)</sup>.

Thalassemia major is the most prevalent hereditary blood disorder around the

world, Regular blood transfusion particularly in patient with thalassemia , has improve their overall survival , but carries a definitive risk of acquisition of blood-borne virus infections , specially viral hepatitis C <sup>(4)</sup>.

Infections is the second prevalent cause of death among thalassemic patients <sup>(4)</sup>, Although, improvement in screening of blood products since 1990 , decrease the risk of transmission of blood-borne

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diseases, however, hepatitis C is still remain as an important problem in patient with thalassemia<sup>(6,7)</sup>.

Using screening methods specially advanced methods like ELISA generation 1, 11, and 111 associated with decrease risk of both hepatitis C and HIV<sup>(8)</sup>, Nevertheless for some reason, hepatitis C was observed in many thalassemic patients that is necessate more attention, since it is not only affect the life of thalassemic patient and family but can affect also other especially than relatives who are at risk of transmission. WHO studies show that, 170 million of people infected with hepatitis C<sup>(9)</sup>.

Blood transfusion is the common transmission pathway<sup>(10)</sup>, Chronic hepatitis flows acute hepatitis C in about 60-70% of cases, progression to cirrhosis accurse in about 20% and hepatoceller carcinoma in rate 3-5% per year<sup>(11)</sup>.

Hepatitis C is an enveloped single stranded RNA virus, classified in the family Flaviridea and was discovered at 1988 and published in flowing year<sup>(12)</sup>.

There is six HCV genotypes, which are subdivided in to subclasses or subtypes identified by Lower-Case letter (1a, 1b, etc) and these of value in response to treatment<sup>(13)</sup>.

Incubation period of HCV about 7-9 weeks (average 1-24 weeks), however the individual with suspected HCV are typically tested for HCV- Antibodies by Enzyme Immunoassay (EIA), if positive it should be confirmed by HCV RNA assay by polymerase chain reaction (PCR).

Polymerase chain reaction (PCR) testing has been available sience 1995, Most patient with have detectable level of HCV RNA in plasma 1-2 weeks after exposure, 10-12 weeks prior ALT elevation and 10-24 weeks before seroconversion<sup>(14)</sup>.

Hepatitis C virus (HCV) RNA positive in Anti-HCV negative patient with acute hepatitis strongly indicate acute hepatitis C, which is unlikely when both markers (PCR RNA, Anti-HCV) are

negative. While the presence of both markers indicate either acute infection or acute exacerbation of chronic hepatitis<sup>(15)</sup>.

The highest levels of circulating viral RNA are found during the early course of infection, suggesting that the patient could be highly infectious at that time<sup>(16)</sup>.

People at risk of hepatitis C

1-Long term kidney dialysis

2-Reguler contact with blood (health workers)

3-Reguler blood transfusion program (thalassemia)

4-Reguler blood products infusions (hemophilia)

### **Aim of the study**

To determine the percentage of hepatitis C infection (positive polymerase chain reaction) and possible risk factors in children with B-Thalassemia major in Babylon Center of Hereditary blood disorders.

### **Patients and methods**

A prospective study was done on 226 child with Beta-Thalassemia major (aged from 2 years to 18 years) that have been transfused with blood, as part of their management, at least 10 units of blood (which was routinely screened for Anti-HCV and was -ve) irrespective of their age, sex were included in this study from a period 1 of march 2013 to 1 of July 2013 in Babylon center of hereditary blood disorder in Babylon Gynecology and Children Teaching Hospital. Patient with past history of jaundice, history of surgery or dental procedure, and transfused less than 10 units of blood were excluded from the study. All patients were diagnosed as Beta-Thalassemia major on HPLC. Patient underwent history taking including age, age at diagnosis of beta-thalassemia major, number of bold transfusion units, any risk factors of getting hepatitis (Diabetes Mellitus, other blood disorders, history of contact with hepatitis patient),

type of chelating agent, Clinical examination including the size of liver and spleen, routine investigations including liver enzyme (ALT, AST). Serum was stored at -20 then tested for anti-hepatitis C virus (Anti-HCV) by ELISA (3<sup>rd</sup> generation commercial micro kit (DIA.PRO, Italy), Positive Anti-HCV cases were confirmed by polymerase chain reaction test(PCR HCV) (Roche, Germany).

**Statistical analysis**

The data analysis was performed by descriptive statistic (mean, slandered deviation, and percentage) and analytical statistic (Chi-square, Fischer exact test) using SPSS version 14. The level of significant P value was set at <0.05.

**Results**

Total number of patient was 226, male 129(57%), female 97 (43%) with minimal age 2 years and maximal 18 years with mean age 8.4 with st .deviation 4.3 year. Anti-HCV Ab was positive in 17 (7.5%). From those which is +ve HCV Ab , only 12(70%) were +ve PCR for hepatitis C and 5 (30%) were -ve PCR .Regarding the relation of number of blood transfusion units, We found 9 patients (52.9%) with +ve Anti-HCV with history of blood transfusion from 150-200 units , 6 patients (35.2%) with blood transfusion units(100-150units) and only 2 patients(11.7%) with blood transfusion (50-100 units) with p.value 0.00, while we found 1 patient (8.3%) with +ve PCR HCV with blood transfusion unit from 50-100, 4 patient (33.3%) with blood transfusion units (100-150), and 7 patient(58.3%) with blood transfusion units (150-200) with p.value 0.045 as shown in table (1)

Table 1. Relation of number of blood transfusion units in patient of Anti-HCV +ve and PCR-HCV +ve

Blood transfusion units	Total number(226)	Anti-HCV +ve(NO 17)	p.value	PCR-HCV +ve(NO 12)	P.value
10-50	109(52.1)	0	0.000	0	0.045
50-100	81(37.7)	2(11.7)		1(8.3%)	
100-150	27(10.2)	6(35.2)		4(33.3%)	
150-200	0	9(52.9%)		7(58.3%)	

Regarding organomegaly , we found 10 patients(83.4) with +ve PCR -HCV , had splenomegaly with size more than 8 cm below costal margin, 1 patient(8.3%) with size of splenomegaly 4-8cm, and 1 patient (8.35) with size less than 4 cm with p.

value 0.002, as shown in table(2). Also we found 9 patients(75%) with +ve PCR-HCV , had hepatomegaly with size 4-8 cm , and 3 patients(25%) with hepatomegaly less than 4 cm, with p. value 0.107, as shown in table(2).

Table 2. Relation of hepato-splenomegaly among patients with +vePCR-HCV

Organ	<4 cm	4-8cm	>8cm	P value	Total NO
liver	3(25%)	9(75%)		0.107	12
spleen	1(8.3%)	1(8.3%)	10(83.4)	0.002	12

Abnormal liver enzymes level (ALT,AST) was reported in 9 patients(83.4%) with +ve PCR-HCV , while we found

3patients(16.6) had normal level of ALT and AST , with p.value 0.032, as shown in table(3).

Table 3. Relation of liver enzymes level (ALT,AST) among patients with +ve PCR-HCV

Liver enzyme	Normal	Abnormal	p.value	Total NO
ALT	2(16.6%)	10(83.4%)	0.032	12
AST	2(16.7%)	10(83.4%)	0.032	12

Male with +ve PCR-HCV account 8(66.7) , and female with +ve PCR-HCV account 4(43.3), while female with –ve PCR-HCV account 5(100%), with no male with –ve PCR-HCV , with p,value 0.029 as shown in table (4).

Table 4. Relation of sex among patients with +ve and –ve PCR-HCV

Sex	+ve PCR-HCV	-ve PCR-HCV	P.value
Male	8(66.7%)	0	0.029
Female	4(43.3%)	5(100%)	
Total	12	5	

We found in this study , 9patients (75%) with +ve PCR-HCV had age more than 10 year , while only 3patients (25%) with +ve PCR-HCV had age less than 10 years, as shown in table (5).

Table 5. Frequency of age group in years with +ve PCR-HCV

Age in year	frequency	Percentage%
7	2	16.7%
13	2	16.7%
14	2	16.7%
5	1	8.3%
15	3	25%
16	1	8.3%
17	1	8.3%
<b>Total</b>	<b>12</b>	<b>100%</b>

Regarding the type of chelating agent and its relation with +ve PCR-HCV, we found 9 patients (75%) on Desferal therapy, while 3 patients (25%) on Exgade therapy with p. value 0.107.

## Discussion

In our study, the percentage of +ve Anti-HCV antibodies among children of Beta-Thalassemia major was 7.5%, and this rate was lower than what is reported in different parts of our country. Anti-HCV antibodies were detected in 67.3% among thalassemic children in Ibn Al Balady hospital <sup>(17)</sup>, and 37.6%in other study was done in the same hospital, 30% in Mosil, 10% in Thigar (18), and 9.5% in Al Basrah <sup>(19)</sup>. In different parts of the world the prevalence of HCV infection in

thalassemic children is different. In India it was 16.7%, <sup>(20)</sup> in Malaysis 22.4% <sup>(21)</sup>, 47% in Italy <sup>(22)</sup>, 41.7 in Pakistan<sup>(23)</sup>, and 20.2% in Thiland<sup>(24)</sup>.

This variation between our results and other results perhaps due to other study were done on different age group(adult and children), and variation of the sensitivity of kits (ELISA) and its type of generation were used in detection of Anti-HCV antibodies in different studies.

In this study, we found from 12 patients +ve Anti-HCV(12/17) were also +ve RNA HCV. The agreement between the Anti-HCV and RNA HCV detection was 70%, a finding to less extent similar to those reported for other populations, i.e rates ranging from 65 to 86% <sup>(25,26)</sup>, while 92.6% in other study <sup>(27)</sup>. Failure to detect HCV RNA in serum may be due to various factors such as inactivation of viral RNA during serum collection and storage, fluctuating viremia levels, resolved infection, or false-positive anti-HCV results <sup>(25, 28)</sup>.

In this study, we found the percentage of +ve Anti-HCV and +ve PCR HCV were increase with increasing number of blood transfusion units (more than 150 units) with significant p. value (0.000 and 0.045 respectively), and this is what was found in different studies <sup>(29-32)</sup>. In other study, no relation was found between the prevalence of hepatitis C infection and increasing the rate of blood transfusion units <sup>(24)</sup>.

Our finding suggests the blood transfusion is the important risk factors for acquisition of HCV among thalassemic patients in spite of routinely screening blood donor for Anti-HCV. Thalassaemic patients may acquire hepatitis C through the administration of HCV infected blood collected during the donor window period. This is one reason in thalassaemic children because the risk due to drug abuse and sexual activity are reasonably low in them <sup>(33)</sup>.

In this study, we found patients with hepatitis C infection (+ve PCR HCV) had more abnormal liver enzyme (ALT,AST)

in compare with patient with –ve PCR HCV with significant p.value (0.032), and this is goes with result of other study, Wanachiwanawin , et al 2003<sup>(24)</sup>. Patients with +ve PCR HCV who have elevated serum liver enzymes , are more likely to have significant liver disease than those of have –ve PCR HCV , and this elevation could be due to viral replication <sup>(34,35)</sup>, or due to iron overload <sup>(7)</sup>.

Also we found that patients with +ve PCR HCV have huge splenomegaly (more than 8 cm) with significant statistical p. value (0.002) and this is mostly also related to large number of blood transfusion units and this also was observed the study of Triantos, et al, 2013 <sup>(36)</sup>. While hepatomegaly (4-8cm) was observed in 9 patients with +ve PCR HCV but without significant statistical value (p. value 0.107).

In our study the age of patients with +ve PCR HCV mostly above the 10 years (75%) and this also was observed in other study <sup>(7)</sup> and this is logically explained due to with advanced age group more blood exposure and eventually more risk to get blood-borne transmitted infections like hepatitis C virus.

Our results showed that 66.7% of patients with +ve PCR HCV were male and 43.3% female with significant p. value 0.029, and this is goes with results of study was done in Diyala <sup>(37)</sup>, while female more in other study of Tamaddoni et. Al <sup>(31)</sup>.

In the present study showed that 9 patients (75%) with +ve PCR HCV on Desferal therapy, while only 3 patients (25%) on Exgade but without statistical significant (p.value 0.107), and this is possibly due to majority of thalassemic children with very high serum ferritin (which is indictor of more blood units transfusion) were on Desferal rather than Exgade.

## Conclusion

Children with B- Thalassemia major are more prone to get Hepatitis C infection specially if get blood transfusion more

than 150 units , huge splenomegaly , abnormal liver enzymes and male gender.

## Recommendation

To reduce the risk of transmission of HVC infection through blood transfusion , we should introduce a sensitive survey methods that my detect the infection during the window period with use of PCR test specially in advanced economy <sup>(38)</sup>.

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