Prevalence of hepatitis C viral infection among multi-transfused thalassemic patients.

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Abstract
A prospective study was conducted on 325 patients (187 males and 138 females) with B-thalassemia major & sickle-thalassemia who were attending the thalassemia center at Maternity and Children Teaching Hospital in Al-Diwaniya, from the period of September 2007 till April 2008. Their ages ranged from 1-20 years.

They were studied for Anti HCV sero positivity among patients with B-thalassemia major & sickle-thalassemia (12 patients (4.01%) and 1 patient (3.84%) respectively). Anti HCV sero positivity was significantly higher among patient's ages from (5-15) years and also sero positivity was more among males. Statistically, there were significant differences among both sexes.

The study has revealed also that anti HCV sero positivity was directly related to the frequency of blood transfusion. Also they were studied for HCV antibodies. 13 patients (4%) were found to have antibodies in comparison to zero among the 325 children of the control
The risk of exposure to HCV was higher than HBsAG and HIV among the same patients.

**Introduction**

Approximately 600 structural variants of hemoglobin have been identified (1). The commonest hemoglobinopathies that need frequent, regular blood transfusion are thalassemia (alpha and beta) and sickle-cell thalassemia (2). Thalassemia is classically associated with increased susceptibility to infection in general, mainly through blood transfusion. These patients interval can be directly infected with a number of viruses with increase risk of infection by HBV, HCV, CMV, and EBV (3).

With the development of serological assay to detect antibodies against HCV, we now know that HCV cause more than 90% of cases of post-transfusion hepatitis (4).

Before the introduction of universal screening of blood donors for anti-HCV, thalassemic patients on long term transfusion therapy were at high risk of exposure to HCV. In addition 50% of HCV infected patients will develop chronic hepatitis and 20% of them go on to develop cirrhosis within 10 years (5).

**Hepatitis C-virus (HCV)**

The existence of transfusion related hepatitis is not caused by known hepatitis virus A and B. Thus the coined non A, non B hepatitis has been known since 1970 (8). However, it was until 1989 that Choo and co-worker at Chiron successfully cloned a portion of genome of a new virus that transmitted hepatitis to chimpanzees using the technique of molecular biology. The entire genomic sequence of HCV has been delineated allowing the development of advanced diagnostic tests. HCV is now known as an important cause of chronic hepatitis and liver disease (8).

**Organism**

HCV is newly classified member of flavi viruses; it has a single stranded, positive RNA genome of approximately 9.4 kilo bases. The virion is small, 30 to 34 nm, enveloped, and thought to be icosahedrons shape (8-10). It was first isolated from animals infected with contaminated factor VIII 1989 (11). The types classified as I, II, III, IV, and V corresponded to types la, lb, 2a, 2b, and 3a respectively (12). The major genotypes show distinct geographic clustering and may be associated with different rates of transmission and different levels of pathogenicity (13). HCV type I has been reported from all countries, including Europe, North America, Japan and Australia (14) HCV type III has been reported from Japan (15). HCV type III has been reported
from Europe, the United States, Thailand and India but not from Japan (9). HCV type IV has been reported from blood donor populations in Middle East (appears to be the most common in Saudi Arabia) [9, 15]. HCV type V has been reported from South Africa [14]. Determination of HCV genotypes is important to assist serological diagnosis, identify mixed infections due to multiple blood transfusions, predict response to interferon in patients with chronic infection and assist vaccine development [15].

**Pathogenesis**

Antibody produced against hepatitis C virus is not protective, thus, infected individuals can be reinfected with different strains. In addition, post exposure prophylaxis pooled immune globulin has not been effective in preventing disease and is not recommended after accidental exposure [8].

**Epidemiology**

Hepatitis-C-Viral infection is an important cause of non A, non B hepatitis throughout the world and it can lead to chronic liver disease and liver cirrhosis. Approximately 50% of infected patients have biochemical evidence of chronic hepatitis and approximately 20% of them have histological evidence of cirrhosis [16]. It was estimated that 3.5 million people in the United States are infected with HCV and 150,000 new infections occur annually [8]. Based on epidemiological and serological studies, it was estimated that 1-2% of world population were chronically infected with HCV and the range of sero prevalence is 0.4-15% [17]. HCV was transmitted in 80-90% through blood hem dialysis, frequent transfusions of blood products, organ transplantation and occupational exposure such as needle sticks [8].

Sexual and vertical transmission also may occur, but are not common. Vertical transmission occurs in approximately 6% of infants born to antibody positive mothers, and risk of transmission is correlated with the amount of virus present in the mother [8].

Transmission of HCV from patient to health-care worker has been generally documented following percutaneous exposure to blood [9]. There has also been one case report of transmission through mucous membrane exposure, via a blood splash to the conjunctiva [9]. Patients co-infected with both HIV and HCV have a higher risk of progression to chronic liver disease than those infected with HCV alone [18, 19]. And HIV infection appears to enhance HCV replication.
Clinical Features

Symptoms from acute HCV infection occur between 1-24 weeks after infection with a mean of 8 weeks. They range from none to those indistinguishable from other causes of hepatitis including anorexia, malaise, nausea and right upper quadrant pain [8].

Incubation period of HCV 30-60 days [20]. Fulminant disease does not appear to be caused by HCV alone; infection with other blood born pathogens is not uncommon and may contribute to the severity of disease [8].

The most important feature of HCV is that approximately 60-80% of infected patients develop chronic hepatitis (elevated ALT lasting longer than 6 months) [8,16] During this time patients are often clinically a symptomatic. Despite minimal or no symptoms, progressive destruction of liver ensures and approximately 20% of patients with chronic HCV infection develop cirrhosis [16]. Late symptoms often are associated with liver failure [8] and include bleeding esophageal varices and portal hypertension. It is also clear that HCV infection play a role in the development of hepatocellular carcinoma. It is thought that the cycle of inflammation, necrosis, regeneration and cirrhosis rather than a directional oncogenesis are responsible for the relationship [8].

Recently, a number of extra hepatic clinical findings have been linked with hepatitis C-infection. They include mixed cryoglobulinemia, membrano proliferative glomerulo nephritis (MPGN), porphyria cutanea tarda, autoimmune thyroiditis and idiopathic pulmonary fibrosis [8].

Diagnosis

HCV infection can be diagnosed nowadays by using serological assay through third generation ELISA (Enzyme-Linked Immune-Sorbet Assay) which detect antibody against viral infection. Patterns of the anti HCV response in patients with hepatitis have a typical delayed response in which the antibody was not detected until 20-22 weeks after exposure and 14-16 weeks after the onset of hepatitis. Once the antibodies are present, its level rises to a plateau and persists for more than 10 years [16]. Previously at 1990, first generation ELISA was used. This assay detected antibody to one HCV antigen. This assay was fraught with lack of sensitivity and specificity. False negative results were likely due to sequence variation and false positive results were associated with immunologic interference from a variety of conditions including hyper globulinemia and recent influenza immunization. Second and nowadays third generation assays have improved the sensitivity, specificity and ability to detect early infection by adding multiple antigens of the virus. Current antibody testing has 99% sensitivity and 99.5% specificity [8].
Direct detection of viral antigen rather than antibody can be done using the HCV RNA polymerase chain reaction (PCR). This can detect virus as early as 1-2 weeks after infection and may be useful in following response to therapy [8, 21]. Since the major location of virus replication is the liver, it has been suggested that detection of HCV RNA is better performed on cells from liver tissue rather than serum [15].

**Aim of the study**
This study was carried out to determine:
1- The prevalence of HCV antibodies in thalassemic children in Al-Diwanyia city.
2- The correlation of anti-HCV sero-positivity with certain variables including age, sex, and number of blood transfusions.

**Patients and Methods**

1. **Patients**
A total of 325 (m=187 f=138) known patients with B-thalassemia major and sickle cell thalassemia who were attending Maternity & children teaching hospital in Al-Diwaniya from the period of September 2007 till April 2008 were included in this study, their age ranged from 1-20 year 299 patients included had B-thalassemia major and 26 patients had sickle cell thalassemia.

11. **Control group**
325 healthy children visiting the out patient department for vaccination or minor illness like upper respiratory tract infection were randomly selected as a control group, their ages ranged from 1 month – 19 years. All of them did not have a history of previous blood transfusion and they had negative family history of haemoglobinopathies.

**III**- Questionnaire form filled by a single observer including a detail history and physical examination was obtained for all children for both groups.

**IV**- A simple important investigation, i.e reticulocyte count, hemoglobin concentration, and total serum bilirubin and combs test were done for the inclusion of children in the control group.

**V**- A blood sample was aspirated from patients for the following tests:
- HCV Antibodies by ELISA
- HIV antibodies (25) by ELISA
- HBS Ag (26) by ELISA
**Results**

**Table -1: Distribution of anti HCV sero positivity among patients and control**

<table>
<thead>
<tr>
<th></th>
<th>Sero+ve</th>
<th>Sero-ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Patients</td>
<td>13</td>
<td>4%</td>
<td>312</td>
</tr>
<tr>
<td>Control</td>
<td>zero</td>
<td>0%</td>
<td>325</td>
</tr>
</tbody>
</table>

$X^2_{chi}=0.0215$

$X^2_{tab, 0.05}=5.991$

Significant

**Table- 2: Distribution of anti HCV, HBsAg, and anti- HIV among multi-transfused patients**

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Sero+ve</th>
<th>Sero-ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>HCV</td>
<td>13</td>
<td>4%</td>
<td>312</td>
</tr>
<tr>
<td>HBsAg</td>
<td>5</td>
<td>1.5%</td>
<td>320</td>
</tr>
<tr>
<td>HIV</td>
<td>-----</td>
<td></td>
<td>325</td>
</tr>
</tbody>
</table>

$X^2_{chi}=0.02158$

$X^2_{tab, 0.05}=5.991$

Significant

**Table -3: Distribution of anti- HCV sero positivity according to Hb-electrophoresis pattern**

<table>
<thead>
<tr>
<th>Hb-elect.</th>
<th>Sero+ve</th>
<th>Sero-ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>B-thal.</td>
<td>12</td>
<td>4.01%</td>
<td>287</td>
</tr>
<tr>
<td>SC-thal.</td>
<td>1</td>
<td>3.84%</td>
<td>25</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td>13</td>
<td>4%</td>
<td>312</td>
</tr>
</tbody>
</table>

$X^2_{chi}=0.0001725$

$X^2_{tab, 0.05}=5.991$

Significant

**Table -4: Distribution of anti HCV sero positivity according to the number of blood transfusion**

<table>
<thead>
<tr>
<th>No.of transfusion</th>
<th>Sero+ve.</th>
<th>Sero-ve.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>&gt;20</td>
<td>11</td>
<td>5.21%</td>
<td>200</td>
</tr>
<tr>
<td>&lt;20</td>
<td>2</td>
<td>1.75%</td>
<td>112</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13</td>
<td>4%</td>
<td>312</td>
</tr>
</tbody>
</table>

$X^2_{chi}=0.05214$

$X^2_{tab, 0.05}=5.991$

Significant
Table- 5: Distribution of anti HCV sero positivity according to the age.

<table>
<thead>
<tr>
<th>Age(years)</th>
<th>Sero+ve</th>
<th>Sero-ve</th>
<th>Total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>1-5</td>
<td>0</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td>5-15</td>
<td>8</td>
<td>61.53%</td>
<td>196</td>
</tr>
<tr>
<td>&gt;15</td>
<td>5</td>
<td>38.46%</td>
<td>48</td>
</tr>
<tr>
<td>total</td>
<td>13</td>
<td>4%</td>
<td>212</td>
</tr>
</tbody>
</table>

$X^2$chi=0.02034
$X^2$tab, o.05=5.991

Significant

Table- 6: Distribution of anti HCV sero positivity according to the sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Sero+ve</th>
<th>Sero-ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>53.84%</td>
<td>180</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>46.15%</td>
<td>132</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td></td>
<td>312</td>
</tr>
</tbody>
</table>

$X^2$chi=0.0320
$X^2$tab, o.05=5.991

Significant

Discussion

Before the introduction of routine testing for HCV antibody in blood donation, the risk of exposure to HCV directly depended on the number of transfused blood units and on the prevalence of HCV in blood donor population (27). 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% (28).

The results of this study show that 4% of thalassemic children were anti HCV sero positive, 1.5% of them were seropositive for HBsAg & none of them was anti HIV sero positive. Anti HCV sero positivities reported in some Arabian countries like Egypt, Bahrain, and Saudi Arabia, in addition to other countries like China were higher than our result, whose anti HCV sero positivities in these countries were 44% (29) ,40% (30) , 70% (31) , 34% (32) , respectively. However, in USA and since the introduction of screening of blood products, transfusion now accounts for less than 5% of new hepatitis C-cases (8).
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 (31), in addition to high frequency of blood units used in the treatment. In this study, we use the third generation ELISA with the very high sensitivity & specificity (8). In addition to HCV, multi-transfusion thalassemic patients are at risk also of acquiring HBV & fortunately, our study has revealed a low prevalence rate of HBsAg which is 1.5%, in comparison to a study done in Saudi Arabia in 1993, where the exposure rate of thalassemic children to HBV was 26.7%. This low prevalence rate of HBV may be due to the use of third generation ELISA technique for screening of donated blood which was started several years ago in addition to the strict precautionary measures applied at hospital against spread of HBV infection and the use of hepatitis B- vaccine in the immunization schedule. The prevalence of HCV was higher in patients with B-thalassemia major (4.01%) in comparison to patient with sickle cell thalassemia (3.8%). The sickle thalassemia syndrome are a heterogeneous group of disorder whose clinical features depend on the severity of the B- thalassemia components ranging from the more severe form B0 to the mild form B+ (33). Therefore, our results can be explained for the treatment of patients with B-thalassemia major, similar results were obtained in previous study in Basrah (34) & in Saudi Arabia (31). The prevalence rate of HCV was also directly related to the number of transfusion units of blood, 5.2% of patients who had received blood more than 20 times were sero-positive, compared to 1.7% of patients who had received of less than 20 times, other studies have revealed similar results (31, 35, 36) & these result can be explained by the increased risk of transmission of infection with increasing number of blood units transfused. There is significant difference of Anti HCV sero-positivities prevalence rate among males and females as the transmission of virus is mainly through blood transfusion & drug injection rather than other routes. The prevalence of HCV infection increases in the age group 5-15 years, ranging from zero% in patient less than 5 years of age to 3.92% in patients between ages 5-15 years & 9.43% in patients older than 15 years. This attributed to number of blood unite transfused as children getting older due to growth, development of antibody to red blood cells & possibility of developing hypersplenism. This result was compatible to studies done in different countries of world (35, 36, and 37).
Conclusions
1- This study revealed that the prevalence of HCV antibodies among thalassemic children (including patients with B- thalassemia major and sickle cell thalassemia) was 4% and this rate is lower than that reported in many countries.
2- The prevalence of anti HCV sero positivity is higher than that for other viral infection transmitted mainly through the blood like HBV and HIV infection.
3- Anti HCV sero positivities was directly related to the frequency blood transfusion and age of patients.

Recommendations
1- It seems that until a vaccine against HCV becomes available, preventable measures such as a blood screening for anti HCV before transfusion and stringent infection control measures are crucial for the control of spread of HCV infection.
2- All thalassemic patients should be screened for HCV infection every 6 months.
3- Transmission of HCV from patients to health care worker has been generally documented following percutaneous exposure to blood; therefore, it is advisable that all health care workers have to take precautions measures.
4- Availability of ELISA that detect antibody rather then antigen after 14-16 weeks from onset of infection (20-22week from blood transfusion). May results 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.

References