

# Prevalence of Hepatitis C among Multi-transfused Thalassaemic Patients in Oman

## Single centre experience

Khalid Al-Naamani,<sup>1</sup> Ibrahim Al-Zakwani,<sup>2</sup> Siham Al-Sinani,<sup>3</sup> Fauzia Wasim,<sup>4</sup> \*Shahina Daar<sup>5</sup>

## مدى انتشار التهاب الكبد الوبائي سي في مرضى التلاسيميا متعددي نقل الدم في سلطنة عمان

### خبرة مركز واحد

خالد النعماني، ابراهيم الزكواني، سهام السنانية، فوزية وسيم، شاهينة داعر

**ABSTRACT: Objectives:** Regular blood transfusions are essential for patients with thalassaemia major. However, infections with hepatotropic viruses remain a major concern. The objective of this study was to evaluate the prevalence and characteristics of hepatitis C viral (HCV) infection among patients with homozygous beta thalassaemia in a single centre in Oman. **Methods:** A retrospective chart review of 200 patients treated at the Thalassaemia Unit of Sultan Qaboos University Hospital (SQUH) in Muscat, Oman, between August 1991 and December 2011 was performed. Relevant demographic and clinical characteristics were collected, including age, gender, HCV status and the presence of endocrinopathies. **Results:** A total of 81 patients (41%) were found to be anti-HCV-antibody (anti-HCV)-positive. HCV ribonucleic acid tests were performed on 65 anti-HCV-positive patients and were positive among 33 (51%); the remaining 16 patients died before these tests were available. Anti-HCV-positive patients were significantly older than anti-HCV-negative patients ( $P < 0.001$ ) and were more likely to be diabetic than anti-HCV-negative patients (27% versus 8%;  $P < 0.001$ ). A total of 100 patients had been transfused before they were transferred to SQUH in 1991; of these, 70 (70%) were anti-HCV-positive. Only 11 (11.5%) of the 96 patients who were seronegative in 1991, or who were transfused later, became seropositive. **Conclusion:** It is likely that the high prevalence of HCV among multi-transfused thalassaemic patients in Oman is due to blood transfusions dating from before the implementation of HCV screening in 1991 as the risk of HCV-associated transfusions has significantly reduced since then. Additionally, results showed that anti-HCV-positive patients were more likely to be diabetic than anti-HCV-negative patients.

**Keywords:** Hepatitis C; Anti-HCV Antibodies; Beta Thalassaemia; Seroprevalence; Blood Transfusions; Blood Safety; Oman.

**الملخص:** الهدف: نقل الدم المنتظم ضروري لمرضى التلاسيميا الكبرى، لكن تظل التهابات الكبد الفيروسيّة مصدر قلق كبير. الهدف من هذه الدراسة هو تقييم مدى انتشار التهاب الكبد الوبائي سي والتعرف على خصائصه بين المرضى الذين يعانون من مرض التلاسيميا بيتا في مركز واحد في سلطنة عمان. **الطريقة:** تمت مراجعة ملفات 200 مريض ممن تمت متابعتهم في وحدة التلاسيميا في مستشفى جامعة السلطان قابوس في مسقط، سلطنة عمان، في الفترة ما بين أغسطس 1991 وديسمبر 2011. جمعت الخصائص الديموغرافية والسريرية ذات الصلة، بما في ذلك العمر والجنس وحالة الفيروس (HCV) وجود اختلال هرموني. **النتائج:** مضادات الأجسام ضد (HCV) ضد وجدت في 81 مريض (41%). تم فحص الحمض النووي (RNA) في 65 مريضا ممن كانت لديهم مضادات الأجسام ضد (HCV) وكان الفحص إيجابيا في 33 مريضا (51%). توفي 16 مريضا قبل أن تكون هذه الاختبارات متوفرة. المرضى الذين كانت نتائج مضادات الأجسام لديهم إيجابية كانوا أكبر سنا بكثير من المرضى الذين كانت نتائج مضادات الأجسام عندهم سلبية ( $P < 0.001$ ) وكانوا أكثر عرضة لداء السكري (27% مقابل 8%;  $P < 0.001$ ). 100 مريض تم نقل الدم لهم قبل تحويلهم لمستشفى جامعة السلطان قابوس في عام 1991، من هؤلاء 70 (70%) كانت نتائج مضادات الأجسام ضد (HCV) إيجابية. أحد عشر مريضا فقط (11.5%) من أصل 96 مريضا والذين كانت نتائج مضادات الأجسام عندهم سلبية أو تم نقل الدم لهم بعد عام 1991 تحولت نتائجهم إلى إيجابية. الخلاصة: من المرجح أن ارتفاع معدل انتشار فيروس التهاب الكبد الوبائي سي بين مرضى التلاسيميا متعددي نقل الدم في عمان سببه عمليات نقل الدم التي يعود تاريخها قبل بدء تنفيذ فحص (HCV) في عام 1991 حيث إنه انخفض معدل خطر الإصابة بالفيروس منذ ذلك الحين. بالإضافة إلى أن النتائج تشير إلى أن المرضى الحاملين لمضادات الأجسام ضد الفيروس أكثر عرضة للإصابة بداء السكري مقارنة بالمرضى الذين لا يحملونها.

**مفتاح الكلمات:** التهاب الكبد الوبائي سي: الأجسام المضادة للفيروس: تلاسيميا بيتا: الانتشار المصلي: نقل الدم: سلامة الدم: عمان.

**ADVANCES IN KNOWLEDGE**

- The results of this study indicate that the prevalence of hepatitis C viral (HCV) infections in multi-transfused homozygous beta thalassaemic patients in Oman is high, especially among older patients. This is likely due to blood transfusions occurring before the implementation of HCV screening in 1991.
- This study found that anti-HCV-antibody (anti-HCV)-positive patients were more likely to be diabetic than anti-HCV-negative patients.

**APPLICATION TO PATIENT CARE**

- The results of this study support public health efforts to reduce HCV in Oman. National health authorities in Oman can contribute to the further reduction of HCV by supporting and recommending advanced blood screening procedures, such as nucleic acid testing. Furthermore, promoting endogenous blood donations as well as screening blood products will lead to the further reduction of transfusion-related HCV among thalassaemic patients in Oman.
- As diabetes is becoming increasingly prevalent in the Omani population, long-term health plans will need to take into account the significant correlation between HCV and diabetes found in this study.

**H**OMOZYGOUS BETA THALASSAEMIA ( $\beta$ -thalassaemia) is an inherited anaemia characterised by deficient synthesis of the beta globin subunits of the haemoglobin. Oman has a high prevalence of thalassaemia in both the beta and alpha forms.<sup>1</sup> The incidence of homozygous  $\beta$ -thalassaemia in Oman is approximately eight in 10,000, while the global prevalence is reported to be around 0.07%.<sup>2,3</sup>

Regular blood transfusions administered from early childhood onwards are the mainstay of treatment in  $\beta$ -thalassaemia major patients, while thalassaemia intermedia patients only require occasional transfusions. Most thalassaemia major patients require 2–4 blood transfusions per week while those with thalassaemia intermedia can usually maintain reasonable haemoglobin levels without regular transfusions. However, the latter group may occasionally need blood transfusions during pregnancy, prior to surgery or if they develop an infection. Chronic hepatitis C viral (HCV) infections are a comorbidity associated with regular blood transfusions; up to 80% of multi-transfused thalassaemic patients around the globe are infected with transfusion-related viral hepatitis.<sup>4–6</sup> Transfusion-related hepatitis infections are therefore a major concern for those with  $\beta$ -thalassaemia.

In addition to the risk of viral hepatitis transmission, multiple blood transfusions may lead to iron overload. The rate of liver fibrosis reportedly accelerates in patients with iron overload and HCV when compared to patients with either one alone.<sup>7</sup> Zurlo *et al.* found that advanced liver fibrosis is one of the most common causes of death in transfusion-dependent thalassaemia patients over 15 years old.<sup>8</sup> In comparison to the previous two decades, recent advances in the treatment of thalassaemia, iron overload and HCV have led to improved outcomes and survival rates. The early identification and treatment of infections among thalassaemic patients, before the development

of advanced fibrosis, can yield better patient responses and outcomes.

The prevalence of HCV among multi-transfused patients varies from one area to another and depends on the endemicity of viral hepatitis in different regions. The highest reported prevalence was in Egypt, where 75% of patients with homozygous  $\beta$ -thalassaemia are infected with HCV, while the prevalence ranges from 33–67.3% in the neighbouring countries of Kuwait and Iraq.<sup>9–11</sup>

As reported in a study from Iran, the introduction of screening blood products for HCV in 1990 led to a marked reduction in the incidence of new transfusion-related chronic HCV cases,<sup>12</sup> while the majority of currently infected cases are due to infected blood products transfused before the implementation of screening. Testing for HCV was introduced at Sultan Qaboos University Hospital (SQUH) in Muscat, Oman, in 1991. This initially began with the first-generation enzyme-linked immunosorbent assay (ELISA); in subsequent years, new generations of both the ELISA and recombinant immunoblot assay (RIBA) were used as they became commercially available. The use of real time-polymerase chain reaction (RT-PCR) technology was introduced in 2000.<sup>13</sup>

The objective of the current study was to evaluate the prevalence and characteristics of HCV among multi-transfused  $\beta$ -thalassaemia patients in Oman.

## Methods

This retrospective chart review was conducted between August 1991 and December 2011. A total of 200 homozygous  $\beta$ -thalassaemia patients treated and followed up at the SQUH Thalassaemia Unit during the study period were evaluated. The SQUH Thalassaemia Unit is the largest single centre in Oman for thalassaemia management. All patients with either thalassaemia major (transfusion-dependent; n = 180) or thalassaemia intermedia (transfusion-independent;

**Table 1:** Demographic characteristics of homozygous beta thalassaemia patients in Oman between 1991 and 2011 (N = 200)

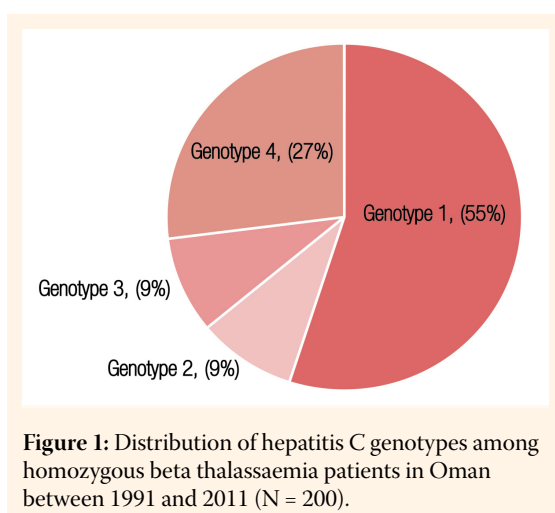
Characteristics	n (%)
Mean age in years	23 ± 7
Male gender	104 (52.5)
Thalassaemia major	180 (90)
Thalassaemia intermedia	20 (10)
HCV infections before 1991	70 (70)
Deaths	41 (20.5)

HCV = hepatitis C virus.

n = 20) who had been managed in the Thalassaemia Unit at SQUH from 1991 onwards were included in the study. Patients with missing or irretrievable data were excluded. Relevant demographic and clinical characteristics were collected.

The following equipment was used to test for HCV. In 1991, the first-generation ELISA (Ortho-Clinical Diagnostics, Inc., Raritan, New Jersey, USA) was used to measure anti-HCV antibody. Between 1992 and 1996, second-generation enzyme immunoassays (Abbott Laboratories, Abbott Park, Illinois, USA) were then used. A second-generation RIBA (HCV 2.0, Chiron Corp., Emeryville, California, USA) was introduced in the SQUH laboratory in mid-1994. It was then replaced by third-generation RIBAs, the HCV 3.0 (Chiron Corp.) and HCV Blot (Genelab Diagnostics Pte. Ltd., Singapore) at the end of 1996. Between 1997 and 2001, sera screening was performed by a third-generation microparticle enzyme immunoassay (AxSYM, Abbott Laboratories). Sera that were initially reactive by ELISA were retested and the results were interpreted according to the manufacturers' specifications. All sera which were repeatedly reactive by ELISA were subjected to additional testing by RIBA. HCV ribonucleic acid (RNA) was tested on samples using the RT-PCR kit COBAS® AMPLICOR HCV Test, Version 2.0 (Roche Molecular Systems Inc., Pleasanton, California, USA). In 2009, SQUH started testing samples for HCV RNA using quantitative COBAS® TaqMan® analysers (Roche Molecular Systems Inc.) with a linear quantification range between 43 and  $6.9 \times 10^7$  IU/mL and a lower limit of detection of 12.6 IU/mL.

Descriptive statistics were used to present the data. For categorical variables, frequencies and percentages were reported. Analyses were performed using Pearson's Chi-squared test. For continuous variables, means and standard deviations were used to summarise the variables while analysis was conducted using Student's t-test. A *priori* was set at 0.05. Analyses were performed using Stata, Version 13.1 (Stata Corp.,



**Figure 1:** Distribution of hepatitis C genotypes among homozygous beta thalassaemia patients in Oman between 1991 and 2011 (N = 200).

College Station, Texas, USA).

Ethical approval for this study was obtained from the Medical Research & Ethics Committee at the College of Medicine & Health Sciences, Sultan Qaboos University (MREC#402).

## Results

Of the 200 homozygous  $\beta$ -thalassaemia patients included in the study, 52.5% were male and the mean age was  $23 \pm 7$  years (range: 7–50 years). The majority of the patients had thalassaemia major and the rest had thalassaemia intermedia [Table 1]. A total of 81 patients (41%) were found to be anti-HCV-antibody (anti-HCV)-positive; including 77 with thalassaemia major and four with thalassaemia intermedia. There were 100 patients (50%) who had been transfused before 1991; of these, 70 tested positive for anti-HCV. Additionally, 11 of 96 patients with thalassaemia major (11.5%) who were anti-HCV-negative in 1991, or who started transfusions after that date, became seropositive. Four patients with thalassaemia intermedia had never been transfused.

Repeat anti-HCV testing using a third-generation ELISA confirmed the positive results of the first- and second-generation ELISAs. Anti-HCV-positive patients were significantly older than their anti-HCV-negative counterparts (28 versus 20 years;  $P < 0.001$ ) and were significantly more likely to be diabetic than the anti-HCV-negative patients (27% versus 8%;  $P < 0.001$ ).

No hepatitis B co-infections were noted among the patients. Four patients were co-infected with HCV and human immunodeficiency virus (HIV). There were 41 deaths (20.5%) during the study period; all but 10 of these patients were infected with HCV. The most common causes of death were cardiac complications (41%) followed by sepsis (24%). Before HCV RNA testing was available, 16 of the 81 patients who tested

positive for anti-HCV died; four from HIV-related causes, eight from cardiac iron load-related causes and four due to bacterial sepsis. Therefore, RT-PCR to detect HCV RNA was performed for 65 anti-HCV-positive patients. HCV RNA was detected in 33 patients (51%); 18 (55%) had genotype 1, three (9%) had genotype 2, three (9%) had genotype 3 and nine (27%) had genotype 4 [Figure 1]. Half of these patients had advanced stage fibrosis (stages three and four) according to Ludwig *et al.*'s staging system.<sup>14</sup> Of the 33 patients with HCV RNA, 16 (48.5%) underwent a liver biopsy.

The mean serum ferritin level in patients who tested positive for anti-HCV was  $3,381 \pm 3,290$  ng/mL (normal range: 20–300 ng/mL). Of the 200 patients, high liver enzymes were found in 76 (38%). Mean alanine aminotransferase levels were significantly higher in thalassaemic patients who were positive for anti-HCV than in those who were negative ( $82 \pm 111$  versus  $46 \pm 39$  U/L;  $P = 0.011$ ) and significantly higher in those who were HCV RNA-positive than those who were HCV RNA-negative ( $106 \pm 150$  versus  $45 \pm 35$  U/L;  $P = 0.042$ ).

## Discussion

Unlike hepatitis B, there is as yet no vaccination for HCV and multi-transfused thalassaemic patients who are at high risk of acquiring viral hepatitis.<sup>4</sup> There has been a marked reduction in transfusion-related viral hepatitis worldwide since the implementation of screening tests for HCV in the early 1990s.<sup>15</sup> As observed in a study by Velati *et al.*, transfusion-related viral hepatitis cases have also decreased due to the introduction of advanced screening methods such as nucleic acid testing.<sup>16</sup>

The current study revealed that 41% of investigated  $\beta$ -thalassaemia patients had been infected with HCV. This is comparable to the prevalence of anti-HCV observed in a similar cohort of patients in Jordan (40.5%) but higher than those reported in India (30%), Pakistan (35%) and Kuwait (33%).<sup>10,17–19</sup> However, active HCV infections were found in 33 of the 65 anti-HCV-positive patients (51%) in the current study who could be tested using RT-PCR to detect HCV RNA.

Important factors to keep in mind when evaluating the prevalence of HCV in different countries include its prevalence in specific populations, the source of blood utilised during transfusions, the blood product screening methods employed and the age of the cohort being studied. There is currently no true population-based prevalence of HCV in Oman. One study reported the seroprevalence of hepatitis C antibodies using a second-generation enzyme immunoassay which detected antibodies to three HCV antigens in

26.5% of patients undergoing haemodialysis, 13.4% of kidney transplant patients and 1% of non-dialysed non-transplanted patients with various renal diseases.<sup>20</sup> In another study, Al Dhahry *et al.* investigated the prevalence of HCV among Omani blood donors between 1991 and 2001 using three generations of ELISAs and RIBAs to confirm positive ELISA tests.<sup>13</sup> Out of 30,012 samples, 272 (0.91%) were positive for anti-HCV and 46.5% of these were confirmed positive by RIBA, giving a true prevalence of 0.42%. The proportion of sera that were confirmed to be anti-HCV-positive varied from 95% among intravenous drug users to 81% in patients with hepatitis and 70% in those with haemoglobinopathies.<sup>13</sup>

The proportion of positive anti-HCV findings among the multi-transfused thalassaemic patients in the current study is much higher than the 0.42% prevalence reported by Al Dhahry *et al.*<sup>13</sup> It is likely that this discrepancy is due to the fact that healthy blood donors were used rather than patients with thalassaemia. Furthermore, Oman relied on imported blood from the USA from the mid-1970s to the early 1990s; during this time, the National Health and Nutrition Examination Survey III (1988–1994) showed HCV to be the most common chronic blood-borne infection in the USA.<sup>21,22</sup> There was also a lack of HCV testing for blood products during the specified period.<sup>22</sup> In addition, the authors of the current study observed that many patients travelled to the Indian subcontinent for splenectomies in the 1970s and had transfusions performed there. In 1984, the screening of imported blood for HBV and HIV using HBV surface antigen and HIV antigen tests was initiated with the help of the World Health Organization.<sup>22</sup> In the early 1990s, the Omani Ministry of Health decided to rely on local blood donations to meet the required demand and by July 1991 no more imported blood was being used in Oman.<sup>22</sup>

The majority of the thalassaemic patients assessed in the current study were infected with HCV before the implementation of screening in 1991. This is also reflected in the finding that anti-HCV-positive patients were significantly older than their counterparts. This is in agreement with data from a neighbouring country—Alavian *et al.* showed that the pooled odds ratio of the HCV infection rate for patients transfused before the era of screening in Iran was 7.6 (95% confidence interval [CI]: 4.7–12.3).<sup>23</sup> The current study shows that promoting endogenous blood donation and instituting a policy of screening blood products has led to a reduction of transfusion-related HCV infection in Oman.

False-positive anti-HCV results have been reported with the use of first- and second-generation ELISAs.<sup>24</sup> However, the patients found to be positive in

the present study were tested with a third-generation ELISA which confirmed their anti-HCV status. Of the anti-HCV-positive patients, 41% had positive HCV RNA tests, implying active viral replication. More than half were infected with genotype 1. This is fitting considering the source of imported blood in Oman before 1991, as genotype 1a was common in the USA in the early 1990s.<sup>25</sup> Genotype 4 was the second most common genotype in the current study; this genotype is reportedly common in Africa.<sup>25</sup> Its high prevalence among the studied Omani patients is therefore no surprise considering the historical and cultural relationship between Oman and East Africa and the migratory patterns of many families between these two regions. In comparison to other genotypes, genotypes 1 and 4 have been associated with a reduced response to the combination treatment of pegylated interferon and ribavirin.<sup>26</sup>

One interesting finding of the current study was the significant correlation between those who were anti-HCV-positive and the presence of diabetes mellitus (DM) when compared to anti-HCV-negative thalassaemic patients. DM is a well-known complication of iron overload in multi-transfused thalassaemic patients. The worldwide incidence of impaired glucose tolerance among thalassaemic patients is approximately 4–24% while the incidence of DM ranges between 0–26%.<sup>27,28</sup> Factors such as insulin resistance and insulin deficiency secondary to iron deposition in islet cells have been suggested to explain the high incidence of DM among thalassaemic patients.<sup>29</sup> The association between HCV and DM has been well-established in the literature.<sup>30,31</sup> The National Health and Nutrition Examination Survey III, which included more than 9,000 subjects aged  $\geq 20$  years, demonstrated that the odds ratio of DM developing in anti-HCV-positive subjects  $\geq 40$  years old is 3.77 (95% CI: 1.80–7.87). This association was independent of gender, race and body mass index.<sup>32</sup> The main mechanism that may explain the onset of DM in patients with HCV is the development of insulin resistance secondary to alterations in the intracellular signalling of insulin in such patients.<sup>33</sup> The synergic effect of HCV and thalassaemia in the development of DM has been described in a study by Labropoulou-Karatzas *et al.*<sup>34</sup> Their findings demonstrated that thalassaemic patients with HCV were more likely to be diabetic compared to thalassaemic patients without HCV infections (45.3% versus 11.3%;  $P < 0.001$ ). The current study also confirms this association.

Anti-HCV-positive patients were significantly older than their counterparts. However, it is important to note that these older patients were poorly chelated and thus had significantly higher iron loads. In fact,

it is likely that the advanced stage fibrosis present in a group of relatively young patients (mean age: 28 years) is secondary to the synergistic effect of HCV and iron overload.

In the current study, four patients were co-infected with HCV and HIV. They were born in the early 1980s and thus were infected before the implementation of blood screening. All of these co-infected patients died of HIV-related complications. The main causes of death in the study (cardiac complications and sepsis) were similar to those reported by Ladis *et al.* while investigating survival rates and causes of death in thalassaemic patients from Greece.<sup>35</sup> They found that heart failure was the most common cause of death (71.3%) followed by sepsis (7.8%). Similar results were also reported from Italy and Iran.<sup>8,36</sup> In the current study, 75.6% of the patients who died were infected with HCV. This finding affirms the results of previous studies describing the morbidity and mortality associated with HCV infection in multi-transfused thalassaemic patients.<sup>37</sup>

## Conclusion

A total of 200 homozygous  $\beta$ -thalassaemia patients were included in the study and 41% were anti-HCV-positive. Anti-HCV-positive patients were significantly more likely to be diabetic than anti-HCV-negative patients. In addition, anti-HCV-positive patients were significantly older compared to their counterparts. This indicates that the high prevalence of HCV among multi-transfused thalassaemic patients in Oman is likely due to transfusions of HCV-infected blood before the implementation of HCV screening in 1991, suggesting that blood screening implemented after this time has significantly reduced the risk of HCV associated with blood transfusions.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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