

Hepatitis B and C Infections and Different Genotypes of HCV Among Sickle Cell Anemia Patients in Ahvaz, South-Western Iran

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Background: Although it is life-saving, blood transfusion therapy has resulted in risk for transfusion-transmitted infections (TTIs) in the majority of sickle cell anemia being patients.

Objectives: The current study aimed to determine the prevalence of HBV, HCV and different genotypes of HCV among sickle cell anemia (SCA) patients in Ahvaz city, South-western Iran.

Materials and Methods: A cross-sectional study was conducted on 56 SCA patients who referred to the Hemoglobinopathy and Thalassemia research centre during January 2009 to February 2010. Patients' sera were tested for HBsAg and anti-HCV using ELISA and confirmed by PCR (HBV) and RT-PCR (HCV). HCV genotypes were determined with HCV genotype specific primers using HCV genotyping kit.

Results: The overall prevalence rate of HBsAg and anti-HCV were 1.8% (95% CI: 0-5.26) and 12.5% (95% CI: 3.8 - 21.1), respectively. Five of the anti-HCV patients (71.4%) were also HCV RNA positive. The predominant HCV genotype in the patients was 1a (60%), though genotype 1b was found in 40% of the subjects.

Conclusions: It seems that stringent donor selection procedures reduce HCV infection in multi-transfused patients, but there is still serious risk for these patients.

Keywords: Hepatitis B; Hepatitis C; Prevalence; Genotype; Anemia, Sickle Cell

1. Background

Sickle cell anemia (SCA) is one of the most common hereditary hematologic diseases Worldwide. Patients with SCA require multiple blood transfusions and therefore are exposed to transfusion-transmitted infections (TTIs) such as hepatitis B virus (HBV) and particularly hepatitis C virus (HCV). Regular blood or blood products transfusion in patients with sickle cell anemia, thalassemia and haemophilia has improved their overall survival, but carries a definite risk of acquisition of blood-borne virus infections (1-5). Improvement of the people's knowledge about TTIs risk factors, blood screening strategies, national HBV vaccination program since 1992 for all neonates have led to a dramatic decrease in the prevalence of TTIs particularly HBV during the last decades (6, 7). However, post transfusion transmission of HCV has still remained a major health concern in multi-transfused patients (1).

Ahvaz city, the capital of Khuzestan province, located

in the south-western of Iran, is a tropical area with an approximate population of 1.4 millions (census 2006). Khuzestan has suffered the heaviest damage of all Iranian provinces during a 28-year period including: the Iran-Iraq War (1980-1988), the Gulf War (1990-1991), and the 19-year crisis in Iraq (1990-2009) (8). This geographical location, mass immigration from Iraq, frequent travels to Iraq and neighbouring Arabian countries have all affected prevalence of TTIs in Khuzestan province (9, 10). Moreover, hemoglobinopathies are important health problems throughout Iran particularly in this region (3). The epidemiology of HBV, HCV and different genotypes of HCV among sickle cell anemia (SCA) patients in Ahvaz city has not been extensively investigated, and few data are available on prevalence of the diseases in this area.

2. Objectives

Due to the lack of sufficient reported data from the re-

Implication for health policy/practice/research/medical education:

Due to the large number of blood transfusions received, sickle cell anemia patients are at high risk for acquiring HCV and HBV infections. This study is the first report on prevalence of HCV genotypes among sickle cell anemia patients in Iran and provides information that will assist in developing intervention guidelines to reduce the risk of acquiring transfusion-transmitted infections, which continue to be a significant public health problem in Khuzestan province.

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gion, the current study, as the first of its kind in south-western Iran, was conducted to investigate the prevalence of HBV, HCV infections and different genotypes of HCV among SCA patients in Ahvaz.

3. Materials and Methods

3.1. Subjects

This cross-sectional study was performed from January 2009 to February 2010 on SCA patients referring to Research Centre of Thalassemia and Hemoglobinopathies (RCTH) in Ahvaz city, South-western Iran. The study was approved by the institutional review board.

Patients participated in the study after giving written, informed consent (n = 56). Peripheral blood samples were obtained from patients and then serum was separated, aliquoted and stored at -20° C. Demographic factors, such as age, duration and number of blood transfusions were obtained from patient records. Furthermore, no patient was receiving interferon therapy or other antiviral drugs.

3.2. Laboratory Assays

All sera were screened using HBsAg and anti-HCV assays with 3-rd generation of immune enzymatic test (ELISA-III). Positive samples were confirmed using DNA polymerase chain reaction (PCR) and nested RT-PCR for HBV and HCV, respectively. All samples were submitted to DNA and RNA extraction, using high pure nucleic acid kits (Roche, Germany) according to the manufacturer's

instructions. HCV RNA was transcribed into cDNA by random primer (Fermentas, Lithuania). The cDNA was targeted using a nested-PCR with specific primers for the conserved sequences in the 5' non-coding region (5'-NCR) of HCV. HCV genotypes were determined with HCV genotype specific primers using HCV genotype kit (Sacace, Italy).

3.3. Statistical Analysis

Prevalence and 95% confidence intervals (95% CI) were calculated by SPSS software version 13.0 (SPSS Inc., Chicago, IL).

Data comparisons were performed using the Chi-square test, Fisher's exact and two-tailed t-test. The differences were considered significant if $P < 0.05$.

4. Results

Fifty six SCA patients including 32 (57.1%) males and 24 (42.9%) females were tested. The mean age (\pm SD) was 22.0 ± 10.62 years (range 2 – 48 years) (Table 1). Seven patients were positive for HCV antibodies, for a prevalence rate of 12.5% (95% CI: 3.8-21.1) (Table 1). One HBsAg-infected patient (1.8%, 95% CI: 0-5.26) was positive for HBV DNA. Furthermore, HCV RNA was detected in 71.4% (5/7) of the anti-HCV-positive patients. The current study found that the prevalence rate of anti-HCV seropositivity was significantly high ($P = 0.03$) among patients who had started to receive transfusions before 1996 when serological screening for anti-HCV antibody had not been introduced to blood banks in Iran.

Table 1. HCV Infection Among Sickle Cell Anemia Patients Receiving Multiple Transfusions

Characteristics	Anti-HCV		P value	HCV RNA		P value
	Positive, No.(%)	Negative, No.(%)		Positive, No.(%)	Negative, No.(%)	
Prevalence	7 (12.5)	49 (87.5)		5 (8.9)	51 (91.1)	
Age			0.07			0.05
Mean \pm SD	28.8 \pm 10.1	21.1 \pm 10.4		30.0 \pm 10.5	21.1 \pm 10.3	
Sex			0.1			0.09
Male	6 (18.8)	26 (81.2)		5 (15.6)	27 (84.4)	
Female	1 (4.2)	23 (95.8)		0	24 (100)	
First transfusion			0.03			0.1
Before 1996	7 (20.5)	26 (79.5)		5 (15.1)	28 (84.9)	
Starting in 1996	0	23 (100)		0	23 (100)	
No. of units transfused			0.008			0.04
< 100	0	21 (100)		0	21 (100)	
100-200	0	12 (100)		0	12 (100)	
> 200	7 (30.4)	16 (69.6)		5 (21.7)	18 (78.2)	

The prevalence of anti-HCV and HCV RNA was significantly higher in patients who received blood transfusion more than 200 times compared to subjects with blood

transfusion less than 100 times ($P = 0.008$ and $P = 0.04$, respectively). No statistically significant difference was observed between anti-HCV or HCV RNA positive and

negative patients with respect to age or sex. In the end, predominant HCV genotype among sickle cell anemia patients in the region was 1a (3/5, 60%), whilst genotype 1b was found in 40% (2/5) of the patients.

5. Discussion

Due to the large number of blood transfusions received, sickle cell anemia patients are at high risk for acquiring HCV and HBV infections. Prevalence of hepatitis infection in these patients is reported by several authors ranging between 4.5 to 21% for HCV (2, 11-13) and 3 to 39% for HBV (14-16). The current study found that the prevalence rates of anti-HCV and HBsAg were 12.5% (7/56) and 1.8% (1/56), respectively. Since serologic tests became available, the blood donors screening has been performed in most countries. In Iran, mandatory anti-HCV screening was introduced to blood banks in 1996 (1, 5, 9). The current study results showed that 58.9% of SCA patients had been transfused for more than 12 years. It was suggested that the patient who received blood products could be exposed at higher risk to HCV.

In the current study the frequency of anti-HCV positive among patients with blood transfusion was significant ($P = 0.008$). Recent studies of SCA patients show that the prevalence of anti-HCV varied (4.5 to 21%) (2, 11-13), which suggests that the geographical area may affect the potential risk of HCV in patients (17-19). Moreover, one of the most common causes of HCV transmission by transfusion is the occurrence of new infections in blood donors. Hajiani et al. reported that blood transfusion is the leading risk factor for HCV acquisition in Khuzestan province as 52% of HCV-positive individuals were diagnosed with chronic haemolytic anemia and received regular blood transfusion (19).

Prevalence of HCV in blood donors in different provinces of Iran has been reported to be 0.3-0.97% (1, 5, 19, 20). Nevertheless, a recent study on 2376 blood donors in Ahvaz, showed that 2.3% of the subjects were positive for anti-HCV (21). It is noteworthy to mention that the most important problem with the studies is the possibility of selection bias and low positive analytical value of anti-HCV detection by ELISA (1, 22). However, in the current study anti-HCV positivity was detected in 7 (12.5%) of the 56 SCA patients. This rate is higher than that of the blood donors in the region. Furthermore, the anti-HCV prevalence in the current study (12.5%) was lower than that of the multi-transfused patients in some neighbouring Arabic countries (29.4 to 44%) (23-25) and other countries such as Pakistan (48.6%) (26) and Brazil (14.1%) (13).

However, in the previous studies the male preponderance in HCV-infected patients was reported (19, 20). The results of the present study were consistent with the previous studies though they were not statistically significant (Table 1). HCV RNA was detected in 71.4% of the anti-HCV-positive patients. In different studies, this rate

was a wide range between 48-82% (13, 21, 27). Although the use of 3-rd generation of enzyme immunoassays (EIAs) is often appropriate and practical, particularly in routine HCV diagnosis, more care should be taken about ELISA test limitations to avoid unnecessary distress to patients, as a result of false-positive HCV-antibody detection.

Studies of some neighbouring Arabic countries reported an HCV infection rate of 29.4 -44% among multi-transfused patients (23 - 25). Khuzestan province shares a land, river and sea border with these countries. In addition, the Iran-Iraq War of 1980-1988, has had a devastating impact on public health. Moreover, during a period of 18 years, due to poor security and living conditions, many Iraqi refugees have crossed over the Iraqi border to Iran, mainly to the south western regions (8, 10). The geographical situation, mass immigration from Iraq, where a significantly higher prevalence of anti-HCV has been found among different populations (28, 29) and frequent travels between Khuzestan province and neighbouring Arabic countries, all could affect prevalence of TTIs in the region.

In the current study, HBsAg positivity and HBV DNA was found at the rate of 1.8% among the SCA patients which was less than those of the blood donors in Iran (1.8% vs. 3%) (6). This could be due to vaccination against HBV for newborn and particularly high-risk groups since 1992 and the compulsory screening of donated bloods by the local blood banks since 1995 (6, 7). There is no previous study regarding the frequency of HBsAg in SCA patients in the region. However, it seems that the rate of HBV infection in multi-transfused patients has been declining in Iran since 1995 (17).

To our best knowledge, the current study was the first report on prevalence of HCV genotypes among SCA patients in Iran. Two HCV genotypes were identified in these patients in Ahvaz city: subtypes 1a and 1b. Subtype 1a was predominant (60%) similar to the recent studies in different populations in the region (18, 21). This finding is different from HCV genotypes distribution and the high frequency of genotype HCV 1a/1b plus HCV 4 and HCV 2/2a plus HCV 4 in multi-transfused patients living in neighbouring countries, such as Bahrain and Saudi Arabia respectively, sharing sea boarder with Khuzestan province (4).

In conclusion, the current study provided information that will assist in developing intervention guidelines to reduce the risk of acquiring TTIs, which continue to be a significant public health problem in Khuzestan province. The prevalence of HCV infection decreased after introduction of screening tests and stringent donor selection procedures, but TTIs in patients with SCA is still serious risk for these patients.

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Authors' Contribution

None declared.

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