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Hepatitis C virus infection in a Brazilian population with sickle-cell anemia

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Abstract

Patients with sickle-cell anemia submitted to frequent blood transfusions are at risk of contamination with hepatitis C virus (HCV). Determination of HCV RNA and genotype characterization are parameters that are relevant for the treatment of the viral infection. The objective of the present study was to determine the frequency of HCV infection and the positivity for HCV RNA and to identify the HCV genotype in patients with sickle-cell anemia with a history of blood transfusion who had been treated at the Hospital of the HEMOPE Foundation. Sera from 291 patients were tested for anti-HCV antibodies by ELISA 3.0 and RIBA 3.0 Chiron and for the presence of HCV RNA by RT-PCR. HCV genotyping was performed in 19 serum samples. Forty-one of 291 patients (14.1%) were anti-HCV positive by ELISA and RIBA. Both univariate and multivariate analysis showed a greater risk of anti-HCV positivity in those who had started a transfusion regime before 1992 and received more than 10 units of blood. Thirty-four of the anti-HCV-positive patients (34/41, 82.9%) were also HCV RNA positive. Univariate analysis, used to compare HCV RNA-negative and -positive patients, did not indicate a higher risk of HCV RNA positivity for any of the variables evaluated. The genotypes identified were 1b (63%), 1a (21%) and 3a (16%). A high prevalence of HCV infection was observed in our patients with sickle-cell anemia (14.1%) compared to the population in general (3%). In the literature, the frequency of HCV infection in sickle-cell anemia ranges from 2 to 30%. The serological screening for anti-HCV at blood banks after 1992 has contributed to a better control of the dissemination of HCV infection. Because of the predominance of genotype 1, these patients belong to a group requiring special treatment, with a probable indication of new therapeutic options against HCV.

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- Hepatitis C
- Anti-HCV antibodies
- HCV RNA
- HCV genotype
- Sickle-cell anemia

Introduction

Since hepatitis C virus (HCV) was first identified (1,2) one of the best known and most extensively studied routes of HCV transmission has been blood or blood derivative transfusion (3,4). Individuals submitted to frequent blood transfusions such as patients with sickle-cell anemia are at risk for HCV infection, with a prevalence ranging from 2 to 30% (5-11), especially those who were submitted to blood transfusion before serological screening for anti-HCV antibodies at blood banks (3,4). The identification of HCV ribonucleic acid (HCV RNA), which indicates viremia, is an important step in the diagnosis of HCV infection (12), as also is genotype characterization, a relevant predictive parameter of the response to antiviral treatment since genotype 1 is associated with a lower sustained virologic response (30%) compared to genotypes 2 and 3, whose sustained virologic response is 65% with the therapeutic scheme currently used (interferon and ribavirin) (13-17). However, few data are available in the literature about the determination of HCV RNA in serum or about genotype characterization in patients with sickle-cell anemia (18,19).

The objectives of the present study were to determine the frequency of HCV infection and the positivity for HCV RNA and to identify the HCV genotype in patients with sickle-cell anemia with a history of blood transfusion followed up at the Hospital of the Hematology and Hemotherapy Foundation of Pernambuco (HEMOPE), a reference service for patients with hematological diseases in the State of Pernambuco, in the northeastern region of Brazil.

Material and Methods

Study population

A total of 458 individuals with a diagnosis of sickle-cell anemia were registered at the Hospital of the HEMOPE Foundation from January to December 1998, 311 of whom had received blood transfusions and were homozygotes (Hb SS) for sickle-cell anemia. Of these, 291 participated in the study (135 men and 156 women). Median age was 17 years (range: 1 to 84 years). Twenty patients could not be located.

Patient data (sex, age, origin, year when the transfusion regime had started, and amount of blood components received) were obtained from their medical records. With respect to origin, patients were divided into individuals residing in the metropolitan area of Recife and individuals from the remaining regions of the State of Pernambuco, denoted interior region of the state. Patients were considered to have been submitted to blood transfusion when they had received red cell concentrate or other blood derivatives since the time of registration with the Hospital of the HEMOPE Foundation or when they reported transfusion at another service.

In view of the fact that serological screening for HCV was started in 1992 at blood banks in the State of Pernambuco, this year was considered for the evaluation of the risk of contamination with HCV.

The study was approved by the Medical Ethics Committee of the Hospital of the HEMOPE Foundation and informed consent was obtained from each participant or person responsible.

Standardization of the techniques

Serum sample. Ten milliliters of whole blood was collected and two serum samples were separated after a maximum of 2 h at room temperature, one for serological study and the other stored frozen at -80ºC for molecular biology studies.

Detection of anti-HCV. Patient sera were tested by a 3rd-generation immunoenzymatic test (ELISA 3.0) and those with positive results were submitted to the recombinant im-

munoblot test (Chiron RIBA HCV 3.0 SAI, Ortho-Clinical Diagnostics, Raritan, NJ, USA). Both tests were carried out according to manufacturer instructions. The results were considered to be positive for anti-HCV when both the ELISA and RIBA tests were positive.

Detection of HCV RNA. Anti-HCV-positive serum samples were examined for the presence of HCV RNA using the reverse transcriptase-polymerase chain reaction (RT-PCR) with the commercial kit Amplicor™ Hepatitis C Virus (HCV) Test (Roche Diagnostics, Mannheim, Germany) according to manufacturer instructions.

Determination of HCV genotype. Nineteen of 34 HCV RNA-positive serum samples were submitted to genotyping by strip immunoblotting (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium) according to manufacturer instructions.

Detection of anti-HIV1 HIV2. ELISA was performed on 291 serum samples using the commercial kit Genelavia MIX (Sanofi Diagnostics Pasteur, Chaska, MN, USA) according to manufacturer instructions.

Statistical analysis

Data were analyzed statistically using the Epi-Info 6.0 and SPSS-PC software. The association between each variable (sex, age, origin, year when the transfusion regime had started, and amount of blood components received) and the dependent variable (anti-HCV and HCV RNA) was first tested. To test statistically significant differences, the chi-square, chi-square with Yates correction and Fisher exact tests were used for the categorical variables, and the Kruskal-Wallis test and F statistic were used for the continuous variables. The variables that were associated significantly with the dependent variable by univariate analysis were introduced in a multivariate model (multiple logistic regression) and the statistical significance of removal of each variable was tested. The level of significance was set at $P<0.05$ for all tests.

Results

Forty-one of the 291 patients were anti-HCV positive by 3rd-generation ELISA and RIBA, corresponding to a prevalence of 14.1%. Two patients were RIBA negative and three yielded indeterminate results. The mean and median age of the 41 anti-HCV-positive patients was higher than that of negative patients ($P<0.00001$). No significant difference in prevalence was observed between males and females ($P = 0.22$). The prevalence of HCV infection among individuals residing in the metropolitan area of Recife (17.6%) was significantly higher ($P = 0.01$) than among residents in the interior region of the State of Pernambuco (6%). The prevalence of HCV infection (19.7%) was significantly higher ($P = 0.00008$) among

Table 1. Distribution of patients with sickle-cell anemia studied from January to December 1998 at the Hospital of the HEMOPE Foundation according to demographic and clinical characteristics and to the result of anti-HCV antibody (RIBA) and HCV RNA (RT-PCR).

Characteristics	Anti-HCV (RIBA)		HCV RNA (RT-PCR)	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
Prevalence	41/291 (14.1)	247/291 (84.9)	34/41 (82.9)	7/41 (17.1)
Age (years)				
Mean ± SD	25.4 ± 7.7*	17.34 ± 11.6	25.7 ± 7.6	24 ± 8.3
Median	26*	15	27.5	22
Range	10-37	1-84	10-37	13-37
Sex				
Male	23 (17.3)	110 (82.7)	18 (78.3)	5 (21.7)
Female	18 (11.6)	137 (88.4)	16 (88.9)	2 (11.1)
Origin				
Metropolitan region of Recife	36 (17.6)*	168 (82.4)	29 (80.6)	7 (19.4)
Interior region of the state	5 (6)	79 (94)	5 (100)	0 (0)
Beginning of transfusion				
Before 1992	40 (19.7)*	163 (80.3)	33 (82.5)	7 (17.5)
Starting in 1992	1 (1.2)	84 (98.8)	1 (100)	0 (0)
Transfused units				
≤10 units	15 (8.7)	158 (91.3)	12 (80)	3 (20)
>10 units	26 (22.6)*	89 (77.4)	22 (84.6)	4 (15.4)

RIBA, recombinant immunoblot test; RT-PCR, reverse transcriptase-polymerase chain reaction.
*$P<0.05$ for comparison of anti-HCV-negative and -positive results (chi-square, chi-square with Yates correction, Kruskal-Wallis H test).

individuals who had started to receive transfusions before 1992 when serological screening for anti-HCV antibody had been started in blood banks in the State of Pernambuco, than among individuals (1.2%) who had started to receive transfusion after 1992. The prevalence of HCV infection was higher (P = 0.003) among individuals who had received more than ten units of blood components (22.6%) than among those who had received up to ten units of blood components (8.7%) (Table 1).

The prevalence of HCV RNA was 82.9% (34/41 patients) among anti-HCV-positive individuals. Among the five ELISA-positive individuals who were RIBA negative (2/5) or indeterminate (3/5), only one RIBA-indeterminate individual was positive for HCV RNA. Mean age was higher among HCV RNA-positive patients than among HCV RNA-negative patients (P = 0.57). The prevalence of HCV RNA was higher among females (88.9%) than among males (78.3%), although the difference was not significant (P = 0.32). The prevalence of HCV RNA was higher (100%) among individuals from the interior regions of the state than among individuals residing in the metropolitan area of Recife (80.6%) (P = 0.37). There was no significant difference in the prevalence of HCV RNA (P = 0.82) between individuals who had started to receive transfusions before 1992 (82.5%) and those who had started to receive them after 1992 (100%). The prevalence of HCV RNA was higher among the individuals who had received more than ten units of blood components (84.6%) than among those who had received less than ten units (80%), but the difference was not statistically significant (P = 0.50) (Table 1).

HCV genotyping revealed that 12 of the 19 patients tested (63%) had genotype 1b, 4 (21%) had genotype 1a, and 3 (16%) genotype 3a.

No patient was found to be HIV seropositive.

Discussion

Since the 1950's, reports have been published stating that hepatitis is one of the causes of liver disease in individuals with sickle-cell anemia (20,21). With the demonstration that the C virus was the major etiologic agent of post-transfusional hepatitis (2,22), patients with sickle-cell anemia started to be considered as a population at high risk to acquire HCV infection (23), as demonstrated by the frequency of HCV infection in the world population in general, which is estimated at 3% (24), while the frequency of HCV infection among patients with sickle-cell anemia submitted to transfusion of blood or blood derivatives reported in the literature ranges from 2 to 30% (5-11). However, molecular biology tests confirming viremia and permitting characterization of HCV genotype are scarce in patients with sickle-cell anemia (18,19), an important factor when treatment of infection is indicated (13). In the present study, a high prevalence (14.1%) of HCV infection was detected among these individuals, as well as a high agreement between anti-HCV and HCV RNA results (82.9%), emphasizing once more the importance of the C virus as a possible agent triggering liver damage in these patients.

In the patients studied here there was no sex difference in frequency of HCV infection, although a predominance of HCV infection among males has been reported for the general population (25).

In the State of Pernambuco, 41% of the population resides in the capital (Recife) and in its metropolitan region (26), where the Hospital of the HEMOPE Foundation is located and from where most of the participants in the study originated (71%). The C virus occurred more frequently among individuals from Recife and its metropolitan region.

Donahue et al. (3) reported a lower frequency of HCV infection among individuals

submitted to blood transfusion after the implantation of serological screening for anti-HCV at blood banks in the United States. In the present study we obtained a similar result, with a lower frequency (1.2%) of anti-HCV among individuals whose blood transfusions had started after 1992, when serological screening for anti-HCV was started at blood banks in the State of Pernambuco, a factor that contributed to a better control of the dissemination of HCV infection among patients with sickle-cell anemia.

The 2nd- and 3rd-generation ELISA test for the detection of anti-HCV is highly sensitive but does not provide full safety in terms of the prevention of post-transfusional hepatitis C (3,4). The estimated risk of HCV infection is 0.01 to 0.001% per unit of blood or blood component transfused (27). In the present study we identified a patient with positive anti-HCV and HCV RNA tests who had received transfusions after 1992, a year when the search for anti-HCV was already part of the serological screening routine in the State of Pernambuco. The patient contaminated by blood transfusion after 1992 was not submitted to detection of C virus antibody before blood transfusion. It was not possible to survey the donors of blood products received by this patient.

The wide variation (2 to 30%) (5-11) in the prevalence of anti-HCV among patients with sickle-cell anemia can be explained as a function of the various risk factors to which the patients in the different studies were exposed, such as amount of blood components transfused (7,8) and geographic distribution of the C virus (27). In the present study the larger number of units transfused implied a greater risk for HCV infection, data similar to those reported by other investigators for patients with sickle-cell anemia (5-8).

In the present study, the agreement between anti-HCV and HCV RNA detection was 82.9%, a finding similar to those reported for other populations, i.e., rates ranging from 65 to 86% (25,28-31). 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 (12). In the present study, care was taken to avoid some of these problems according to the guidelines of Kwok and Higuchi (32). The commercial kit Amplicor™ Hepatitis C Virus (HCV) Test was used, whose sensitivity is 95% (30).

The importance of genotype identification in HCV infection is in its value as a predictor of the response to antiviral treatment since genotype 1, and 1b in particular, is associated with a poorer response to treatment with interferon and ribavirin (13-17). In the present study, the most frequent genotype was genotype 1 (84% of infected patients), as also observed in several other geographic regions of Brazil (33-38). The fact that genotype 1b was more frequent in the present study placed these patients in the group of individuals with the worst response to currently available antiviral treatment (13-17), who are likely to be assigned to the group of pegylated interferon monotherapy since the use of ribavirin is contraindicated (17,39).

The low prevalence of anti-HIV among patients with sickle-cell anemia has been described in some studies (11,40). In the present investigation, no patient was anti-HIV positive although all had received blood transfusion as a risk factor for double infection (HCV and HIV).

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