Epidemiological aspects of hepatitis C virus infection among renal transplant recipients in Central Brazil

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An investigation was conducted involving 255 renal transplant recipients in the state of Goiás, Central Brazil, to determine the prevalence of hepatitis C virus (HCV), its risk factors, the genotypes involved, and the level of alanine aminotransferase (ALT) present in the patients. All serum samples were tested for anti-HCV antibodies and HCV RNA. Forty-one patients were anti-HCV and/or HCV RNA positive, resulting in an overall HCV infection prevalence of 16.1% (95% CI: 11.9-21.3). A multivariate analysis of risk factors showed that a history of blood transfusions without anti-HCV screening, the length of time spent on hemodialysis, and renal transplantation before 1994 are all associated with HCV positivity. In HCV-positive patients, only 12.2% had ALT levels above normal. Twenty-eight samples were genotyped as genotype 1, subtypes 1a (62.5%) and 1b (31.3%), and two samples (6.2%) were genotype 3, subtype 3a. These data show a high prevalence of HCV infection and low ALT levels in the studied population. The risk factor analysis findings emphasize the importance of public health strategies such as anti-HCV screening of candidate blood and organ donors, in addition to the stricter adoption of hemodialysis-specific infection control measures. The present study also demonstrates that HCV genotype 1 (subtype 1a) is predominant in this population

Key words: hepatitis C virus - renal transplant recipients - prevalence - risk factors - ALT - genotypes

Hepatitis C virus (HCV) is a well-known agent of liver diseases, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Chen & Morgan 2006). Patients with chronic kidney disease are at an increased risk for acquiring HCV either because of their frequent exposure to blood from transfusions, or by exposure to HCV by nosocomial transmission, either during hemodialysis or at the time of renal transplantation. HCV infection has been established as a factor in reduced patient and graft survivals following renal transplantation (Meyers et al. 2003, Aroldi et al. 2005, Fabrizi et al. 2005, Pedroso et al. 2006, Einollahi et al. 2007).

The determination of alanine aminotransferase (ALT) levels as well as HCV RNA tests have been utilized in both the diagnosis and follow-up of patients with HCV infection. The latter also plays an important role in monitoring the virological response to antiviral treatment in addition to HCV genotype determination, which is a relevant predictive parameter of the response to treatment; it is thus used for selecting therapeutic regimens (Scott & Grech 2007). This virus is classified into six genotypes (1-6), each comprising multiple subtypes (designated a, b, c, etc). These genotypes have distinct geographical distributions. Furthermore, the genotyping of HCV isolates is a useful tool for establishing the source of outbreaks in hemodialysis centers and other nosocomial settings (Zein 2000, Simmonds et al. 2005).

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The prevalence of HCV infection has been reported to range from 7.2% among renal transplant recipients (RTP) in Switzerland to 63.8% in Saudi Arabia (Mitwalli et al. 2006, Fehr et al. 2003). In Brazil, a continental country, epidemiological data concerning HCV infection in renal transplant patients are still rare (Corrêa et al. 2003, Giordano et al. 2003), and little is known about the genetic diversity of HCV isolates in these patients (Giordano et al. 2003, Perez et al. 2003). In this study, the prevalence of HCV infection among RTP in Central Brazil was estimated and risk factors associated with HCV infection in this population were analyzed. In addition, ALT levels and HCV genotypes were determined in these patients.

PATIENTS, MATERIALS AND METHODS

Patients - This study was carried out in the Santa Casa de Misericórdia in Goiânia city, the largest renal transplantation unit in the state of Goiás (GO), Central Brazil. A pilot study found a prevalence of 16% for anti-HCV. In this investigation, the sample was calculated according to the size of the population (420 RTP), on the basis of an alpha error of 5%, a power of 80%, an expected HCV prevalence of 16% and a precision of 3%. In accordance with these data, the minimum sample size necessary would be 243 patients. In 2004, 255 renal transplant patients were interviewed regarding sociodemographic characteristics and possible risk factors for HCV infection using a standardized form. Blood samples were collected from all individuals and sera were stored at -20ºC. The sample population had an age range of 17 to 75 years, with an average of 41.3 years. Most patients were males (60%), born in GO (70%), married (60%), had received a low level of education (less than 9

years of formal education) (65%), and reported income between two and five Brazilian minimum wages/month (~US$ 330-825) (55%). Concerning the etiology of chronic renal failure, the main identified causes were chronic glomerulonephritis (n = 56) and hypertensive nephrosclerosis (n = 52), followed by chronic pyelonephritis (n = 15), polycystic kidney disease (n = 11), diabetes mellitus (n = 8), renal litiasis (n = 5), and interstitial nephropathy (n = 1); the etiology could not be determined in 107 patients. All transplantations were performed between 1982 and 2003. These patients received a kidney from either living (70%) or deceased (30%) donors. All patients were recipients of a first renal transplant and were using immunosuppressive drugs such as prednisone (pred), cyclosporine (cyc), mycophenolate mofetil (mmf), azathioprine (aza), tacrolimus and sirolimus (the main schemes used were a combination of pred + cyc + mmf in 31.8%, and pred + cyc + aza in 29.8%).

HCV serological and molecular tests - Serum samples were screened by ELISA for the presence of anti-HCV antibodies (INNOTEST HCV Ab III, Innogenetics NV, Belgium). Positive samples were retested for confirmation using a line immunoassay (INNO-LIA HCV Ab III, Innogenetics). All samples were subjected to RNA extraction, reverse transcription, and a nested PCR with primers complementary to the conserved area of the 5'NC region of HCV, essentially as described by Ginabreda et al. (1997). HCV genotyping was carried out for all HCV-RNA-positive samples. A line probe assay (Inno-LiPA HCV II, Innogenetics) was used to determine the genotype of the amplicons of the 5' NC region according to the procedure described by the manufacturer.

ALT - Serum ALT levels were determined monthly by an automated kinetic method within a period of three months from the date of the interview and blood sample collection for this study. The mean of the ALT levels from each patient was calculated. ALT levels below 40 IU/L were considered normal.

Statistical analysis - The prevalence and 95% confidence intervals (95% CI) were calculated. Chi-square and Fisher tests were used to evaluate risk factors associated with HCV infection (defined as positive for anti-HCV and/or HCV RNA). Statistical significance was assessed at the 0.05 probability level in all analyses. Risk factors, first estimated by odds ratio in univariate analysis, were analyzed subsequently by multiple logistic regression to identify possible confounders. Statistical analyses were performed using the Epiinfo program, version 2000 package developed by the Centers for Disease Control and Prevention (Atlanta, USA).

Ethical issues - The study protocol was approved by the Ethics Committee of the Santa Casa de Misericórdia, and written informed consent was obtained from all participants.

RESULTS

Of the 255 RTP, 43 were found to be anti-HCV positive by ELISA. Of these, 39 were subsequently confirmed as being positive by LIA and four were indeterminate, resulting in an anti-HCV prevalence of 15.3%. HCV RNA was detected in 32/255 (12.5%) patients: 30 were positive for anti-HCV and two were negative. Thus, 41 patients were positive for either anti-HCV and/or HCV RNA, giving an overall HCV infection prevalence of 16.1% (95% CI: 11.9-21.3). Twenty-eight samples were genotyped as genotype 1, subtypes 1a (62.5%) and 1b (31.3%), and 2 samples (6.2%) as genotype 3, subtype 3a (Table I).

Fig. 1 shows the mean of ALT levels from each RTP. The majority of the HCV-positive, indeterminate and negative patients had normal ALT levels. Of the HCV-positive patients, only 12.2% (5/41) had an abnormal ALT profile that was almost 1.5 times the upper normal limit (40 IU/L) or slightly greater.

Univariate analysis of risk factors showed that a history of blood transfusions without screening for anti-HCV, the length of time spent on hemodialysis, and renal transplantation before 1994 were all associated with HCV-positivity in the studied population. In a multivariate analysis, renal transplantation before 1994 (adjusted OR = 20.2; 95% CI: 4.6-87.5) and a length of time on hemodialysis of more than three years (adjusted OR = 11.9; 95% CI: 2.3-61.8) were strongly associated with HCV infection. A history of blood transfusions without anti-HCV screening (adjusted OR = 3.7; 95% CI: 1.5-9.5) also remained an independent risk factor for HCV positivity when confounders were taken into account (Table II).

TABLE I

Prevalence of hepatitis C virus (HCV) markers among 255 renal transplant recipients in the state of Goiás, Central Brazil

	Positive	
Markers	n (%)	95% CI
Anti-HCV		
ELISA	43 (16.9)	12.5-22.0
LIA	39 (15.3)	11.1-20.3
RNA-HCV	32 (12.5)	8.7-17.3
Anti-HCV and/or RNA-HCV	41 (16.1)	11.9-21.3
Genotypes		
1a	20 (62.5)	43.7-78.3
1b	10 (31.3)	16.7-50.1
3a	2 (6.2)	1.1-22.2

CI: confidence interval.

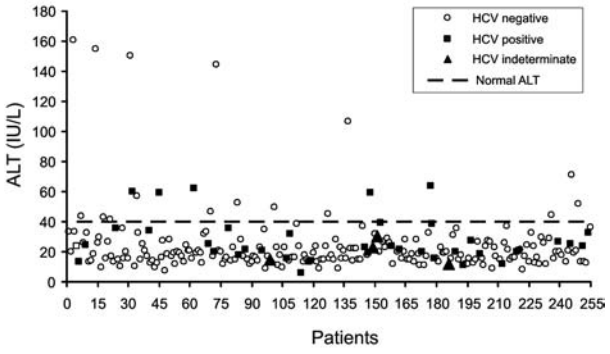


Fig. 1: mean of alanine aminotransferase (ALT) levels in hepatitis C virus (HCV) positive, negative and indeterminate renal transplant patients, Central Brazil.

TABLE II

Risk factors associated with hepatitis C virus (HCV) infection in renal transplant recipients in the state of Goiás, Central Brazil

Risk factor	HCV Pos./Total[a] (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Transfusion of blood screened for anti-HCV[b]			
Yes	17/152 (11.2)	1.0	1.0
No	22/50 (44.0)	6.2 (2.7-14.2)	3.7 (1.5-9.5)
Length of time on hemodialysis[c]			
< 1 year	3/58 (5.2)	1.0	1.0
1- 3 years	11/105 (10.5)	2.1 (0.5-10.2)	1.6 (0.3-8.5)
> 3 years	27/72 (37.5)	11.0 (2.9-48.9)	11.9 (2.3-61.8)
Renal transplantation before 1994			
No	27/234 (11.5)	1.0	1.0
Yes	14/21 (66.7)	15.0 (5.0-46.2)	20.2 (4.6-87.5)

a: anti-HCV indeterminate patients (n = 4) were excluded; *b*: the denominator represents the number of patients who received blood transfusion; *c*: the denominator represents the number of patients who received hemodialysis; CI: confidence interval, adjusted for gender, age, transfusion of blood not screened for anti-HCV, length of time on hemodialysis and renal transplantation before 1994; OR: odds ratio.

DISCUSSION

The present study is the first concerning HCV infection in RTP in Central Brazil. The prevalence of HCV infection of 16.1% found in these patients in GO is almost 12 times greater than that observed among local blood donors (1.4%) (Martins et al. 1994). Nevertheless, relative to other Brazilian renal transplant recipient populations, this prevalence is lower than those reported in the cities of Porto Alegre (33%) (Corrêa et al. 2003) and Campinas (54%) (Giordano et al. 2003); it was, however, in accordance with those carried out in other countries, where rates have been found to range from 7.2% to 63.8% (Fehr et al. 2003, Mitwalli et al. 2006). In addition, the prevalence among RTP we determined is similar to that reported for hemodialysis patients in GO (16.4%) (Carneiro et al. 2007). These data demonstrate that HCV infection is a significant problem among RTP.

The HCV genotype distribution in the studied population, with the predominance of subtype 1a, followed by 1b and 3a, is similar to that reported in RTP in São Paulo city, where genotypes 1a (68%), 1b (27%) and 3 (5%) were found (Perez et al. 2003). In addition, genotype 1, subtype 1a was also predominant in RTP in Campinas (Giordano et al. 2003) and in hemodialysis patients in the cities of São Paulo (Moreira et al. 2003, Perez et al. 2003), Recife (Albuquerque et al. 2005) and Campo Grande (Freitas et al. 2008), and in the states of Tocantins (Souza et al. 2003) and GO (Espírito-Santo et al. 2007). These data suggest that this subtype is more likely to disseminate in a hemodialysis environment. It could also be more adapted to situations of immunosuppression, or it may have been selected from a mixed infection (Perez et al. 2003).

In HCV-positive RTP, only 12.2% had ALT levels above normal. Moreover, only discrete changes in ALT levels were observed in these patients, who had 1.5 times the upper normal limit or greater. Similarly, other studies found normal ALT levels in the majority of the renal transplant patients evaluated (Fabrizi et al. 2001, Perez et al. 2005, Unal et al. 2006, Contreras et al. 2007), but the mechanism underlying this phenomenon remains unknown. However, immunosuppressive drugs, such as pred and cyc, could be responsible for reduced ALT levels in RTP (Morales et al. 1993, Fong et al. 1994). In addition, low ALT levels can be related to uremia (Yasuda et al. 1995).

Studies have also indicated that blood transfusion before screening for anti-HCV is associated with HCV infection in hemodialysis and RTP (Albuquerque et al. 2005, Aroldi et al. 2005, Pedroso et al. 2006, Carneiro et al. 2007, Fabrizi et al. 2007). In fact, patients who had received transfusions of blood not screened for anti-HCV in the past presented a 3.7-fold (95% CI: 1.5-9.5) greater risk for HCV infection compared to those who were transfused with screened blood. In addition, a length of time on hemodialysis of more than three years (adjusted OR = 11.9; 95% CI: 2.3-61.8) and renal transplantation before 1994 (adjusted OR = 20.2; 95% CI: 4.6-87.5) were strongly associated with HCV infection. As observed elsewhere, while the first risk factor reinforces the hemodialytic environment as source of HCV dissemination in this population (Morales & Campistol 2000, Corrêa et al. 2003, Giordano et al. 2003, Aroldi et al. 2005, Pedroso et al. 2006, Gheith et al. 2007), the latter clearly shows the impact of anti-HCV screening in candidates for blood and organ donations (Abbott et al. 2005, Carneiro et al. 2005, Fabrizi et al. 2007). Among the HCV-positive patients studied, renal transplantation was performed in 16 (39%) subjects between 1982 and 1993, prior to the beginning of anti-HCV screening for candidate blood and organ donors/recipients in Brazil. Thus, these patients could have been infected by either blood transfusion or by nosocomial transmission during hemodialysis or at the time of renal transplantation. The remaining patients (n = 25, 61%) were anti-HCV positive before transplantation, suggesting that the most probable risk factors for these RTP were blood transfusion and hemodialysis treatment.

In conclusion, although the prevalence of HCV infection is high among RTP in GO, Central Brazil, the pattern of ALT levels is normal in most of these patients. The findings of risk factor analysis in the studied population emphasize the importance of public heath strategies such as anti-HCV screening for candidate blood and organ donors in addition to the stricter adoption of hemodialysis-specific infection control measures. The present study also demonstrates that HCV genotype 1 (subtype 1a) is predominant in this population.

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