GB Virus C/Hepatitis G Virus Infection in Dialysis Patients and Kidney Transplant Recipients in Central Brazil

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In order to investigate the prevalence of GB virus C (GBV-C)/hepatitis G virus (HGV) infection in dialysis patients and kidney transplant recipients in Central Brazil and also to analyze the virus genotypes distribution, a total of 123 patients including 98 on hemodialysis, 13 on continuous ambulatory peritoneal dialysis treatment, and 12 who received kidney transplantation were interviewed in one unit of dialysis treatment in Goiânia city. Blood samples were collected and serum samples tested for GBV-C/HGV RNA by polymerase chain reaction. Genotypes were determined by restriction fragment length polymorphism (RFLP) analysis. Eighteen samples were GBV-C/HGV RNA-positive, resulting in an overall prevalence of 14.6% (95% CI: 9.2-21.7). A high positivity for GBV-C/HGV RNA was observed in patients who had received kidney transplant (16.7%), followed by those on hemodialysis (15.3%), and peritoneal dialysis (7.7%). RFLP analysis revealed the presence of genotypes 1, 2, and 3 of GBV-C/HGV; more precisely, 9 (50%) samples were found belonging to the 2b subtype, 4 (22%) to the 2a subtype, 3 (17%) to genotype 1, and 2 (11%) to genotype 3. The present data indicate an intermediate prevalence of GBV-C/HGV infection among dialysis patients and kidney transplant recipients in Central Brazil. Genotype 2 (subtype 2b) seems to be the most prevalent GBV-C/HGV genotype in our region.

Key words: hepatitis G virus - GB virus C - dialysis - kidney transplant - Central Brazil

GB virus C (GBV-C) and hepatitis G virus (HGV) are different isolates of the same virus which were identified by two independent research groups (Simons et al. 1995, Linnen et al. 1996). Although it was initially identified as possible aetiological agent of viral hepatitis in humans, and despite its similarity in genome structure with hepatitis C virus (HCV), it is unlikely that GBV-C/HGV is a cause of liver diseases (Zhu et al. 2003). Nevertheless, several recent studies have addressed that patients infected with human immunodeficiency virus (HIV) showed a beneficial effect of co-infection with GBV-C/HGV. The benefit is demonstrated by lower progression to acquired immunodeficience syndrome (AIDS) and prolonged survival time after its development (Yeo et al. 2000, Tillmann et al. 2001, Xiang et al. 2001, Muerhoff et al. 2003).

GBV-C/HGV is an envelope positive-stranded RNA virus with a genome of about 9.4 kb belonging to the *Flaviviridae* family. At least five major genotypes of this virus have been proposed by sequence analysis of the 5' non-coding region (5' NCR) or E2 gene. Genotype 1 is found mainly in West Africa, while genotype 2 is the most common in the US and Europe. Genotype 3 is frequently observed in parts of Asia (Muerhoff et al. 1996, 1997, Mukaide et al. 1997, Okamoto et al. 1997, Katayama et al. 1998). Recent studies have identified genotype 4 in samples from Myanmar, Vietnam, and Indonesia (Naito et al. 2000, Handajani et al. 2000), and genotype 5 in South Africa (Tucker et al. 1999, Tucker & Smuts 2000).

GBV-C/HGV is transmitted mainly by parenteral route. Thus, patients with chronic renal failure are at high risk of acquiring this virus because they need frequent blood transfusions and undergo medical procedures that accompany bleeding. There are conflicting data about GBV-C/HGV RNA prevalence in chronic hemodialysis patients; studies showed rates ranging from 3.1%, in Japan (Masuko et al. 1996), to 57.5%, in France (de Lamballerie et al. 1996). On the other hand, few reports are available in patients on peritoneal dialysis treatment or in kidney transplant recipients. In Brazil, data concerning GBV-C/HGV infection in hemodialysis patients are still rare (Lampe et al. 1997, Watanabe et al. 2003), and little is known about the genetic diversity of GBV-C/HGV strains circulating in our country (Gallian et al. 1998, Lampe et al. 1998, Oliveira et al. 2002). In the present study we aimed at knowing the prevalence of GBV-C/HGV RNA in hemodialysis patients in Central Brazil and also to analyze the virus genotypes distribution. Moreover, we studied the GBV-C/HGV epidemiology in an additional group of patients on peritoneal dialysis treatment and kidney transplant recipients.

MATERIALS AND METHODS

Subjects - This study was carried out in one unit of dialysis treatment in Goiânia city (1,000,000 inhabitants), Central Brazil. Between January and March 2000, all 123 patients including 98 on hemodialysis, 13 on continuous ambulatory peritoneal dialysis treatment, and 12 who received kidney transplantation were asked to take part of

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this study, and informed consent was obtained from all of them. The study was approved by the Ethical Committee of the Federal University of Goiás.

A standardized form was used to collect sociodemographic data as number of previous blood transfusions, length of time on dialysis, kidney transplantation, acupuncture, tattooing, intravenous drug use, dental procedure with non-licensed dentist, multiple sex partners, sexually transmitted diseases, and possible household contact with hepatitis.

The studied population ranged in age from 16 to 86 years (average 52.5 years). Sixty (48.8%) were females and 63 (51.2%) were males. The aetiology of chronic renal failure was diabetic nephropathy (n = 30), hypertension and nephrosclerosis (n = 25), chronic glomerulonephritis (n = 17), polycystic kidney disease (n = 11), pyelonephritis (n= 5), renal diseases of unknown aetiology (n = 27), and others (n = 8).

Serological tests - Blood samples were collected from all patients and sera were stored at –20ºC. They were screened for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), hepatitis B surface antibody (anti-HBs), and hepatitis C antibody (anti-HCV) by enzyme-linked immunosorbent assays (ELISA) (Abbott Laboratories, US). All samples were also tested for alanine aminotransferase (ALT) levels by a colorimetric method (Dolles Laboratory, Brazil).

Detection of GBV-C/HGV and HCV RNA - All samples were submitted to RNA extraction, reverse transcription, and a nested PCR with primers complementary to the conserved area of the NS5 region of the GBV-C/HGV genome, essentially as described by Lampe et al. (1997). For HCV RNA detection, primers complementary to the conserved area of the 5' NCR of HCV were used (Ginabreda et al. 1997).

GBV-C/HGV genotyping - GBV-C/HGV RNA-positive samples were amplified by PCR using primers complementary to the 5' NCR. Genotypes were determined by means of RFLP method (Quarleri et al. 1999). Briefly, amplicons were initially cleaved with *Hin*f I, and depending on the restriction pattern observed, a second digestion was performed either with *Aci* I or *Aat* II. Restriction fragments were resolved in ethidium bromide-stained 3% agarose gels.

Statistical analysis - Prevalence and 95% confidence intervals (CI) were calculated. Chi-square test or Fisher's exact test were performed to evaluate the distribution of characteristics associated with GBV-C/HGV infection. Statistical significance was assessed at the 0.05 probability level in all analyses. Statistical evaluations were performed using Epiinfo 6.0 program developed by the Centers for Disease Control and Prevention (Atlanta, GA).

RESULTS

As shown in Table I, an overall prevalence of 14.6% (95% CI: 9.2-21.7) was found for GBV-C/HGV infection in patients with chronic renal failure. A higher positivity for GBV-C/HGV RNA was observed in patients who had received kidney transplant (16.7%), followed by those on hemodialysis (15.3%) and peritoneal dialysis (7.7%).

There was no association between GBV-C/HGV RNA and age, sex, history of blood transfusion, length of time on dialysis ($p > 0.05$). Analysis of the characteristics of GBV-C/HGV RNA-positive patients is shown in Table II. These individuals ranged in age from 23 to 71 years. Eleven (61.1%) were men. The majority of them was on hemodialysis and had less than one year of treatment. Two subjects received kidney transplant. Only one patient was on peritoneal dialysis. Almost 90% of these infected patients had history of blood transfusion. Four (22.2%) of the GBV-C/HGV RNA-positive patients were co-infected with HCV (anti-HCV and HCV RNA positive). Two of them, were also positive for anti-HBc and anti-HBs markers. All but one GBV-C/HGV infected individuals had normal ALT levels.

All 18 GBV-C/HGV RNA-positive samples were genotyped by RFLP pattern. It was observed that 3 (17%) were of genotype 1, 13 (72%) of genotype 2, and 2 (11%) of genotype 3. In samples identified as genotype 2, 4 (22%) belonged to subtype 2a and 9 (50%) to subtype 2b (Figure).

DISCUSSION

The present study represents the first investigation of GBV-C/HGV infection in patients with chronic renal failure in Goiânia city, Central Brazil. A high overall prevalence (14.6%) was found, when compared to that observed in blood donors (7.1%) from the same region (Oliveira et al. 2002).

A positivity rate of 7.7% was detected on patients on peritoneal dialysis, which was similar to that reported in local blood donors (Oliveira et al. 2002). These patients are on continuous ambulatory peritoneal dialysis, a home therapy that reduces the risk for nosocomial transmission. As the number of patients studied was small, further investigations are necessary to provide more information on the epidemiology of GBV-C/HGV infection among patients on peritoneal dialysis treatment.

TABLE I

Prevalence of GB virus C/hepatitis G virus RNA in dialysis patients and kidney transplant recipients in Central Brazil

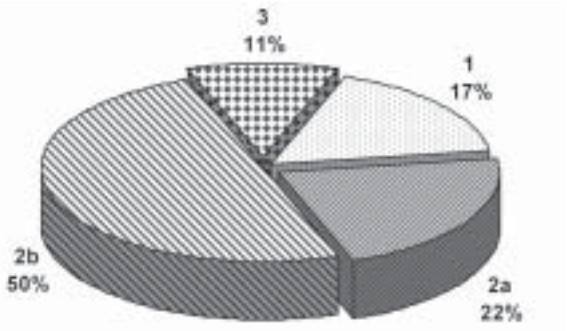
Renal replacement therapy	n	Prevalence (%)	95% CI
Peritoneal dialysis	13	7.7	0.4-32.5
Hemodialysis	98	15.3	9.2-23.5
Kidney transplantation	12	16.7	2.9-45.1
Total	123	14.6	9.2-21.7

CI: confidence interval

TABLE II
Characteristics of 18 GB virus C/hepatitis G virus RNA-positive patients with chronic renal failure

Patient	Age (yr)	Gender	Nr of transfusions	Renal therapy		HBV	HCV	ALT
				Type	Duration			
1	38	M	1	HD	< 1year	-	-	25
2	36	M	> 10	TX	> 3 years	+	+	73
3	23	M	8	PD	< 1year	-	-	6
4	48	M	2	HD	1- 3 year	-	-	22
5	59	M	9	HD	< 1year	-	-	28
6	67	M	8	HD	< 1year	-	-	16
7	41	M	4	HD	< 1year	-	-	18
8	59	M	8	HD	1- 3 year	-	-	40
9	52	F	3	HD	1- 3 year	-	+	14
10	33	M	1	TX	> 3 years	+	+	19
11	63	M	0	HD	< 1year	-	-	5
12	66	F	0	HD	< 1year	-	-	32
13	47	M	3	HD	< 1year	-	-	23
14	28	F	3	HD	< 1year	-	-	10
15	51	F	3	HD	< 1year	-	-	27
16	69	F	1	HD	< 1year	-	-	14
17	70	F	3	HD	< 1year	-	+	21
18	71	F	3	HD	< 1year	-	-	14

TX: kidney transplantation; PD: peritoneal dialysis; HD: hemodialysis; HBV: hepatitis B virus; HCV: hepatitis C virus; ALT: alanine aminotransferase



Frequency of GB virus C/hepatitis G virus genotypes in patients with chronic renal failure in Central Brazil.

Regarding hemodialysis patients, GBV-C/HGV RNA prevalence rates range from 3.1% to 15% in Japan (Masuko et al. 1996, Shibuya et al. 1998), from 11.5% to 20% in the US (Bastani et al. 1998, Medina et al. 1998) and from 6% to 57.5% in Europe (de Lamballerie et al. 1996, Fabrizi et al. 1997). It has also been reported a high rate (55%) in Indonesia (Tsuda et al. 1996). In South America, a prevalence of 17.6% was observed in Argentina (Fernandez et al. 2000). In addition, rates of 15% and 12.8% were detected in Rio de Janeiro and Ribeirão Preto, Brazil (Lampe et al. 1997, Watanabe et al. 2003), which were similar to the prevalence of 15.3% found in Goiânia. Thus, GBV-C/HGV prevalence infection in Brazilian hemodialysis patients could be placed in an intermediate position.

In the present study, the frequency of detectable GBV-C/HGV RNA in serum was higher in kidney transplant recipients (16.7%) than among hemodialysis patients. Similar results were observed in Germany (15.2% x 9.7%) (Szabo et al. 1997) and Italy (6% x 3.6%) (Fabrizi et al. 1997). It is possible that the immunosuppressive regimen used may enhance GBV-C/HGV replication, either directly or indirectly through its activity on the host's immune system. In addition, this infection may occur during dialysis treatment, at the time of or after kidney transplantation, or due to blood transfusions (Fabrizi & Martin 1999). However, as the number of kidney transplant recipients studied was small, more studies are needed to confirm this observation.

These differences in the prevalence of GBV-C/HGV infection may also be explained by epidemiological variations, including the size and the clinical features of the patients, methods of detection of GBV-C/HGV RNA (especially the use of different primers), duration of dialysis treatment, infection control measures practiced in the dialysis unit, and geographic factors (Hassan & Bastani 2000).

Almost 90% of the GBV-C/HGV RNA-positive patients had history of blood transfusion. This finding rate was in agreement with the studies of Masuko et al. (1996) and Fabrizi et al. (1997) who found a history of transfusion in 75% and 80% of hemodialysis patients infected with GBV-C/HGV, respectively. On the other hand, the GBV-C/HGV RNA was detected in two hemodialysis patients with no transfusion history and without any other risk of parenteral exposure, supporting the hypotesis of nosocomial transmission.

Prevalence rates of 26% and 37.4% were found for hepatitis B and C, respectively (data not shown). Of the 18 GBV-C/HGV RNA-positive patients, 4 (22.2%) were co-infected by HCV. Among them, two were positive for anti-HBc and anti-HBs. In addition, no association was found between GBV-C/HGV RNA positivity and serum ALT lev-

els. These data indicate that this virus has been independently widespread in patients with chronic renal failure in Central Brazil.

All GBV-C/HGV RNA-positive samples were genotyped by RFLP. Genotype 2 (72%) was the most prevalent, followed by genotype 1 (17%), and 3 (11%). Genotypes 1 and 2 were detected by Gallian et al. (1998) in a rural population in the state of Ceará and also by Lampe et al. (1998) in individuals living in Rio de Janeiro. The presence of both genotypes in Brazil is likely to reflect the European and African origin of the population. In this study, genetic diversity of genotype 2 revealed the predominance of subtype 2b in patients with chronic renal failure. Recently, this subtype was also dominant (53.8%) in blood donors in Goiânia, it was followed by subtype 2a (28.6%) and genotype 1 (17.6%) (Oliveira et al. 2002). On the other hand, 2 (11%) patients with chronic renal failure were infected with genotype 3. This is the first related of the occurrence of genotype 3 in Brazil. Although this genotype was not detected previously in our country (Gallian et al. 1998, Lampe et al. 1998, Oliveira et al. 2002), it was observed in Colombia (Tanaka et al. 1998), Argentina (Quarleri et al. 1999), and Bolivia (Konomi et al. 1999). Probably, its presence in South America reflects the immigration from Asia, where genotype 3 is prevalent (Tucker & Smuts 2000).

In conclusion, our data point out an intermediate endemicity of GBV-C/HGV infection in dialysis patients and kidney transplant recipients in Central Brazil. The presence of strains belonging to genotypes 1, 2a, 2b, and 3 reveals a large genetic diversity of GBV-C/HGV circulating in Brazil.

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