ARTIGO ORIGINAL

HEPATITIS B AND C INFECTIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

INFECÇÃO PELOS VÍRUS DA HEPATITE B E C EM PACIENTES COM LÚPUS ERITEMATOSO SISTÊMICO

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SUMMARY

OBJECTIVE: determine the seroprevalence of HBV and HCV in SLE patients attended at the University Hospital from October 2009 to July 2010. **METHOD**: the serological markers for HBV (HBsAg, anti-HBc total and anti-HBs) and HCV (anti-HCV) were investigated by enzyme-linked immunosorbent assay (ELISA). In HBsAg and/or anti-HBc total and anti-HCV positive samples were analyzed for HBV-DNA and HCV-RNA by PCR. **RESULTS**: one hundred and sixty-nine SLE patients were studied and the prevalence of anti-HBc total was 10.1% (17/169) and all were negative for HBV-DNA. The anti-HCV was present in 1.8% (3/169) and only one was HCV-RNA positive, presenting a viral load of 212.000 copies/mL. **CONCLUSIONS**: considering the absence of data on HBV in SLE patients in Brazil, the prevalence found in this study was high when compared to that reported in the general population in the same geographical area. With regard to the seroprevalence of HCV, it was lower than that observed in other Brazilian SLE patients.

KEY WORDS: Systemic lupus erythematosus, hepatitis B, hepatitis C and prevalence.

INTRODUCTION

Infectious diseases are considered the second leading cause of death and account for 30% to 50% of the morbidity and mortality in systemic lupus erythematosus (SLE) patients[1]. In these individuals, there is an increased risk of infection because of immune disorders associated with SLE, such as the presence of leukopenia, immunosuppressive treatment and hospitalization[2]. Although bacteria are the most common etiological agents, viruses, fungi and protozoa have also been reported in these patients[3].

Various genetic, environmental, and hormonal factors have been implicated to justify the diversity of clinical presentation and course of SLE. Clinical and laboratory evidences suggest that persistent viral infections could lead to a sustained polyclonal activation of B cells in individuals with genetic predisposition. Thus, hepatitis B and C could facilitate the emergence or modify the natural history of SLE[4].

The infection by the hepatitis B virus (HBV), classified in the genus *Orthohepadnavirus*, family *Hepadna-*

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viridae[5], can also trigger an autoimmune reaction, because DNA polymerase of the virus has high homology with four nuclear and two smooth muscle human proteins[6]. Furthermore, autoantibodies against proliferating cell nuclear antigen were detected in chronic hepatitis B patients and were also present in SLE patients, but were absent in other autoimmune diseases[7].

Previous studies have demonstrated the presence of HBsAg in renal biopsies of lupus nephritis patients by hemagglutination[8]. Surveys in Asia reported HBsAg seroprevalence ranging from 1% to 3.5% in SLE patients[9]. In Brazil, there are no data on the seroprevalence of this infection in these patients.

Moreover, the virus hepatitis C (HCV) may be related with cryoglobulinemia and Sjögren's syndrome. Chronic infection by this virus can mimic an SLE-like syndrome with several clinical manifestations being HCV considered as a possible etiological agent in the development of SLE[10]. Worldwide, the HCV seroprevalence in SLE ranges from 1% to 14,1%[11,12].

There are therapeutic implications in the treatment of HCV infection and autoimmune diseases. The therapy of SLE, based on corticosteroids and immunosuppressants, can lead to an increase in HCV replication, accentuating hepatocytes infection, resulting in more severe and fulminant hepatitis[13].

However, interferon used routinely in the treatment of hepatitis C can precipitate or exacerbate a variety of autoimmune diseases in addition to causing side effects, such as arthralgia, leukopenia and thrombocytopenia, which is common in some rheumatic diseases, such as SLE, thus making it difficult to evaluate patients[14].

Rare are studies showing the clinical course of rheumatic diseases in HCV patients. Perlemuter et al. (2000)[15], analyzing SLE patients infected with HCV, observed a severe clinical picture with the involvement of multiple organs, in which a therapy with corticosteroids does not alter the course of chronic hepatitis.

Thus, considering the paucity of data in Brazil and the clinical and therapeutic implications of these infections in these individuals, this study aimed to determine the prevalence of HBV and HCV markers in SLE patients.

METHOD

Study site and patient selection

We enrolled 169 patients who attended the Rheumatology Outpatient Clinic at the Clinics Hospital, Federal University of Pernambuco, Recife, Brazil, from October 2009 to July 2010.

The study included patients of both sexes that presented at least four of the eleven diagnostic criteria for SLE as proposed by the American College of Rheumatology[16].

Informed consent was obtained from all participants of this study. The study protocol was approved by the Ethics Committee of Research from the Health Sciences Center, Federal University of Pernambuco, Brazil (research protocol number: 354/08).

Data Collection

Through a standardized questionnaire, data were collected from each patient, including gender, age, race, education, income and risk factors for transmission of HBV and HCV: history and period (before or after 1993) of blood transfusions, history of hemodialysis, history of surgery, history of transplant of organ/tissue, history of dental treatment, presence of tattoos and/or piercing and a history of injecting and/or inhalable drug use.

Collection of blood samples and serology

Blood samples (10 mL) were collected and the serum obtained by centrifugation and stored at -80°C until the serologic and molecular tests were performed in the Division of Virology, Laboratory of Immunopathology Keizo Asami (LIKA), UFPE.

Serological markers for HBV and HCV were investigated by enzyme-linked immunosorbent assay (ELISA) of the third generation, according to the manufacturer's instructions. All samples were tested for anti-HBc total Monolisa Plus Assay (Bio-Rad Laboratories), anti-HBs Monolisa Plus Assay (Bio-Rad Laboratories), Hepatitis B HBsAg (Wiener Laboratories). The presence of anti-HCV was investigated using the Hepatitis C anti-HCV (Wiener Laboratories).

All samples that were anti-HCV positive were retested by Microparticles Enzyme Immunoassays (MEIA) using AxSYM HCV 3.0 kit (Abbott, Max-Plack-Ring 2) according to the specifications of the manufacturer.

Detection of viral genetic material

The samples HBsAg and/or anti-HBc total positive were subjected to DNA extractions using Brazol® (LGC Biotecnologia), including 3 mg/mL of glycogen. The S sequence of HBV was amplified according to published protocol [17] by qualitative nested PCR assay with a sensitivity of 300 copies/mL on THERM 1000/Maxygene thermocycler® (Axygen).

The anti-HCV positive samples were extracted by the QIAamp Viral RNA Mini Kit ® (Qiagen) and followed by quantitative real-time PCR assay, using the One-Step RT-PCR Kit ® (Qiagen), according to the manufacturer's instructions. The reaction, with a sensitivity of 20 IU/mL, was performed on a CFX-96 Real-Time Thermal Cycler ® (Bio-Rad Laboratories).

Statistical analysis

Initially, descriptive analyses of socio-demographic and clinical characteristics of patients were performed. The 95% confidence interval of the respective prevalence of each serological marker was set up by the exact binomial.

The association between the positivity of serological markers and risk factors was verified using the odds ratio (OR) and the 95% confidence interval.

The Chi-square (χ^2) and Fischer's exact test were used, when appropriate, to determine whether the associations were statistically significant. Statistical significance was considered for *p*-values <0.05. In order to monitor the confusion effect, all of these associations were adjusted for age.

Data from each patient were analyzed using the Epi Info software version 6.04d.

RESULTS

Among the patients, 93.5% (158/169) were females. The average age was 39 ± 10.9 years. In respect to the age group distribution, 55.6% (94/169) were <40 years. As for skin color, 37.9% (64/169) of patients were black.

Household income ranged between 1 and 3 basic wages in 53.6% (91/169). The most common educational level was primary school incomplete in 34.9% (59/169) and 49.7% (84/169) of patients came from rural towns in the state of Pernambuco, Brazil.

At the time of the study, all patients were using varying doses of corticosteroids and immunosuppressive agents, depending on the severity and disease activity.

The seroprevalence of HBV was 10.1%, considering the positivity of anti-HBc total (Table 02). The qualitative nested PCR assay did not detect HBV-DNA in any of the 17 samples with anti-HBc total positive.

On the other hand, the prevalence of anti-HCV was 1.8%, based on ELISA and confirmed by MEIA (Table 02). The quantitative real-time PCR assay detected the HCV-RNA in one sample with 212.000 copies/mL; therefore, 0.6% was the prevalence.

TABLE I – Distribution of SLE diagnostic criteria proposed by the American College of Rheumatology in patients investigated.

SLE criteria	Number (n = 169)	Frequency (%)
Malar rash	101	59.8
Chronic cutaneous lesions (discoid)	38	22.4
Photosensitivity	122	72.2
Oral ulcers/nasopharyngeal	25	14.8
Non-erosive arthritis	108	63.9
Pleuritis/pericarditis	17	10.1
Renal disorder[a]	55	32.5
Seizure/psychosis	14	8.3
Hematological disorder[b]	112	66.3
Immunological disorder[c]	51	30.2
ANA +, absent of drug-induced	147	87.0

FONTE: Protocolo de pesquisa. [a]proteinuria > 0.5g or cylinders; [b]hemolytic anemia with reticulocytosis and/or leukopenia (<4.000 cells) and/or thrombocytopenia (<100.000 platelets.); [c]anti-DNA and/or anti-Sm and/or VDRL positive.

TABLE II – Prevalence of HBV and HCV serological markers in SLE patients.

Serological markers	Positive	Prevalence (%), (95% CI[a])
HBV		
HBsAg alone	00	–
Anti-HBc total	17	10.1 (5.4 – 14.6)
Anti-HBc total alone	06	3.5 (1.3 – 7.6)
Anti-HBc total + Anti-HBs	11	6.5(3.3 – 11.3)
Anti-HBs alone	23	13.6 (8.8 – 19.7)
HCV		
Anti-HCV	03	1.8 (0.2 – 3.8)

FONTE: Protocolo de pesquisa. (95% CI[a]) = 95% confidence interval.

TABLE III – Association of variables related to SLE patients, according to positivity for anti-HBc total

Variable	Anti-HBc total				OR[a] (95% CI[b])	*p-value*
	Positive		Negative			
	n	%	*n*	%		
Gender						
Male	01	9.1	10	90.9	–	–
Female	16	10.1	142	89.9	1.21 (0.14 – 10.2)	0.855
Educational level						
Until to primary school	12	15.6	65	84.4	–	–
More than primary school	5	5.4	87	94.6	0.34 (0.11 – 1.02)	0.056
Family Income						
Less than one basic wage	09	13.2	59	86.8	–	–
One or more basic wages	08	8.9	93	89.9	0.65 (0.23 – 1.79)	0.407
Blood transfusion						
No	11	9.3	110	90.7	–	–
Yes	06	12.5	42	87.5	1.46 (0.50 – 4.23)	0.485
Hemodialysis						
No	16	9.9	148	90.1	–	–
Yes	01	20	04	80	3.13 (0.31 – 31.5)	0.333
Surgery						
No	02	3.9	50	96.1	–	–
Yes	15	12.8	102	87.2	3.16 (0.67 – 14.7)	0.142
Organ/tissue transplant						
No	16	9.3	151	90.7	–	–
Yes	01	50	01	50	7.04 (0.40 – 123.2)	0.181
Dental treatment						
No	10	13.1	114	86.9	–	–
Yes	07	15.6	38	84.4	1.39 (0.45 – 4.32)	0.560
Tattoo						
No	16	9.6	151	90.4	–	–
Yes	01	50.0	01	50.0	9.09 (0.53 – 155.0)	0.127
Piercing						
No	17	10.2	149	89.8	–	–
Yes	00	00	03	100	–	–
Drug injectable/inhalable						
No	17	10.2	151	89.9	–	–
Yes	00	00	01	100	–	–

FONTE: Protocolo de pesquisa. OR[a] = odds ratio; 95% CI[b] = 95% confidence interval

This serological marker was more frequently observed in females, representing 94.12% (16/17) of the positive cases for anti-HBc total. The variable age, which was grouped by age group, had a higher frequency of patients ≥ 40 years (OR: 2.52, 95% CI: 0.88 to 7.17, p = 0.083), however, this difference was no statistically significant.

The three patients with anti-HCV positive were female and the average age was 45.33± 10.69 years. All had received blood transfusion, one of them before 1993. One of these has submitted to dental treatment and another underwent hemodialysis due to chronic renal failure.

DISCUSSION

There are about 116 different types of autoantibodies in SLE directed against a variety of self-antigens. Many of these are known, whereas others still have uncertain immunopathogenesis. Therefore, the interpretation of serological assays in SLE patients requires caution, because cross-reactions can occur, generating false-positive results and, consequently, overestimation of the seroprevalence of these markers[18].

In this work, there were no positive results for HBsAg, however, 2.3% positivity for this marker was detected in China[9]. However, being a country of high endemicity for hepatitis B, these data may be influenced by a prevalence bias, increasing the frequency of serological markers of viral infection in SLE patients.

Besides the detection of HBsAg in the serological screening in SLE patients, is important search for anti-HBc total in order to evaluate the past exposure to HBV. Thus, the prevalence of anti-HBc total in this study was higher than that found by Ram et al. (2008)[19] (2.5%) in Colombia and Berkun et al. (2009)[20] (1.7%) in Israel. Nevertheless, a lower than that prevalence reported by Chng et al (1993)[21] (19.7%) in Singapore, which may also be influenced by the prevalence bias discussed earlier.

In Brazil, there are no data on the prevalence of HBV in SLE patients. However, the seroprevalence of anti -HBc in our study was higher than that found in the general population in the same geographical area (9.8%)[22]. The absence of HBV-DNA in anti-HBc total positive samples may have been influenced by the sensitivity of technique applied in this study because Tse et al (2009)[23] detected the HBV-DNA in lupus nephritis patients, using real-time PCR with a detection limit of 1.00×10^2 copies/mL.

Although no statistically significant association was detected between the presence of anti-HBc total and the risk factors analyzed, we obtained interesting findings. A higher frequency of this marker in women aged ≥ 40 years, which reflects the profile of patients treated at the study site.

In Brazil, an annual incidence of 8.7 cases per 100.000 population of SLE was reported, most often in women than in men (14.1:2.2)[24]. The largest number of cases aged ≥ 40 years may suggest a cumulative exposure to risk for HBV infection throughout life.

In our study, low education and low income in patients with anti-HBc total positive may be responsible for poor access to information about the modes of transmission of HBV, favoring viral contagion. Pereira et al. (2009)[22] reported that the lower level of education was a risk factor for HBV infection in northeastern Brazil.

There was a high frequency of history of surgery in patients with anti-HBc total positive. This finding can be explained because invasive procedures may be contributing factors to a greater exposure of these individuals to HBV infection. Additionally, being an illegal practice, the frequency of injecting drugs/inhaled use may be underestimated in our sample.

In Brazil, the only two studies evaluating the anti-HCV in SLE patients showed seroprevalence of 2.3% in Goiânia-GO[25] and 6.6% in Rio de Janeiro-RJ[18], and both are superior to that observed in this study. Such differences may be due to the different criteria for patient selection, use of different techniques in search of infection and variation in the prevalence of hepatitis C observed in different geographical regions.

Considering the results of quantitative real-time PCR assay, the prevalence of hepatitis C in SLE patients was 0.6%, similar to that found by Mercado et al (2005)[26]. The only sample positive PCR showed a viral load of 212.000 copies/mL. It should be noted that cases with a viral load >800.000 copies/mL predict worse clinical prognosis[27].

Blood transfusion represents one of the main risk factors for transmission of HBV and HCV. However, the time of transfusion is also important, since prior to November 1993 there was no serological screening for HCV in blood banks of Brazil. Meanwhile, due to the low frequency of anti-HCV in the samples, it was not possible to perform statistical analysis with this variable.

CONCLUSION

Considering the absence of data on HBV in SLE patients in Brazil, the prevalence found in this study was high when compared to that reported in the general population in the same geographical area. With regard to the seroprevalence of HCV, it was lower than that observed in other Brazilian regions. Therefore, it was emphasized the importance of research on these viruses in this population, since, patients with SLE are more susceptive to acquiring infections.

RESUMO

INFECÇÃO PELOS VÍRUS DA HEPATITE B E C EM PACIENTES COM LÚPUS ERITEMATOSO SISTÊMICO

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OBJETIVO: determinar a soroprevalência do HBV e HCV em pacientes com LES atendidos em Hospital Universitário de Outubro de 2009 a Julho de 2010. **MÉTODO**: pesquisaram-se os marcadores sorológicos para o HBV (HBsAg, anti-HBc total e anti-HBs) e HCV (anti-HCV) através de ensaio imunoenzimático (ELISA). Nas amostras HBsAg e/ou anti-HBc total e anti-HCV positivas foram pesquisados o HBV-DNA e HCV-RNA, pela reação em cadeia da polimerase (PCR). **RESULTADOS**: 169 pacientes lúpicos foram estudados e a prevalência do anti-HBc total foi 10,1% (17/169), com negatividade para o HBV-DNA. O anti-HCV esteve presente em 1,8% (3/169) dos pacientes e em apenas um o HCV-RNA foi positivo, com carga viral de 212.000 cópias/mL. **CONCLUSÃO**: em virtude da inexistência de outros trabalhos brasileiros que relatem a prevalência do HBV em pacientes lúpicos, verificou-se que a prevalência encontrada na pesquisa foi superior a da população local. Com relação à soroprevalência do HCV, esta foi menor do que a verificada em pacientes lúpicos brasileiros.

DESCRITORES: lúpus eritematoso sistêmico, hepatite B, hepatite C e prevalência

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Acknowledgements

The authors thank Prof. Dr. Angela Luiza Branco Pinto Duarte, Rheumatologist and Medical Director of Rheumatology Service, HC-UFPE, for permission to access the service and recruitment of patients, to Prof. Dr. Vera Magalhães da Silveira for collaboration with molecular biology tests for HCV.

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Recebido em 13.11.2013 – Aprovado em 04.12.2013