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Prevalence of non-organ-specific autoantibodies in a rural community from northeastern Brazil: a population-based study

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ABSTRACT

Non-organ-specific autoantibodies (NOSA) are well-recognized diagnostic markers of autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), but can also be observed in patients with viral hepatitis as well as in healthy subjects. The aim of this study was to evaluate the prevalence of NOSA in subjects living in a rural community in Brazil and to correlate their occurrence with the presence of liver disease. Seven hundred twenty-five apparently healthy subjects were randomly selected for assessment of antinuclear (ANA), anti-smooth muscle (SMA), antimitochondrial (AMA), anti-liver/kidney microsome type 1, and anti-liver cytosol type 1 antibodies. Subjects with those NOSA were evaluated for the presence of AIH, PBC, and viral hepatitis. Reactivities for all NOSA, SMA, ANA, and AMA were detected, respectively, in 14, 10, 4, and 0.1% of subjects, with a mean titer of 1:40. NOSA-positive subjects were significantly older and more frequently females. No correlation was observed between the occurrence of NOSA and PBC, AIH, or viral hepatitis. The prevalence of NOSA in Brazilians was 14%. They were usually low titer. NOSA were more frequently observed in females and older subjects and their presence was not correlated with the presence of AIH, PBC, or viral hepatitis.

1. Introduction

Non-organ-specifc autoantibodies (NOSA) may be observed in healthy subjects as part of the circulating repertoire of naturally occurring autoantibodies (Nabs). By contrast, several NOSA are also recognized as diagnostic markers of several autoimmune diseases [1]. In this regard, antinuclear (ANA), anti-smooth muscle (SMA), antimitochondrial (AMA), antisoluble liver antigen/liver-pancreas, anti-liver/kidney microsome type 1 (anti-LKM1), and anti-liver cytosol type 1 (anti-LC1) antibodies are employed, usually when titers are high, for the diagnosis of autoimmune liver diseases such as autoimmune hepatitis (AIH) [2] and primary biliary cirrhosis (PBC) [3]. However, they are also usually reported, particularly ANA and SMA, in 1–43% of apparently healthy subjects [4–8], in 14–66% [9] of patients with chronic hepatitis C, and in up to 90% of patients with autoimmune rheumatic diseases [10].

In contrast to disease-associated NOSA, Nabs were demonstrated to be germline-type antibodies, usually but not exclusively of immunoglobulin (Ig)-M isotype, and displaying multireactivity and low affinity for antigens [11–13]. They tend to cluster in females [7,14] and in the elderly [4,5,14–16]. By contrast, the occurrence of NOSA may be associated with an enhanced predisposition or even the presence of occult liver disease, as previously reported for AMA as well as for other NOSA, such as SMA, ANA, or anti-LKM1, respectively, for PBC [17,18], and for hepatitis C [9,19–26]. However, with the exception of AMA, data concerning the significance of those aforementioned NOSA as surrogate markers of autoimmune or viral diseases of the liver are lacking.

The purpose of the present study was to investigate the prevalence of NOSA in apparently healthy subjects from a rural community in the northeastern region of Brazil, to analyze their occurrence according to age and gender and the presence autoimmune liver diseases, as well as with evidence of past or present infection with hepatitis A (HAV), B (HBV), and C (HCV) virus.

2. Subjects and methods

2.1. Study population

This study was conducted in the district of Cavunge in Ipacaeta County in the semiarid region of Bahia, Brazil. The district had a population in 1999 of 2,049 subjects living in an area of 63.5 km^2 composed of 1 small village and several rural settlements scattered

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Table 1
Demographic features of subjects from Cavunge County[a]

N	725
% of the estimated population (n = 2,049)	35%
Female sex	369 (51%)
Age (years)	23 (0.2–91)
Age less than 16 years	39%
Place of residence	
Urban area	27%
Rural area	73%

[a]According to the 1999 census.[27]

in distinct farms. The demographic features and socioeconomic status of this population were previously described [27,28]. Briefly, its inhabitants were of highly admixed origin with differing percentages of Caucasoid, Negroid, and Amerindian ancestries. Most of the population lived in the rural settlements and were of poor socioeconomic status, with their main income derived from subsistence farming, relying on growing corn, beans, and cassava. Nearly half were illiterate.

The present study is part of a large project sponsored by the Brazilian National Health Foundation to perform a sentinel surveillance of HAV, HBV, and HCV infection in northeastern Brazil. According to the methodology employed, data collection was carried out in the Cavunge district at 2 different time periods.

In the first round of the project, carried out between 1999 and 2000, 1,800 subjects from Cavunge were enrolled to assess the prevalence of past or current infection with HAV, HBV, and HCV [28].

The second part of the project was carried out after 2004 to provide data collection for the prevalence of HAV, HBV, and HCV infection at least 5 years apart. During this time period, participants of this sentinel surveillance study were randomly invited by local agents from the Brazilian Family Health Program to be enrolled in the present investigation concerning the prevalence and significance of NOSA in the Cavunge District.

All subjects or their legal guardians who agreed to take part in the study had given their informed consent. Participants were required to state residence in the Cavunge District for more than 6 months.

Subjects who were unable to understand the principles of the study, such as those with chronic degenerative cerebrovascular disorders or psychiatric illness, were excluded from the current investigation, as well as all subjects less than 18 years of age without formal consent of their parents or legal guardians. Subjects who either required hospitalization or needed medical care in the previous 6 months were also excluded. Subjects less than 16 years of age were arbitrarily defined as children.

The study was approved by the Ethics Committee in Research of the Prof. Edgard Santos University Hospital and by the National Ethics Research Council.

Seven hundred twenty-five subjects were accepted to participate and underwent an interview to gather demographic data such as age, gender, and local address. Subsequently, blood samples were collected and processed into 1 to 4 aliquots of 1 mL of plasma, subsequently frozen, and stored at – 80°C. Only a 1-mL aliquot was separated for NOSA determination.

2.2. Autoantibodies

All subjects were screened for SMA, ANA, AMA, anti-LKM1, and anti-LC1 antibodies by indirect immunofluorescence (IIF) at a screening dilution of 1:40 on rat liver, kidney, and stomach tissue sections according to international criteria [29]. SMA reactivity was further characterized as vessel (V), glomeruli (G), and tubule (T) patterns according to Bottazzo et al. [30]. All SMA$^+$ subjects had their sera subsequently tested for antiactin (microfilament) reactivity by IIF at a screening dilution of 1:20 using the heat serum inactivation technique [31]. All sera were also tested for ANA on HEp-2 cells using a commercially available kit (code 44509, Biosystems, Barcelona, Spain). Reactivities for anti-LKM1 and/or anti-LC1 as well as for AMA, whenever present, were further confirmed by immunoblotting, respectively, using rat liver microsomal fractions [32,33], human liver cytosol [34], and submitochondrial fractions of beef heart as the source of antigens [35–37], as previously described.

2.3. Viral markers

Serologic markers of past or present hepatitis A, B, and C infection, including anti-HAV antibody (anti-HAV) IgG, hepatitis B surface antigen (HBsAg), anti-hepatitis B core antigen antibody (anti-HBC IgG), anti-hepatitis B surface antigen antibody (anti-HBs), and anti-HCV antibody (anti-HCV; ELISA III, Roche, Basel, Switzerland) were performed in the first round of the project. All anti-HCV$^+$ samples were subsequently examined for HCV-RNA quantification and genotyping.

2.4. Clinical follow-up

Subjects with reactivity to SMA, ANA, or anti-LKM1 at titers equal to or higher than 1:80 and to AMA at titers equal or higher than 1:40 were submitted to subsequent clinical and laboratory evaluation to assess the presence of AIH, PBC, and primary sclerosing cholangitis, as well as other viral and metabolic liver disorders. The titers were arbitrarily chosen as clinically relevant based on published guidelines for the diagnosis of AIH and PBC [2,3]. All patients with evidence of current infection with HBV or HCV were further investigated and treated whenever indicated according to established clinical, laboratory, and histologic criteria.

2.5. Statistical analysis

Clinical and laboratory data were compared using the χ^2 test with Yates' correction or the Mann–Whitney test when appropriate. A p value < 0.05 was considered significant. Clinical data are presented in the text and tables as median and range.

3. Results

Seven hundred twenty-five subjects (369 females, median age of 23 [0.2–91] years) from the rural community of Cavunge were evaluated. All were apparently healthy. Demographic data concerning those subjects are summarized in Table 1. Thirty-nine percent were less than 16 years of age and were considered children. Most subjects were living in the rural area of Cavunge County.

One hundred one subjects (14%) exhibited reactivity for NOSA. Positivity of SMA, ANA, and AMA was observed in 74 (10%), 32 (4%), and 1 (0.1%) subject, respectively. Six had concurrent reactivity for SMA and ANA and none of the subjects had either anti-LKM1 or anti-LC1 by IIF. Seventy-two (71%) subjects had NOSA at titers equal

Table 2
Frequency of non-organ-specific autoantibodies (NOSA) according to titers and immunofluorescence patterns in subjects from Cavunge County

	NOSA titers (%)		
	1:40	1:80	1:320
NOSA (n = 101)	72 (71)	28 (28)	1 (0.9)
SMA (n = 74)	51 (69)	23 (31)	0 (0)
V pattern (n = 59)	43 (58)	16 (22)	
G pattern (n = 15)	8 (11)	7 (9)	
ANA (n = 32)	26 (81)	5 (16)	1 (3)
Nuclear coarse speckled pattern (n = 27)	26 (81)	1 (3)	
Nucleolar homogeneous pattern (n = 2)		1 (3)	1 (3)
Fibrillar cytoplasmatic pattern (n = 2)		2 (6)	
Nuclear homogeneous pattern (n = 1)		1 (3)	
AMA (n = 1)		1 (100)	