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Absence of *Borrelia burgdorferi* antibodies in the sera of Venezuelan patients with localized scleroderma (morphea).

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Key word: *Borrelia burgdorferi*, Lyme Borreliosis, localized scleroderma (morphea), Venezuela.

Abstract. A possible association of *Borrelia burgdorferi* (Bb) with localized scleroderma (LS) has been postulated. However, the published data do not provide unequivocal results. Previous serologic analysis in LS patients in South American countries yielded positive results. The present study in our laboratory looked for evidence of Bb in LS patients in Venezuela, by antibacterial antibodies detection using the two-tiered approach as recommended by the Center for Disease Control and Prevention of Atlanta (United States). Twenty one serum samples from LS patients and twenty one samples from healthy individuals were analyzed. Four serum samples were ELISA positive: three from patients with LS and one from a healthy control. All ELISA positive samples were negative by IgG Western Blot. Our data do not support an association of Bb infection and the sclerotic lesions of LS; but do not rule out the possibility of a relationship between LS and an unknown geno-specie of Bb *sensu lato complex*, a different Borrelia *specie* or a different spirochetal organism, as the etiological agents of the skin lesions in the area.

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Ausencia de anticuerpos contra *Borrelia burgdorferi* en suero de pacientes venezolanos con esclerodermia localizada (morfea).
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Palabras clave: *Borrelia burgdorferi*, Borreliosis de Lyme, esclerodermia localizada (morfea), Venezuela.

Resumen. En los últimos años se ha postulado una posible asociación entre *Borrelia burgdorferi* (Bb) y esclerodermia localizada (morfea). Sin embargo, los datos publicados no han proporcionado pruebas inequívocas de tal asociación. En Suramérica se han realizado estudios serológicos que han evidenciado resultados positivos. El objetivo de este estudio fue buscar evidencia de la infección por Bb en pacientes venezolanos con morfea, mediante la detección de anticuerpos antibacterianos, usando el protocolo de dos pasos recomendado por el Center for Disease Control and Prevention de Atlanta (Estados Unidos). Veintiún muestras de suero de pacientes con morfea y veintiún muestras de sujetos sanos (control) fueron analizadas. Cuatro muestras resultaron ELISA positivas: tres correspondieron a pacientes con morfea y una, a un sujeto control. Todas las muestras ELISA positivas fueron negativas en la prueba IgG Western Blot. Los resultados obtenidos no soportan una asociación entre la infección por Bb y las lesiones escleróticas de morfea, pero tampoco descartan la posibilidad de una relación entre morfea y una genoespecie desconocida del complejo *Bb sensu lato*, entre morfea y una especie diferente de *Borrelia* o entre morfea y otra espiroqueta, como agente etiológico de la lesión en la localidad.

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INTRODUCTION

Localized scleroderma (LS), also known as morphea, is a disorder characterized by thickening and induration of the skin and subcutaneous tissues due to excessive collagen deposition (1). The disease has an incidence rate of 27 new cases per 1 million population per year. The actual incidence may be higher because many cases may not come to medical attention due to the benign nature of the disease (2).

The etiology of morphea is still unknown. However, a possible association of *Borrelia burgdorferi* (Bb) with LS has been postulated. But the published data do not provide unequivocal results (3).

In South America, antibodies against Bb have been detected in LS patients in Venezuela (Zulia State) and Colombia (4, 5). In the study conducted in Venezuelan patients, enzyme-linked immunosorbent assay (ELISA) positive results were not confirmed by immunoblot.

To improve the accuracy of testing for antibodies to Bb, a two-tiered approach testing is currently recommended by the Center for Disease Control and Prevention (CDC) of Atlanta (United States). The two-steps protocol relies on a sensitive screening test (e.g. an ELISA), which is followed by specific immunoblotting assay of all samples with equivocal and positive screening test results (6).

Considering that the detection of antibodies against Bb is a diagnostic method frequently used to analyze the association between LS and Bb (7), the aim of this study was to evaluate the presence of antibacterial antibodies in the sera of LS patients in Zulia State of Venezuela, using the two-tiered approach for antibodies detection against Bb as recommended by the CDC.

MATERIALS AND METHODS

Between July, 2002 and August, 2003, twenty one serum samples from LS patients in Zulia State of Venezuela were collected. The mean age of the patients was 21.7 years (range, 6-65 years). The clinical diagnosis of LS was confirmed by histological criteria. The onset of the clinical lesions was between 2 months and 7 years before sera samples were drawn. One of the patients remembered a tick bite. In addition, sera from 21 individuals (mean age, 30.5 years; range 17-61 years) without any symptoms of Lyme Borreliosis and who did not have morphea were taken as the control group. This study was approved by an ethics committee and the patients gave their informed consent.

All samples were analyzed by ELISA using the C6 Lyme ELISA Kit, (Inmunetics, Inc. Cambridge, MA). The antigen used in this kit is a synthetic peptide, C6 peptide, derived from the VlsE protein of Bb. This peptide sequence is conserved and equally antigenic in humans infected with Bb *sensu stricto* or the European genospecies *(B. garinni, B. afzelii)*. This assay detects total antibodies (IgM/IgG) and exhibits a sensitivity and specificity of 97%. Results were interpreted following the recommendations of the manufacturer.

ELISA positive samples were analyzed by IgG Western Blot (WB) using QualiCode Lyme disease WB Kit, (Inmunetics, Inc. Cambridge, MA), which is elaborated with a low passage Bb B31 strain. This assay exhibits a sensitivity of 83% and a specificity of 97%. Results were interpreted following the recommendations of the manufacturer.

RESULTS

Four serum samples were ELISA positive: three of them were from patients with LS. The fourth positive sample corresponded to a healthy control. All ELISA positive samples were negative by IgG WB (Table I).

TABLE I
IgG WB RESULTS OF STUDIED SAMPLES

Specimen Identification	WB Bands													Interpretation
	18*	23*	28*	30*	31	34	39*	41*	45*	58*	60	66*	93*	
HC														Negative
LS								+						Negative
LS								+						Negative
LS								+						Negative
NC														Negative
PC	+	+	+	+	+		+	+		+	+	+	+	Positive

* Diagnostic Bands. HC= Healthy control. LS= Localized Scleroderma. NC = Negative Control. PC= Positive Control.

DISCUSSION

Since the relationship of Bb with morphea was first suggested by Aberer *et al.* (8) many investigations have been conducted to establish a possible association between the spirochetal infection and the skin lesion. Some studies, mostly European, using different techniques such as immunohistochemistry (9), culture (10-12), serology (10, 13-15) and polymerase chain reaction (16, 17) have demonstrated an association. On the contrary, North American studies have failed to do so (18-20). The association of the different clinical manifestations of Lyme Borreliosis and each genospecies of Bb (21) could partially explain these controversial results. Balmetti and Pifferetti (21), reported an association between cutaneous symptoms (e.g. erythema chronicum migrans and acrodermatitis chronica atrophicans) and the Bb genospecie, *B. afzelii.* Fujiwara *et al.* (20) detected *B. afzelii* and *B. garinii* (European genospecies), but not Bb *sensu stricto*, in skin biopsies of German and Japanese patients with morphea, using polymerase chain reaction assay. It is noteworthy to point out that the predominant strain in North America, Bb *sensu stricto,* has never been related with late dermatologic manifestations of Lyme Borreliosis (22).

Although any of the Bb genospecies has been isolated in South America, positive serologic tests have been described in Venezuelan and Colombian patients with dermatologic manifestations associated with Lyme Borreliosis (4, 7, 23). Arocha *et al.* (4) detected antibodies against Bb in 6 of 14 Venezuelan patients suffering LS using ELISA. Sánchez *et al.* (23) reported a case of a young infant from Táchira State (Venezuela) with erythema chronicum migrans and antibodies against Bb. Few cases of erythema chronicum migrans have been detected in Zulia State of Venezuela (data not published), but an association between this condition and Bb infection has not been demonstrated yet. In Colombia, Palacios *et al.* reported IgG WB positive samples in 2 of 9 patients with morphea (5).

Burkot *et al.* (24), reported that the interpretation of positive serology results in tropical countries, where neither Bb genospecies nor competent vectors have been isolated, should be done with caution, because of the possibility of cross-reactivity. Elevated immunoglobulin levels and cross-reactive antibodies against other pathogens (*Treponema pallidum*, *Borrelia recurrentis*, *Helycobacter pylori*, *Ehrlichia*, *Babesia*, Epstein-Barr virus) and diseases like systemic lupus erythematosus are likely sources of false reactivity in ELISAs of residents of tropical regions. The ability of WB to detect antibodies to individual proteins, including some specific of Bb, reduces the number of false positive test results obtained with ELISA (25).

According to CDC criteria a negative result on the WB or ELISA indicates there is no serologic evidence of infection by Bb at the time the samples were drawn (6). Based on these criteria and considering the IgG WB assay negative results obtained in this study coupled with the lack of immunoblot confirmation of the ELISA positive results previously detected in the state, we considered the possibility that results reported by Arocha *et al.* were false-positives, due to cross-reactivity.

Our data do not support an association of Bb infection and the sclerotic lesions of LS, but do not rule out the possibility of a relationship between LS and an unknown genospecie of Bb *sensu lato complex*, different Borrelia *specie* or a different spirochetal organism, as the etiological agents of the skin lesions. A study conducted in Colombia reported a partial

shared reactivity of sera from patients with syphilis and LS, providing evidence for a possible association of sclerotic lesions with a spirochetal organism (7).

Our long-term goals include new studies using other methodologies such as polymerase chain reaction in skin biopsies of LS patients to further investigate the association of LS and Bb infection in the area and a survey of competent vectors in Zulia State.

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