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BRIEF REPORT

Frequency of antinuclear antibodies in mestizo Mexican children with morphea

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Abstract Morphea is a disease that affects connective tissue and microvessels. Its pathogenesis is unknown, but several autoimmune factors participate. Our objective was to determine the frequency of antinuclear antibodies (ANAs) in pediatric patients with morphea and to establish their relation with the clinical variants and disease activity. A cross-sectional study was carried out from January 1999 to January 2008 in patients with morphea seen at the Instituto Dermatologico de Jalisco. ANAs were determined through an indirect immunoflourescent method, and the immunospecificity was done with a double immunodiffusion technique in agarose gel. A total of 34 children were included in the study, 74% of the female gender. Plaque morphea was the most common variant, present in 44% of the cases, followed by linear morphea in 38%, and generalized morphea in 18%. ANAs were positive in 29%, with homogenous immunoflourescense as the most frequent pattern (70%). Of the ANA-positive patients, 83% had generalized morphea, and in 70% of the cases the disease were considered as active. The frequency of ANA-positive children with morphea was 29%, and seems to be related to more extensive disease. No previous studies exist on this topic in the mestizo Mexican population.

Keywords Antinuclear antibodies · Children · Juvenile · Localized scleroderma · Morphea

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Introduction

Morphea, also known as localized scleroderma, is a chronic disease that affects the connective tissue, characterized by skin hardening and fibrosis, without affecting the internal organs. According to the Mayo Clinic classification, morphea can be classified in one of the following types: plaque, generalized, bullous, linear, or deep morphea [1]. Other authors include guttate, keloideal, en coup de sabre, and nodular morphea [2–4].

In its pathogenesis, three main alterations have been described: (1) dysregulation in the extracellular matrix metabolism, (2) damage to microvessels with secondary ischemia, and (3) activation of inflammatory and autoimmune factors, with endothelial lesions being one of the most significant histopathological findings. It has been observed that during periods of ischemia, the tissues release reactive forms of oxygen which cause fragmentation of the autoantigens with epitope exposure that then break the immunological tolerance and generate autoantibodies [5–9].

At this time, there is no clear explanation regarding the association between antinuclear antibodies (ANAs), with

Table 1 Demographic, clinical, and serological characteristics of the pediatric patients with morphea

Variables	Plaque *N*=15 (44%)	Linear *N*=13 (38%)	Generalized *N*=6 (18%)	Total *N*=34 (100%)
Gender, M/F	6/9	3/10	0/6	9/25
Average age, years±SD	10±5	10±4	11±5	10±4
Average evolution, months±SD	30±17	26±16	35±40	23±33
Activity, # (%)	4 (27)	8 (53)	3 (20)	15 (44)
Antinuclear antibodies, # (%)	1 (7)	4 (31)	5 (83)	10 (29)

few studies done on pediatric populations [3, 4, 10–20]. In such population, the frequency varies between 26% and 81% [14–16] while for some authors no association exists between ANAs and the clinical characteristics of the disease [10, 11]. Others mention that ANAs are associated with more severe and disseminated disease [3, 12, 21].

Our objectives were to determine the frequency of ANAs in pediatric mestizo Mexican patients with morphea and to establish the relation with the clinical variants and disease activity.

Material and methods

A cross-sectional study was done in the outpatient department of the Instituto Dermatologico de Jalisco. Patients 18 years of age or younger, with clinical and histopathological diagnosis of morphea were included. Data obtained included: demographic information, clinical variables, and medical history of rheumatic or other autoimmune diseases. Body segments were described as head, neck, trunk, upper limbs, and lower limbs. Plaque morphea was defined by the presence of no more than four lesions, linear morphea in lesions with definite linear pattern, deep morphea when lesions extended into the hypodermis or muscle, and generalized morphea when more than four lesions were present and/or when two or more segments were affected during physical examination [22, 23]. Active disease was defined as an increase in the size or numbers of lesions during the 6 months previous to the dermatology consultation, all patients were diagnosed by the same medical team (EGG and ATP).

The presence of ANAs was determined through indirect immunoflourescence, using commercially available HEp-2 cells as the antigenic substrate (Inmunomex®), considering positive titers ≥1:40 [24]. Blood serum from the patients who were positive for ANA was subjected to a double immunodifussion in agarose gel to determine the immunospecificity (anti-Sm, anti-RNPn, anti-RNPr, and anti-SSB/La) [25]. To determine the difference in proportions between the groups, the Chi-squared test was used, or Fisher's exact test, considering $p \leq 0.05$ to be statistically significant.

The study protocol was approved by the Ethical Committee of the Institution and written informed consent was obtained from parents or guardians.

Results

Of the 34 patients included, 25 (74%) were female and nine (26%) were male. The average age was 10±4 years (3–18 years). The average time of evolution of the disease was 23±33 months (1–108 months). The observed variants were: plaque morphea in 15 cases (44%), linear morphea in 13 cases (38%) and generalized morphea in six cases (18%). We did not observe patients with deep or bullous variants, there were no cases on the coexistence of two or more types of morphea in the same patient, nil other autoimmune diseases in the study group.

As to the time of evolution in relation to the clinical variant of the disease, plaque morphea was 30±17 months (1–61 months), linear 26±16 months (4–84 months) and generalized 35±40 months (4–108 months). Active disease was observed in 15 patients (44%); of which four (27%) had plaque morphea, eight (53%) had linear morphea, and three (20%) had generalized morphea. Table 1 shows the demographic, clinical, and serological characteristics of the study population.

The ANAs were positive in ten patients (29%), all of them female and with an average age of 11±4 years (5–18 years). The average time of disease evolution was 23±32 months (ranging from 4–108 months). Of the 15 patients with plaque morphea, one (7%) was positive for ANA; of the 13 with linear morphea, four (31%) were positive, and of the six with generalized morphea, five (83%) were ANA-positive (p= 0.0004, OR=70 [95% CI: 2.60–1111]). In the ANA-positive group, seven (70%) showed active disease and only three (30%) were inactive (p=0.04, OR=4.6 [95% CI: 1.7–47.4]). With regard to the number of affected segments, four (40%) were affected in only one body segment, and six (60%) in more than one segment (p=0.05, OR=4.5 [95% CI: 1.36–47.7]). The ANA immunoflourescence pattern was homogenous in seven cases (70%), nucleolar in two (20%), and fine speckled in one case (10%). The immunospecificity was negative in all ten patients. Table 2.

Table 2 Clinical demographic characteristics of the ten patients with morphea and positive ANA

Patient number	Type of morphea	Gender	Age (years)	Evolution (months)	Number of affected segments	Activity	ANA pattern/titer
4	Generalized	Female	7	6	>1	Yes	Homogeneous/1:40
5	Linear	Female	16	12	1	Yes	Homogeneous/1:280
7	Linear	Female	6	4	1	Yes	Nucleolar/1:40
8	Generalized	Female	11	4	>1	Yes	Nucleolar/1:640
12	Linear	Female	12	4	1	Yes	Fine speckled/1:280
19	Generalized	Female	5	10	>1	Yes	Homogeneous/1:80
20	Linear	Female	14	12	1	Yes	Homogeneous/1:40
24	Generalized	Female	18	108	>1	No	Homogeneous/1:40
27	Plaque	Female	13	26	>1	No	Homogeneous/1:40
29	Generalized	Female	10	48	>1	No	Homogeneous/1:40

Discussion

In our study, of the 34 included patients, 74% were female, similar to what Zulian reported and who stated that women were three times more affected than men [2]. The average age observed was 10±4 years, older than the average of eight years reported by Uziel in his series of 30 cases [10]. As to the clinical variants of morphea, in our series the most frequent was plaque morphea, 44% of the cases. Linear morphea was seen in 38% and generalized morphea in 18% of the cases. This differs from other pediatric series, where the linear variant is reported as the most frequent, up to 87% of the cases [3, 10]. This difference could be due to the time of evolution of the disease. When each of the variants was analyzed, we found that the time of evolution was directly proportional to the extension of the lesions (generalized morphea 35±40 months vs. linear 26± 16 months), although the difference between the groups was not statistically significant.

As to the frequency of ANA, 29% of our patients were positive, a percentage similar to that observed in the Marzano and Bodemer's series, who reported a frequency of 26% and 28%, respectively [14, 15]. But still, this differs from other publications, where a frequency of 42% to 73% was reported [3, 4, 11, 13, 17]. The variable frequency of morphea in pediatric patients could be explained in part by the different inclusion criteria used in each series, the number of patients and the substrate used to determine the ANA.

Table 3 Relation between the presence of ANAs and the morphea variants

Author/Year	Number of patients	Percentage of ANAs (+)	Percentage of ANAs in plaque morphea	Percentage of ANAs in linear morphea	Percentage of ANAs in generalized morphea
Takehara/1983 [17]	22	73	50	67	100
Bernstein/1985 [20]	8	66	Not realized	Not realized	Not realized
Larregue/1986 [19]	27	37	Not included	37	Not included
Falanga/1987 [18]	22	50	54	Not included	44
Fontan/1988 [16]	11	81	Not included	81	Not included
Sato/1994 [13]	49	61	5	50	93
Uziel/1994 [10]	30	76	Not specified	Not specified	Not specified
Rosenberg/1995 [11]	27	63	60	67	0
Bodemer/1999 [15]	56	28	Not specified	Not specified	Not specified
Marzano/2003 [14]	126	26	Not included	Not included	60
Zulian/2006 [2]	671	42	34	47	31
el-Azhary/2006 [3]	35	38	Not included	42	Not included
Christen/2008 [4]	32	59	20	76	80
Leitenberger/2009 [21]	122	30	Not specified	Not specified	Not specified
Actual series	34	29	7	30	83

In our study, the frequency of ANA was directly related to the extension of the disease, being more frequent in the generalized morphea (83%) than in the plaque variant (7%) (p=0.0004, OR=70 [95% CI: 2.60–1111]). This finding is similar to that reported by Christen-Zaech, Sato, and Takehara [4, 13, 17], who reported an ANA frequency in generalized morphea of 80%, 93%, and 100% respectively. However, these results cannot be really compared to other published studies on children with morphea, as they did not include all the clinical variants [10, 14–16, 18–20]. Among our patients, those with only one affected segment had a 40% positive rate for ANA, while those with more than one affected segment had a positive frequency of 60%. (p= 0.05, OR=4.5 [95% CI: 1.36–47.7]).

Our results showed that in the ANA-positive group, the titer was higher and more commonly observed in patients with active disease, four out of seven (58%), Table 2. The reason for this is unknown, although the titer may be related to the surface area of involvement, as has been suggested previously [3, 13, 21]. Table 3 shows the relation between the presence of ANA and the morphea variants from previously published studies.

In the localized variants of scleroderma, several serologic markers have been identified, but none is specific, including ANA, anti-topoisomerase IIα, antiphospolipid, and rheumatoid factor [21], anti-single-stranded DNA has been described in high titers in patients with linear morphea [18]. Antihistone antibodies, which are thought of as markers of drug-induced lupus, appears to correlate with surface area and extent of disease involvement, as well as the activity of the disease in linear scleroderma [3, 13]. In our group, the most frequent immunoflourescent pattern was the homogeneous type, similar to that reported by Sato and Falanga [13, 18]. However, it was not possible to determine by double immunodiffusion which cellular proteins the auto-antibodies were directed to, as the immunospecificity (anti-Sm, anti-RNPn, anti-RNPr, and anti-SSB/La) was negative in our study patients.

Double Immunodiffusion takes advantage of the ability of molecules (both antigen and antibody) to migrate through agarose gel, bind together, precipitate, and produce a visible line that indicates the presence of antibodies. This test is less sensitive than immunofluorescence and ELISA but is more specific. A positive ANA test does not indicate the specific type of antibody, although the patterns of fluorescence of the nuclei are usually associated with specific antinuclear antibodies. The homogeneous pattern could be associated with antibodies to native DNA or antibodies to histones [13, 25].

This is, to our knowledge, the first study in a mestizo Mexican pediatric population that determines the frequency of ANA in morphea and its frequency in the different clinical variants, as well as high titers of ANA with disease activity. We recognize that the present study represent a single-center population, and further studies are needed with a greater number of patients and with long-term follow-up in order to confirm our findings.

Disclosures None

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