Neurología. 2010;**25(2)**:71-77

NEUROLOGÍA

www.elsevier.es/neurologia

ORIGINAL ARTICLE

Clinical characteristics and presence of antiphospholipid antibodies (anticardiolipin-β2GP-1) cerebrospinal fluid and serum of in a series of patients with multiple sclerosis in Mexico

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Received on 22nd June 2009; accepted on 22nd December 2009

KEYWORDS
Antiphospholipid antibodies;
β2GP-1;
Cardiolipin;
Multiple sclerosis;
Mexico

Abstract
Introduction: The differential diagnosis of multiple sclerosis (MS) includes a wide variety of autoimmune diseases (systemic lupus erythematosus, Sjögren syndrome, antiphospholipid syndrome, etc.). The presence of antiphospholipid antibodies (APLA) in serum of MS patients has been reported to be as low as 10%, or sometimes as high as 88% of the cases, although its significance in the pathogenesis of the disease, or its diagnostic usefulness is still unknown. The goal of this study was to describe the clinic and demographic characteristics of a sample of patients with MS from the Hospital General de México (HGM), as well as to determine the presence and frequency of APLA in cerebrospinal fluid (CSF) and serum samples of these patients.
Patients and methods: A prospective study with patients from the Neurology Department at the HGM was performed. These patients were diagnosed with MS over a one-year period. Clinical and demographic characteristics were compiled. VDRL and anti-cardiolipin-β2GP-1 complex antibodies were analyzed in CSF and serum samples.
Results: Twelve patients were included in the study, the majority females (58%). The predominant clinic feature was optic neuritis (66.6%) followed by medullary involvement (58%). Most of patients were ambulatory (< 4 EDSS points). Auto-antibody levels were found in negative ranges in all cases, both in CSF and serum.
Conclusions: The clinical-demographic characteristics in patients studied in this work were similar to those previously reported, and the levels of anti-cardiolipin-β2GP-1 were negative, thus indicating the existence of different clinical and demographic variables influencing their detection.

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PALABRAS CLAVE
Antifosfolípidos;
Anti-β2GP-1;
Anticardiolipina;
Esclerosis múltiple;
México

Características clínicas y anticuerpos antifosfolipídicos (anticardiolipina-β2GP-1) en líquido cefalorraquídeo y suero en una muestra de pacientes con esclerosis múltiple en México

Resumen
Introducción: El diagnóstico diferencial de la esclerosis múltiple (EM) incluye una gran variedad de enfermedades autoinmunitarias (lupus eritematoso sistémico, síndrome de Sjögren, síndrome antifosfolipídico, etc.). Los anticuerpos antifosfolipídicos (AAFL) en el suero de pacientes con EM se encuentran en cifras tan bajas como en el 10% y tan altas como en el 88% de los casos, pero su significación en la patogenia de la enfermedad o su utilidad diagnóstica aún no se han establecido. El objetivo fue conocer las características clínicas y demográficas de una muestra de pacientes con EM del Hospital General de México (HGM), y determinar presencia y frecuencia de anticuerpos antifosfolipídicos en líquido cefalorraquídeo y suero de estos pacientes.
Pacientes y métodos: Se realizó un estudio prospectivo con pacientes captados en la consulta externa de neurología del HGM, con diagnóstico definido de EM a lo largo de 1 año. Se analizaron sus características clinicodemográficas y se determinó VDRL y anticuerpos anticomplejo cardiolipina-β2GP-1 en LCR y suero.
Resultados: Se incluyó a 12 pacientes, con predominio del sexo femenino (58%). El cuadro clínico predominante fue la neuritis óptica (66,6%) seguida de la afección medular (58%). La mayoría de los pacientes fueron ambulatorios (< 4 puntos EDSS). Los títulos de los autoanticuerpos fueron negativos en todos los casos, tanto en LCR como en sangre.
Conclusiones: Las características clinicodemográficas en la muestra de este estudio son semejantes a las comunicadas con anterioridad y las concentraciones de anticardiolipina-β2GP-1 fueron negativas, lo cual puede indicar que hay diversas variables clínicas y demográficas que influyen en su detección.

Introduction

Multiple sclerosis (MS) is a neurological disease mediated by immune mechanisms that is characterised by clinically episodes of multifocal neurological symptoms of very different kinds: motor, sensory, cerebellar, visual, cognitive, etc., which evolve with spontaneous remissions and periodic exacerbations[1,2]. These symptoms are caused by a process of inflammatory demyelination of the white matter in the central nervous system, which may also evolve with secondary axonal damage and progressive neuronal loss[3]. It is estimated that worldwide prevalence of the disease varies by geographic region, being as high as 80-300/100,000 inhabitants in the Northern countries and as low as 5/100,000 inhabitants in some regions of Africa, Asia and South America[3,4]. The first studies on this disease carried out in Mexico in the seventies showed a very low incidence (1.6 cases per 100,000 inhabitants)[5,6]; however, more recent studies have shown an increase in the incidence in Mexico[2,7-12]. In a recent systematic review of epidemiological studies carried out in South America, the estimated global MS prevalence in this region was 1.48-17/100,000 inhabitants[13]. Despite this, the information available is still quite limited for properly estimating the true epidemiological situation of MS in Mexico[11].

Current diagnostic criteria for MS require the exclusion of a number of neurological diseases, both primary and systemic, that may present similar symptoms or paraclinical findings; among these are various autoimmune diseases such as antiphospholipid syndrome (APS)[1,14,15]. Furthermore, because MS shares many clinical features with autoimmune diseases in general, it is not difficult to find the coexistence of MS and other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, autoimmune thyroiditis, type 1 diabetes mellitus, Sjögren's syndrome or APS[16-17]. One of the main haematological disorders that cause thrombo-occlusive disease, especially in young women, APS is characterised by the presence of autoantibodies against various blood antigens (phospholipids, proteins, anticoagulants, etc.); these generate a prothrombotic state and, consequently, vaso-occlusive and ischemic events as well as infarctions in various tissues, including the nervous system[18]. There are several studies that have shown the presence of antiphospholipid antibodies in the serum of patients with MS, although the results so far show vary widely; a range of positivity between 10 and 88% has been reported[19-26]. This situation complicates the differential diagnosis of MS, in addition to the fact that the real pathological significance of these antibodies in patients with MS remains unknown[24,27-32]. The objectives of this study were to ascertain the clinical and demographic characteristics of a sample of MS patients at the Hospital General de Mexico (HGM), to determine the presence and frequency of antiphospholipid antibodies in the cerebrospinal

fluid and serum of these patients and, ultimately, to establish a possible relationship of these antibodies with certain clinical and paraclinical variables in the patients studied.

Patients and methods

We performed a prospective study that included all patients who were newly diagnosed with probable MS in the outpatient department of the Neurology Service at the HGM over the course of a year, from October 2002 to October 2003. We included in the final study only those patients who, after an appropriate diagnostic study, met the characteristics required to consider them as having definite MS, according to revised McDonald criteria[14]. We excluded patients who did not meet the criteria of definite MS for any reason, as well as patients in whom we suspected a different disease or who were suffering from a concomitant systemic or neurological disease. All patients underwent a complete medical history and all signed an informed consent prior to being included in the study, together with an approval for the lumbar puncture procedure and removal of cerebrospinal fluid (CSF), as was done in all cases as part of the diagnostic protocol for the analysis of CSF and oligoclonal bands[33]. The variables considered were: age, gender, clinical variety (outbreak-remission, primary-progressive, secondary-progressive), time of evolution of the disease, points on the Kurtzke extended disability status scale[34] (EDSS) at the time of definitive diagnosis, alteration of the multimodal potentials (auditory, visual, somatosensory), number of demyelinating lesions in imaging studies (cerebral and spinal magnetic resonance imaging [MRI], when available, through a 1.5 T device; the imaging protocol included sagittal sections in T1, axial sections in T1, T2 and FLAIR), location of demyelinating lesions, oligoclonal bands in CSF/serum (through electrophoresis by isoelectric focusing), anticardiolipin-β2GP-1 antibody titers, both for IgM and for IgG (through the ELISA test according to the previously communicated and standardised methodology[35], building an 8-point standard curve expressed in arbitrary units [U]), and, finally, a venereal disease research laboratory (VDRL) test. Normal antibody titers were taken from the average of blood samples obtained from 10 healthy volunteer donors. Antibody titers were considered positive if they were beyond two standard deviations of the normal average found for each antibody (IgG <6 U; IgM <4 U).

Results

A total of 16 patients with probable MS were selected and diagnosed at the HGM during the period referred; of these, only 12 met the predefined criteria for definite MS. Of these, seven were women (58%) and 5 (42%) were male. The average age was 32.9 years, with an interval of 17-51 years. For females the average age was 31 (range 17 to 41) years and for males it was 35.6 (range 19 to 51) years. As for the variety of clinical behaviour, the most frequent type was outbreak-remission in 7 cases (58.3%), recurrent-progressive in 2 (16.6%), secondary-progressive in 2 (16.6%) and primary-progressive in 1 case (8.3%). The average time of evolution of the disease at the time of the study was 5.8 years, with an interval of 10 months to 19 years; there were no significant differences in the time of evolution between genders (5.8 compared to 5.6 years). In the category of disability points (EDSS), the average was 4.75 points and the interval ranged from 1.5 to 8. The locations of clinical lesions were distributed as follows: lesion in optic nerve (optic neuritis), 8; in the spinal cord, 7; in the cerebellum, 7; in the hemisphere, 5, and in the brain stem, 3 (table 1).

In relation to paraclinical studies, visual evoked potentials were performed in 8 patients (66.6%), of which six were abnormal (75%) and 2 were normal. The brain stem auditory evoked potentials were performed in 5 patients (41.6%) and were abnormal in 3 cases (60%). Somatosensory evoked

Table 1 Clinical characteristics of patients

Case	Gender	Age (years)	Clinical variety	Evolution	EDSS	Lesion location
1	Male	51	OR	3 years	5	M, ON
2	Female	25	OR	2 years 7 months	6	M, ON, S
3	Female	17	RP	1 year	4	ON, C, M
4	Female	25	OR	8 years	1.5	H, ON, C
5	Female	35	SP	19 years	8	M, ON
6	Female	39	OR	4 years	6	M, C, S, ON
7	Male	34	OR	8 years	3	H
8	Female	41	OR	3 years	4	H, S
9	Male	32	SP	5 years	5	C, M, H
10	Male	19	PP	10 months	3	C, H
11	Male	42	RP	12 years	6.5	M, ON, C
12	Female	35	OR	2 years	5	ON, C

OR: outbreak, remission; C: cerebellum; H: hemispheres; M: medulla; ON: optic nerve; PP: primary progressive; RP: recurrent progressive; SP: secondary progressive; S: stem.

potentials were performed in 7 patients (58.3%) and were abnormal in all cases (100%). In MRI studies, the injury rate evidenced (compatible with MS, according to the McDonald criteria) was 3.8 lesions (range, 1 to 10). Lesion distribution by MRI was as follows: medullar in 7 patients (63.6%), periventricular in 6 (54.4%), cerebellar in 3 (25%) and in the brain stem in 2 patients (18.1%), and even a pseudotoumoural frontal lesion in 1 (Case 7). The study of oligoclonal bands was determined in only 7 patients (58.3%), and was considered positive in 5 (71.4%). In these cases, the electrophoretic pattern found was polyclonal in serum versus oligoclonal in CSF (table 2). As for immunological determinations, all patients had negative VDRL in serum and CSF. In addition, all presented negative titers for all the autoantibodies (both in CSF and in serum); in fact, the titers were significantly lower than in controls (fig. 1). In patients with MS, the antibody titers were significantly lower in CSF than in serum (fig. 2). Titer distribution was as follows: for the cardiolipin-β2GP-1 anti-complex in serum, the average for IgG titers was 1,939 (range, 0 to 3.82) U, and for IgM, 1,547 (range, 0 to 4.3) U. As for the determination in CSF, the average for IgG was 0.014 (range 0.010 to 0.026) U; for IgM, it was 0.015 (range, 0 to 0.158) U.

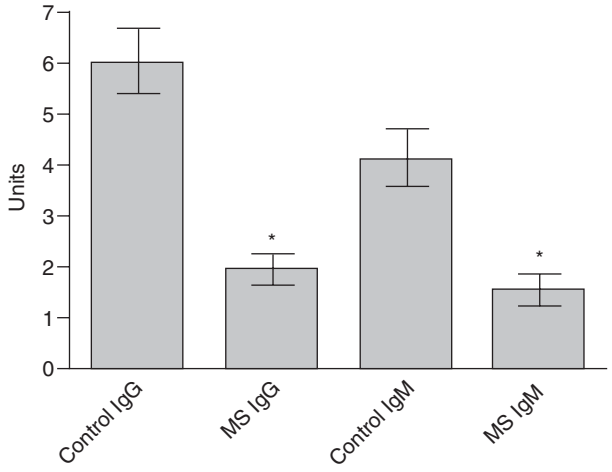

Figure 1 Comparative titers of anticardiolipin-β2GP-1 antibodies (IgG/ IgM) in serum. Patients with multiple sclerosis (MS) compared to controls. *ANOVA and Tukey with respect to control, p = 0.001.

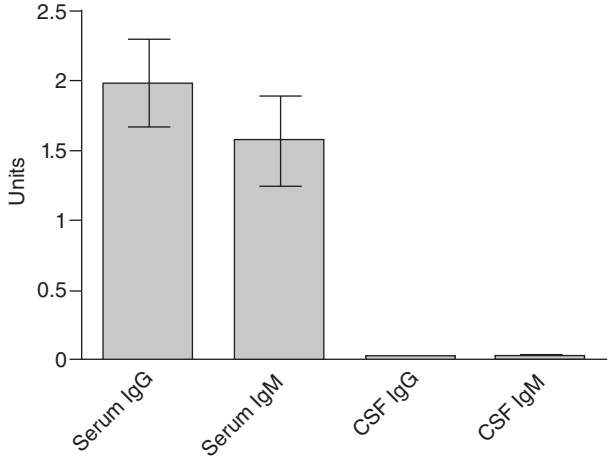

Figure 2 Titers of anticardiolipin-β2GP-1 antibodies in serum and cerebrospinal fluid (CSF) in patients with multiple sclerosis (MS) (IgG and IgM).

Discussion

Mexico has been considered a country with a low incidence of MS[5,36], but recent studies have reported an increase in case frequency[2,7-12]. This increase may be explained by several factors: *a*) an increase in the number of patients diagnosed due to increased awareness of the disease on the part of the medical community and the general public[9]; *b*) increased availability of imaging studies with high resolution for diagnosis, in particular structural and functional MRI; *c*) a reduction in infant feeding with breast milk, which can confer passive immunity against certain infectious diseases[4]; *d*) an increase in the frequency of viral diseases, such as herpes and the virus of varicella zoster in the Mexican

Table 2 Paraclinical characteristics

Case	VEP	BAEP	SSEP	Lesions by MRI (n)	Sitio de lesiones por RM	BOC
1	NA	Abnormal	NA	4	S, M, ON	NA
2	Abnormal	Abnormal	Abnormal	3	S, M	NA
3	Abnormal	NA	Abnormal	6	P, M	Positive
4	Abnormal	NA	Abnormal	6	P, C, ON	Negative
5	NA	NA	NA	2	M, ON	NA
6	NA	NA	NA	2	M, S	NA
7	Normal	Normal	Abnormal	1	LFS	Positive
8	Normal	Normal	Abnormal	4	P	Negative
9	Abnormal	Abnormal	Abnormal	3	M, P	Positive
10	Abnormal	NA	Abnormal	6	P, C	Positive
11	NA	NA	NA	5	M, P, C	NA
12	Abnormal	NA	NA	NA	NA	Positive

OCB: oligoclonal bands; C: cerebellum; PFL: pseudotoumoural frontal lesion, M: medulla; NA: not available; ON: optic nerve; P: periventricular; BAEP: auditory potentials; SSEP: somatosensory evoked potentials; VEP: visual evoked potentials; S: stem.

population, both considered as a risk factor for the development of MS[37,38]; and *e*) the global increase in autoimmune and allergic diseases associated with, among other things, the widespread use of antibiotics and antibacterial measures (hygiene hypothesis)[39]. However, this reported increase in the incidence of MS is based on only the few existing studies in the Mexican population, which are still insufficient for estimating the real situation of MS in Mexico[11].

This article presents a prospective study carried out on the Mexican population, at a concentration hospital, over 1 year, that includes only patients with a diagnosis of certainty, according to the revised McDonald criteria[14], and completely excluding other concomitant diseases. It is important to point out that there was of a prior descriptive study on the same hospital population, over five years, in which an annual incidence of 12.6 cases was obtained[40].

In this study, we obtained an annual incidence of 12 patients. However, we did not include 4 patients who had not completed their diagnostic protocol at the closing of the study, and others (n = 5), in whom MS was suspected, but who were suffering from other intercurrent disease.

With regard to patient clinical characteristics, there was a female predominance (58%), as well as an outbreak-remission form of presentation in the majority of cases (58%), with only 8% of cases having a primary-progressive form, which was expected since the patients had been newly diagnosed, results consistent with previous national and international reports[12,41]. The average age found was 32 years, slightly higher than that previously reported in Mexican patients (Gonzalez et al.[9], 1995, 27 years; Velázquez et al.[12], 2002, 27 years). The average time of disease progression at diagnosis was 5.8 years, a longer delay in the diagnosis than had previously been reported (Luna[40], 4.1 years; González et al.[9], 3.8 years). These figures may derive from the fact that most of the patients included in the study had already received prior treatment for other medical instances before reaching the HGM, and some were even being treated for another aetiology.

The most common symptoms coincided with those in previous reports, with a high frequency of optic neuritis (66.6%) (Luna[40], 61.9%; Velázquez-Quintana et al.[2], 56%; González et al.[9], 28%) and spinal affectation (58.3%). Notably, this characteristic is similar to what has been reported in Asian populations[42], but higher than has been observed in other South American countries (Vizcarra-Escobar et al.[43], 2005, 36%) or in Europe[41]. This emphasises the genetic heterogeneity of the Mexican population and its genealogical relationship with the Asian population, rather than with the European[44].

Most of the patients included in the study had low scores on the disability scale (EDSS, 4.75 points). This has already been reported in previous studies, and has been associated with an earlier age at onset and with the symptoms of optic neuritis, which may carry a better prognosis[9].

As for immunological findings, no titers in positive intervals were found in this study for anticardiolipin-β2GP-1 antibodies in any of the cases, neither in CSF nor blood. In the literature, obtaining antiphospholipid antibodies in patients with MS presents great variability, from 10%[19,21,45] to 88%[3]; however, methodology also varies considerable in patient determination and clinical conditions, which could also explain these large discrepancies.

Studies seek different antibodies directed against various antigens: cardiolipin, β2GP-1, thrombin, specific phospholipids, factor VII, etc., as well as different immunoglobulin IgG and IgM isotypes. In this case, the variability in the results may reflect a differential expression of these autoantibodies in patients with MS under different circumstances[26]. Another variable to consider are the clinical conditions of patients studied, since some studies do not mention whether the patients are in an acute outbreak of the disease or in remission, or if the patients suffer from progressive or recurrent forms of the disease, which may affect the expression of these autoantibodies. Another confusing factor is the possible use of immunomodulatory therapies (interferon, immunosuppressants, steroids, etc.), which can also modify antibody expression[31]. Studies in this regard have shown that using beta interferons can increase the expression of serum autoantibodies, such as anti-thyroglobulin and anti-microsomals although anti-phospholipid antibodies have not been detected[46]. In the present study, we decided to carry out the determination of antibodies against the β2GP-1 complex because it is the main antigen to which the autoantibodies that are observed in APS bind[47].

Unfortunately, the results of β2GP-1 antibodies in MS in the studies reported so far are also highly variable. There have been positive cases reported ranging from 82% in patients with acute exacerbation[26], to 6% in some cases[20] and none[48]. This variability may be related to the clinical and methodological factors already mentioned, since at present there are no consistent reports on the frequency of these antibodies[22-24,26-28,32]. In this study, we did not document the status of patient disease activity at the time of the study, and most of the cases had already received some form of immunomodulatory treatment when we tested them, which may partly explain the negativity of the immunological studies. Another limitation of our study is that we did not count with a control group of healthy patients or patients with another condition for the determination of antibodies in CSF. This limited our ability to analyse the behaviour of these antibodies in the CSF (data still unpublished at present)[27].

To our knowledge, this is the first study communicating APL antibody titers, both in blood and CSF, in a sample of Mexican patients with MS. We do not know whether ethnic origin plays a role in the presence of these antibodies, an issue to investigate in future studies using molecular tools. Meanwhile, the significance of these antibodies in MS pathogenesis is still uncertain, although some authors suggest that they may have a role in altering the permeability of the blood-brain barrier during acute MS exacerbations[26].

Lastly, it is also important to mention that, despite the positivity of these antibodies, the fact that they generate blood clotting disorders that result in clinical vaso-occlusive phenomena has not been documented in any MS case. Despite the obvious methodological limitations of this study, we believe that our results are relevant because the findings are consistent with some studies that have reported a low incidence of APL antibodies in patients with MS, thus supporting the hypothesis that these antibodies are not

present in all cases, but only in some cases that have specific characteristics. Nevertheless, further studies will be needed on larger patient populations, considering all the clinical, methodological and technical variables, to establish the frequency of presentation, as well as the role that these antibodies have in the pathogenesis of the disease and its relationship with different clinical variables.

Acknowledgements

The authors wish to thank Drs. Francisco J. Mena Barranco, Rosalía Vázquez Alfaro, Minerva López Ruiz and Ricardo Ramos Ramírez for their valuable support and cooperation in the implementation and review of this study.

Conflict of interests

The authors declare no conflict of interests.

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