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Controlled study of the frequency of anti-HSP 70 with the ELISA and the Western Blot methods in patients with Ménière Disease

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Abstract

Objectives: To establish the frequency of auto-antibodies anti-HSP 70 using ELISA and Western Blot (WB) methods and to compare the results of each method among patients with the Ménière's Disease (MD) and internal ear diseases (IED) who do not fulfill criteria for MD. Sensibility, specificity and predictive values of anti-HSP70 test in diagnosis of MD were calculated. **Study:** Prospective, case-control. **Methods:** Blood samples were collected from 31 patients with MD and 78 patients with non Ménière IED. Data regarding cochlear and vestibular symptoms were obtained and blood sample was tested. **Results:** ELISA tests results were positive in 4(13%) patients and results of WB were positive in 8(26%) patients. Among patients with positive ELISA results, 1 patient presented active disease and in the remaining 3 patients the disease was inactive. Among the 8 WB positive patients, only 2 patients presented active disease. Statistical analyses did not establish any association between serologic findings and clinical factors of MD. **Conclusion:** The presence of anti-HSP70 using the ELISA and the WB methods did not demonstrate clinical value for the diagnosis of MD. We did not find association between idiopathic MD nor unspecific etiology MD and the presence of anti-HSP70 auto-antibodies.

Keywords: endolymphatic sac, immune system, ménière disease, sensorineural hearing loss.

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INTRODUCTION

In 1861, during a medical conference, Prosper Ménière described for the first time a set of symptoms that up to then were attributed to a cerebral condition defined as cerebral apoplexy[1]. He identified and proved that the disease was a result of alterations in the homeostasis of the inner ear (IE) causing sensorineural hearing loss (SNHL), vertigo episodes from twenty minutes to hours of duration and tinnitus in the absence of neurological symptoms or sequel, the patient remaining oriented and conscious[1,2]. The disease carrying his name presents variations in its clinical presentation, characterized by remissions and exacerbations, leading to a difficult and many times frustrating disease management[3].

The histopathology of the endolymphatic hydrops was described for the first time in articles of Hallpike and Cairns in England and of Yamakawa in Japan, both published in 1938, reporting characteristic alterations in the labyrinth and temporal bones associated to the Ménière Disease (MD). It is a histopathologic term employed in the description of an alteration of the IE arising from the progressive distension of the endolymphatic space, especially of the cochlear and saccule duct, though the utricle and the ampulla of the semi-circular channels may also be involved[4-6]. When the cause of the hydrops can not be identified we name it MD, whereas cases with symptoms that may be related to a known cause are named Ménière's Syndrome[7,8].

Historically, MD is one of the most studied otorhinolaryngological pathology, it remained for several years a disease with scarce treatment possibilities, of difficult management and many times frustrating[9]. As well as the MD, some other inner ear diseases (IED), such: sudden hearing loss and progressive hearing loss[10], remain frequently with unknown etiology[11]. It is possible that a hidden immunological reaction may be the cause of these diseases[8,12,13].

The IE was considered to be segregated from the immune system as it is isolated inside the optical capsule and as it does not have lymphatic drainage[14]. Researches of the past thirty years have changed this notion. A recent study of Yimtae and colleagues has found evidences of an IE's drainage through the medium ear to cervical lymph nodes. There are evidences that the endolymphatic saccule has lymphatic vases that may function as a drainage path to regional lymph nodes[15].

The immunomediated etiopathogeny was described in researches conducted by some authors suggesting this is the cause for part of MD's cases previously classified as of idiopathic etiology[7,12,16-18]. The difficulty to establish an assured diagnosis of the immunomediated disease remains, as it is not possible to collect patient's cochlear tissue samples.

The clinical course of the MD presenting hearing fluctuations, asymptomatic periods and oscillations in the illness' severity, which results in slow and progressive deterioration of the affected organ, is similar to the clinical course observed in several autoimmune diseases. These are exacerbated by physical and psychological stresses, an observation also valid for the MD[19,20].

This study aim was to evaluate the relation between anti-HSP 70 antibody and clinical factors of MD, if this relation varies during the course of the disease, and if the presence of antibodies is useful to distinguish a subgroup of MD. We compared serological findings of patients with MD with a group of patients with non Ménière's disease IED. Sensibility, specificity and predictive values of anti-HSP 70 test in diagnosis of MD, by both ELISA and Western Blot methods, were calculated.

PATIENTS AND METHODS

We conducted a prospective case-control study[21] to evaluate the frequency of anti-HSP 70 antibodies with the ELISA and the WB methods. The study encompassed the above mentioned analyses in a *study group* of MD patients, according to the criteria defined by the American Academy of Otorhinolaryngology and Head Neck Surgery[2,22], compared to the *control group* of IED patients without criteria for the MD.

Cases (Study Group)

We invited all patients from Ménière Disease Clinics of the Otorhinolaryngology Department at the Clinics Hospital of Porto Alegre to participate in the study, through invitation letters sent by mail. The patients, then, received orientation about the objectives of the study and were free to participate or not in it, with no changes or ties attached to their regular treatment plan at the hospital.

Patients were included in the study group if they had the syndromic diagnosis of MD according to the American Academy of Otorhinolaryngology and Head Neck Surgery criteria[2,22].

Patients with history of treatment with immune suppressor or anti-inflammatory corticosteroids within the last three months, burns, diabetes mellitus, Alzheimer disease, ulcerative colitis, cancer, fever or recent infection by influenza, were excluded from the study as these conditions alter the values of the HSP70[23]. In the study group patients presenting evidence of compromise of the central nervous system, such as VIII cranial nerve tumor and evidence of other clinical entities that may mime the clinical manifestation of MD (Ménière-like) were excluded.

Control Group

The control group encompassed patients who were seen at the Otology Clinics of the Otorhinolaryngology Department at the Clinics Hospital of Porto Alegre

with diagnosis of other IED, without criteria to MD. Patients with history of treatment with immune suppressor or anti-inflammatory corticosteroids within the last three months, burns, diabetes mellitus, Alzheimer disease, ulcerative colitis, cancer, fever or recent infection by influenza, were excluded from the study as these conditions alter the values of the HSP70[23].

Study Protocol

Clinical investigation in both groups included anamneses, otorhinolaryngological examination, otoneurological examination, etiological search examination, auto-antibodies' research via ELISA and WB methods, imaging examinations in instances of unilateral hearing loss. The glycerol test was conducted only in the study group.

During testing with immunological methods we counted on the cooperation of a nurse technician, who drew the 5 ml blood samples of each patient from both groups, following duly guidance provided by the researcher regarding the completion of the informed consent form.

In addition to sex and age, the clinical symptoms, such as characteristics of the vertigo, hearing loss, presence of aural plenitude, tinnitus, family history, duration of the disease, laterality and activity of the disease were evaluated.

The time elapsed from the most recent vertigo episode or the hearing fluctuation was evaluated and the disease was considered to be active whenever there had been an episode of vertigo or hearing fluctuation within the 30 day period prior to the drawing of blood samples[24]. The duration of the disease was defined as the moment of onset of the patient's symptoms, which fulfilled the criteria for the MD[2,22].

Otorhinolaryngologycal physical examination

The otorhinolaryngological examination consisted of a review of the clinical history to detect the presence, previous or current, of relevant clinical manifestations. A standard protocol was used for all patients encompassing: general otorhinolaryngological, audiologic and otoneurological examination.

Physical examination was characterized by inspection and handling of the head and neck, otoscopy, anterior rhinoscopy and acumetry.

Radiological examinations

Imaging examinations, such as computed tomography with contrast and, if necessary, the magnetic resonance imaging (MRI) were performed to exclude lesions on the VIII cranial nerve in patients who presented unilateral SNHL or involvement of the central nervous system and, also, in those who either presented results of the audiometric tests of the brain stem or presented clinical data suggesting retro-cochlear involvement.

Audiological examination

The audiologic characteristics were established according to the conventional tonal and vocal audiometry with the determination of the aerial-osseous thresholds via an acoustic cabin and an AD 17 model audiometer of the Interacoustics brand.

Still, the immitance audiometry was conducted, including the study of tympanometry, acoustic reflexes and of the static complacence, using an AZ 26 immitance audiometry of the Interacoustics brand.

The study group patients underwent a glycerol test, which was considered to be positive whenever there was either a 10dB improvement in three frequencies (500, 1000 e 2000 Hz) or an increase of 8 % of the hearing discrimination (data not shown).

Otoneurological examinations

Patients were submitted to a detailed examination of the cranial pairs following the methodology referred by Ganança et al.[25] A complete electronystagmographic study following a methodology referred by Ganança et al.[26,28], was also done in all patients included in the study (data not shown).

Etiologic search examinations

To evaluate the serology for syphilis, laboratorial tests were employed. Globular Sedimentation Rate (GSR), an unspecific examination to verify the inflammatory activity, examinations to evaluate the specific immunity, anti-nuclear antibody (ANA), rheumatoid factor (RF), seric complement 3 and 4 dosage (C3 and C4) and research for metabolic alterations via a lipid profile (total cholesterol and fractions, triglycerides) and the glycemic and insulinemic curves after fasting and following 100 g of glucose and in 60, 90, 120, 180, 240 and 300 minutes were evaluated [27](data not shown).

Auto-antibodies research

The anti-HSP70 test was conducted using ELISA and WB methods[29]. The study was double-blind: the lab team tested serum without the knowledge of the group of referred patients and the medical team managed the patients according to the clinical diagnosis, without knowledge of the results of the tests conducted in the Laboratory of Immunorheumathology of the Biomedical Research Institute of PUCRS.

ELISA and WB tests results were analyzed considering time of the onset of the symptoms, presence of unilateral or bilateral disease, and age and disease activity.

Blood samples and cellular separation

Five ml of blood were drawn from each patient, and from these 2ml were placed in a non heparinazed tube for coagulation and to secure serum; the remaining blood was transferred to heparinazed tubes. Following the procedure, the remaining blood was diluted in a RPMI (Sigma) medium and centrifuged over 1077 (Sigma) histopaque to set aside circulating cells, which were frozen at –80°C.

The serum was initially kept at 4°C for auto-antibodies' analyzes, azida at 0.01% being added to prevent the growth of microorganisms.

Stress Proteins

The HSP70 of human origin was produced at the Laboratory of Immunorheumathology of the Biomedical Research Institute of PUCRS in a recombinant fashion. As there may be a difference in the reactivity of patients to the protein made in human cells as opposed to a recombinant synthesized in bacteria, we tested a second strategy using cellular extracts of mammal lineage bacteria, such as, for example, the K562, a lineage of human myelocytic leukemia before and after a heat-shock at 42°C followed by two hours of recuperation at 37°C. The HSC70 is present at a larger concentration in cells before the heat-shock and the HSP70 I is present in cells that were subjected to the 42°C. The cells were cultivated in a RPMI medium with 10% of bovine fetal serum at 37°C with 5% CO_2. We purified the HSP70 of the cellular extract of K562, using the same chromatography method of ATP-agarose used to purify the recombinant.

Western blot (WB)

The WB allows the identification of an antigen in a complex mixture of proteins. Both purified proteins, commercially secure, and cultivated cells, as described above, were placed in solution in a tampon of samples of SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and applied onto a gel of SDS-PAGE 12%. Following the separation of gel proteins, they were transferred to a nitrocellulose membrane. This phase was completed via the overlapping of the membrane to the gel and an electric current of 150 volts was applied for 1 hour. The proteins were transferred from the gel to the membrane of nitrocellulose after 2 hours at 100 volts. The dye (ponce red) confirms the transference.

On the membrane that was incubated with the patients' serum and with the positive control of WB, was used a monoclonal antibody of Sigma, that recognizes the HSP as well as the HSC70. The reaction was revealed with a solution of DAB, H_2O_2 and PBS (Phosphate Buffered Saline). The visualization of one band allows the determination of the presence of specific antibodies to a protein extract antigen.

ELISA

We tested the patients, also, with the method of ELISA that we developed, using a HSP70 protein. The method of ELISA detects the presence of antibody in serum with the aid of an enzymatic reaction.

Polystyrene plaques of ELISA, with 96 wells were sensitized with human HSP70, blocked with PBS 5% of milk and incubated with different serum dilutions of the tested subjects, allowing specific antibodies to link to the antigen. After an incubation period, the plaque was washed to retrieve antibodies that had not linked. To detect antibodies linked to antigen a conjugated (anti-imoglobulin antibody chemically linked to an enzyme) was applied. Again, after an incubation period the plaque was washed to retrieve the conjugated that were not linked. A chromogenic substrate was used to detect the presence of the antigen-antibody link. The revelation was made with a human anti-IgG conjugated to peroxidase, followed by OPD and the reading was made in a reader of ELISA at 490 nm. The intensity of the color developed by the substrate was proportional to the specific antibodies for the antigen present in the serum. The intensity was evaluated by a spectrophotometer, allowing a quantitative analyzes.

Statistical Analysis

Data were entered and analyzed by the SPSS 11.0 Statistical Software. To verify the association among categorical variables, Chi-square and Fisher's exact tests were used whenever appropriate. For the comparison of averages of continuous variables, the t test of Student was used. In the instance of the comparison of the evolutions' time variable (ordinal) the non-parametric test of Mann-Whitney was applied. The agreement between the ELISA and WB tests was verified by the McNemar's Kappa and Chi-square tests. The significance level adopted was of 0.05. Calculation of the sensibility, specificity, positive and negative predictive value as well as its respective confidence intervals (95%) were made via Epi Info 6.04d.

RESULTS

From the 64 MD patients invited to participate in the study, 15 did not show up for the medical evaluation. Among the remaining 49 individuals, 18 were excluded as they did not fulfill the standard criteria for MD. Thus, 31 MD cases were included.

A total of 81 patients were selected as controls. Three of theses patients were excluded as they presented diabetes.

Table 1 describes characteristics of cases and controls.

Table 1. Characteristics of Ménière Disease (MD) and controls patients.

	MD patients *n=31*	Controls *n=78*
	n (%) or mean±SD	n (%) or mean±SD
Male sex	12 (39)	47 (60)
Age (years)	39,5±15,5	46±15,9
Bilateral disease	13 (42)	50 (64)
Duration of disease (>12 months)	29 (94)	54 (69)
Positive family history	8 (26)*	4 (5)**
Typical vertigo	27 (87)	10 (13)
Non rotational dizziness	4 (13)	27 (35)
Sensorioneural hearing loss	31 (100)	78 (100)
Unilateral progressive fluctuating	15 (48)	4 (5)
Bilateral progressive fluctuating	7 (23)	5 (6)
Progressive unilateral	2 (6,5)	5 (6)
Progressive bilateral	6 (19)	7 (9)
Tinnitus	31 (100)	61 (78)
Aural plenitude	14 (45)	15 (19)
Positive glycerol test	15 (48)	-
Positive syphilis serology	1 (3)	4 (5)

*Positive family history for MD; ** Positive family history for hearing loss.

Investigation of anti-HSP70

Positive anti-HSP70 was an infrequent finding among cases and controls. Results (ELISA and WB) are described in Table 2. Among MD patients, three individuals (10%) presented positive results for both tests and 22 (71%) presented negative results in both tests.

Table 2. Anti-HSP70 (ELISA and Western Blot) tests results among 31 Ménière Disease (MD) patients and 78 controls.

	MD patients n (%)	Controls n (%)	P value
ELISA Positive	4 (12.9)	19 (24.4)	0.36
Western Blot Positive	8 (25.8)	14 (17.9)	0.19

Anti-HSP70 by ELISA had a sensibility of 12,9% (CI 95% 4,2 - 30,8%), specificity of 75,6% (CI 95% 64,4 - 84,3%), positive predictive value of 17,4% (CI 95% 5,7 - 39,5%) and the negative predictive value of 68,6% (CI 95% 57,6 - 77,9%) for diagnosis of MD. Anti-HSP70 by Western Blot presented the sensibility of 25,8% (CI 95% 12,5 - 44,9%), the specificity of 82,1% (CI 95% 71,4 - 89,5%), positive predictive value of 36,4% (CI 95% 18,0 - 59,2%) and negative predictive value of 73,6% (CI 95% 62,8 - 82,2%) for diagnosis of MD, as presented in Table 2.

Clinical diagnosis of MD was considered the golden-standard and based on it we compared the results of the tests ELISA and Western Blot.

Etiological diagnosis

Etiological diagnosis of MD was metabolic (n=24), otosclerosis (n=1), otitis media (n=3), chronic otitis media associated with carbohydrate metabolism disorder (n=2), and undefined etiology (n=5). Patients with the diagnosis of chronic otitis media did not present either suppuration or infection of the middle ear for over three months.

In the control group, etiological diagnosis of inner ear disease were considered unknown in 32 (41%), sudden hearing loss in 14 (18%), presbycusis in 11 (14%), otosclerosis in 12 (15%), and other causes in 9 (12%) patients.

Correlation between Findings of the Etiologic Investigation and the Methods for Auto-Antibodies Research (WB and ELISA)

A higher prevalence of anti-HSP70 (WB and/or ELISA) positive results was not found in MD patients of unknown etiology when compared to MD with established etiology (Fisher's exact test p = 0.3 for WB; Fisher's exact test p = 1.0 for ELISA).

The 5 patients with MD and undefined etiology presented negative serology in both WB and ELISA tests. Among the 4 patients who presented ELISA positive results, disease was unilateral and carbohydrate metabolism disorder was the etiology. Among the 8 patients with positive WB results, 1 patient was diagnosed as idiopathic MD, and other 7 as carbohydrate metabolism disorder etiology.

Immunologic Tests

Unspecific and Specific Immunity Tests

Among MD patients, Globular Sedimentation Rate (GSR) and specific immunity tests (ANA, RF, C3, C4) were normal, except in 1 (3%) patient who presented an elevated C3. In the control group, 10 (14%) patients presented elevated GSR. Other tests (ANA, RF, C3 e C4) were normal. There was no association between unspecific immunity tests and the anti-HSP70 results (Fisher's exact test p = 0.7 for ELISA versus GSR).

Anti-HSP70 results and MD activity

One patient with a positive ELISA presented active disease, while 3 patients with positive results presented inactive disease. A positive WB result was found in 2 patients with active disease, and in 6 patients with inactive disease.

Correlation of unspecific immunity tests with ELISA and WB tests.

The 4 patients of the study group with ELISA positive results and the 8 WB positive from study group do not present alterations in the inflammatory activity examinations.

In the control group, 10 patients presented altered GSR. These patients had the following etiologic diagnosis of the 10 patients: otosclerosis (n=1), bilateral SNHL of unknown etiology (n=3), and sudden deafness (n=6). Among these GSR altered patients, 5 presented positive results in one or both anti-HSP70 methods.

DISCUSSION

Characteristics of the study group and of the control group

Considering the choice of the control group, Soliman suggested that upon conducting a study to evaluate the validity of auto-immunity tests of IE, controls with otological pathologies would be more appropriated than healthy patients; not only hearing-loss patients, but also patients with idiopathic hearing loss not suggestive of immunomediated etiology. Patients with other known autoimmune diseases may be included[30].

Regarding clinical characteristics of the evaluated groups, we did not observe statistical significant association between the higher positive results in the tests of anti-HSP70 and younger patients, such as Garcia Berrocal et al.[31] found in a prior study.

In the control, group the bilateral hearing loss was predominant, differently from the study group, in which the unilateral vestibulocochlear disease was more frequent. However, there was no significant difference in disease's laterality between the groups. It is believed that the bilateral disease could be associated to the systemic processes, thus one could expect a more prevalent presence of auto-antibodies in the bilateral disease. Among MD patients, the floating progressive hearing loss was present in 71%, data similar to the one found in the literature[16]. Data regarding aural plenitude, tinnitus and vertigo's characteristics were also similar to the ones found in literature[16].

Etiologic Investigation in the Ménière Disease Group

The most prevalent etiology in patients with MD was the carbohydrate metabolism disorder, data in accordance with some studies that were published[32-34].

Our study did not find association between MD with unknown etiology and the presence of auto-antibodies. This could be explained by the reduced size of the sample. The 5 patients of the MD group with idiopathic etiology, presented negative serology in the WB and in the ELISA. Such was not expected as it was believed that these patients with disease of unknown etiology could present an immunomediated mechanism with a causal factor for the disease. All patients with positive serology presented an etiologic definition for the disease.

Immunologic Tests

Unspecific and Specific Immunity Tests

Among MD patients there was no association between the results of inflammatory activity examinations and the anti-HSP70. The specific immunity laboratorial tests did not detect instances of auto-immune disease.

Hirose and cols[35, 36] evaluated the usefulness of laboratorial tests in the IED of probable immunomediated etiology. Authors concluded that GSR was able to detect the presence of acute inflammatory activity of immunomediated IED.

Our study demonstrated that the request for specific examinations upon the routine of the etiologic investigation without symptoms of auto-immune disease was not useful. Even though the laboratorial tests may aid investigation of the immunomediated IED, the diagnosis still is based upon the improvement of the symptoms as a response to corticosteroids, which is the golden-standard considering the lack of a test with these characteristics[37].

Duration and activity of the disease

Duration of disease was longer than one year for 83 patients. No significant difference was found when comparing ELISA and WB results, disease activity and duration of disease among study group and control group patients. The longer duration of disease and high prevalence of inactive disease could explain the low level of positive auto-antibodies.

In our MD group there was no association between the presence of auto-antibodies (ELISA and WB), disease activity and bilateral disease. The agreement between the tests of ELISA and WB was low (coefficient of Kappa 0,396).

Considerations on the WB method compared to the ELISA method

The test of the anti-HSP 70 by WB was conducted at the Immunorheumathology laboratory of PUC-RS and some issues were observed. The first issue was that the test was positive for some of the patients when tested with a whole cellular lysate and negative when the antigen used was purified. The second relevant issue was that if large quantities of purified HSP70 were used, both the patients and the controls would be positive.

The major issue of the WB conducted in the tracking of the IED is that it generally uses a whole cellular lysate potentially leading to false positive results that recognize other proteins of 70 KD, which are not actually the HSP70. In a previous study from our group, we verified that the amount of HSP70 present is crucial in the WB and that high amounts of HSP70, even in normal patients, may lead to a positive test. In truth, the anti-HSP70 is present at a low level in all individuals. Another

issue is the denaturation of the protein, which affects the recognition by the patient's serum[29].

A third, and even more important issue, is the antigen's lack of purity associated to the fact that the serum of both patient and control presents antibodies reactive to additional antigen present in the raw extract, such as bovine albumin that has a molecular weigh slightly lower than 68 kDa, frequently distorting this region of the Blot[38,39]. The solution for the issue of antigen's impurity would be the conduction of tests with recombinant protein. Results suggest that the WB is not a suitable method to identify auto-antibodies for the HSP70 and that ELISA is a more trustworthy alternative for diagnosis[25].

The ELISA[29] test is semi-quantitative and therefore more appropriate to identify positive and negative results. Also some auto-immune diseases are associated to auto-antibodies that are specific for organs and tissues, consequently it may be more difficult to establish the significance of the auto-antibodies' positivism in the etiology of the disease.

The study of Shin et al. revealed a higher prevalence of anti-HSP 70 in the serum of patients with MD than in controls, even though the anti-antibody is not specific of IE[39]. The doubt regarding the function of auto-antibodies remains. We do not know if IED is a manifestation of the immune response against the increased production of HSP70 or if the antibodies could be an epi-phenomena of the disease that may be associated to other causal factors such as: prior surgery, viral infection [37,39,41] .

Anti- HSP70 among MD patients and controls

Prevalence of positive WB and ELISA tests were similar in both groups of patients. Thus, these tests do not seem to be useful in the diagnosis of MD.

In a study published by Harris and cols, anti-HSP70 by WB presented a sensibility of 42%, a specificity of 90% and positive predictive value of 91% among patients with positive response to corticoids, as they fulfilled the criteria for immunomediated IED and improved their condition using the immune suppressor treatment. However, according to the author, patients with negative results in the WB may also benefit from corticoids. However, patients in this study were treated less than two weeks from the onset of symptoms, a period during which the disease was in activity[11]. Other authors confirmed such findings. Atlas et al. reported that the presence of these antibodies was significantly related to the activity of disease[37].

The study of Munari et al. evaluated the results of the ELISA method in 13 patients with immunomediated SNHL and in 30 controls and found a sensibility of 84%, a specificity of 93%, positive predictive value of 84% and negative predictive value of 92,8%. These results that are superior to the results found using WB[29].

Rahman et al.[18] report that about one third of the patients with MD presented positive anti-HSP-70 auto-antibodies, fact also reported by other authors. Rauch et al. have also found association between the anti-HSP70 and MD[24].

In our study, in the MD patients, the sensibility and positive predictive value of the tests were low, while the specificity and negative predicted value were high. These characteristics would be useful for the confirmation of diagnosis in a diagnostic test. However, we are not able to make such statement regarding the test of the anti-HSP70 via ELISA and WB in patients with MD, as we did not find significance in the statistical analysis.

The role of the tests is not clear as there was small or no link between the activities of the human HSP in serum of patients with MD. The prognostic and therapeutic value of anti-HSP70 auto-antibodies in the IED, including MD, was not yet established. Only prospective studies with a larger number of patients of different subgroups of the MD will allow the determination of if such origin of disease's subgroups is based upon different pathogenic mechanisms and if there is really participation of the immune response in this process.

CONCLUSIONS

The presence of anti-HSP 70 by ELISA and WB methods did not show clinical usefulness in the diagnosis of the MD. We did not find association between idiopathic MD nor unspecific etiology MD and the presence of anti-HSP70 auto-antibodies.

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