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EC NEUROLOGY
Research Article

# Association Study between Multiple Sclerosis (MS) and Serum Levels of IgG in a Admixed Population of Salvador, BA, Brazil

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**Received:** July 11, 2018; **Published:** July 25, 2018

## Abstract

**Introduction:** Multiple Sclerosis is a chronic neurological disorder, with a tendency to affect young women. Of multifactorial etiology, it associates genetic and environmental factors. The detection of immunoglobulin G oligoclonal bands helps to diagnose disease, however, characteristics antibody specificities have not yet been identified.

**Objective:** To compare serum IgG levels in healthy individuals and MS patients and associate them with clinical-demographic variables.

**Methodology:** Case-control research, result of a PhD thesis, approved by ethics committee of the Federal University of Bahia (CAAE: 3517134.0.0000.5577). It involved a sample of 197 individuals, whose clinical-demographic data were collected through a specific questionnaire. Serum IgG levels were quantified by nephelometry.

**Results:** Serum IgG among patients reached a means of 1,410 ± 323 mg/dL, and in controls, 1,532 ± 310 mg/dL. Most of the individuals in the sample who presented altered serum IgG levels reported early diagnosis of the disease (40%) and Interferon type immunomodulator use (34,4%).

**Conclusion:** In this population, serum IgG levels do not appear to serve as a marker of progressivity, but may be useful for monitoring the therapy used.

***Keywords:*** *Multiple Sclerosis; Immunoglobulin G; Diagnosis; Prognosis*

## Introduction

Multiple sclerosis (MS) is the most common central nervous system (CNS) inflammatory disease [1,2], which tends to affect women in the early stages of their life. Its etiology is related to be caused by demyelination of nerve fibers and perivascular infiltrate of plasma cells, lymphocytes and macrophages [3,4]. Several factors - geographic, sociocultural, demographic and biological - including hereditary and constitution aspects, as well as, lifestyle (stress and smoking), have been related to the development and geographical and racial distribution of MS worldwide [4,5].

Given the variety of clinical conditions that the disease may present, the diagnosis become quite difficult [1]. This stage is based on a deep clinical investigation associated with image exams, specifically the magnetic resonance imaging (MRI) [6], were lesions in the

periventricular white matter, center-semiovals and corpus callosum areas are quite frequent [3]. The analysis of the cerebrospinal fluid is considered standard protocol during the diagnosis/prognosis of the disease, according the European Committee Against MS [7,8]. The presence of two oligoclonal bands in the cerebrospinal fluid (CSF) of a MS patient is one the parameters for diagnosis of MS, when associated with clinical and imaging evidence [9-11].

The identification of biological markers with diagnostic, prognostic and monitoring applicability allows the development of therapeutic modalities at the molecular level. On the other hand, the identification of specific molecules for MS makes its diagnosis faster. Thus, the aim of this study is to evaluate the serum levels of Immunoglobulin G in patients with MS, comparing them with those obtained in healthy individuals.

## Literature Review

Multiple sclerosis is considered one of the most intriguing neurological diseases due to its autoimmune and chronic characters, frequency and tendency to affect young adults [12,13]. Their chronic and incapacitating characteristics determine substantial costs to the public coffers, since they temporarily or definitively restrict the economic and social activities of their bearers, impacting still on the life of their relatives [14,15]. In addition, the clinical and epidemiological characteristics of MS can vary according to environmental and genetic factors [16-18]. The annual incidence rate of MS varies from 0.15 to 19/100.000 cases/100.000 inhabitants in equatorial countries [19].

Several factors - geographic, sociocultural, demographical and biological, and aspects like hereditary, constitution and lifestyle, have been related to the development and the geographic distribution of the MS in the world. These aspects directly influence the individual genetic composition, consequently acting on the etiology of the disease, by activation of the immune system [4,5,20]. Evidences suggest that some environmental factor such as a virus that persists in the CNS or disappears after the aggression, seems to interact, in specific circumstances with a genetically susceptible organism [21,22], confirming the evolution of the disease in the form of outbreaks and remissions. This interaction causes elevation of IgG, IgM and oligoclonal bands in the CSF protein dosage, as well as a decrease in suppressor T lymphocyte population [21-23]. The increase in C3c Synthesis in serum and CSF is also observed and coincides with the occurrence of neurological disability in individuals diagnosed with MS [24]. Additionally, the detection of high rates of G-types oligoclonal bands in the CSF of MS patients determine, by itself, a trend towards for progressivity [25,26].

In view of the variety of clinical conditions that the disease can present the diagnosis is quite complex. It is based on the determination of clinical findings and exclusion of all others disorders that have similar clinical characteristics to those of the MS [1]. Thorough analysis of the cerebrospinal fluid of patients with MS including cytology, blood-brain function and intrathecal synthesis is considered standard protocol during the diagnosis/prognosis of disease, according to the European Committee Against Multiple Sclerosis [7,8].

## Methodology

This case-control study involved 100 MS patients, assisted at the Francisco de Magalhães Neto outpatient clinic, at the University Hospital of the Federal University of Bahia, from October 2016 to April 2017, diagnosed by neurologists through magnetic resonance imaging (MRI), laboratory tests (intrathecal synthesis of oligoclonal bands) and clinical evaluation. For comparative levels of IgG quantification, a sample of 97 individuals who sought the immunology laboratory of the federal University of Bahia during the same period was considered. As an exclusion criteria for these individuals were used special health conditions such as gestation, diabetes, arterial hypertension and heart disease.

The social-clinical-demographical data such as sex, age, race/ethnicity, clinical form of MS, age of diagnosis of the disease, presence or absence of inability and immunomodulatory medication in use were obtained through the use of a specific questionnaire prepared for this research, after the signing of the free and informed consent form.

The blood samples were obtained through peripheral intravenous puncture and after the collection the blood samples were submitted to serum quantification of IgG by nephelometry, an automated procedure using IMMAGE® equipment [27]. This technique is indicated for the detection of soluble antigens and is based on increasing the light scattering rate caused by particles in suspension in a solution as result of the formation of antigen-antibody complexes. After all volumes of reagents are located into the apparatus, the system calculates the turbidity of the sample and outputs the serum IgG concentration in mg/dL. The reference interval values for human serum IgG were determined by the Array® 360 system, which considers concentrations of serum IgG between 650 - 1600 mg/dL [27], within normal limits.

The social-clinical-demographical informations and serum IgG dosage were stored in a database of the statistical analysis software IBM SPSS STATISTICS 22 for windows. These data were submitted to statistical testes to calculate the simple frequencies and the association between the variables with 95% confidence power. Descriptive analyzes of the information obtained were performed, demonstrating relative and absolute frequencies of social-clinical-demographical characteristics and serum IgG levels. This study was submitted and approved by the Ethics Committee of the Faculty Medicine of Federal University of Bahia (CAAE: 3517134.0.0000.5577). Prior to the collection of social-clinical-demographical data and blood samples, participants or their legal representatives provided written consent.

## Results

### Description of the study sample

Of the 100 individuals with MS involved in this research, the predominance was female (83,0%), black or brown (75,0%), aged 30 to 39 years (29,3%). The majority of the sample (55,0%) had the diagnosis of the disease established from the age of 30 years (55,0%), with a relapsing-remission form (76,0%) and no clinical sequels (70,0%). Most patients (58,0%) reported using Interferons for control of the disease. The table 1 below, shows the social-clinical-demographical data.

| **Variable** | | |
|---|---|---|
| | **%** | **N** |
| **Sex** | | |
| Female | 83 | 83 |
| Male | 17 | 17 |
| **Ethnicity** | | |
| White | 25 | 25 |
| Black/Brown | 75 | 75 |
| **Age group** | | |
| ≤ 19 years | 4 | 4 |
| 20 - 29 years | 21 | 21 |
| 30 - 39 years | 28 | 28 |
| 40 - 49 years | 26 | 26 |
| ≥ 50 years | 21 | 21 |
| **Age of diagnosis of MS** | | |
| ≤ 19 to 29 years | 45 | 45 |
| 30 to ≥ 50 years | 55 | 55 |
| **Clinical form of MS** | | |
| Surto - remissão | 76 | 76 |
| Progressivas | 24 | 24 |
| **Sequels** | | |
| None | 70 | 70 |
| More than one | 30 | 30 |
| **Specific Medications for MS** | | |
| Não | 7 | 7 |
| Interferons (IFN-β1-b ou IFN-β1-a) | 58 | 58 |
| Acetato de glamatirama | 28 | 28 |
| Natalizumab | 4 | 4 |
| Total | | 100 |

***Table 1:*** *Number and proportion of patients with multiple sclerosis (MS) (N = 100), according to social-clinical-demographic variables. n= number of individuals; % = Relative frequencies of individuals.*

The serological analysis of Immunoglobulin G revealed for those with MS, a mean of 1,410 ± 323 mg/dL. On the other hand, the group of healthy individuals presented an average of 1,532 ± 310 mg/dL. Higher percentages of individuals of the control group presented a serum elevation of this immunoglobulin when compared to those with MS, although, this data didn`t present statistical significance (p = 0,065). The disease had a tendency to affect women (p = 0,043), of the black or brown races (p = 0,0001). The data founded for this analysis as well as the characteristics of the groups in question are explained in table 2 below.

| Group | Age (years) | Gender | | | | Race | | | | IgG (mg/dL) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Female | | Male | | White | | Black/brown | | 650 - 1.600 | | > 1.600 | |
| | | n[1] | %[2] | n[1] | %[2] | n[1] | %[2] | n[1] | %[2] | n[1] | %[2] | n[1] | %[2] |
| MS (N = 100) | 39 ± 11 | 83 | 83,0 | 17 | 17,0 | 25 | 25,0 | 75 | 75,0 | 75 | 75,0 | 25 | 25,0 |
| Healthy (N = 98) | 41 ± 16 | 69 | 70,4 | 29 | 29,5 | 05 | 5,1 | 93 | 94.8 | 25 | 25,5 | 73 | 74,5 |
| [p]3 | | 0.043 | | | | 0.0001 | | | | 0.065 | | | |

***Table 2:*** *Characteristics of the group of individuals with multiple sclerosis (MS) (N = 100) and the control group (N = 97), and percentage of individuals by IgG serologic range.*

*[1]n = Number of individuals ; [2]%= Relative frequencies of individuals; [3]p= Statistical significance. Significant values: p ≤ 0,05.*

Among the subjects of the sample that showed serum IgG level above 1,600 mg/dL, there was a higher prevalence in women (29,0%) of the brown race (25,4%). Although, these finding were not statistic inference. Additionally, in this same group, a small percent of individuals (12,7%) exhibited levels of IgG serum above 1,600 mg/dL, and this data had statistical significance (p = 0,0001).

The use of glatiramer acetate immunomodulator appeared to reduce the number of patients (3,0%) who had elevated serum levels of the same immunoglobulin (p = 0,035). The analysis of other variables that influence regulatory mechanisms, not included in the study, may help to clarify this serum reduction associated with immunomodulatory medication. The table 3, below, shows the clinical-laboratory correlation found in MS patient investigated.

| | Serum Levels of IgG (mg/dL) | | | | Total | p[3] |
|---|---|---|---|---|---|---|
| | 650 - 1.600 | | > 1.600 | | | |
| | n[1] | %[2] | n[1] | %[2] | | |
| **Gender** | | | | | | 0,06 |
| Male | 16 | 94,0 | 1 | 6,0 | 17 | |
| Female | 59 | 71,0 | 24 | 29,0 | 83 | |
| **Race/ethnicity** | | | | | | |
| White | 19 | 76,0 | 9 | 24,0 | 25 | 0,31 |
| Black/Brown | 56 | 74,6 | 16 | 25,4 | 75 | |
| **Clinical form of MS** | | | | | | 0,29 |
| Relapsing-remitting | 54 | 71,0 | 22 | 28,9 | 76 | |
| Progressives | 20 | 83,3 | 04 | 16,6 | 24 | |
| **Presence of sequel** | | | | | | 0,80 |
| None | 53 | 75,7 | 17 | 24,2 | 70 | |
| One or more | 22 | 73,3 | 8 | 26,6 | 30 | |
| **Age of diagnostic** | | | | | | 0,0001* |
| < 19 - 29 years | 27 | 60,0 | 18 | 40,0 | 45 | |
| 30 - > 50 years | 48 | 87,2 | 07 | 12,7 | 55 | |
| **Immunomodulator** | | | | | | 0.035* |
| Interferon | 38 | 65,5 | 20 | 34,4 | 58 | |
| Glamatiramer acetate | 25 | 89,2 | 03 | 10,7 | 28 | |

***Table 3:*** *Clinical-laboratory correlation of the group with multiple sclerosis (MS) (N = 100).*

*[1]n = Number of individuals; [2]%= Relative frequencies of individuals; [3]p= Statistical significance. Significant values: p ≤ 0,05.*

## Discussion

Measurement of IgG levels in the CSF of MS patients is of fundamental importance because it tells a lot about the dysfunction of blood-brain barrier, a more evident sign of the primary progressive form of the disease. Thus, serum IgG levels may be a predictor of disease activity.

For a correct analysis of IgG levels, it is important to correlate the IgG index, a quotient between the immunoglobulin values found in the CSF and the serum of the patients [23]. Regarding serum IgG levels, statistical analysis showed significant differences (Fisher's exact test) in the composition of the subgroups "white race" and "black or brown race" and in relation to the variable genre. This find may be justified by insufficient sample size, and may be confirmed by future studies where large samples should be considered.

The serological examination of the subjects in the present study revealed a mean level of IgG around 1,410 ± 323 mg/dL for the patients with MS, whereas in control group, these levels reached values of 1,532 ± 310 mg/dL. Immunoglobulin serum levels depend on a variety of factors involved in the development of the immune system such as genetic, socio-demographic (age, sex), geographic and environmental (history of allergy, recurrent, bacterial, viral and parasitic infections), especially in the case of the G-type family [28]. These variables may explain the higher values at these levels in the healthy individuals of the control group.

This study found a percentage of 25% (n = 25) of the total number of MS patients with IgG serologic levels above 1,600 mg/dL. Data from epidemiological studies of the detection G-type oligoclonal bands in MS people vary widely, but in general higher percentages of patients with G-cell immunoglobulin bands are reported in countries with large Caucasian influence, as it case of northern Europe, which reaches rates of up 95% prevalence [29]. In the Western of Europe the prevalence reaches 70 - 75% [30], and for Brazil the prevalence was 54% of MS patients who expressed at least two oligoclonal bands of immunoglobulin G-type in the CSF when compared with another populations of Caucasian women [23]. The association of variables such as geographical location, clinical characteristics of the disease as well as immunogenic factors of the population in question as localization of specific structural proteins, may justify this variation in the IgG synthesis in different populations.

On the other hand, the pathogenesis of MS, which has a characteristic Th1-type profile immune response, a pattern of response that among others cytokines, stands out due to the production of Interferon Gama (IFN-γ), mediator of disease progression. This assertive may justify the increase detected in serum of the individuals in question from the molecular point of view, since some experimental models of MS have demonstrated the production of some IgG isotypes induced by (IFN-γ) [23].

Among the individuals that exhibited serum IgG increase, percentages around 25,4% occurred among black/brown people, when compared to those of the with race. Non-caucasian MS individuals tend exhibit increase in IgG index and higher percentages for the presence of oligoclonal bands [26]. In this context the investigation of the integrity of the blood-brain barrier in individuals of black or brown race may be considered, once they may posse molecular and structural peculiarities in this brain region that can make it more susceptible to disfunction.

When analyzed from a clinical-serological point of view, the sample showed that among the individuals that exhibited IgG levels above 1,600 mg/dL, higher percentages were of women with early onset of MS and who maintained more than one clinical sequel. High IgG levels in patients with MS have been associated with female who also had persistence of more than one clinical sequel, however, in these patients, the onset of the disease was later [18,25]. This difference in the age of diagnosis can be justified by the higher prevalence of the milder relapsing remitting clinical form verified in the sample [31].

## Conclusions

The maintenance within the physiological patterns of serum IgG in Brazilian individuals with MS has previously been reported in the literature. The serum increase of this immunoglobulin demonstrated a strong association between the early age of diagnosis of MS and

therapy with Interferons. However, in the population in question, it is not an indicator of progressivity. The identification of IgG levels either in the serum or in the CSF of people with this condition, makes a great contribution to the monitoring of MS activity, prognosis of its clinical course and also provides information about the effectiveness of the therapeutics instituted. Future studies may improve the use of this molecule as an aid in the diagnosis, prognosis and monitoring of MS.

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**Volume 10 Issue 8 August 2018**
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