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POTENTIAL RISK FACTORS FOR MULTIPLE SCLEROSIS IN RIO DE JANEIRO

A case-control study

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Abstract – **Purpose:** To evaluate potential risk factors for the development of multiple sclerosis in Brazilian patients. **Method:** A case control study was carried out in 81 patients enrolled at the Department of Neurology of the *Hospital da Lagoa* in Rio de Janeiro, and 81 paired controls. A standardized questionnaire on demographic, social and cultural variables, and medical and family history was used. Statistical analysis was performed using descriptive statistics and conditional logistic regression models with the SPSS for Windows software program. **Results:** Having standard vaccinations (vaccinations specified by the Brazilian government) (OR=16.2; 95% CI=2.3–115.2), smoking (OR=7.6; 95% CI=2.1–28.2), being single (OR=4.7; 95% CI=1.4–15.6) and eating animal brain (OR=3.4; 95% CI=1.2–9.8) increased the risk of developing MS. **Conclusions:** Results of this study may contribute towards better awareness of the epidemiological characteristics of Brazilian patients with multiple sclerosis.

KEY WORDS: multiple sclerosis, risk factors, immunization, tobacco, single person, consumption of animal brain, Brazil.

Potenciais fatores de risco para o desenvolvimento de esclerose múltipla no Rio de Janeiro: um estudo caso controle

Resumo – **Objetivo:** Avaliar os potenciais fatores de risco para o desenvolvimento de esclerose múltipla em pacientes brasileiros. **Método:** Um estudo caso-controle incluiu 81 pacientes atendidos no Departamento de Neurologia do Hospital da Lagoa, no Rio de Janeiro, e 81 controles. Um questionário padronizado incluiu variáveis demográficas, sociais e culturais, além da história familiar e clínica. A análise dos dados foi realizada por meio do programa SPSS para Windows e foi constituída de estatísticas descritivas e de um modelo de regressão logística condicional. **Resultados:** Pacientes com história de imunização (OR=16,2; IC95%=2,3–115,2), fumantes (OR=7,6; IC95%=2,1–28,2), solteiros (OR=4,7; IC95%=1,4–15,6) e que consumiam cérebro de animal (OR=3,4; IC95%=1,2–9,8) tiveram risco mais elevado de desenvolver esclerose múltipla quando comparados aos controles. **Conclusão:** Os resultados deste estudo podem contribuir para um melhor entendimento das características epidemiológicas dos pacientes brasileiros com esclerose múltipla.

PALAVRAS-CHAVE: esclerose múltipla, fatores de risco, epidemiologia, Brasil.

Multiple sclerosis (MS) is the most common idiopathic inflammatory demyelinating disease of the central nervous system[1]. One of the most intriguing aspects of MS is its characteristic racial distribution, since it affects predominantly caucasians, while rarely affecting black or indigenous populations. Genetic and environmental factors have been extensively evaluated in epidemiological studies carried out worldwide, mainly in the northern hemisphere where prevalence rates of this illness are higher[2]. Many factors have been assessed in relation to the devel-

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opment and the geographical and racial distribution of MS worldwide : geographical[3,4]; demographic[5-10]; socio-cultural[3-8,10-17] and biological factors[8-13,15,16] comprising inheritance and constitution, and directly referring to the role genes and the immunological system may play in the disease's etiology.

Brazil is a low prevalence area for MS[18]. Data from the first Brazilian multicenter study, referred to as the Atlântico Sul Project[19] and carried out between 1995 and 1997 by the Neuroimmunology Group of the Brazilian Academy of Neurology (ABN), showed that although 30% of patients were mullatos or black, both the clinical course and outcome of MS and the genetic susceptibility were similar to cases described in Europe and North America.

In view of the scarcity of research on risk factors for MS in mixed-race populations living in tropical regions, a case-control study was carried out in MS patients in Rio de Janeiro to evaluate some of the geographical, demographic, biological, social and cultural factors of this disease.

METHOD

One hundred consecutive patients attending the Neurology Department of the *Hospital da Lagoa*, Rio de Janeiro, Brazil, who had clinically defined MS according to the criteria established by Poser et al.[20], were enrolled to this case-control study between October 1996 and January 1998.

This city is located in the southeastern region of Brazil, latitude 22º 54' 10" and longitude 43º 12' 27". It has a tropical climate, a wide coastal plain with mountains and forests, fertile soil and a high average annual temperature. Its capital is Rio de Janeiro, which in 2001 had 6,051,399 inhabitants, predominantly of mixed race and of middle to low socioeconomic level[21].

All patients had taken part in the Atlântico Sul Project[19] and had been registered in the Brazilian MS SIAPEM database[22] by specialists of the Neuroimmunology Group of the ABN. Patients who had not undergone specific screening tests for demyelinizing diseases (cerebrospinal fluid and a magnetic ressonance imaging scan of the brain) were excluded from this study.

In order to determine the minimum sample size to be analyzed, a 80% power, a significance level of 5% and the ability to adequately get a significant odds ratio of 3 – considering a 20% prevalence of exposure in not ill group, which is the estimate prevalence for smoking, one of the most important epidemiological risk factors for non-communicable diseases – were taken into consideration. The sample size required was 72 cases and 72 controls.

The final case group consisted of 81 patients. The control group was composed of 81 friends or neighbors of these patients, all non-relatives, who were paired with patients according to gender, age (±5 years) and place of birth. These controls had the advantage of being of similar socioeconomic and educational status as the patients.

Patients and controls were interviewed according to a standardized questionnaire in which the following factors were evaluated: demographic characteristics, medical history, family history, social and cultural factors, and diet. All participants (controls and cases) were interviewed face-to-face in the hospital by the one of the investigators (KRPS), who had been trained at the Institute of Clinical Neurosciences, *Carlos Haya* Regional Teaching Hospital, Málaga, Spain. The questionnaire was based on that used in a previous study carried out in Malaga, Spain[14]. Race and ethnicity were defined in our database[19,22] according to the following characteristics: the african-brazilians did not know white ancestry up to three generations, and the white brazilians denied african ancestry for the same generation time. Smokers were defined as individuals who were current smokers at the time of the interview; individuals who had quit smoking or who had never smoked were classified as non-smokers. With respect to diet, the patients and controls were questioned regarding breast and bottle feeding in infancy, and consumption of meat, fruit, vegetables; special attention was paid to the consumption of liver, brain and thymus of animal origin. Alcohol consumption was defined as current daily use of alcoholic beverages. Standard immunization included the vaccinations specified by the Brazilian government: BCG (tuberculosis), DTP (diphtheria, whooping cough, tetanus), Polio (infantile paralysis), HBV (hepatitis B), Hib (haemophilus influenzae b), MMR (measles, mumps, rubella). The presence of unhealthy working conditions and humid household conditions were defined by the patients' subjective assessment. Information on past history of of mumps, measles, chickenpox, rubella, surgical intervention, traumatic spinal or brain injury and other clinical aspects were obtained from the patients' interview. Answers refer to the time before diagnosis in the case of the study group or to the date the controls were interviewed.

Means and standard deviations (SD) were calculated for continuous variables, and frequencies and percentages for categorical variables. The percentages reflect valid numbers that are a product of the exclusion of the missing values. Quantitative variables were compared using Student's t-test. Proportions were compared using the χ^2 test or Fisher's exact test when an expected value was below 5. A forward stepwise conditional logistic regression analysis of the explanatory variables was performed to assess the independent effects on the odds of developing MS. Any variable whose univariate test had a p-value <0.15 was considered for inclusion in the multivariate model, as recommended by Hosmer and Lemeshow[23]. Variables were entered into the model with a p-value of 0.05 being the probability for stepwise inclusion and a p-value of 0.10 being the probability for stepwise exclusion. All p-values reported are 2-sided. In all analyses, probability values of <0.05 were considered significant. Data were analyzed using the SPSS for Windows software program, version 10.0.1 (SPSS Inc.).

This study protocol was approved by the Internal Review Board of the *Hospital da Lagoa*. All patients and controls signed the consent form for participation in this study.

RESULTS

There was no significant difference between cases and controls in relation to age, sex, race / ethnicity, marital status, place of residence prior to 16 years of age, educational level, place of birth or nationality (Table 1).

With respect to the medical history of the subjects (Table 2), patients in the study group were less likely than controls to report having had rubella in the past (20.5% *vs.* 39.0%; p=0.01). Concerning blood type, an individualized analysis comparing the percentage of cases and controls of blood group AB versus the other blood groups showed a higher frequency of cases of MS in individuals of blood group AB and this difference was statistically significant (9.8% *vs.* 0%; p=0.01). Moreover, a history of surgical intervention and standard immunization (vaccinations specified by the Brazilian government) was more common in the study group compared to the control group; however, these differences were not statistically significant (p=0.07 and 0.09, respectively).

Although all cases and all controls were born in Brazil, more subjects in the study group reported having foreign ancestry (parents or grandparents) compared to individuals in the control group (44.9% *vs.* 18.9%; p<0.001). Most of these ancestors came from Portugal, Spain or Italy (data not shown). There was also a statistically significant difference between the two groups with respect to having another case of MS in the family (8.6% *vs.* 0% for the case and control groups, respectively; p=0.01).

An analysis of social and cultural factors showed that cases were more likely to be current smokers compared to controls (34.6% *vs.* 21.0%; p=0.05). On the other hand, controls were more likely to consume alcoholic beverages (55.6% *vs.* 27.2%; p<0.001) and to work in unhealthy conditions (39.5% *vs.* 16.0; p<0.001) compared to individuals in the study group. In addition, there were no statistically significant differences in dietary patterns in childhood or in adulthood between cases and controls, and no statistically significant difference between the two groups in relation to any of the types of food evaluated.

As a final point, a logistic regression model (Table 3) including marital status (single *vs.* others), place of residence up to 16 years of age (state of Rio de Janeiro *vs.* other places), blood type (AB *vs.* others), working conditions (unhealthy *vs.* others), history of surgical intervention, history of rubella, history of standard vaccinations, having siblings, having foreign ancestors, having another case of MS in the family, smoking habit, alcohol consumption and consumption of animal brain (yes *vs.* no) showed that, in this case series, a statistically significant association with risk of developing MS was found only for the following factors: history of standard vaccinations (OR=16.2; 95% CI=2.3–115.2), cigarette smoking (OR=7.6; 95% CI=2.1–28.2), being single (OR=4.7; 95% CI=1.4–15.6) and history of consumption of animal brain (OR=3.4; 95% CI=1.2–9.8). On the other hand, patients with a history of alcohol consumption (OR=0.2; 95% CI=0.1–0.4) and rubella (OR=0.2; 95%

Table 1. Demographic characteristics of MS patients and controls, Rio de Janeiro, Brazil.

Characteristics	Cases (n=81)	Controls (n=81)	P-value[a]
Mean age (years)	39.5 (±11.3)	38.5 (±11.3)	0.56b
Sex, N (%)			
Male	26 (32.1)	26 (32.1)	1.00
Female	55 (67.9)	55 (67.9)	
Race / ethnicity, N (%)			
White Brazilians	51 (63.0)	55 (67.9)	0.51
African-Brazilians	30 (37.0)	26 (32.1)	
Marital status, N (%)			
Single	31 (38.3)	17 (21.0)	0.02
Others	50 (61.7)	64 (79.0)	
Residence up to the age of 16 years, N (%)			
State of Rio de Janeiro	67 (83.8)	59 (72.8)	0.09
Other state	13 (16.2)	22 (27.2)	
Education (years of schooling) , N (%)			
≤7 years	13 (16.0)	19 (23.5)	0.24
≥8 years	68 (84.0)	62 (76.5)	
Born in Rio de Janeiro, N (%)			
Yes	65 (80.2)	58 (71.6)	0.20
No	16 (19.8)	23 (28.4)	

[a]Values calculated using chi-square test unless otherwise indicated; [b]Student's t-test used for comparison of means.

Table 2. Potential risk factors for MS (81 cases versus 81 controls), Rio de Janeiro, Brazil.

Characteristics	Cases N (%)	Controls N (%)	OR (95% CI)	P–value[a]
Normal delivery *versus* caesarean / forceps	66 (85.7)	71 (88.8)	0.8 (0.3–2.1)	0.57
History of surgical intervention	58 (71.6)	47 (58.0)	1.8 (0.9–3.7)	0.07
Use of oral contraceptives	38 (70.4)	34 (63.0)	1.3 (0.6–3.1)	0.50
History of traumatic brain or spinal injury	13 (24.1)	10 (18.5)	1.4 (0.5–3.9)	0.41
History of mumps	52 (68.4)	55 (71.4)	1.1 (0.5–2.3)	0.69
History of measles	56 (73.7)	54 (69.2)	1.2 (0.6–2.7)	0.54
History of chickenpox	55 (72.4)	60 (77.9)	0.7 (0.3–1.7)	0.43
History of rubella	16 (20.5)	30 (39.0)	0.4 (0.2–0.9)	0.01
History of standard vaccinations[c]	70(93.3)	60 (84.5)	2.6 (0.8–9.1)	0.09
History of transfusion of blood or blood products	6 (7.4)	4 (5.0)	1.5 (0.4–6.7)	0.75[b]
Blood group AB versus others	6 (9.8)	0 (0)	1.5 (0.4–6.7)	0.01[b]
Sibling present	9 (11.1)	16 (19.8)	0.5 (0.2–1.3)	0.13
First child in the family	33 (42.3)	24 (31.6)	1.6 (0.8–3.3)	0.17
Foreign ancestors	35 (44.9)	14 (18.9)	3.5 (1.6–7.8)	<0.001
Another case of MS in the family	7 (8.6)	0 (0)	Undefined	0.01[b]
Contact with pets	71 (87.7)	71 (87.7)	1.0 (0.4–2.8)	1.00
Contact with wild animals	10 (12.3)	5 (6.2)	2.1 (0.6–7.6)	0.18
Current cigarette smoking	28 (34.6)	17 (21.0)	2,0 (0.9–4.3)	0.05
Alcohol consumption	22 (27.2)	45 (55.6)	0.3 (0.2–0.6)	<0.001
Use of illegal drugs	7 (8.6)	5 (6.2)	1.4 (0.4–5.5)	0.55
Unhealthy working conditions	13 (16.0)	32 (39.5)	0.3 (0.1–0.7)	<0.001
Humid household conditions	7 (8.8)	13 (16.0)	0.5 (0.2–1.5)	0.16
Breast feeding *versus* bottle feeding	32 (43.2)	34 (45.3)	0.9 (0.5–1.9)	0.80
Meat versus a vegetarian diet	80 (98.8)	76 (93.8)	5.3 (0.6–121.8)	0.21[b]
Consumption of animal brain	40 (50.0)	30 (38.0)	1.3 (0.7–2.7)	0.13
Consumption of thymus	63 (78.8)	59 (74.7)	1.3 (0.6–2.8)	0.54
Consumption of liver	74 (92.5)	68 (86.1)	2.0 (0.6–6.5)	0.19

[a]Values calculated using chi-square test unless otherwise indicated; [b]Fisher's two-tailed test; [c]Vaccinations specified by the Brazilian government.

CI=0.1–0.5) were found to have a lower risk of developing MS. None of the remaining factors were independently associated with the risk of developing MS.

Table 3. Conditional logistic regression model for factors associated with MS (81 cases versus 81 controls), Rio de Janeiro, Brazil.

Characteristics	OR (95% CI)	P–value
History of standard vaccinations[a]	16.2 (2.3–115.2)	0.005
Cigarette smoking	7.6 (2.1–28.2)	0.002
Being single	4.7 (1.4–15.6)	0.01
Consumption of animal brain	3.4 (1.2–9.8)	0.02
Foreign ancestors	2.6 (0.9–7.6)	0.09
Alcohol consumption	0.2 (0.1–0.4)	0.001
History of measles	0.2 (0.1–0.5)	0.001

[a]Vaccinations specified by the Brazilian government.

DISCUSSION

MS was considered a very rare disease in Brazil until the 1990's. However, during the last 10 years, various case studies have shown that, despite the low prevalence of the disease, the hot climate and racial miscigenation, this illness now appears with clinical manifestations similar to those observed in Western countries[24-26].

Although numerous studies have been carried out on risk factors for MS, their results are conflicting with respect to almost all the factors evaluated. Due to the low incidence of the disease and to the long latency period between a potentially relevant exposure and the onset of clinical symptons, case-control studies are the most common method of assessing these factors, despite their limitations, particularly the difficulty in establishing a temporal relationship.

In this study, with respect to geographical factors, no

difference was found between patients and controls, since all subjects were Brazilians, who in most cases had been born in the state of Rio de Janeiro and lived there up to 16 years of age. Moreover, there was no difference between cases and controls with respect to race / ethnicity or education level.

MS has been reported to become clinical apparent or be exacerbated following immunization with a variety of vaccines[9,15,27]. In the meantime, in a recent systematic review conducted by Hernán, Alonso and Hernández-Díaz[28] the OR of MS associated with history of tetanus vaccination was 0.67 (95% CI: 0.55 to 0.81). In addition, an 8-year cohort study of nearly 500 children shown that the risk of MS was not increased by HBV vaccination[29]. Also, in a nested case-control study including 163 cases of MS and 1,604 controls no increased risk of MS was associated with tetanus and influenza vaccinations[30]. The current study demonstrates that having standard immunization was statistically associated with higher risk of MS (OR=16.2).

Surgery has been considered a potential risk factor for developing MS[12-15]; however, this was not confirmed in the present study. With respect to a history of traumatic events, no association was found between MS and these variables in the present study.

The studies conducted by Boiko et al.[3] and Granieri et al.[7] failed to find statistically significant differences between MS patients and controls with respect to a history of infection. In this study, MS was not related to past history of of mumps, measles, chickenpox, surgical intervention, traumatic spinal or brain injury. On the other hand, we reported an inverse association (OR=0.15) between history of rubella and MS.

Kurtzke and Page[8] observed that the blood groups B and AB seemed to be associated with MS, but the difference reported was not statistically significant. In our study, there was also a higher frequency of cases of blood group AB compared to controls, although statistical significance was not reached in the multivariate analysis. Blood transfusion was not found to be associated with MS in the present study, and this is in agreement with data published by Bansil et al.[6].

With respect to family history, a statistically significant difference was found between patients and controls with respect to having foreign ancestors (OR=2.6). Among the cases in this series, most of these ancestors came from Europe, a high-risk area for this disease[24,31,32].

Analysis of the social and cultural factors of the individuals in the two groups showed that smoking increased the risk of developing MS 7.6-fold. Ghadirian et al.[9], Zorzon et al.[27] and Riise et al.[17] also reported a direct and statistically signficant relationship between smoking and MS. Tardieu and Mikaeloff[29] also have shown that the risk of MS was increased by passive smoking at home, but this aspect was not studied in the current work.

Another factor associated with MS in this study was ingestion of animal brain (OR=3.4). Although this hypothesis had been raised by Tarrats et al.[16] in a Mexican study, these authors found no association between ingestion of pig or bovine brain and MS. Contact with animals (dogs, birds and farm animals) was found to be associated with the disease according to reports published by Bobowick et al.[13], Bansil et al.[6] and Ghadirian et al.[9]. However, a history of contact with cats was inversely associated with MS in the last two studies. In our study, no statistically significant association was found with cats and this is in agreement with data published by Kurtzke and Page[8]. On the other hand, no difference was found in our study with respect to type of residence, corroborating the findings of Granieri and Casetta[7]. Other factors, such as artificial baby milk[16] and a predominance of meat and animal fat in the diet[4,15], have been associated with this illness; however, these findings were not confirmed in the present study nor in the one conducted by Kurtzke and Page[8].

Finally, a history of alcohol consumption was reported by more controls than patients in our population, indicating that this factor was inversely related to the risk of developing MS. Since exposure is measured retrospectively in case-control studies, the possibility of a recall bias cannot be excluded as patients may over- or underestimate their history of exposure. In addition, the inclusion of prevalent cases into the study may have introduced a selection bias, as patients may stop drinking following diagnosis. If the patient stopped drinking due to MS symptoms, this may explain our finding of a reduction in risk in drinkers.

Moreover, the small number of patients (81 cases and 81 controls) in the present study resulted in poor power to satisfactorily detect the risk factors for MS. In addition, since our study was conducted in Brazilian patients, extrapolation to other ethnic groups must be done with an appropriate degree of caution. Other limitations to this study include the fact that no adjustments were made for multiple comparisons and that the interviewer was not blinded with respect to the patient/control groups. Because of MS is a disease that can have a long sub clinical phase, there is also the possibility of a selection bias in the control group (the controls may included people who had sub clinical disease). This possible bias may be minimized by the low frequency of disease in the general population.

It should be emphasized that, since this is the first case-control study to evaluate risk factors for MS in Brazil, other epidemiological studies need to be carried out with larger numbers of cases and controls, and in other regions of the country, to confirm these findings and identify others that were not assessed in the present study. Such studies would contribute towards increasing knowledge on the epidemiological profile of MS in Brazil.

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