

A Genetic Study of Celiac Disease in Patients with Multiple Sclerosis in Comparison with Celiac Patients and Healthy Controls

Fateme Barazandeh Ahmadabadi¹, Bijan Shahbazkhani^{2,*}, Abbas Tafakhori³,
Asghar Khosravi⁴, Marzie Maserat Mashhadi⁵

¹ Fellow of Gastroenterology, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

² Associate Professor, Department of Gastroenterology, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

³ Assistant Professor, Iranian Center of Neurological Research, Neurology Department, Tehran University of Medical Sciences, Tehran, Iran

⁴ Assistant Professor, Lorestan University of Medical Science, Khorramabad, Iran

⁵ Nurse, Iranian Center of Neurological Research, Neurology Department, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Background:

Studies have reported the controversial association between multiple sclerosis (MS) and celiac disease (CD). Thus, we aimed to conduct a case control study on patients with MS, CD, and controls to investigate CD in patients with MS by means of comparing CD genetic markers in patients with MS and controls. We also evaluated serological markers in patients with MS.

Materials and Methods:

This is a case control study conducted on 60 patients with MS, 140 patients with CD, and 151 healthy controls in 2015 in Tehran, Iran. HLA typing was done to identify the carriers of the DQB1*02, DQB1*0301, DQA1*05, or DQA1*0201 alleles for HLA-DQ2, DQB1*0302, or DQA1*03 for HLA-DQ8. All data were analyzed using SPSS software (version 23, IBM Corp). Serological markers including anti-gliadin antibodies (AGA) (IgA, IgG), anti-tissue trans glutaminase antibodies (Anti tTG) (IgA, IgG), anti-endomysial antibody (EMA) (IgA, IgG), and total IgA were assessed in MS group by enzyme immunoassays.

Results:

The data of 60 patients with MS (26.7% male, mean age = 34.83 years), 140 patients with CD (33.6% male, mean age = 38.37 years) and 151 controls (48.3% male, mean age = 40.43 years) were analyzed. The results of serological markers were not positive in any of the patients with MS. The prevalence of IgA deficiency ($IgA \leq 0.7$) was 13.3% in patients with MS. 34 (56.7%) patients with MS, 90 (59.6%) controls, and 135 (96.4%) patients with CD had positive results in either or both HLA DQ2 and DQ8. There was a significant difference in the HLA typing results between the patients with MS and controls with CD group ($P < 0.001$), while comparing the MS group with the controls there was not a significant difference in the result of HLA typing ($P = 0.69$). Four (50%) patients with MS and IgA deficiency had positive DQ2 and/or DQ8.

Conclusion:

Our results did not show any correlation between MS and CD, which was similar to other studies.

Keywords: Multiple sclerosis, Celiac disease, HLA-DQ2, HLA-DQ8

please cite this paper as:

Barazandeh Ahmadabadi F, Shahbazkhani B, Tafakhori A, Khosravi A, Maserat Mashhadi M. A Genetic Study of Celiac Disease in Patients with Multiple Sclerosis in Comparison with Celiac Patients and Healthy Controls. *Govaresh* 2018;22:256-260.

*Corresponding author:

Bijan Shahbazkhani, M.D
Department of Internal Medicine, Division of
Gastroenterology and Hepatology, Imam Khomeini
Hospital, Tehran University of Medical Sciences,
Keshavarz Blvd., Tehran, Iran
postal code: 1419853976
Tel: + 98 21 61192639
Fax: + 98 21 66581650
E-mail: shahbaz@ams.ac.ir

Received: 19 Sep. 2017

Edited: 10 Dec. 2017

Accepted: 11 Dec. 2017

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune chronic demyelinating neurological disorder, which involves central nervous system including brain, spinal cord, and optic nerves (1-6).

The prevalence of MS has been calculated as 1% of world population predominantly in young people and women. Most of the patients with MS are characterized by relapsing-remitting form of MS (RRMS) (7).

Studies have shown the role of osteopontin gene haplotypes, a pleiotropic cytokine, which is responsible for different autoimmune diseases by its effect on response of Th1 and Th17 proinflammatory cells, in

the onset and progression of MS. The presence of osteopontin in plasma and intestinal tissue of patients with celiac disease (CD) have also been reported (8-11).

However, dysregulation in immune system and impaired apoptosis are the mutual characteristics in CD and MS, which can possibly describe their association together and with other autoimmune disorders such as diabetes and thyroiditis (2-4).

CD is a chronic immunological gluten sensitivity enteropathy resulting in villous atrophy and crypt hyperplasia in the proximal part of the small intestine and following malabsorption (1,2,4,12,13).

CD has been defined to be associated with neurological manifestation such as neuropathy, nevertheless, its etiology has not been elucidated yet (2-4). Moreover hereditary occurrence of CD demonstrates the importance of genetic factors in the etiology of CD other than environmental and immunological factors. HLA complex specifically HLA class-II has been shown to be associated with CD, though HLA-DQ2.5 expression is the most common in the patients with CD and more than 90% of such patients possess one or two copies of HLA-DQ2.5 (1,14).

Thus, according to this information, we aimed to conduct a case control study to investigate the genetic markers of CD in MS and CD patients and healthy controls. We also studied serological markers in MS patients.

MATERIALS AND METHODS

This is a case control study conducted on patients with MS, CD (case groups), and healthy controls in 2015 in Tehran, Iran. Demographic and clinical data as well as disease history were collected by checklist. Informed consent was obtained from each patient or patient's guardian prior to the study enrollment.

We included patients with MS and CD and control groups after age matching. The age range of the participants was 5-70 years. After applying inclusion and exclusion criteria, the individuals for control and cases groups were selected randomly.

Patients:

60 patients with MS who were referred to a neurology clinic in Imam Khomeini Hospital, Tehran, and were diagnosed according to Mac Donald criteria (15) were

recruited as case group. In addition 140 patients with CD [according to celiac guidelines (16)] who were referred to gastrointestinal clinic were recruited as the other case group. The patients with CD were recruited over a period of 12 months from 2013 to 2014, based on the previous study in Iran (17).

Control group:

151 donors in National Blood Transfusion Organization of Iran without any symptom of CD (chronic diarrhea, weight loss, and osteoporosis) and history of health problems such as history of Crohn's disease, cancer, and immunological disorders were screened and recruited to the study as healthy control group.

Laboratory tests:

We investigated genetic markers in patients with MS, and CD, and control groups. Also, we measured serological markers in MS group.

Serological markers:

Serological markers including anti-gliadin antibodies (AGA) (IgA, IgG), anti-trans tissue glutaminase antibodies (anti-tTG) (IgA, IgG), anti-endomysial antibody (EMA) (IgA, IgG), and total IgA were assessed in MS group by enzyme immunoassays (EIA, Euroimmun AG kit, Germany).

AGA level more than 12 unit/mL and anti-tTG and EMA more than 20 unit/mL were considered as positive. This is while total IgA levels equal or less than 0.7g/L was considered as IgA deficiency.

Genetic markers:

For HLA typing 5 mL of venous blood was drawn from all the participants and collected in ethylene-diamino-tetra-acetic (EDTA) tubes and stored at -20°C until further analysis. Genomic DNA was extracted from the whole blood using QIAmap DNA Blood Mini Kit (Qiagen, Hilden, Germany). HLA DQ2/ DQ8 haplotypes were determined in cases and controls using PCR-SSP technique.

BAG kits (BAG, Germany) were used for HLA typing to identify individuals who were carriers of the DQB1*02, DQB1*0301, DQA1*05, or DQA1*0201 alleles for HLA-DQ2, DQB1*0302, or DQA1*03 for HLA-DQ8.

Table 1: The characteristics of MS, CD, and control groups

Group	MS	Celiac	Control
Mean age (years)	34.83	38.37	40.43
Male	16 (26.7%)	47(33.6%)	73 (48.3%)
Female	44 (73.3%)	93 (66.3%)	78 (51.7%)

CD: Celiac disease, MS: Multiple sclerosis

Table 2: The comparison of HLA DQ frequency in MS, CD, and control groups

HLA Classification	MS	Celiac	Control
DQ2 Positive	17 (28.3%)	66 (47.1%)	61 (40.4%)
DQ8 Positive	11 (18.3%)	24 (17.1%)	21 (13.9%)
DQ2/DQ8 Positive	6 (10%)	45 (32.1%)	8 (5.3%)
DQ2/DQ8 Negative	26 (43.3%)	5 (3.6%)	61 (40.4%)
Total	60	140	151

HLA: Human leukocyte antigen, MS: Multiple sclerosis, CD: Celiac disease

Table 3: Serological markers in MS group

Serologic Marker	Mean±SD
AGA IgA	2.50±1.30
AGA IgG	2.44±0.98
Anti tTG IgA	2.35±1.37
Anti tTG IgG	2.34±0.63
Anti EMA IgA	2.47±1.01
Anti EMA IgG	2.39±0.70

MS: Multiple sclerosis, SD: Standard deviation

Statistics:

All data were analyzed using SPSS software (version 23, IBM Corp). Descriptive statistics was used as mean ± standard deviation (median) for continuous variables and frequency (percentage) for categorical variables. For bivariate correlation for categorical data we used Chi square and Fisher’s exact test and for continuous data Mann-Whitney U test was used. P value less than 0.05 was considered as statistically significant.

RESULT

Patients’ characteristics:

The characteristics of MS, CD, and control groups are summarized in table 1. The data of 60 patients with MS (26.7% male), 140 patients with CD (33.6% male), and 151 controls (48.3% male) were analyzed. Mean age was 34.83 years in patients with MS, 38.37 years in patients with CD, and 40.43 years in controls.

Of the patients with MS, 54 had relapsing-

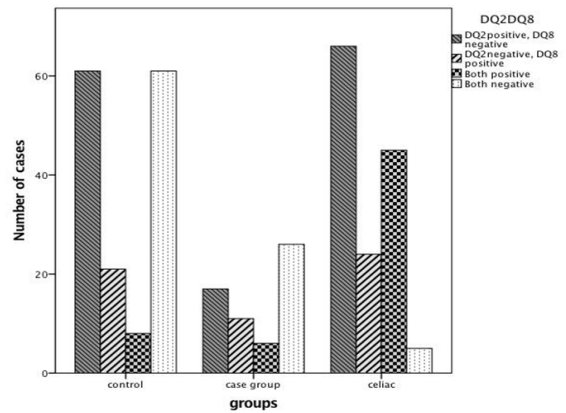


Fig.1: Distribution of DQ2 and DQ8 in patients with multiple sclerosis (case group), and celiac disease, and in controls

remitting multiple sclerosis (RRMS), 4 patients had secondary progressive MS (SPMS), and 2 patients had progressive-relapsing MS (PRMS).

Serological and genetic markers results:

The descriptive results of genetic markers are summarized in table 2 and figure 1. 34 (56.7%) patients with MS, 90 (59.6%) controls, and 135 (96.4%) patients with CD had positive results in either or both HLA DQ2 and DQ8 ($p < 0.001$). There was a significant difference in the HLA typing results between the MS and control groups with the CD group ($p < 0.001$), while comparing the MS group with the controls there was not a significant difference in the result of HLA typing ($p = 0.69$).

The serological markers did not show positive results in any of the patients with MS. The mean level of serological markers is summarized in table 3. The prevalence of IgA deficiency ($IgA \leq 0.7$) was 13.3% in patients with MS. Four (50%) patients with MS and IgA deficiency had positive DQ2 and/or DQ8.

DISCUSSION

Association of gluten sensitivity and MS is a very controversial issue. Some researchers recommended IgG AGA as a part of the routine survey for all patients with MS-like symptom of obscure etiology. On the other hand, other investigators decisively criticized this approach.

In this study, 34 (56.7%) patients with MS, 90 (59.6%) controls, and 135 (96.4%) patients with CD had positive results in either or both HLA DQ2 and DQ8

($p < 0.001$). There was a significant difference in the HLA typing results between the MS and control groups with the CD group ($p < 0.001$), while comparing the MS group with the controls there was not a significant difference in the result of HLA typing ($p = 0.69$). We did not find any positive results in the serological markers the patients with MS. The prevalence of IgA deficiency ($\text{IgA} \leq 0.7$) was 13.3% in the patients with MS.

Our results did not show any correlation between MS and CD, which was similar to other studies. Borhani and colleagues, studied 161 clinically definite patients with MS who referred to neurology outpatient clinic of Namazee Hospital, Shiraz, south of Iran from March 2004 to October 2005. The test of IgA anti-tTG and duodenal biopsy were carried out in patients with either IgA or IgG AGA positive sera. Anti-tTG antibody and histopathological studies were negative in all patients with positive IgG or IgA AGA results. Conclusion was that gluten sensitivity was not associated with MS in Iran (18).

Nicoletti and co-workers evaluated the frequency of CD among patients with MS. They evaluated the presence of IgA and IgG celiac disease-related antibodies in a sample of 217 patients with MS and in a sample of 200 controls not affected by neurological disorders. None of the 217 patients with MS presented IgG and IgA AGA, anti-EMA, anti-tTG, and anti-reticulon, whereas only one of the selected controls presented specific antibodies. This subject resulted to be effectively affected by CD. The data did not show an increased frequency of CD among patients with MS (4). In a study by Salvatore and colleagues (3), on 95 patients with MS measuring anti-tTG (IgA), none of the patients showed positive results. In our study, none of the patients with MS had positive serological markers for celiac, therefore similar to the three latter studies, we did not find increased frequency of CD among patients with MS, either.

In contrast, in a study by Rodrigo and co-workers, the researchers analyzed the prevalence of serological, histological, and genetic CD markers in a series of 72 patients with MS and in their 126 first-degree relatives, compared with 123 healthy controls. Tissue IgA-anti-transglutaminase-2 antibodies were positive in seven patients with MS (10%), compared with three healthy controls (2.4%, $p < 0.05$). No differences were found in HLA-DQ2 markers between the patients with MS

(29%) and controls (26%). Mild or moderate villous atrophy (Marsh III type) was found in duodenal biopsies of eight patients with MS (11.1%) and also a high proportion of CD was found among the first-degree relatives (32%). This finding represents a prevalence of gluten intolerance as 5.5 to 11 times higher in patients with MS compared with the general population (7).

IgA deficiency is more common in CD (2-5%) than in the general population ($< 0.5\%$). In a study by Khoshbaten and colleagues, the prevalence of CD was evaluated in MS in the northwest, Iran. All subjects (100 patients with MS and 121 controls) were negative for anti-tTG. IgA deficiency was found in 14% of the patients with MS and 11% of controls ($p > 0.1$) (19). In our study the prevalence of IgA deficiency was 13.3% among the patients with MS.

CONCLUSION

Our study revealed that none of MS patients had celiac disease. Also, prevalence of HLA DQ2 and/or DQ8 positive was similar control, %56.7 and %59.6, respectively. Therefore, the present study failed to demonstrate a positive relationship between MS and celiac disease.

One of the main limitations of our study was the small number of our patients. This was mainly the result of conducting this study in a localized geographic area and limited time. The second limitation was the unavailability of laboratory facilities to assess other heterotypes of HLA-DQ2 and HLA-DQ8.

CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

REFERENCES

1. Mormile R. Multiple sclerosis and susceptibility to celiac disease: an osteopontin gene haplotypes affair? *Immunol lette* 2015;163:132-3.
2. Salvatore S, Tozzo A, Nespoli L. Celiac disease in multiple sclerosis: a controversial issue. *Int J Neurol Neurother* 2014;1:1.
3. Salvatore S, Finazzi S, Ghezzi A, Tosi A, Barassi A, Luini C, et al. Multiple sclerosis and celiac disease: is there an increased risk? *Mult scler* 2004;10:711-2.
4. Nicoletti A, Patti F, Lo Fermo S, Sciacca A, Laisa P, Liberto

- A, et al. Frequency of celiac disease is not increased among multiple sclerosis patients. *Mult scler* 2008;14:698-700.
5. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci* 2008;31:247-69.
 6. Chou IJ, Whitehouse WP, Wang HS, Tanasescu R, Constantinescu CS. Diagnostic modalities in multiple sclerosis: perspectives in children. *Biomed J* 2014;37:50-9.
 7. Rodrigo L, Hernández-Lahoz C, Fuentes D, Alvarez N, López-Vázquez A, González S. Prevalence of celiac disease in multiple sclerosis. *BMC neurol* 2011;11:31.
 8. Neuman MG. Osteopontin biomarker in inflammatory bowel disease, animal models and target for drug discovery. *Dig dis and sci* 2012;57:1430-1.
 9. Kourepini E, Aggelakopoulou M, Alissafi T, Paschalidis N, Simoes DC, Panoutsakopoulou V. Osteopontin expression by CD103- dendritic cells drives intestinal inflammation. *Proc Natl Acad Sci USA* 2014;111:E856-65.
 10. Shimizu Y, Ota K, Ikeguchi R, Kubo S, Kabasawa C, Uchiyama S. Plasma osteopontin levels are associated with disease activity in the patients with multiple sclerosis and neuromyelitis optica. *J neuroimmunol* 2013;263:148-51.
 11. Comi C, Cappellano G, Chiocchetti A, Orilieri E, Buttini S, Ghezzi L, et al. The impact of osteopontin gene variations on multiple sclerosis development and progression. *Clin Dev Immunol* 2012;2012:212893.
 12. Borhani Haghighi A, Ansari N, Mokhtari M, Geramizadeh B, Lankarani KB. Multiple sclerosis and gluten sensitivity. *Clin Neurol Neurosurg* 2007;109:651-3.
 13. Batur-Caglayan HZ, Irkec C, Yildirim-Capraz I, Atalay-Akyurek N, Dumlu S. A case of multiple sclerosis and celiac disease. *Case Rep Neurol Med* 2013;2013:576921.
 14. De Oliveira P, de Carvalho DR, Brandi IV, Pratesi R. Serological prevalence of celiac disease in Brazilian population of multiple sclerosis, neuromyelitis optica and myelitis. *Mult Scler Relat Disord* 2016;9:125-8.
 15. Lauret E, Rodrigo L. Celiac disease and autoimmune-associated conditions. *Biomed Res Int* 2013;2013:127589.
 16. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292-302.
 17. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of Gastroenterology. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013;108:656-76.
 18. Khosravi A, Mansouri M, Rostami-Nejad M, Shahbazkhani B, Ekhlasi G, Kalantari E. The likelihood ratio and frequency of DQ2- DQ8 haplotypes in Iranian patients with celiac disease. *Gastroenterol Hepatol Bed Bench* 2016;9:18-24.
 19. Khoshbaten M, Farhoudi M, Nikanfar M, Ayromlou H, Shaafi S, Sadreddini SA, et al. Celiac disease and multiple sclerosis in the northwest of Iran. *Bratisl Lek Listy* 2012;113:495-7.