Original Article

# Risk factors of Multiple sclerosis and their Relation with Disease Severity: A Cross-sectional Study from Iran

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**Abstract**

**Background:** Many risk factors have been investigated in multiple sclerosis (MS); however, few studies have focused on the association between risk factors and the disease severity. The aim of our study was to evaluate the association between some of these risk factors and MS severity in a population sample of Iranian patients.

**Methods:** This cross-sectional study was conducted on 660 patients with MS. In addition to demographic variables, many potential risk factors were recorded. To compare the severity, progression index (PI) was calculated. This index is created by current Expanded Disability Status Scale (EDSS) /disease duration.

**Results:** Univariate analysis revealed that active smoking status is related with MS severity. (*P*-value = 0.012). Furthermore, our findings demonstrated that age at the disease onset [*P* < 0.001; OR = 1.05 (95% CI: 1.03–1.07)], female gender [*P* = 0.002; OR = 1.86 (95% CI: 1.24–2.77)] and marital status [*P* = 0.002; OR = 1.71 (95% CI: 1.21–2.41)] correlated with the severity of MS in the adjusted model. MS severity was observed to be related with high school and academic studies ([*P* = 0.004; OR = 0.56 (95% CI: 0.38–0.83)], [*P* = 0.001; OR = 0.52 (95% CI: 0.35–0.78)]) (Primary/secondary school studies are used as reference). Moreover, there was an association between MS severity and occupation (white collar, pink collar) ([*P* = 0.006; OR = 0.32 (95% CI: 0.14–0.73)], [*P* = 0.007; OR = 0.47 (95% CI: 0.27–0.81)]) (Student is used as reference). Furthermore, the results showed a significant correlation between vision and motor symptoms as an initial symptom and PI (*P* = 0.001, *P* = 0.025).

**Conclusion:** Due to high cost of MS care and its moderate to severe disability, identification of factors influencing the MS severity is important. Our results demonstrated that the major modifiable factors related with MS severity in Iranian population, some protective and some promotive, were smoking, education, marital status and occupation. Prospective studies on larger scale are needed for further proof of these results.

**Keywords:** Multiple sclerosis (MS), risk factors, severity

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## Introduction

Multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS).[1] It is a chronic disabling CNS disorder, which is more commonly observed in young adults. The disease is defined by inflammation, variable degrees of axonal injury and demyelination. These pathological patterns show clinical disability and a highly various and unpredictable course.[2–4]

Recent data has shown that the prevalence of MS has been increasing in the Middle East.[5]

Similar to other countries in this region, data has demonstrated rapid increase in the incidence and prevalence of MS in Iran.[6] Previous studies have reported the prevalence of MS to be 5.3 to 74.28/100,000.[6]

MS is a highly costly chronic disorder with high burden on individuals, families and the society. Early onset and chronicity of the disease lead to decrease in quality of life and working efficiency.[7] Torabipour *et al*. showed that MS imposes about 11.48 billion Rials on patients annually in the Khuzestan province of Iran.[8]

The exact pathophysiology of MS is not clear, but it seems to be a multifactorial disorder related to genetic susceptibility and environmental triggers. There is a strong evidence showing the importance of environmental triggers in the pathogenesis of multiple sclerosis. Many studies have demonstrated that about 80% of MS patients do not have affected relatives, and that about 75% of twins with an affected identical twin do not develop multiple sclerosis.

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The increasing prevalence of MS in developing countries and its high cost have attracted researchers towards finding the potential factors that might affect the incidence and severity of MS. Some probable environmental factors are latitude, immunization, viruses, smoking, diet and toxic chemicals.[9,10]

These environmental factors might play a role in MS severity as well as the disease onset. Until now, few studies have assessed the role of environmental risk factors in MS severity.[10,11]

Therefore, the aim of our study was to evaluate the association between environmental risk factors and MS severity.

## Materials and Methods

### Study design and Subjects ascertainment

The study was a cross-sectional study in Iran between 2012 and 2013. A total of 660 patients with MS were enrolled using nonprobability sampling. Patients from different parts of Iran who consented to participate in study and were treated with the same protocol were enrolled. Any individual with memory and/or cognition dysfunction was excluded. To obtain the appropriate sample, the authors divided Iran into four parts according to climate: the Central plateau, Coastal areas of South Caspian Sea, Coastal areas of North Persian Gulf, and Mountainous regions. Then, data was gathered according to MS prevalence reported in each part.[6] Most MS prevalence belonged to the central part of Iran. Therefore, most cases were gathered from central part (n = 586).[6] Other cases were enrolled from other parts according to MS prevalence.[6] The major cities involved in this study were Isfahan, Tehran, Arak (central plateau) Tabriz, Kermanshah (Mountainous region) Ahvaz (Coastal areas of North Persian Gulf) and Sari (Coastal areas of South Caspian Sea). Participants were selected from those referred to university clinics in each region. The onset of MS was determined based on McDonald criteria.[12] The median age of disease duration at the time of enrollment was 4 years (IQR = 8).

The researchers were committed to the ethical guidelines of the Declaration of Helsinki.[13] Ethical approval for the study was obtained from the Institutional Review Board at Shahed University. Signed consent forms were also obtained from all participants.

### Assessments

We examined the probable effective environmental risk factors using a structured check-list in a face-to-face interview. The interview was performed and the check-list was completed by neurologists.

Data were recorded concerning demographic, socio-economic, ethnicity, fetal status, childhood and infancy status, climate of birth place, climate of living place, sun exposure, dairy consumption, conserved food, microwave usage, cigarette smoking, supplementation, comorbidity, adulthood problems, pet exposure, toxin exposure, heavy metals exposure, Expanded Disability Status Scale (EDSS) and disease duration. To increase the accuracy of the gathered data, documents such as vaccination card or others documents were used. To determine the severity of MS, "Progression Index" (PI) was calculated. This index was created by current EDSS/disease duration.[14] EDSS is a method of quantifying disability in MS.[15]

### Statistical Analysis

SPSS Software version 22.0 was used for statistical analysis (IBM, Chicago, IL, USA). Relative frequency percentages were reported to describe nominal and/or categorical variables. Considering the skewed distribution of the numeric variables, we used median and interquartile range (IQR) for descriptive report. Univariate associations between progression index and probable risk factor were assessed using one-way ANOVA, Mann-Whitney U-test, Pearson Chi-square or Fisher exact test, wherever appropriate. Multivariate ordinal regression model was applied to adjust the potential confounding effect of age, sex, ethnicity, income and marital status on the association between different risk factors and the quartiles of the PI. For each comparison, the corresponding adjusted odds ratio (OR) and its 95% confidence interval (CI) were calculated. In all statistical procedures, a two-tailed $P$-value $< 0.05$ was considered to represent significant association.

## Results

A total of 660 patients with MS were enrolled in this study. The clinical characteristics of participants are shown in Table 1. The median age of patients at the time of enrollment was 37 years (IQR = 12). Most study population were female (n = 521, 84.6%). The most common MS clinical feature was relapsing-remitting (RR) (n = 430, 74.0%) and the most prevalent initial symptom was sensory (n = 298, 49.5%). The median of current EDSS and progression index (PI) were 2.0 (IQR = 3.0) and 0.4 (IQR = 0.8), respectively.

Table 2 compares the demographic and socio-economic characteristics between patients with MS and different severity of PI. According to the results of the multivariate ordinal regression model, older age at disease onset [OR = 1.05 (95% CI: 1.03–1.07), $P < 0.001$], male gender [OR = 1.86 (95% CI: 1.24–2.77), $P = 0.002$] and being single [OR = 1.71 (95% CI: 1.21–2.41), $P = 0.002$] were significantly associated with a higher quartile of PI (more rapid progression). After statistical adjustment for onset age, sex, ethnicity and marital status, higher level of education was associated with a lower quartile of PI [for high school: OR = 0.56 (95% CI: 0.38–0.83), $P = 0.004$; for university education: OR = 0.52 (95% CI: 0.35–0.78), $P = 0.001$ compared to primary/secondary school as the reference level]. In addition, slower progression was demonstrated by the lower quartiles of PI in white collar [OR = 0.32 (95% CI: 0.14–0.73), $P = 0.006$] and pink collar [OR = 0.47 (95% CI: 0.27–0.81), $P = 0.007$] job categories when compared to the student group even after multivariate adjustment.

Possible associations between a comprehensive list of environmental, familial, dietary and comorbidity profile from on the one hand and severity of MS (here PI) on the other are shown in Table 3. Univariate analysis revealed that active smoking is related with higher PI ($P = 0.012$).

Comparison of the season of symptoms onset between MS patients with different quartiles of the PI showed no significant findings ($P = 0.562$) (Figure 1). Nevertheless, as illustrated in Figure 2, significant associations were observed between the prevalence rate of vision and motor symptoms as initial manifestations of MS and the PI ($P = 0.001$, $P = 0.025$, respectively).

## Discussion

We evaluated the association between some probable risk factors and MS severity. Our findings demonstrated that age at the disease

**Table 1.** Clinical characteristics of recruited patients with multiple sclerosis (*n* = 616).

| | |
|---|---|
| **Age at disease onset** (yr) median (IQR) | 30 (11) |
| **Current age** (yr) median (IQR) | 37 (12) |
| **Gender** NO (%) | |
| Female | 521 (84.6) |
| Male | 95 (15.4) |
| **Ethnicity** NO (%) | |
| Persian | 521 (85.7) |
| Others | 87 (14.3) |
| **Marital status** NO (%) | |
| Married | 403 (65.5) |
| Single | 207 (33.7) |
| Divorced | 5 (0.8) |
| **Level of education** NO (%) | |
| Primary/Secondary School | 150 (25.5) |
| Diploma/College | 189 (32.1) |
| University | 249 (42.3) |
| **Consanguinity in parents** NO (%) | 115 (21.3) |
| **Number of children** median (IQR) | 2 (2) |
| **Disease duration** (yr) median (IQR) | 4 (8) |
| **Season of symptoms onset** NO (%) | |
| Spring | 132 (24.3) |
| Summer | 153 (28.2) |
| Autumn | 106 (19.5) |
| Winter | 152 (28.0) |
| **MS clinical feature** NO (%) | |
| Relapsing-remitting (RR) | 430 (74.0) |
| Progressive-relapsing (PR) | 1 (0.2) |
| Secondary-progressive (SP) | 142 (24.4) |
| Primary-progressive (PP) | 8 (1.4) |
| **Onset symptom** NO (%) | |
| Sensory | 298 (49.5) |
| Motor | 159 (26.3) |
| Vision | 216 (35.8) |
| Brain stem | 100 (16.4) |
| Cognitive | 2 (0.3) |
| Sphincter | 11 (1.8) |
| **First presentation** NO (%) | |
| Mono-symptomatic | 444 (75.0) |
| Poly-symptomatic | 148 (25.0) |
| **Current EDSS** median (IQR) | 2.0 (3.0) |
| **Progression index (PI)** (/yr) median (IQR) | 0.4 (0.8) |
| Progression index (PI) = current EDSS/disease duration (time between disease onset and latest assessment) | |

onset, female gender and marital status were related with severity of MS in the adjusted model. MS severity was related to high school and academic education (Primary/secondary school education are used as the reference). Also, there was an association between MS severity and occupation (white collar, pink collar) (Student is used as the reference). Furthermore, univariate analysis revealed that active smoking was associated with MS severity. Moreover, the results showed a significant correlation between vision and motor symptoms as an initial symptom and PI.

MS is an autoimmune disease of CNS with the age at onset between 20–40 years. MS prevalence is higher among females.[16] In our study, the median age of the disease onset was 30 years and the most prevalent subtype was the relapsing-remitting similar to the results of Etemadifar *et al.*[6]

The results illustrated that exacerbation and symptom onset occurred more frequently in summer. Other studies have also revealed that T2 lesion activity and more clinical exacerbations occurred during warmer weather and summer.[17,18] Changing in environmental factors or metabolic activity associated with the season may be the reason.

Based on the findings of this study, most patients with MS were from the central part of Iran. Also, Ebadifar *et al.* in their systematic review showed that the total number of patients in Isfahan and Tehran as central region of Iran was greater than other cities such as Khuzestan and Mazandaran. So, it seems that MS is more prevalent in central parts of Iran.[6]

In our study, univariate analysis showed that active smoking is related with MS severity. Similar to our study, others also showed that smoking has an association with the risk of MS severity and promotion to the progressive form.[19,20] Smoking induces inflammatory factors (e.g. IL-6, C-reactive protein and fibrinogen) and leads to a dysregulation of B-cell and T-cell homeostasis.[21] Furthermore, carbon monoxide in cigarette smoke results in blocking tissue oxygenation and demyelination.[22] Also,

**Table 2.** Comparison of the demographic and socio-economic factors between multiple sclerosis patients with different progression index (total *n* = 616).

| Disease Category | 1st Quartile (*n* = 154) | 2nd Quartile (*n* = 191) | 3rd Quartile (*n* = 142) | 4th Quartile (*n* = 129) | Univariate *P*-Value # | Adjusted OR (95% CI) | Multivariate *P*-Value¶ |
|---|---|---|---|---|---|---|---|
| **Age at disease onset** (yr) Mean (SD) | 27.1 (8.4) | 26.8 (7.4) | 28.8 (8.9) | 30.7 (9.1) | <0.001* | 1.05 (1.03–1.07) | <0.001* |
| **Sex** NO (%) | | | | | | | |
| Female | 137 (89.0) | 171 (89.5) | 113 (79.6) | 100 (77.5) | 0.004* | Ref. (1) | 0.002* |
| Male | 17 (11.0) | 20 (10.5) | 29 (20.4) | 29 (22.5) | | 1.86 (1.24–2.77) | |
| **Marital status** NO (%) | | | | | | | |
| Married | 107 (70.4) | 125 (65.8) | 85 (61.2) | 86 (66.7) | 0.424 | Ref. (1) | 0.002* |
| Single | 45 (29.6) | 65 (34.2) | 54 (38.8) | 43 (33.3) | | 1.71 (1.21–2.41) | |
| **Ethnicity** NO (%) | | | | | | | |
| Persian | 126 (83.4) | 168 (88.9) | 122 (86.5) | 105 (82.7) | 0.359 | Ref. (1) | 0.761 |
| Others | 25 (16.6) | 21 (11.1) | 19 (13.5) | 22 (17.3) | | 1.07 (0.70–1.63) | |
| **Education** NO (%) | | | | | | | |
| Primary/secondary school | 27 (18.2) | 40 (21.4) | 42 (30.9) | 41 (35.0) | 0.011* | Ref. (1) | |
| High school diploma | 48 (32.4) | 65 (34.8) | 47 (34.6) | 29 (24.8) | | 0.56 (0.38–0.83) | 0.004* |
| University | 73 (49.3) | 82 (43.9) | 47 (34.6) | 47 (40.2) | | 0.52 (0.35–0.78) | 0.001* |
| **Occupation** NO (%) | | | | | | | |
| Student | 13 (8.6) | 20 (10.8) | 18 (13.2) | 17 (14.0) | 0.046* | Ref. (1) | |
| Self-employment | 9 (5.9) | 15 (8.1) | 20 (14.7) | 21 (17.4) | | 0.91 (0.45–1.85) | 0.800 |
| White collar | 16 (10.5) | 7 (3.8) | 7 (5.1) | 8 (6.6) | | 0.32 (0.14–0.73) | 0.006* |
| Pink collar | 108 (71.1) | 135 (72.6) | 86 (63.2) | 71 (58.7) | | 0.47 (0.27–0.81) | 0.007* |
| Blue collar | 2 (1.3) | 1 (0.5) | 1 (0.7) | 0 | | 0.18 (0.03–1.25) | 0.082 |
| Retired/jobless | 4 (2.6) | 8 (4.3) | 4 (2.9) | 4 (3.3) | | 0.46 (0.18–1.15) | 0.095 |
| **Income** NO (%) | | | | | | | |
| <6,000,000 IRR | 18 (21.4) | 25 (22.3) | 13 (16.3) | 24 (30.0) | 0.588 | Ref. (1) | |
| 6,000,000-10,000,000 IRR | 28 (33.3) | 36 (32.1) | 35 (43.8) | 26 (32.5) | | 0.88 (0.51–1.52) | 0.647 |
| 10,000,000-20,000,000 IRR | 29 (34.5) | 42 (37.5) | 24 (30.0) | 24 (30.0) | | 0.72 (0.41–1.26) | 0.249 |
| >20,000,000 IRR | 9 (10.7) | 9 (8.0) | 8 (10.0) | 6 (7.5) | | 0.63 (0.29–1.38) | 0.244 |

# Chi Square statistics for categorical and one way ANOVA for quantitative variables; ¶Ordinal regression model; * Statistical significant. Adjustment is made for onset age, sex, ethnicity and marital status.

**Table 3.** Comparison of the environmental, familial, dietary, and comorbidity profile between multiple sclerosis patients with different progression index (total *n* = 616).

| Disease category | 1st Quartile (*n* = 154) | 2nd Quartile (*n* = 191) | 3rd Quartile (*n* = 142) | 4th Quartile (*n* = 129) | Univariate *P*-value # | Adjusted OR (95% CI) | Multivariate *P*-value ¶ |
|---|---|---|---|---|---|---|---|
| **Mother pregnancy** NO (%) | | | | | | | |
| Singleton | 146 (100) | 173 (100) | 131 (100) | 119 (99.2) | 0.289 | - | - |
| Twin | 0 | 0 | 0 | 1 (0.8) | | | |
| **Delivery** NO (%) | | | | | | | |
| Natural vaginal | 138 (92.0) | 165 (90.2) | 125 (91.2) | 110 (90.9) | 0.950 | Ref. (1) | 0.691 |
| Cesarean section | 12 (8.0) | 18 (9.8) | 12 (8.8) | 11 (9.1) | | 1.16 (0.56–2.41) | |
| **Fetal problems** NO (%) | | | | | | | |
| Post-dated | 1 (0.7) | 1 (0.6) | 2 (1.5) | 2 (1.7) | 0.754 | 2.45 (0.31–19.51) | 0.396 |
| Prematurity | 7 (5.0) | 8 (4.7) | 3 (2.3) | 3 (2.5) | 0.502 | 1.15 (0.41–3.24) | 0.799 |
| Perinatal infection | 1 (0.7) | 1 (0.6) | 0 | 0 | 0.647 | - | - |
| Low birth weight | 2 (1.4) | 4 (2.4) | 0 | 2 (1.7) | 0.387 | 0.99 (0.21–4.78) | 0.992 |
| **Infancy feeding** NO (%) | | | | | | | |
| Breast feeding | 125 (91.2) | 155 (91.7) | 114 (95.0) | 108 (92.3) | 0.673 | 0.52 (0.19–1.41) | 0.198 |
| Dried milk | 24 (17.5) | 26 (15.4) | 14 (11.7) | 15 (12.8) | 0.542 | 0.94 (0.51–1.73) | 0.847 |
| Cow milk | 5 (3.6) | 5 (3.0) | 2 (1.7) | 1 (0.9) | 0.455 | 0.49 (0.14–1.68) | 0.258 |
| Goat milk | 3 (2.2) | 4 (2.4) | 1 (0.8) | 0 | 0.320 | 0.47 (0.13–1.71) | 0.254 |
| Water & Sugar | 3 (2.2) | 5 (3.0) | 1 (0.8) | 0 | 0.215 | 0.53 (0.16–1.80) | 0.310 |
| **Childhood infection** NO (%) | | | | | | | |
| Measles | 33 (25.2) | 55 (33.3) | 34 (30.4) | 31 (29.2) | 0.503 | 0.76 (0.47–1.22) | 0.250 |
| Rubella | 15 (11.5) | 24 (14.5) | 16 (14.5) | 17 (16.0) | 0.769 | 1.06 (0.57–1.96) | 0.863 |
| Chickenpox | 71 (54.2) | 86 (52.4) | 55 (49.1) | 49 (46.2) | 0.616 | 0.91 (0.58–1.41) | 0.663 |
| Mumps | 48 (36.6) | 58 (35.8) | 47 (42.0) | 37 (34.9) | 0.687 | 0.87 (0.55–1.38) | 0.558 |
| **Childhood health problems** NO (%) | | | | | | | |
| Head trauma | 6 (4.4) | 8 (4.7) | 4 (3.3) | 4 (4.1) | 0.944 | 0.76 (0.28–2.09) | 0.592 |
| Hospital admission | 2 (1.5) | 0 | 0 | 1 (1.0) | 0.262 | 0.21 (0.01–3.17) | 0.258 |
| **Climate of birth place** NO (%) | | | | | | | |
| Central Plateau | 127 (82.5) | 171 (90.0) | 122 (87.1) | 103 (79.8) | | Ref. (1) | - |
| Coastal areas of South Caspian Sea | 0 | 3 (1.6) | 0 | 2 (1.6) | **0.044*** | 0.73 (0.14–3.87) | 0.709 |
| Coastal areas of North Persian Gulf | 15 (9.7) | 8 (4.2) | 5 (3.6) | 10 (7.8) | | 0.68 (0.11–4.25) | 0.683 |
| Mountainous regions | 12 (7.8) | 8 (4.2) | 13 (9.3) | 14 (10.9) | | 0.99 (0.16–6.33) | 0.991 |
| **Childhood vaccination** NO (%) | | | | | | | |
| Complete | 124 (86.7) | 147 (81.7) | 116 (87.9) | 108 (92.3) | 0.065 | Ref. (1) | 0.280 |
| Incomplete | 19 (13.3) | 33 (18.3) | 16 (12.1) | 9 (7.7) | | 0.71 (0.38–1.32) | |
| **Adulthood vaccination** (one month prior to diagnosis)NO (%) | 6 (4.1) | 4 (2.1) | 4 (3.0) | 4 (3.3) | 0.787 | 1.27 (0.42–3.86) | 0.671 |
| **Climate of living place** NO (%) | | | | | | | |
| Central Plateau | 134 (87.6) | 179 (94.2) | 128 (91.4) | 110 (85.3) | | Ref. (1) | - |
| Coastal areas of South Caspian Sea | 0 | 0 | 0 | 2 (1.6) | **0.018*** | 0.75 (0.41–1.35) | 0.335 |
| Coastal areas of North Persian Gulf | 13 (8.5) | 6 (3.2) | 3 (2.1) | 9 (7.0) | | 0.69 (0.37–1.29) | 0.248 |
| Mountainous regions | 6 (3.9) | 5 (2.6) | 9 (6.4) | 8 (6.2) | | 0.61 (0.26–1.41) | 0.243 |
| **Sun exposure** NO (%) | 103 (89.6) | 104 (84.6) | 84 (91.3) | 77 (88.5) | 0.449 | 0.74 (0.35–1.57) | 0.435 |
| **Timing of sun exposure** NO (%) | | | | | | | |
| 10–12 A.M. | 63 (55.3) | 60 (48.8) | 54 (59.3) | 42 (48.8) | 0.365 | 0.83 (0.51–1.35) | 0.454 |
| 12–2 P.M. | 53 (46.5) | 49 (39.8) | 35 (38.5) | 41 (47.7) | 0.457 | 0.87 (0.53–1.42) | 0.564 |
| 2–4 P.M. | 52 (45.6) | 54 (43.9) | 37 (40.7) | 30 (34.9) | 0.450 | 0.82 (0.50–1.35) | 0.436 |
| **Sun screen** NO (%) | 32 (23.2) | 41 (25.0) | 28 (22.4) | 21 (18.1) | 0.591 | 0.84 (0.49–1.44) | 0.523 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Dairy consumption** NO (%) | | | | | | | |
| Type: | | | | | | | |
| Milk | 41 (42.7) | 54 (41.9) | 47 (48.5) | 36 (40.0) | 0.666 | 0.82 (0.50–1.34) | 0.422 |
| Yogurt | 46 (48.4) | 70 (54.3) | 56 (57.7) | 51 (56.7) | 0.575 | 1.12 (0.68–1.83) | 0.666 |
| Cheese | 57 (59.4) | 76 (58.9) | 58 (59.8) | 54 (60.0) | 0.999 | 0.97 (0.59–1.61) | 0.914 |
| Timing: | | | | | | | |
| Daily | 92 (62.6) | 112 (60.5) | 73 (56.2) | 72 (61.5) | Ref. (1) | | 0.863 |
| Weekly | 55 (37.4) | 73 (39.5) | 57 (43.8) | 45 (38.5) | 0.721 | 0.96 (0.64–1.45) | |
| **Conserved food** NO (%) | | | | | | | |
| Less than 3 times a week | 146 (98.6) | 181 (98.4) | 121 (96.0) | 113 (95.0) | 0.176 | Ref. (1) | 0.383 |
| More than 3 times a week | 2 (1.4) | 3 (1.6) | 5 (4.0) | 6 (5.0) | | 1.85 (0.47–7.31) | |
| **Microwave use** NO (%) | | | | | | | |
| Less than 3 times a week | 57 (56.4) | 67 (52.3) | 51 (57.3) | 51 (57.3) | 0.853 | Ref. (1) | 0.339 |
| More than 3 times a week | 44 (43.6) | 61 (47.7) | 38 (42.7) | 38 (42.7) | | 0.79 (0.49–1.28) | |
| **Smoking** NO (%) | | | | | | | |
| Active | 8 (5.2) | 16 (8.5) | 14 (10.1) | 21 (16.8) | **0.012*** | 1.34 (0.68–2.63) | 0.402 |
| Passive | 39 (26.2) | 42 (22.8) | 28 (20.3) | 29 (24.2) | 0.692 | 0.79 (0.47–1.34) | 0.384 |
| Amount (pack year)median (IQR) | 10 (12.5) | 15 (11.6) | 7 (7.5) | 30 (30) | 0.084 | 0.95 (0.87–1.04) | 0.271 |
| **Supplementation** NO (%) | | | | | | | |
| Vitamin D | 14 (10.6) | 23 (13.4) | 18 (15.7) | 14 (13.1) | 0.708 | 1.16 (0.61–2.18) | 0.657 |
| Calcium | 20 (15.2) | 23 (13.4) | 21 (18.3) | 11 (10.5) | 0.404 | 0.86 (0.46–1.60) | 0.623 |
| **Comorbidities** NO (%) | | | | | | | |
| Diabetes Mellitus | 0 | 0 | 0 | 1 (0.9) | 0.296 | - | - |
| Thyroiditis | 8 (5.9) | 15 (9.0) | 6 (4.7) | 3 (2.6) | 0.132 | 0.57 (0.23–1.44) | 0.234 |
| Hypertension | 3 (2.2) | 5 (3.0) | 5 (3.9) | 2 (1.7) | 0.730 | 0.53 (0.17–1.66) | 0.273 |
| Inflammatory bowel disease (IBD) | 2 (1.5) | 1 (0.6) | 1 (0.8) | 1 (0.9) | 0.874 | 0.60 (0.12–3.16) | 0.551 |
| Migraine | 23 (17.2) | 16 (9.7) | 19 (14.7) | 10 (8.5) | 0.106 | 0.55 (0.30–1.04) | 0.065 |
| Collagen vascular diseases | 0 | 3 (1.8) | 1 (0.8) | 0 | 0.213 | 0.45 (0.06–3.42) | 0.442 |
| Asthma | 2 (1.5) | 3 (1.8) | 0 | 2 (1.7) | 0.522 | 0.78 (0.09–6.96) | 0.822 |
| Seizure | 0 | 0 | 0 | 1 (0.9) | 0.302 | - | - |
| **Family history of MS** NO (%) Positive | 25 (17.7) | 24 (14.3) | 32 (24.1) | 20 (18.7) | 0.189 | 1.15 (0.55–2.42) | 0.708 |
| **Family history of other comorbidities** NO (%) | | | | | | | |
| Diabetes Mellitus | 25 (21.6) | 45 (31.3) | 22 (20.0) | 23 (23.0) | 0.143 | 1.08 (0.62–1.88) | 0.794 |
| Thyroiditis | 21 (15.4) | 26 (16.0) | 21 (16.8) | 21 (18.1) | 0.949 | 1.55 (0.84–2.86) | 0.164 |
| Inflammatory bowel disease (IBD) | 13 (9.8) | 15 (8.9) | 15 (12.1) | 15 (12.8) | 0.688 | 1.29 (0.70–2.39) | 0.413 |
| Migraine | 27 (20.5) | 31 (19.1) | 30 (24.6) | 23 (21.1) | 0.729 | 1.23 (0.73–2.06) | 0.444 |
| Collagen vascular diseases | 16 (11.6) | 21 (13.5) | 23 (18.5) | 10 (8.8) | 0.146 | 1.23 (0.69–2.18) | 0.481 |
| **Adulthood health problems** NO (%) | | | | | | | |
| Trauma | 8 (5.3) | 16 (8.6) | 11 (8.0) | 11 (8.8) | 0.657 | 1.34 (0.62–2.87) | 0.456 |
| Stressful events | 100 (66.2) | 116 (62.4) | 84 (60.4) | 81 (64.8) | 0.744 | 1.01 (0.66–1.54) | 0.972 |
| **Pet exposure** NO (%) | 27 (18.0) | 34 (18.4) | 22 (16.7) | 23 (18.9) | 0.971 | 1.05 (0.62–1.79) | 0.847 |
| **Toxin Exposure** NO (%) | 2 (1.3) | 4 (2.2) | 4 (3.0) | 3 (2.4) | 0.803 | 1.32 (0.43–4.08) | 0.631 |
| **Heavy metals exposure** NO (%) | | | | | | | |
| Mercury | 0 | 2 (1.1) | 1 (0.8) | 0 | 0.430 | 1.14 (0.13–9.83) | 0.906 |
| Lead | 0 | 1 (0.5) | 1 (0.8) | 1 (0.8) | 0.760 | 2.66 (0.37–19.16) | 0.331 |
| **Working in mines** NO (%) | 0 | 0 | 0 | 1 (0.8) | 0.280 | - | - |

# Chi Square, Fisher's Exact or Mann-Whitney U-test; ¶Ordinal logistic regression model; * Statistically significant; Adjustment is made for onset age, sex, education, ethnicity, income and marital status.

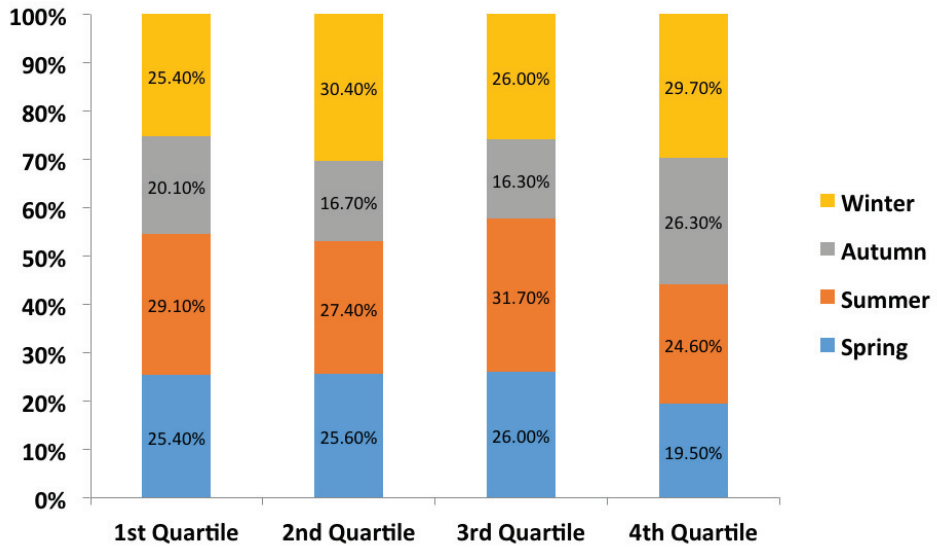


**Figure 1.** Comparison of the season of symptoms onset between multiple sclerosis patients with different quartiles of progression index (Chi square = 7.72, *P* = 0.562).

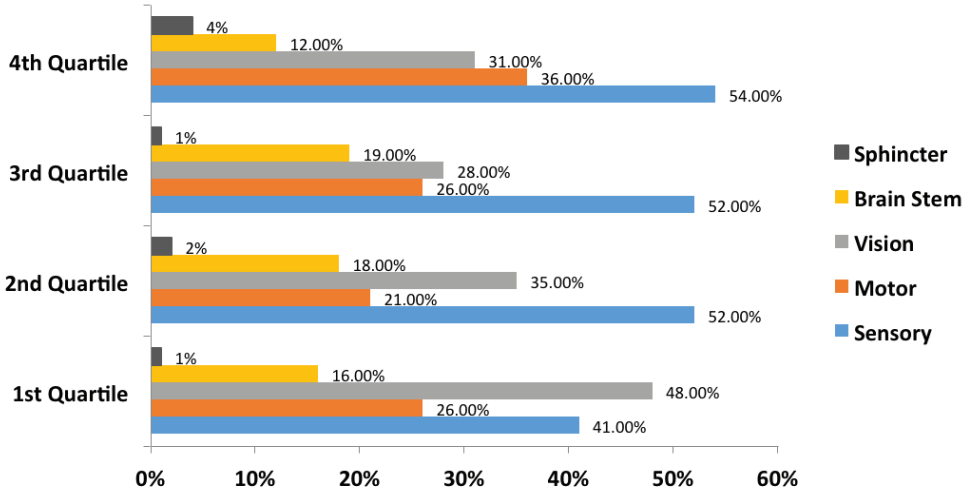


**Figure 2.**Comparison of the frequency of sensory (*P* = 0.118), motor (*P* = 0.025), vision (*P* = 0.001), brain stem (*P* = 0.364) and urinary sphincter (*P* = 0.217) symptoms between multiple sclerosis patients with different quartiles of progression index.

nitric oxide in cigarette smoke causes mitochondrial damage, oligodendrocyte necrosis and axonal degeneration.[23]

Our findings revealed that age at the disease onset, female gender and marital status might be associated with severity of MS. Previous studies have shown that younger age at the disease onset is less suffering.[24] This might be due to CNS repair and better recovery in younger patients.

The relation between sex and age at the disease onset and disease severity is still under investigation. Horakova *et al.* showed that younger age at the disease onset and female gender are associated with increased number of relapses over the 2-year period.[25] Furthermore, other studies have mentioned female sex as a probable risk factor for progression of clinically isolated syndrome (CIS) to MS.[4] On the other hand, multivariate analysis demonstrated that gender did not have a major effect on long term prognosis.[4] Moreover, in primary progressive subtype of MS, the female-to-male ratio is close to one with a small male predominance.[16] Compared to the study by Abedini *et al.* in Iran that showed most patients were married, our results showed an association between marriage and slower disease progression in MS. Although most of patients were married, the severity of disease in this group was less than the singles.[26]

Our results showed no association between calcium and vitamin D supplementation, dairy consumption or sunlight radiation and MS progression. Over 5 years of follow-up, Ascherio *et al.* demonstrated that higher serum 25(OH)D levels were related with

a lower degree of MS activity, clinical progression, MRI lesion load and brain atrophy.[27] On the other hand, Mandia *et al*.[11] showed an inverse association between lower vitamin D concentrations and disease severity. Furthermore, studies about vitamin D supplementation or dairy consumption and MS severity are rare. Munger KL *et al.* in a prospective study on dietary vitamin D intake reported that high vitamin D levels are related with a protective effect against MS progression.[28] Vitamin D, known as a modulator of the immune system, suppresses inflammatory cytokines such IL-17 and IFN-γ and stimulates Tregs.[29]

Previous studies have indicated that more than two hours sunlight exposure daily is related with less MS progression.[29] On the other hand, Woolmore JA *et al.* demonstrated no association between UVR exposure and MS severity.[30] The probable mechanism of sunlight radiation effect is explained by vitamin D production and then IL-10, TNF-α and Treg cells, all of which have anti-inflammatory effects.[31]

Our results showed no association between immunization and MS severity. Other studies demonstrated no association between vaccines against tetanus and risk of MS exacerbation. [32] On the other hand, some studies have reported an association between Hepatitis-B vaccine and both new and relapse cases of MS.[33] It may be concluded that immunization may initiate MS exacerbation in the same way that vaccines trigger other autoimmune diseases such as the Guillain-Barre syndrome.[34–35] Overall, more studies on large scale are required to determine the effect of vaccination on MS severity.

Studies about initial symptom as prognostic factor have yielded inconsistent results. Our results demonstrated a significant correlation between vision and motor symptoms as an initial symptom and PI. We could not find any association between sphincter symptoms and MS severity. Similar to our study, Trojano *et al.* found no association between sphincter symptoms and disease severity.[36] On the other hand, some studies have illustrated sphincter involvement as an initial symptom increasing the risk of disease severity.[37–39]

Regarding motor symptoms, similar to our study, some studies showed that motor symptoms at the disease onset increased the risk of disease severity.[37–40] It seems that different types of initial symptoms are not strong predictors for MS severity.

Studies on factors affecting MS exacerbation and severity are limited. Although previous studies have evaluated some factors such infection[41] or latitude,[42] we could not find more factors in the present study. It seems that prospective studies on large scale are required.

The strengths of this study were the large sample of patients from different parts of Iran and different subtypes of MS. The present study had some limitations, including its cross-sectional design and validity information. Similar to other cross-sectional studies, there is no evidence that the exposure caused the outcome, because this study assessed the exposure and outcome simultaneously. Therefore, causality is unclear. In addition, this type of study has the risk of misclassification bias. The exact time at which the pathological changes of MS started was not clear. To reduce the risk for this misclassification bias, a time lag between the history of exposure and MS diagnosis was considered to search for the probable factor when any MS-related symptom had not started yet. However, this was not completely preventive.

Another limitation of this study was the ambiguous association between MS severity and some factors such as education, whether high education level is a factor for MS severity or patients with less disability are likely to get more educated. However, other risk factors of MS should be evaluated. In addition, genetics and other environmental factors have important roles in the incidence of MS.[43]

In order to increase the validity of the information, the checklist answer was compared with patients' families and their medical documents. Moreover, any individual with memory and/or cognition dysfunction was excluded. Therefore, the risk for recall bias is not high in this study.

In conclusion, our results reveal that younger age at disease onset, female gender, marital status and smoking could be correlated with MS severity. Furthermore, obtaining high school diploma, university education, white collar and pink collar occupation seemed to be related with decreased MS severity. Previous data have shown an increase in the incidence and prevalence of MS in Iran.[6] Due to the high cost and possible disability of MS, identification of factors influencing MS severity is important. Prospective studies on large scale are required for further proof of these associations.

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