Environmental Risk Factors for Multiple Sclerosis: A Case-control Study in Kerman, Iran

Abstract

Background: Several studies have addressed the environmental risk factors for multiple sclerosis (MS). Concerning contradictory results and change of epidemiologic patterns and the role of environmental factors, in the present study, some risk factors, especially environmental factors, on MS were studied. **Materials and Methods:** This was a retrospective case-control study conducted among 120 patients with MS and 360 healthy individuals in Kerman, Iran. Inclusion criteria included (1) MS disease, diagnosed by a neurologist according to the McDonald criteria, and (2) tendency to participation in the study. Exclusion criteria included (1) suffering from cognitive disorders; (2) incomplete questionnaire; and (3) continuous migration. Data were collected using a questionnaire consisting of personal information and some environmental factors. Data were analyzed using descriptive and inferential statistics. **Results:** The results showed that diet was associated with a higher risk of MS with the odds ratio (OR) of 14.46 and 95% confidence interval (CI) of 3.02–69.21 ($p < 0.001$) for vegetarian and OR of 11.74, 95% CI of 4.66–29.57 ($p < 0.001$) for animal diets. Similarly, vitamin D supplementation contributed to MS risk (OR: 2.27, 95% CI: 1.32–3.89; $p < 0.001$). In contrast, history of using cow's milk during infancy resulted in a lower risk of MS (OR: 0.33, 95% CI: 0.20–0.52, $p < 0.001$). **Conclusions:** This study suggests that different lifestyles including using cow's milk during infancy and avoiding only vegetarian and animal diets may reduce MS risk in southeastern Iran. More studies are suggested to investigate the controversial finding of the negative effect of vitamin D supplementation in this area.

Keywords: Case-control study, environmental exposure, multiple sclerosis

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Introduction

Multiple sclerosis (MS) is an autoimmune, chronic, and progressive disease of the central nervous system.[1-3] It is considered as one of the most prevalent disabling diseases in young people, especially women.[4] It has been estimated that more than two million individuals suffer from this disease throughout the world.[2] Prevalence of MS on a global scale varies between 2 and 150 per 100000 people. Countries such as USA, Canada, and New Zealand are considered to be regions with a high prevalence of MS.[4] MS prevalence is different across Asian countries. Western Asia and the Middle East are encountering higher number of MS cases in comparison with east and southeast areas.[5] There are no accurate statistics about the number of people suffering from MS in Iran. According to the reports released by the ninth International MS Congress in Iran

in 2012, MS prevalence is 60 per 100000 people.[6] Ebrahimi and Sedighi (2013) reported that the prevalence of MS in Kerman province is 31.5 per 100000 people and is 57.5 per 100000 people in the city of Kerman.[7]

Although the main cause of MS has not been identified yet,[8] studies have shown that different factors may cause developing MS. Some of these include genetic, environmental, climatic, immune, viral factors, high fat diet, lack of vitamin D, lack of sunlight exposure, exposure to organic solvents, smoking, and contact with pet animals.[4,9-11] Also, according to some evidence in infancy, cow's milk is the most frequently encountered dietary allergen and may induce autoimmune processes such as MS.[12] In fact, the definite effects of these factors have not been proved yet. For example, there is inconsistent evidence regarding animals including pets as risk factors for the development of MS.[13] Due to the fact that lifestyle has considerably

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changed during the recent decade in the world,[14] it could lead to the change of MS epidemiological risk factors patterns.[15] Such changes could be unique in each region of the world, indicating the necessity of new studies. Also, given the controversial findings of environmental risk factors, the relatively high prevalence of MS in Kerman and the presence of some environmental factors such as high cigarette smoking,[16] the present study aimed to determine the risk factors for developing MS, especially environmental factors, in Kerman, Iran.

Materials and Methods

This was a retrospective case-control study performed during 2015 and 2016. This study was conducted in three educational hospitals and MS charity association in Kerman. Kerman is the largest city in southeastern Iran, with a population of more than 722,000 (latitude 25.55 to 32°N).

Convenience sampling was done for both control and case groups. Inclusion criteria included (1) MS disease diagnosed by a neurologist according to the McDonald criteria[17] and (2) tendency to participate in the study. Exclusion criteria included (1) suffering from cognitive disorders; (2) incomplete questionnaire; and (3) continuous migration (i.e., changes of living place in each year). Furthermore, the control group was selected among individuals who were matched with the patient group regarding age (±2 years) and gender. The control group included patients' companions in other wards of educational hospitals of Kerman. It is noteworthy that the ratio of the control group to case group was 3:1. Sample size was 480 with a confidence level of 95% and power of 80% (MS group = 120 and healthy group = 360). For illiterate individuals, the questionnaire was completed by interview in three Kerman educational hospitals and MS charity association. In the present study, the response rate for MS patients was 71.42%; five questionnaires were omitted because of missing data. In control group, the response rate was 86.11% and no questionnaire was omitted due to missing data.

Data was collected using a researcher-made questionnaire consisting of two parts: (1) sociodemographic characteristics including age, birth season, gender, marital status, education, job, living place, and income; (2) information related to disease and its environmental risk factors including smoking, second smoker, opium user, alcohol user, sunlight exposure, having chronic diseases, use of vitamin D supplementation before developing disease (yes/no), diet, and use of cow's milk in childhood.[4,9-12] To determine content validity the questionnaire was given to five neurologists and faculty members. Test-retest was used to determine the reliability. The questionnaire was given to 20 individuals and intraclass Correlation (ICC) was calculated which was excellent (ICC = 0.93).

Data were analyzed by SPSS (version 19, SPSS Inc., Chicago, IL, USA). Descriptive statistic (frequency distribution tables, percentage, mean, and standard deviation) was applied to describe sample demographic characteristics. Chi-square and Mann–Whitney U-test were performed to compare study variables between the two groups. Univariate and multivariate logistic regression were used to determine the association between significant variables and the risk of MS. Significance level of the p value was considered to be 0.05.

Ethical considerations

Kerman University of Medical Sciences approved this project (ethical code:94/115). After approval, permission was issued to the management of educational hospitals and MS charity association. The researcher offered some oral information to the participants including the goals and objectives of the study. The confidentiality and anonymity of the data was maintained. Participants were free to withdraw from the study at will. Written informed consent was taken from all individuals.

Results

In total, 120 patients and 360 healthy subjects participated in the study. The two groups were matched according to age, sex, marital status, birth season, and job [Table 1]. Even though most patients (73.30%) and most healthy subjects (55.80%) were educated (above diploma degree), there was a significant difference between the two groups in this regard. Approximately 9.20% of patients compared to 3.30% of healthy subjects lived in a village, which was statistically significant. In addition, family income was significantly different between two groups [Table 1]. The mean (SD) year of having MS was 37.73 (32.14) months (minimum = 1 month to maximum = 180 months; 18.30% one year, 33.30% two years, 17.50% three years, 11.70% four years, 4.20% five years and the rest more than five years).

Table 2 presents the differences between two groups regarding lifestyle characteristics and some probable environmental risk factors. According to the results, there were significant differences between the two groups in case of sunlight exposure, history of chronic illness, diet, vitamin D supplementation, and use of cow's milk in infancy [Table 2].

For further evaluation of significant variables, we used logistic regression. First, univariate logistic regression was calculated. We used further multivariate logistic regression. According to multivariate logistic regression, educational level, family income, diet, vitamin D supplementation, and use of cow's milk were associated with a risk of MS [Table 3]. The results showed that only vegetarian diet and only animal diet were related to higher risk of MS in comparison with vegetarian-animal diet. People who used vitamin D supplementation were 2.27 times more at risk of MS than people who did not. Furthermore, participants who were used to drinking cow's milk in childhood were at lower risk of MS compared with those who were not

Variables	Multiple sclerosis group (n=120)		Healthy group (n=360)		Statistics test	p
	Frequency / Mean (SD)	Valid Percent	Frequency / Mean (SD)	Valid Percent		
Age (yr)	30.92 (3.51)		31.56 (5.51)		$Z = -0.69$	0.49
Sex						
Female	97	80.80	270	75.00	$\chi^2=1.70$	0.19
Male	23	19.20	90	25.00		
Marital status						
Single	51	42.50	137	38.10	$\chi^2=4.65$	0.10
Married	54	45.00	198	55.00		
Others	14	11.70	25	6.90		
Birth season						
Spring	28	24.80	103	28.70	$\chi^2=1.97$	0.58
Summer	44	38.90	121	33.70		
Autumn	26	23.00	75	20.90		
Winter	15	13.30	60	16.70		
Job						
Unemployed	65	54.2	156	43.30	$\chi^2=4.35$	0.23
Employed	22	18.30	86	23.90		
Self-employed	32	26.70	115	31.90		
Retired	1	0.80	3	0.80		
Educational level						
Under diploma	4	3.30	64	17.80	$\chi^2=18.16$	<0.001
Diploma	32	23.30	95	26.40		
Above diploma	88	73.30	201	55.80		
Living place						
City	109	90.80	348	96.70	$\chi^2=6.71$	0.01
Village	11	9.20	12	3.30		
Family Income (monthly)						
<357 Dollars	23	19.30	77	21.40	$\chi^2=26.75$	<0.001
238 to 357 Dollars	81	68.10	156	43.30		
>357 Dollars	15	12.60	127	35.30		

used to doing so [odds Ratio (OR): 0.33, 95% confidence interval (CI): 0.20–0.52]. Note that the logistic model had an acceptable goodness-of-fit according to Hosmer and Lemeshow test ($\chi^2 = 1.40$, df = 7, and p value = 0.98).

Discussion

In the present study, both groups were matched regarding age and gender. Based on the results of the study, no significant differences were found between both groups regarding marital status, birth season, and job. Using multivariate logistic regression results showed no significant differences between groups concerning primary and secondary smoking (passive smoker), opium user, alcohol user, the level and duration of sunlight exposure, living place, and history of chronic illness. Given controversial results about smoking, many studies have shown that smoking increases the risk of developing MS,[18-21] while some other studies suggest that smoking had no significant effect on developing MS.[12,22] Therefore, this result is not in agreement with many studies. This contradiction may be because of a low number of smokers

in this study. This contradiction can be probably due to the low number of cigarettes per day and the short history of smoking in most participants. Drinking alcohol and drug abuse may increase the risk of MS,[23,24] but it is not popular among Iranian women. Therefore, such results are not in agreement with the literature. In addition, studies suggest a protective effect of exposure to sunlight in MS.[25-27] Evidence indicates that exposure of the body to sunlight can increase synthesis of vitamin D. High level of vitamin D reduces the risk of developing MS.[28] However, the present study indicates that exposure to sunlight has no significant effect on reducing the development of MS. Given that both study groups were chosen from one place with the same sociocultural background and similar clothes, it can be acceptable that the level of exposure to sunlight is similar for both groups. Furthermore, Kerman province has a warm and dry climate so that most people are commonly exposed to sunlight. In addition, evidence shows that chronic disorders are considered to be one of the risk factors for developing MS.[29] However, the results of the present study did not approve this evidence. It may

Table 2: Compression of risk factors between two groups

Variables	Multiple sclerosis group (*n*=120)		Healthy group (*n*=360)	Statistics test		*p*
	Frequency	Valid Percent	Valid Percent			
Cigarette smoking						
Yes	16	13.30	52	14.40	χ^2=0.09	0.76
No	104	86.70	308	85.60		
Passive smoker						
Yes	32	26.70	123	34.20	χ^2=2.32	0.13
No	88	73.30	237	65.80		
Opium user						
Yes	7	5.80	34	9.40	χ^2=1.50	0.22
No	113	94.20	326	90.60		
Alcohol user						
Yes	7	5.80	33	9.20	χ^2=1.31	0.25
No	113	94.20	327	90.80		
Sun exposure surface						
Face and two hands	99	82.50	249	69.40	χ^2=7.82	0.005
More than face and hands	21	17.50	110	30.60		
Sun exposure (time)						
<30 min	6	5.00	38	10.60	χ^2=5.49	0.14
30-60 min	43	35.80	133	36.90		
60-120 min	51	42.50	120	33.30		
>120 min	20	16.70	69	19.20		
Family history of MS						
Yes	34	28.30	83	23.10	χ^2=1.36	0.24
No	86	71.70	277	76.90		
History of chronic illness						
No	89	74.20	215	59.70	χ^2=8.09	0.004
Yes	31	25.80	145	40.30		
Diet						
Both vegetarian and carnivore	74	61.70	279	77.90	χ^2=65.57	<0.001
Vegetarian	8	6.60	3	0.80		
Carnivore	25	20.80	8	2.20		
Less vegetarian more carnivore	11	9.20	50	14.00		
More vegetarian less carnivore	2	1.70	18	5.10		
Vitamin D supplementation						
Yes	46	38.30	98	27.20	χ^2=5.29	0.02
No	74	61.70	262	72.80		
Use of cow's milk in infancy						
Yes	54	45.00	259	71.90	χ^2=28.80	<0.001
No	66	55.00	101	28.10		
Exercise						
Yes	26	21.70	104	28.90	χ^2=2.38	0.12
No	94	78.30	256	71.10		
Stress						
Low	11	9.20	32	8.90	χ^2=2.45	0.29
Moderate	90	75.00	247	68.60		
High	19	15.80	81	22.50		

be due to the way of measuring this variable in the present study. More study with precise monitoring is needed to confirm this result.

Concerning results, vitamin D supplementation does not have a protective effect rather it significantly increases the risk of developing MS. However, evidence indicates that vitamin D reduces recurrence of MS.[30] Lack of vitamin D supplementation has been questioned in the present study while the concentration of serum level of vitamin D has been studied in some other studies,[31-34] which is a more accurate criterion than its consumption. In the present study, only vegetarian diet or only animal diet had a significantly higher association with risk of MS. Evidences showed that vegetarian diet is rich in fiber, low calorie, and may reduce

Table 3: Univariate logistic regression and multivariate logistic regression model of some variables

Variables	Univariate logistic regression			Multivariate logistic regression		
	OR*	CI**	*p*	OR	CI	*p*
Educational level						
Under diploma	1			1		
Diploma	4.72	1.58-14.09	<0.001	5.98	1.82-19.61	0.003
Above diploma	7.00	2.47-19.83	<0.001	9.04	2.84-28.78	<0.001
Living place						
City	1			-	-	-
Village	2.93	1.26-6.82	0.01			
Family income (monthly)						
>357 Dollars	1			1		
238 to 357 Dollars	0.93	1.24-5.14	0.01	0.81	0.42-1.58	0.54
<357 Dollars	1.48	2.41-8.00	<0.001	0.18	0.08-0.43	<0.001
Sun exposure surface						
More than face and hands	1			-	-	-
Face and two hands	2.08	1.24-3.51	<0.001			
History of chronic illness						
Yes	1			-	-	-
No	1.94	1.22-3.07	<0.001			
Diet						
Both vegetarian and carnivore	1			1		
Vegetarian	10.05	2.60-38.84	<0.001	14.46	3.02-69.21	0.001
Carnivore	11.78	5.10-27.19	<0.001	11.74	4.66-29.57	<0.001
Less vegetarian more carnivore	0.83	0.41-1.67	0.60	0.93	0.42-2.07	0.86
More vegetarian less carnivore	0.42	0.10-1.85	0.25	0.50	0.11-2.41	0.39
Vitamin D supplementation						
No	1			1		
Yes	1.66	1.08-2.57	0.02	2.27	1.32-3.89	0.003
Use of cow's milk in childhood						
No	1			1		
Yes	0.32	0.21-0.49	<0.001	0.33	0.20-0.52	<0.001

*Odds ratio **Confidence interval

vitamin D input.[3] Because serum level of vitamin D has a protective effect on MS,[31] such a diet may increase the risk of developing MS, considering that obesity is one of the risk factors of MS. According to the controversial findings on diet and vitamin D and their effect on MS, it is suggested to conduct further research with more accurate measuring tools.

Our results indicated that the use of cow's milk in childhood was significantly higher in the control group than the case group. Therefore, this factor has a protective effect on the risk of MS. In contrast with our results, some studies results showed the increased risk of MS in adults that consumed cow's milk in infancy.[12] Consumption of cow's milk protein may cause an auto-immune process before maturity resulting in increasing the risk of MS in adults. Despite this, another study indicated that the use of cow's milk in childhood did not increase the risk of MS.[35] This disagreement may be due to recall bias and retrospective nature of our study.

One of the important limitations of our study is convenience sampling that reduced generalization of the results. Therefore, it is suggested that future studies to be conducted by the random sampling method and the other

limitation were the retrospective nature of this study, which may cause recall bias. However, we requested our participants to respond to questions carefully and we paid attention to their comfort in replying to the questionnaire.

Conclusion

The results of this study showed the protective effects of consuming cow's milk in infancy and the risky effect of diet (vegetarian and animal diet) and vitamin D supplementation in developing MS. Concerning contradictory results of this study in comparison with previous studies, it is suggested that other case-control studies should be conducted in this region. Future studies with a more accurate measurement of variables such as time of vitamin D consumption and its serum concentration should be conducted to determine whether a true association exists between vitamin D and the risk of MS in this region. Results of this study are useful for designing interventions to empower nurses for educating patients regarding healthy lifestyle for preventing MS. Moreover, health systems policies should be adopted to promote community health.

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