Iranian Journal of Neurology

Official Journal of Iranian Neurological Association

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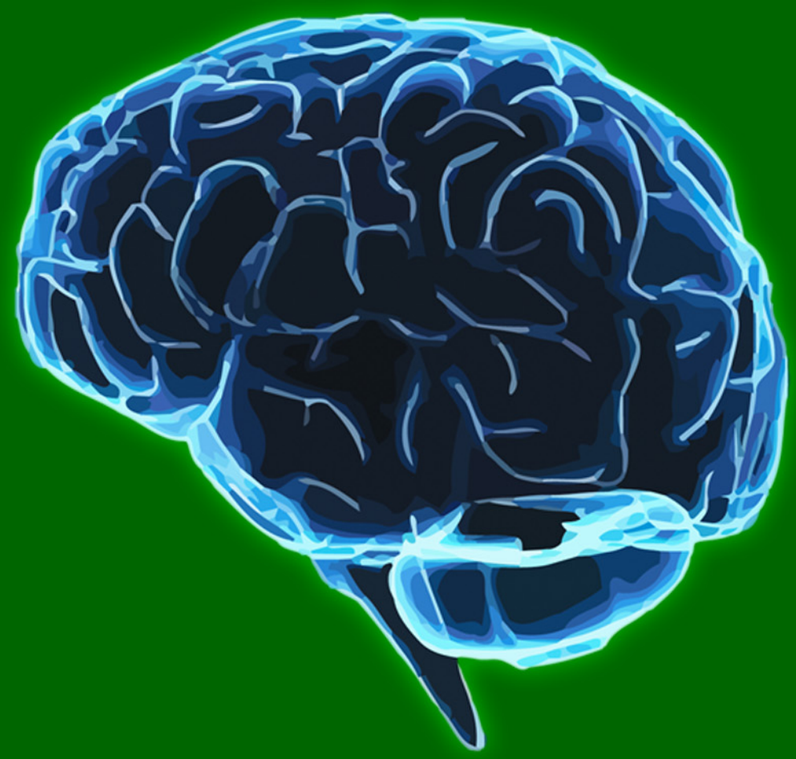

Volume 16, Issue 1, Winter 2017

Volume 16, Issue 1, Winter 2017
ISSN: 2008-384X
ijnl.tums.ac.ir

Iranian Journal of Neurology

Volume 16, Issue 1, Winter 2017

Email: ijnl@tums.ac.ir **http://ijnl.tums.ac.ir**

Copy Edit, Layout Edit, Proof Reading, Design, Print and Online Support: FaRa Publishing House (Farzanegan Radandish)
http://farapub.com Email: farapublications@gmail.com
Tel/fax: +98 31 32224335, +98 31 32224382

Indexed in

- PubMed,
- PubMed Central,
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The Iranian Journal of Neurology is dedicated to the Iranian Neurological Association. The journal is a peer-reviewed journal published quarterly and publishes neurological experiences in basic or clinical fields in *English Language*. *The Iranian Journal of Neurology* aims to publish manuscripts of a high scientific quality representing original clinical, diagnostic or experimental works or observations in neurological sciences. Papers in *English* are welcomed, particularly those which bring novel information and researches in clinical or basic fields from the neurological disorders. All received manuscripts coving the scope of the journal will be evaluated by properly competent referees.

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Original Article
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Details

Original and review papers: The maximum length of original and review papers (including tables and figures materials) is 3000 words.

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Each section of the paper should begin on a new page

The manuscript must include:

Page 1: Title Page

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Page 3 and subsequent pages: manuscript body including Introduction, Materials and Methods, Results, Discussion, Conclusion, References, Tables, Figures

1. Title page:

Title page should contain paper title, full names of authors, authors' place of work, full name and address of the corresponding author (including e-mail address and telephone number), given in that order.

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Proofs: The proofs will be sent via email and must be accordingly corrected and get back within 48 hours.

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Original Paper

Iran J Neurol 2017; 16(1): 1-6

Comparison of the effects of low dose interferon and high dose interferon on reduction of the number and size of plaques in patients with Multiple Sclerosis: A historical cohort

Received: 22 Sep 2016
Accepted: 10 Dec 2016

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Keywords
Multiple Sclerosis; High Dose Interferon; Low Dose Interferon; Magnetic Resonance Imaging

Abstract

Background: This study was performed to compare the effects of low dose interferon beta-1 (IFN-β-1) (CinnoVex, 30 mcg) and high dose IFN-β-1 (REBIF, 44 mcg) on the reduction of the number and size of plaques in magnetic resonance imaging (MRI) in patients with multiple sclerosis (MS).

Methods: This historical cohort study, which was performed in 2014 in Sanandaj (western part of Iran). 43 MS patients in two groups were investigated. The first group, which included 19 patients, was treated using high dose IFN (44 mcg) and the second group, which was consisted of 24 patients, was treated using low dose IFN (30 mcg). Patients' data were collected and analyzed by the Stata version 11 software; the analyses were performed using statistical t-test, chi-square test, Fisher test, and logistic regression.

Results: Both drugs were effective in controlling active demyelinating plaque and in preventing plaque activation (P = 0.633). The impact of both drugs in the reduction of the number and size of plaques was evaluated. Based on the results of the MRI, high dose IFN therapy was more effective than the low dose IFN drugs and had a better performance in terms of reducing the number of plaques and in stop-and-recovery (P = 0.039), as well as in reducing the plaque size (P = 0.050).

Conclusion: The high dose IFN therapy was more effective than the low dose IFN therapy in reducing the number and size of brain plaques in patients with relapsing-remitting MS (RRMS).

Introduction

Multiple sclerosis (MS) is a chronic, recurrent inflammatory and demyelinating disease which involves the central nervous system. It mostly affects young women and causes disabilities in patients.[1] There are several different types of MS

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including relapsing-remitting form of MS (RRMS), primary progressive MS, secondary progressive MS, and isolated clinical syndrome.[2]

The first line of treatment for RRMS is consisted of interferon beta (IFN-β) and is glatiramer acetate.[3] They have a good effect on reducing relapse and a variety of disabilities and on magnetic resonance imaging (MRI) criteria. Effect of IFN-β for the treatment of RRMS has been proved. IFN therapy can make its effect via its anti-proliferative effects and reduces the permeability in the blood–brain barrier.

MRI has a high capacity for early diagnosis of MS, particularly if the clinical diagnosis is uncertain, monitoring of treatment, evaluation of disease progression, and response to treatment.[4]

Abnormalities in brain MRI are observed in more than 95% of newly diagnosed patients. There are 5-10 new or large plaques enhanced with gadolinium; on the other hand, T2 lesions show attacks in any patient with RRMS.[5] Brain MRI can show the areas of edema, demyelination, damaged axons, gliosis, and repair of myelin in areas with high signal in T2.[5,6]

Beta IFN compounds include beta IFN-β-1a (Avonex) or low dose IFN and high dose IFN-β-1a (REBIF) and beta IFN-β-b called (Betaseron).[7-10]

IFN-β1a lowers the rate of attacks in MS patients by 33%.

High dose IFN-β-1-a (REBIF) is one of two available formulations of IFN-β1. This drug is used and injected subcutaneously at doses of 22 and 44 μg 3 times a week.[11] Low dose IFN-β-1a (Low dose IFN) is prescribed for intramuscular injection at a dose of 30 mg once a week low dose IFN (CinnoVex is the commercial name of IFN-β-1a and it is manufactured in Iran as the world's third largest manufacturer in the market). It is a biosimilar or biogeneric of Avonex drug.[12]

Some studies have shown that IFN-β compounds can reduce MS attacks, brain atrophy, and the number and volume of brain lesions.[3,11] Other studies have suggested the better effects of low dose IFN-β-A and high dose (REBIF) in reducing plaques in MRI compared with placebo.[13]

Although the low dose IFN drug is used abundantly by MS patients in Iran, few study has been conducted on the effects of the drug (especially CINOVEX) on MS patients in terms of reducing the number of plaques or to compare it with high dose IFN drugs. Furthermore, we assume this survey may be a view about Kurdish patients with MS and an effect of Iranian products of IFN-β-1-a (CinnoVex) on them, which is an important medical issue in our area.

Accordingly, this study examines the impact and efficacy of low dose IFN (CinnoVex) on reducing the number of MRI plaques in MS patients and compares it with high dose IFN (REBIF).

Materials and Methods

This study was a historical cohort and it was conducted on patients with RRMS who were under the treatment with low dose IFN drugs (CinnoVex) or high dose IFN (REBIF); the patients had a profile in the Clinic of Kurdistan University of Medical Sciences or in Sanandaj MS Society, Iran. The study was conducted in 2014.

Although clinical trial with randomization is the best way to test the hypothesis of this study, because of budgetary limitations and the long duration of the project, it became difficult for the researchers to use this method.

IFN-β-1a is sold under the trade names Avonex (Biogen) and Rebif (Merck Serono), (Pfizer); CinnoVex (CinnaGen) is biosimilar of Avonex. Rebif, it is co-marketed by Merck Serono and Pfizer in the US.

CinnoVex is the trade name of recombinant IFN-β-1-a, which is manufactured as biosimilar/biogeneric in Iran. It is produced in a lyophilized form and sold with distilled water for injection. CinnoVex was developed at the Fraunhofer Institute in collaboration with CinnaGen. Dosage of both drugs in this study was 44 mcg (REBIF) and 30 mcg (CinnoVex), respectively.

According to inclusion criteria patients with the following features were included in the study: men and women aged between 18 and 50 years, patients who had been under treatment with low dose or high dose IFN for at least a year before the study, patients who were not pregnant and did not breastfeed a child, patients who were using a reliable contraception method, patients who were identified as RRMS according to the latest amendments to the Revise McDonald 2010 criteria, patients whose Expanded Disability Status Scale (EDSS) was ≤ 5.5, patients who were not concurrently taking drugs that had interference with low dose or high dose IFN, and patients who consented and were able to cooperate until the end of the project. Exclusion criteria included the following: patients whose

Table 1. Association of Interleukin 6 (IL-6) level with National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS) and other infarcts

Variables	Low dose IFN	High dose IFN	P
Sex [n (%)]			
Male	4 (17)	4 (21)	0.714*
Female	20 (83)	15 (79)	
Age (year) (Mean)	33.58	29.84	0.070**

*Chi-square, **t-test. IFN: Interferon

symptoms were the likely signs of other diseases other than MS, patients who had fully transverse myelitis or bilateral optic neuritis, patients with clinically isolated syndrome, and patients who had enhanced plaque in the initial MRI.

With regarding difference between 2 groups based on the effects of outcome, it was equal to 40% and p_1 = 30% also, with regarding p_1 = 70% with 5% alpha and beta 20% sample size (based on the below formula) was 21 patients for each group.

$$n = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 (p_1(1-p_1) + p_2(1-p_2))}{(p_1 - p_2)^2}$$

Because of our limitation in this study, we considered 24 patients in 1 group and in other group 19 patients entered in the study.

In this study, all patients with RRMS who were under treatment with low dose or high dose IFN and referred to the Neurology Clinics and/or were the member of the MS Society of Sanandaj in 2014 and met the inclusion criteria were enrolled in the study. Data were collected through questionnaires and interviews with patients and conducting MRI at the beginning and end of the project.

To conduct the study first, size and enhanced plaques on initial MRI were recorded. Then again MRI was done after a year, and the results were compared in terms of the number, size, and enhanced plaques. All MRI tests were performed in a specified imaging center. Drug side effects and relapse of disease were measured during the follow ups using questionnaires and interviews with patients and phone calls.

The collected data were entered in STATA 11 (Stata Corporation, College Station, TX, USA) software. Chi-square and Fisher exact tests and logistic regression were used for analysis of data. The researchers in this study were committed to the principles of research ethics and observed research ethics issues for patients.

Results

Patients

A total of 43 patients were enrolled in this study, and 24 patients (55.8%) were assigned to the group treated with low dose IFN and 19 patients (44.2%) were assigned to the group treated with high dose IFN. The mean age of patients treated with low dose IFN was 33.58 years, and the mean age of the patients treated with high dose IFN was 29.84; they had not a statistically significant difference (P = 0.073). Of patients treated with low dose IFN, 20 patients were female (46.51%) and 4 patients were male (9.30%). Of patients treated with high dose IFN, 15 patients were female (34.88%) and four patients were male (9.30%), and there was no statistically significant difference (P = 0.714). Table 1 shows demographic variables in 2 groups of study.

MRI findings and relapse

Based on the results of Fisher exact test, the P value obtained from this relationship (P = 0.039) was significant. In addition to the above test, we also used logistic regression. Based on the results of logistic regression analysis, compared with the low dose IFN therapy, treating patients with high dose IFN (with odds ratio of 5.19 and confidence interval of 2.1-32.4) had a better impact on the reduction of the number of plaques (Table 2).

Table 3 compares the two groups in terms of the impact of drugs on the size of the plaque. During the course of treatment, six patients in each of the two groups suffered from relapse and the difference was not statistically significant (P = 0.633).

Table 2. Comparison of changes in plaque size based on the results of magnetic resonance imaging (MRI) in the two groups treated with low dose interferon (IFN) and high dose IFN

Group	Reduction or stabilization in the size of plaques [n (%)]	Increase in the size of plaques [n (%)]	P
The group treated with low dose IFN	17 (71)	7 (29)	0.059*
The group treated with high dose IFN	18 (95)	1 (5)	

*Fisher exact test. IFN: Interferon

Table 3. Comparison of side effects between two groups

Side effect	Low dose IFN [n (%)]	High dose IFN [n (%)]	χ^2	P
Yes	21 (87.5)	18 (95.0)	0.658	0.417
No	3 (12.5)	1 (5.0)		

IFN: Interferon

Side effects

Of all, 21 patients in the low dose IFN group and 18 patients in the high dose IFN group had some degrees of side effects, and the difference was not statistically significant (P = 0.417).

Table 4 shows the relationship between the levels of reduction in the number of MRI plaques in the two groups. Based on the results of Fisher's exact test, the P value obtained from this relationship (P = 0.048) was significant.

Discussion

The aim of this study was to determine the effect of low dose IFN and high dose IFN and compare their effects on changes of demyelination plaques in brain MRI of patients with RRMS.

Based on the results of this study, compared with low dose IFN, high dose IFN was more efficient in stopping and healing patients in terms of the number of plaques. In addition, compared with low dose IFN, high dose IFN had better performance in stopping and curing patients in terms of the reduction in the size of plaques.

Our study has similarities with some other studies that have been conducted in this field. In Bastianello, et al.'s study,[14] a total of 520 patients with RRMS were selected and were treated using IFN-β-1a with two different doses. The results showed that subcutaneous IFN-β-1a clearly played a role in reducing MRI plaques; in addition, using a higher dose was more effective in the treatment of patients and reduction of the plaques. This study is in line with our study as it showed that higher doses of the drug were more effective. Our study is also consistent with Mori, et al.'s study[15] which showed that high dose IFN had a better impact on the improvement of some patients suffering from disorders caused by MS. The results of Lowery-Nordberg, et al.'s study[16] also confirm our findings; they showed that high dose IFN had an impact on the levels of biological factors such as the PMP (CD31+) and PMP (CD54+) and it was also able to make changes in these markers.

The results of a study that was conducted by Hartung[17] showed that treatment with high dose IFN is more effective for the prevention of relapse and it is the most important indicator. In a study by Schwid, et al.,[18] which was conducted on the effects of IFN-β therapy in the management of relapsing MS, the results were consistent with the results of our study and showed that, compared with IM IFN-βa-1a 30 mcg QW, using SC IFN-β-1a 44 mcg TIW for the treatment of MS patients was associated with a significant reduction in clinical and imaging measures of disease activity over 1-2 years. In addition, the study also showed that patients who changed from low dose QW treatment to high dose TIW treatment experienced more benefits of treatment without a substantial increase in adverse events. The results of our study are different from Li, et al.'s study,[19] as they reported that all types of IFN therapy can make changes in all parameters of the MRI. However, in our study, the difference in IFN dose was clear and that there were differences in the effects of high dose and low dose IFN.

In a systematic review study by Oliver, et al.,[20] which investigated IFN-β treatments in adults with RRMS, the results indicate the high dose IFN therapy was more effective than lower doses in reducing relapse. This finding was not consistent with our results but it was in line with our study in terms of increased number of plaques and plaque stability.

Table 4. Comparison of reductions in the number of plaques based on the results of magnetic resonance imaging (MRI) in the two groups treated with low dose interferon (IFN) and high dose IFN

Group	Reduction or stabilization in the number of plaques [n (%)]	Increase in the number of plaques [n (%)]	P
The group treated with low dose IFN	14 (58)	10 (42)	0.048*
The group treated with high dose IFN	17 (89)	2 (11)	

*Fisher exact test. Based on the results of Fisher's exact test, the P value obtained from this relationship (P = 0.048) was significant.
IFN: Interferon

In a study by Prosperini, et al.,[21] 121 patients with RRMS switched to high dose IFN-β and they were followed up for 2 years. The results of their study showed that switching from the low dose to the high dose IFN-β did not reduce the risk of further relapses or increased disability in the 2-year follow-up period. As a result, the findings of their study were different from ours. However, as in our study, they also recommended further studies to obtain more evidence.[19] Unlike our results, in a study by Etemadifar, et al.[13] no significant difference was observed between low dose IFN and high dose IFN in terms of the reduction in disease relapse.

As one of the limitations of this study, although clinical trial with randomization is the best way to test the hypothesis of this study, due to budgetary limitations and the long duration of the project, it became difficult for the researchers to use this method. A lack of implementation of clinical trials for this study may result in estimation errors. It is recommended to conduct clinical trial studies to investigate the effect of different pharmaceutical brands.

Conclusions

This study has three key messages: At first, the two drugs were similar in terms of reducing disease relapse and complications (including flu-like symptoms, injection site reaction, injection site redness, and slight increase in liver enzymes). In addition, both drugs were effective in controlling active and demyelinating plaques and preventing the activation of plaques. However, high dose IFN-β-1a was more effective in reducing the number and size of MRI plaques in patients with RRMS. Second, it is recommended to conduct more properly designed clinical trials. To better assess the effects of low dose IFN drug especially for Iranian-manufactured IFNs, it is recommended to carry out similar studies with more patients and with a longer time periods to assess the reduction of disability, relapse, and complications and to evaluate the improvements in the results of brain and spinal cord MRI; such studies can also assess the effect of the time of initiating treatment process. Third, as a practical suggestion, it is recommended to use high dose IFN-β-1a for RRMS patients as high dose IFN-β-1a drug is more effective in reducing the number and size of MRI plaques.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The authors thank the MS Society of Kurdistan for their contributions in this study and Research Affairs of Kurdistan University of Medical Sciences for the grant supporting of this study. This article provided based on the medical student thesis submitted to the Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

How to cite this article: Khomand P, Moradi G, Ahsan B, Abtahi S. Comparison of the effects of low dose interferon and high dose interferon on reduction of the number and size of plaques in patients with Multiple Sclerosis: A historical cohort. Iran J Neurol 2017; 16(1): 1-6.

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Iranian Journal of Neurology

Original Paper

Iran J Neurol 2017; 16(1): 7-14

Awareness toward stroke in a population-based sample of Iranian adults

Received: 15 Sep 2016
Accepted: 21 Nov 2016

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Keywords
Awareness; Stroke; Signs and Symptoms; Risk Factors

Abstract

Background: Stroke is the leading cause of death and functional disability. While there have been major advances regarding the management of stroke, a significant proportion of people are still unaware of stroke-related symptoms and risk factors. This study was performed to assess the awareness of stroke's warning signs and risk factors among a sample of Iranian population.

Methods: A total of 649 participants were randomly selected using systematic randomization from the list of telephone numbers obtained from the telephone directory. Demographic characteristics were recorded. Participants were asked to answer questions regarding the awareness about stroke, its warning signs and risk factors.

Results: Patients' mean age was 32.0 ± 12.2 years old, and 56.4% were women. Hypertension and history of stroke were major risk factors, and loss of consciousness, vertigo and ataxia were major warning signs of stroke correctly identified by respondents. Multiple linear regressions showed that age (β = 0.277, P < 0.001), academic level of education (β = 6.41, P = 0.01), housewifery (β = 8.9, P < 0.001), jobs related to medical care (β = 13.17, P = 0.016) and previous information about stroke (β = 18.71, P < 0.001) were significant predictors of the overall awareness about stroke.

Conclusion: The awareness of people about stroke, its risk factors and warning signs were good in this study. The awareness toward stroke can be associated with factors such as age, academic level of education, job and previous information about stroke. Further studies are recommended to program public multimedia and health education in academies and colleges.

Introduction

Stroke is the second cause of death and the leading cause of long-term disability worldwide.[1-3] While there has been a decrease in the incidence of stroke over the past forty years in western countries, the incidence of stroke in the same time period has increased in developing countries.[1,4] Recent study showed a considerably higher incidence of stroke among Iranian population than most other regions of the world.[5] A number of reasons have been suggested to explain the higher incidence and mortality rate in low to middle-income countries,

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including population growth, aging, adoption of a sedentary lifestyle and poor dietary habits, increased disease-related risk factors and lack of public knowledge about stroke.[6-8]

Lack of knowledge regarding main clinical presentations of the disease is a major health problem which leads to prolonged time elapsed from the onset of stroke to hospitalization, late diagnosis and therefore delayed start of appropriate treatment. The knowledge and awareness of people about symptoms, warning signs and risk factors of stroke is crucial to prevent stroke by reducing the number of patients who are at a higher risk of the disease and to help patients to seek immediate medical care and receive timely diagnosis and life-saving treatment by the rapid detection of those at a higher risk of developing neurovascular events.[8,9]

While there have been major advances toward the management of stroke since few years ago, significant proportion of people are still unaware of stroke-related symptoms and risk factors.[10,11] In addition to developing countries,[7,8,10,12,13] in many developed nations like Canada,[14] the USA,[15-19] South Korea,[20] Australia,[21] France,[22] and Denmark[11] lots of stroke patients are not presented to the emergency department in timely manner to receive appropriate treatment due to their inadequate knowledge about major warning signs and risk factors of the disease.[10] Cossi, et al.[8] demonstrated that more than 33.0% of participants were able to recognize at least one stroke symptom, and more than 55.2% were aware of stroke risk factors. They found that paralysis or hemiplegia was the most frequent symptom identified by 34.4%, and hypertension was the major risk factor identified by 34.5% of individuals.

Although abundant studies have been conducted to survey the public knowledge of stroke in western nations, far too little attention has been paid to this important issue in developing countries, especially in Iran where the average age of population, epidemiologic features and risk factors of stroke, availability of information, education, training, and medical care facilities are different. In addition, considering the marked increase in the incidence of stroke in developing countries, especially in Iran,[4,5] findings of such study are of great interest that help us to know the knowledge of our population about stroke and understand the extent of the problem. This will help us to adopt more effective, comprehensive, educational programs to increase the public knowledge of stroke and therefore reduce the burden of stroke. This study was therefore conducted to evaluate the public awareness regarding risk factors and warning signs of stroke among a sample of Iranian population. To the best of our knowledge, this study is the first population-based survey that assesses the level and the factors related to stroke awareness among Iranian population.

Materials and Methods

Guilan province is located in the northern part of Iran and forms the southwest border of the Caspian Sea. The province extends over 14000 km^2 and has inhabitants of about 2.5 million people. Rasht, the capital of Guilan, is the most populous and the largest city along the Caspian Sea coast.

This cross-sectional, population-based telephone survey was carried out between May and July 2012 in Rasht, Iran. The study was approved by the ethic and faculty research committee of the Guilan University of Medical Sciences (GUMS). A total of 649 households were randomly selected using a systematic randomization from the list of telephone numbers obtained from the information service of the telephone directory and then contacted by telephone call. To avoid probable selection bias, the participants were randomly selected from the three socioeconomically different districts of the city. Individuals who were 15 years or more and consented to participate in our research were initially included in the study. The other phone number was substituted when the eligible person was not available on the first phone call to answer our questions. The interviewers first introduced themselves and briefly explained the aim of the study to respondents and then asked them if they were interested to participate in this research. Two medical interns of GUMS were trained and given instructions to implement a telephone interview and clarify any ambiguous question if needed. Respondents' answers were recorded without the direct intervention of interviewers. All questions were closed-ended and were asked in Persian.

The sample size of the study was calculated with a confidence interval (CI) of 95% and an acceptable error of 2.5% on the basis of the study by Borhani Haghighi, et al.[6] in which the knowledge of people about warning signs of stroke was assessed. We calculated that the study can be performed with 649 individuals using the following formula:

$$n = \frac{Z_{1-\alpha}^2 P(1-P)}{d^2}$$

The questionnaire was modified to suit individual local socio-cultural condition. For assessing the content validity of questionnaire, 10 independent academic experts were invited to review questionnaire based on content validity ratio (CVR) indexes and content validity index (CVI). CVR was used for assessing the importance and accuracy of items. Based on Lawshe table, the CVR value of all items were higher than 62%. Therefore, all items considered as necessary items in the questionnaire. CVI was used for assessing congruency of each item. The CVI for each item was in range of 0.7-1.0. CVI less than 0.7 was unacceptable; 0.7-0.8 needed major revision; 0.8-0.9 needed minor revision and modification; and CVI ≥ 0.9 was acceptable without any revision. According to expert's opinions, all questions had high CVI and CVR values for quantitative validity. Reliability of the questionnaire was also assessed using simultaneous method based on results of a pilot study (n = 25) (reliability more than 90%). The internal consistency of the questionnaire as calculated by Kuder-Richardson 20 coefficients was considered acceptable (α = 0.79). Kuder-Richardson 20 coefficients > 0.70 was considered acceptable for internal consistency.

In addition to demographic characteristics (i.e., age, gender, profession, and educational level), the questionnaire composed of 36 questions in three sections. The first section consisted of six closed-ended questions about the source of information and approach to stroke (i.e. stroke in relatives or friends, previous information about stroke, interest to have information about stroke, sources of information, recommended sources of information, and encountering patients with symptoms suggesting stroke). The second section included fifteen closed-ended questions evaluating the awareness of the participants about symptoms and warning symptoms (i.e., numbness or weakness of one side of the body, difficulty speaking or understanding speech, double or blurred vision, severe headache, and dizziness). The third section included fifteen closed-ended questions regarding the awareness of the participants about the risk factors (i.e., hypertension, hyperlipidemia, smoking, obesity, previous stroke, diabetes mellitus (DM), alcoholism, oral contraceptives, heart disease, and positive family history for stroke). The individuals' awareness of stroke warning signs and risk factors is classified into three categories: poor (equal or fewer than 5 correct answers), moderate (6-10 correct answers), and good (more than 10 correct answers). The overall awareness level was defined as a percentage score of the number of correct answers in all sections divided by the total number of answers.

Statistical analysis was done by SPSS for Windows (version 18, SPSS Inc., Chicago, IL, USA). Descriptive data were reported as percentages, frequencies, or mean ± standard deviation (SD). The normality of variable distribution was checked by the Kolmogorov-Smirnov test. Mann Whitney U Test was used to determine differences between mean values. Kruskal-Wallis Test was used to compare the frequencies of variables with more than two groups. Multiple linear regression model was used to examine the predictors of the overall level of stroke knowledge. Variables with a P-value ≤ 0.01 were included in the final stepwise model. P-value less than 0.05 was considered significant.

Results

In this study, 649 subjects with the mean age of 32.0 ± 12.2 years (ranging from 15 to 80 years) were interviewed; and 75.0% of respondents were younger than 43 years old. Women constituted 56.4% of the study population; 29.6% of subjects were self-employed and 51.6% had academic education (Table 1).

Table 1. Demographic data of subjects (n = 649)

Variables	n (%)
Gender	
Male	283 (43.6)
Female	366 (56.4)
Occupation	
Studies	98 (15.1)
Housewife	164 (25.3)
Employee	181 (27.9)
Medical care jobs	14 (2.2)
Self-employment	192 (29.6)
Education	
College education	335 (51.6)
Diploma	179 (27.6)
Less than diploma	135 (20.8)

This study showed that 26.8% of subjects knew someone in their family who had a stroke. About 55.8% of subjects had previous information about stroke and 92.0% were interested to obtain information about stroke.

The most common sources of information in respondents were family (21.1%) and media (17.3%). Most subjects (65.5%) recommended "mass media" as the best source of information about stroke. When encountering a patient with symptoms consistent with stroke, 82.4% of subjects would notify emergency medical systems (EMS); 92.8% of them would refer the patient to a neurologist and 90.9% believed that the patient should immediately be transferred to a specialized healthcare center in less than 3 hours to receive adequate treatment. The source of information and approach of respondents about stroke is shown in table 2.

The awareness of participants toward risk factors and warning signs of stroke is shown in table 3. Hypertension (82.3%) and previous history of stroke (78.6%) were the major factors reported by participants, while oral contraceptive pill (OCP) (12.8%) and DM (38.8%) were not reported commonly. In addition, opium use (82.1%) and depression (91.0%) were the most common factors not correctly identified as major risk factors. The awareness about risk factors was poor in 48.8%, moderate in 39.9% and good in 11.3% of respondents.

Table 2. The source of information and approach of respondents about stroke (n = 649)

Variables	n (%)
Stroke in relatives or friends	
Yes	174 (26.8)
No	475 (73.2)
Previous information about stroke	
Yes	362 (55.8)
No	287 (44.2)
Interest to have an information about stroke	
Yes	597 (92.0)
No	59 (8.0)
Sources of information	
Family members and friends	137 (21.1)
Television and radio	112 (17.3)
Reading book	38 (5.9)
Newspapers	15 (2.3)
Others	240 (37.0)
Multisource	107 (16.5)
Recommended sources of information	
Mass media audiovisual	425 (65.5)
Educational booklets	100 (15.4)
Others	52 (8.0)
Multisource	72 (11.1)
Encounter patients with symptoms suggesting stroke	
Telephone EMS	535 (82.4)
Refer to neurologist	602 (92.8)
Need medical help immediately (before 3 hours)	590 (90.9)

EMS: emergency medical systems

Table 3. Awareness of subjects about risk factors and warning symptoms

Variables	Correct	Incorrect	No information
Risk factors [n (%)]			
Hypertension	534 (82.3)	16 (2.5)	99 (15.3)
Smoking	397 (61.2)	62 (9.6)	190 (29.3)
DM	252 (38.8)	111 (17.1)	286 (44.1)
Hypercholesterolemia	288 (44.4)	361 (55.6)	-
Alcohol abuse	332 (51.2)	72 (11.1)	289 (44.5)
History of cardiac disease	260 (40.1)	128 (19.7)	261 (40.2)
Family history of stroke	477 (73.5)	46 (7.1)	126 (19.4)
Past history of stroke	510 (78.6)	25 (3.9)	114 (17.6)
Aging	482 (74.3)	39 (6.0)	128 (19.7)
OCP	83 (12.8)	184 (28.4)	382 (58.9)
Opium	116 (17.9)	207 (31.9)	326 (50.2)
Peptic ulcer	286 (44.1)	252 (38.8)	286 (44.1)
Hair coloring	20 (32.0)	57 (8.8)	384 (59.2)
Depression	56 (8.6)	328 (50.2)	265 (40.8)
Obesity	327 (50.4)	56 (8.6)	266 (41.0)
Warning symptoms [n (%)]			
Sudden weakness of a leg	279 (43.0)	88 (13.6)	282 (43.5)
Sudden weakness of an arm	268 (41.3)	88 (13.6)	293 (45.1)
Sudden pain of unilateral limbs	141 (21.7)	172 (26.5)	336 (51.8)
Sudden severe abdominal pain	290 (44.7)	44 (6.8)	315 (48.5)
Sudden ataxia and vertigo	418 (64.4)	25 (3.9)	206 (31.7)
Sudden epistaxis	270 (41.6)	99 (15.3)	280 (43.1)
Sudden difficulty to speak	407 (62.7)	41 (6.3)	201 (31.0)
Sudden difficulty to understand	374 (57.6)	44 (6.8)	231 (35.6)
Sudden perspiration	89 (13.7)	245 (37.8)	315 (48.5)
Sudden chest pain	263 (40.5)	96 (14.8)	290 (44.7)
Sudden visual defect in one eye	322 (49.6)	79 (12.2)	248 (38.2)
Sudden and severe headache	330 (50.8)	50 (7.7)	269 (41.4)
Sudden diplopia	278 (42.8)	68 (10.5)	303 (46.7)
Sudden loss of consciousness	424 (65.3)	30 (4.6)	195 (30.0)
Seizure	75 (11.6)	328 (50.5)	246 (37.9)

OCP: Oral contraceptive pill; DM: Diabetes mellitus

Table 4. Results of multiple linear regressions in variables related to subjects awareness

Variables	β-coefficient	SE	P	95% CI of coefficient	
				Upper limit	Lower limit
Age	0.27	0.07	< 0.001	0.13	0.4
Previous information about stroke	18.70	1.59	< 0.001	15.60	21.8
Students	6.41	2.49	0.010	1.51	11.3
Housewives	8.90	1.89	< 0.001	1.51	12.6
Medical care jobs	13.20	5.44	0.016	2.48	23.9

CI: Confidence interval; SE: Standard error

The loss of consciousness (65.5%), as well as vertigo and ataxia (64.4%), were reported as the most common warning signs of stroke. On the other hand, sudden perspiration (86.3%) and unilateral pain in limbs (78.3%) were the most correctly identified incorrect responses. In addition, the weakness of an arm (41.3%) and diplopia (42.8%) were less commonly identified as the warning signs of stroke. Totally, the awareness of stroke warning signs was poor in 51.8%, moderate in 34.8% and good in 13.4% of respondents.

The approach to patients with stroke was not significantly associated with age (P = 0.700), sex (P = 0.345), educational level (P = 0.084), job (P = 0.340), family history of ischemic stroke (P = 0.100), previous knowledge about stroke (P = 0.130), and the source of information (P = 0.060). However, the awareness of people regarding the risk factors of stroke was significantly related to age (P < 0.001), sex (P = 0.029), educational level (P = 0.006), job (P < 0.001), family history of ischemic stroke (P < 0.001), having previous knowledge about stroke (P < 0.001), and the source of information (P = 0.050). Moreover, the awareness of people about stroke warning signs was significantly associated with age (P < 0.001), sex (P = 0.008), educational level (P = 0.004), job (P < 0.001), family history of ischemic stroke (P < 0.001), having previous knowledge about stroke (P < 0.001), and the source of information (P = 0.018).

Totally, the overall mean percentage score of subjects' awareness about risk factors and the warning signs of stroke was 54.4 ± 22.8 (ranging from 3.03 to 93.9). In addition, the total awareness in 75 % of participants was more than 70%. In total, the overall awareness of people about stroke was associated with gender (P = 0.017), educational level (P = 0.016), job (P = 0.001), family history of ischemic stroke (P = 0.001), previous knowledge about stroke (P = 0.001), and the source of information (P = 0.050). Multiple linear regressions showed that age (β = 0.277, P < 0.001), the academic level of education (β = 6.41, P = 0.010), housewifery (β = 8.9, P < 0.001), jobs related to medical care and the previous information about stroke (β = 18.71, P < 0.001) were significant predictors of the overall awareness of patients about stroke (Table 4) as the overall awareness level of subjects increased by 0.27% in proportion to every year of increase in age. It also increased by 18.7% when people had previous information about stroke.

Discussion

To propagate efficient treatment-seeking behavior and to bring the correct message appropriately, the assessment of public needs for information should precede the development and implementation of educational campaigns for the public.[9] The early detection of stroke risk factors and warning signs has an important role in the prevention and management of patients with stroke.[6] The lack of information about stroke can disarrange the prevention programs and delay the rapid medical intervention. This study was the first population-based telephone survey in Iran which assessed the public awareness of stroke, warning signs and risk factors in Rasht. This study has shown that the awareness about stroke, its risk factors and warning signs is adequate, and it can be related to significant factors such as education and the source of information.

Consistent with a study by Borhani Haghighi, et al.,[6] hypertension was a major risk factor reported by our study population. In a study by Alaqeel, et al.,[23] the awareness regarding stroke's risk factor was found to be low and only 33 percent of participants reported hypertension as a risk factor. It seems that in Iran people are appropriately informed about hypertension and its complications. In this study, depression and a history of opium use were incorrectly considered as risk factors by considerable percentage of participants; while DM and hyperlipidemia were not identified as major risk factors. Considering the role of multimedia as one of the most important sources of information in our study,

further public education using various media sources including television, radio, newspaper, magazine, and educational pamphlets is needed to improve the awareness of community regarding stroke's risk factors.

The loss of consciousness as well as vertigo and ataxia were the major warning signs of stroke identified by participants. However, a few number of respondents reported paresthesia and aphasia as warning signs. In addition, sudden chest pain and perspiration were reported by some respondents. In the only community-based, face-to-face interview survey conducted in Iran, Borhani Haghighi, et al. revealed that abdominal pain are one of the most commonly identified symptoms of stroke.[6] While in another study in Korea,[24] participants identified paresthesia as the main warning signs. Therefore, most of the educational efforts in future should be focused on increasing the awareness of Iranian community about stroke's warning signs.

In our study, the mean percentage score of public awareness about risk factors and warning signs was 54.4% and it was more than 70.0% in 75.0% of cases. In a telephone survey by Pancioli, et al. 57% of subjects knew at least one warning sign and 68% of them named at least one risk factor.[18] In the only community-based, face-to-face interview survey in Korea by Kim, et al.,[24] 62.0% reported at least one stroke symptom and 56.0% reported at least one risk factor for stroke in open-ended questioning. In Saudi Arabia, Alaqeel, et al.[23] revealed that 21.7% of the respondents correctly chose ≥ 5 risk factors and made ≤ 1 error and 18.4% of the participants were able to correctly identify ≥ 3 symptoms of the list and make ≤ 1 error.

In another large population-based telephone survey, Sug Yoon, et al.[21] found that 76.2% of Australian individuals could name one or more risk factors of stroke; however, just 49.8% of them could identify at least one stroke warning sign. Moreover, smoking and visual disturbance were two most common risk factors and symptoms of stroke listed by 39.4% and 24.1% of respondents, respectively. The high rate of correct answers in our study is likely to be related to use of closed-ended questions in our questionnaires, in contrast to most previous studies. Therefore, further studies will need to be performed to survey the public awareness when using open ended questionnaire.

In a study by Travis, et al. in the USA, 42.0% of persons would first call EMS if having a stroke.[25] In our study, 82.4% of respondents would immediately call EMS, 92.8% would refer to a neurologist and 90.9% suggested receiving adequate treatment in less than three hours, when they see patients with symptoms suggesting stroke. There are several possible explanations for this relatively high percentage of correct responses, compared to similar studies. First, participation of more educated people in this study. Second, higher general medical knowledge of our population. Third, identifying loss of consciousness as the most common warning sign by participants. It is therefore likely that the fear of loss of consciousness sign alone may be related to high rate of calling EMS in our study.

The most common sources of information in our study were friends and then multimedia; moreover, the highest awareness was seen in respondents that studied books as sources of information. Kim, et al.[24] revealed that the major source of information about stroke was television (59%), and the most reliable sources were the respondents' physicians (55%); however, among the respondents of 20 to 39 years of age, the Internet (37%) was the second greatest source of information. Alaqeel, et al.[23] reported that 49.9% of respondents named mass media as the source of their knowledge. In a study by Stern, et al.[26] in the USA, 657 adults were examined for the effectiveness of the slide/audio community education program lonely or accompanied by facilitation led by a trained individual. They reported that slide/audio program is effective in increasing the knowledge of stroke risk factors, warning signs, and necessary action but facilitation did not significantly affect the short-term acquisition of information.[26] Different findings in source of information can be related to increased number of Internet users; also it showed that multimedia programs can be effective in all developing and developed countries.

This study showed that the overall awareness of people about stroke, its risk factors and warning signs was related to age, academic level of education, job and previous information about stroke. In addition, the awareness of subjects increased 0.3% in proportion to the age increase in every year, and it increased 18.7% when people had the previous information about stroke. Travis, et al. reported that the level of knowledge was higher in females, young adults, subjects with quality education, previous history of stroke or hypertension, smokers and high-

income people.[25] Borhani Haghighi, et al. showed that the attitude and knowledge were related to age, education and income but not to gender and domicile.[6] Stern, et al. expressed that multimedia, family and friends, health professionals and educational campaigns can successfully increase stroke awareness. However, they also showed that race or educational level could not increase the knowledge.[26] These differences can be related to cultural and other influential factors among different nations. According to the important effect of age and educational level in this study, more educational programs, especially in school age, should be planned in this region to increase the level of awareness of students.

As a limitation, limited-sample telephone-based survey instead of face-to-face interview was done in our study. Telephone call can affect the responses of subjects. Although interviewers were trained on how to avoid leading questions, the interviewer bias might have influenced the participant response. In addition, a number of people in this region might not have had access to telephone; thus, people with low socioeconomic status may not have been included.

Conclusion

This study concludes that the awareness of people about stroke, its risk factors and warning signs was adequate and can be related to significant factors such as education and the source of information. So it is suggested to program public multimedia and health education in academies and colleges in future to increase the knowledge and awareness of people.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The authors would like to thank Guilan Trauma Research Center, and Dr. Kazemnezhad for his statistical advice.

How to cite this article: Hosseininezhad M, Ebrahimi H, Seyedsaadat SM, Bakhshayesh B, Asadi M, Ghayeghran AR. Awareness toward stroke in a population-based sample of Iranian adults. Iran J Neurol 2017; 16(1): 7-14.

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Iranian Journal of Neurology

Original Paper

Iran J Neurol 2017; 16(1): 15-25

Circulating concentrations of interleukin (IL)-17 in patients with multiple sclerosis: Evaluation of the effects of gender, treatment, disease patterns and IL-23 receptor gene polymorphisms

Received: 16 Sep 2016
Accepted: 27 Nov 2016

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Keywords
Multiple Sclerosis; Interleukin-17; Gender; Treatment; Interleukin-23 Receptor; Gene Polymorphisms

Abstract

Background: Interleukin (IL)-17/IL-23 axis performs a prominent role in the pathogenesis of several autoimmune disorders. This study aimed to investigate the concentrations of IL-17 in patients with multiple sclerosis (MS) and its relationship with gender, medication, disease forms and single nucleotide polymorphisms (SNP) in IL-23R gene, including rs11209026 and rs1004819.

Methods: The blood specimens were obtained from 135 healthy individuals and 135 MS patients. The patients exhibited relapsing-remitting (RRMS; n = 65), primary progressive (PPMS; n = 19), secondary progressive (SPMS; n = 35) or progressive relapsing (PRMS; n = 14) MS. The DNA was analyzed for SNPs using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and IL-17 concentrations were measured by enzyme-linked immunosorbent assay (ELISA).

Results: We have observed elevated serum IL-17 concentrations in MS patients compared with healthy individuals ($P < 0.001$). The men with MS had higher IL-17 concentrations than women patients ($P < 0.050$). Untreated patients had significantly higher IL-17 concentrations than healthy individuals and treated patients ($P < 0.001$ and $P < 0.010$, respectively). The IL-17 concentrations were significantly decreased in patients treated with

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interferon-β (IFN-β), methylprednisolone or both drugs as compared with untreated MS patients ($P < 0.050$, $P < 0.020$ and $P < 0.050$, respectively). The IL-17 concentrations were also significantly higher in patients with RRMS and PRMS compared with healthy individuals ($P < 0.005$ and $P < 0.010$, respectively). The genetic variations at SNPs rs11209026 and rs1004819 were not significantly different between healthy individuals and patients. The IL-17 concentrations were not influenced by genetic variations at investigated SNPs.

Conclusion: These results indicated higher levels of IL-17 in MS patients that may be influenced by disease patterns, medication and gender. No association was observed between investigated SNPs and MS.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune-mediated disease of the central nervous system (CNS) that leads to neuronal demyelination and axonal degeneration.[1] The clinical courses of MS are defined as relapsing-remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS) or progressive relapsing (PRMS).[1] The lymphoid cells, in particular, $CD4^+$ T-helper (Th) lymphocytes play a fundamental role in the development of MS and its animal model named experimental autoimmune encephalomyelitis (EAE).[2] Functionally, separate Th lymphocytes are differentiated from naïve T cells after antigenic stimulation including Th1, Th2, Th17 or regulatory T (Treg) lymphocytes.[2] Both interferon (IFN)-γ-producing Th1 cells and interleukin (IL)-17-producing Th17 cells are accountable for demyelination in MS and EAE diseases.[2,3] In contrast, the Treg cells may confer protection against diseases while the Th2 role remains obscure.[2,4] Elevated concentrations of a Th17 cell-associated chemokine (CCL20) and diminished levels of a Th2/Treg cell-related chemokine (CCL22) were indicated in patients with MS.[5,6]

Th17 cells are characterized by the production of a large number of pro-inflammatory cytokines which include mainly IL-17A (also called IL-17), IL-17F, tumor necrosis factor-α (TNF-α), IL-21, IL-22, CCL20 and granulocyte monocyte-colony stimulating factor (GM-CSF).[3,7] IL-17 can influence various cell types such as epithelial cells, endothelial cells, fibroblasts, myeloid cells and synoviocytes.[8] IL-17 elicits the release of different pro-inflammatory mediators such as CXCL1, CXCL6, CXCL8 IL-1b, IL-6, TNF-α, GM-CSF, macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1) and granulocyte colony-stimulating factor (G-CSF).[3,8] IL-17 also acts as a powerful inducer of neutrophil aggregation into the inflammatory tissues.[3] Th17 cells perform an essential function in the development of a number of autoimmune disorders (such as MS, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus and psoriasis) and allergy and asthma.[9]

IL-23 is a heterodimer cytokine that is composed of two polypeptide including P19 subunit (specific for IL-23) and P40 subunit (shared with IL-12).[10] The IL-23 receptor is also a heterodimeric molecule and composed of IL-23R and IL-12Rβ1 which bind to P19 and P40 subunits, respectively.[10] The main producers of IL-23 are dendritic cells and macrophages and its function is to increase the full activation and maintenance of Th17 lymphocytes.[10] The antigenic stimulation in presence of both IL-6 and transforming growth factor-β (TGF-β) induces the early differentiation of naive $CD4^+$ T cells to Th17 lymhocytes.[11] However, subsequent interaction with IL-23 is needed for reinforcement and pathogenic activities of Th17 lymhocytes. Indeed, TGF-β and IL-6-induced Th17 lymphocytes are less pathogenic and more exposure to IL-23 is needed for development of inflammatory Th17 lymphocytes.[10,12] The relationship between IL-23/IL-17 axis and a number of autoimmune and inflammatory disorders including systemic lupus erythematosus,[13] spondyloarthritis,[14] psoriatic arthritis,[15] Graves' disease,[16] Crohn's disease,[17] and EAE and MS[18] has been reported.

The IL-23R gene is located on the chromosome 1p31.3 that encodes the receptor for IL-23.[19] The association of the IL-23R gene polymorphisms with several inflammatory and autoimmune diseases such as ankylosing spondylitis,[20] psoriatic arthritis,[21] allergic rhinitis,[22] rheumatoid arthritis,[23] and systemic lupus erythematosus[24] has been reported. Recently, in one study the associations of several single nucleotide polymorphisms (SNPs) within IL-23R gene (rs2201841, rs10889677 and rs7517847) with MS have been investigated in a Chinese population and showed no association between these SNPs and MS disease.[25]

The SNP rs11209026 (Arg381Gln) leads to replacement of Arg with Gln within the IL23R-binding domain for the JAK2 kinase, and

therefore may change the downstream signaling pathways and responses to IL-23.[19,26] The much less common glutamine allele seems to protect against some autoimmune diseases such as inflammatory bowel disease, psoriasis and ankylosing spondylitis.[19,27] The association of SNP rs11209026 with a number of immune-related diseases such as inflammatory bowel disease,[28] psoriasis,[29] Crohn's disease[30] has also been reported. The SNP rs1004819 is located in the intronic region of the IL-23R gene and may exert its influence by regulating the splicing of IL-23R mRNA.[19] However, a considerable association between SNP rs1004819 with ulcerative colitis[31] and ankylosing spondylitis[19] has also been indicated in other investigations. The aforementioned SNPs may influence the expression and function of the IL-23R; however, their relationship with MS have not been investigated in Iranian populations, yet.

Although a number of studies have evaluated the contribution of IL-17 in the development of MS, its concentrations have not been assessed adequately in MS patients with respect to gender, medication, forms of MS and IL-23R gene polymorphisms,. Accordingly, this study was conducted to assess the concentrations of IL-17 in patients with MS and its relationship with gender, medication, disease patterns and SNPs in IL-23R gene, such as rs11209026 and rs1004819.

Materials and Methods

A total of 135 MS patients (29 men and 106 women) referring to the Shephah Hospital of Kerman (a city placed in southeast Iran) were enrolled into the investigation. Sixty five patients presented with RRMS, 19 presented with PPMS, 14 had PRMS and 35 presented with SPMS. The diagnosis of MS was verified by expert neurologists based on the reliable diagnostic findings including clinical and paraclinical examinations [magnetic resonance imaging, presence of oligoclonal bands in cerebrospinal fluid (CSF) and evoked potentials according to the McDonald's criteria.[32] The MS patients were classified as newly diagnosed (untreated) patients (n = 47) who were not treated with any drug, and previously diagnosed (treated) patients (n = 88) who were treated with methylprednisolone (intravenously by a dose of 1000 mg/day for 3 to 5 days after acute MS attack), IFN-β [Avonex (Biogen Idec Co, USA; 30 µg intramuscularly, one time weekly), CinnoVex (CinnaGen Co, Iran; 30 µg intramuscularly, once time weekly) or Rebif (Merck Biopharma Co, Germany; 44 µg subcutaneously, three times weekly)] for at least 3 months. A number of MS patients initially received IFN-β (during silent phase of disease) and later methylprednisolone after having an acute attack of MS.

Also 135 healthy individuals including 35 men and 100 women were enrolled into the investigation as a control group. The healthy individuals were selected among blood donors referring to the Blood Transfusion Organization of Kerman and were interviewed regarding CNS disorders and none of them had CNS or other related disorders. All control individuals were basically healthy, with no acute or chronic sickness. Indeed individuals with illness (such as any suspected immunological disorders, history of recurrent infections, asthma, allergy and atopic diseases), cigarette smoking and those taking any medication were excluded from the study. The other exclusion criteria were malignancy, surgery and major trauma within 6 months prior to blood collection.

The Ethical Committee of Kerman University of Medical Sciences evaluated and approved the investigation. In addition, the participants were enrolled into the study on their own wish and informed written consent was also obtained from all of them. A peripheral blood specimen (2-4 ml) was taken from all participants and the sera were separated and stored at -70 °C until analysis.

DNA extraction and genotype analysis: Genomic DNA was separated from peripheral white blood cells by salting out technique as previously described by Miller, et al.[33] The purity and the quantity of DNA specimens were determined by the detection of the optical density at 260 and 280 nm wavelengths using a spectrophotometry system (Ependorf, Germany). DNA specimens were kept at -20 °C until testing. The genetic variations at SNPs rs11209026 and rs1004819 in IL-23R gene was determined by utilization of polymerase chain reaction-restriction length polymorphism (PCR–RFLP) technique.

The PCR reaction mixture was formed by adding following reagents to a 0.2-ml microcentrifuge tube on ice: 2.5 µl of Taq DNA polymerase buffer (10×), 0.5 µl of MgCl2 (stock concentration 1.5 mM), 0.5 µl of each dNTP [dATP, dCTP, dGTP, and dTTP (stock concentration of 10 mM)], 1 µl of each primer, 1 µl of prepared DNA, and sterile double-distilled water to a final volume of 25 µl.

For SNP rs11209026, the sequences of primers were 5′-AGTCACTCTGTGGCCTAAAGTAAAG-3′ for the forward primer and 5′-AGATTTTTCTAGTAAACAACTGAAATGA-3′ for the reverse primer. Amplification was done with the following thermocycler program:

One early phase of 94 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, 61 °C for 1 minute and 72 °C for 90 seconds. The amplified PCR product of IL-23R gene covers the SNP rs11209026 with a molecular size of 350 bp. The Hpy1 88I (Fermentase, Finland) restriction enzyme had been used for digestion of the PCR product. The digested products were electrophoresed on a 2.5% agarose gel after adding 4 µl of loading buffer (Cinnagen, Iran) and studied on Chemi-Doc model XRS (Bio-Rad, USA) after staining with ethidium bromide. As reported previously,[19] in the situation of heterozygotic form (AG), four different fragments with 285, 250, 65 and 35 bp are produced. In the GG homozygotic form, three fragments (250, 65 and 35 bp) and in the AA homozygotic form, two fragment (85 and 65 bp) are formed.

For SNP rs1004819 the sequences of primers were 5′-GCATTCTAG GACCGTTTTGG-3′ for the forward primer and 5′-ATCTGGTGGAAATATGTGAAACCTAA-3′ for the reverse primer. The amplification was performed with the following thermocycler program:

One primary phase of 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, 61 °C for 1 minute and 72 °C for 90 seconds. The amplified PCR product of IL-23R gene covers the SNP rs1004819 with a molecular size of 270 bp. The TaaI (Fermentase, Finland) restriction enzyme had been used for digestion of the PCR product. The digested products were electrophoresed as mentioned above. As reported previously,[34] in the situation of homozygotic form (GG), three different fragments with 185, 71 and 13 bp are produced. In the AA homozygotic form, two fragments (185 and 65 bp) and in the GA heterozygotic form four fragment (257, 185, 71 and 13 bp) are observed.

Cytokine assay: The serum IL-17A concentrations were measured in duplicate by commercial enzyme-linked immunosorbent assay (ELISA) kits (Mabtech, Sweden) based on the manufacturer's instructions. The sensitivity of the kits was less than 4 pg/ml and the intra-assay variation was < 5%.

The differences in variables were analyzed using parametric statistical tests (including ANOVA and Student's t-tests for normal distribution of data) and non-parametric statistical tests (including Kruskal-Wallis and Mann-Whitney U tests for non-normal distribution of data) as appropriate. A P-value of less than 0.05 was considered significant. The data were analyzed using SPSS (version 15, SPSS Inc., Chicago, IL, USA).

Results

The mean ages for MS patients and healthy individuals were 35.70 ± 7.90 years and 36.50 ± 7.79 years, respectively (P = 0.410). The gender distribution was 106 women (78.5%) and 29 men (21.5%) in MS patients, and 100 women (74.1%) and 35 men (25.9%) in healthy individuals (P = 0.470).

Serum IL-17 concentrations in MS patients and healthy individuals: We have observed elevated serum IL-17 concentrations in MS patients compared with healthy individuals (P < 0.001) (Table 1). The men with MS had higher IL-17 concentrations compared with women patients (P < 0.050). In healthy individuals, no significant difference was observed between men and women regarding the IL-17 concentrations. In both men and women patients the IL-17 concentrations were significantly higher than the healthy individuals with same gender (P < 0.002 and P < 0.010, respectively) (Table 1) (Figure 1).

Table 1. The serum interleukin-17 (IL-17) concentrations in multiple sclerosis (MS) and healthy groups according to gender

Groups	Gender	n	IL-17 levels† (mean ± SD)	P
MS patients	Male	29	56.30 ± 28.60	0.050*
	Female	106	17.00 ± 7.03	0.001**
	Total	135	25.50 ± 8.31	-
Healthy (control)	Male	35	4.50 ± 0.80	0.260*
	Female	100	7.11 ± 1.35	
	Total	135	6.43 ± 1.03	

†The serum levels of cytokine expressed as pg/ml, *Represent the difference between men and women in each group, **Represent the difference between MS and healthy groups

IL: Interleukin; MS: Multiple sclerosis; SD: Standard deviation

Table 2. The serum interleukin-17 (IL-17) concentrations in newly untreated and treated multiple sclerosis (MS) patients according to their gender

Groups	Sex	n	IL-17 levels† (mean ± SD)	P
Untreated MS patients	Male	12	121.10 ± 66.00	0.050*
	Female	35	30.90 ± 19.10	0.001**
	Total	47	53.90 ± 22.40	-
Treated MS patients	Male	17	10.70 ± 2.45	0.950*
	Female	71	10.20 ± 4.60	0.240**
	Total	88	10.30 ± 3.74	-
Healthy	Male	35	4.50 ± 0.80	
	Female	100	7.11 ± 1.35	0.260*
	Total	135	6.43 ± 1.02	

†The serum levels of cytokine expressed as pg/ml, *Represents the difference between men and women in each group, **Represents the difference between indicated and healthy groups
IL: Interleukin; MS: Multiple sclerosis; SD: Standard deviation

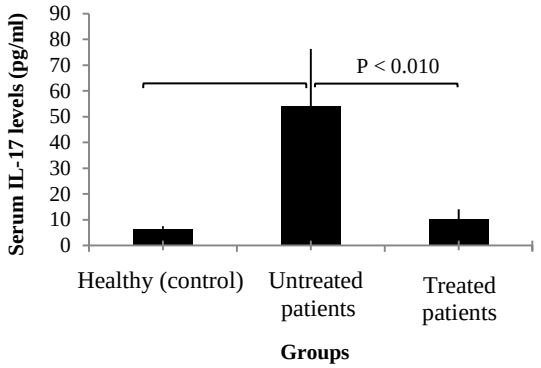

Figure 1. Comparison of the serum interleukin-17 (IL-17) levels between healthy control group, untreated and treated multiple sclerosis (MS) patients
The IL-17 levels in untreated patients was significantly higher than healthy group and treated patients (P < 0.001 and P < 0.010, respectively)

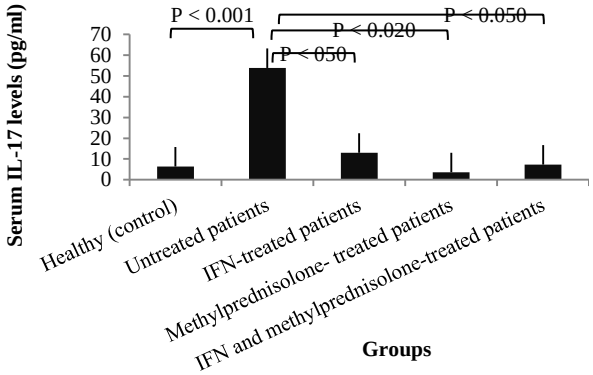

Figure 2. The serum levels of interleukin-17 (IL-17) in multiple sclerosis (MS) patients according to treatment program
The mean serum levels of IL-17 in untreated patients was also significantly higher than that in healthy group (P < 0.001). The mean serum levels of IL-17 was significantly lower in patients treated with IFN-β, methylprednisolone or both IFN-β and methylprednisolone as compared to untreated

Serum IL-17 concentrations in untreated and treated MS patients: The mean serum IL-17 concentrations in newly diagnosed MS patients was significantly higher than healthy individuals and treated MS patients (P < 0.001 and P < 0.010, respectively) (Table 2) (Figure 2). However, the difference of the mean serum IL-17 concentrations in treated MS patients and healthy individuals was not statistically significant (P = 0.240). The newly diagnosed patients had RRMS (n = 20), PPMS (n = 14), PRMS (n = 10) or SPMS (n = 3) patterns of disease. There were no significant differences between serum IL-17 concentrations from newly diagnosed patients with regard to their pattern of disease.

The mean serum IL-17 concentrations in untreated MS men was significantly higher than healthy men or treated men with MS (P < 0.010 and P < 0.050, respectively). Serum IL-17 concentrations in treated men with MS was also significantly higher than healthy men (P < 0.010) (Figure 3).

The mean serum IL-17 concentrations in untreated women with MS was also significantly higher than healthy women (P < 0.050). The difference of the mean serum IL-17 concentrations between untreated-and treated MS women was not significant (P < 0.160). The difference of the serum IL-17 concentrations in untreated women with MS and healthy women was also not significant (P < 0.400). Serum IL-17 concentrations in untreated men with MS was markedly higher than untreated women with MS (P < 0.070). In treated MS patients, no significant difference was observed between men and women regarding the IL-17 concentrations (Table 2).

IL-17 concentrations in MS patients according to medication program: The serum IL-17 concentrations in MS patients, according to their medication program are demonstrated in table 3.

Table 3. The serum interleukin-17 (IL-17) concentrations in multiple sclerosis (MS) patients according to treatment programs

Groups	Treatment	n	IL-17 levels* (mean ± SD)	P
MS patients	Interferon	55	12.97 ± 5.92	
	Methylprednisolone	15	3.65 ± 0.90	0.120**
	Methylprednisolone + interferon	10	7.30 ± 3.47	
	No treatment	47	53.90 ± 22.40	-
Healthy control	-	135	6.43 ± 1.02	-

*The serum levels of cytokine expressed as pg/ml, **Represent the difference between MS patients with different treatment
IL: Interleukin; MS: Multiple sclerosis; SD: Standard deviation

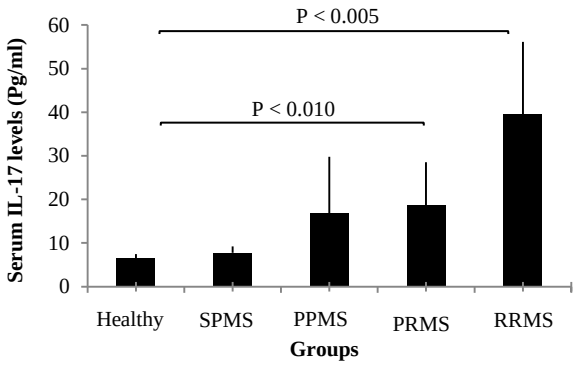

Figure 3. The serum levels of IL-17 in MS patients according to disease patterns
In patients with RRMS and PRMS patterns the mean serum levels of IL-17 was significantly higher than that observed in healthy control group (P < 0.005 and P < 0.01, respectively).

The IL-17 concentrations were significantly decreased in patients treated with IFN-β, methylprednisolone or both drugs as compared with untreated MS patients (P < 0.050, P < 0.020 and P < 0.050, respectively) (Table 4). The differences of the serum IL-17 concentrations between patients treated with methylprednisolone or IFN-β plus methylprednisolone and healthy individuals were not significant (Table 4).

IL-17 concentrations in MS patients according to disease patterns: Statistical analyses indicated that the differences of the serum IL-17 concentrations between MS patients with different disease forms were not significant (Table 5). In patients with RRMS and PRMS patterns, serum IL-17 concentrations were significantly higher than healthy group (P < 0.005 and P < 0.010, respectively) (Table 6). Although, in patients with PPMS serum IL-17 concentrations were higher than healthy individuals but the difference was not significant (P < 0.060). The mean serum IL-17 concentrations were similarly expressed in patients with SPMS and healthy individuals (Tables 5 and 6).

The relation between SNPs rs11209026 and rs1004819 and MS: The genetic variations at SNPs rs11209026 and rs1004819 in the IL-23R gene in the patients with MS and the healthy individuals are demonstrated in tables 7 and 8. There was no deviation from equilibrium of Hardy-Weinberg neither in patients nor in healthy individuals regarding the genotype frequencies. There were no significant differences between MS patients and control subjects regarding the frequencies of genotypes and alleles at SNPs rs11209026 and rs1004819. Moreover, there were no significant differences between patients with RRMS, SPMS, PPMS and PRMS patterns regarding the frequencies of genotypes and alleles at SNPs rs11209026 and rs1004819.

Serum IL-17 concentrations according to the genetic variations at SNPs rs11209026 and rs1004819 in IL-23R gene were demonstrated in table 9 and table 10. No significant differences were observed between individuals with different genotypes or alleles at SNPs rs11209026 and rs1004819 with respect to the serum IL-17 concentrations neither in MS patients nor in healthy individuals.

Table 4. Statistical comparison of the serum interleukin-17 (IL-17) concentrations between multiple sclerosis (MS) patients, according to their treatment program

Treatment program	IFN-β	Methylprednisolone	IFN-β + Methylprednisolone	No treatment
IFN-β	-	0.12*	0.41	0.050
Methylprednisolone	0.12	-	0.24	0.020
IFN-β + Methylprednisolone	0.41	0.24	-	0.050
No treatment	0.05	0.02	0.05	-
Healthy (control)	0.28	0.05	0.81	0.001

*The symbol represents P-values; IL: Interleukin; IFN: Interferon

Table 5. Serum interleukin-17 (IL-17) concentrations in multiple sclerosis (MS) patients according to disease patterns

Groups	Diseases patterns	n	IL-17 levels* (mean ± SD)	P
MS patients	RRMS	65	39.50 ± 16.60	0.001**
	SPMS	37	7.73 ± 1.46	
	PPMS	19	16.80 ± 13.00	
	PRMS	14	18.70 ± 9.79	
	Total	135	16.00 ± 4.21	
Healthy (control)	-	135	6.43 ± 1.02	

*The serum levels of cytokine expressed as pg/ml, **Represent the difference between all groups (healthy subjects and MS patients with various disease patterns)

RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis; PPMS: Primary progressive multiple sclerosis; PRMS: Progressive relapsing multiple sclerosis; IL: Interleukin; MS: Multiple sclerosis; SD: Standard deviation

Table 6. Statistical comparison of serum interleukin-17 (IL-17) concentrations between multiple sclerosis (MS) patients, according to their disease patterns

Disease patterns	RRMS	SPMS	PPMS	PRMS
RRMS	-	0.15*	0.28	0.28
SPMS	0.150	-	0.34	0.28
PPMS	0.280	0.34	-	0.90
PRMS	0.280	0.28	0.90	-
Healthy (control)	0.005	0.53	0.06	0.01

*The symbol represents P-values

RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis; PPMS: Primary progressive multiple sclerosis; PRMS: Progressive relapsing multiple sclerosis

Discussion

Results from our study indicated that increased serum concentrations of IL-17 in MS patients that is consistent with findings reported by other investigators. In both male and female patients the concentrations of IL-17 were significantly higher in comparison with healthy individuals with the same gender. According to these results, increased concentrations of IL-17 might have a role in the pathogenesis of MS in either males or females.

Table 7. The frequencies of genotypes and alleles at rs11209026 in interleukin-23 (IL-23R) gene in patients with multiple sclerosis (MS) and healthy control group

Genotype	MS patients [n (%)]	Healthy subjects [n (%)]	P
GG	126 (93.3)	124 (91.90)	0.640
GA	9 (6.70)	11 (8.10)	0.640
G	261 (96.67)	259 (95.90)	0.640
A	9 (3.33)	11(4.07)	0.640

MS: Multiple sclerosis

The autoreactive Th17 lymphocytes have shown to be at high frequencies in the CNS of EAE mice and in the peripheral blood mononuclear cells (PBMCs) of MS patients.[35,36]

Table 8. The frequencies of genotypes and alleles at rs1004819 in IL-23R gene in patients with multiple sclerosis (MS) and healthy control group

Genotype	MS patients [n (%)]	Healthy subjects [n (%)]	P
GG	44 (32.6)	36 (26.7)	0.280
GA	63 (46.7)	73 (54.1)	0.220
AA	28 (20.7)	26 (19.3)	0.760
A	119 (44.1)	125 (46.3)	0.220
G	151 (55.9)	145 (53.7)	0.260

MS: Multiple sclerosis

Table 9. The serum interleukin-17 (IL-17) concentrations according to the genetic variations at rs11209026 in IL-23R gene

Groups	Genotypes	n (%)	IL-17 levels* (mean ± SD)	P
MS	GG	126 (93.3)	26.60 ± 8.89	0.590
	GA	9 (6.7)	8.95 ± 4.45	
	G	261 (96.7)	26.00 ± 6.06	0.600
	A	9 (3.3)	8.95 ± 4.45	
Healthy	GG	124 (91.9)	6.54 ± 1.11	0.700
	GA	11 (8.1)	5.15 ± 1.74	
	G	259 (95.9)	6.49 ± 0.75	0.710
	A	11 (4.1)	5.15 ± 1.74	
Total	GG	250 (92.6)	16.70 ± 4.55	0.540
	GA	20 (7.4)	6.86 ± 2.19	
	G	520 (96.3)	16.30 ± 3.09	0.550
	A	20 (3.7)	6.86 ± 2.19	

IL: Interleukin; SD: Standard deviation

Table 10. The serum interleukin-17 (IL-17) concentrations according to the genetic variations at rs1004819 in IL-23R gene

Groups	Genotype	n (%)	IL-17 levels* (mean ± SD)	P
	GG	44 (32.59)	29.11 ± 16.83	0.950
	GA	63 (46.66)	23.40 ± 11.30	
MS	AA	28 (20.74)	24.38 ± 16.76	
	G	151 (55.92)	25.75 ± 9.65	0.440
	A	119 (44.07)	23.70 ± 1.37	
	GG	36 (26.66)	4.13 ± 0.73	0.250
	GA	73 (54.04)	6.58 ± 1.35	
Healthy	AA	26 (19.25)	9.19 ± 3.60	
	G	145 (53.70)	5.77 ± 0.94	0.450
	A	125 (46.29)	7.27 ± 1.37	
	GG	80 (29.62)	17.87 ± 9.32	0.730
	GA	136 (50.37)	14.37 ± 5.31	
Total	AA	54 (20.00)	17.06 ± 8.84	
	G	296 (109.62)	15.67 ± 4.97	0.790
	A	244 (90.37)	15.14 ± 4.54	

The elevated IL-17 concentrations in the CSF of patients with MS also represent the contribution of this cytokine in the development of disease.[36] In addition, RORγt (a Th17-specific transcription factor)-deficient mice were more resistant to EAE induction.[2] Low EAE scores, delayed onset and low histological changes with early disease recovery were also reported in IL-17-deficient mice.[2] However, the mice treated with the monoclonal antibody against IL-17 were reported to have the ability to develop EAE, even with a decreased disease severity.[37] In addition to IL-17A, other cytokines including IL-17F, GM-CSF, IL-6, IL-21, IL-22 and TNF-α are produced by Th17 lymphocytes, which can play a prominent role in the development of EAE and MS diseases.[3,8]

The Th17 lymphocytes may be involved in the establishment of MS and EAE by recruitment of neutrophils into the CNS, induction of the reactive oxygen species (ROS) in CNS endothelial cells, activation of microglia cells to secrete pro-inflammatory mediators, and the induction of astrocytes to release CXC chemokines.[3] Some Th17 lymhocyte-related cytokines (including TNF-α) elicit the expression of matrix metalloproteinases, which may play a considerable role in rupture of blood-brain barrier (BBB) during MS.[38]

In the current study, the men with MS had significantly more serum IL-17 concentrations compared to the female patients. This difference may attribute largely to the effect of sex hormones. In a number of experimental models of inflammatory diseases, it has been indicated that the frequency of Th17 lymphocytes was higher in male as compared with female gender and this phenomenon has been attributed to the inhibitory effects of estrogen on the Th17 cells differentiation.[39]

The results obtained from our study demonstrated that the patients presenting RRMS and PRMS patterns had significantly higher serum IL-17 concentrations in comparison with healthy individuals. Although serum IL-17 concentrations in patients presenting with PPMS were also markedly higher than healthy individuals, but the difference did not reached to a significant value ($P < 0.060$). These results represented that IL-17 may contribute at least to the pathogenesis of RRMS and PRMS patterns. Consistent with our results, higher frequency of Th17 lymphocytes were reported in patients presenting with RRMS, SPMS and PPMS.[40,41] Serum IL-17 concentrations were similar in patients presenting with SPMS and control individuals. In agreement with our finding, the results reported by Frisullo, et al. also showed no significant difference between patients presenting SPMS and healthy individuals regarding the IL-17 production by their PBMC. Therefore, it has been proposed that the initial phase of MS disease is strongly associated with IL-17.[42]

Based on the present results, the serum concentrations of IL-17 were significantly lower in patients treated with IFN-β and/or methylprednisolone compared with untreated patients. It has been reported that IFN enhances activation-induced apoptosis in Th17 lymphocytes through binding to its receptor that is expressed on these cells.[43] Moreover, IFN-β down-regulates the

expression of RORγt, IL-17A and IL-23R, but up-regulates the expression of IL-10 in the $CD4^+$ T lymphocytes.[44] The diminished expression of IL-27 in the CNS of EAE mice was observed in our previous study.[45] IFN-β may mediates its therapeutic effects through the induction of IL-27 production, which in turn inhibits Th17 cell-related responses.[46] The PBMC from non-responder MS patients to IFN-β therapy produced lower IL-27 concentrations than responder patients following in vitro stimulation.[46]

The modulatory effects of methylprednisolone on Th17 lymphocytes were reported in a number of studies. methylprednisolone down-regulates the production of IL-17 by PBMC from asthmatic children or patients with rheumatoid arthritis.[47,48] Moreover, the reducing effects of methylprednisolone on the frequency of Th17 lymphocyte were demonstrated in patients with MS disease.[49] In addition, IFN-β and methylprednisolone may up regulate the expression of IL-35 (a Treg-type cytokine) in MS patients.[50] Based on the present results, the immunomodulatory effects of IFN-β and/or methylprednisolone may perform in part, via the inhibition of the IL-17 production.

We found no significant differences for frequencies of genotypes and alleles at SNPs rs11209026 and rs1004819 between patients with MS and healthy individuals. These data represent that investigated SNPs may have no association with MS disease.

Several mechanisms have been suggested by which polymorphisms can change the function of the receptor. The SNPs may also influence the expression of the IL-23R (e.g. by enhancing mRNA stability), therefore, they reinforce the differentiation of native T cells towards a Th17 cells which results in an increased secretion of other inflammatory cytokines. However, our results represent that MS may develop through the mechanisms independent of SNPs rs11209026 and rs1004819. The results of a number of studies have reported no association between the SNP rs1004819 and immune-related diseases including ankylosing spondylitis,[51] systemic lupus erythematosus,[52] inflammatory bowel disease[53] and rheumatoid arthritis.[23] It should be also noted that a SNP may be in disequilibrium linkage with other SNPs, unlikely conferring independent influence.

No significant differences were observed between subjects with various genotypes or alleles at SNPs rs11209026 and rs1004819 with respect to serum IL-17 concentrations neither in MS patients nor in healthy individuals. These results represent that the serum concentrations of IL-17 were not influenced by genetic variations at SNPs rs11209026 and rs1004819 in MS patients. It has been reported that the SNP rs11209026 influences serum IL-17 concentrations in patients with rheumatoid arthritis.[26] Whether SNPs rs11209026 and rs1004819 may affect IL-17 concentrations in CNS or CSF during the development of MS remains to be determined in future studies.

Conclusion

In conclusion, the results obtained from the current research showed higher IL-17 concentrations in MS patients, especially in patients with RRMS and PRMS patterns. Treatment of MS patients with IFN-β and/or methylprednisolone had reducing effects on the IL-17 levels. The serum levels of IL-17 may also be influenced by the gender of patients. However, there was no association between the investigated SNPs rs11209026 and rs1004819 and MS. Also the serum levels of IL-17 were not influenced by genetic variations at SNPs rs11209026 and rs1004819.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

This work was supported by a grant from the Neurology Research Center, Kerman University of Medical Sciences, Kerman, Iran.

How to cite this article: Ghaffari SA, Nemati M, Hajghani H, Ebrahimi H, Sheikhi A, Jafarzadeh A. Circulating concentrations of interleukin (IL)-17 in patients with multiple sclerosis: Evaluation of the effects of gender, treatment, disease patterns and IL-23 receptor gene polymorphisms. Iran J Neurol 2017; 16(1): 15-25.

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Iranian Journal of Neurology

Original Paper

Iran J Neurol 2017; 16(1): 26-9

Knowledge, attitude, and practices among Iranian neurologists toward evidence-based medicine

Received: 17 Sep 2016
Accepted: 22 Nov 2016

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Keywords
Evidence-Based Medicine; Knowledge; Attitude; Neurologist; Iran

Abstract

Background: Evidence-based medicine (EBM) is a current practice in medicine to produce clinical practice guidelines from well-designed, randomized, controlled trials. We studied knowledge, attitude, and practice of EBM of neurologists who participated in the Iranian congress of neurology.

Methods: A self-administered anonymous questionnaire was distributed and filled by neurologists.

Results: A total of 200 neurologists were randomly sampled and with response rate of 56%. 33.9% of responder had previously participated in EBM courses. The average total knowledge score was 4.05 ± 0.80 out of a maximum possible score of 5.0. Textbooks were still the most favorite source of knowledge for our neurologists. A lack of time was the highest, and motivation the least mentioned barrier in using EBM.

Conclusion: Overall, the Iranian neurologist had acceptable knowledge and attitude toward EBM and had same similar as found in other studies.

Introduction

As neurologists, we are living in an era of exponential growth of knowledge. Needless to say that practicing medicine is very different today than it was 15 years ago and even is going to be more different in 2020. This will be challenging, especially with the current rate of medical knowledge doubling every 3 years. The rate of doubling is expected to be every 73 days by 2020.[1]

The evidence-based medicine (EBM) is the process of producing clinical guidelines from well-designed, randomized and controlled trials, published in literature databases.[2,3] Hence, the main goal of EBM is to optimize clinical decision-making and keep health practitioners' knowledge up-to-date.[4]

The knowledge of medicine is growing very rapidly and using all available resources by medical professionals to be up-to-date is becoming more difficult (if not impossible). Therefore, the physician's attitude toward EBM has increased enormously and positively.[5]

By learning how to practice EBM and adopting evidence-based practice protocols, medical professionals can put themselves in tandem of

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medical advances, and this can be helpful to enhance their clinical performances.[6]

Neurologists, like other medical professionals worldwide, are being encouraged to apply EBM to improve their clinical care.

To the best of our knowledge, little is known about knowledge, attitude, and practice of EBM among Iranian neurologists.

To gain more information, we designed and conducted a survey.

The aim of our study was to answer five research questions about these topics:

- What are main resources for Iranian neurologist to find their clinical questions?
- Do Iranian neurologists know enough about important clinical aspects of EBM?
- How frequently they use EBM methods in their clinical practice?
- What are their limitations to use EBM?

Have they ever participated in EBM workshops?

Materials and Methods

A cross-sectional observation study was conducted on Iranian neurologists who participated in the 22nd Iranian Congress of Neurology and Electrophysiology, held in Tehran, in 2014. We chose this congress because it was popular and the participation level was expected to be high.

We used convenience-sampling procedure to select participants, and a self-administered anonymous questionnaire was distributed to participants.

The questioner had been translated, validated and used by Navabi, et al.[7] The scientific reliability of the questionnaire was confirmed and labeled as optimal by previous studies, and all of the items were deemed highly appropriate to use for this group of physicians.

The questionnaire included 22 questions and evaluated background data, knowledge, attitude, and practice, in four sections. The first section covered background data, including gender, age, level of education, and type of practice.

The second section evaluated their knowledge level of EBM. They were asked to answer five statements with a three-point Likert scale: "correct," "incorrect," or "do not know." The total knowledge score was calculated by scoring the answers: one point for correct answer and zero point for wrong or for "do not know" answers. The sum of the scores was the basis of calculating their level of knowledge.

In the next section, neurologists were asked to state their most favorite sources for getting their medical information. They were also tested for their knowledge about EBM terms such as EBM, clinical effectiveness, relative risk, systematic review, critical appraisal, and Cochrane collaboration. They had to state their knowledge level by "know well," "little is known," or "do not know anything."

The participants were given enough time, and the questionnaires were done anonymously.

One of the objectives of the survey was to understand their level of online medical resources usage, and their limitations in using EBM. We were also interested to know if they had participated in other EBM workshops.

Data were analyzed using Pearson's chi-squared test for the comparison of frequencies and mean scores and the SPSS (version 16, SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered significant.

Results

A total of 200 survey questioners were distributed among neurologists who attended the congress, 113 of them returned the survey questioners, making 56% response rate. 81.4% of the responders were male with mean age of 44.4 years. 33.6% of them were university faculty members with 11.8 years of work experience after finishing their neurology training.

The responders got the mean score of 4.05 ± 0.80 out of a maximum score of 5, regarding their knowledge.

Our survey responders mentioned their sources of acquiring professional knowledge. 84.1% of our responder used textbooks to get their clinical answers. Other sources of medical knowledge were online resources (79.6%), expert opinion (61.9%) and personal experiences (24.8%), respectively.

Only 33.9% of the responders admitted that had attended EBM courses before. The "Clinical Effectiveness" was the most familiar and the "EBM" was least familiar term among the participants. More detail is shown in figure 1.

We also asked about limitations in using EBM by Iranian neurologists. Figure 2 shows the frequencies of these barriers. By far the highest mentioned barrier was lack of time (69.9%) and on the other hand, motivation was the least barrier (0.9%).

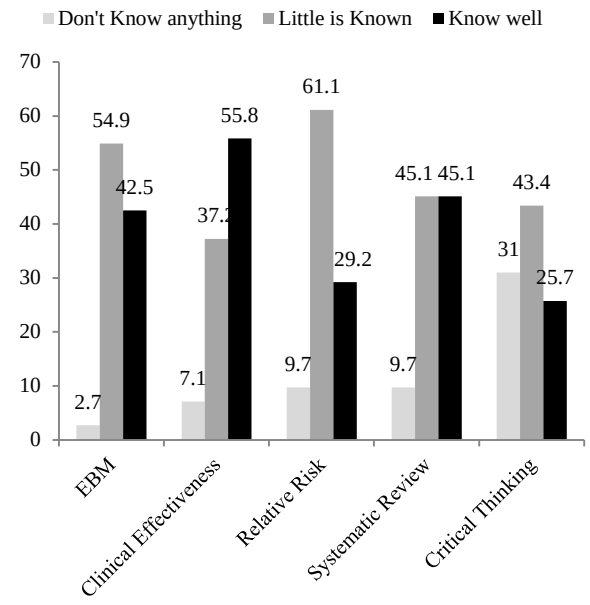

Figure 1. The statement of the Iranian neurologists regarding selected evidence-based terms. (n = 113)

On average participants in our survey spent 1-5 hours/week online to find their clinical answers.

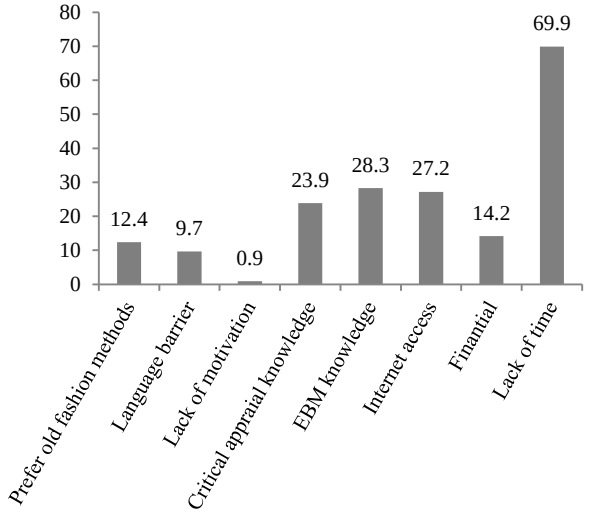

Figure 2. Frequency of mentioned barriers in using evidence-based medicine (EBM) by Iranian neurologists

Discussion

This was a questionnaire-based survey on the Iranian neurologist who participated in the annual congress of Iranian Neurology Associations. An acceptable numbers of them kindly returned the self-administered questioner.[8]

In general, neurologists who participated in our survey positively welcomed EBM and had acceptable basic knowledge and understanding of EBM. Compared to the same survey on dentists by Navabi, et al.,[7] neurologists had better mean total knowledge score and were more likely to have participated in EBM workshop before. Both groups admitted that they usually prefer textbooks than other sources to resolve uncertainty in clinical practice. A lack of time was expressed as the predominant barrier to use EBM.

Ghahremanfard, et al.[9] studied knowledge and attitude toward EBM among medical students in Semnan, Iran in 2014. Based on their results, only 24.5% of medical students had good basic information and familiarity with the term of EBM. The positive attitude toward EBM existed in 89.3% of participants. Only 29% of medical students reported having had formal training in search strategies.

Our findings were quite similar to those of other studies conducted in the Middle East, which assessed the healthcare provider's attitude toward EBM. They also showed that the major mentioned barriers to practicing EBM were lack of free personal time.[10,11]

Barghouti, et al.[8] assessed Jordanian family practitioners' attitudes toward and awareness of EBM in 2009. Only 20.4% of them had received formal training in research and critical appraisal. They also found that lack of personal time was the main perceived barrier to practicing EBM.

In a study on Australian general practitioners, the most commonly cited barrier to EBM was patient demand for treatment despite the lack of evidence for effectiveness. In those groups of physicians, the next most highly rated barriers were lack of time. They mentioned that lack of time was rated as a "very important barrier" by significantly more participants than lack of skills.[12] Same findings have been reported in a Norwegian study in 2009.[13]

The evidence-based approach can be rationalized as the best treatment in resource-limited countries like Iran. It can also be the most cost-effective approach by reducing clinical practices that have no proven benefit. At present, EBM has major barriers, such as its inherent complexity, misperceptions, absence in medical curriculum, and unawareness of practicing clinicians.[14]

In general, physicians experience significant barriers to integrate EBM into clinical practice.[15]

These steps are recommended to overcome

these barriers: effective teaching of skills of EBM during residency, motivating the established clinicians, formulating locally applicable guidelines, increasing the accessibility to internet, availing telemedicine facility at remote center and disseminating appropriate information via free journals or even newspapers. A strong political commitment is needed so that these steps can help to lay the foundation of EBM in Iran.

Conclusion

- Textbooks were main resources for Iranian neurologist to find their clinical questions. They less relied on expert opinion for that reason. We think, it is a major change in their view
- Iranian neurologists knew well enough about important clinical aspects and term in EBM practice
- They were using EBM methods in their clinical practice more than other medical practitioners in similar studies

They had good motivation on using EBM, but the lack of time was a major barrier in that way.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

Authors would like to express our sincere gratitude to all neurologists who had accepted to participate in our study. The authors are also grateful to the neurology research center for supporting and Mr. Samadani for editing our article.

How to cite this article: Shafiei K, Sedaghati F. Knowledge, attitude, and practices among Iranian neurologists toward evidence-based medicine. Iran J Neurol 2017; 16(1): 26-9.

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Iranian Journal of Neurology

Original Paper

Iran J Neurol 2017; 16(1): 30-3

Polymorphisms at activated protein C cleavage sites of factor V: Are they important in the absence of factor V Leiden?

Received: 26 Sep 2016
Accepted: 05 Dec 2016

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Keywords
Cerebral Venous Thrombosis; Factor V; Activated Protein C Resistance; Cleavage Sites

Abstract

Background: Activated protein C (APC) inactivates factor V (FV) by cleavage of its heavy chain at Arg306, Arg506, Arg679, and Lys994. Mutational changes, which abolish APC cleavage sites, may predispose thrombosis by altering the inactivation process of FV. FV Leiden (FVL) (Arg506Glu) has been demonstrated as a strong risk factor for thrombosis. In the current study, we have studied whether mutations in the cleavage sites of FV for APC, not due to FVL, would have a role in presenting APC resistance (APCR) and initiation of a cerebral thrombotic event.

Methods: A group of 22 patients with a history of cerebral venous thrombosis (CVT), who were not carriers of FVL enrolled in the study. The patients who had conditions associated with acquired APCR were excluded from the study. APCR test was performed on the remaining 16 patients, which showed APCR in 4 plasma samples. DNA sequencing was performed on four exons of FV of APCR patients, encoding Arg306, Arg506, Arg679, and Lys994.

Results: Mutations were not found within nucleotides encoding the cleavage sites; neither was found within their close upstream and downstream sequences.

Conclusion: Our results show that polymorphisms affecting cleavage sites of FV other than Arg506Glu it would be less likely to be the basis for APCR and its increased thrombosis susceptibility. In addition, it emphasizes on the importance of screening for APCR in the patients diagnosed with CVT.

Introduction

Cerebral venous thrombosis (CVT) is an uncommon but serious type of cerebral vascular events which mostly affects young adults and children.[1] It can be caused or predisposed by several medical and surgical disorders such as head trauma, central nervous system infections, malignancies, hematologic and inflammatory disorders. Furthermore, the risk for developing a CVT is higher in patients with hypercoagulable states such as hyperhomocysteinemia, protein C, protein S and antithrombin III deficiencies, as well as careers of prothrombin gene and factor V Leiden (FVL) mutation.[1,2] Accordingly, the prothrombotic screening for hypercoagulable

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states due to genetic and acquired disorders is an important part of investigating the etiology of CVT.[1,2]

Activated protein C (APC) plays a key role in the anticoagulant pathway by inactivating FVa and Factor VIIIa. There are several proteolytic cleavage sites on FV that is responsible for activation and inactivation of the molecule.[3] APC can down regulate FV procoagulant activity by cleavage at Arg306, Arg506, Arg679, and Lys994.[3,4] Mutational changes which abolish APC cleavage sites may dilute its effect on inactivation of FV. FVL, which is the result of a single point mutation at Arg506, is a common and strong hereditary risk factor for thrombosis in Caucasians.[5,6] The APC resistant (APCR) phenotype which is associated with a 7-fold increase in the risk of deep vein thrombosis, is considered to be a consequence of FVL in 95% of cases.[5]

Acquired conditions including cancer, pregnancy, using oral contraceptive pills (OCP) and hormone replacement therapy (HRT), elevated Factor VIII levels ≥ 150 IU/dl and positive lupus anticoagulant results may also be the cause of APCR.[7,8]

The functional assays that are being used for screening of APCR are widely considered equal to FVL screening test, especially in populations with high prevalence of FVL.[9] However, DNA-analysis has shown that some cases with APCR do not carry an R506Q mutation.[10] Therefore, in these individuals, the inactivation of FVa by APC may be influenced by polymorphisms other than Leiden mutation. In the current study, we have studied whether mutations in the cleavage sites of FV for APC, not due to FVL, would have a role in presenting APCR and initiation of a cerebral thrombotic event.

Materials and Methods

A group of 38 ≤ 65 year-old adults with a past diagnosis of CVT was recalled for this study. These patients had previously been checked for the presence of FVL using restriction fragment length polymorphism (RFLP) method, and the results were negative.[11] 22 patients accepted to take part in the current study performed at the Neurology Clinic of Alzahra University Hospital (Isfahan, Iran). Of each patient, a 5 cc sample of venous bloodstream was collected into a citrated tube. Poor platelet plasma was extracted after centrifuging and stored at −20 °C. Another 5 cc sample was collected in ethylenediamine tetraacetic acid tube and stored at −20 °C until molecular lab assessments.

Factor VIII, lupus anticoagulant and APCR tests were performed on 22 plasma samples of patients. To eliminate the conditions that could induce APCR without a Leiden mutation, the patients who had ≥ 150 IU/dl of Factor VIII or positive lupus anticoagulant test results were excluded from the study. None of them were pregnant, OCP consumers or under HRT at the time of sampling.

Using HEMOCLOT Factor V-L kit (Aniara, USA), APCR was measured in the plasma samples of the patients. The ratio of clotting time was calculated in the presence and absence of APC. If this ratio was ≥ 2, the plasma is considered normal. In patients with significant APCR such as R506Q mutation, this ratio is lowered to ≤ 1.80. The ratio > 1.80 and < 2 is considered as borderline.

DNA was extracted from blood samples of 4 APCR patients with a standard DNA isolation kit (PrimePrep, Genet Bio, South Korea). Four primers were used for running PCR on the cleavage site regions:

A 228 bp fragment of exon 7, encoding Arg306 cleavage site, was amplified by the following primer: Forward: 5′-CTTGAACCTTTGCCCAGTGG-3′ and reverse: 5′-TTGTCTTTCTGTCCTAACTCAGC-3′.[12] A 267 bp fragment of exon 10, encoding Arg506 cleavage site, was amplified by following primer: Forward: 5'-TGCCCAGTGCTTAACAAGACCA-3′ and reverse: 5'-TGTTATCACACTGGTGCTAA-3′.[5] A 226 bp fragment of exon 13, encoding Arg679 cleavage site, was amplified by the following primer: Forward: 5′-TGTCTGTCTCTCTTCTGTAAGAACT-3′ and reverse: 5′-CTGGTAATCATAGTCAGCATCAC-3′.[12] The last primer: Forward: 5′-CAGCCCCCAGAATGCCTCA-3′ and reverse: 5′- AGACCTGGAGGACAGCTTGC-3′ was designed to synthesize a 432 bp fragment of exon 13, that encodes Lys994 cleavage site.

Polymerase chain reaction products were collected into sterile 500 µl tubes and stored in −20 °C until sequencing procedures (ABI 3730XL DNA Analyzer, Bioneer, South Korea). The results were compared with sequence information of FV in the National Centre for Biotechnology Information (NCBI) gene database.

Results

A total of 22 patients with a history of CVT, who

were negative for FVL, were studied. There were 16 females (72.7%) and 6 males (27.3%) with a mean age of 36.77 ± 13.35 years. Three patients had a positive lupus anticoagulant test and were excluded from the study. The level of Factor VIII was increased in 4 patients, and they were excluded too (one patient excluded for both).

Four patients out of remaining 16 patients (25%) showed resistance to APC in the absence of other acquired conditions by showing a clotting time ratio under 2. Two of them represented APCR by 1.4 and 1.6 clotting time ratio. The other two patients were in the borderline range (ratio 1.9).

DNA sequencing was done on four regions of FV that encode Arg306, Arg506, Arg679, and Lys994 cleavage sites in four APCR patients. Mutations were not found within nucleotides encoding the cleavage sites; neither was found within their close upstream and downstream sequences.

Discussion

In this series of patients, the FV-mediated APCR was investigated in a selected group of CVT patients with no evidence for acquired causes of this thrombogenic condition. The sequencing study of four known cleavage sites of FV for APC, as potential sites for generating APCR, failed to find any polymorphism. Similar to the results of the previous testing with RFLP method, DNA sequencing on Arg506 confirmed the absence of FVL mutation. Thus, the APCR could not be explained by a molecular mechanism affecting the APC cleavage sites other than R506Q.

Mutated FVL which decreases the anticoagulant activity of APC by altering the main cleavage site of APC on FV, has been proposed to be strongly associated with CVT.[13,14] It has been reported that 10-25% of CVT patients are carriers of this mutation.[14,15] Among Iranian population, Rahimi, et al.[16] found a significant correlation between FVL mutation and CVT in Iranian patients with Kurdish ethnic background. In contrast a study by Ashjazadeh, et al.[17] indicated that frequency of FVL mutation were not significantly increased in CVT patients who lived in southern Iran with predominant Fars ethnicity.

Similarly, we observed a frequency of 5% in a group of 40 CVT patients and could not find a significant difference compared with controls.[11] As APCR was observed in some of our patients in the absence of FVL, looking for other genetic polymorphisms appeared to be the most logical approach. Mostly the polymorphisms that potentially act in a similar way as FVL does.

Our exclusion criteria resulted in a small group of 4 patients to be investigated out of 22 recalled CVT patients without FVL, but simultaneously, it would raise the possibility that APCR was a result of a genetic defect.

The cleavage of FV by APC at the cleavage site Arg506 is the most important part in the inactivation of FVa and results in a partial inactivation. It is also essential for optimal exposure of cleavage sites at Arg306 and Arg679 and subsequent inactivation of FVa.[4,18] The latter two cleavage sites are associated with cofactor inactivation in which cleavage at Arg306 is responsible for 70% loss of cofactor activity and the subsequent cleavage at Arg679 that occurs at a slower rate than that of Arg306 and Arg506, is responsible for the remaining 30%.[18] Although there is a lack of studies on kinetics and clinical significance of APC-mediated cleavage at Lys994 in the literature, it appears to be a location of minor importance on cofactor activity of the molecule.[19]

Although the mutation at position Arg506 leads to a significant APCR and associated with 3- to 7-fold increase in the risk of thrombosis as heterozygous and up to 80 folds as homozygous,[20] two reported polymorphisms at Arg306 have shown a milder APCR phenotype. Arg306→Thr (FV Cambridge) makes a mild APCR and Arg306→Gly (FV Hong Kong) appeared to have no change in the susceptibility to APC cleavage.[21] Mutations at Arg306 are probably associated with low thrombotic risk.[21,22]

Theoretically, the mild APCR observed in our patients can be attributed as a result of other unknown cleavage sites on FV. The recent studies have demonstrated some novel cleavage sites close to Arg306 and Arg506.[23,24] Hence, the APC cleavage is occurred very slower in these sites and to our knowledge have no physiologic significance in normal situation.[23,24] They may have a role in the presence of mutant factors (Leiden, Cambridge, Hong Kong), but still remains to be studied.

The observed APCR could be attributed to other variants of v that display APCR phenotype without interfering its cleavage sites on FV. FV Liverpool[25] is a mutated variant that carries aIle359Thr substitution which provides an additional glycosylation at Asn357 that results in impaired inactivation of FVa. In addition, it appears to have a poor APC cofactor activity.[25,26]

The FV R2 haplotype, which is a collection of mutations in FV, also may represent a mild APCR in homozygous carriers.[27]

Conclusion

Our study could not find any genetic polymorphism of FV, at its multiple cleavage sites for APC. It shows that polymorphisms affecting cleavage sites of FV other than Arg506Glu it would be less likely to be the basis for APCR and its increased thrombosis susceptibility. In addition, as FVL and APCR are considered as independent risk factors for thrombosis,[28] our results stress the importance of APCR screening in patients with CVT.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

This project has been funded by a research grant from Isfahan University of Medical Sciences. (Grant number: 190059).

How to cite this article: Kheradmand E, Haghjooy-Javanmard S, Dehghani L, Saadatnia M. Polymorphisms at activated protein C cleavage sites of factor V: Are they important in the absence of factor V Leiden? Iran J Neurol 2017; 16(1): 30-3.

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Iranian Journal of Neurology

Review Article

Iran J Neurol 2017; 16(1): 34-40

Multiple sclerosis-A disease on a dramatically rising trend in Iran: Review of possible reasons

Received: 18 Sep 2016
Accepted: 03 Dec 2016

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Keywords
Multiple Sclerosis; Causality; Prevalence; Incidence; Environmental Factors; Iran

Abstract
There has been a global rising trend in recent years in the incidence of multiple sclerosis (MS). Despite being an MS low-risk region, this disease has also been recently on the rise in the Middle East. As part of the Middle East, Iran has not been spared either; however, the cause of this dramatic increase remains to be discovered. This study reviews possible reasons for this increase in Iran. Although many factors such as the increased rate of smoking, lifestyle changes, modernization, and contact with toxic solvents can be proposed as reasons for this sudden rise in the prevalence of MS in Iran, these factors cannot be taken as definite causes and further studies are required to prove their impact.

Introduction

About 5-10% of the population in developed countries is affected by autoimmune diseases. These diseases are also a major cause of mortality and disability and increased medical costs.[1,2] In terms of causality, autoimmune diseases can be created through a combination of various factors such as genetic, immunity, hormonal, and environmental factors.[3-5] Multiple sclerosis (MS) is a neuronal infection in the central nervous system with a heterogeneous clinical and pathological composition that can develop suddenly and lead to death within only a few weeks or months. In many patients, it has a gradually increasing progress with a long and severe clinical course.[6,7] Nevertheless, the progress of MS might cease or slow down after the first or second phase of its progress in benign cases.[8]

About 2 million people were estimated to have MS in 2003 across the world, about 400000 of whom were reported to live in the US. The majority of people with MS are in the 20-50 age range, and the disease tends to affect women more than men. Eastern Europeans are more affected by MS compared to Asians, Africans, and Latinos. This disease can cause death, disability, depression,

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physical impairment, and reduced quality of life. For instance, 50% of those affected require help with mobility and 10% need to use wheelchairs 15 years after the onset of the disease. In general, there are no treatments for MS.[9-12] In addition to the absence of a treatment method, the cause of this disease is also still unknown. So far, various causes have been proposed by researchers for this disease, including infectious, environmental, and social factors. Many infectious diseases with various viral and bacterial causes have been investigated so far. Recent studies have been heavily focused on Epstein-Barr virus (EBV) infection. Acute EBV infection can remain in the body for life and its weaker form affects the B lymphocytes in 90% of the youth infected. In a number of case-control studies, EBV antibodies were noticed to have increased in the case group compared to the control group. In several well-designed studies, this virus was proposed as a risk factor for MS; however, in biological terms, this assumption remains only a possibility.[13]

Moreover, this disease is caused by various factors, including geographical latitude, vitamin D intake, skin color, immigration, meals, smoking, occupational contact with toxins and stress. The main factors involved in the development of this disease for which researchers have clear evidence include vitamin D, geographical latitude, and immigration.[3,14] In the past, the pattern and distribution of MS were dependent on geographical latitude and was less prevalent in regions with higher latitudes. Overall, countries with a high prevalence of MS were mostly located in North America or Europe and countries closer to the equator boasted a lower prevalence of MS.[15] Some current studies indicate that the global pattern of the prevalence of this disease has been changing. Some regions that used to be in the low-prevalence MS zone are now becoming moderate to high prevalence zones. The World Health Organization (WHO) published a report in 2008 on the global distribution of MS.

According to figure 1, despite the prevalence gradient of the disease across the world, geographical distribution models previously proposed no longer apply to some regions such as the Middle East and particularly Iran.[16,17] Although Iran is in the low-risk MS zone, studies indicate a dramatic rise in the prevalence of MS in Iran in recent years. This study was therefore conducted to investigate the possible reasons for the increase in the prevalence of MS in Iran.

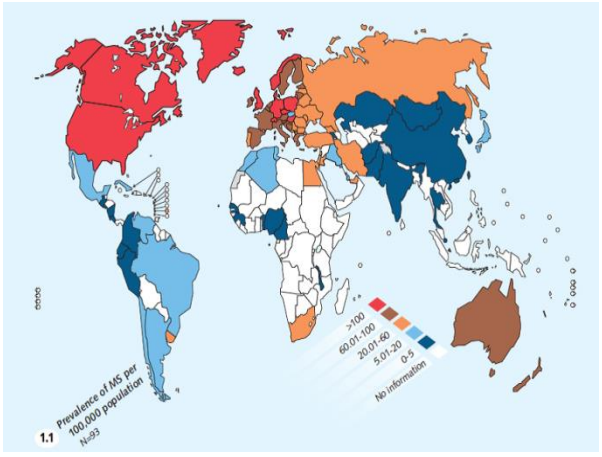

Figure 1. The global distribution of the prevalence of multiple sclerosis (MS) in 2008

MS status in the Middle East

According to a study by Aljumah, et al., there are no proper epidemiological data in the Middle East about the prevalence, incidence or history of MS. Based on the Kurtzke Classification, the Middle East is located in a low-risk zone for MS. Nonetheless, recent studies argue that the prevalence of MS in the Middle East is increasing to moderate or high levels, and women are more affected by the disease.[18]

Other data available on MS and the Middle East have been presented in table 1 (given the full section to be presented on the status of MS in Iran, Iran has not been included in the list in this table).

According to table 1, there is an extensive time and place variation in the distribution of MS in the Middle East, and the geographical gradient previously detected in the region is changing.

Status of MS in Iran

Various studies conducted in different cities of Iran show a dramatic increase in the prevalence of MS in recent years.[29,30] According to a study by Sahraian, et al., Iran has been switching from a low-prevalence zone for MS into a moderate to high prevalence zone. The results of the study show an estimated prevalence of about 52 per 100000 people in Tehran, Iran, 72.3% of whom are women and 27.7% men. The study also showed that the women to men ratio of the disease have been increasing from 2 in 2002 to 3.14 in 2007. The mean age of infection with MS is 27.24 years.[31]

In their study, Moghtaderi, et al.[32] reported the prevalence and incidence of MS in southeast Iran as 13.96 per 100000 women and 2.67 per 100000 also reported a significant rise in the prevalence of

Table 1. The prevalence of multiple sclerosis (MS) in countries in the Middle East

Authors (references)	Country	Prevalence of MS
Inshasi and Thakre[19]	Emirates	54.77 per 100000 people in 2007
Al-Hashel, et al.[20]	Kuwait	14.77 per 100000 people in 2000
Bohlega, et al.[21]	Saudi Arabia	40 per 100000 people in 2008
Al-Araji and Mohammed[22]	Iraq	300 patients diagnosed in the country in 2000
Yamout, et al.[23]	Lebanon	Estimated 1200-1800 patients in the country in 2008
El-Salem, et al.[24]	Jordan	39 per 100000 people in Amman
Radhakrishnan, et al.[25]	Libya	5.9 per 100000 people in 1982-1984
Tharakan, et al.[26]	Oman	4 per 100000 people in 1990 to 2000
Attia Romdhane, et al.[27]	Tunisia	12 per 100000 people in 1985
Dehghani, et al.[28]	Iran	44.53 per 100000 people in 2011

men in 2010, with women to men ratio of 2.18, and MS in Iran compared to previous years.

The dramatic rise in the prevalence of MS was also noticed in Isfahan, Iran, in a study conducted by Etemadifar and Abtahi.[33] Recent reports have proposed Isfahan as a city with the highest risk of MS in Asia and Oceania. The authors also suggested the design and implementation of control and screening programs for the prevention of this indiscriminately increasing prevalence. An ecological study conducted by Dehghani, et al.[28] also revealed the dramatically growing trend of MS throughout Iran, with a prevalence increasing from 26.24 per 100000 people in 2006 to 44.53 per 100000 in 2011, and of the total of 31 provinces, 19 showed a moderate prevalence (5 to 30 patients per 100000 people) and 8 had a high prevalence (more than 30 patients per 100000 people), and only 3 had a low prevalence (< 5 patients per 100000 people); meanwhile, in 2011, only 11 provinces showed a moderate prevalence and the rest showed a high prevalence (Figure 2).

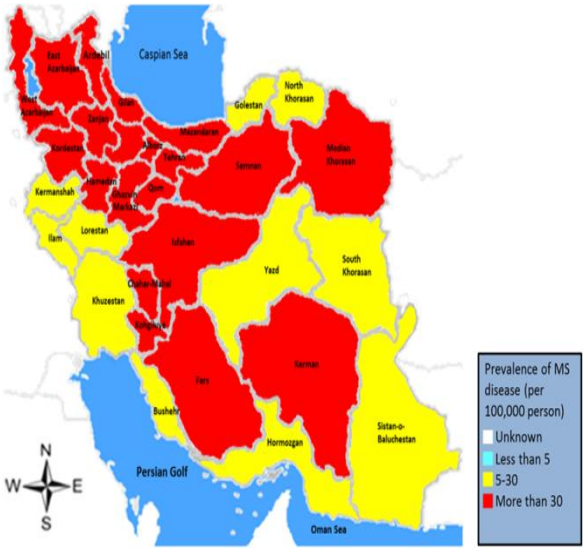

Figure 2. Status of multiple sclerosis (MS) in different provinces in 2011

Lifestyle Changes associated with Modernization, Industrialization, and Urbanization

According to a WHO report, the use of tobacco and diets rich in fat, salt and sugar, which can lead to hypertension and obesity, and the increased use of packaged foods along with a sedentary lifestyle, might have emerged more as consequences of industrialization, urbanization, economic growth and globalization and can contribute to the development of chronic diseases.[34] Based on a national census in Iran, there has been a tremendous rise in the rate of urbanization over the past few decades.[35] A study by Ghassemi, et al. confirmed the rapidly changing nutrition pattern in Iran as well as the changing pattern of mortality and birth rates. The tendency toward urbanization was also shown to have occurred speedily among the Iranian population due to the unstable socioeconomic conditions. Furthermore, a poor nutrition pattern has taken over the entire Iranian population and overeating has become an integral part of life in one-third of the population. All these factors can predispose the individual to a variety of diseases. In the female population of Iran, obesity is a serious risk factor, which may be associated with many chronic diseases.[36] In a study conducted in the US on 8983 patients with MS, 25.0% were obese and 31.3% were overweight, and 18.2% were exposed to the risk of alcohol abuse either through themselves or their relatives.[37]

In addition to the change in nutrition patterns, the quality of food items has also been dramatically changing. Some individuals and industries commit food frauds for making greater profits, which may lead to people's deprivation of good food or the intake of harmful food products, causing various diseases. Food fraud was an existing crime in Iran as confirmed by Dehghani, et al.[38]

Reduced vitamin D intake

Many studies have proposed vitamin D as the key factor in the prevention of MS.[39] Vitamin D deficiency is also the cause of many other chronic diseases aside from MS.[40] A cohort study conducted on 95310 women from 1991 to 2002 conducted a regular investigation of the subjects' vitamin D intake and their other dietary features using a validated nutrition questionnaire. Of the entire study population, 173 women developed MS in the course of the study and vitamin D intake was found to be inversely related to the risk of developing MS.[41] Studies indicate that vitamin D deficiency is an epidemic condition in 20-25% of the population in the US, Canada, Europe, Asia, and Australia.[42] Considering the reduced physical activity levels in countries of the Middle East as a result of lifestyle changes caused by the rise of industrialization and urbanization, sun exposure has been decreasing in this region (according to a WHO report). The lower exposure to the sun can be a factor in this region reduced rate of daily sunlight intake,[43] which can significantly increase the development of MS in residents of this region and subsequently of Iran.[44] It should be noted that although vitamin D is most likely the best dietary composition for the prevention of MS, other nutritional factors and even lifestyle-related tainting factors, too, can have a decisive role. Tainting factors may include other vitamin sources aside from vitamin D since vitamin D is normally absorbed in the presence of other vitamins.[41]

Economic growth and living standards

The increasing rate of urbanization and economic growth in recent years in the Middle East may be involved in the improved lifestyle of the residents of this region.[28] Some studies suggest a greater risk of developing MS in individuals with higher living standards so that the immune system's adaptation to foreign agents is poorer in individuals with a better economic status during childhood, which can itself be a factor for the increased risk of the development of MS.[3] According to a report published by the WHO, higher income countries were shown to have a higher prevalence of MS compared to poorer countries (Figure 3).

The factors involved in the countrywide increasing trend of the prevalence of MS have been listed and explained.

Figure 3 presents two diagrams showing the prevalence of MS in different continents and by income levels in different countries.[17] It should also be noted that there is a poorer access to diagnostic facilities in the less developed countries compared to the developed countries, which might be a cause for underestimation in the reports. These differences are large enough to somewhat dismiss the poorer access to diagnostic facilities.

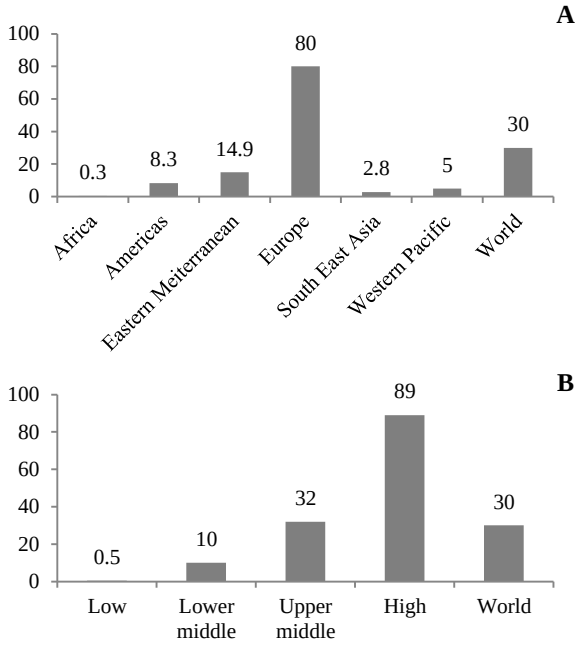

Figure 3. The prevalence of multiple sclerosis (MS) in different continents and by income level in different countries

A: Prevalence of MS per 100,000 population in WHO regions and the word (N-93); B: Prevalence of MS per 100,000 population in different income groups of countries (N-93)

Smoking

Smoking is a major cause of many different chronic diseases.[45] Many studies have associated smoking with the development or the increased risk of the development of MS.[46] A study conducted in Norway showed that the risk of developing MS was 1.81 times greater in smokers than in those who have never smoked.[47] Smoking increases the frequency and duration of respiratory infections and might, therefore, cause the recurrence of MS. However, there is a gap in reports on the relationship between MS and smoking or problems associated with pathological diseases. For instance, the results of a study conducted in Isfahan suggested a significant increase in the rate of the development of MS in women compared to men in recent decades; however, only 1.4% of the

population of women were smokers in Iran, which does not suggest a significant relationship between smoking and MS.[48] The absence of such relationship can be attributed to the different effects of smoking on the immune system. One of the major factors contributing to the development of chronic diseases is passive or second-hand smoking.[49,50] The rate of smoking has been dramatically increasing in Iran; according to reports, about 60 billion cigarettes are smoked in Iran every year. An investigation conducted by the Iranian Ministry of Health confirms the growing trend of smoking cigarettes in recent years.[51,52] A study conducted by Dehghani, et al.[28] on the relationship between the prevalence of MS and lifestyle showed the greater prevalence of MS in provinces with a larger number of male smokers; however, this study was ecological and further studies are required to better demonstrate the impact of smoking.

Air pollution

Another possible factor for the development of MS is the greater air pollution in urban areas. The role of this factor is becoming more pronounced by the day, as studies have shown that increased urbanization leads to increased air pollution. Investigations have shown that air pollution (especially with particles such as PM10) can increase the risk of the development of MS.[53-55] In recent years, air pollution (especially with PM10) has become a cause for great concern in many areas of Iran.[56,57] For instance, in a study conducted in 2011 in Kashan, located in the high MS prevalence province of Isfahan, Dehghani, et al.[58] found that the city's air quality was acceptable only in 177 days of the year and that particulate matters were the main cause of air pollution in this city.[59] The adverse effects of this factor require further investigations, especially in developing countries.

Radon

The majority of studies conducted to date on the effect of radiation absorption have been concerned with ultraviolet rays and their protective effects against MS. However, a limited number of studies state the lesser considered hypothesis that radon can be a potential risk factor for the development of MS.[60] Radon is naturally emitted from the soil in some areas of Iran such as Ramsar, Iran. Some rocks and stones can naturally absorb the emitted rays, including decorative stones, especially granites, which tend to be commonly used in buildings in Iran without any supervision or prior evaluation.[61,62] Although this element is only proposed as a potential risk factor for the development of MS, further investigations are required to fully understand the particular conditions in Iran.

Occupational and Nonoccupational Contact with Chemicals

Studies conducted in various parts of the world consider contact with industrial solvents as a factor contributing to the development of MS. However, the evidence seems insufficient, and this hypothesis has not yet been proven.[63-65] No proper studies have been conducted in Iran on the link between contact with chemicals or special industrial solvents and MS. Nevertheless, according to some studies, contact with chemicals could entail a high risk for the development of this disease.[66,67] For instance, Dehghani, et al.[68] suggested that toxic chemicals are too easily available to people and emphasized the lack of specific rules and regulations for the purchase of these substances in Iran, which can increase the likelihood of developing certain chronic diseases, such as MS, which can be caused by contact with chemicals.[69,70]

Conclusion

This study reviewed factors that can increase the prevalence of MS in Iran. The results obtained suggest a close link in Iran between MS and lifestyle changes, modernization, industrial growth, and urbanization. In recent years, the risk of developing diseases such as MS has been increasing in urban areas of Iran due to the changes in lifestyle, the increase in the urban population and the subsequent increase in air pollution. These factors have only been suggested as potentially effective, and future controlled studies could help further examine the relationship between MS and these factors.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The authors would like to express their gratitude to the authorities at Kashan University of Medical Sciences and Hormozgan University of Medical Sciences, Iran, for their financial and moral assistance.

How to cite this article: Sahraian MA, Sahebkar M, Dehghani R, Derakhshan-Jazari M, Kazami-Moghaddam V, Kouchaki E. Multiple sclerosis-A disease on a dramatically rising trend in Iran: Review of possible reasons. Iran J Neurol 2017; 16(1): 34-40.

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Iranian Journal of Neurology

Letter to Editor

Iran J Neurol 2017; 16(1): 41-2

The first attack of multiple sclerosis presented immediately after voluntary and intensive weight loss: A case series

Received: 05 Sep 2016
Accepted: 11 Nov 2016

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Keywords
Multiple Sclerosis; Weight Loss; Intensive; Diet

There are many studies have reported that nutritional deficiencies (macro and micronutrients) are involved in the etiology of multiple sclerosis (MS).[1]

Some neurological complications such as polyneuropathy and optic neuropathy that are common in MS have been observed in consequential severe weight loss (WL) after bariatric surgery. There are two viewpoints to explain this event; nutritional deficiencies and releasing of inflammatory cytokines after severe WL.[2-4]

In this retrospective study, we reported four fascinating cases with definite diagnosis of MS presented after intended and intensive WL. These patients had been referred to "Nutrition Clinic" for consultation about WL. Here, we describe the past medical history of their WL diets and the short time intervals between WL and first presentation of MS.

Case one was a 27-year-old woman with MS for over 7 years. Body mass index (BMI) was 29 at the time of going to clinic. She had a past history of obesity and usage of WL diets several times and recurrent weight gains from adolescence. The first presentation of MS was blurred vision due to optic neuritis. 2 months before presentation of this sign; she had lost 20 kg of her weight with an inappropriate WL diet. Owing to severe reduction in the amount of calorie consumed and the elimination of carbohydrate resources arbitrarily, she experienced 20 kg losing weight within 2 months (16 kg within the 1st month and 4 kg within the 2nd month). The mean of WL per month was 10 kg. She reported that she did not take any supplements and medications and had no exercise program.

Case two was a 32-year-old man with MS for over 6 years. BMI was 35 at the time of going to clinic. He had a past history of obesity since early adolescence and tried several unsuccessful WL diets. He had gone on a 6 months WL diet up to 1 month before the first presentation of MS. The

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first presentation of MS was blurred vision due to optic neuritis. He had lost 30 kg within the first 3 months. During the 3 months of continuing with the same WL diet, he had lost another 10 kg. The mean of WL per month was 6.7 kg.

Case three was a 30-year-old woman with MS for over 4 years. BMI was 33 at the time of going to clinic. The first presentation of MS was blurred vision due to optic neuritis. She had a past history of inappropriate WL diet just before the first presentation of MS without consuming any supplements or having any scheduled exercise programs. She had lost 13 kg within 45 days. The mean of WL per month was 8.6 kg.

Case four was a 26-year-old woman diagnosed with MS for over 2 years. BMI was 28 at the time of going to clinic. The first presentation of MS was paresthesia of the lower limbs. She had a past history of several WL diets from the onset of adolescence. She had a hard dietary regimen without consuming any supplements or having any exercise programs just before the first presentation of MS. She lost 40 kg within 4 months. The mean of WL per month was 10 kg.

Conclusion

Presented cases had experienced intended and intensive WL (mean of WL was 8.8 kg/month overall) in the closest time to expression of MS symptoms. They all had a history of obesity and made concerted efforts to reduce their weight with disordered and abnormal WL diets. We considered that severe or rapid WL with intensive food intake restriction may be etiologic or accelerating factor for MS due to nutritional deficiencies. In addition, inflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α, and interleukin-1 release due to intensive WL and massive lipolysis probably are involved in the incidence of MS.[3,4] Our observations buttressed earlier studies suggesting that neurological adverse effects have been observed after severe WL in obese people.[4]

The bariatric surgery is one of the common ways of severe WL in which some nutritional deficiencies such as vitamin A, B, D, and E have been identified.[5,6]

Neurological complications affected both central and peripheral nervous system due to axonal loss and demyelination that observed in MS can occur after bariatric surgery through nutritional and inflammatory mechanisms.[4,6]

We express a new hypothesis in etiology of MS related to nutritional deficiencies and inflammatory processes accompanied severe WL. Further studies are recommended to investigate the accuracy of this hypothesis.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

This research was supported by Tehran University of Medical Sciences and Health Services grant number 93-03-54-27090.

How to cite this article: Bitarafan S, Amani K, Sahraian MA, Sarraf P, Soltani D, Naser Moghadasi A, et al. The first attack of multiple sclerosis presented immediately after voluntary and intensive weight loss: A case series. Iran J Neurol 2017; 16(1): 41-2.

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Iranian Journal of Neurology

Letter to Editor

Iran J Neurol 2017; 16(1): 43-4

The effects of intensive language therapy in aphasic patients

Received: 04 Sep 2016
Accepted: 13 Nov 2016

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Keywords
Aphasia; Intensive Language Therapy; Mississippi Aphasia Screening Test

Aphasia defined as an acquired communication disorder caused by brain damage and characterized by an impairment of language modalities including speaking, listening, reading, and writing.[1] There are many treatments to restore language functions. Intensive language therapy is one of the most effective treatment approaches. Studies demonstrated that intensive aphasia therapy delivered over 2-3 months were critical to maximize the aphasia recovery and also they reported that higher-intensity therapy provided over a short period results in a significant change in outcome. One novel method of intensive language treating is constraint-induced aphasia therapy (CIAT). In this protocol, patients with aphasia who receive short-term, intensive speech therapy is forced to communicate verbally, and all compensatory strategies (e.g., gesturing, writing, pointing) are restricted.

Effect of CIAT approach on patients naming skill was published previously.[2] In this study, we decided to evaluate the impact of intense therapy using the CI paradigm on the expressive and receptive index in patients with chronic aphasia.

One of the participants was a 57-year-old male who suffered a left cerebrovascular injury for 7 years before the current investigation. The other participant was a 45-year-old woman who suffered a left cerebrovascular injury for 5 years before the current investigation. In this study, the Mississippi screening aphasia test was the main outcome measure.

Mississippi Aphasia Screening Test (MAST) examines three subtests: (1) expressive index include; naming; automatic speech, repetition, verbal fluency and writing/spelling to dictation, (2) receptive index include: Yes/no accuracy, object recognition, verbal instructions, reading instructions, and (3) total score consists of the expressive and receptive score. The MAST was

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administered during two phases: (1) baseline (1 time per week for 3 weeks) and (2) treatment (1 time per week for 4 weeks).

The score mean of the receptive index of the first patient was 24.33 at the baseline evaluation which was increased to 31.75 after the intervention, and for the second patient was 29-34. Furthermore, the score mean of the expressive index of the first participant was 9-20.5 and for second patient 22.33-32. Thus, total score mean made an improvement about 18.92 for the first participant and about 14.67 for the second participant.

We found that CIAT with its characteristics is useful in improving expressive and receptive skills of chronic aphasia patient. Our finding is in consistent with the report of Kurland, et al. which showed that CIAT has a positive effect even in patients with chronic aphasia. CIAT has been shown that is more effective in improving verbal outcome due to cortical reconstruction and neuroplasticity.[3-5]

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

We thank Tehran University of Medical Sciences for its support. Also, authors thank Dr. Marcus Meinzer and Professor Nickels for their helpful comments. Special thanks to our participants for taking part in this study.

How to cite this article: Khatoonabadi AR, Kaviani S, Nakhostin-Ansari N, Saadati M, Shahverdi E. The effects of intensive language therapy in aphasic patients. Iran J Neurol 2017; 16(1): 43-4.

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Iranian Journal of Neurology

Clinical Note

Iran J Neurol 2017; 16(1): 45-9

Psoriasis, bulbar involvement, and diarrhea in late myoclonic epilepsy with ragged-red fibers-syndrome due to the m.8344A > G tRNA (Lys) mutation

Received: 12 Sep 2016
Accepted: 16 Nov 2016

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Keywords
Mitochondrial Disorders; Metabolic Myopathy; Lactate; Epilepsy; Psychiatric; Multi-Organ Disorder Syndrome

Myoclonic epilepsy with ragged-red fibers (MERRF) syndrome was first described by Tsairis, et al. in 1973.[1] The phenotypic spectrum broadened with the description of > 320 MERRF-patients since then (Table 1).[2] Here, we report further phenotypic variability.

The patient is a 71 years, HIV-negative, Caucasian female from nonconsanguineous parents, height 161 cm, weight 55 kg, with a history of a multiple organ disorder syndrome (MODS). Since early childhood she suffered from psoriasis. Since the age of 38 years recurrent diarrhea (1-2 times/day) developed. After a fall over a staircase at the age of 42 years she experienced a traumatic brain injury. Shortly after the trauma she developed left-sided peripheral facial palsy, which never resolved completely. In addition, mild but transient paraparesis of the lower limbs and stocking-type hypesthesia bilaterally were found. Since the age of 53 years bilateral progressive hypoacusis developed. At the age of 54 years diabetes was diagnosed. Since the age of 57 years she noticed myoclonic jerks which could be triggered by light, pain, touch, or fear. Myocloni were associated with recurrent falls (initially 3-4/month, at the age of 58 years 10-15/day) without losing consciousness. Wasting of the entire musculature shortly afterward resulted in weight loss of 10 kg in 1 year. She also reported blurred vision, recurrent double vision, and mild cognitive impairment (MCI) since the age of 57 years. Since the age of 62 years she required nocturnal noninvasive positive-pressure-ventilation and since the age of 65 years mechanical ventilation. Myoclonic seizures resolved only after application of piracetam at the age of 67 years. At the age of 69 years a tracheostoma was implanted. Her last medication included piracetam (24 g/day), glimepiride (1 mg/day), pantoprazole (40 mg/day),

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fluticasone (3 × 2 H), and bisoprolol (5 mg/day). The patient died at the age of 71 years from gastrointestinal bleeding after implantation of a percutaneous endoscopic gastrostomy. Her history was also positive for anemia, palpitations, hyperlipidemia, hypertension, early morning muscle cramps, easy fatigability, and multiple, large-scale, subcutaneous lipomas in the cervical, shoulder, and thoracic region. Her son had myoclonic epilepsy since the age of 12 years, hypoacusis, and experienced sudden death at the age of 17 years. The father's family had psoriasis. No information about her mother was available.

Clinical neurologic exam at the age of 58 years revealed left-sided ptosis, mild wasting of tongue edges, mild dysarthria, left-sided peripheral facial palsy, hypoacusis, weak head anteflexion (M5-), on the upper limbs right predominant diffuse weakness (M5-), diffuse wasting, reduced reflexes, bilateral dysdiadochokinesia, and on the lower limbs proximal weakness, diffuse wasting, absent reflexes, and recurrent myoclonic jerks. She was unable to stand or walk because of sudden loss of muscle tone during myocloni.

Table 1. Phenotypic manifestations of the m.8344A > G tRNA (Lys) mutation

Manifestation	Current patient	Previously reported*	References
Myopathy	Yes	67	Catteruccia, et al.[3]
Respiratory involvement	Yes	67	Catteruccia, et al.[3] and Blakely, et al.[4]
Lactate acidosis	Yes	67	Catteruccia, et al.[3] and Lorenzoni, et al.[5]
Cardiac involvement	Yes	53	Catteruccia, et al.[3]
Polyneuropathy	Yes	47	Catteruccia, et al.[3]
Myocloni	Yes	20-40	Catteruccia, et al.[3]
Epilepsy	Yes	40	Catteruccia, et al.[3]
Cerebellar ataxia	No	13-83	Catteruccia, et al.[3] and Lorenzoni, et al.[6]
Hypoacusis	Yes	25-35	Mancuso, et al.[2]
Exercise intolerance	Yes	15-25	Mancuso, et al.[2]
Migraine	No	5-15	Mancuso, et al.[2]
Elevated creatine-kinase	Yes	UK	Chinnery, et al.[7]
Elevated CSF protein	No	2-8	DiMauro, et al.[8]
Ptosis	Yes	UK	Blakely, et al.[4]
Cognitive impairment	Yes	UK	Mancuso, et al.[2]
Multiple lipomatosis	Yes	UK	Mancuso, et al.[2]
Diabetes	Yes	UK	Mancuso, et al.[2]
Myalgia	Yes	UK	Mancuso, et al.[2]
Visual impairment	Yes	UK	Chen, et al.[9]
Arterial hypertension	Yes	UK	Austin, et al.[10]
Arrhythmias	Yes	UK	Wahbi, et al.[11]
Optic atrophy	Yes	UK	Mancuso, et al.[2]
Short stature	Yes	UK	Lorenzoni, et al.[6]
Tremor	No	UK	Mancuso, et al.[2]
Leigh syndrome	No	UK	Scalais, et al.[12] and Monden, et al.[13]
Stroke-like episode/strokeno	No	UK	Vastagh, et al.[14] and Zaganas, et al.[15]
Leukoencephalopathy	No	UK	Biancheri, et al.[16]
Depression	No	UK	Molnar, et al.[17]
Fibrous bone dysplasia	No	UK	Chen, et al.[9]
Ophthalmoplegia	No	UK	Wiedemann, et al.[18]
Parkinson syndrome	No	UK	Mancuso, et al.[19] and Horvath, et al.[20]
Pigmentary retinopathy	No	UK	Lorenzoni, et al.[6]
Chronic pancreatitis	No	UK	Toyono, et al.[21]
GI dysfunction**	Yes	UK	Tanji, et al.[22]
Bulbar involvement	Yes	UK	NR
Hyperlipidemia	Yes	UK	NR
Psoriasis	Yes	UK	NR
Diarrhea	Yes	UK	NR

*Figures are in percent and relate to the respective size of the cohort investigated, **Gastrointestinal dysfunction manifested as paralytic ileus.

UK: Unknown; NR: Not reported; CSF: Cerebrospinal fluid; GI: Gastrointestinal

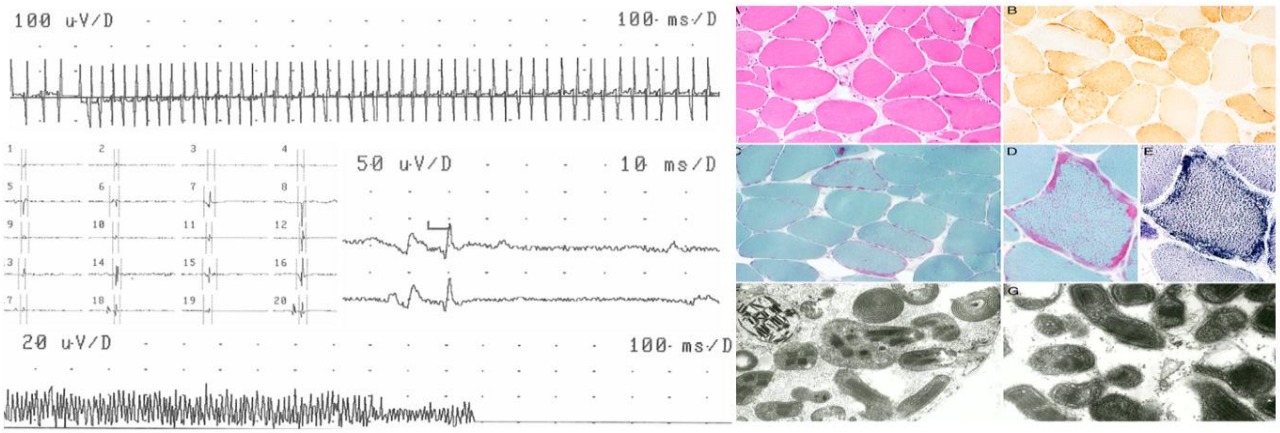

Figure 1. Left part: Myogenic needle electromyography (EMG) of the right anterior tibial muscle showing a pseudomyotonic discharge (upper panel), shortened motor unit action potentials (MUAP) with a mean of 7.9 ms (middle left), satellite potentials (middle right) and a dense, low-amplitude interference pattern (lower panel). Right part: Muscle biopsy from the left deltoid muscle revealed caliber changes and ragged-red fibers in hematoxylin and eosin staining (A), accompanied by cytochrome oxidase-negative fibers (B), Gomori trichrome staining revealed ragged-red fibers (C and D), which were visible in the succinate dehydrogenase enzyme histochemistry staining, (E) electron microscopy confirmed the SS accumulation of mitochondria showed concentrically arrayed tubular cristae and the presence of typical paracrystalline inclusions (F and G)

Blood chemical investigations revealed elevated resting lactate of 2.8 mmol/L (n: < 2.3 mmol/l), hyperlipidemia, fasting blood sugar of 157 mg/dl, and mild hyperCKemia of 113 U/l (n: < 71 U/l). Lactate stress testing was highly abnormal (3.3, 3.6, 6.5, 8.9, 9.2 mmol/l). Nerve-conduction-studies at the age of 58 years revealed reduced nerve conduction velocity (left peroneal nerve). Needle-electromyography (EMG) of the right brachial-biceps-muscle was myogenic with numerous fibrillations and pseudomyotonic discharges, some satellite potentials, mean motor unit action potential (MUAP) duration of 11.4 ms, 15% polyphasia, and a dense, reduced interference-pattern. Needle-EMG of the right anterior-tibial-muscle was myogenic with 4/20 fibrillations, 2/20 pseudomyotonic discharges, a mean MUAP-duration of 7.9 ms, and a dense, low-amplitude interference pattern (Figure 1). Visually-evoked potentials revealed a prolonged P100-latency bilaterally. Cerebral magnetic resonance imaging showed numerous lacunas in the basal ganglia and the left pedunculus cerebri, and mild diffuse atrophy. Electroencephalogram (EEG) at the age of 51 years revealed left predominant generalized spikes and short spike-wave complexes. EEG at the age of 56 years showed recurrently generalized spikes. EEG at the age of 57 years revealed occasional, paroxysmal activity over the frontal projections. Neuropsychological testing demonstrated MCI. Muscle biopsy from the left deltoid muscle at the age of 58 years revealed ragged-red fibers, increase of fat droplets in some fibers, ring-shaped hyperreactive subsarcolemmal (SS) in ragged-red fibers on staining for oxidative enzymes, and some cytochrome oxidase-negative fibers (Figure 1). Electron microscopy showed SS accumulation of mitochondria, increased accumulation of free glycogen, subsarcolemmally and between myofibrils. The mitochondria showed concentrically arrayed tubular cristae and typical paracrystalline inclusions (Figure 1). Genetic testing at the age of 59 years revealed the tRNA (transfer RNA) (Lys) mutation m.8344A > G with a heteroplasmy rate of 70%.

The presented patient is interesting for the multiple organ nature of the phenotype, including the previously unreported phenotypic features psoriasis, chronic diarrhea, bulbar involvement, and hyperlipidemia. Phenotypic manifestations previously reported and present or absent in the presented patient are listed in table 1. Only for some of the previously reported phenotypic manifestations the frequency is known (Table 1).[23] Her son had developed MERRF-syndrome as well and probably died from sudden cardiac death or sudden unexplained death in epilepsy.

Phenotypic features unreported so far in MERRF-syndrome were hyperlipidemia, psoriasis, bulbar involvement, and diarrhea (Table 1). Whether psoriasis should be regarded

as a true manifestation of the disorder remains speculative since she might have inherited it from her father but the m.8344A > G mutation cannot be excluded as cause of the dermatological abnormality. An argument for a causal relation is that dermatological abnormalities are a frequent feature of mitochondrial disorders (MIDs).[24] Although there are some reports indicating that arterial hypertension could be associated with MID,[25,26] this association is not generally accepted. Although many patients with MID also manifest with hyperlipidemia, it is not convincing to regard it as a manifestation of the underlying mutation in each case since today hyperlipidemia is endemic in the Western world. However, the high prevalence of hyperlipidemia in MID patients suggests that it can be a phenotypic feature. Bulbar involvement was mild but has not been previously reported in MERRF. An argument for bulbar involvement as a feature of MERRF is that it has been reported in other MID patients.[27] Although diarrhea has not been reported as a phenotypic feature of MERRF, it is a common feature of other MIDs.[28] The patient is also noteworthy for the late onset of the syndrome, which usually starts in childhood and rarely in adulthood. The commonly early onset is an argument for psoriasis to belong to the clinical spectrum of MERRF. It is also noteworthy that diarrhea was an early manifestation of the syndrome. The phenotypic variability could be explained with the variable heteroplasmy rates or other modifying factors.

This case shows that the phenotypic spectrum of MERRF syndrome is broader than previously reported and has to be classified as mitochondrial MODS. Diarrhea, psoriasis, bulbar involvement, and hyperlipidemia should be included in the phenotypic spectrum of MERRF-syndrome.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

None.

How to cite this article: Finsterer J, Kovacs GG. Psoriasis, bulbar involvement, and diarrhea in late myoclonic epilepsy with ragged-red fibers-syndrome due to the m.8344A > G tRNA (Lys) mutation. Iran J Neurol 2017; 16(1): 45-9.

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Iranian Journal of Neurology

Clinical Note

Iran J Neurol 2017; 16(1): 50-2

Endovascular management of chronic internal carotid occlusion with Penumbra system

Received: 21 Sep 2016
Accepted: 26 Nov 2016

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Keywords
Carotid Occlusion; Revascularization; Penumbra System

Carotid occlusion is a common disease and its incidence is about 6 in 100000. Annual risk of stroke is between 3% and 10% in older than 60 years with carotid occlusion and recurrent ischemic event have been reported in 25% of symptomatic carotid occlusion.[1] Since many years ago it has been advocated in complete arterial occlusion, there was no chance for emboli to pass into the distal circulation. Therefore, a complete occlusion of the internal carotid artery (ICA) has been regarded as no need to follow-up and the natural history and optimal management of this condition remain undetermined up to now.[2]

Nowadays, chronic occluded vessels could be revascularized by endovascular techniques. It has been successfully used in peripheral and coronary arteries.[3] The recent development of endovascular therapy has enabled the recanalization of carotid occlusion technically although it needs more study to evaluate its efficacy.

In this case presentation, we describe new technique in opening symptomatic chronic carotid occlusion with the aim of reducing risk of distal embolization using the Penumbra thromboaspiration system (Alameda, California, USA).

A 56-year-old man was refereed with history of repeated stroke in the territory of left anterior circulation since 1 month ago. He was treated medically and evaluated for risk factors of stroke. He was heavy smoker. Carotid duplex study showed bilateral carotid occlusion. Digital subtraction angiography confirmed bilateral ICA occlusion from the origin. ICAs were filled in cavernous part by retrograde flow from ophthalmic arteries of external carotid arteries (ECAs). The patient had repeated transient ischemic attack in spite of best medical treatment. Due to the deterioration of the patient, it was decided to treat the patient by endovascular revascularization. The risks and benefits of the procedure were explained to the patient and his relatives. An informed consent was obtained from the patient.

The patient underwent endovascular revascularization, he was under aspirin and plavix for 2 weeks. The procedure was performed under general anesthesia and via the percutaneous transfemoral route. Heparin was injected intravenously to maintain activated clotting time between 200 and 250 seconds. Guiding catheter 8 Fr was placed in the left common carotid artery (CCA). With contrast injection it was shown that there was left ICA

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occlusion at origin (Figure 1-A). For reducing the risk of dissection because it is impossible to show the roadmap for determining the right way to pass the true lumen (Figure 1-B). With the coaxial system 3 Max reperfusion catheter (Alameda, California, USA) was placed near to the occlusion and microwire 0.014 pilot was introduced to the catheter to approach to the occlusion part. Microwire probing the occlusion part to find a passing way. During navigating and probing the occlusion the Penumbra system was working for thromboaspiration continuously. It was applied through the Penumbra system. After passing the microwire from occlusion part (Figure 1-C), the 3 Max catheter was enhance over the microwire in association with negative pressure through catheter by Penumbra system (Figure 1-D). Navigation of catheter with suction decreased the risk of distal embolization. With passing the catheter to distal part of ICA, it was evaluated the patency of distal ICA (Figure 1-E). It was confirmed that catheter is in true lumen by control angiography (Figure 1-F). Fortunately, the rest of the lumen was opened.

3 Max catheters was retrieved under suction of Penumbra system (Figure 1-G). Then WALLSTENT 7 mm × 40 mm was deployed in left ICA to CCA (Figure 1-H). Then balloon post dilatation 5 × 20 was performed. ICA occlusion was repaired completely (Figure 1-J). Carotid occlusion revascularization was successful.

The patient condition was good and stable after recovery. There was no new neurological deficit, and he was referred to neurologic intensive care unit.

Brain magnetic resonance imaging (MRI) showed no new lesion and carotid duplex and magnetic resonance angiographic showed patency of left ICA. The patient got better during hospitalization. He could walk independently after 1 week and he was discharged in good condition.

It seems still revascularization of carotid occlusion is a taboo. Unfortunately, the natural history of patients with carotid artery occlusion is poorly understood, so there are continuing areas of debate in decision to recanalization of the occluded ICA. It is preferred to treat them medically as the difficulty and risks of surgery are thought to outweigh the natural history of the condition. Why carotid occlusion is abandoned to treat. It may be due to previous study which could not prove the efficacy of ECA/ICA bypass surgery.[4]

There is growing case reports and case series of carotid occlusion revascularization with intervention in recent years. They showed carotid occlusion revascularization is feasible and safe.[5-7]

There is always risk of dissection and distal emboli. Kao, et al.[7] advocated that excessive rotational or drilling motion of the wire was avoided, but successive small penetrate-and-advance steps carefully along the imaginary tract of the occluded vessel segment can pass the occlusion. Namba, et al.[3] suggest that initial penetration of the occluded stump from the anterior side will provide maximal chance to access the "true lumen." Rostambeigi, et al.[8] used ultrasonography to navigate and pass the carotid occlusion.

The major concern in recanalizing chronic ICA occlusion is the possibility of causing distal embolism from the stump. Terada, et al.[9] believe that embolic complication appear during angiography just after angioplasty for the occluded point from the guiding catheter placed in the CCA. Forceful injection of the contrast agent might cause the emboli remaining in the stump of the recanalized lumen to migrate. Although Spearman, et al.,[10] believed in chronic ICA occlusion, the thrombotic content in the stump is further organized, and the possibility of releasing embolic debris during device manipulation should be minimal. In addition, the antegrade flow after guide wire crossing and gentle undersized predilatation is usually sluggish, with low risk of carrying emboli downstream.

For decreasing the risk of distal emboli there was two strategies that was applied by different authors. Distal protection and proximal protection is recommended.[6] In this case, the different strategy was applied by Penumbra system to decrease the risk of distal emboli. Using the Penumbra system, it seems to reduce the risk of distal emboli and facilitate navigation and passing through the occlusion. Penumbra system is safe and effective in patients experiencing acute ischemic stroke secondary to large vessel occlusive disease, it also has been used for other occlusive disease like sinus thrombosis.[11] Penumbra system can be used to revascularization of chronic carotid occlusion with low risk of distal emboli. Although, this is a preliminary report but it seems this technique is safe and feasible.

Conflict of Interests

The authors declare no conflict of interest in this study.

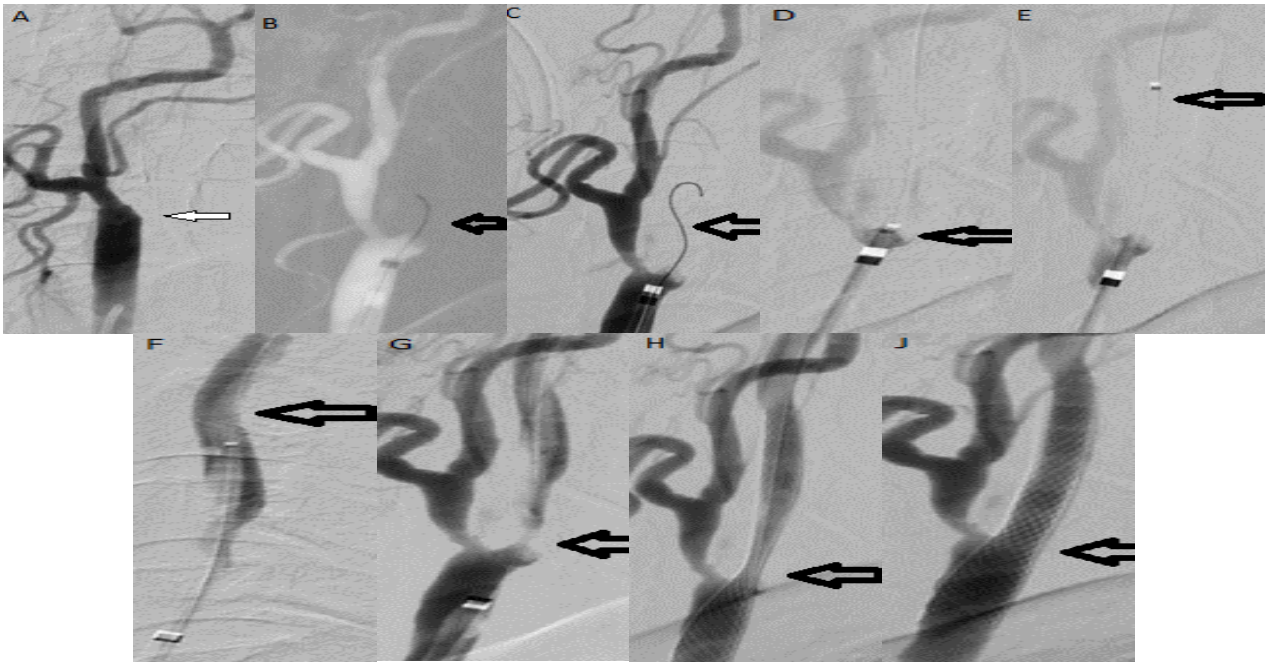

Figure 1. (A) Left internal carotid artery (ICA) complete occlusion (arrow), (B) passing microwire through the occlusion, (C) control digital subtraction angiography after microwire position, (D) approaching 3 Max catheter with Penumbra system, (E) passing through the occlusion, (F) control catheter position in ICA, (G) retrieve 3 Max, (H) deploying the stent, (J) post ballon angioplasty final result

Acknowledgments

I am grateful to Dr. Kadkhodazadeh, and Dr. Sara Mokhtar for their comments on preparing this manuscript.

How to cite this article: Mehrpour M. Endovascular management of chronic internal carotid occlusion with Penumbra system. Iran J Neurol 2017; 16(1): 50-2.

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Iranian Journal of Neurology

Neurological Images

Iran J Neurol 2017; 16(1): 53-4

Fahr disease: Idiopathic basal ganglia calcification

Received: 26 Sep 2016
Accepted: 02 Dec 2016

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Keywords
Fahr Disease; Idiopathic Basal Ganglia Calcification; Tomography; X-Ray Computed

A 23-year-old man presented with progressive bulbar and oromandibular dystonia, in addition to distal limbs involvement. In neurological exam, the patient had diverse manifestations, most commonly movement disorder, cognitive impairment, ataxia and speech disorder. Other minor neurologic manifestations included pyramidal signs, psychiatric features, and gait disorders. He had no positive family history, and his parents were not relative. Calcifications in the brain computed tomography scan (CT scan) are seen primarily in the basal ganglia and in other areas such as the cerebral cortex (Figure 1).

Fahr disease (FD) is a rare genetically dominant, neurodegenerative disorder characterized by idiopathic bilateral deposits of calcium in the striopallidodentate area.[1] Symptoms may include deterioration of motor function, dementia, seizures, headache, dysarthria, spasticity, eye impairments, and athetosis.[2] After ruling out the medical calcium metabolism abnormalities, FD is diagnosed by the presence of extensive bilateral symmetric intracranial calcifications and developmental defects.

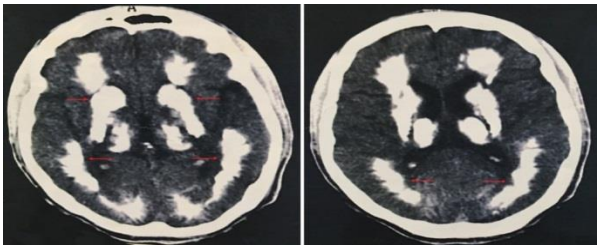

Figure 1. Brain computed tomography scan (CT scan) of the patient with Fahr disease (FD) showing bilateral symmetrical calcifications in the basal ganglia and the cerebral cortex (Arrows)

The major differential diagnosis includes hypoparathyroidism. Bilateral symmetric calcification involving striatum, pallidum, dentate nucleus, thalamus, and white matter is reported from asymptomatic individuals to a variety of neurological conditions. The key point is that there is no known calcium metabolism abnormality in autosomal dominant or sporadic bilateral striopallidodentate calcinosis. Movement disorders, especially Parkinsonism is the most common presentation followed by cognitive impairment and ataxia.[3]

Magnetic resonance imaging (MRI) is sensitive in detecting brain abnormalities; however, it is difficult to identify calcifications by routine MRI because calcifications. Therefore, brain CT scan is considered to be critical for detecting and localizing the extent of intracranial calcifications.[4]

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Conflict of Interests

The authors declare no conflict of interest in this study.

How to cite this article: Shahidi GA, Safdarian M. Fahr disease: Idiopathic basal ganglia calcification. Iran J Neurol 2017; 16(1): 53-4.

Acknowledgments

None.

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