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Original Articles

Clinical Notes

Letter(s) to Editor

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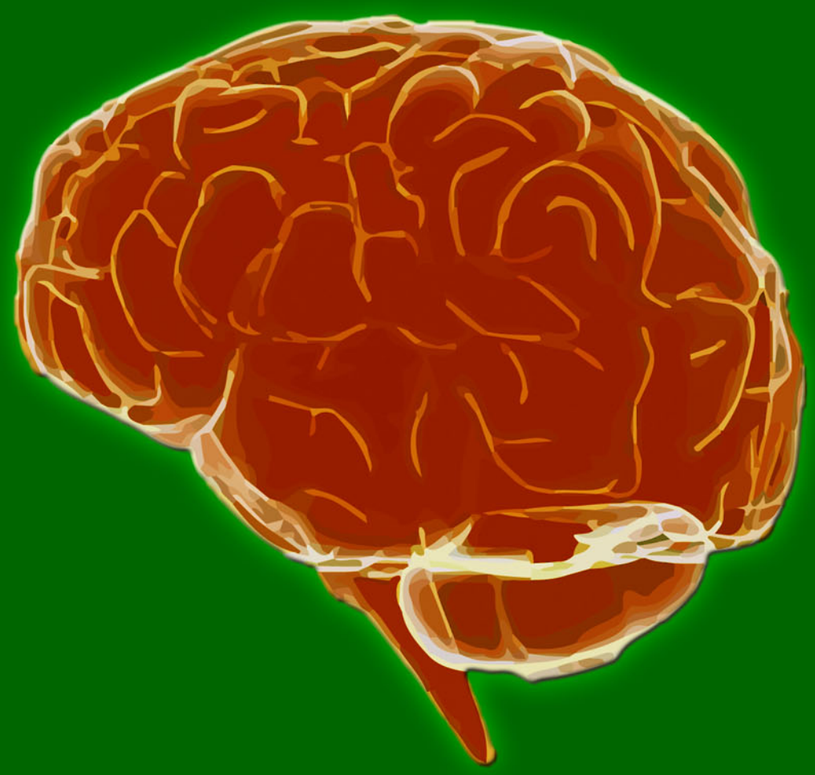
Volume 14, Issue 4, Fall 2015



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Iranian Journal of Neurology

INFORMATION FOR AUTHORS

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Submissions should be accompanied by a cover letter including a declaration by the first author on behalf of the others to the effect that

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(3) All persons listed as the authors have read it and approved it for publication. The cover letters should be submitted in section "Comments for the Editor".

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The journal publishes:

- Original Article
- Review Article
- Case Report
- Short Communication
- Clinical Notes
- Editorial
- Letters to Editor
- Neurological Images
- Neurological Videos
- Iranian Neurological Events
- Clinical Quiz

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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Neurological images or videos: Interesting cases as **neurological images** or **videos** are welcome. They should be maximally 400 words with legends without abstract and unstructured. The videos should be uploaded as supplementary files.

Letter to the Editor: May concern short scientific reports and comments. The maximum number of words should be below 800 words with maximum 5 references, no abstract, no table or figure, and unstructured.

Clinical notes: Refer to important interesting observations which are imperative for reminders in clinical practice. The maximum number is 1000 words with maximum 5 references, 1 table and 1 figure with no abstract.

Iranian neurological events: Include the brief description of major regional events (congresses or seminar) implemented in Iran.

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- Manuscripts should be submitted in 12 points, Arial font, with double line spacing and sufficient margins of 2.5 cm.
- The text should not be formatted.
- Each section of the paper should begin on a new page

The manuscript must include:

- Page 1: Title Page
- Page 2: Abstract and Key Words
- 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.

2. Abstract page:

- The length of the abstract should be at least 200 and not more than 250 words for original papers and not more than 150 words for review papers and case reports. Abstracts of original papers should be structured to include the background, methods, results and conclusion.
- Below the abstract authors should provide between three and six keywords conforming to Medical Subject Headings (Index Medicus).

3. Page three and subsequent pages of the original paper and short communication should include the text arranged in the following order (for other mansucript type, see above):

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3. **Results:** Present results directly related to the topic of the paper only; tables and/or figures are recommended.
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Proofs: The proofs will be sent via email and must be accordingly corrected and get back within 48 hours.

Table of Contents

Original Article(s)

Clinical Notes

Letter(s) to Editor

Iranian Journal of Neurology

Original Paper

Iran J Neurol 2015; 14(4): 185-189

Assessment of clinicopathologic features in patients with pituitary adenomas in Northeast of Iran: A 13-year retrospective study

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Accepted: 18 Jun 2015

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Keywords
Pituitary Adenoma, Functional Adenoma, Survival Rate

Abstract

Background: Intracranial lesions of the pituitary gland are common pituitary adenomas, accounting for 6-10% of all symptomatic intracranial tumors. In this retrospective study, the clinicopathologic features and survival rate of pituitary adenomas were evaluated.

Methods: The present retrospective study was conducted on 83 patients with pituitary adenomas, referring to radiation oncology departments of Ghaem and Omid Hospitals, Mashhad, Iran, over a period of 13 years (1999-2012). Data obtained from clinical records including clinical features, type of surgery (if performed), treatment modality, overall survival rate, and progression-free survival rate were analyzed.

Results: Eighty-three patients including 44 males (53%) and 39 females (47%) participated in this study. The median age was 40 years (age range: 10-69 years). Chiasm compression was reported in 62 patients (74.4%), and 45.78% of the subjects suffered from headaches. Functional and non-functional adenomas were reported in 44 (53.01%) and 39 (46.99%) patients, respectively. In cases with functional and non-functional adenomas, the disease was controlled in 95 and 84.5% of the subjects for 3 years, respectively. Furthermore, 1- and 3-year survival rates for functional adenoma were 84.6 and 23%, respectively; the corresponding values were 90.9 and 22.7% in non-functional adenomas, respectively.

Conclusion: In this study, a significant correlation between headache severity and type of adenoma was observed. So, application of surgery and radiotherapy together could be a highly effective approach for treating functional adenomas, although it is less efficient for the non-functional type.

Introduction

Intracranial lesions of pituitary gland are common pituitary adenomas, accounting for 6-10% of all symptomatic intracranial tumors.[1-3] Pituitary adenomas are defined as abnormal growth of tumors in pituitary glands (benign adenomas,

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invasive adenomas, and carcinomas).[4,5] Recent studies have shown that invasive adenomas may approximately affect 1 in 1000 people of the general population.[6]

The most frequent pituitary adenomas are microadenomas with an estimated incidence of 16.7%. Pituitary adenomas are also categorized as active-functioning and non-functioning adenomas; two-thirds of clinically diagnosed lesions are functional adenomas.[7]

Symptoms of pituitary disorders are often non-specific and may differ given the effects of space-occupying lesions, increased hormonal release or both.[8,9]

Among patients with pituitary adenomas, different types of headaches such as chronic, episodic migraines and unilateral headaches including primary stabbing headache, short-lasting unilateral neuralgiform headache, cluster headache, and hemicrania continua are common.[10-13] Pituitary adenomas are also associated with psychiatric disorders including hostility, anxiety, apathy, depression, emotional instability, and irritability.[14,15]

Although the treatment of pituitary adenoma depends on the size and type of tumor, surgery is the common treatment modality. Transsphenoidal adenomectomy is a method for tumor removal, though recently, endoscopic surgery has been commonly applied.[16] Due to the importance of pituitary disorders and insufficient research in this field, this study aimed to assess the clinicopathologic features and treatment outcomes of patients with pituitary adenomas over a 13-year period in the Northeast of Iran.

Materials and Methods

We studied the clinicopathologic features of all patients, presenting with pituitary adenomas. The subjects had referred to the departments of radiation oncology at Omid and Ghaem hospitals in years 1999-2012.

The sample size included 83 patients, according to inclusion criteria. Since all eligible subjects were recruited within a specific time span, use of a formula for calculating the sample size was not necessary. The inclusion criteria were as follows: (1) Pathological evidence of pituitary adenoma; (2) essential information including age, gender, treatment modality, and type of surgery and medical records; and (3) undergoing medically proposed treatments. The exclusion criterion was unfinished complementary treatment (the recommended treatment).

In this retrospective, cohort study, all patients' medical records were collected and examined after review and pathological assessment for the selection of definitive or complementary treatments. Data obtained from the clinical records such as clinical signs, type of surgery (if performed), treatment modality, overall survival rate, and progression-free survival were examined; in addition, previous medical histories and clinical variables were recorded. We contacted the patients in case the data needed to be corrected or completed.

Patients with pituitary adenomas, diagnosed via pathological assessment were included in this study after meeting the inclusion criteria. All patients' records were included in the predesigned questionnaires.

Data obtained from patients' records and recorded calls were analyzed by SPSS software (version 16, SPSS Inc., Chicago, IL, USA). For descriptive data, statistical indices, tables, and diagrams were used. Conventional methods of survival analysis including Kaplan–Meier and Cox regression were employed in order to study the effects of variables on disease-free survival rate.

Since no medical interventions were performed in this study and the patients' records were kept confidential, no written consents were obtained.

Results

Eighty-three patients including 44 males (53%) and 39 females (47%) participated in this study. 4, 12, 24 and 19 patients were within the age range of 10-20, 20-29, 30-39, and 40-49 years, respectively; in addition, 19 patients were 50-59 years old, and five subjects were within the age range of 60-69 years. The median age was 40 years (age range: 10-69 years), and the majority of the subjects (28.9%) were 30-39 years old.

Chiasm compression was reported in 62 patients (74.4%) and 45.78% of the subjects suffered from headaches. Functional and non-functional adenomas were observed in 44 (53.01%) and 39 (46.99%) patients, respectively.

Prolactin (PRL) and growth hormones (GH) were the most secreted hormones (19.3% and 20.5%, respectively). Of all patients, one showed an increased insulin-like growth factor-1 (IGF-1) level, 16 experienced prolactin elevation and 17 cases had elevated growth hormone; in addition, adrenocorticotropic hormone (ACTH) level increased in 4 cases (Figure 1).

Galactorrhea and acromegaly were observed in 6% and 22% of the patients, respectively, and Cushing's syndrome was reported in 4.82% of the subjects; this disease recurred in 25 patients (30%).

Among 44 patients (53%) with functional adenomas, 22 subjects were males (50%) and 22 were females (50%). Thirty-nine (47%) individuals

suffered from non-functional adenomas, including 22 males (50%) and 17 females (43.6%). According to the findings of the present study, no significant association was found between adenoma type and gender (P = 0.540).

Among 44 patients with functional adenomas, 25 patients aged < 40 years (56.8%) and 19 cases were > 40 years (43.2%). Furthermore, in patients with non-functional adenomas, 17 and 22 individuals were < 40 and > 40 years of age, respectively (43.6% and 56.4%, respectively). There was no significant association between age and type of adenoma (P = 0.229).

Chiasm compression, accompanied by adenoma, was assessed in patients with functional and non-functional adenomas. Signs of compression were reported in 31 patients with functional adenomas (70.5%) and 31 subjects with non-functional adenomas (79.5%); no significant relationship was observed between chiasm compression and tumor type (P = 0.345).

The headache was reported in 13 individuals with functional adenoma (29.5%) and 25 patients with non-functional adenoma (64.1%). The findings showed that headache was prevalent among patients with non-functional adenomas; therefore, there was a significant association between adenoma type and headache (P = 0.001, r = −0.346).

Adjuvant radiotherapy, as a complementary treatment, was applied for 60 patients (72.3%). Radiotherapy was also performed for 22 subjects (26.5%) after surgical failure; in addition, the mean duration of follow-up was 16 months (6-120).

Active disease was reported in only one patient (2.3%) with functional adenoma. Among patients with non-functional adenomas, five cases (12.8%) showed signs of active disease; in both groups, the disease was controlled satisfactorily (P = 0.064). In the evaluation of the relationship between tumor type and disease activity, no significant difference was observed between patients undergoing primary radiotherapy and those receiving adjuvant radiotherapy (P = 0.534).

Out of 27 patients with non-functional adenoma, 24 individuals (88.9%) had non-active disease and 3 patients (11.1%) showed signs of active disease. As to the findings, there was a significant relationship between the type of adenoma and disease control; in patients with functional adenoma, disease control was significantly higher (P = 0.004).

Analysis of overall survival rate

The mean follow-up duration was 30 months, with a median of 16 months (range: 6-125). We compared the overall survival rate of patients, based on the type of adenoma. Three-year survival rate was 95% in patients with functional adenomas and 84.5% in patients with non-functional adenomas. According to these results, there was no correlation between patients' overall survival rate and adenoma type (Table 1).

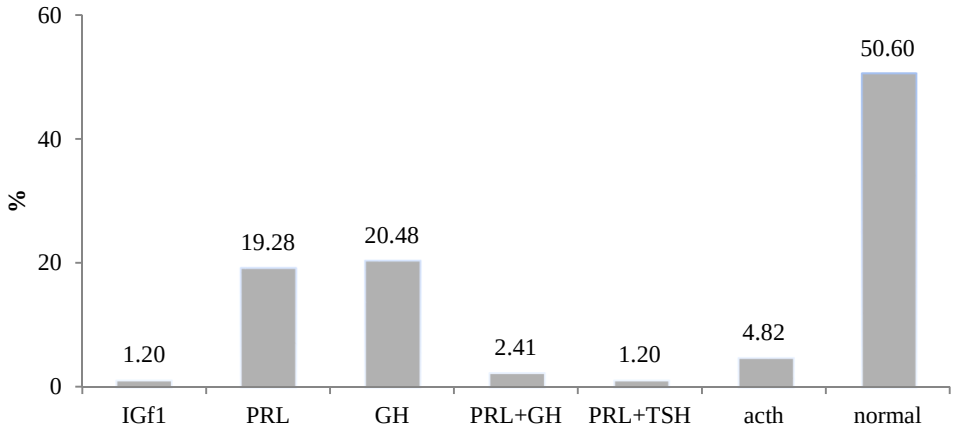

Figure 1. Increased secreted hormones
IGF1: Insulin-like growth factor-1; PRL: Prolactin; GH: Growth hormone; TSH: Thyroid stimulating hormone

Table 1. Relationship between type of adenoma and mean ± SD of overall survival rate

Type of adenoma	Number	Mean ± SD
Functional	44	31.659 ± 29.953
Non-functional	39	30.282 ± 28.799
Total	83	31.012 ± 29.246

SD: Standard deviation

Discussion

Pituitary adenomas account for 10-15% of primary intracranial neoplasms. In the current study, we examined the clinicopathologic features of 83 patients, presenting with pituitary adenomas during 13 years. The most common signs were vision impairment and chiasm compression; also, galactorrhea was more prevalent than Cushing's disease or acromegaly. Functional adenoma was observed in more than half of the patients. Three-year survival rate was 95% for functional adenoma and 84.5% for non-functional adenoma, although no association was found between survival rate and type of adenoma.

Rim et al.[17] evaluated patients with pituitary adenoma, using external beam radiation therapy (EBRT). Correspondingly, it was revealed that headache, vision impairment, and hormonal disorders were the most common signs. In the mentioned study, EBRT was considered to be an effective method for controlling the pressure effects of non-functional adenoma; similarly.

Furthermore, Shao and Li[18] in a similar study reported that headache and hormonal disorders were more prevalent among patients with pituitary adenomas; however, lower levels of hormones were observed, compared to our findings; this may be due to differences in the studied populations.

In the current study, 74.4% of the studied patients suffered from vision impairment. Another similar study performed in Egypt showed that 57% of patients had optical disorders, which is different from the findings of the present study; the mean follow-up duration was 44 months in El-Shehaby et al's.[19] study, while it was 31 months in our study.

In the present study, adenoma type was not significantly associated with gender, age or signs of chiasm compression, though a significant relationship was observed between adenoma type and headache severity; this could be due to hormonal activity in patients with functional adenoma. Furthermore, prolactin, IGF-1, growth hormone and ACTH levels were high in some patients in the present study.

In our study, there was a significant relationship between adenoma type and disease activity in patients undergoing complementary adjuvant radiotherapy. Becker et al.[20] in a review study reported that non-functional adenomas are more significantly affected by radiotherapy, compared to functional adenomas (80-90%), while our findings showed that functional adenomas can be better controlled.

Rim et al.[17] in another study showed that external radiotherapy plays a critical role in the recurrence of non-functional adenomas; correspondingly, as it is presented in this study, use of radiotherapy is recommended for controlling adenoma, although with a different impact.

Mecca et al.[21] studied the efficacy of external conventional radiotherapy (CRT) in short- and long-term control of acromegaly; they indicated the long-term effects of CRT on active acromegaly. Similar to our study, active adenoma was controlled in several cases.

As the results indicated, 3-year survival rate was 95% for functional adenoma and 84.5% for non-functional adenoma; therefore, there was no relationship between survival rate and adenoma type.

Also, in our study, 1- and 3-year survival rates for functional adenoma were 84.6 and 23%, respectively; however, regarding the non-functional type, these values were 90.9 and 22.7%, respectively. Rim et al.[17] reported 10-year control rates to be 96% and 66% for functional and non-functional adenomas, respectively.

We also showed that survival in functional adenoma was more than that observed in non-functional tumors. Puataweepong et al.[22] and Wilson et al.[23] reported the 5 years survival rate to be 91% and 87%, respectively. Zargar et al.[24] studied the clinical and endocrine aspects of pituitary tumors. As they indicated, in an endocrine center, functional pituitary tumors were more prevalent than non-functioning tumors; similarly, we showed that functional adenomas are more frequent than the non-functional type.

Limitations

Unfortunately, in the present study, the exact time of disease recurrence was not recorded and many patients did not refer for follow-up sessions after the initial treatment.

Conclusion

In the present study, type of adenoma was not associated with age, gender or signs of chiasm compression, although there was a significant association between headache and type of adenoma. Application of surgery and radiotherapy together could be a highly effective approach for treating functional adenoma, although it is less efficient for the non-functional type. It is suggested that further research with different methods be performed on larger populations to obtain better outcomes.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

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Original Paper

Iran J Neurol 2015; 14(4): 190-194

Mutation analysis in exons 22 and 24 of SCN4A gene in Iranian patients with non-dystrophic myotonia

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Keywords
Nondystrophic Myotonia, Mutation, SCN4A, Polymerase Chain Reaction-Single Strand Conformational Polymorphism

Abstract

Background: Non-dystrophic myotonias are a heterogeneous set of skeletal, muscular channelopathies, which have been associated with point mutations within sodium channel α-subunit (SCN4A) gene. Because exons 22 and 24 of SCN4A gene are recognized as hot spots for this disease, the purpose of the study is to identify mutation in exons 22 and 24 of SCN4A gene in Iranian non-dystrophic myotonias patients.

Methods: In this study, 28 Iranian patients with non-dystrophic myotonia analyzed for the mutation scanning in exons 22 and 24 of SCN4A gene by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) and sequencing.

Results: We found 29073G>C substitution in SCN4A gene in one case and 31506A>G substitution in seven cases. The 29073G>C substitution causes a missense mutation G1306A, located in the conserved cytoplasmic loop connecting repeat III and IV of the SCN4A channel but, 31506A>G substitution do not alter amino acid in SCN4A protein.

Conclusion: G1306A residue is located in functionally important protein region. In "hinged-lid model" for Na^+ channel inactivation in which glycines[1306] act as the hinge of the lid occluding the channel pore. Mutation in this region slowed fast inactivation. Therefore, it might be a pathogenic mutation. The causal relationship of this mutation with the disease is an object for further discussion.

Introduction

Muscle channelopathies (the inherited muscle ion channel diseases) are rare disorders of the skeletal muscle. Non-dystrophic myotonias are a heterogeneous set of skeletal, muscular channelopathies, which have been associated with specific point mutations within sodium channel α-subunit (SCN4A) or Cl–channel (CLCN1) genes.[1,2] The prevalence of non-dystrophic myotonia has been estimated to be ~1 in 100,000 in the worldwide.[3] Voltage-gated sodium channels are prominent transmembrane proteins in excitable tissues and are responsible for the rising phase of the action potential in the membranes of neurons and most electronically excitable cells.[1,4]

The skeletal muscle sodium channel comprises a principal pore-forming and voltage sensing subunit (the alpha subunit), which is associated with an

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accessory beta-1 subunit. The beta-1 subunit has not been reported to be linked to any disease. It's alpha is encoded by the SCN4A gene, which is located on chromosome 17q23-25, comprises 24 exons with a 5.5 kb open reading frame, is associated with various neuromuscular disorders.[5] The alpha subunit consists of four homologous domains, and each domain possesses six hydrophobic putative transmembrane segments (S1–S6).[4] Conserved sequences in these channels promote specific functions.[6,7]

SCN4A mutations produce several clinically distinct skeletal muscle disorders including hyperkalemic periodic paralysis, paramyotonia congenita, potassium-aggravated myotonia, hypokalemic periodic paralysis, and congenital myasthenic syndrome.[5]

The similarities between sodium channel myotonia and myotonia congenita can lead to difficulty in prioritizing genetic testing. Clinical history and examination considered in conjunction with electromyogram findings can improve the ability to distinguish between the two and guide genetic analysis, but in some cases screening of SCN4A genes will be required.[8]

More than 40 mutations have been reported in SCN4A gene, but exons 22 and 24 of SCN4A gene are recognized as hot spots for myothonia,[9] and there is no study investigating on Iranian patients with non-dystrophic myotonia, so the aim of this study was to screen this hotspot exon of SCN4A gene in Iranian patients with non-dystrophic myotonia by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) and sequencing.

Materials and Methods

Twenty-eight Iranian patients with non-dystrophic myotonia were included in the present study (Table 1). The control group comprised 30 healthy controls that matched for age, sex, and ethnicity. Control subjects had no signs of the neuromuscular disease when enrolled in the study. All of the patients and the control group were informed of the aims of the study and gave their informed consents for the genetic analysis. Patients were referred for assessment by consultant neurologists in Iran.

DNA was obtained directly from peripheral blood samples by chloroform extraction and ethanol precipitation. Samples of genomic DNA were amplified by the polymerase chain reaction (PCR) with specific primers. The experimental conditions were optimized for each pair of primers.

The following primer pairs were designed to amplify the exon 22 of the SCN4A gene and the exon 24 of SCN4A gene (Table 2). Primers were designed by Primer Design Software (Primer Premier 5.0; Premier Biosoft Inc., Canada), and their secondary structure was examined with Gene Runner version 3.05 (Hastings Software Inc. Hastings, NY, USA, http://www.generunner.com).

PCR was performed in a total volume of 25 µl containing 100 ng of template DNA, 10 pmol of each primer and 1× PCR Master Mix (Yekta Tajhiz Azma, Tehran, Iran). The PCR was performed based on the following conditions: initial denaturation at 94° C for 2 minutes; followed by 35 cycles including denaturation at 94 °C for 35 seconds, annealing at 64 °C (exon 22) and 62 °C (exon 24) for 50 seconds, and extension at 72 °C for 30 seconds; and a final extension at 72 °C for 5 minutes followed by a final extension for 5 minutes. The PCR products were electrophoresed on an ethidium bromide-stained 2% agarose gel.

The amplified PCR products were analyzed using SSCP analysis.[10] 6 µl of the amplified samples were diluted with 6 µl of SSCP loading buffer dye, denatured at 94° C for 3 minutes and then kept on ice for 5 minutes until loaded onto 8% polyacrylamide gels. Gels were run at 120 V for 12 hours in a buffer containing TBE ×0.5 (pH = 8.3). After electrophoresis; the gels were stained by silver nitrate.

Table 1. Clinical data for non-dystrophic myotonia patients

Group	Sex	Number	Age	Age of onset	Effected of family
Patient	Male Female	14 14	36 ± 12	9 ± 5	16 famillial 12 sporadic
Healthy control	Male Female	17 13	34 ± 14	-	-

Table 2. Primers used for amplification of sodium channel α-subunit (SCN4A) gene

Exon	Primer sequence (5'-3')	Temperature (°C)	Size (bp)
22	F: TGGAGGCAGGAAGGGGAACT R: GGCAGCACACACAGGACAGG	64	197
24	F: TTCGAGACCTTCGGCAACAG R: CGGCTGTAGGCGATGAACTG	62	362

F: Forward; R: Reverse

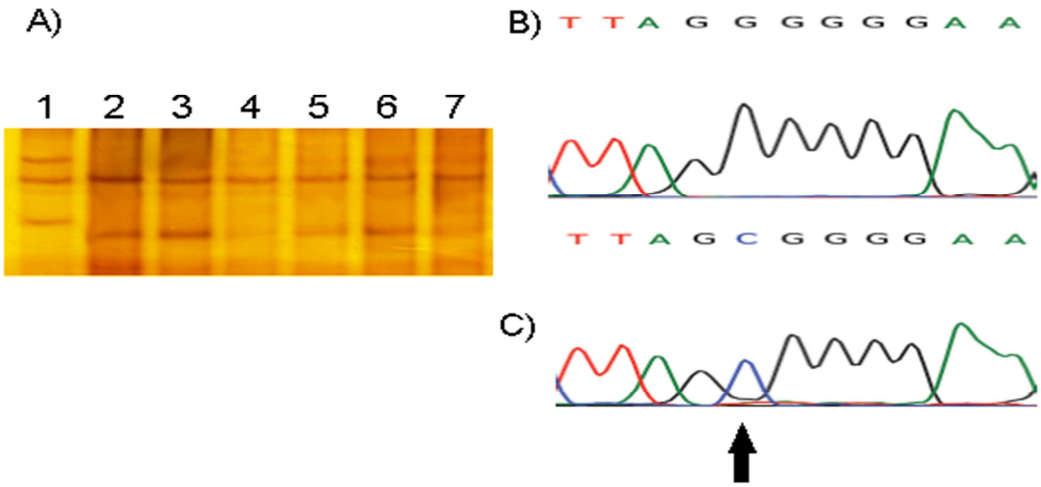

Figure 1. (A) Single strand conformational polymorphism gel electrophoresis of exon 22 of the SCN4A gene. Line 1 shows different pattern banding regard to lines 2, 3, 4, 5, 6, and 7. Line 7 is normal control. (B) Chromatogram of sample 7 (without mutation) and (C) Chromatogram of sample 1 (without 29073G>C mutation).

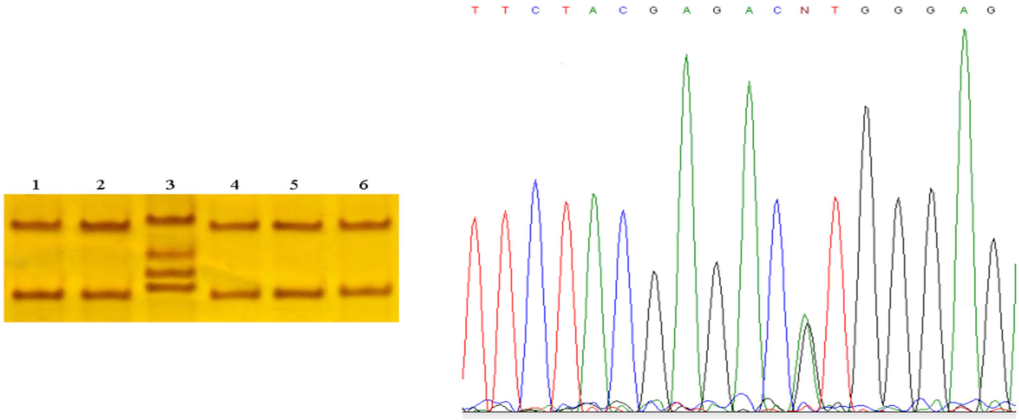

Figure 2. Single-strand conformational polymorphism results. Line 3 shows shift bands in a patient with a heterozygous variation. Lines 1, 2, 4, and 5 show patients without any variations and line 6 is a control sample. Sequence analysis of the genomic DNA of the patient revealed the heterozygous transition of an A to G at position 31506 in exon 24 SCN4A genes in a patient with non-dystrophic myotonia (left)

PCR products of samples with various band patterns in SSCP gel were sent to a commercial agency (Macrogene Seoul, South Korea) for sequencing. The online multiple sequence alignment software; ClustalW2 (http://www.ebi.ac.uk/tools/msa/ clustalw2/) and Blast analysis was used to find the percent homology of the sequences that has been obtained in the study and with all other sequences of the other species.

Levels of the quantitative variables are presented as a mean ± standard deviation. Student's t-test was used for comparison of continuous variables; Fisher's exact test was used for comparison of categorical variables. The GraphPad Prism software (version 3.00, GraphPad Software, La Jolla, CA, USA) was used for statistical analysis, with $P < 0.05$ considered indicative of statistical significance.

Results

The mobility of single-stranded DNA fragments in SSCP gel was conducted on 28 patients and 30 healthy controls. Mean age was 36 ± 12 and 34 ± 14 years for patients and controls, respectively. The screening of exon 22 of SCN4A gene led to the identification of one mutation in one out of 28 patients. DNA sequencing revealed 29073G>C variant (Figure 1). This variant causes a missense mutation G1306A (Glycine to Alanine).

The SSCP and DNA sequencing of exon 24 revealed a synonymous heterozygous 31506A>G variation in seven out of 28 patients. This variation does not change an amino acid in SCN4A protein and has not been

previously reported in SCN4A gene (Figure 2).

The results of multiple sequence alignment with various species showed that G1306 is conserved during evolution (Figure 3).

Discussion

The clinical signs and electrophysiological indicators are used to prioritize genetic testing in the non-dystrophic myotonias but, the detection of sodium channel myotonia from dominant myotonia congenital is difficult. Hence, it requires that a proportion of patients to the screen of both SCN4A and CLCN-1 genes.[11] Lerche et al. found three point mutations at the same nucleotide position 29073 of the SCN4A gene in three families with a form of myotonia. These mutations change glycine 1306 to glutamic acid, valine, or alanine in SCN4A protein.[12]

Furthermore, McClatchey et al. found one of the three substitutions at location 1306 (glycine-to-valine) in a family characterized by chronic myotonia.[13] Vicart et al. demonstrated that the most common sodium channel myotonia mutations are V1589M and G1306 position.[14]

Matthews et al. studied the clinical and genetic features a long cohort of UK patients with non-dystrophic myotonia and they demonstrated that 3 of their patients had mutations (G1306A, G1306E) that previously described and identified two novel mutations (R1448L, L1436P) in SCN4A gene.[9]

Here, we found one homozygous missense mutation G1306A (Glycine to Alanine) in SCN4A gene in one patient with mild painful myotonia. Multiple sequence alignment with various species showed that G1306 is conserved during evolution (Figure 3). Data of Polyphen-2 software (with 0.54 score) predicted this mutation is a possible pathogen. This substitution has been previously predicted in the conserved cytoplasmic loop connecting repeat III and IV domains of the sodium channel α-subunit could cause to inactivation of gate of the sodium channel. The glycine 1306 confers a good flexibility of the hinge that could restricted by side-chains of other amino acids.[12,15,16]

Second nucleotide variation, the heteroplasmic 31506 A>G polymorphism in exon 24 SCN4A gene, which no alter amino acid sequences, has not been previously described. This synonymous mutation is located in a moderately conserved amino acid of the C-terminal loop of SCN4A protein.

Study limitations

No accessibility to tissue samples from our patients is the major limitation of our study. Another limitation is the lack of classification of the patients according to their clinical findings. Further studies with larger cohorts of patients are warranted to reveal the relationship of these nucleotide changes with non-dystrophic myotonias.

Conclusion

Our mutational analysis confirms the role of single nucleotide polymorphisms in SCN4A gene in Iranian patients with non-dystrophic myotonias. Hence, to find out and understand the nature of pathogenesis and predisposition effects of these variations on non-dystrophic myotonias, further genetic, and functional studies are necessary.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

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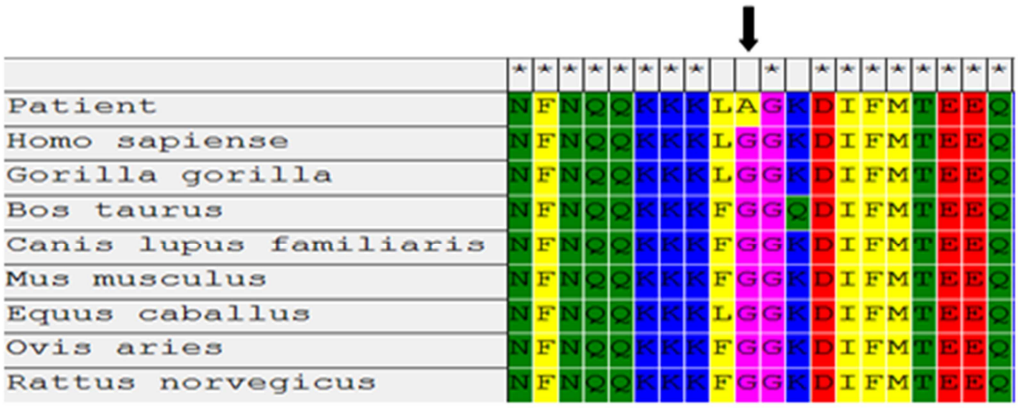

Figure 3. Multiple sequence alignment of a part of exon 22 from various species. Glycine 1306 is completely conserved among all Na^+ channel α-subunits

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Effects of L-arginine pre-treatment in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's diseases in Balb/c mice

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Keywords
Parkinson Disease, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, BALB C Mice, Protective Agents

Abstract

Background: Parkinson's disease (PD) is a common neurodegenerative disease resulting from the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). Increasing evidence demonstrated that mice treated intranasally with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) suffered impairments in motor functions associated with disruption of DA neurons in SNc conceivably analogous to those observed in PD. L-arginine has been proposed as a novel neuroprotective agent that plays protective roles in several models of neuronal cellular damage. This study aimed to evaluate the effects of L-arginine on the numerical density of dark neurons (DNs) in the SNc of Balb/c mice subjected to MPTP administration.

Methods: In the present study, we demonstrated that repeated treatment with L-arginine (300 mg/kg, i.p.) during 7 consecutive days attenuated the production of DNs in SNc of adult male Balb/c mice infused with a single intranasal administration of MPTP (1 mg/nostril).

Results: Pre-treatment with L-arginine significantly decreased the numerical density of DNs in SNc of mice 21 days after intranasal MPTP administration.

Conclusion: This investigation provides new insights in experimental models of PD, indicating that L-arginine represents a potential neuroprotective agent for the prevention of DA neuron degeneration in SNc observed in PD patients.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative and age-related disease that usually affects people over the age of 50.[1,2] PD is a slowly progressing disorder resulting in degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc), a region of the brain that controls movement. The neuronal death in SNc leads to impaired motor functions such as tremor, bradykinesia, akinesia, rigidity, and postural instability.[3] While great advances are being made in our understanding of the risk factors underlying PD

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that may soon allow for the clinical use of preventive pharmaceuticals, at this time these do not exist.[4] Even though etiology of disease remains unknown, there are well-known mechanisms involved in the pathogenesis of DA nigrostriatal degeneration of PD patients, including apoptosis, oxidative stress, and mitochondrial dysfunction.[3,5] These interrelated events finally lead to neuronal death by apoptosis; hence, anti-apoptosis strategies could, in principle, prevent, or delay the progression of PD.[6]

Recent experimental and epidemiological studies suggest that intranasal (i.n.) infusion of several environmental agents, including viruses[7] or cadmium,[8] or inhalation of aluminum[9] or manganese, may contribute to PD pathogenesis.[10] Sometimes such agents may enter the brain via the olfactory neuroepithelium, a concept termed the olfactory vector hypothesis.[11] In accordance with this hypothesis, several studies have shown that approximately 90% of patients with early-stage PD exhibit olfactory dysfunction[11,12] and that the olfactory bulb is among the first brain structures to exhibit PD-related pathology, occurring pre-clinically before the classic disease motor signs.[13]

In a series of earlier human and experimental studies, administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a systemic neurotoxin, causes a specific loss of DA neurons in the nigrostriatal system, that recapitulated the DA neuron degeneration seen in idiopathic PD patients.[14] Therefore, in the recent works, the MPTP mouse model has become the most commonly used animal model of PD.[6,15-20] In addition, the some of them demonstrated that low concentrations of MPTP can enter the brain via the olfactory mucosa and alter DA function in a range of brain structures.[16-20] Because of safety considerations, the i.n. administration of MPTP, which is not constrained by such factors, may be more effective in getting higher levels of MPTP into the brain and to induce alterations in central nervous system structure and function.[16-20]

When MPTP is injected into animal, the chemical penetrates the brain through the blood-brain barrier and is metabolized to 1-methyl-4-phenyl1-2,3-dihydropyridium (MPP+) by monoamine oxidase-B enzyme in glia. MPP+ has high affinity for the dopamine transporter (DAT) on DA cells, and is taken up into the cell.[21] MPP+ is then released from the glia and enters neurons via the DAT on DA cells.[22] MPP+ is then accumulated in the mitochondria and creates further neuronal damage through the activation of reactive microglia and subsequent generation of free radicals.[5,23] By seven days post-administration of MPTP, a significant loss of DA neurons in the SNc is evident, along with a significant reduction of DA production in the terminal field within the striatum.[24] Thus, MPTP administration to animals induces a DA neuron loss that mirrors the loss seen in end-stage PD.

L-arginine is a semi-essential amino acid and has different roles in the normal brain functioning. L-arginine is oxidized to nitric oxide (NO) in a NADPH-dependent reaction by the action of the enzyme nitric oxide synthase (NOS). L-arginine and NO play a modulatory role in the brain, and are involved in synaptogenesis, synaptic plasticity, neurogenesis, neuroprotection, memory and learning function, and neuroendocrine secretion.[25,26] It also has been shown that NO is synthesized by neurons as a response to the activation of N-methyl-D-aspartate (NMDA) receptors by the excitatory amino acid glutamate,[27-29] and leads to the formation of guanosine 3',5'-cyclic monophosphate (cGMP) in the brain.[28,30] Further, other studies have demonstrated the feedback inhibition of NMDA receptors by NO.[31,32] Experimental evidence has demonstrated that NO is involved in NMDA receptor-mediated neurotoxicity[33] and in the neuronal death that occurs after focal cerebral ischemia.[34,35]

L-arginine and NO can also influence the immune system by playing a key role in regulating inflammatory processes and redox stress.[36,37] It also promotes easy and efficient flow of blood through the blood vessels going to the brain.[38,39] L-arginine is also implicated in the pathophysiology of some neurodegenerative disease (i.e., Alzheimer's disease), although it's precise role remains to be determined.[40,41] Moreover, there are no reliable proofs yet with the use of L-arginine to prevent or treat PD disease. Although, it had shown some ability in improving certain conditions.[42]

In previous experimental studies, dark neurons (DNs) productions have been reported in the brain of animals exposed to various pathological conditions.[43,44] DNs are the final product of a series of physico-chemical reactions initiated from extracellular milieu and propagate into the neuron.[45-49] Morphologically DNs are characterized by at least six features namely: hyperbasophilia, argyrophilia, disappearance of antigenicity, ultrastructural compaction, volume reduction and increased electron density.[43] In addition, the morphological study of DNs by transmission electron microscopy showed chromatin changes, darkness, and shrinkage and swelled mitochondria.[46] It is believed that these types of neurons are in recovering phase (reversible type) in contrast to real DN (dead or irreversible).[45,47-49] These kind of degenerating neurons

have been reported in Huntington disease, epilepsy, spreading depression, and also in aging process.[44,50]

Since L-arginine and its product, NO, exert such a range of critical roles in regulating physiological functions of the brain, we hypothesize that L-arginine can possibly prevent the MPTP-induced neurodegeneration in the SNc of mice. So, this study was designed to evaluate the effects of L-arginine on the numerical density of DNs in the SNc of Balb/c mice subjected to MPTP administration.

Materials and Methods

Healthy adult male Balb/c mice (20-30 g body weight, 6-8 weeks old) were purchased from the Experimental Animal Facility of Birjand University of Medical Sciences, Iran. The animals were housed in polypropylene cages (four per cage) under controlled temperature and light conditions (22 ± 3 °C, 40-70% relative humidity, 12 hours light phase with daylight). They were fed with standard pellet diet (Javaneh co., Iran) and water ad libitum. All procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Birjand University of Medical Sciences. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Mice were randomly assigned to four equal groups (n = 7 each):

1. Model control (MPTP) group: mice were administrated intranasally with a single dose of MPTP (Sigma-aldrich, St. Louis, MO, USA; dissolved in saline 0.9%) at the dose of 1 mg/nostril.[18,51]

2. Sham control group: mice were administrated intranasally with a same dose of vehicle (saline 0.9%).

3. L-arginine - treated model (L-arginine treated PTP) Group: mice received intraperitoneally L-arginine (Sigma-aldrich, St. Louis, MO, USA; 300 mg/kg dissolved in saline 0.9%) once daily for 1 week starting from 3 days before MPTP administration.

4. L-arginine control group: mice only received intraperitoneally L-arginine (Sigma-aldrich, St. Louis, MO, USA; 300 mg/kg dissolved in saline 0.9%) once daily for 1 week.

MPTP (1 mg\nostril) was administered by i.n. route according to the procedure previously described[16-18] and modified in our laboratory. Briefly, mice were lightly anesthetized with xylazine/ketamine (10-75 mg/kg body weight, intraperitoneal injection) and a 7 mm piece of PE-10 tubing was inserted through the nostrils. The tubing was connected to a calibrated peristaltic pump set at a flow rate of 12.5 IU/minutes (Figure 1). The MPTP was dissolved in saline at a concentration of 20 mg/ml, after which it was infused in 1 minute intervals for 4 minutes (6 seconds pump on and 54 seconds pump off).

The control solution consisted of saline. Animals were given a 1 minute interval to regain normal respiratory function and then this procedure was repeated with infusions administered through the contralateral nostrils.

Twenty-one days after the MPTP administration, mice were anesthetized with chloral hydrate (100 mg/kg). The mice were subjected to thoracotomy and perfusion with ice-cold 0.9% sodium chloride 50 ml, then with 4% paraformaldehyde 100 ml in 0.01 M phosphate buffered saline (PBS) through the left ventricle. After fixation, the brains were removed immediately and post-fixed overnight at room temperature in the following fixative: 10% formaldehyde in 0.01 M PBS.

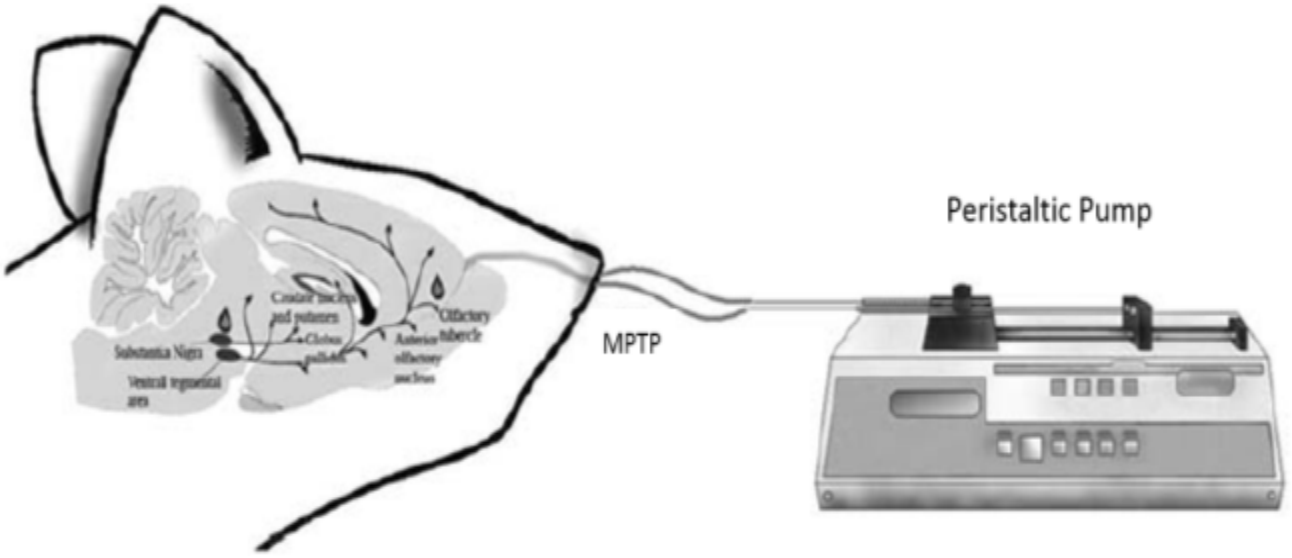

Figure 1. A schematic procedure of the intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice

Following fixation, samples were dehydrated using an ascending ethanol series, cleared in xylene and infiltrated with paraffin. They were then embedded in paraffin and sectioned through the SN coronally at 5 µm thickness using rotary microtome (Leica, Germany). All of the sections containing SN[52] were mounted on slides. Sections were stained with 1% toluidine blue in 1% sodium borate for 1 minute at 60 °C.

DNs in SNc were counted by an investigator blinded to the protocol treatment, using the optical dissector technique described in detail by Gundersen et al.[53] The optical dissector technique eliminates bias in counting as a result of cell size and shape. Briefly, DNs were counted as they came into focus while scanning through the section.

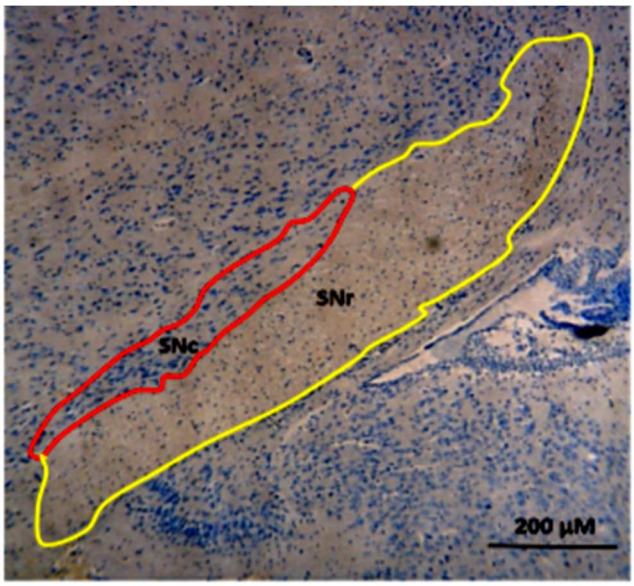

Figure 2. Photomicrograph of coronal section from the mice substantia nigra (SN) sub-regions [SNc: Substantia Nigra pars compacta (Red); SNr: Substantia Nigra pars reticulata (Yellow)] illustrating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dark neurons production in SNc sub-region where used for the stereological study; Regional boundaries were determined by cross-referencing with the atlases of Paxinos and Watson

For each section, 4-6 unbiased counting frames were sampled in a systematically random fashion inside the area of SNc. The preparations were examined under a light microscope using a ×60 objective lens (UPlanFI, Japan) and images were transferred to a computer using a high-resolution camera (BX51, Japan). The number of DNs was counted using a 10,000 µm² counting frame. The mean numbers of neurons per unit area (NA) in SNc were calculated using the formula as follows:

$$N_A = \frac{\sum \bar{Q}}{a|f.\sum P}$$

In this formula "$\sum \bar{Q}$"is the summation of counted DNs appeared in sections, "a/f" is the area associated with each frame (10,000 µm²), "$\sum P$" is the sum of frames associated points hitting the reference space.

The numbers of 8-10 sections from each animal were averaged, and the data from 7 animals of each group were presented as means ± standard deviation. Results were analyzed using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons between different groups studied. The level of statistical significance was set at $P < 0.05$. SPSS software for Windows (version 19, SPSS Inc., Chicago, IL, USA) was used to perform the total statistical analysis.

Results

To explore the neuroprotective effects of L-arginine against MPTP-induced neuronal loss, Toluidine Blue staining was used to examine the numerical density of Dark degeneration neurons in the SNc of Balb/c mice. Normal cells showed round and pale stained nuclei with a distinct nucleolus. The shrunken cells after MPTP administration with the morphological features of pro-apoptosis such as nuclear shrinkage and condensed chromatin were counted as DNs. To determine the numerical density of DNs in the SNc of Balb/c mice, we traced the boundaries for SNc as in figure 2. The numerical density of DNs were stereologically counted in SNc of mice in different studied groups.

In sham-control and L-arginine-control groups, there were a few numbers of DNs in SNc of the Balb/c mice (Figures 3 a, c, and 4). MPTP administration induced severe DNs production. Our results revealed a marked increase in the number of DNs in SNc of the Balb/c mice in MPTP group when compared with both sham-control and L-arginine-control groups ($P < 0.05$ and $P < 0.01$, respectively) (Figures 3 a, b, d, and 4). In addition, the number of DNs in the L-arginine plus MPTP group also increased significantly when compared with both control–sham and control groups ($P < 0.05$ and $P < 0.01$, respectively) (Figures 3 a, c-d, and 4).

Nevertheless administration of L-arginine (300 mg/kg; i.p.) once daily for 7 days starting from three days before MPTP administration significantly decreased the numerical density of dark degenerating neurons in SNc sub-region of SN of the Balb/c mice ($P < 0.05$) (Figures 3a, d, and 4). We found a statistical decrease in the number of DNs in the SNc in the L-arginine plus MPTP group Balb/c mice comparing to the MPTP group ($P < 0.05$) (Figures 3a, d, and 4).

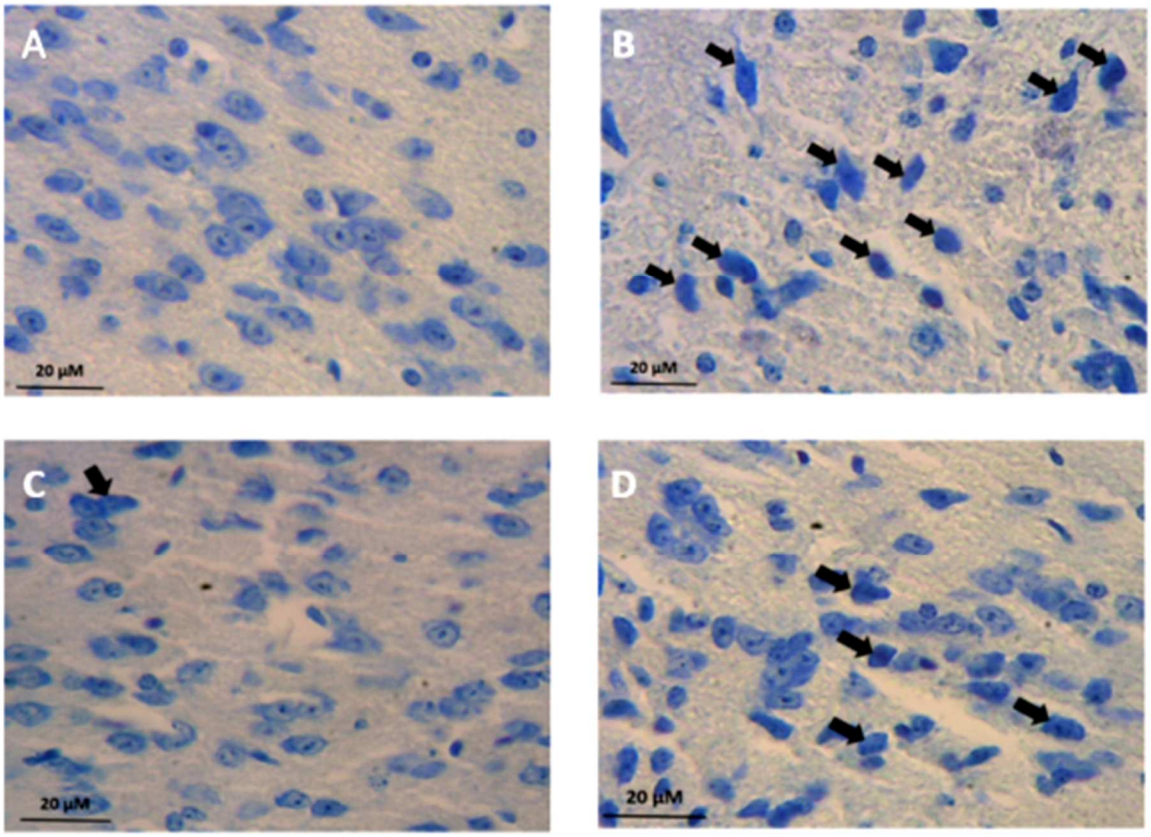

Figure 3. Photomicrographs showing distribution of dark neurons (DNs) in Substintia Nigra pars compacta (SNc) subdivisions of Balb/c mice in the Sham-control (a), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (b), L-Arginie-control (c), and L-arginine plus MPTP (d) groups. DNs pointed with black arrows. As shown the distribution of DNs in SNc sub-region were strikingly increased in MPTP and L-arginine plus MPTP group animals, compared to Sham-control and L-arginine-control Balb/c Mice

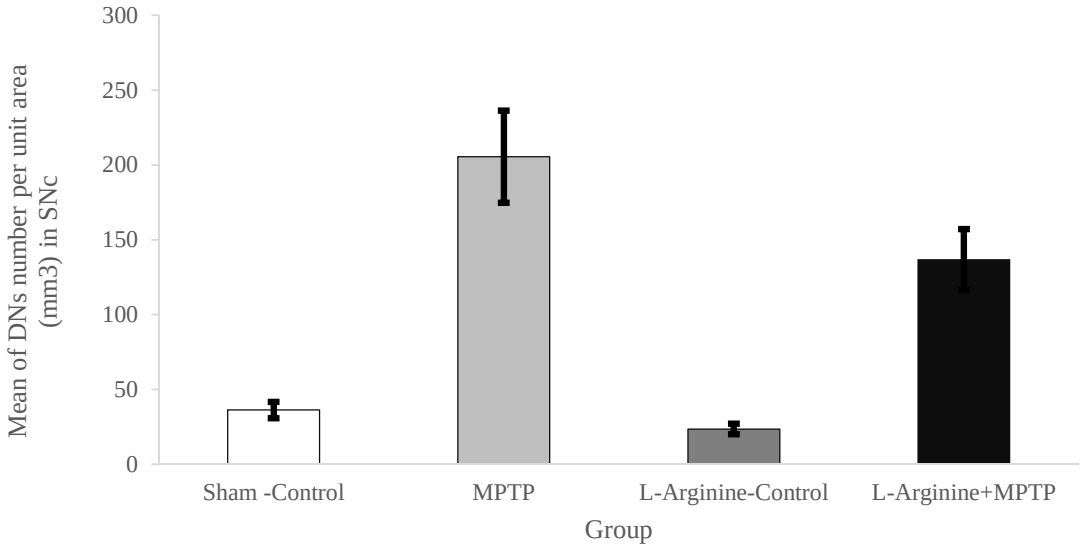

Figure 4. Mean of dark neuron (DN) numbers per unit area in the Substintia Nigra pars compacta (SNc) subdivisions of Balb/c mice and its comparison in the different studied groups. The data show that the mean number of DNs per unit area in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) group significantly increased in SNc comparing to L-arginine-Control and Sham-Control Balb/c Mice. Evaluation of neuroprotective effects on DN production in SNc sub-region revealed a significant reduction in the mean number of DNs in L-arginine plus MPTP groups

* $P < 0.05$ significant difference

Discussion

PD is one of the most common neurological disorders that is mainly characterized by problems with body movements.[1-3] Regarding PD symptoms, an increasing number of studies have demonstrated that PD seems to be a multidimensional disease, and besides motor deficits, it is associated with a number of sensorial, cognitive and emotional disturbances.[54] In this context, a recent series of studies demonstrated that a single i.n. infusion of MPTP in rodents produces diverse signs of PD such as impairments in motor, cognitive, emotional, and olfactory functions.[16,18,19]

Preventive or therapeutic strategies that stop or even slow the progress of neurodegenerative disorders such as PD are expected to have a major impact on the prevention or treatment of these diseases.[55] The results of this study show novel neuroprotective effects of L-arginine against MPTP-induced neurodegeneration in SN of Balb/c mice. To our knowledge, this is the first report investigating the neuroprotective effects of L-arginine in animal model of PD.

The current hypothesis about the mechanisms by which neurons come into apoptotic or necrotic process of degeneration has led to the belief that the use of drugs modulating the function of glutamate NMDA receptors may have beneficial effects in PD cases.[56] In this context, there is increasing evidence of the neuroprotective effects of L-arginine, which among other possible targets blockades NMDA receptors such as Mg^{2+} ions, against different insults of the CNS.[57-59] Recent studies on laboratory animals revealed that the administration of L-arginine has potential therapeutic importance, including anticonvulsant, anxiolytic and antidepressant-like actions.[57-59]

L-arginine is a normal constituent of the body and is found in both enteral and parental nutrition formulas, little experience is available about the pharmacology of L-arginine administration in the doses given in experimental studies, especially in neurodegenerative diseases.[42,60] Nevertheless, there is sufficient evidence suggesting that the 300 mg/kg dose of L-arginine in the rodents provides the best results.[60]

In this study, the repeated treatment with L-arginine (300 mg/kg, i.p.) during 7 consecutive days was able to decrease significantly the numerical density of DNs in SNc of Balb/c mice administrated intranasally with MPTP (1 mg/nostril). These data corroborate the neuroprotective potential of L-arginine (300 mg/kg, i.p.) in PD, since it attenuated the DA cell loss in the SNc of Balb/c mice infused intranasally with MPTP (1 mg/nostril).

In earlier investigations, administration of L-arginine has been shown to increase cerebral blood flow (CBF) and reduce neurological damage after experimental traumatic brain injury (TBI).[60-63] A study by Cherian et al., the researchers found that the L-arginine administration (300 mg/kg, i.v., 5 minutes after the brain injury) restores CBF to near pre-injury levels and significantly reduces the volume of contused brain.[63] In experimental TBI models and in some cerebral ischemia models also similar neuroprotective effects have been observed with administration of L-arginine.[60,62,63] As a result of these observations, L-arginine has become an interesting potential therapeutic agent for improving cerebral perfusion after TBI.

The neuroprotective effects of L-arginine may result from different mechanisms including blocking of NMDA receptors,[57-59] inhibition of NOS,[64] oxygen radical scavenging[65] and protection against mitochondrial membrane potential collapse.[66] However, the sequence of events leading to the protective effects of L-arginine against cell damage has not been fully elucidated. Previous studies have demonstrated that MPTP decreases glutamate uptake by astrocytes in cell culture.[67] Therefore, one possible mechanism by which L-arginine may exert protective effects against MPTP neurotoxicity may be due to the modulation of glutamate reuptake into neural cells, the main mechanism responsible for decreasing extracellular glutamate levels, thus attenuating glutamate neurotoxicity.

In addition, the neuroprotective effects of L-arginine administration could occur from its effects on the vasculature,[68] including that L-arginine is essential for the function of certain KATP channels.[69] Some of neuroprotective effects L-arginine are also presumed to occur via production of NO, as L-arginine is the precursor of NO in the reaction mediated by the enzyme NOS. NO is produced by many different tissues and has numerous physiological and pathological effects.[39,42,62] In the brain, NO plays a role as a neurotransmitter by stimulating soluble guanylyl cyclase to form the second messenger molecule, cGMP in the target cells.[26] Experimental studies have well documented the synthesis of NO in the brain, and its role in a variety of neuronal functions including learning and memory processes, cortical arousal, and blood vessel dilatation and immune response.[26]

NO is also a potent vasodilator and inhibits the platelet aggregation and leukocyte adhesion and may improve blood flow by preventing microvascular plugging by platelets and leukocytes.[70] NO inhibits Ca^{2+} influx through the NMDA receptor and may limit glutamate

neurotoxicity in cerebral ischemia.[70,71]

On the other hand, agmatine, formed by the decarboxylation of L-arginine by arginine decarboxylase, has been shown to be neuroprotective in experimental brain trauma and ischemia models.[51]

Recently, agmatine has been proposed as a novel neuromodulator that plays protective roles in several models of neuronal cellular damage.[51,72] A study by Matheus et al.[51] demonstrated that treatment with agmatine (30 mg/kg, i.p.) during 5 consecutive days increased the survival rate of old C57BL/6 female mice infused with a single i.n. administration of MPTP (1 mg/nostril), improving the general neurological status of the surviving animals. Moreover, pretreatment with agmatine was found to attenuate memory and locomotor activity, impairments observed at different periods after i.n. MPTP administration. They also reported that behavioral benefits of agmatine were accompanied by a protection against the MPTP induced loss of DA neurons in the SNc of aging mice. The researchers claimed that agmatine represents a novel potential therapeutic tool for the management of cognitive and motor symptoms of PD, together with its neuroprotective effects.

Of high importance, the administration of L-arginine demonstrated its neuroprotective properties as previously described in several models of neuronal damage.[63,73] These results corroborate recent findings on L-arginine neuroprotection in cellular models of neurodegenerative diseases.[42] Taken together, these results suggest that L-arginine may represent a potential disease-modifying therapy for PD.

Conclusion

The present findings reinforce i.n. MPTP administration as a valuable rodent model for testing novel palliative and neuroprotective compounds for PD. More importantly, the present study provides the first preclinical data indicating that repeated systemic treatment with L-arginine prevents DA cell loss in the SNc of mice submitted to an experimental model of PD. These results provide new insights in experimental models of PD, indicating that L-arginine may represent a new neuroprotective agent for the prevention of DA neuron degeneration observed in PD patients.

Conflict of Interests

The authors declare no conflict of interest in this study.

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A descriptive study of prevalence, clinical features and other findings of neuromyelitis optica and neuromyelitis optica spectrum disorder in Khuzestan Province, Iran

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Keywords
Neuromyelitis Optica, Multiple Sclerosis, Epidemiology, Prevalence, Iran

Abstract

Background: Neuromyelitis optica (NMO) is an uncommon neuro-inflammatory syndrome that has shown to be distinct from multiple sclerosis (MS) and associated with the autoantibody marker NMO-immunoglobulin G (IgG). There are still only a few studies regarding the epidemiology of NMO in Iran. In the present study, we tried to describe the epidemiology of NMO in Khuzestan as one of the densely populated regions in Iran.

Methods: A cross-sectional study was performed during the period 2013-2014. Multiple regional sources of data were used including hospital records, details from neurologists and MS society database. The diagnosis of NMO was based on clinical presentation, abnormal findings on neuroimaging and serological tests.

Results: A 51 Caucasian patients (36 patients with NMO and 15 with NMO-spectrum disorder) were identified with a female/male ratio of 7.5:1.0. The crude prevalence of NMO was 1.1/100,000 population. The mean age at onset was 29.2 ± 6.1 years and the mean duration of symptoms was 5.0 ± 0.4 years. The majority of patients (60.8%) were classified as having mild disability (Expanded Disability Status Scale = 0-3.5). Among of 35 patients whose titer of NMO-IgG was measured, 19 (54.2%) were seropositive.

Conclusion: Our study suggests that NMO prevalence rate in South West Iran (Khuzestan Province) is much lower than that reported for MS prevalence rate (16.2/100,000) and our patients had a lower age at onset presentation and milder course of the disease than western countries.

Introduction

Neuromyelitis optica (NMO) or Devic's disease is a severe inflammatory demyelinating disease of the central nervous system that preferentially targets the spinal cord and optic nerves. For a long time, this disease was regarded as a severe variant of multiple sclerosis (MS), but recent investigations show that it is a distinct disease with humoral pathogenic

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mechanisms. Devic and his student Gault, in 1894, first reported the clinical characteristics of NMO, optic neuritis (ON), and acute transverse myelitis (TM), based on 16 cases from the literature as well as a fatal case from his own experience.[1] Since the first description of NMO, it was considered as severe monophasic syndrome characterized by bilateral ON and myelitis occurring simultaneously or in rapid succession, but subsequent studies reported a relapsing course which ultimately resulted in paraplegia and blindness. NMO spreads worldwide and poor prognosis is still a challenge.[2] In addition to clinical, laboratory, immunological, and pathological characteristics which are mainly used to distinguish NMO from MS, a serum autoantibody termed NMO-immunoglobulin G (IgG), which targets the astrocytic water channel aquaporin-4 (AQP4), has recently been characterized for more differentiation. Therefore, detection of this highly specific marker in the serum of patients with NMO, has helped to define a NMO spectrum of disorders (NMOSD).[3]

Although this disease has been reported in several countries and racial groups, knowledge on the incidence and prevalence of NMO is still limited. The disease is 3-9 times more prevalent in women than it is in men, and the average age of onset ranges from 35 to 45 years among adults. Monophasic form of NMO affects females and males equally, however in the relapsing form, females (ratio 5:1-10:1) are over represented.[4] Population based studies from Japan,[5] Cuba,[6] Denmark,[1] Mexico,[7] and the French West Indies[8] calculated prevalence rates of 0.52-4.40 per 100,000 patient-years and incidence rates of 0.053-0.400 per 100,000 people. The disease is more common in Asian, Indian and black populations, in which NMO forms 15-57% of demyelinating diseases.[4] It is estimated that NMO prevalence in the United States is approximately 1-2% that of MS, with a female/male ratio of 6.5:1.0 in and a seropositivity of 68.3%.[9] The disease is mainly sporadic; however, a few studies have reported familial cases.[10]

There's little epidemiological study about NMO in Iran. Khuzestan is the most densely populated province in southwestern Iran where, to date, no epidemiological data on NMO exist, which emphasizes the importance of epidemiological assessments in this area. The present study aims to description of prevalence, clinical characteristics and other finding of NMO and NMOSD patients in Khuzestan.

Materials and Methods

The Khuzestan province is situated in the southwest of Iran with a population of over 4.5 million of which 2.28 million are male and 2.24 million are female according to the national census in 2010.[11] Most of the population is between 15 and 35 years, mainly 20-25 years. Khuzestan is inhabited by a number of ethnic groups: Lors (including Bakhtiari people) Arabs, Persians, Turks, and Kurds. This province is located in a subtropical/tropical area with humid and hot weather in summer and dry-cold weather in winter. The province covers an area of approximately 64,055 km^2 with most of the area situated over 10 m above sea. Khuzestan borders Iraq and the Persian Gulf and its capital is Ahvaz. The population data used for calculating the prevalence rate was based on the 2010 census, from the Iranian Central Bureau of Statistics. To evaluate the geographical distribution of NMO we divided the province into five regions: North, South, West, East, and Center.

All the patients in our investigation were residents of Khuzestan Province. The study was conducted on patients registered with diagnosis of NMO (during 2006-2014) from the following sources: (1) The records from MS clinic registry of Ahvaz Golestan Hospital, Iran, as the only referral center for NMO and MS patients in the province; (2) the records of patients referred to neurology department of the hospital; and (3) the records from neurologists across the province. We reviewed the medical records of the patients with ON, TM, or NMO, and contacted them to obtain the required details. The patients were also personally re-examined by neurologists to confirm the diagnosis.

We used diagnostic criteria for NMO that required ON and TM plus 2 of the following three supportive elements: (1) Longitudinally extensive TM (LETM) (≥ 3 vertebral segments in length), (2) magnetic resonance imaging (MRI) of the brain with normal findings or with findings not consistent with MS, and (3) NMO-IgG seropositivity.[12] Requirement of presence of either ON or LETM is challenged in the current definition of NMOSD. It is now evident that brain symptoms are not only frequent during disease course, but may antedate ON or TM for a long time. To diagnosis NMOSDs at least one the following clinical settings were met:

1. Single, recurrent or simultaneous bilateral ON
2. LETM (≥ 3 vertebral segments)
3. Recurrent brainstem symptoms
4. Recurrent hypothalamic symptoms
5. Recurrent cerebral symptoms.

Plus at least 1 of the following:

1. Positive AQP4-IgG serum status
2. Brain MRI lesions typical of NMO.[13]

In our study, only MRIs obtained at the initial clinical event were assessed. Brain MRIs classified

as being normal or atypical for MS. Lesions in the spinal cord were characterized as extending over three or more vertebral segments and mainly located in the cervical and thoracic cord with a central gray pattern. NMO-IgG test was done on the serum samples and results were recorded as seropositive or seronegative.[4] The degree of neurologic impairment in NMO patients was evaluated by Expanded Disability Status Scale (EDSS).[14] Patients were classified to three groups for EDSS: 0-3.5 (mild disability), 4.0-5.5 (moderate disability), and ≥ 6 (severe disability). Other demographic and clinical parameters which were recorded included: Patient age, gender, ethnicity, age at onset of illness, geographical distribution of illness, disease duration, presentation at onset, course of the disease, family history of NMO, visual evaluation, the use immunosuppressive therapies, type of attacks, history of attacks during pregnancy. Statistical analysis was conducted using SPSS software (version 20, SPSS Inc., Chicago, IL, USA). The frequency or percentage for the nominal variables was calculated. Mean ± standard deviation was calculated for each continuous variable. Exact Poisson confidence intervals (CI) were calculated. The prevalence rate was calculated on November 2013.

Results

A total of 51 patients were identified with NMO/NMOSD. One of the patients died by time of this report. Patients were exclusively Caucasian and predominantly female, with a female to male ratio of 7.5:1.0. Comparison of the clinical patterns and other finding in our patients based on sex are summarized in table 1. Mean age of patients, mean age at onset and mean EDSS was lower in males than females. The average age at onset of all patients was 29.20 ± 1.55 years (range: 8-58 years), with 10 patients (19%) having an age of onset below 20 years. The mean age of patients was 35.20 ± 1.55 and the duration of the symptoms had a mean of 6.0 ± 1.3 years.

Among the 51 patients, 30 cases (58.8%) were Lors, 13 cases (25.5%) were Arabs, 6 cases (11.8%) were Persians, and 2% were of other ethnic groups (Kurd and Turk). The most cases of NMO disease occurred in the central part (26 patients) as compared to 8 cases in the north, 8 cases in the south, 1 case in the west, and 8 cases in the east of Khuzestan. The crude prevalence of NMO/NMOSD among the Caucasian population living in Khuzestan was 1.1/100,000 (95% CI = 1.04-1.16). Crude prevalence for females and males was 2 (95% CI = 1.92-2.09) and 0.26 (95% CI = 0.23-0.29) per 100,000, respectively. The crude prevalence rate for NMO and NMOSD patients were calculated 0.8 and 0.3 per 100,000 patients (Table 2).

Comparison of the clinical features of the NMO/NMOSD patients are summarized in table 2. Among the 51 patients, 36 cases (70.6%) were identified with NMO and 15 cases (29.4%) with NMOSD. 19 patients (37.3%) presented with ON, 31 (60.8%) with TM and 1 (2%) with simultaneous ON and TM. The disease course was monophasic in 27.5% and a relapsing course was seen in 72.5% of the patients.

Only 2 patients (3.9%) had family history of NMO, who were sisters. Of 4 patients who were attacked during pregnancy, 3 patients had TM and 1 had ON. In our study, 88.2% of patients were on immunosuppressive therapies; of these, 51% used both azathioprine and prednisolone and remaining patients received azathioprine (15.7%), methotrexate (3.9%), cellcept (3.9%), prednisolone (9.8%), and cellcept + prednisolone (3.9%) one of the patients presented NMO with other manifestations such as intractable vomiting. Approximately 12% of our patients received psychiatry therapy.

Table 1. Comparison of the clinical patterns and other findings in our patients by sex

Findings	**Men (%)**	**Women (%)**
Number of patients	6 (11.7)	44 (86.3)
NMO	6 (16.6)	30 (83.4)
NMO-Ab positive	1 (16.6)	18 (40.9)
Normal brain MRI	5 (83.3)	31 (70.4)
NMOSD	0	15 (100.0)
Family history of NMO	0	2 (3.9)
EDSS mean	2.25	3.87
Prevalence rate(per 100,000)	0.26	2.00
Age (mean in years)	30.80	35.90
Age at onset (mean in years)	26.00	30.20

NMO: Neuromyelitis opticam; NMOSD: Neuromyelitis optica spectrum disorder; NMO-Ab: NMO antibody; EDSS: Expanded Disability Status Scale; MRI: Magnetic resonance imaging

Table 2. Comparison of the patients by type of the disease

Findings	NMO (%)	NMOSD (%)
Number of patients	36 (70.6)	15 (29.4)
Patients with TM at presentation	19 (52.7)	12 (80.0)
Patients with ON at presentation	16 (44.4)	3 (20.0)
Patients with simultaneous ON + TM	1 (2.9)	0
NMO-Ab positive	15 (42.0)	4 (27.0)
Age (mean in years)	36.3	34.1
Age at onset presentation	31.2	27.2
Female/male ratio	5.0	15.0
Prevalence rate (per 100,000)	0.8	0.3
Mean of EDSS	3.8	3.1

NMO: Neuromyelitis optica; NMOSD: Neuromyelitis optica spectrum disorder; NMO-Ab: NMO antibody; ON: Optic neuritis; TM: Transverse myelitis; EDSS: Expanded Disability Status Scale

Table 3. Magnetic resonance imaging (MRI) characteristic of neuromyelitis optica (NMO)/ neuromyelitis optica spectrum disorder (NMOSD)

Findings	NMO (%)	NMOSD (%)
Brain MRI		
Normal	26 (72.2)	11 (73.3)
Atypical plaques for MS	10 (27.8)	4 (26.7)
Spinal cord MRI		
Normal	1 (3.0)	1 (7.0)
Cervical LETM	32 (82.0)	10 (67.0)
Thoracic LETM	4 (10.0)	2 (13.0)
Cervical plus thoracic LETM	2 (5.0)	2 (13.0)

NMO: Neuromyelitis optica; NMOSD: Neuromyelitis optica spectrum disorder; LETM: Longitudinal extensive transverse myelitis; MRI: Magnetic resonance imaging

Table 4. Comparison of the patients by Expanded Disability Status Scale (EDSS)

Findings	Mild (0-3.5) n (%)	Moderate (4-5.5) n (%)	Severe (≥ 6) n (%)
Number of patients	31 (60.8)	9 (17.6)	11 (21.6)
Type of disease			
NMO	21 (67.7)	6 (66.7)	8 (72.8)
NMOSD	10 (32.3)	3 (33.3)	3 (27.2)
NMO-Ab positive	12 (63.0)	3 (16.0)	4 (21.0)
Female/male ratio	26/5 (5.2)	8/1 (8.0)	11

NMO: Neuromyelitis optica; NMOSD: Neuromyelitis optica spectrum disorder; NMO-Ab: NMO antibody

The neuroimaging, neuro-electrophysiological and laboratory findings are listed in table 3. NMO-IgG antibodies were checked for 35 patients. The result was positive in 19 patients and negative in the remaining 16 patients. 15 NMO cases and 4 NMOSD cases were among seropositive patients. Brain MRI findings were characterized as normal findings in 72.5% of patients and atypical plaques for MS was seen in 27.5%. Among the 51 patients underwent spine MRI, 49 (96%) had spinal cord lesions; of these 82.4% had cervical cord lesions; 5.9% had thoracic cord lesions; and 5.9% had lesions spanning the cervical and thoracic cord. Nearly 61% of the all patients had mild disease (EDSS = 0-3.5), 17.5% moderate disease (EDSS = 4.0-5.5), and 21.6% severe disease (EDSS = 6 and above). Because EDSS scores were recorded at the time of clinical visit, the scores for 5 patients with severe disability were obtained when they were experienced an attack (Table 4).

Twenty-five patients underwent visual evoked potential (VEP) test of which 80% had abnormal results. Cerebrospinal fluid oligoclonal bands (OCB) were detected in 4/51 patients (7.8%) and one case was positive for OCB (2%). Three patients showed clinical symptoms and laboratory findings of hypothyroidism. One patient was positive for HLA-B5, which was followed up. Two cases were positive for anti-dsDNA; however in later laboratory tests both had negative results.

Discussion

In our study, the prevalence of NMO/NMOSD in Khuzestan province was determined to be 1.1/100,000, all of whom were Caucasian. The

prevalence of NMO in this region is similar to the prevalence rates in Cuba,[6] Japan,[5] South Wales,[15] and Merseyside (UK),[16] but lower than Denmark[1] and Martinique.[8] A recent report by Etemadifar et al.[17] in Isfahan, reported a prevalence of 1.9/100,000 for NMO, which is similar to our findings. Our prevalence rate was much lower than that reported for MS in Khuzestan in which a prevalence rate of 16.2/100,000 was observed in 2009[18] and then prevalence rate of MS in Qom.[19] The prevalence rates by gender showed a much higher rate in females than in males our results is consistent with the previously reported results of a higher female frequency of NMO.[20-22] Female to male rate in our study was 2 times more than the ratio for MS patients living in Khuzestan.[18]

The mean age at onset of illness in this study was 29.2 years (range 8-58) which is very close to those of previous studies in Isfahan and Tehran in which the means of 30 and 27.16 years were suggested.[17,23] However, our estimation was lower than the average age of onset in other counties[24-27] as well as in the US where a mean of 41.1 years (range 3-81) were reported.[9]

We observed a considerable frequency difference between ethnic groups living in Khuzestan over half of the patients (58.8%) were Lors followed by 25.5% for Arabs. A recent study conducted in Saudi Arabia, found a low frequency prevalence of NMO and a low NMO-IgG seropositivity in a cohort of Saudi patients with Arab ethnic background.[28] It is believed that Iraq, which has common border with Khuzestan, as well as other Arab countries located in Asia and sub-tropical Africa are classified as low risk regions for MS.[29-31] Sharafaddinzadeh et al.[18] compared the characteristics of Arab and Persian patients with MS in Khuzestan and observed a much lower prevalence of MS in the Arab ethnic group. The observed ethnic differences between patients with NMO suggest that genetic factors may influence susceptibility to NMO.[5-8]

In this study, the relapsing form of NMO was more common than the monophasic form (72.5% vs. 27.5%). These findings were similar to prior studies in which 80-90% of the patients followed a relapsing course.[2,6,32] A high proportion of relapsing NMO in Iran was also reported by Sahraian et al.[23] however Etemadifar et al.[17] observed monophasic course in 60% of patients. The majority of our patients (60.8%) had EDSS score below 3.5, suggesting a mild course of NMO in our patients similar to the previous reports from Iran[17,23] as well a study in Saudi Arabia[27] but do not support the existing data from around the world which estimated a higher score.[32-35] This indicates an aggressive course of NMO in other populations than our population. More patients with MS in Khuzestan had a mild EDSS (< 3.5)[18] which is in line with NMO patients in our study. In fact, the mean EDSS score in Iranian NMO patients are very close to MS.

In our cohort of NMO patients, there was a family history of NMO in only 2 patients (3.9%) who were two sisters. Similarly, the proportion of patients with positive family history in other studies is low. It is strongly evident that NMO cases appear sporadically; however, recently a few familial NMO cases were reported.[36] Forty-five patents with NMO received immunosuppressive drugs after diagnosis of NMO of whom 51% used both azathioprine and prednisolone current evidence suggests that the attack prevention is achieved with effective immunosuppressive therapy.[37]

Analysis of the clinical data showed that 4 patients with NMO (7.8%) had an attack while pregnant; one of the patients developed TM 2 months after from delivery and the resting three patients experienced attack during pregnancy. A few reports showed an increase in attack rate in the 1st month after birth.[38,39] Several case reports of NMO emerged or became active during pregnancy.[40-42] However, there are no studies to indicate the influence of pregnancy on the long-term course of NMO.

It has been demonstrated that endocrinopathies including hypothyroidism is associated with NMO. Our results showed that 3 patients had symptoms and signs of hypothyroidism and 3 patients were found to be HLA-B5 and anti-dsDNA positive, although this finding was ruled out in serial tests, but indicating a general susceptibility to antibody-mediated autoimmune disease. VEP was performed on 25 patients; of these 20 (80%) had an abnormal VEP response. Previously published data confirms our results by indicating that VEP are frequently altered in NMO.[43,44] Existing studies found over 60% of patients with NMO having VEP abnormal results.[28,43,44] Although VEP is not necessary in the diagnosis of NMO, VEP can be helpful and are used commonly in patients with demyelinating disease.[46] Among 35 patients who took NMO-IgG test, 19 (54.3%) were NMO-IgG seropositive. This rate was close to the range reported from other studies in Caucasian populations suggesting a seropositivity of over seventy percentage.[9,15,16] Other studies from several Asian countries showed a seroprevalence of 27-39%.[45-49] Recent studies of seroprevalence of NMO-IgG antibody in NMO from Iran reported proportions of 30-66%.[23,50,51] Which are relatively similar to our estimation. Harirchian et al.,[51] using cell-based immunofluorescence assay to measure NMO-IgG, demonstrated that NMO antibody is

highly specific for NMO but it is not highly sensitive for diagnosing NMO patients. The variable sensitivity across studies can be due to a number of factors including ethnic background, characteristics of patients and more important method of measurements.[52] The limitations of our study were that the exact population of Lors and Arabs was not available therefore we could not study the relationship between the prevalence of the disease and ethnicity, also some patients may refer to larger city for treatment so we do not have the exact number of patients.

Conclusion

Our results are in line with other data from western counties in terms of prevalence, NMO features, MRI findings, laboratory results, and female preponderance. However, the lower age of onset and milder course of the disease are exceptions in our patients. Overall, patients in Khuzestan appear to have similar characteristics to Caucasians living in Asia and western countries.

Conflict of Interests

The authors declare no conflict of interest in this study.

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Policy interventions to improve rural retention among neurosurgeons in Iran: A discrete choice experiment

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Discrete Choice Experiment, Stated Preference, Health Manpower, Rural Areas

Abstract

Background: Health workforce shortages in rural and remote areas are a global challenge that almost every health system has to deal with. This study aimed to discover neurosurgeons' job preferences and propose policy interventions that could possibly increase their retention in rural, remote, or underserved areas.

Methods: A discrete choice experiment (DCE) was conducted in November 2014 with a sample of Iranian neurosurgeons selected from five contrary's provinces representing the geographical diversity. Job attributes included income, dual practice opportunities, workload, proximity to family, clinical infrastructure, housing, educational facilities, and work location. Probit regression model was used to estimate the importance of different job attributes and examine the extent to which neurosurgeons were willing to tradeoff between monetary and nonmonetary attributes.

Results: Findings indicated that increased salary, permission to undertake dual practice and access to adequate clinical infrastructure were the most important retention policies. Provision of subsidized housing and educational facilities also increased neurosurgeons' attraction and retention in rural areas.

Conclusion: A range of policy interventions focusing on both monetary and nonmonetary incentives are required to increase neurosurgeons' retention in rural, remote, or underserved areas.

Introduction

Inequitable distribution of physicians between large metropolitan cities and remote or noncapital areas has become a serious concern and a priority to deal with.[1,2] Despite being a universal concern,[3,4] low and middle income countries made great efforts to give a best possible answer to the question of how to attract physicians and improve their retention in underserved areas.[5,6] World Health Organization (WHO) has identified 16 retention strategies, including regulation and compulsory policies, financial motivations, education and training, personal and job-related support plans.[7] All around the world different policies have been developed to cope with uneven distribution of physicians. Some were related to monetary incentives[8] and some

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nonfinancial motivations,[9] although the successfulness of strategies were highly dependent on the local context and type of health cadres.[1,10,11]

One method to determine effective strategies for attracting and retaining health workforce in underserved areas is discrete choice experiment (DCE). This method has recently been used in health care as a form of stated preferences methods.[12,13] DCE helps policy makers to determine a ranking of health providers' preferences toward possible incentive packages in a way that each attribute's value is comparable to another.[14] Recently, DCE has increasingly been used in several developing countries for rural retention problems.[5,15-19]

Islamic Republic of Iran is a middle income country located in south west of Asia. Iran Ministry of Health and Medical Education has recently faced with challenges in attracting and retaining health professionals particularly physicians in rural and remote areas. Nearly, 2000 general practitioners and 1700 specialists are lacking to serve in such areas of the country.[20] In addition, there are around 2200 specialists working in 270 underserved areas who are not satisfied with their income and work condition and seek an opportunity to leave their workplace in a shortest time possible.[21] Therefore, a particular attention must be paid to this issue and appropriate action plans should be proposed to encourage physicians to serve in rural, remote and nonmetropolitan areas of the country. To this aim, we conducted a DCE with technical assistance from WHO guidelines to inform the selection of appropriate recruitment and retention strategies based on physicians' preferences.[22,23] In this study, we chose neurosurgeons as they play important role in diagnosis, treatment and rehabilitation of neuro-spine illnesses or injuries and their shortage in some areas would cause irreparable damages.

The study objective was to examine physicians' preferences for different job attributes and to find out important determinants for deciding to serve in a rural remote area. We also evaluated the effects of different policy interventions on the probability of choosing to serve in rural or underserved areas.

Materials and Methods

A DCE was conducted in November 2014 with a sample of Iranian neurosurgeons selected from five contrary's provinces representing the geographical diversity. Neurosurgeons under study were asked to choose between hypothetical job profiles describing as a combination of job attribute levels.

In the DCE literature, it is suggested to have at least 50 participants for each subgroup of interest.[17,24-26] Following this rule, we considered two main subgroups of interest: those under 35 compared to those over 35, and those with rural background compared with those with urban background. Regarding to potential difficulties in data collection we increased target sample size by 20%, which led to 120 neurosurgeons. We used three stage sampling. First, five provinces were selected in a way to include geographical diversity. Second, remote and nonurban areas of each province where neurosurgeons were working in were defined and all included in the study. In the last stage, all in service neurosurgeons from public facilities were selected to reach the target sample.

Prior to conduct DCE, 17 in-depth interviews were done with in service neurosurgeons working in nine provinces of Iran to generate job attributes and corresponding levels from their own point of view. Physicians were asked about factors that could be influential in their retention in nonmetropolitan and remote areas.[27] The qualitative data were used to decide on attributes and corresponding levels. Levels of each attribute were created in a way to reflect the existing work condition. Then additional levels were produced from a rational increase in the base levels. Appendix 1 depicts job attributes and levels determined as a result of the qualitative study.

Once the eight attributes and related levels were determined, alternative job profiles were generated (25.9.4 = 900) consisting of all possible combinations of attribute levels. To reduce this to a practical number, SPSS software (version 22, SPSS Inc., Chicago, IL, USA) orthoplan procedure was used and produced a fractional factorial design with 16 scenarios.[24,28] The criteria of level balance, orthogonality and minimum overlap among attribute levels were considered in designing an efficient experimental design.[29] One of the job profiles in an urban city with average attribute levels was selected as a constant scenario comparing to the remaining job profiles and provided 15 choice sets for each physician to regard. The questionnaire also included a number of socio demographic questions to collect background characteristics of the physicians. The sequence of presenting job pairs and the attributes was different in the questionnaire to reduce any risk of careless response and boredom of respondents.[30] Face and content validity of the questionnaire was tested in a pilot study conducted on 17 neurosurgeons who had participated in the interviews to identify any modification needed to be applied in a prior questionnaire. Final DCE questionnaire was applied to 120 in service neurosurgeons working in public health facilities of selected districts during November and December 2014. An example of a choice set is shown in table 1.

Ethical issues related to the research were taken in to account. Permission to conduct the research was obtained from Tehran University of Medical Sciences, Iran, and participation in the study was considered to be voluntarily.

Data were entered in SPSS and checked for consistency. To analyze survey results, questionnaire data were transformed in to STATA (version 13, Stata Corp LP, College Station, USA) wherein dependent variable (whether to choose Job A or Job B) was defined in a binary form. Probit regression model was used to analyze preference data and determine whether physician selected Job A or Job B. To investigate the correlation between physicians' responses to different job choices, we used a random effect probit model. Estimated coefficients in the model equation would illustrate the marginal utility of each attribute level from physicians' point of view. They also provided information about the significance and direction of the effects related to change in levels of attributes:

Prob [U job B > U job A] = β_1 location + β_2 income (2-4) + β_3 income (4-5) + β_4 income (5-6) + β_5 dual + β_6 workload (average-light) + β_7 workload (average-heavy) + β_8 family + β_9 education + β_{10} clin + β_{11} housing (basic-none) + β_{12} housing (basic-superior) + $\varepsilon_i + \mu$

Where location, income (2-4), income (4-5), income (5-6), dual, workload (average-light), workload (average-heavy), family, education, clin, housing (basic-none) and housing (basic-superior) represent the difference in attribute levels between job A and B and corr (ε_i, μ) stands for the correlation among individual choices.

As evaluation of different policies to identify most effective interventions in physicians' retention is one of the most interesting analyses for policy makers we used regression results to calculate willingness to pay (WTP) and policy impact measures in our study. First we measured the amount of monetary attributes physicians were willing to overlook in obtaining more of nonmonetary attributes using marginal rate of substitution (MRS). This way, WTP for each attribute was calculated by dividing the attribute regression coefficient by income coefficient. Although WTP could be calculated for all income levels, but we only reported it for the lowest level to best reflect current situation.

$$MRS_{iq}^{kh} = -\beta_h|\beta_k$$

Where MRS_{iq}^{kh} is the individual q's marginal WTP for attribute h, β_h represents the coefficient of attribute h and β_k corresponds to the coefficient of attribute k (income) of the model.

Finally, we simulated the impact of various policy interventions on probability to choose rural job positions among physicians.[30] To do so, we considered a baseline job (a job with the lowest level of attributes) and estimated the change in the probability of taking it up following a change in one of the job attributes. Marginal effect analysis was conducted for the purpose.[31]

Results

In total, 120 neurosurgeons working in public hospitals of 5 country provinces' 15 districts completed the questionnaire (response rate = 83.6%). Respondents were mainly male and married (96.7%), and had urban background (55.8%). All respondents have chosen the best job profile with superior attribute levels verifying the internal consistency of responses.

The results for random effects probit model on which job neurosurgeons would choose confirmed that regression coefficients were statistically significant for seven of the attributes (location, income, dual practice, workload, educational facilities, clinical infrastructure, and housing) verifying their importance for neurosurgeons to make decision about different job choices. We tested the theoretical validity of the model by checking out the attribute levels' sign and determined whether they were in compatible with anticipated sign or not. At 5% of confidence interval level, physicians positively valued working in urban area, higher salary, opportunity for dual practice and more number of surgeries per month, having access to adequate clinical infrastructure, educational facilities, and proper subsidized housing. Relative impacts of the attributes were also estimated using partial log-likelihood analysis.

Table 1. An example of a choice set

Job A	Job B
Location: Rural, remote or underserved area	Location: National capital or urban developed city
Income: 200% increase in current income	Income: 150% increase in current income
Dual practice: Yes	Dual practice: No
Workload: Heavy	Workload: Heavy
Proximity to family: No	Proximity to family: No
Clinical infrastructure: Adequate	Clinical infrastructure: Adequate
Educational facilities: Superior	Educational facilities: Basic
Housing: Basic	Housing: No
Which job profile do you prefer to choose?	Job A ○ job B○

Table 2. Probit regression results and monetary value of different job attributes

Independent variables	From … to …	Coef.	SD	P	WTP (%)
Location	Urban to rural	-0.17	0.08	0.040	-8.5
Income 1	2000-4000 $	1.00	0.17		-
Income 2	4000-5000 $	1.29	0.15	< 0.001	-
Income 3	5000-6000 $	1.49	0.21		-
Dual practice	Not permitted to permitted	2.79	0.11		139.5
Workload 1	Low to moderate	0.37	0.08		18.5
Workload 2	Moderate to high	0.17	0.06	0.006	8.5
Family proximity	Near to far	0.10	0.08	0.200	-
Educational facilities	Basic to superior	0.20	0.08	0.001	14.0
Clinical infrastructure	Inadequate to adequate	0.70	0.11		36.5
Housing 1	None to basic	0.63	0.10	< 0.001	31.5
Housing 2	Basic to superior	0.58	0.11		29.0

WTP: Willingness to pay; SD: Standard deviation

Table 3. Estimated take up rates for a rural job under different policy options

Independent variables	From … to …	Marginal effect	Take up rates (%)	P
Location	Urban to rural	-0.046	4	0.040
Income 1	2000-4000 $	0.205	20	
Income 2	4000-5000 $	0.280	28	
Income 3	5000-6000 $	0.335	33	< 0.001
Dual practice	Not permitted to permitted	0.654	65	
Workload 1	Low to moderate	0.103	10	
Workload 2	Moderate to high	0.047	4	0.005
Family proximity	Near to far	0.028	-	0.200
Educational facilities	Basic to superior	0.080	8	0.001
Clinical infrastructure	Inadequate to adequate	0.178	18	
Housing 1	None to basic	0.163	16	< 0.001
Housing 2	Basic to superior	0.149	15	

Results showed that the most important attribute was dual practice, which physicians put the greatest value on it and the least important attribute was workload from their point of view.

The magnitude and sign of estimated attribute levels' coefficients suggested that physicians regarded a higher level of utility for superior attribute levels except for workload that respondents preferred a moderate level rather than superior. The negative sign of location coefficient confirmed that respondents desired a job in urban area rather than rural, remote, or underserved town (Table 2). The sixth column of the table 2 reports the WTP estimates for different attribute levels. As data shown, neurosurgeons required a remuneration of 170 $ per month (8.5% of their income) to work in a rural area. They were willing to give up (2790 $ or 139.5% of income) to obtain the possibility of working in a private sector. To attain a moderate level of workload, having access to superior educational facilities and adequate clinical infrastructure, they were willing to sacrifice (370 $ or 18.5%), (280 $ or 14%) and (730 $ or 36.5%), respectively. In terms of housing, there was a WTP (630 $ or 31.5% of income) to be provided with basic subsidized housing.

The impact of improvement in the level of nonmonetary attributes as policy intervention on the probability to choose a rural job was also analyzed in the study. The marginal effect estimates in table 3 showed that an increase in workload from low to moderate level was associated with 10% increase in probability of choosing such a job.

Providing a chance to undertake dual practice and having access to basic subsidized housing would respectively raise the probability by 65% and 16%. If physicians were supposed to have access to superior educational facilities and adequate clinical infrastructure, 8 and 18% increase could be achieved in the likelihood of choosing a remote job. Raising the monthly income up to 4 and 5 thousand dollars increased the probability of taking a rural job by 20% and 28%. Finally, enlarging the salary up to 6000 $ enhanced the probability by 33%. The results showed that although wage increases were important incentives, but they became less effective as income increased. In addition to check for the effects of promotion in single attribute levels; we also examined the impact of different scenarios combined of a group of attribute levels on the

probability of choosing a rural job among neurosurgeons. As table 4 depicts, a combination of 150% salary increase, opportunity for dual practice and access to adequate clinical infrastructure would result in 99% increase of rural uptake.

By providing the opportunity to access adequate clinical infrastructure and subsidized housing, rural uptake would increase up to 54%. If neurosurgeons in rural areas were provided with superior educational facilities and moderate level of workload, the uptake rate would be very similar to the effect of 200% increase in salary and providing a superior housing for physicians.

Discussion

This study revealed important findings about neurosurgeons' job preferences and policy interventions that could possibly influence their attraction and retention in rural, remote and underserved areas. Data analysis found that all job attributes except proximity to family had statistically significant effect on neurosurgeons' preferences in their job choices. This finding implied that there could be a range of policy interventions to improve the probability of choosing a rural job among physicians. To consider an attractive job profile, physicians had strong preference to have opportunity for dual practice and to gain higher levels of salary. Besides monetary bonuses, there were some nonmonetary incentives, which played an important role in physicians' recruitment and retention in underserved areas. Literature supports the findings and emphasizes on the importance of different nonmonetary factors such as opportunity to undertake dual practice, access to subsidized housing, adequate clinical infrastructure, and decent educational facilities as important issues in physicians' preferences.[5,15,32-37] Rockers et al.[33] indicated that regulations in favor of dual practice would make rural jobs attractive. Another study acknowledged that lack of resources and medical equipment acted as a barrier for health workforce to accept a rural job.[38] Kolstad[16] and Mangham and Hanson.[17] considered decent housing as a main incentive to work in rural areas. Hanson and Jack[39] recognized that physicians put the maximum value on the ability to work in private sector. Improved housing, adequate medical equipment, and reduced time commitment were other key factors in their decision to retain.

In our study, participants were willing to tradeoff between different job attributes. They were ready to give up some amount of income in return to obtain subsidized housing and opportunity for dual practice. To work in a national capital or large developed cities rather than rural or remote areas, they requested an increase in current level of income. Similar to our findings, Kolstad[16] found that respondents were willing to give up some amount of income to work in a place with sufficient clinical infrastructure and subsidized housing. Mangham and Hanson.[17] also confirmed that nurses would sacrifice some pay increases to obtain the opportunity for continuous education and basic government housing.

The study findings revealed that individual incentives or mixtures of them could improve neurosurgeons' recruitment and retention in rural or remote areas. Miranda et al.[40] confirmed that combinations of incentives could lead to higher rates of attraction or retention in rural jobs. In a study conducted in Uganda with the purpose of determining retention policies to attract and retain health workforce in rural areas, Rockers et al.[33] found that salary increase, quality improvement of health facilities and monetary support for continued education would make rural jobs more attractive. Kruk et al.[18] in a study of rural practice preferences among medical students in Ghana declared that the joint of three non-financial attributes, including better housing, adequate infrastructure and shorter contract duration would increase the uptake rate of rural areas. A similar research in Zambia suggested government housing, adequate clinical infrastructure, car loans, and educational facilities as important role players for the purpose.[41]

Table 4. Prediction of the uptake rate for rural jobs under different policy scenarios

Policy scenarios	Take up rates (%)	P
Permission for dual practice + 150% salary increase + adequate clinical infrastructure	99.0	
Permission for dual practice + subsidized/government housing	96.0	
Adequate clinical infrastructure + subsidized/government housing + Permission for dual practice	98.0	
100% salary increase + permission for dual practice + subsidized/government housing	97.0	< 0.001
100% salary increase + moderate workload + subsidized/government housing	52.0	
Adequate clinical infrastructure + subsidized/government housing	54.0	
Rural location + superior educational facilities + moderate workload	58.6	
200% salary increase + superior housing	58.4	

Vujicic et al.[14] directed a research to estimate the impact of different policies on improving rural recruitment and retention. They recognized that increasing the amount of salary and possibility for long-term education could play an important role in increasing the take up rate of rural jobs.

Strengths and limitations of the study

There are some strength points regarding to this study. The fact that little studies have been conducted on health human resource management in developing countries,[16] we have attempted to close the research gap and provide valuable information about neurosurgeons' job preferences to work in rural areas. Second, we have assigned the attributes and related levels on the basis of information obtained from a qualitative study conducted among neurosurgeons. Third, we piloted the study with 17 neurosurgeons to ensure that the hypothetical alternatives were well defined and respondents understood how to make their choices. Finally, we collected information from those neurosurgeons currently in service to provide useful information about their perceived incentives rather than medical students or those who have not entered in to the work market yet.

On the other hand, some limitations could be mentioned for the study. First of all we limited our research to one group of specialists because of practical matters. Second, we did not consider costs information and monetary valuations of different policies to determine the most cost effective one affecting physicians' job choices. Finally, because of Inaccessibility to experimental design software solutions to construct efficient designs for choice experiments, we used a constant comparator.

Conclusion

The study findings recommended that in order to resolve geographical imbalances, policy makers should respect not only appropriate reimbursement, but also improvement in a number of non-financial job attributes in rural or remote areas. Analysis revealed some retention strategies to make rural jobs more appealing for neurosurgeons. Increase in salary, legislation in agree with dual practice, improvement in clinical infrastructure, providing subsidized housing and opportunity for continuous education were some of the main inputs suggested to be included in retention policy packages. As there are relatively inadequate numbers of researches on job preferences of health workforce in developing countries, this study can have a significant contribution to the literature.

Conflict of Interests

The authors declare no conflict of interest in this study.

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Appendix 1. Job Attributes and levels

Attribute	Definition	Level
Location	This attribute provides an alternative for respondents to choose between two job profiles (rural and urban areas). Rural areas represent remote, underserved, and nonmetropolitan districts of the country. Urban areas stand for national capital, regional, or district headquarters	Rural Urban
Income	This is the income obtained from governmental sources such as salary, allowances, fee for service, but not those from private practice. Four levels had been defined for income attribute. First level represents the base income; the second, third, and the forth levels stand for 100%, 150%, and 200% increase in base level of income, respectively	Base income Base + 100% Base + 150% Base + 200%
Dual practice	This means whether physicians are permitted to work in private sector besides public facilities or not	No Yes
Workload	This attribute identifies three levels. Low level relates to 5-15 surgical operation per month, moderate level relates to 15-25 operation and high level relates to more than 250 operations per month	Light Moderate Heavy
Proximity to family	This attribute identifies whether physician has to work in a place apart from family or live together in a same place	No Yes
Educational facilities	This attribute is defined in two levels. Basic refers to having access to a general medical library with few specialized books and journals and wireless internet access. Superior level refers to availability of a specialized library with up to date scientific references and fast internet access also possibility to hold journal clubs and training sessions	Basic Superior
Clinical infrastructure	This attribute is defined in two levels. Basic refers to availability of simple diagnostic and treatment facilities in an area. Superior refers to availability of MRI, CT scan and specialized operating rooms	Inadequate Adequate
Housing	This attribute identifies three levels. None depicts a situation which government does not provide a free housing for physician. Basic refers to a situation which government provides a suite with shared kitchen and bathroom for physician. Superior refers to a situation which government provides an apartment with bedroom, kitchen, and bathroom	None Basic Superior

CT: Computed tomography, MRI: Magnetic resonance imaging

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Clinical Note

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Cortical tumor presenting with Parkinsonism

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Parkinsonism, Brain Tumors, Magnetoencephalography, Motor Cortex, Primary Motor Area, Supplementary Motor Area

Parkinsonism is a syndrome with six major characteristics: Tremor at rest, rigidity, bradykinesia, loss of postural reflexes, flexed posture, and freezing.[1] Parkinson's disease (PD) is the most common form of Parkinsonism,[1] but there are many other causes (i.e., drugs),[2] clinician should be alert to alternative diagnoses, especially if patients with Parkinsonism have atypical findings for PD.

Parkinsonism due to brain tumor is very rare.[3,4] Postulated mechanism was the compression of the basal ganglia.[3] We would like to report an interesting case with intra-axial brain tumor presenting with Parkinsonism. In our case, the brain tumor did not compress the basal ganglia. We will discuss the possible mechanism.

A 55-year-old, right-handed man visited our hospital because he had a 1-month history of subjective motor weakness in the right extremities. He described that his handwriting became slow on writing long sentences and that he felt dragging of his right leg when walking for a long time. He said that he did not drag his foot at the beginning of the walk. He denied sudden onset and reported that the symptom had become worse. There were no vascular risk factors. There was no medication history. On detailed neurological examination, he was alert and fully oriented. There was no motor weakness. Mild sensory deficits for all modalities in the right extremities were seen. There was no cerebellar dysfunction. Deep tendon reflex was normal. Pathologic reflexes were not seen. He showed motor slowness in right finger tapping test and hand movements during the repetitive movements (bradykinesia). Rigidity was also observed on the right arm and leg. On his gait, there was no noticeable problem (i.e., hemiparetic gait, freezing, hesitation, etc.). There was no tremor. Brain magnetic resonance imaging (MRI) showed an enhancing mass lesion in the left paracentral area (Figure 1). We could not proceed further evaluation, because he wanted to go another tertiary hospital.

Our case illustrates two important clinical points. The first, the brain tumor in the paracentral area can cause Parkinsonism. The second, a high index of clinical suspicion is important for proper diagnosis and management, in particular, in the case that patients have unusual findings. In our case, unilateral sensory deficits and too short disease duration might raise suspicion of secondary Parkinsonism.

Parkinsonism caused by brain tumor is uncommon.[3-7] Brain tumors showing Parkinsonism were various such as astrocytoma, meningiomas, craniopharyngiomas, and metastasis. They were usually supratentorial lesions involving the basal ganglia or the nigrostriatal tract, directly or indirectly.[3,6] We think that the Parkinsonism of our patient may be associated with a brain tumor in the motor cortex. There is no direct involvement of the

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basal ganglia or no compression to the basal ganglia. Patients with Parkinsonism involving motor cortex were rarely reported, but the patients also had several unusual symptoms and signs, including headache, cognitive decline, motor weakness, and seizure.[3,6] The tumors invaded other regions because the size was large or it was metastasis.[3,6] Our patient was alert and did not complain any cognitive impairment. There was no definite motor weakness, normal tendon reflexes, and negative Babinski reflexes. The tumor was localized to the pericentral area.

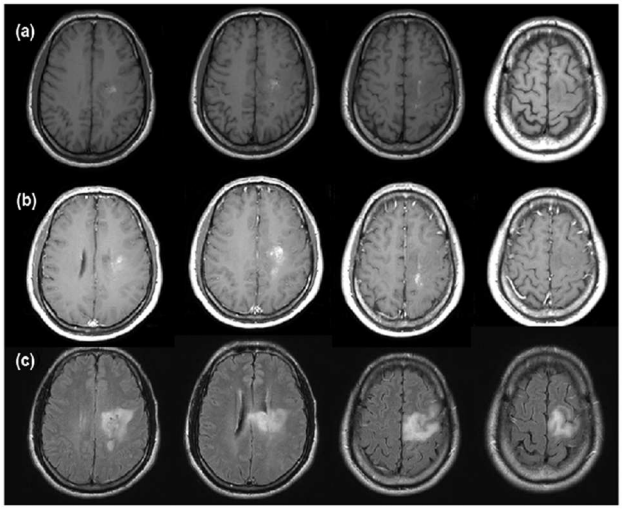

Figure 1. Brain magnetic resonance imaging (MRI) findings, (a) Axial T1-weighted; (b) axial, gadolinium-enhanced, T1-weighted; (c) axial fluid-attenuated inversion recovery. Brain MRI showed an enhancing infiltrating mass lesion in the left paracentral area (a, b), focal high signal is also noted on T1-weighted images, suggesting hemorrhage in the mass (a), MRI findings suggest malignant glial tumor such as malignant astrocytoma or malignant oligodendroglioma.

On initial neurological examination, it was hard to guess whether there was pyramidal tract involvement because there were normal tendon reflexes and negative Babinski reflexes.

The functional activity in the motor cortex is associated with bradykinesia and rigidity in PD.[8-10] In generation of voluntary movements, the basal ganglia connection to motor cortical area is activated: The supplementary motor area (SMA) and the primary motor cortex (MI). The SMA is the main target of basal ganglia output and sends conspicuous projection to the MI. The role of SMA is known to prepare and execute the voluntary movement. The MI is associated with highly skilled movements.[8] In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine animal model, there was the disruptive neuronal activity of SMA and MI.[8] It was reported that pyramidal tract involvement may contribute to bradykinesia. Pyramidal-tract type neurons showed abnormal firing rate in the parkinsonian monkey.[11] The functional imaging studies showed altered activation of the motor cortex in PD.[9,10] One study showed the hypoactivation of the motor cortex,[9] and another, hyperactivation.[10] These contradictory results may be due to different types of motor task.[9,10] PD patients showed overall under activation of brain areas (including the motor cortex). The regional cerebral blood flow of these areas was positively correlated with a movement rate of the task.[9] This hypoactivation may be explained by reduced thalamocortical output in PD.[12] In the case of over-activation, it was explained with compensatory mechanism to decreased basal ganglia activity or reflection of rigidity, not bradykinesia.[10]

Our observations have some limitations. First of all, there is a lack of an appropriate follow-up. In fact, subacute onset or short duration of unilateral Parkinsonism is a strong red flag to the diagnosis of PD. We cannot exclude the possibility that my patient might be a patient with recent onset PD with an incidental brain mass on brain MRI, because we do not have any information about whether his Parkinsonism were improved after tumor treatment (i.e., tumor decompression), or after levodopa therapy. The improvement of his symptoms after levodopa treatment may suggest dopaminergic deficiency. Second, there might be argued that the description of parkinsonian features might not be enough, and we did not show his video clip, Unified PD Rating Scale, and dopamine transporter imaging, but unfortunately, these data are not available.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

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Letter to Editor

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Cerebellar infarction and aneurysmal subarachnoid hemorrhage: An unusual presentation and rare complications of rhinocerebral mucormycosis

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Keywords
Stroke, Mucormycosis, Subarachnoid Hemorrhage

The most common clinical presentation of mucormycosis is rhino-orbital-cerebral infection, which is supposed to begin with inhalation of spores into the paranasal sinuses of a vulnerable host. Hyperglycemia, with an associated metabolic acidosis, is the most common underlying state.[1]

The hallmarks of spread outside the sinuses are tissue necrosis of the palate.[1,2]

We present a 57-year-old man admitted to the emergency department of Ghaem Hospital affiliated to Mashhad University of Medical Sciences, Iran, with fever, decreased the level of consciousness, and left facial swelling from 1 week before admission. He was known the case of diabetes mellitus from 2 years before, and he was under treatment with oral hypoglycemic agents. Initiation of his symptoms was with a common cold and headache from 20 days before.

He was smoker and opiate addict. His drug history was glibenclamide, amlodipine, acyclovir ophthalmic drop and oral diazepam tablets. On physical examination, the patient was febrile (temperature: 39 °C). Ear nose and throat examination showed facial asymmetry due to complete ptosis, significant edema of the right eyelid, palatal necrosis and left alveolar ridge necrosis. On the left side of the nose middle and inferior conchae necrosis was observed. The right side of the nose was normal.

On mental status examination, he was drowsy but he was conscious and obeyed the commands. Examination of cranial nerves showed that he had multiple cranial nerve palsy. Fundoscopy was normal. The left eye had ptosis and pupil was mydriatic and non-reactive. Left frozen eye (3, 4, 6 cranial neuropathies) hypoesthesia of the left side of the face (V1, V2 of left fifth cranial neuropathy) left peripheral seventh nerve palsy (previous bell's palsy). Other cranial nerves were normal. The motor examination was normal. Examination of sensory and gait of the patient was unremarkable because of his drowsiness. Ophthalmology and otolaryngology consult performed urgently.

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The patient was started on intravenous amphotericin-B and wide spectrum antibiotics. Debridement of the left nasal cavity was subsequently performed. Biopsy samples obtained from the nasal eschar illustrated the picture of mucormycosis with some foci of non-septate fungal hyphae and hyphal branches typically at right angles.

The level of consciousness suddenly decreased 1 week after initiation of treatments. Computed tomography (CT) of the head showed extensive subarachnoid hemorrhage. The patient underwent emergent ventriculostomy and brain CT angiography performed 7 days after surgery. Brain CT angiography showed two consecutive fusiform aneurysms in a superior cerebellar artery (5.17 × 5.50 mm) and (4.17 × 5.55 mm). The patient finally died 2 months after admission in stroke intensive care unit (Figure 1).

Mucormycosis is a rare invasive fungal infection with a high rate of morbidity and mortality. Mucor organisms show aggressive characteristics regarding vascular and cranial nerve invasion and extension; thus, imaging and explanations of areas of involvement are key.[3,4] In addition, rhinocerebral mucormycosis should be considered in the appropriate patient presenting with symptoms of unilateral cranial nerve involvement suggesting Garcin syndrome.[2,5] In our patient, the ruptured aneurysm was diagnosed after a subarachnoid hemorrhage and the patient finally died after 2 months on March 26, 2014. The present case emphasizes an atypical presentation of fungal infection that can perplex any physician and thus delay diagnosis. Subarachnoid hemorrhage due to true mycotic aneurysmal rupture is consistently associated with a fatal outcome.[4,5] Another aspect of this case was infarction of the cerebellum in the territory superior cerebellar artery at the first magnetic resonance imaging (MRI). This finding was misleading as an ischemic atherosclerotic infarction because of risk factors of the patient, but actually it was related to superior cerebellar artery aneurysm. This patient first treated for rhinocerebral mucormycosis but an aneurysm ruptured suddenly because of undiagnosed mycotic aneurysm, which was fatal for our patient. It is noteworthy to emphasize on an early angiographic study in patients with rhinocerebral mucormycosis and acute ischemic stroke on presentation because the delay in early diagnosis of a concealed aneurysm may be lethal for the patient.

In conclusion, recognition and early intervention of the underlying vascular complication that predispose patients to fatal outcome are critical in order to avoid serious complications of this rare infection especially when the acute infarction is evident in brain MRI.

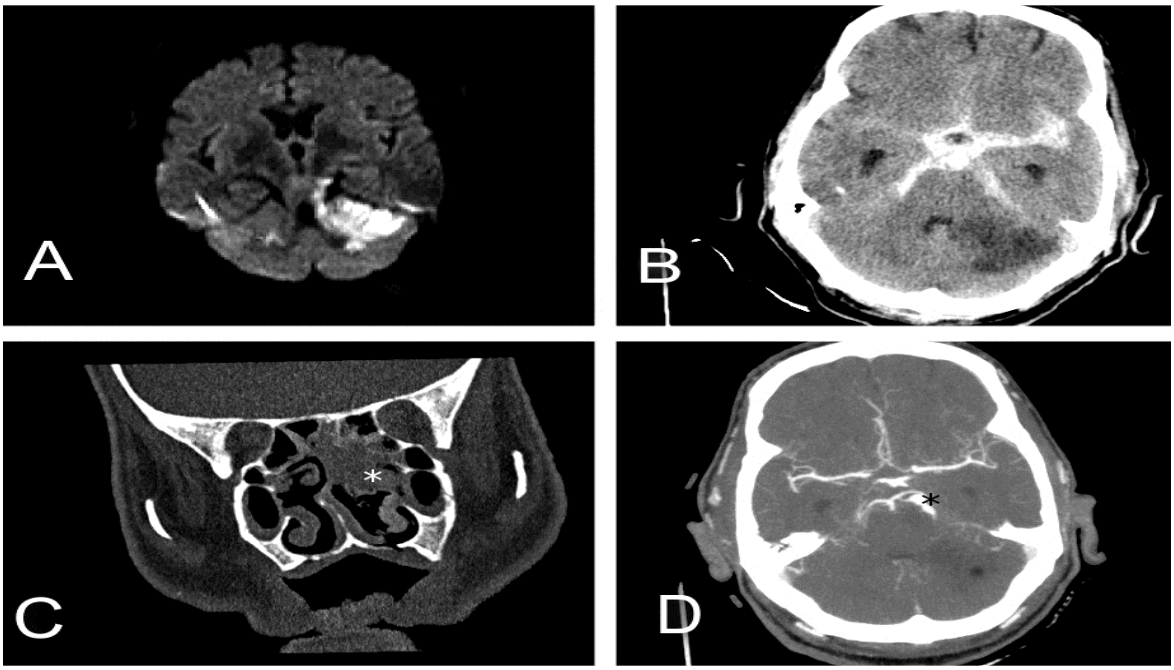

Figure 1. (A) Axial diffusion-weighted magnetic resonance imaging shows increased signal in left cerebellar hemisphere, representing acute ischemic infarction with restricted diffusion pattern, (B) axial computed tomography (CT) of the head shows extensive subarachnoid hemorrhage and cerebellar ischemic stroke, (C) CT coronal image shows an inflammatory process involving nasal cavity and ethmoid cells (white star), (D) Two consecutive fusiform aneurysms in superior cerebellar artery are evident in axial brain CT-angiography (black star).

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

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Iranian Journal of Neurology

Letter to Editor

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Complicated orolingual angioedema after recombinant tissue plasminogen activator treatment in stroke patients under angiotensin converting enzyme inhibitor: Report of two cases

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Keywords
Orolingual Angioedema, Stroke, Angiotensin-Converting Enzyme Inhibitors, Recombinant Tissue Plasminogen Activator

Currently, the intravenous (IV) recombinant tissue plasminogen activator (rt-PA) is approved as a relatively safe treatment approach to improve the outcomes following the acute ischemic stroke.[1] As an uncommon complication, angioedema occurred in 1.3-5% of stroke patients after IV rt-PA;[2] however, previous reports warn that patients taking angiotensin converting enzyme (ACE) inhibitors (ACEIs) are at increased risk of developing angioedema after rt-PA administration.[3] In most patients, this side effect presents as a mild and transitory orolingual angioedema.[3] In general, orolingual angioedema is defined as a localized edematous vascular reaction of either deep dermis or subcutaneous or submucosal tissues caused by dilatation and increased permeability of the capillaries, which pertains to the oral cavity including mouth, lips, and tongue. Following thrombolysis, this unpleasant event has been described in 2% of stroke patients,[4] However, this prevalence rate has been recently shown in a systematic review to be as high as 17% within the first 4 hours of receiving rt-PA.[5] The effect of rt-PA on activation of plasminogen into plasmin and the consequent increase in the serum level of bradykinin has been proposed as one probable etiology for orolingual angioedema.[5] Moreover, it seems that some of the members of the complement system namely C3a, C4a, C5a, and C2-kinin also contribute in the formation of angioedema in patients under rt-PA treatment.[6] As a clinical experience, we present the two stroke patients using ACEI in whom the orolingual angioedema was developed after rt-PA

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and even complicated to respiratory distress in one of them.

Case I

A 55-year-old obese woman with the previous history of hypertension and cerebrovascular accident (3 years ago) was admitted to Firoozgar General Hospital, Tehran, Iran, complaining from acute weakness in the right limbs. The symptom was started in 2 hours before admission with acute onset. In drug history, she was found under treatment with captopril 25 mg due to the history of hypertension for 6 months. On the initial evaluation, her blood pressure was 170/90 mmHg. She was awake and alert with a left gaze preference, dense right hemiplegia with the National Institutes of Health Stroke Scale (NIHSS) of 19. The serum level of C3 and C4 as the components of the complement system was above the normal range. Full laboratory data is shown in table 1. A non-contrast brain computed tomography (CT) scan showed no hemorrhage, but a left dense middle cerebral artery (MCA) sign was seen. Based on neither historical nor laboratory contraindication for thrombolysis, she received a total of 90 mg IV rt-PA approximately 3 hours after symptom onset with 10% of total dose given as a bolus and 90% of it was given within 1 hour. Within minutes after completing the rt-PA infusion, she developed the edematous lip and tongue swelling. She was treated with 0.5 mg subcutaneous epinephrine and 200 mg hydrocortisone (100 mg twice per day for 48 hours). After 30 minutes, the improvement of her orolingual angioedema was started and completely resolved within 36 hours. However, the neurologic deficits the improved dramatically resulting in an NIHSS of 10 in the 2nd day. A repeated brain CT scan at 24 hours post rt-PA infusion showed an improvement with changes of infarction restricted to the superior division of the left MCA.

Case II

A 63-year-old man with a history of hypertension was referred to the emergency ward of the Firoozgar General Hospital presenting with acute left side weakness. The patient was under treatment with captopril for 6 months and his blood pressure was measured as 140/80 mmHg on the initial evaluation. He was alert with dense left hemiplegia and his NIHSS was 13. Elevated serum level of both C3 and C4 was detected in laboratory investigation (Table 1). A non-contrast brain CT scan showed neither hemorrhage, nor early ischemic changes, and nor hyperdense vessel signs. In total, he received 54 mg IV rt-PA with 10% given as a bolus approximately 3.5 hours after symptom onset. Thirty minutes after completing the rt-PA infusion, he developed the left lower lip and tongue swelling (Figure 1). Physical examination revealed an edematous tongue and asymmetric edema in the left lower lip with increased respiratory distress. He was treated with hydrocortisone and epinephrine; however, we had to intubate the patient in order to decrease his respiratory distress. The next day, he was extubated and the repeated brain CT scan also revealed an infarction in the right MCA territory. The patient improved neurologically to an NIHSS of 11 on the discharge day.

Table 1. Laboratory data in two reported cases

Test	Value	Normal range
C3 (mg/dl)	Case I: 198 Case II: 212	90-180
C4 (mg/dl)	Case I: 66 Case II: 74	10-40
WBC (total cells/mcl)	Case I: 8600 Case II: 11200	3500-10500
PMN (%)	Case I: 62 Case II: 59	40-80
Eosinophils (%)	Case I: 4 Case II: 6	1-6
Lymphocytes (%)	Case I: 33 Case II: 33	20-40

WBC: White blood cells; PMN: Polymorphonuclear

Though mild and spontaneously reversible in most patients,[3] orolingual angioedema can become a life-threatening complication of alteplase therapy in stroke patients.[7]

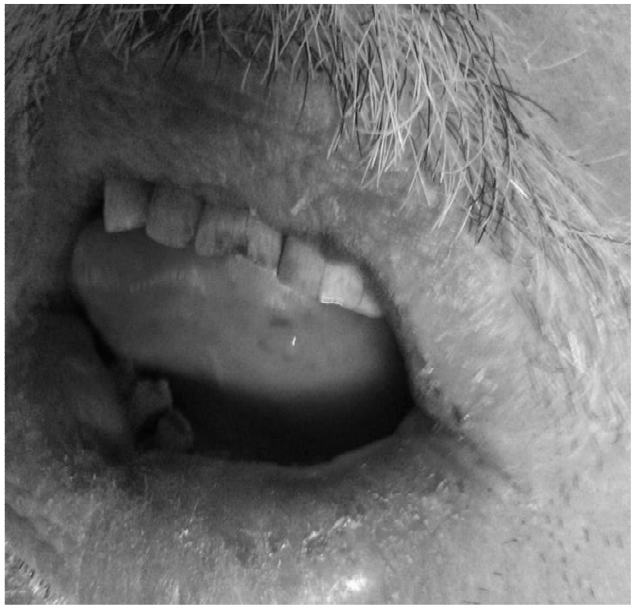

Figure 1. Orolingual angioedema (left lower lip and tongue swelling) after recombinant tissue plasminogen activator infusion in a stroke patient under treatment with captopril

The risk of this unexpected complication is increased to higher rates among those under ACEI medication. The underlying mechanism by which angioedema is produced by rt-PA is explained through the increased level of bradykinin resulting from the cleavage of high-molecular-weight kininogen.[8] This increased bradykinin has vasodilatory properties, increases vascular permeability, and might be induce angioedema.[6] While the ACE normally inactivates bradykinin, the use of ACEIs accumulates bradykinin level that consequently makes stroke patients more susceptible to develop the angioedema following rt-PA infusion.[9] Of note, elevated serum level of the components of complement system (C3 and C4) was observed in both reported cases in our study, which could be in favor of the involvement of complement system in the etiology of angioedema in these patients.

In our report, both patients were consuming captopril to control their hypertension. The orolingual angioedema was rapidly progressive during the 1st minutes after the infusion of rt-PA. Similar to previous reports,[3,8] angioedema was asymmetric in our cases and was occurred in the same side as their ipsilateral hemiparesia. It might be related to the infarction of the contralateral insular cortex, which promotes the autonomic dysfunction and vasomotor changes in the hemiparetic side.[8,10]

We administered the epinephrine and corticosteroid to manage the angioedema. However, the severity of respiratory distress necessitated intubation in one of the patients. This intervention is not required the most often because the course of orolingual angioedema is benign and self-limited in the majority of patients.[2,3,11] In conclusion, orolingual angioedema must be taken into account as one probable post rt-PA complication in stroke patients who are under treatment with ACE inhibitors. Although they often answered to epinephrine and corticosteroid, the physicians should be aware of respiratory distress in severe cases in which the airway management is necessary. We hereby presented the first two reported Iranian stroke patients who developed orolingual angioedema following rt-PA therapy. Regarding the increasing trend of rt-PA administration in Iran,[12] it seems necessary to pay more attention toward orolingual angioedema as a potential adverse event that can become even a life-threatening complication in stroke patients, especially among those under ACEIs medication. Therefore, patients may require the urgent lifesaving procedures such as intubation or tracheotomy.

Conflict of Interests

The authors declare no conflict of interest in this study.

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Iranian Journal of Neurology

Letter to Editor

Iran J Neurol 2015; 14(4): 228-230

Nasopharyngeal carcinoma presenting as Garcin's syndrome: A rare case report

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Keywords
Nasopharyngeal Carcinoma, Multiple Cranial Neuropathies, Radiotherapy

Garcin syndrome consists of unilateral palsies of almost all cranial nerves without either sensory or motor long-tract disturbances and without intracranial hypertension, and it is caused by a malignant osteoclastic lesion at the skull base.[1] The underlying cause of Garcin's syndrome is usually a sarcoma, lymphoma, metastasis, chemodectoma, or carcinoma of the skull base. The literature on Garcin's syndrome presenting as an early sign of nasopharyngeal carcinoma (NPC) is limited, and this makes the present case rare and interesting.

A 70-year-old man, smoker, presented to our institute with complain of headache, drooping of right eyelid, double vision, facial asymmetry, and dysphagia to solid and liquid, dysarthria, hoarseness of voice for 4 months (Figure 1). He had developed difficulty in hearing for 1 month and episodes of syncope in last 15 days.

General physical examination revealed cervical lymphadenopathy. On central nervous system examination, there was complete ptosis and no ocular movements were possible on the right side. Diplopia was present on all gazes. Pupils were bilaterally equal and reacting normally to light. There was decreased sensation over the right side of the face with absent ipsilateral conjunctival and corneal reflex. There was a facial asymmetry with the loss of forehead wrinkle on the right side. Pure tone audiometry showed a conductive hearing loss in the right ear. Indirect laryngoscopy examination revealed right-sided vocal cord palsy, uvula deviated to left side and gag reflex was also absent. The tongue was atrophied on the right side and on protrusion deviated to the right. Thus, 9 out of 12 cranial nerve were involved in this patient. The rest of the systemic examination was within normal limits. Fundus examination and cerebrospinal fluid examination was within normal limits.

A magnetic resonance imaging of the head and neck region revealed primary nasopharyngeal mass lesion of size 49 × 62 × 47 mm extending inferiorly, obliterating Eustachian tube; laterally to pterygomaxillary fissure, sphenopalatine fossa; cavernous sinus, internal carotid artery; right inferior orbital fissure with perineural invasion through right foramen ovale and foramen rotundum (Figure 2). Histopathological examination of the excision biopsy specimen revealed high-grade malignant tumor cells in syncytial arrangement, with large nucleus, vesicular chromatin, prominent nucleoli, and moderate amount of cytoplasm with indistinct cytoplasmic borders suggesting poorly differentiated primary NPC (Figure 3).

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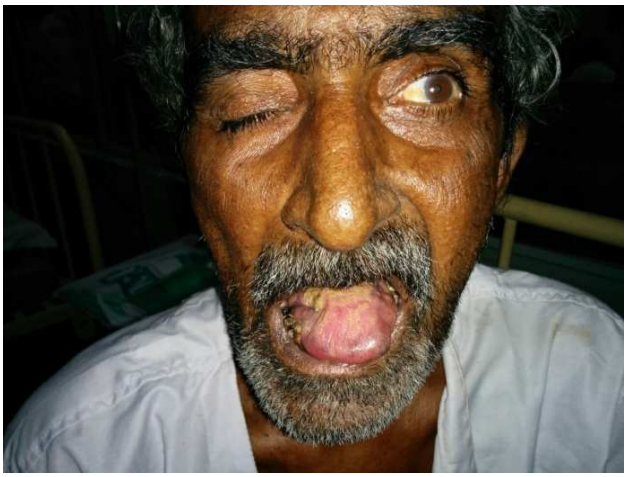

Figure 1. Clinical photograph showing ptosis, loss of nasolabial folds, and deviation of angle of mouth to opposite side

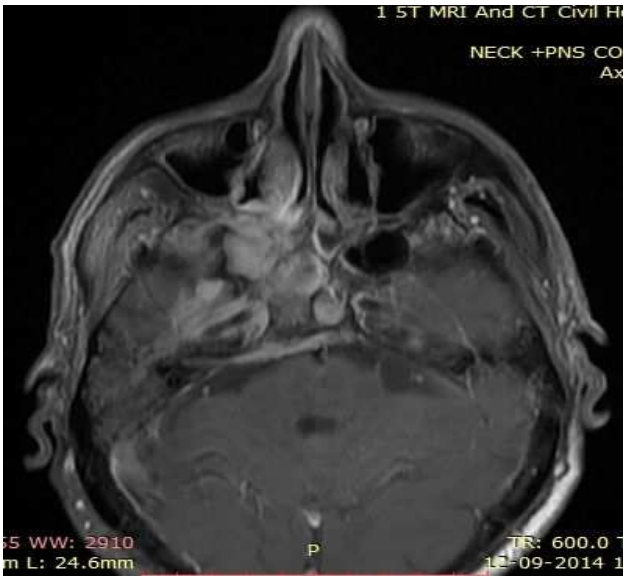

Figure 2. A gadolinium enhanced magnetic resonance imaging of the head and neck region revealed primary nasopharyngeal mass lesion of size 49 × 62 × 47 mm

Thus, the combination of signs, symptoms, radiological and histopathological findings enabled us to diagnose as a case of Garcin's syndrome secondary to NPC. The patient was managed with two cycles of neo adjuvant chemotherapy (DCF protocol: docetaxel, cisplatin and 5-flurouracil) along with growth factor support which he tolerated very well. Concurrent chemo radiotherapy was given with weekly cisplatin along with radiotherapy at a dose of 1.8 Gy per fraction for 30 fractions. There was a dramatic improvement in his symptoms in the form of improvement in the hoarseness, facial weakness, headache, giddiness, dysphagia, dysarthria and diplopia. Now the patient is under regular surveillance at oncology clinic since 6 months.

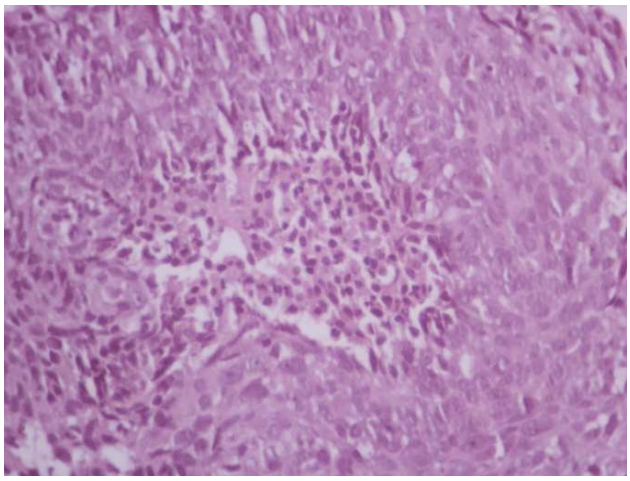

Figure 3. Histopathological examination revealed high grade malignant tumour cells in syncytial arrangement, with large nucleus, vesicular chromatin, prominent nucleoli, and moderate amount of cytoplasm with indistinct cytoplasmic borders suggesting poorly differentiated nasopharyngeal carcinoma

Garcin syndrome is an ipsilateral step-by-step deterioration of all 12 cranial nerves, first described in 1927. This rare progressive condition is generally associated with skull based malignant osteoclastic lesions but has also been described with pachymeningitis secondary to otitis media, rhinocerebral mucormycosis, hypertrophic pachymeningitis, lymphomatous meningitis, carcinomatous leptomeningitis, chemodectoma, meningioma, and giant internal carotid aneurysm. NPC rarely comes to medical attention before it has spread to regional lymph nodes. Enlargement and extension of the tumor in the nasopharynx may result in symptoms of nasal obstruction, changes in hearing, and cranial nerve palsies. The most common physical finding is a neck mass consisting of painless firm lymph node enlargement (80%).

Cranial nerve palsy at initial presentation is observed in 25% of patients. This tumor may involve the cranial nerves in many ways. First, the tumor may extend superiorly through foramen lacerum, which is an unimpeded pathway near the fossa of Rosenmuller into the cranium 3, 4, 5, thus involving the cranial nerves in the middle cranial fossa and cavernous sinus. Cancer may break through the pharyngo-basilar fascia and spread along vascular sheaths, that is, facial planes surrounding the jugular vein and carotid artery. However, NPC solely presenting as Garcin's syndrome is rarely represented in the literature.[2] Lateral and posterior extension of the primary growth itself may involve the lower cranial nerves exiting from jugular and hypoglossal foramina. These lower cranial nerves

may also be involved on their course while traversing the neck by the secondary deposits in the lymph nodes.

The treatment options for NPC are radiotherapy, chemotherapy and concurrent chemoradiotherapy. Chemoradiation significantly improved progression-free survival and overall survival.[3]

Conflict of Interests

The authors declare no conflict of interest in this study.

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Letter to Editor

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The prevalence of Martin-Gruber anastomosis in Iranian subjects by electrodiagnostic criteria

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Introduction

Median–ulnar anastomosis [Martin–Gruber anastomosis (MGA)] is a common anatomic variant. The crossover often occurs in mid-forearm. Median fibers that have crossed then run with distal ulnar nerve to innervate any of following ulnar muscle: (i) Abductor digiti minimi (ADM), (ii) first dorsal interosseous (FDI), (iii) adductor pollicis, (iv) deep head of flexor pollicis brevis, or (v) combination of these. The FDI is the most common termination (34%), followed by hypothenar (15.5%) and then Thenar (12%) musculature.[1] This anomaly is asymptomatic. It seems to have less prevalence in our population, however, we did not find any published document regarding the prevalence of this anomaly, so the main goal of this study was to estimate the frequency of MGA in referred subjects to academic electrodiagnostic (EDX) clinics of physical and rehabilitation (PM and R) department.

This descriptive cross sectional study was performed in subjects referred to our EDX clinics of PM and R department. Ninety subjects were recruited who had normal neurological exam. The subjects with median or ulnar nerves injuries due to trauma or polyneuropathy were excluded by supra max stimulate at wrist and elbow. The median nerve compound muscle action potential (CMAP's) was recorded from abductor pollicis brevis (APB), ADM, and FDI. Afterward, the ulnar nerve was stimulated at wrist and below elbow recording from abductor ADM and FDI and APB muscles. The examinations were conducted in both sides.

After recording these CMAP's, four conditions may have occurred: (1) All the tests were within normal range; (2) while stimulating ulnar nerve and recording from FDI, > 20% decline in amplitude between wrist and elbow was detected and recording from ADM was normal, then median was stimulated in antecubital fossa whereas recording from FDI; provided that MGA was present, the amplitude difference of ulnar stimulation between elbow and wrist was recorded from FDI and when median was stimulated in wrist whereas recording from FDI a small positive wave record from volume conduction of nearby muscles; (3) during stimulating ulnar nerve and recording from ADM, more than 20% of amplitude decline was detected between elbow and wrist; then median was stimulated in antecubital fossa whereas recording from ADM; provided that MGA was present, the amplitude difference of ulnar stimulation between elbow and wrist was recorded from ADM and when median was stimulated in wrist whereas recording from ADM, a

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small positive wave record from volume conduction of nearby muscles; (4) during stimulation of median at wrist and recording from Thenar muscle, the amplitude in proximal was higher than distal. By above mentioned criteria, conditions 2, 3, and 4 were regarded as presence of MGA.

In this research, our study population consists of 90 individuals including 59 women (65.6%), 31 men (34.4%), with an average age range of 40.72 ± 11.69 years and number of subjects with anastomosis was 13 cases. Therefore, the anastomosis frequency was 14.4%. Interpreting the results according to the number of hands for anastomosis, 15 hands (6 cases had the left hands and 5 cases had the right hands and 2 cases had the both hands) had anastomosis and the frequency of anastomosis was 8.33% for all hands. The types of anastomosis for these 15 hands, was as follows: 77 cases had normal first condition (85.6%), 9 cases had the second condition (10%) end to FDI, 2 cases had third condition (2.2%) end to ADM and 2 cases had fourth condition (2.2%) end to Thenar muscles.

Our results are similar to other findings from Iran that estimated the prevalence of MGA as 10.3%.[2] In the study was performed by medical students, the prevalence of MGA was around 20% indicating a high prevalence.[3]

In another study from Korea, MGA was found in 39.2% from 102 upper limbs. Among 12 instances of MGA between the branches innervating the flexor digitorum profundus muscle, 8 anastomotic branches solely innervated the muscle without crossover from median to ulnar nerve.[4]

The major risk in not recognizing MGA is that of mistakenly interpreting the findings as ulnar conduction block. Apart from it, MGA has clinical significance for understanding median nerve lesion and carpel tunnel syndrome.

It appears from the present study that identification of MGA is extremely crucial before labeling the condition as ulnar neuropathy as the mode of treatment differs accordingly (former needs no treatment, whereas later needs it appropriately). Furthermore, we concluded that nerve conduction study is a reliable tool in diagnosis of MGA prevalence of, which we reported as 14.4% in EDX clinics of PM and R department Isfahan University of Medical Sciences, Iran. For future, the studies with larger samples may be required.

Conflict of Interests

The authors declare no conflict of interest in this study.

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