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ORIGINAL ARTICLE

Evaluation of NOS3 Gene rs1800779 Polymorphism in Iranian Patients affected by Migraine and Normal individuals

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ABSTRACT
Migraine is a regular neurological disorder affecting between 10 and 20% of the population this disease is a serious, annoying headache which may be preceded or followed closely by sensory warning symptoms for instance flash of light, blind spots. There are many common risk factors such as NOS3 gene rs1800779 polymorphism that play key roles in the development of migraine disease. Polymorphisms in the endothelial nitric oxide synthase (NOS3) gene have been associated to migraine in some populations. Facts proposed that NOS3 might have a role in this disorder; as a result we studied NOS3 gene rs1800779 polymorphism in Iranian patients affected by Migraine and normal individuals to realize the association between them. The present research was conducted including number of 68Iranian patients affected by Migraine and 100 normal individuals by employing ARMS-PCR process. To conclude, the data were analyzed by SPSS software. To sum up, the end outcome of present study explains substantial relation between NOS3 gene rs1800779 polymorphism in Iranian patients affected by Migraine and normal individuals. It could be an important genetic predisposition feature.
Key words: *Migraine, rs1800779, NOS3, headache, polymorphism*

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INTRODUCTION

A migraine is generally a rigorous headache felt as a throbbing pain at the front or side of the head [1]. Usually the headache affects half of the head, is pulsating naturally, and may last from 2 hours to 3 days. Related symptoms could contain nausea, vomiting, and sensitivity to light, sound, or smell [2]. The pain is usually made worse by physical activity. Up to one-third of men and women with migraine headaches perceive an aura: a temporary visual, sensory, language, or motor trouble which signals that the headache will quickly happen. Infrequently an aura could occur with minimum headache following it [3].

Migraines are considered to be as a result of combination of environmental and genetic aspects. About two-thirds of instances run in family units [4, 5]. Changing hormone levels could also play an important role, as migraines affect slightly more boys than girls before puberty, but about two to three times more women than men. The chance of migraines frequently is reduced during pregnancy [4]. The precise mechanisms of migraine aren't recognized. It's however, thought to be a neurovascular disorder. The principal theory relates to increased excitability of the cerebral cortex and abnormal control of pain neurons in the trigeminal nucleus of the brainstem [6, 7].

NO molecule contributes in cell signaling importantly. In the presence of oxygen, L-arginine, is converted to L- citrulline and release NO. The responsible enzyme for catalyzing this reaction is Nitric oxide synthase (NOS), and it occurs in three isoforms. In the CNS, the neuronal isoform (nNOS) is extensively distributed in neurons, astrocytes and blood vessels. The endothelial isoform (eNOS) is placed in the hippocam-palpyramidal neurons, endothelial cells and some astrocytes. (iNOS) expression is typically low but is increased in microglia and astrocytes during neuro- inflammation. Under physiological conditions, it is supposed that NO regulates the release of neurotransmitters and hormones and promotes cell

survival and long term potentiating. Even though, high levels of NO are made in inflammatory conditions, which might contribute to synaptic dysfunction, protein and lipid oxidative damage, and neuronal death [9, 10].
Consequently, we conducted a research to study NOS3 gene rs1800779 polymorphism in Iranian patients affected by Migraine and normal individuals.

MATERIAL AND METHODS
DNA was obtained from 67 patients with migraine. A total of 100 healthy controls aged 42-65 years or younger were also analyzed to define a possible role for the NOS3 gene rs1800779 polymorphism in Iranian patients with migraine. Genomic DNA was amplified by polymerase chain reaction (PCR) with proper primers and then, the data were analyzed by (SPSS) software.
This polymorphism was genotyped by ARMS-PCR, with the primer pairs:

Primer	Sequence (5'->3')	Length	Tm	GC%
F_C	AAGGCAGGAGACAGTGGATG	20	59.38	55.00
R_N	TGAAGGAAGAGTTCTGGTGGC	21	59.93	52.38
R_M	TGAAGGAAGAGTTCTGGTGGA	21	58.31	47.62

Specifications primers used for internal control:

Primer	Sequence (5'->3')	Length	Tm	GC%
F	GTGTACCCCACCTGCATTCT	20	59.67	55.00
R	CCCAGCAAGGATGTAGTGAC	20	57.97	55.00

PCR program used for ARMS-PCR polymorphism G-T894:

Cycle	temperature (Celsius)T	Time
First	95	7 Minutes
Two to thirty-five	94 59 72	1 minute and 15 seconds 55 Seconds 30 seconds
thirty-six	72	5 Minutes

Gel Electrophoresis
The electrophoresis was carried out using 1% Gel Red stained agarose gel, at 80V for 35 min We Use Horizontal Electrophoresis Cell (Model: JY-SPAT) with TBE Buffer (PH=8.3) , Ladder Were Used 50bp DNA Ladder (JenaBioscience) After electrophoresis, the amplified PCR products were Perceive under U. V. light.
Statistical analysis
Statistical analyses were conducted using with the SPSS software (Statistical Package for Social Sciences) version18. Chi- square test ($\chi 2$), was used to check the association between two categorical variables or even to detect difference between several proportions. Pearson chi-square was used to investigate the connection involving the NOS3 gene rs1800779 polymorphism polymorphism and Migraine.

RESULTS
We analyzed 68 genotype patients with Migraine, and 100 healthy controls, for the NOS3 gene rs1800779 polymorphism polymorphism.
NOS3 gene rs1800779 polymorphism frequencies were in equilibrium in patients and controls. Patients showed an extensively increased frequency of the NOS3 gene rs1800779 polymorphism allele compared

with controls. Thus the NOS3 gene rs1800779 polymorphism allele would confer a slightly increased risk of developing late onset Migraine.

Genotype Table:

Table1: Genotype Table of NOS3 gene rs1800779 polymorphism:

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype* Group	168	100.0%	0	.0%	168	100.0%

Table2: Genotype * Group Cross tabulation

Genotype * GroupCross tabulation

Count

		Group		
		Case	Control	Total
Genotype	GG	56	93	149
	GT	12	6	18
	TT	0	1	1
Total		68	100	168

Table3: Chi-Square Tests

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.322[a]	2	.042
Likelihood Ratio	6.577	2	.037
Linear-by-Linear Association	3.206	1	.073
N of Valid Cases	168		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .40.

Table4: Symmetric Measures

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.139	.081	-1.803	.073[c]
Ordinal by Ordinal	Spearman Correlation	-.162	.078	-2.118	.036[c]
N of Valid Cases		168			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Table5: Risk Estimate

Risk Estimate

	Value
Odds Ratio for VAR00001 (1 / 2)	[a]

Risk Estimate

	Value
Odds Ratio for VAR00001 (1 / 2)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

ALLELE Table:

Table6: ALLELE Table of NOS3 gene rs1800779 polymorphism

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Allele * Group	336	100.0%	0	.0%	336	100.0%

Table7: Group Cross tabulation and Chi-Square Tests

Allele * Group Cross tabulation

Count

		Group		
		Case	Control	Total
Allele	G=1	124	192	316
	T=2	12	8	20
Total		136	200	336

Table8: Chi-Square Tests for ALLEL of NOS3 gene rs1800779 polymorphism

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.365[a]	1	.067		
Continuity Correction [b]	2.558	1	.110		
Likelihood Ratio	3.288	1	.070		
Fisher's Exact Test				.098	.056
Linear-by-Linear Association	3.354	1	.067		
N of Valid Cases	336				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 8.10.

b. Computed only for a 2x2 table

Table9: Symmetric Measures

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Interval by Interval	Pearson's R	-.100	.055	-1.838	.067[c]
Ordinal by Ordinal	Spearman Correlation	-.100	.055	-1.838	.067[c]
N of Valid Cases		336			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Table10: Risk Estimate for ALLEL of NOS3 gene rs1800779 polymorphism

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Allele (1 / 2)	.431	.171	1.083
For cohort Group = 1	.654	.446	.959
For cohort Group = 2	1.519	.882	2.617
N of Valid Cases	336		

DISCUSSION

The evidence exposed in the piece of writing confirms that NOS3 gene rs1800779 polymorphism plays an important role in Iranian patients affected by Migraine. In accordance with this, an increased frequency of the allele among patients with Migraine has been seen.

By analyzing a group of Iranian patients, it is understood that the NOS3 gene rs1800779 has been connected with this disorder. As a result NOS3 gene rs1800779 polymorphism is actually a noteworthy genetic tendency factor for in Iranian Migraine patients. Therefore, NOS3 gene rs1800779 polymorphism may be a genetic predisposing factor for Migraine disorder treatment in Iranian population.

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