Artículo de investigación

Investigation of Estrogen (ESR) Receptor Gene Polymorphism in Multiple Sclerosis (MS) Disease

Investigación del polimorfismo en el gen del receptor de estrógeno (ESR) en la enfermedad de esclerosis múltiple (EM)

Investigação do Polimorfismo do Receptor de Estrogênio (ESR) na Doença da Esclerose Múltipla (EM)

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Abstract

MS is the disease of the central nervous system in which myelin sheath of the optic nerves is degraded. According to the role of estrogen in the embryonic distinctions and that the estrogen is a female sex hormone and considering the high prevalence of MS in women and in regard of the fact that the sex hormones carry out their effects using their own specific receptors, the present study aims at determining the role of estrogen receptor gene in patients with MS in Azerbaijan-e-Gharbi Province and comparing the gen in both the patients and the healthy individuals so as to make it clear what role do estrogen and its receptor genes play in MS disease.

The present study is a case-control study that was conducted on MS patients who had referred to Orumia's Imam Khomeini (may Allah consecrate the soil of his tomb) Hospital. The study sample volume was consisted of two groups: one group comprised of 30 women with MS and another group composed of 30 healthy women who had been randomly assigned to these groups. Blood samples, 2cc, were taken from all the patients following which the DNAs of the patients were extracted and the estrogen hormone receptor gene, named ESR1, was subjected to examination based on RFLP method. Next, the information was analyzed using SPSS software, version 21.

In patients with MS, eight cases (26.7%) had homozygous gene, five cases (16.6%) had heterozygous gene and 17 cases (56.7%) had

Resumen

La EM es la enfermedad del sistema nervioso central en la cual la vaina de mielina de los nervios ópticos se degrada. De acuerdo con el papel del estrógeno en las distinciones embrionarias, el hecho de que estrógeno es una hormona sexual femenina, en consideración de la alta prevalencia de EM en las mujeres y en relación con el hecho de que las hormonas sexuales llevan a cabo sus efectos utilizando sus propios receptores específicos, el objetivo del estudio es determinar el papel del gen del receptor de estrógeno en pacientes con EM en la provincia de Azerbaiyán-e-Gharbi y comparar el gen tanto en los pacientes como en los individuos sanos para aclarar qué papel desempeñan los genes del estrógeno y sus receptores en la EM. enfermedad.

El presente estudio es un estudio de casos y controles que se realizó en pacientes con EM que se habían referido al hospital Imam Khomeini de Orumia (que Allah consagra el suelo de su tumba). El volumen de la muestra del estudio consistió en dos grupos: un grupo compuesto por 30 mujeres con EM y otro grupo compuesto por 30 mujeres sanas que habían sido asignadas al azar a estos grupos. Se tomaron muestras de sangre, 2cc, de todos los pacientes, después de lo cual se extrajeron los ADN de los pacientes y el gen receptor de la hormona estrógeno, llamado ESR1, se sometió a un examen basado en el método RFLP. A continuación, la información se analizó utilizando el software SPSS, versión 21.

healthy gene; in the healthy individuals of the control group, zero cases had homozygous gene, 3 cases (10%) had heterozygous gene and 27 cases (90%) had healthy genes.

The genotypic and allelic frequencies of Era rs2234693 (C/T) polymorphism shows a significant difference between the patient and control groups.

Keywords: multiple sclerosis, estrogen receptor, polymorphism, PCR-RFLP

En los pacientes con EM, ocho casos (26.7%) tenían un gen homocigoto, cinco casos (16.6%) tenían un gen heterocigoto y 17 casos (56.7%) tenían un gen sano; en los individuos sanos del grupo control, cero casos tenían un gen homocigoto, 3 casos (10%) tenían un gen heterocigoto y 27 casos (90%) tenían genes sanos.

Las frecuencias genotípicas y alélicas del polimorfismo Era rs2234693 (C / T) muestran una diferencia significativa entre los grupos de pacientes y de control.

Palabras clave: esclerosis múltiple, receptor de estrógeno, polimorfismo, PCR-RFLP

Resumo

A EM é a enfermaria do sistema nervoso central na doença da vuelva de mielina dos nervos ópticos se degrada. De acordo com o papel do estrogênio em distúrbios embrionários, o hecho de que o estrógeno é uma hormona sexual feminina, uma consideração de alta prevalência de EM e as mulheres e a relação com o hecho de que as hormonas sexuais são um cabo de seus efeitos Utilizando os receptores específicos, o estudo do estado do papel do gene do receptor de estrógeno em pacientes com EM é a província do Azerbaijão-e-Gharbi e comparando o genérico dos pacientes com os sintomas específicos para o papel desempeñan los genes del estrógeno y sus receptores en la EM. enfermedad.

The present estudio of cases in control control and patients in the development of hospital Imam Khomeini de Orumia (que Allah consagram o suelo de su tumba). O volume da música do estúdio consistiu em dois grupos: um grupo composto por 30 mulheres e o grupo composto por 30 mulheres que tinham sido submetidas a todos os grupos. Se tomaron a sangre de sangre, 2cc, de todos os pacientes, despiste-se do extra para o ADN dos pacientes e o receptor genérico do estrogênio, chama-se ESR1, é considerado um exame baseado no RFLP do método. A continuação, a informação é utilizada com o software SPSS, versão 21.

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As frecuencias genotípicas e alélicas do polimorfismo Era rs2234693 (C / T) muestrana importante entre os grupos de pacientes e controle.

Palavras-chave: esclerose múltipla, receptor de estrogênio, polimorfismo, PCR-RFLP

Introduction

MS is a chronic CNS disease that has influenced more than two million individuals worldwide (Hauser, 1994) and it occurs more frequently in women than in men and it is considered as one of the most common reasons giving rise to abnormal nervous disability in adults. Although there is not a clear-cut reason identified for the occurrence of MS, a multifactorial model, incorporating the interactions between the genetic infections, epigenetic infections, nutritional factors, climatic factors as well as an array of the other environmental factors, including infections with Barr Virus, has been designed to make predictions in this regard (Hedström, Alfredsson & Olsson, 2016; Olsson, Barcellos & Alfredsson, 2017; Rhead et al., 2016). In MS, the myelin sheath is firstly degraded and then the cells producing the sheath, i.e.

oligodendrocytes, are damaged (Richardson, 1994). MS usually affects the adults or young adults ranging in age from 20 to 40 years and it has been recognized as the most substantial disease disabling the individuals below 65 years of age (Sundqvist et al., 2014). It is believed that the sex hormones exert a considerable effect on the inflictions with MS. Although the mechanism by which the hormones influence autoimmunity is yet to be clarified, it seems that the female hormones are effective on the antigenic processes and antigen secretion as a result of which the immunity responses are intensified and corroborated (Tanriverdi et al., 2003). The studies performed by Tanriverdi et al indicated that estrogen exerts a remarkable effect on the leukocytes in such a way that they stimulate the immune system while androgens play a curbing role. So, it can be stated that androgens play a protective MS counteracting role, whereas the estrogens set the ground for their growth and this can justify their more prevalence in the female gender (Tanriverdi et al., 2003). Estrogen is one of the two steroid female sex hormones secreted by the ovary. The majority of the pituitary regulations of the ovary take place through the effect of two hormones, named estrogen and progesterone. Estrogen plays a significant role in the embryonic development, the expression of secondary sex traits, fertility cycles and gestation maintenance. Moreover, estrogen regulates the growth and distinction of endometrium cells (Meldrum, 1993). Also, estrogen is influential in the pathological processes of diseases associated with hormones like ovary cancer, breast cancer, intestine cancer and bone cancer (Santen et al., 2008). Estrogen receptors (ER) are a collection of transcription factors activated through attaching to the ligand and they are considered as members of nuclear receptor (NR) families (Kumar & Thompson, 1999; Klings, 2000). Two main groups of estrogen receptors are Era and ERB (Koehler et al., 2005; Herynk & Fuqua, 2004). Era resides on chromosome 6 on 6q25.1 site. The gene is comprised of eight axons and 40 kilobases (Kuiper et al., 1996; Kuiper & Gustafsson, 1997). The present study aims at determining ESR1 polymorphism in patients with multiple sclerosis (MS) and comparing the frequency of ESR1 gene polymorphism in patient and control groups.

Materials and Methods

The present study is a case-control research that was conducted on the MS patients who had referred to Orumia's Imam Khomeini (may Allah consecrate the soil of his tomb) Hospital. The study sample volume was consisted of 30 female patients with multiple sclerosis and 30 healthy female patients. Blood samples, 2cc each, were taken from all the patients; next, knowing the possibility of storing them for three days in refrigerator and then freezer, they were transferred to genetic laboratory of Imam Khomeini (may Allah consecrate the soil of his tomb) Hospital in Ardabil City. In Ardabil's Imam Khomeini (may Allah consecrate the soil of his tomb) Hospital, the samples were subjected to DNA extraction following which the estrogen receptor gene, named ESR1, was examined using RFLP method. Afterwards, the effect of the genotype (homozygous or heterozygous) on the emergence of the disease and the expression of the clinical symptoms of the disease were investigated following which the information was inserted in SPSS software, version 21, to undergo further statistical analyses.

Results

The present study takes advantage of Fisher's exact test to investigate the relationship between ESR1 genotype and the MS occurrence. The study examined 30 patients with MS and 30 healthy individuals.

Spectrophotometry was the method of choice for quantitative study of the extracted DNA so that the DNA concentrations can be ascertained. Then, DNA values were calculated in 260 nm and 280 nm using OD measurements. Then the average DNA absorption rate was read from the spectrophotometer for the extracted nucleic acid specimens (table 1).

Table 1: quantitative investigation of DNA

The results of quantitative examination of DNA	
DNA concentration	234 ng/ml
The 260nm to 280nm ratio	1.78
The 260nm to 280nm ratio	1.51
Absorption on 260 nm wavelength	0.154
Absorption in 280nm wavelength	0.123
Absorption in 230 nm wavelength	0.134

The absorption rate in 260nm/280nm wavelength was over 1.5 that is indicative of the idea that the DNA specimen is almost free of any protein and the 260nm/230nm absorption was also found above unity in which case the amount of carbohydrate in the DNA specimen is very low, nearly zero (table 1). In order to perform a qualitative verification of the condensed DNA, the specimen was subjected to electrophoresis on agarose gel. After the electrophoresis of the specimens on 1% agarose gel, the entire samples were showing single strips and the results were reflective of a very good quality of the extracted DNA.

To investigate the polymorphism, a pair of primer was designed (table 2) and incision by restriction enzymes was conducted after performing PCR, RFLP (table 3).

Table 2: the sequence of the primer pair used in the present study

Gene's name	Polymorphism	Primer sequence	PCR product (bp)	Restriction enzyme	RFLP product
ESR1	rs2234693(T/C)	F: AGG GTT ATG TGG CAA TGA CG R: GTT GCA GCA AAA GGT GTT GC	350bp	PvuII	254+96

Table 3: the site of the incision by the enzymes used in the present study

Incision site	Restriction enzymes
CAG CTG	PvuII

After the selection of the primers, the PCR protocol was optimized for each pair of the primers, meaning that the number of cycles, Ta time and proper MgC12 amount were determined in every PCR protocol for every primer pair (table 4).

Table 4: optimized PCR protocol for each primer pair

PCR stages of primer	Number of cycles	Temperature (°C) and time (s) of preliminary denaturation	Temperature (°C) and time (s) of denaturation	Temperature (°C) and time (s) of connection	Temperature (°C) and time (s) of elongation	Temperature (°C) and time (s) of final elongation	MgC12 amount for every Mastermix (1ml)
rs2234693(C/T)	35	95°C 15 min	94°C 1 min	58°C 1 min	72°C 1 min	72°C 10 min	2.0 mM

PCR was conducted after the optimization of PCR protocol of each pair of primer for 30 case-patients and 30 control subjects. To verify the accuracy of PCR, electrophoresis was carried out on PCR products in 1.5% agarose gel stained using safe stain method following which the agarose gel was observed under UV. After it was made sure that the PCR protocol has been undertaken properly, RFLP was conducted on PCR products to investigate the genotype of every individual for polymorphism. To perform RFLP, PvuII restriction enzyme and its suitable buffer and an appropriate amount of PCR product were utilized. To examine the RFLP result, electrophoresis was conducted on agarose gel. In patients with MS, eight specimens (with a frequency of 26.7%) had homozygous genes, five specimens (with a frequency of 16.6%) had heterozygous genes and 17 specimens (with a frequency of 56.7%) were found healthy. In the healthy individuals' group, no specimen was found having homozygous genes, three specimens (with a frequency of 10%) had heterozygous genes and 27 specimens (with a frequency of 90%) were healthy (table 5).

Table 5: the results of Fisher's exact test for the investigation of the relationship between ESR1 polymorphism and MS disease

Group	Frequency/percent	Homozygote	Heterozygote	Healthy	Total sum
MS patients	frequency	8	5	17	30
	Percentage	26.7%	16.6%	56.7%	100%
Healthy subjects	frequency	0	3	27	30
	Percentage	0	10%	90%	100%
Statistic-value					11.311
P-value					0.002
Test result					The existence of relationship

According to the fact that the amount of P-value obtained for Fisher's exact test is equal to 0.002 and smaller than 0.05, it can be concluded that the relationship between ESR1 gene and MS is confirmed in a 95% confidence level ($F=11.311$, P-value<0.05). Based on the frequency values obtained, it is made clear that the presence of the homozygous gene has a great influence on an individual's being diagnosed with MS (table 5).

Discussions

Multiple sclerosis is a likely autoimmune disease that influences the central nervous system (CNS) and it is more prevalent in women in comparison to men and it is usually seen influencing the individuals in an age range from 20 to 40 years (van Munster & Uitdehaag, 2017). Besides accounting for the MS immunity aspects as well as some other cerebral disorders and damages, sex hormones regulate the intrinsic brain cells like estrogen receptors (Khalaj et al., 2013). Sex hormones have a great deal of effect on the inflictions with MS. Although the autoimmunity mechanisms are not well-identified in MS, the female hormones seem to be effective on the antigenic processes and hormone secretions as a result of which the immunity responses are intensified and strengthened (Tanriverdi et al., 2003).

According to what was mentioned up to the current point, the present study aims at the investigation of the possible effects of estrogen hormone receptor gene (ESR) polymorphism on multiple sclerosis. It appears that the individual differences in the rate of estrogen receptors' expression, or at least part of them, are pertinent to the allelic polymorphism inside the regulatory regions of the estrogen receptor gene (Pineda et al., 2010; Abbasi, Nouri & Azimi, 2012). In a study that was carried out by Kikuchi et al on 79 MS patients and 73 healthy individuals and Pvu11 and ESR polymorphism in Xba1 was investigated based on PCR-RFLP on DNA, the results indicated that Pvu11 polymorphism is possibly associated with MS development and Xba1 polymorphism is correlated with the MS development age (Kikuchi et al., 2002). In another study that was performed by Richard on rats with autoimmune encephalomyelitis based on an experimental method, it was demonstrated that the absence of ESR1 and ESR2 restricts the production of inflammatory IFN and TFN Gama and Alpha cytokines and the reduction of these inflammatory cytokines causes decrease in the diseases stemming from immune system in the brain and spinal cord system, including MS; furthermore, the study results showed that immune system can be regulated through ESR1's use of 17-Beta Estradiol (Jones et al., 2008). In another study by Karine Lelu that was conducted on rats, it was indicated that ESR1 expressed in ordinary T-lymphocytes is the primary intermediary of E2 (17-Beta Estradiol)'s anti-inflammatory action through its being capable of controlling Th1 and Th17 cells in secondary lymphocytic tissues (Lelu et al., 2011). It was declared in the study by Sundermann et al that there are strong evidences indicating the relationship between the genetic diversity of ESR1 gene and the cognitive outputs. The relationship between ESR1 and nervous diseases, like cognitive disorder, has been ascertained in women and it has been known to be a major risk for the diseases leading to the loss of reason or dementia. The case-control studies demonstrate that X and P alleles of Pvu11 and Xba1 polymorphism are prone to risk of MS development (Sundermann, Maki & Bishop, 2010). In a study that was conducted by Christianson et al, it was indicated that low estrogen states, as in menopausal and post-delivery period, cause an increase in the intensity of the disease in the patients with multiple sclerosis. Christianson et al reported that estrogen plays a positive role in reducing the symptoms and pathological changes of MS

(Christianson, Mensah & Shen, 2015). In another study by Jessa Haldane, there are numerous proofs confirming the estrogen's treatment potentials in autoimmune diseases.

The mechanisms of action include immune system and nervous paths and this is reflective of the idea that estrogen can usefully influence inflammation and nervous diseases (Haldane, 2012). While the clinical evidences are suggestive of the advantages of using estrogen for preventing the exacerbation of the disease in women with MS, it is well-proved that estrogen causes an increase in the central nerves' effect on the central nervous system (CNS). Thus, every aspect should be taken into account in using estrogen for treating MS (Christianson, Mensah & Shen, 2015).

In the current research paper, the existence of homozygous mutation was evidenced in eight patients and in none of the healthy individuals. All of the study subjects, both patients and the healthy individuals, had PCR products in 350 nm band but, in regard of PCR-RFLP product, eight patients showed homozygous mutation in TC region that was observed in 254+96nm bands. Five patients showed heterozygous mutation and three of the healthy subjects were also identified with heterozygous mutation. Using the calculations made herein, it can be asserted in a 95% confidence level that 26.7% of the patients had homozygous mutation in TC region but no trace of such a mutation was documented in the healthy subjects. Based on the results, it can be stated in a 95% confidence level that there is a relationship between gene polymorphism and MS. Of course, it is worth mentioning that there is a need for further research on the healthy and patient individuals from various populations and with different genders so that more accurate results could be attained. Also, the gene expression's effect on the damaged tissues should also be taken into consideration in future researches.

Conclusion

The genotypic and allelic frequencies of Era rs2234693(C/T) polymorphism causes a significant difference amongst the patient and control groups.

Suggestions

To obtain more precise results, there is a need for performing studies on a larger number of healthy and patient individuals from various populations with different genders and also the gene expression's effect on the damaged tissues is deemed necessary.

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