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RESEARCH PAPER

Molecular Study of *IL-7R* Gene Polymorphism and their Associations with Male Multiple Sclerosis Patients in Erbil Province.

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A B S T R A C T:

Multiple sclerosis (MS) is an autoimmune disease in which immune cells attacks the body cells mistakenly; it is characterized by chronic inflammation that leads to demyelination and conduction of nerve impulse is affected negatively. The cause of the disease is unknown, but it may be partially under the control of genetics, including interleukin 7 receptor alpha (*IL7Rα*). In this case-control study, 40 relapsing-remitting MS (RRMS) male patients, which fulfills McDonald criteria and 40 healthy controls, with matched sex, were compared depending on the rs6897932 polymorphism within the exon 6 of *IL7Rα* gene by Tetra-amplification refractory mutation system polymerase chain reaction (Tetra-ARMS-PCR) method. The frequency of T allele of *IL7Rα* rs6897932 was considerably higher in male MS patients than healthy control males (31.25 *vs* 17.5%). Genotype distributions of the single nucleotide polymorphism (SNP) rs6897932 deviated from Hardy-Weinberg equilibrium with a p-value of 0.80. Both homozygous (TT) and Heterozygous (CT) were non-significantly positively associated with MS male patients (OR = 6.75, 95%CI = 0.73-62.4, p = 0.059, OR = 1.68, 95%CI = 0.64-4.38, p = 0.28) respectively. The distribution of the rs6897932 polymorphism is not significantly different in our case/control study in the Erbil province.

KEY WORDS: Multiple Sclerosis, Interleukin 7 Receptor, Polymorphism, Relapsing-Remitting Multiple Sclerosis
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1. INTRODUCTION

Multiple sclerosis (MS) is a long-standing autoimmune disease that occurs by the inflammation in the central nervous system (CNS) and results in demyelination and impairment of axons (Valcarcel *et al.*, 2018, Gold *et al.*, 2012). It can be classified as an autoimmune disease that impacts CNS and distinguished by numerous brain and spinal cord lesions (Rosati, 2001, Hafler, 2004, Wilkins, 2018).

The disease is expected to influence approximately two and a half million people in the world (Rosati, 2001). Furthermore, MS is more common in females than in males. Currently, the rate of females with this disease to males is 2–1 (Ascherio and Munger, 2016).

MS etiology is unclear, but several studies have already shown that causative agents of MS is reliant on a strong genetic factor (Akkad *et al.*, 2009). Several different methods have been used to determine the genetic bases of MS, such as genetic linkage, gene expression and candidate

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gene association (Gregory *et al.*, 2007, Rizvi *et al.*, 2020).

Amongst the association study is a viable approach toward recognizing the risk genetic loci of genes as a marker such as SNP. Among these was reported the relationship of many *IL-7* receptor SNPs with MS in various ethnic groups (Sahami-Fard *et al.*, 2020, Zhang *et al.*, 2019). The position of *IL7R* is chromosome 5 short arm 13 (5p13). This gene consists of γ chain (*IL7Rγ*) and α chain (*IL7Rα*) usually recognized as CD132 and CD127 respectively. The SNPs throughout the alpha chain of *IL7R* gene have a big role in the impairment of immune system homeostasis and this prone to MS easily (Zhang *et al.*, 2005, Čierny *et al.*, 2015).

Appropriately, association studies for the variants of *IL7Rα* gene revealed that rs6897932 in the exon 6 might be a causative factor for MS evolving in many Japanese and European communities (Gregory et al., 2007, Weber *et al.*, 2008, Fang *et al.*, 2011).

In the exon number 6 of *IL7Rα* gene there is SNP called rs6897932 induces non-conservative amino acid transition at location 244 in which isoleucine shifted to threonine 244 (Ile → Thr) (ATC / ACC) (Teutsch *et al.*, 2003). This amino acid shift affects the expression product of *IL7Rα* which results in the variation amount of membrane-bound isoform and soluble form. Such modifications are accompanied by the regulation of the IL7 signaling pathway and a direct association MS with this SNP (Gregory et al., 2007).

Before many types of researches have been done, most of them have assessed the relationship of T244I variant, rs6897932 with MS pathology, while other researches couldn't find any connection between them. Studies that recently done have shown that the C allele is slightly more prevalent than the T allele. Also, it has been suggested in region rs6897932 the C allele will lead to a higher threat of MS in some population (Gregory et al., 2007, Weber et al., 2008, Fang et al., 2011). Therefore, the opportunity to have MS will increase by 1.5 and 1.3 folds for the genotypes CC and CT respectively, if we deliberated that genotype TT as a normal risk. Dependent on many types of researches with varied results about the polymorphism rs6897932, it is difficult to know for sure concerning the interaction of this SNP with MS (Čierny et al., 2015).

To arrive at a definite conclusion, we decided to have this study in Erbil province-Iraq to evaluate the importance of the *IL7Rα* gene polymorphisms, rs6897932 in such community and investigate their influence on *IL7Rα* gene expression concerning MS pathogenesis.

2. MATERIALS AND METHODS

2.1. Sample Collection

Forty males with MS patients enrolled in this study who visited department of neurology, Rzgari hospital in Erbil city-Iraq from April to June of 2015, and they were diagnosed according to 2005 revised McDonald criteria. Forty control males who were age-matched with MS patients.

2.2. Molecular Technique Analysis

2.2.1. Genomic DNA Extraction from Human Blood Samples

Human Blood samples were taken from peripheral veins using the five-milliliter syringe. The blood put into K_2EDTA (5.4mg) tube for direct DNA extraction. The Genomic DNA extracted from whole blood samples by using spin column method (AccPrep Genomic DNA extraction Kit-Bioneer, South Korea), depending on the manufacturer's instructions. Then the concentration and purity of genomic DNA extracted from each human blood samples were determined using Nano-Drop ™ (Thermo Scientific, USA) spectrophotometer by recording the concentration ranged (11.05-61.60 ng/µl) and purity (1.69-2.27) for each sample.

2.2.2. Genotyping *IL7Rα* Gene Polymorphism (PCR Amplification)

Genotyping of the SNP rs6897932 C>T that located in exon 6 of *IL7Rα* gene performed by a rapid and cost-effective technique called the Tetra-ARMS-PCR method in Tishk International University. In this polymorphism one sequence-specific forward primers: 5′-AAGAAGGGAAGAGAGCATTGG-3′, and three sequence-specific reverse primers that two of them: 5′-GAAAAAACTCAAAATGCTGATGG-3′ (for C allele) and 5′-AGAAAAAACT CAAAATGCTGATGA-3′ (for T allele) and one reverse were a primer for internal control 5′ TTACTTTGGGGACAGCGTTT-3′ used (Majdinasab *et al.*, 2014).

The total of 25 µl volume of PCR master mix reaction achieved containing 3µl of genomic

DNA, 12.5 µl of Taq DNA Polymerase 2x Master Mix RED (Ampliqon, Danish) and 1µl was added for each of the forward and reverse primers for *IL7Rα* gene. Then the mixture was completed by adding 3.5 µl of nuclease-free water (Čierny et al., 2015).

The program of PCR amplification consisted of an initial denaturation at 94 °C for 5 minutes followed by the next 35 cycles 94 °C for 30 seconds (denaturation), 62 °C for 30 seconds (annealing),72 °C for 30 seconds (elongation) and the final step at 72 °C for 5 minutes to extend all PCR fragments (Majdinasab et al., 2014). After PCR amplification, the PCR DNA amplicons separated on 1.5% agarose gel (after applying 5 V/cm) (Magdeldin, 2012), then the separated bands were stained with ethidium bromide to visualize under UV light (Bio-Rad UV-transilluminator) (Brown, 2016). The 301 bp mean the appearance of *IL-7R* gene polymorphism in exon 6 (Figure 1).

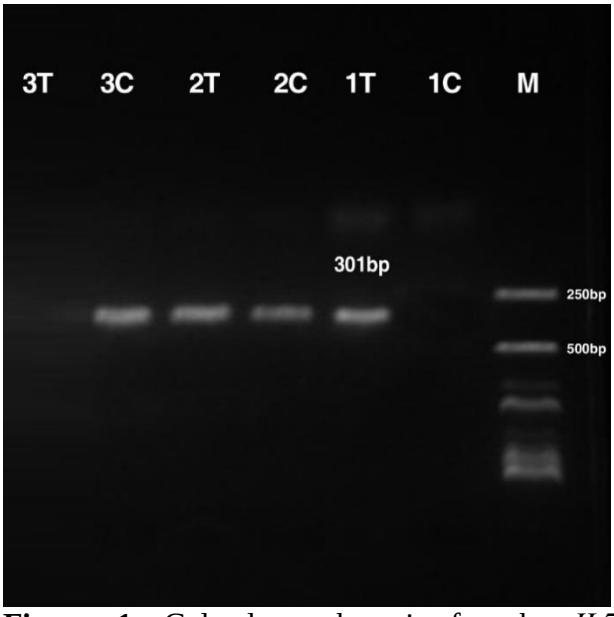

Figure 1. Gel electrophoresis for the *IL7Rα* rs6897932 gene showing the T and C alleles in some MS patients. Band 301bp indicates the presenece of T or C alleles. Sample 1 (lane 1C and 1T) has TT genotypes, sample 2 (lane 2C and 2T) has CT genotype and sample 3 (lane 3C and 3T) has CC genotype, M= 250 bp DNA ladder.

2.3. Statistical Analysis

GraphPad Prism 6 statistical software used for statistical analysis. A student t-test used to compare the ages between MS patients and healthy controls. Genotype and allele frequencies of MS patients and healthy controls were analyzed using the Chi-square ($\chi 2$) test. Both genotype and allelic odds ratio (ORs) and 95% confidence interval (CI) calculated to determine the association of the SNP in exon 6 of the *IL7Rα* receptor gene polymorphisms with MS. A *p-value* of less than 5% ($p < 0.05$) set to be statistically significant.

3. RESULTS

Demographic profiles of both male MS patients and healthy control males had shown in table 1. The mean age of MS patients was 34.85 ± 1.442 years, and of the control group was 32.25 ± 1.375 years; yielding no statistically significant difference in their ages. In this case-control study, 40 MS patients and 40 healthy controls compared depending on the rs6897932 polymorphism within the exon 6 of *IL7Rα* gene by Tetra-ARMS-PCR method. The genotype and allele frequencies of *IL7Rα* rs6897932 are given in (Table 2). The frequency of the T allele of *IL7Rα* rs6897932 was not significantly changed in male MS patients than healthy control males (31.25 *vs* 17.5%). Genotype distributions of the SNP rs6897932 deviated from Hardy-Weinberg equilibrium with a p-value of 0.80. The significance association was not found in both homozygous (TT) and Heterozygous (CT) in MS male patients (OR = 6.75, 95%CI = 0.73-62.4, p = 0.059, OR = 1.68, 95%CI = 0.64-4.38, p = 0.28) respectively (Table 2).

Table 1. Age of MS patients and controls

Variable	Age Range (years)	Mean Age ± SE
MS Patients	20-50	34.85 ± 1.442
Controls	20-50	32.25 ± 1.375

Table 2. Association of MS with carriage of alleles/genotypes of *IL7Rα* rs6897932 SNP

Polymorphism	MS (n=40)		Control (n=40)		OR	95% CI	p-value	HWE
	No.	%	No	%				
CC	20	50.0	27	67.5	1.0	-	-	0.80
CT	15	37.5	12	30.0	1.688	0.64-4.38	0.28	
TT	5	12.5	1	2.5	6.75	0.73-62.4	0.059	
C-Allele	55	68.75	66	82.5	2.143	1.016-4.518	0.0428	
T-Allele	25	31.25	14	17.5				

4. DISCUSSION

Multiple sclerosis (MS) is an autoimmune disease, which is characterized by demyelination of the axon of neurons in the CNS and predominant of inflammation. The etiology of the disease is not well known, but it may be under genetics and environmental factors (Dyment *et al.*, 1997). The genes, which are involved and increased susceptibility to the disease, are divided into two groups: *HLA* genes (Sawcer *et al.*, 2011) and non-*HLA* genes (Rounachi *et al.*, 2009). The most HLA gene which is related to MS is *HLA- DRB1* 1501* class II allele (Alcina *et al.*, 2010), it is located on chromosome 6p21. Besides *HLA* genes there are many genes which are proposed by GWAS that candidates be involved in the development and severity of the MS. Non-*HLA* genes have a role in regulation and modulation of cytokine, cytokine receptor, transcription factor and T lymphocyte receptor (Rounachi et al., 2009). Non-*HLA* genes that are usually involved in MS are *CD 25*, *EVI5*, *CD58*, *CD154* and *IL7Rα*.

Interleukin 7 receptor alpha (*IL7Rα*, also known as *CD127*), is one of the most critical genes which has a role in developing MS. It located on chromosome 5p13.2. It expressed on B and T lymphocytes, it has essential in maturation, developing and survival of them (Manolio *et al.*, 2009). There are many SNP of *IL7Rα* but, the most critical SNP in MS is (rs6897932, p.T244I) which is vital for developing *IL7Rα* in the form of membrane-bound or the way of the soluble

receptor, in this manner it is involved in developing MS (Booth *et al.*, 2005, Zuvich *et al.*, 2009). Since, rs6897932 polymorphism of *IL7Rα* controlling post-transcription modification and alternative splicing the exon 6 of pre-mature mRNA of *IL7Rα* (Booth et al., 2005). The allele of "C" has a role in skipping alternative splicing the exon 6, and developing a soluble form of *IL7Rα* while, The allele of "T" has a role alternative splicing the exon 6 and developing a membrane-bound type of *IL7Rα* (Gregory et al., 2007). The carriers of the "T" allele believed to be a predisposing factor for developing MS because it leads to the formation of the more membrane-bound form of *IL7Rα* and the creation of more auto-reactive T and B lymphocyte (Gregory et al., 2007).

Another explanation for the role of IL7Rα in the pathogenesis of RRMS is that IL7Rα down-regulates the forkhead box P3 (FoxP3) which is, in turn, is the transcription factor for developing CD4+CD25+ regulatory T (T reg) cells, T reg cells is crucial in promoting tolerance and suppress autoreactive T lymphocyte. IL7Rα by decreasing FoxP3 can breakdown tolerance and developing an autoimmune disease, e.g. MS . The results of the current analysis are inconsistent with O'Doherty *et al.* (2008), Alcina *et al.* (2008) and Majdinasab et al. (2014) who found that rs6897932 polymorphism of IL7Rα was related with MS in UK, Spain and Iran, respectively.

CONCLUSIONS

In summary, there is no association between rs6897932 polymorphism of *IL7Rα* and RRMS patients in the Erbil province, by comparing this variant in control and MS groups. This study confirmed that rs6897932 polymorphism of *IL7Rα* is not a predisposing factor for developing the disease in the Erbil province.

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