Med. J. Cairo Univ., Vol. 91, No. 1, March: 33-42, 2023
www.medicaljournalofcairouniversity.net

Association of IL-7Rα Gene Polymorphisms and Serum Interleukin 7 Levels with Multiple Sclerosis and Neuromyelitis Optica in Egyptian Patients

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Abstract

Background: Multiple sclerosis (MS) and neuromyelitis optica (NMO) are autoimmune demyelinating diseases of the CNS. Interleukin-7 (IL-7) and interleukin-7 receptor alpha (IL-7Rα) were suggested to be important in the pathogenesis of both diseases because of the roles they played in the differentiations of autoimmune lymphocytes.

Aim of Study: To investigate the association of IL-7R α (rs6897932) genotypes and serum level of IL7 with MS and NMO in a group of Egyptian patients.

Patients and Methods: A cross-sectional case control study including 90 subjects (40 MS, 20 NMO patients and 30 healthy controls). IL-7R α (rs6897932) genotypes were studied by amplification refractory mutation system-polymerase chain reaction. Serum level of IL7 was measured by sandwich enzyme-linked immunosorbent assay.

Results: This results suggested that CT genotype, combined CT and TT genotypes and T allele increases risk of MS in the sample of Egyptian population studied (OR 5.040, 95% CI 1.293-19.646; OR 5.400, 95% CI 1.395-20.907 and OR 4.75 95% CI 1.316-17.148, respectively). CT genotype, combined CT and TT genotypes and the T allele were found to be significantly higher in MS cases versus controls (*p* 0.02, 0.015 and 0.017, respectively). As for NMO, combined CT and TT genotypes and T allele increases risk of NMO (OR 4.846, 95% CI1.075-21.842 and OR 4.75, 95% CI 1.176-19.18 respectively). Combined CT and TT genotypes and T allele were significantly higher in NMO versus controls (*p* 0.04 and *p* 0.029, respectively). IL7 was significantly lower in the MS patients during the attacks than in MS patients in between attacks (*p* 0.030).

Conclusion: T allele of IL-7R α (rs6897932) could be considered as susceptibility marker of MS and NMO in Egyptian patients. IL7 serum level might be involved in the pathogenesis of MS and NMO but assessment of its level in treatment of naïve patients is essential to prove it.

Key Words: *Multiple sclerosis – Neuromyelitis optica – IL7 – IL7R α gene polymorphism – rs6897932.*

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Introduction

MULTIPLE sclerosis (MS) and neuromyelitis optica (NMO) are autoimmune inflammatory demyelinating diseases of the central nervous system causing lifelong neurological disability in young adults **[1]**. Various genetic approaches are proving to be helpful for better understanding of autoimmune neurological disease aetiology, risk, progression, and pathophysiology. Current research is focused on the identification of new risk factors and the extent to which they contribute to pathogenesis of autoimmune neurological diseases **[2]**.

MS is characterized by demyelination of nerve fibres of the brain, spinal cord, and optic nerves, leading to impaired transmission of nerve impulsesin pathways involving vision, sensation, and movement leading to loss of vision, loss of power in limbs or sensory affection. MS is twice as high in females than males, between the age of 20 and 35 years **[3,4]**.

MS is a heterogeneous, multifactorial, immune-mediated disease that is influenced by both genetic and environmental factors **[5]**. Among the environmental factors are associations of Epstein-Barr virus infection, low vitamin D levels, smoking, and adolescent obesity **[6]**. The genes involved with higher susceptibility to MS include HLA genes (particularly HLA-DRB 1* 1501) and non-HLA genes, which have a role in modulation of cytokines, cytokine receptors, transcription factors and T lymphocyte receptors such as CD25, EVI5, CD58, CD154 and interleukin-7 receptor alpha (IL7Rα) **[7]**.

NMO is an autoantibody mediated chronic inflammatory diseases, where serum antibodies against the aquaporin-4 water channel leads to

recurrent attacks of optic neuritis, myelitis and/or brainstem syndromes [8]. It is more common in females and is characterised by longitudinally extensive spinal cord lesions (>3 vertebral segments), and absence of oligoclonal IgG bands [9]. Various HLA and non-HLA genes were being studied in NMO including HL A-DQB 1 *05:02, SNPs in AQP4 gene, single nucleotide polymorphism (SNPs) in cytokines and cytokine receptors (such as IL 12Rα, IL2Rα, IL7, IL7Rα, IL 17 and TNFRSF1A) [10].

IL-7 is widely considered to be a key cytokine controlling the differentiations and immune responses of several T-cells subsets. It is suggested that the function of IL-7 is not only stimulating IL-7Rα to promote the differentiation of Th1, but also involving in the survival and proliferation of pathogenic Th17 cells in experimental MS patients. The IL7/IL-7Rα interaction has essential roles in some processes in the immune system, including T cell survival, memory T cell development, and homeostasis of T and B cells, particularly CD4+T cells [11].

As the IL-7/IL-7R pathway might be a very attractive therapeutic target for inflammatory disorders, the genetic variants in this pathway were proposed to implicate in the pathogenesis of various autoimmune diseases. IL7Rα gene variations were previously found to be associated with type 1 diabetes, rheumatoid arthritis, and asthma [12-15]. Both IL7 and its receptor IL7Rα emerge as candidate genes for MS and NMO susceptibility [1]. Some studies have shown associations between IL7Rα polymorphisms with MS and NMO with varying results in several populations [7,16,17].

The aim of the current study was to identify the association of single nucleotide polymorphism (SNP) of IL7Rα chain gene (rs6897932) with MS and NMO compared to control group, correlating the resultant genotypes with the clinical and laboratory features of both diseases. It also aimed at evaluating serum IL7 in MS and NMO versus control groups correlating the findings with the clinical and laboratory features of both diseases.

Patients and Methods

Study population:

This cross-sectional case-control study involved 90 subjects divided into 3 groups: 40 MS patients (20 in between attacks and 20 during attacks), 20 NMO patients and 30 ethnically, gender and age matched healthy controls with no personal or family history of autoimmune diseases or neurological disorders. MS and NMO patients were recruited from Kasr Al-Ainy Multiple Sclerosis unit (KAMSU), Neurology Department, Cairo University during the period between September 2021 to September 2022.

The study was approved by the Ethics and Scientific Committee of Cairo University (Number 615-2021), in accordance with the ethical guidelines of the Declaration of Helsinki [18]. An informed consent was obtained from all participants prior to enrolment.

Patients diagnosed with MS according to the McDonald criteria [19] and patients diagnosed with NMO according to the revised diagnostic criteria for NMO [20] of both genders were included in the study. Patients with unconfirmed diagnosis of MS or NMO, those suffering from other autoimmune diseases and those who did not sign the consent form were excluded from the study.

All participants were subjected to detailed history taking including co-morbidities, onset, and duration of the disease, full clinical assessment, Extended Disability Status Scale (EDSS) [21] and brain MRI imaging. Laboratory workup included CBC with differential count, anti-MOG antibodies, anti-aquaporin 4 antibodies and CSF examination for oligoclonal bands and IgG index.

Methods:

Six ml blood were collected and divided into Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainers (3ml) for molecular analysis and sterile vacutainers (3ml) for ELISA. Samples were stored at –20ºC until used.

Detection of IL7R α polymorphism (rs6897932) by amplification refractory mutation system (ARMS-PCR):

DNA extraction was performed from EDTA anticoagulated peripheral blood using a QIAamp DNA Blood Mini kit (Qiagen, catalog number: 51304, US and Canada). PCR with allele specific primers (amplification refractory mutation system [ARMS] primers) was used to detect the IL7Rα (rs6897932) polymorphism at exon 6. Four primers were used as follows: forward primer: 5'-AAGAAGGGAAGAGAGCATTGG-3', reverse primer for the C allele: 5'-GAAAAAACT CAAAATGCTGATGG-3', reverse primer for the T allele: 5'-AGAAAAAACTCAAAATGCT-GATGA-3', reverse primer for internalcontrol: 5'-TTACTTTGGGGACAGCGTTT-3'. Each combination of forward and C or T reverse primers contained 301bp fragment and 577bp fragment for internal control [16].

PCR was performed with 5 µl of extracted DNA in two tubes, one for C allele and one for T allele. Each reaction tube contained 12.5 µl Emi Link TM PCR Master Mix (ELK biotechnology, catalog number: EQ004, China), 1 µl of forward primer and reverse for internal control and1.5 µl of either C primer or T primer, 0.5 µl Easy Taq (Taqman) DNA polymerase (Trans, Cat. No. AP111, China) and 3.5 µl double distilled water.

Thermal cycling conditions were as follows: 1 cycle of pre-denaturation at 94 °C for 3 minutes, 35 cycles of denaturation at 94 °C for 30 seconds, 35 cycles of annealing at 62 °C for 30 seconds, 35 cycles of extension at 72 °C for 30 seconds and a final extension cycle at 72 °C for 10 minutes. PCR product viewed by agarose gel 1 % and the distance the DNA has migrated in the gel was judged visually by monitoring the migration of the loading buffer dye and comparing it to A 100bp DNA ladder (Thermo Fisher Scientific, catalog number: DM003-R500, U.S.A) [16]. Examples from our study results of ARMS-PCR for IL7Rα (rs6897932) on gel electrophoresis are illustrated in Fig. (1).

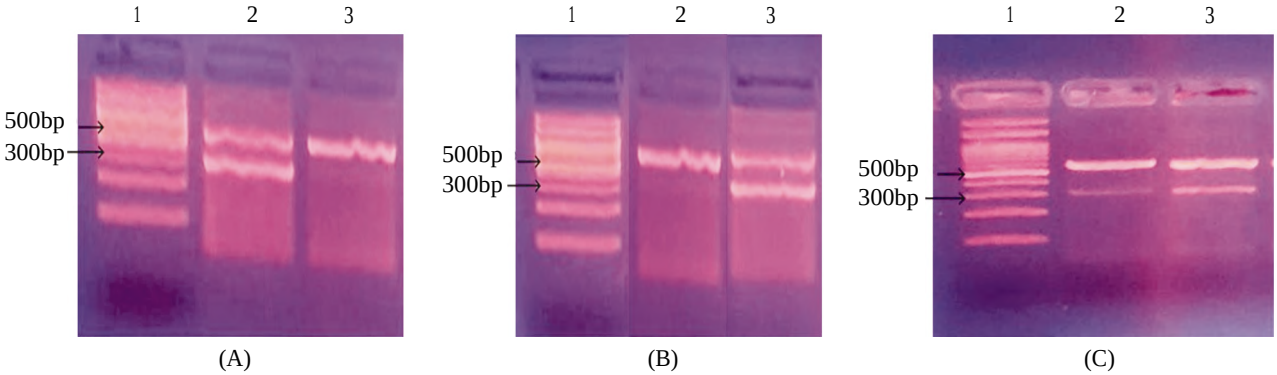

Fig. (1): Examples from our study results of ARMS-PCR for IL7R α (rs6897932) on gel electrophoresis. Lane (1) Shows DNA ladder (100 bp), Lane (2) Shows the result of tube containing C allele primers and Lane (3) Shows the result of tube containing T allele primers. (A): CC homozygous genotype with Lane (2) Showing a band detected at 577 bp (internal control) and a band detected at 301 bp (C allele) and Lane (3) Showing a band detected at 577 bp (internal control) only; (B): TT homozygousgenotype with Lane (2) Showing a band detected at 577 bp (internal control) only and Lane (3) Shows a band detected at 577 bp (internal control) and a band detected at 301 bp (T allele); (C): CT heterozygousgenotype with Lane (2) Showing a band detected at 577 bp (internal control) and a band detected at 301 bp (C allele) and lane (3) showing a band detected at 577 bp (internal control) and a band detected at 301 bp (T allele).

Measurement of serum IL7 levels:

Measurement of serum IL7 was performed by sandwich Enzyme-Linked Immune-Sorbent Assay (ELISA) (ELK Biotechnology, catalog number: ELK4959, China).

Statistical analysis:

Data was coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [22]. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5 [23]. Correlations between quantitative variables were done using Spearman correlation coefficient [24]. Genotype and allele frequencies were compared between the disease and the control groups using logistic regression. Odds ratio (OR) with 95% confidence intervals was calculated. ROC curve was constructed with area under curve analysis performed to detect best cut-off value of IL-7 for detection of MS during attacks. *p*-values less than 0.05 were considered as statistically significant.

Results

Demographic data:

Median age of MS group, NMO group and control group was 35.00 years (range: 15-51), 35.50 years (range: 16-53), and 32.00 years (range: 18-49) respectively (*p* 0.768). Twenty-eight (70%) of patients with MS were females and 12 (30%) were males, 16 (80%) patients of NMO patients were females and 4 (20%) were males versus 16 (53.3%) females and 14 (46.7%) males in the control group (*p* 0.122).

Clinical manifestations, brain MRI findings and laboratory investigations of MS and NMO patients:

Regarding age of onset, disease duration and number of attacks since onset, there was no statistically significant difference between MS and NMO groups, with a median of 31.0 years (range: 15 to 51 years), 2.5 years (range: 1 to 15 years) and 2.0 attacks (range: 1-7) respectively for MS and 32.5 years (range: 15 to 49 years), 2.5 years (range 1-6 years), 2.0 attacks (range: 1-3) respectively in NMO. EDSS was significantly different between MS and NMO patients (p-value <0.001) with a median of 2 (range 1-7) and 3.5 (range 2-8.5) respectively. Table (1) demonstrate scomparis on between clinical manifestations of MS and NMO groups.

MS group showed significantly higher changes in brain MRI than NMO group. Brain MRI showed juxtacortical T2 FLAIR in 23 (57.5%) and 5 (25%) MS and NMO patients respectively, (p 0.017), periventricular T2 FLAIR in 32 (80%) and 15 (25%) MS and NMO patients respectively, (p<0.001) and cerebellar T2 FLAIR 8 (20%) and 0 (0%) MS patients MS and NMO patients respectively, (p 0.043).

Table (1): Comparison between clinical manifestations of MS and NMO patients.

	MS cases (n=40)		NMO Cases (n=20)		p-value
	Count	%	Count	%	
Diminished motor tone	29	72.5	19	95.0	0.04*
Hyperreflexia	22	55.0	8	40.0	0.27
Sensory impairment	16	40.0	14	70.0	0.03*
Optic neuritis	3	7.5	6	30.0	0.04*
Weakness	26	65.0	8	40.0	0.06
Numbness	12	30.0	11	55.0	0.06
Diminution of vision	7	17.5	1	5.0	0.24
Transverse myelitis	0	0.0	5	25.0	0.003*

* Statistically significant (p<0.05=Significant, p<0.001=Highly significant).

Oligoclonal bands in CSF was also significantly higher in MS than NMO, with 37 (92.5%) and 5 (25%) patients respectively (p<0.001). There was no statistically significant difference between MS and NMO regarding CSF IgG index ranging from 0.3-1.7 with a median of 0.90 and from 0.2-1.1 with a median of 0.77, respectively. All NMO patients had positive anti-MOG and aquaporin-4 autoantibodies, while none of the MS patients did. All MS and NMO patients received disease modifying therapies (DMTs), while 25 MS patients (62.5%) and 8 NMO patients (40%) received steroids (p-value 0.09).

Comparison between IL7R α rs6897932 gene polymorphism in MS, NMO and control groups:

There is an overall significant difference between MS, NMO and control groups regarding genotype and allele frequencies which is shown in Table (2) and Fig. (2). Genotype and allele frequencies for MS versus controls are shown in Table (3) while genotype and allele frequencies for NMO versus controls are shown in Table (4).

Table (2): Genotypes and allele frequencies in patients and controls.

	MS cases (n=40)		NMO Cases (n=20)		Control group		p-value
	Count	%	Count	%	Count	%	
IL 7Rα (rs6897932) genotype:							
CC	25	62.5	13	65.0	27	90.0	0.044*
CT	14	35.0	6	30.0	3	10.0	
TT	1	2.5	1	5.0	0	0.0	
IL-7Rα alleles:							
allele C	64	80.0	32	80.0	57	95.0	0.029*
allele T	16	20.0	8	20.0	3	5.0	

* Statistically significant (p<0.05=Significant, p<0.001=Highly significant).

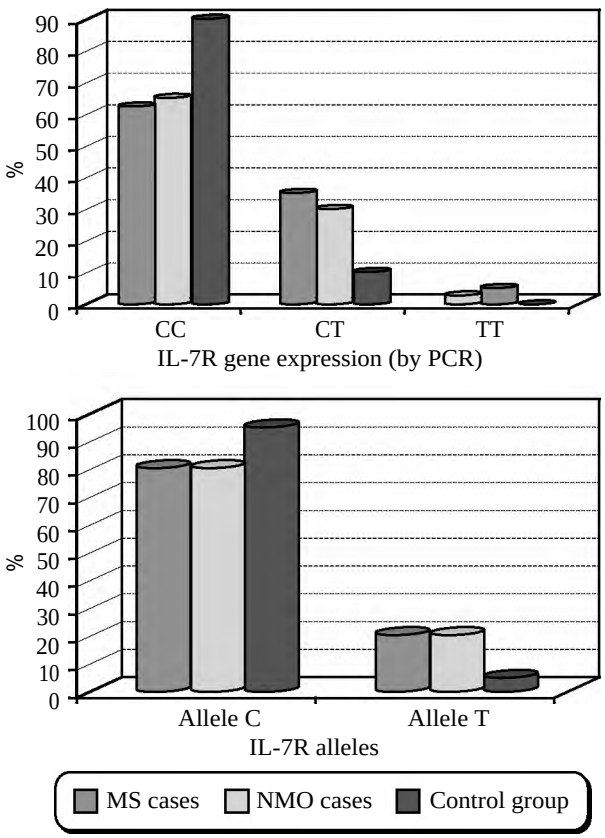

Fig. (2): Frequency of IL7Ra genotypes and alleles.

Table (3): Genotypes and allele frequencies in MS patients and controls.

	MS cases (n=40)		Control group (n=30)		*p*-value	OR	95% CI	
	Count	%	Count	%			Lower	Upper
IL7R α (rs6897932) genotype:								
CC	25	62.5	27	90.0	0.01*		Reference	
CT	14	35.0	3	10.0	0.02*	5.040	1.293	19.646
TT	1	2.5	0	0.0	1		–	
CT+TT	15	37.5	3	10.0	0.01*	5.400	1.395	20.907
IL7R α (rs6897932) alleles:								
allele C	64	80.0	57	95.0	0.01*		Reference	
allele T	16	20.0	3	5.0	0.02*	4.750	1.316	17.148

* Statistically significant (p<0.05=Significant, p<0.001=Highly significant).

Table (4): Genotypes and allele frequency in NMO patients and controls.

	NMO cases (n=20)		Control group (n=30)		*p*-value	OR	95% CI	
	Count	%	Count	%			Lower	Upper
IL7R α (rs6897932) genotype:								
CC	13	65.0	27	90.0	0.03*		Reference	
CT	6	30.0	3	10.0	0.069	4.154	0.894	19.294
TT	1	5.0	0	0.0	1	–	–	–
CT+TT	7	35.0	3	10.0	0.04*	4.846	1.075	21.842
IL7R α (rs6897932) alleles:								
allele C	32	80.0	57	95.0	0.03*		Reference	
allele T	8	20.0	3	5.0	0.03*	4.750	1.176	19.180

* Statistically significant (p<0.05=Significant, p<0.001=Highly significant).

Comparison between clinical manifestations, brain MRI findings and laboratory investigations of MS and NMO patients with IL7R α genotypes:

MS and NMO groups were classified according to the different IL7R α genotypes into 2 distinct groups: The wild (CC) genotype group and the CT + TT genotype group. There was no statistically significant difference between these 2 groups regarding disease course, clinical manifestations, and investigations in both MS and NMO.

Comparison between MS, NMO patients and control group regarding serum IL7 level:

There was no statistically significant difference between the MS, NMO and control groups regarding IL7 levels in serum, with a median of 21.75pg/ml (range: 7.00-1203.00), 22.50pg/ml (range: 0.00-1051.00) and 21.60pg/ml (range: 0.00-733.80) respectively (p 0.978).

There was also no statistically significant difference between IL7 in MS group regarding CC (median 20.20pg/ml, range: 7.00-1203) and CT+TT genotypes (median 22.20pg/ml, range: 11.40-74.60) (p 0.978). Similarly, there was also no statistically significant difference between IL7 in NMO group regarding CC median 19.90pg/ml, range: 0.00-1051) and CT+TT genotypes (median 28.30pg/ml, range: 18.10-64.00 respectively) (p=0.183).

Comparison between MS and NMO patients with serum levels of IL7 as regards their clinical data and investigations:

There was a statistically significant difference between IL7 and diminished motor tone in MS patients (p 0.014). There was also a trend towards significant positive correlation between EDSS and IL7 (r=0.312, p=0.05). Apart from these findings, there was no statistically significant difference or correlation between MS and NMO patients with

serum IL7 regardingdisease course, clinical manifestations, and investigations.

Comparison between MS patients' subgroups:

IL7 level was significantly lower in the MS patients during the attacks (median 18.80pg/ml, range 10.10-73.90) than in MS patients in between attacks (median 26.55pg/ml, range 7.00-1203.00) (*p* 0.030). However, there was no statistically significant difference between the IL7R α genotypes or alleles and the two subgroups of MS (*p* 0.514, 0.264 respectively).

Receiver operating curve (ROC) analysis for MS patients' subgroups and serum level of IL7:

Receiver operator characteristic (ROC) analysis revealed that serum IL7 showed significance as a diagnostic marker for MS subgroups (*p* 0.022), where serum IL7 showed AUC = 0.699 with 95% CI 0.528-0.869. The best cut-off point of serum IL7 was 22.45pg/ml with 75.0% sensitivity and 70% specificity. This is demonstrated in Fig. (3).

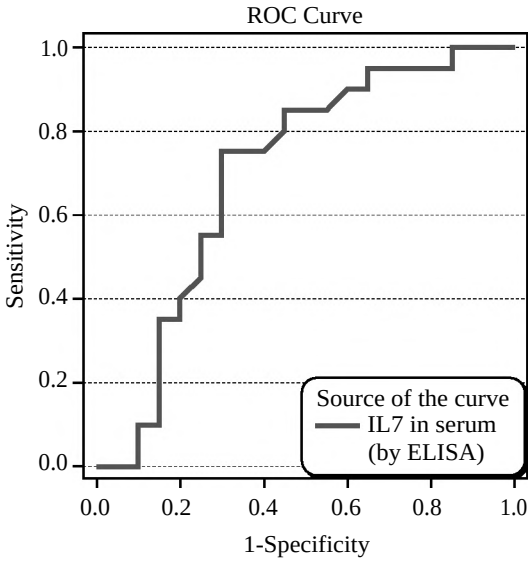

Fig. (3): ROC curve analysis of serum IL7 levels in MS subgroups.

Discussion

Multiple sclerosis (MS) and neuromyelitis optica (NMO) are autoimmune inflammatory demyelinating disorders of the CNS that cause severe neurological disability in young adults. The exact causes of MS and NMO are still unclear, although the diseases are known to result from interplay of genetic susceptibility and environmental risk factors [4].

Our demographic and disease course data for the MS group showed a mean age of onset 30.12 ±9.67, 70% female patients, 30% male patients, median disease duration of 2.5 years (range 1-15) and an EDSS score of 2 (range 1-7). Our results were comparable with results of Elshafie et al. [25] whose study included 31 MS Egyptian patients with a mean age of onset 32.06±5.40 years, 61.3% female patients, 38.7% male patients, median disease duration of 1 year (range 0.20-5) and median EDSS score of 1 (range 1-6).

Our study found an association between rs6897932 polymorphism and MS and NMO patients in Egyptian patients when compared to the control group. Our results suggested that CT genotype, combined CT and TT genotypes and T allele increases risk of MS in the sample of Egyptian population studied (OR 5.040, 95% CI 1.293-19.646: OR 5.400, 95% CI 1.395-20.907 and OR 4.75, 95% CI 1.316-17.148, respectively). CT, combined CT and TT genotypes and the T allele were found to be significantly higher in MS cases versus controls (*p* 0.02, 0.015 and 0.017, respectively).

Some studies have shown associations between IL7Rα polymorphisms and MS with varying results in several populations. According to a recent study of 40 male MS patients in Iraq, the frequency of the C allele and CC genotype of IL7Rα rs6897932 were significantly higher in control group when compared to MS group, which is concordant with our study suggesting the C allele to be the reference allele in both Egyptian and Iraqi population. The frequency of the T allele, CT, and TT genotypes of IL7Rα rs6897932 were non-significantly higher in male MS patients [7], may be owing to the inclusion of only males in their study thus not allowing the results to reach significant values.

Also, in concordance with our results, a study by Zayed et al. [26], conducted on 63 Egyptian MS patients found statistically significant difference regarding IL7Rα (rs6897932) genotype frequency between MS patients and controls, found CC to be the reference genotype and T allele to be a risk for MS. Our study was privileged by studying NMO patients as well and evaluating serum levels of IL7 in all patient groups. Our results were also concordant with Majdinasab et al. [16], who concluded that the T allele and CT genotype were significantly higher in Iranian MS patients.

A meta-analysis study found that the C allele of IL7Rα polymorphism has a significant association with increased MS susceptibility in Europe population and no association was found in Middle East [27]. Another meta-analysis showed similar

results [28]. However, the Middle East countries included in the first meta-analysis were only Iran and Jordan, which does not provide a proper representation of the Middle East and does not include Egypt. Other studies varied in their results, for example, found the C allele to have a significant association with MS risk in Northern Ireland [29], or found no significant association between the rs6897932 and MS susceptibility [17,30].

As for NMO, our results showed that combined CT and TT genotypes and T allele increases risk of NMO (OR 4.846, 95% CI 1.075-21.842 and OR 4.75, 95% CI 1.176-19.18 respectively). Combined CT and TT genotypes and T allele were significantly higher in NMO versus controls (p 0.04 and p 0.029, respectively). A study which was done in Chinese Han population and found that CC genotype and C allele to be significantly higher in control group versus NMO patients, is in line with our findings [1].

There are many SNPs of IL7Rα, but the most critical SNP in MS is rs6897932 which is vital for developing IL7Rα in the form of membrane-bound or in the soluble receptor form, and thus involved in developing MS. rs6897932 polymorphism of IL7Rα controls post-transcription modification and alternative splicing the exon 6 of pre-mature mRNA of IL7Rα. The C allele has a role in skipping alternative splicing the exon 6, and therefore developing the soluble form of IL7Rα while the T allele has a role for alternative splicing the exon 6 and developing a membrane-bound type of IL7Rα [31,32]. The carriers of the T allele are thought to be predisposed for developing MS because it leads to the formation of the more membrane-bound form of IL7Rα and the creation of more autoreactive T and B lymphocyte [27,31].

In the steady state (with low levels of IL7), IL7Rα is slowly internalized and degraded, while large amounts are recycled back to the plasma membrane, thus increasing the membrane bound receptor. On the other hand, in the presence of IL-7, IL7Rα is rapidly endocytosed, the recycling is reduced and most of the receptor is degraded by either lysosome or proteasome, decreasing the amount of membrane bound receptors [32].

In another study, with TT and CT genotypes coded as the reference group, CC genotype was found to be statistically significant in MS cases versus controls [31]. The population studied was of European ethnicity and this explains the difference in the reference alleles. Their results cannot be explained with the mechanism of formation of soluble form of IL7Rα by the C allele.

Another study confirmed increased levels of soluble IL7Rα in individuals with CC homozygous for the at-risk genotype at rs6897932. Furthermore, it demonstrated a dose allele effect with those CC genotype, with a threefold increase in soluble IL7Rα levels over TT genotype, with the heterozygote CT having intermediate levels of soluble IL7Rα. The authors also demonstrated increased IL7 levels in MS patients with the CC homozygous genotype when compared to the other genotypes [33].

Another explanation for the role of IL7Rα in the pathogenesis of MS is that IL7Rα downregulates the forkhead box P3 (FoxP3) which is the transcription factor for developing regulatory T (Treg) cells. Treg cells are vital in promoting tolerance and suppressing autoreactive T lymphocyte. IL7Rα by decreasing FoxP3 can breakdown tolerance and lead to developing MS [7].

When comparing the genotypes of rs6897932 with disease course of MS (age of onset, duration of disease, EDSS score and number of attacks), we did not find any significant association. In line with our results, Gregory et al. [31], showed absence of association between genotypes at rs6897932 and age at onset of MS and multiple sclerosis severity.

In our study, serum levels of IL7 between MS patients, NMO patients and controls showed no statistically significant difference (medians of 21.75, 22.50 and 21.60pg/ml, respectively, p 0.97). We expected serum IL7 levels to be higher in MS and NMO patients versus controls. T allele was significantly higher in MS cases versus controls. It can be hypothesized that serum IL7 failed to increase in MS cases due to the presence of the T allele, which increases the level of membrane bound IL7Rα which in turn leads to decreased serum levels of IL7. This is supported by a study that attempted to evaluate the impact of IL-7/IL7Rα signalling components and function in MS. It showed that the serum IL-7 level is markedly lower and the expression of membrane bound IL7Rα on NK cells is significantly higher in MS patients versus controls [34]. Another study by Kreft et al. [35], proved that the C allele correlated with high levels soluble IL7Rα, which supports the current hypothesis. They also found that MS patients had a significantly higher ratio of membrane bound to soluble IL7Rα and a significantly lower serum level of IL7 when compared to controls.

We found IL7 to be significantly lower in the MS patients during the attacks than in MS patients in between attacks (p 0.030). This can be explained by increase in lines and doses of treatment of the patients during the attack, leading to further reduction in IL7 levels.

There was no significance between the clinical findings in MS and NMO with IL7 levels except for diminished motor tone in MS. This can be attributed to the fact that all of our patients were under treatment, which affected IL7 levels.

A trend towards significant positive correlation between EDSS score and serum level of IL7 (r 0.312, p 0.05) was seen in our results. This suggests that disease severity is linked to IL7 levels. However, lower levels of IL7 were detected in our study as all our patients are on treatment and this affects the level of IL7. Further studies with newly diagnosed and treatment naïve patients is highly recommended.

To our knowledge, this is the first study to evaluate rs6897932 polymorphisms as well as serum IL7 levels in both MS and NMO patients. Very few papers studied the serum level of IL7 in MS patients, while there areno previous records of IL7 in serum in NMO patients. In addition, very few papers studied the association of rs6897932 with both MS and NMO.

Conclusion:

The current study found an association between rs6897932 polymorphism and MS and NMO patients in Egyptian patients when compared to the control group. CT genotype, combined CT and TT genotypes and T allele were found to increase the risk of MS in the sample of Egyptian population studied. Combined CT and TT genotypes and T allele were found to increase the risk of NMO. Serum IL7 was significantly lower in the MS patients during the attacks than in MS patients in between attacks.

It is recommended to replicate the study with a larger sample size and newly diagnosed, treatment naïve patients for better evaluation of IL7 levels in serum. Larger sample size could allow studying the various subgroups of MS such as relapsing remitting MS and primary progressive MS. It is also recommended to measure the membrane bound and soluble IL7 receptor and correlate their levels with the alleles detected and the level of IL7. Studying other SNPs of IL7Rα affecting MS and NMO can help in clarifying the genetic role in the developing of MS and NMO and achieving better treatment.

References

1- ZHUANG J.C., WU L., QIAN M.Z., CAI P.P., LIU Q.B., ZHAO G.X. and WU Z.Y.: Variants of interleukin-7/interleukin-7 receptor alpha are associated with both neuromyelitis optica and multiple sclerosis among Chinese Han population in southeastern China. Chinese medical journal, 128 (22): 3062-3068, 2015.

2- THOMPSON A.J., BANWELL B.L., BARKHOF F., CARROLL W. M., COETZEE T., COMI G., et al.: Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology, 17 (2): 162-173, 2018.

3- VOSKUHL R.: It is time to conduct phase 3 clinical trials of sex hormones in MS-Yes. Multiple sclerosis Journal, 24 (11): 1413-1415, 2018.

4- FILIPPI M., BAR-OR A., PIEHL F., PREZIOSA P., SOLARI A., VUKUSIC S. and ROCCA M.A.: Multiple sclerosis. Nature reviews Disease Primers, 4 (1): 1-27, 2018.

5- MOHAMMADI M.: Brief note on multiple sclerosis. Journal of Multiple Sclerosis, 8 (1): 1-9, 2021.

6- ALFREDSSON L. and OLSSON T.: Lifestyle and Environmental Factors in Multiple Sclerosis. Cold Spring HarbPerspect Med., Apr. 1; 9(4):a028944. doi: 10.1101/cshperspect.a028944, 2019.

7- SMAIL S., AALI M., QADIR F., PIRDAWID A., SHAREEF A., KARIM A., YASEEN R., MUSTAFA M., JALAL, K. and SALIHI A.: Molecular Study of IL-7R Gene Polymorphism and their Associations with Male Multiple Sclerosis Patients in Erbil Province. Zanco Journal of Pure and Applied Sciences, 33 (1): 21-26, 2021.

8- BORISOW N., MORI M., KUWABARA S., SCHEEL M. and PAUL F.: Diagnosis and treatment of NMO spectrum disorder and MOG-encephalomyelitis. Frontiers in neurology, 9: 888, 2018.

9- JACOB A., MCKEON A., NAKASHIMA I., SATO D.K., ELSONE L., FUJIHARA K. and DE SEZE J.: Current concept of neuromyelitis optica (NMO) and NMO spectrum disorders. Journal of Neurology, Neurosurgery & Psychiatry, 84 (8): 922-930, 2013.

10- GHAFOURI-FARD S., AZIMI T. and TAHERI M.: A Comprehensive Review on the Role of Genetic Factors in Neuromyelitis Optica Spectrum Disorder. Front Immunol., Oct 5 (12): 737673. doi: 10.3389/fimmu.2021.737673, 2021.

11- OMRANINAVA M., MEHRANFAR S., VAHEDI P., RAZI B., IMANI D., ASLANI S. and FEYZINIA S.: Association between IL7 Receptor Alpha (IL7R a) gene rs6897932 polymorphism and the risk of Multiple Sclerosis: A meta-regression and meta-analysis. Multiple sclerosis and related disorders, 48: 102687, 2021.

12- BADOT V., DUREZ P., VAN DEN EYNDE B.J., NZEUSSEU-TOUKAP A., HOUSSIAU F.A. and LAUWERYS B.R.: Rheumatoid arthritis synovial fibroblasts produce a soluble form of the interleukin-7 receptor in response to pro-inflammatory cytokines. J. Cell Mol. Med., 15 (11): 2335-2342. doi:10.1111/j.1582-4934.2010.01228.x150, 2011.

13- MONTI P., BRIGATTI C., KRASMANN M., ZIEGLER A.G. and BONIFACIO E.: Concentration and activity of

the soluble form of the interleukin-7 receptor α in type 1 diabetes identifies an interplay between hyperglycemia and immune function. Diabetes, 62 (7): 2500-2508. doi:10.2337/db12-1726120, 2013.

14- SOCIÉ G. and RITZ J.: Current issues in chronic graft-versus-host disease. Blood, 124 (3): 374-384. doi:10.1182/blood-2014-01-514752, 2014.

15- SINHA S., SINGH J. and JINDAL S.K.: Association of interleukin 7 receptor (rs1494555 and rs6897932) gene polymorphisms with asthma in a north Indian population. Allergy Rhinol., 6 (3): 168-176. doi:10.2500/ar.2015.6.0137115, 2015.

16- MAJDINASAB N., HOSSEINI BEHBAHANI M., GALEHDARI H. and MOHAGHEGH M.: Association of interleukin 7 receptor gene polymorphism rs6897932 with multiple sclerosis patients in Khuzestan. Iranian journal of neurology, 13 (3): 168-171, 2014.

17- VINOY N., SHEEJA N., KUMAR S. and BISWAS L.: Class II HLA (DRB1, & DQB1) alleles and IL7Rα (rs6897932) variants and the risk for Multiple Sclerosis in Kerala, India. Multiple Sclerosis and Related Disorders, 50: 102848, 2021.

18- World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA, Nov. 27; 310 (20): 2191-4. doi: 10.1001/jama.2013.281053, 2013.

19- POLMAN C.H., REINGOLD S.C., BANWELL B., CLANET M., COHEN J.A., FILIPPI M., et al.: Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol., 69 (2): 292-302, 2011.

20- WINGERCHUK D.M., BANWELL B., BENNETT J.L., CABRE P., CARROLL W., CHITNIS T. and JARIUS S.: International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology, 85 (2): 177-189, 2015.

21- KURTZKE J.F.: Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology, 33 (11): 1444-1444, 1983.

22- CHAN Y.H.: Biostatistics 102: Quantitative Data - Parametric & Non-parametric Tests. Singapore Med. J., 44 (8): 391-396, 2003a.

23- CHAN Y.H.: Biostatistics 103: Qualitative Data -Tests of Independence. Singapore Med. J., 44 (10): 498-503, 2003b.

24- CHAN Y.H.: Biostatistics 104: Correlational Analysis. Singapore Med. J., 44 (12): 614-619, 2003c.

25- ELSHAFIE S., ABDEL RASOL H.A., HELMY H. and YOUNAN S.: The expression of IL-7RA in Egyptian patients with multiple sclerosis. Comparative Clinical Pathology, 24 (5): 1223-1230, 2015.

26- ZAYED A.M., SHEHATA A.E., GOUDA T.A.A.R. and ABD ELNOUR H.M.: Association of Vitamin D Receptor, Interleukin 7 Receptor Alpha Gene Polymorphisms With The Risk Of Multiple Sclerosis. Zagazig University Medical Journal, 27 (1): 122-131, 2021.

27- SAHAMI-FARD M.H., MOZHDEH M., IZADPANAH F., KASHANI H.H. and NEZHADI A.: Interleukin 7 receptor T244I polymorphism and the multiple sclerosis susceptibility: A meta-analysis. Journal of Neuroimmunology, 341: 577166, 2020.

28- TAVAKOLPOUR S.: Interleukin 7 receptor polymorphisms and the risk of multiple sclerosis: A meta-analysis. Multiple sclerosis and related disorders, 8: 66-73, 2016.

29- O'DOHERTY C., KANTARCI O. and VANDENBROECK K.: IL7Rα A polymorphisms and susceptibility to multiple sclerosis. New England Journal of Medicine, 358 (7): 753-754, 2008.

30- BENEŠOVÁ Y., VAŠKŮ A. and BIENERTOVÁ – VAŠKŮ J.: Association of interleukin 6, interleukin 7 receptor alpha, and interleukin 12B gene polymorphisms with multiple sclerosis. Acta Neurologica Belgica, 118 (3): 493-501, 2018.

31- GREGORY S.G., SCHMIDT S., SETH P., OKSENBERG J.R., HART J., PROKOP A. and PERICAK-VANCE M.A.: Interleukin 7 receptor α chain (IL7Rα) shows allelic and functional association with multiple sclerosis. Nature Genetics, 39 (9): 1083-1091, 2007.

32- WINER H., RODRIGUES G.O., HIXON J.A., AIELLO F.B., HSU T. C., WACHTER B.T. and DURUM S.K.: IL-7: Comprehensive review. Cytokine, 160: 156049, 2022.

33- KOUSIN-EZEWU O.: Investigation of the role of antagonism of the Interleukin-7 Receptor in the treatment of Multiple Sclerosis in Humans and In Vitro differences between genetically stratified subjects based on Interleukin-7 Receptor Genotype (Doctoral dissertation, University of Cambridge), 2020.

34- SU N., SHI S.X., ZHU X., BORAZANCI A., SHI F.D. and GAN Y.: Interleukin-7 expression and its effect on natural killer cells in patients with multiple sclerosis. Journal of Neuroimmunology, 276 (1-2): 180-186, 2014.

35- KREFT K.L., VERBRAAK E., WIERENGA-WOLF A.F., VAN MEURS M., OOSTRA B.A., LAMAN J.D. and HINTZEN R.Q.: Decreased systemic IL-7 and soluble IL-7Rα in multiple sclerosis patients. Genes and Immunity, 13 (7): 587-592, 2012.

علاقة تعدد الأشكال الجينى لمستقبلات انترلوكين ٧ ومستوى انترلوكن ٧ فى الدم بمرضى التصلب المتعدد ومرضى التهاب النخاع والعصب البصرى المصريين

مقدمة : يعد التصلب المتعدد والتهاب النخاع والعصب البصرى من أمراض المناعة الذاتية المزيلة للميالين فى الجهاز العصبى المركزى. انترلوكين ٧ ومستقبلات انترلوكين ٧ ألفا يمكن أن يكونوا ذو أهمية فى التسبب فى كلا المرضين بسبب الأدوار التى لعبوها فى تمايز الخلايا الليمفاوية المناعية.

هدف العمل : هدفنا هو التحقيق فى تعدد الأشكال الجينى لمستقبلات انترلوكين ٧ ومستوى انترلوكن ٧ فى الدم بمرضى التصلب المتعدد ومرضى التهاب النخاع والعصب البصرى المصريين.

الطريقة : الدراسة تشمل ٩٠ شخصاً (٤٠ مريض تصلب متعدد، ٢٠ مريضاً بمرض التهاب النخاع والعصب البصرى و ٣٠ عنصر تحكم سليم). تمت دراسة تعدد الأشكال الجينى لمستقبلات انترلوكين ٧ rs6897932 عن طريق نظام الطفرة الحرارية التضخمية تفاعل البلمرة المتسلسل. تم قياس مستوى انترلوكن ٧ فى الدم بواسطة مقايسة الممتز المناعى المرتبط بالإنزيم.

النتائج : تشير نتائجنا إلى أن الأنماط الجينية CT والمشتركة لـ CT و TT وT تزيد من خطر الإصابة بالتصلب المتعدد فى عينة السكان المصريين. تم العثور على النمط الجينى CT، والأنماط الجينية CT و TT مجتمعة و alleleT أعلى بشكل ملحوظ فى حالات التصلب المتعدد مقابل مجموعة التحكم (0.02 p و ٠.٠١٥ و ٠.٠١٧، على التوالى). بالنسبة التهاب النخاع والعصب البصرى، تزيد الأنماط الجينية مجتمعة CT و TT و alleleT من خطر الإصابة. كانت الأنماط الجينية مجتمعة CT و TT و alleleT أعلى بشكل ملحوظ فى التهاب النخاع والعصب البصرى مقابل مجموعة التحكم (0.04 p و 0.04 و 0.029 p، على التوالى). كان انترلوكين ٧ أقل بشكل ملحوظ فى مرضى التصلب المتعدد أثناء النوبات مقارنة بمرضى التصلب المتعدد بين النوبات (0.030 p).

الملخص : يمكن اعتبار المريض الموجود عنده T allele لـ (rs6897932) أكثر عرضه لمرض التصلب العصبى المتعدد والتهاب النخاع والعصب البصرى فى المرضى المصريين. قد يكون مستوى انترلوكين ٧ متسبباً فى مرض التصلب العصبى المتعدد والتهاب النخاع والعصب البصرى ولكن تقييم مستواه فى المرضى الذين لم يتلقوا أى علاج أمر ضرورى لإثبات ذلك.