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The Relationship of Gene Expression between TNF and TNF-Like Cytokine 1A Genes in Sample of Multiple Sclerosis Iraqi Patients

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Abstract: Multiple Sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative, heterogenic and multifactorial disease. This study was planned to detect the relationship between TNF and TNF-like Cytokine 1A (TL1A) genes expression in sample of Multiple Sclerosis Iraqi patients. Fifty MS patients (18male and 32 female) with age ranged from (23to54years), and 50 age and gender-matched healthy controls were involved in the study, 0.25ml of blood was collected from all individuals, subjected to Trizol preservation for RNA extraction, consequent, TNF and TL1A genes expression by one step RT-qPCR, the Statistical analysis was done by using program of Statistical Analysis System (SAS).The results of comparison of TL1A gene expression in patients and control groups revealed a substantial elevated in TL1A average folding in patients (1.02±0.36 fold) in opposition to control group (0.052±0.02 fold) with significant differences (P≤0.05), as well as TNFα gene expression in patients and control groups showed an extensive increased in TNFα folding in patients (26.55±2.68fold)in vs. control group (4.29±0.88fold)with significant differences (P≤0.01).While results of correlation between TL1A and TNFα genes expression showed a direct relationship in MS patients and control groups. The current study concluded that the incidence of the disease is more in females than in males, and that the family history of autoimmune diseases is not related to incidence of disease, while the TL1A and TNFα genes expression was high folding when compared patients with control, and this rise folding was a direct positive relationship between both of patients and control.

Key word: TNF, TL1A, gene expression, MS.

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Introduction

Multiple sclerosis (MS), also known as encephalomyelitis disseminata, is the most common demyelinating disease (1) in which the insulating covers of nerve cells in the brain and spinal cord are damaged (2) Cytokines are critically involved throughout the course of MS, from the initial pathogenic T-cell differentiation in the periphery, to the resulting inflammation and tissue damage in the CNS. One major cytokine that has been shown to play a pivotal role in MS is tumor necrosis factor (TNF), it is a pleiotropic cytokine regulating many physiological and pathological functions of both the immune system and the central nervous system (CNS). TNF is a member of the TNF superfamily, which consists of various transmembrane proteins with a homologous TNF domain. TL1A is also belong to a subset of the TNF family of cytokines that co-stimulate T cells, which is also known as a vascular

endothelial growth inhibitor because of its ability to induce endothelial cell apoptosis and inhibit angiogenesis(3) ,It has been shown that different forms of TL1A can have different functions and so that TL1A is important for components of both humeral and cellular immune responses (4), consequently it can effect on the pathogenesis of autoimmune diseases such as MS (5). So, and due to the importance of MS health issue, the current study was conducted on the investigation of the relation of TNF and TL1A genes with Multiple Sclerosis in Iraqi MS patient samples.

Material and methods

Current study was conducted in the laboratories of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies University of Baghdad, A group of 50 MS patients with age ranged from 23 to 54 years, and 50 age and gender-matched healthy controls were enrolled in the study. 0.25 ml of blood was collected from all individuals, put into 0.75 ml Trizol preservation for RNA extraction using RNA purification kit (Promega/USA). Estimation RNA concentration and purity was done according to Mohammed (6) by using Nanodrop (Bioneer/Korea). Subsequent, expression of TNF and TL1A genes detection was done by using One Step RT-qPCR according to Mohammed (7) by using specific primer supplied by (Macrogen/ Korea), depending on NCBI as illustrated in table (1).

Table (1): The TL1A and TNF-α primer sequences

Primer Name	Sequence 5`- 3`	Size bp	Reference
TL1A F	CACCACATACCTGCTTGTCAGC	44	Wenxiu *et al* (8)
TL1A R	TCTCCGTCTGCTCTAAGAGGTG		
TNF-α F	CCCAGGCAGTCAGATCATCTTC	42	Enayati *et al* (9)
TNF-α R	GTTTCAGGAGGCTGGCATGA		
β-Globin-F	ACACAACTGTGTTCACTAGC	40	This study
β-Globin-R	CAACTTCATCCACGTTCACC		

Component of PCR mixture reactions of 10 µl volume including qPCR Master Mix (Promega /USA) are shown in Table (2).

Table (2): Reaction component for PCR reactions

Master-mix components	Volume µl
qPCR Master Mix	5
RT mix	0.25
MgCl2	0.25
Forward primer	0.5
Reverse primer	0.5
Nuclease Free Water	2.5
RNA	1
Total	10

The PCR amplification was done by using RT-qPCR (Molecular System / Australia) according to program which clarified in table (3).

Table (3): Amplification fragments PCR program

Steps	°C	m: s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	40
Denaturation	95	00: 20	
Annealing	60-65 55-65	00: 20	
Extension	72	00: 20	

Results analysis was done by using the program of Statistical Analysis System-SAS (10) to estimate the effect of difference factors in work parameters. T-test was used to significant compare between means and Chi-square test was used to significant compare between percentages.

Results and discussion

The results of distribution of MS patients according to gender revealed that females were more affected with MS 32 (64.00%) than males 18 (36.00%) with highly significant difference (P≤0.01) as shown in table in Table (4).

Table (4): Comparison between patients in gender.

Gender	Patients No. (%)
Male	18 (36%)
Female	32 (64%)
Total	50
P-Value	**0.0001
**** (P≤0.01).**	

Cytokines are one of the most important factors in the regulation of inflammatory immune response, therefore they can effect on the pathogenesis of MS disease. Results of current study found that females were more suspected to MS disease than male, it was sure that female immune system effected by many physiological and biological parameters like period, pregnancy, breast feeding and others, all of these, are change the hormones status and subsequently effected the immune system. X chromosome could influence in expression of genes on it, and could lead to inactivation of a gene that protects against autoimmunity, or overexpression of a susceptibility gene, leading to increased autoimmune disease as well as there are gender-specific differences in some of the miRNAs, with a higher than expected miRNA density on the X chromosome that mediating translational repression and m RNA degradation, that have been reported to be associated with MS (11). Study of Al-Hamadani (12), agreed with current results who reported that females outnumbered males in cases with illness beginning as teens. as well study of Khademi *et al*. (13) came in accordance with current result when observed significant differences detected in sex incidence (78% male against 88 % female), or in illness severity.

The outcome of distribution MS patients according to autoimmune disease family history showed high significant (P≤0.01) increase 39 (78%) in patients' number with no family history of autoimmune disease, in contrast 11(22%) of the patients have a family history of autoimmune diseases as clarified in Table (5).

Table (5): Distribution of MS patients according to autoimmune disease family history

Auto immune disease	No	Percentage (%)
Yes	11	22.00 %
No	39	78.00 %
Total	50	100%
P-value	---	0.0001 **
**** (P≤0.01).**		

The result of distribution MS patients according to autoimmune disease family history showed high significant (P≤0.01) increase in patients' number with no family history of other autoimmune disease the study so in compare with others (14), the probability of autoimmune diseases in patients with MS could be higher with several causes (older patients, longer duration of disease, and also in patients with higher age at time of MS diagnosis). Other studies are needed to confirm results and for the main cause of difference in the prevalence and type of autoimmune diseases and MS varying but it could be due to the similarity of genetic factors, immune pathways, and environmental factors, which support the idea that it seems this area from the world could be differ from other areas (15), Present outcome approved with research of Criswell *et al*.(16),who suggested that there was no sign of familial autoimmunity when used 265 families from the Multiple Autoimmune Disease Genetics Consortium (MADGC) to examine the prevalence of autoimmune diseases (ADs) among relatives of MS families.

The comparison of TL1A gene expression in patients and control groups revealed a substantial elevated in TL1A average folding in patients (1.02 ±0.36 fold) in opposition to control group (0.052 ±0.02 fold) with significant differences (P ≤ 0.05) as explained in table (6) and Figure (1).

Table (6): Comparison between B Globin and TL1A genes expression in MS patients and control groups.

Group	Mean ± SE				
	Beta globin	TL1A	Delta CT	Delta Delta CT	TL1A Folding
Patients	14.51	25.79	11.47	4.15	1.02 ±0.36
Control	13.55	26.61	13.07	5.75	0.052 ±0.02
P-value	--	--	--	--	0.0492
T-test	--	--	--	--	0.752 *
*** (P≤0.05)**					

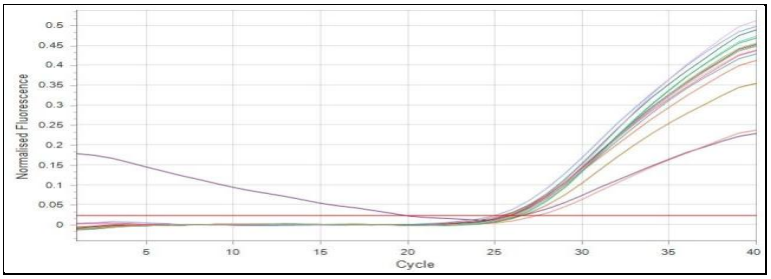

Figure (1): B Globin and TL1A genes expression in patients and control groups

From present results of the comparison of TL1A gene expression in patients and control groups showed a significant elevated in TL1A average folding in patients in against control group, up to available information, there is no previous study for estimation TL1A gene expression from whole blood of MS patients. Elevation TL1A gene expression my become due to several causes such as treatment, patients age , MS duration .Note that TL1A is normally low concentration in WBC with Reads per Kilobase of transcript, per Million mapped reads (RPKM) 0.267, so the results were logically convincing, and matched what was stated in the National Center for Biotechnology Information (NCBI).The TL1A gene functions as suppressed T cells, various cytokines that increase environmental anti-inflammatory activity (17), TL1A conceder as a marker of inflammation it is expressed as membrane bound form in activated T cells, macrophages, monocytes, and dendritic cells, but it is also present in fully active secreted form (18), TNF-like ligand 1A (TL1A) binds with its receptor DR3, and this interaction leads to the activation, proliferation, and production of cytokines by T cells and NK cells, as well as provides the apoptotic signals to lymphocytes (19). A little of previous research dealt with the relationship of the TL1A gene expression with MS disease, including a study of Basnyat *et al* (20), which was compatible with present result and suggested that TL1A found at higher expression among MS patients and among RRMS patients as compared to healthy controls and among patients who were treated with DMTs (Disease Modifying Therapy). Moreover, they reported that TL1A expression was found to be associated with the clinical and MRI findings of MS patients suggesting its possible involvement in the establishment or preservation of immune system homeostasis or in the regulation of inflammatory activity. Immune cells express cytokines at the effect of different factors including hormonal condition, inflammation, infection, and gene polymorphisms of cytokines (21). The increased TL1A expression and/or TL1A gene polymorphisms are associated with the pathogenesis of various autoimmune and inflammatory diseases, thus, TL1A connects innate immune responses to adaptive immune responses and is critically involved in the induction of autoimmune and inflammatory diseases (22), so for these reasons TL1A have direct effect on MS disease.

The comparison of TNFα gene expression in patients and control groups showed an extensive increased in TNFα folding in patients (26.55 ±2.68 fold) in vs. control group (4.29 ±0.88 fold) with significant differences ($P \leq 0.01$) as clarified in table (7) and Figure (2).

Table (7): Comparison between B Globin and TNFα genes expression in MS patients and control groups

Group	Mean ± SE				
	Beta globin	TNFα	Delta CT	Delta Delta CT	TNFα Folding
Patients	15.63	30.51	14.88	-1.65	26.55 ±2.68
Control	13.81	30.33	16.53	0.00	4.29 ±0.88
P-value	--	--	--	--	0.041**
T-test	--	--	--	--	2.14
** (P≤0.01).					

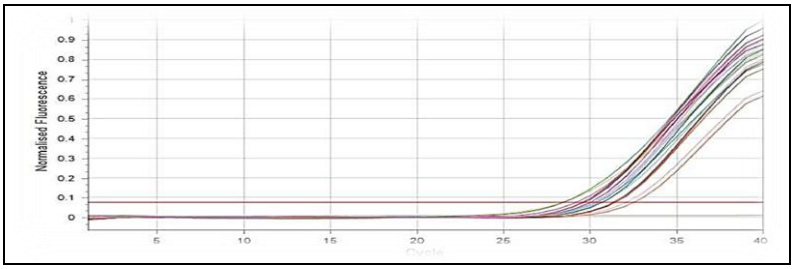

Figure (2): B Globin and TNF α genes expression in patients and control groups.

Also the present results of the comparison of TNFα gene expression in patients and control groups suggested that a high elevated in TNFα expression in patients vs. control group with significant differences. It seems that TNF superfamily with diverse functions in cell like differentiation, inflammation, immunity, and apoptosis. It has primary role of in the regulation of immune cells and its overproduction has been implicated in a variety of human diseases including autoimmune disorders and cancer (23). TNF is primarily secreted from activated macrophages, also by other cell types including monocytes, T-cells, mast cells, NK cells, keratinocytes, melanocytes, fibroblasts, and neurons. Especially neurons cells are involve in MS disease and any disturbances in TNF-a metabolism is associated with several autoimmune and infectious diseases. Current results showed increase in TNF-α gene expression in MS patients than control, this result came in accordance with study of Ahmed et al (24) who found elevated in TNF gene expression in MS patients.

The results of correlation between TL1A and TNFα genes expression in MS patients and control groups showed a direct relationship as illustrated in table (8,27).

Table (8): Correlation between TL1A and TNFα genes expression in MS patients and control groups

Group	Mean ± SE	
	TL1A(Fold)	TNFα (Fold)
Patients	1.02 ±0.36	26.55 ±2.68
Control	0.052 ±0.02	4.29 ±0.88
T-test	0.752 *	2.14
P-value	0.0492	0.041**
* (P≤0.05), ** (P≤0.01).		

Current study found a direct correlation between the TL1A and TNFα genes expression in MS patients, but unfortunately, did not find previous studies to determine the relationship. Thus, present study may be considered

as first study to demonstrate this relationship and comparison the expression of these two genes in MS. The study was correlated TNF-α gene expression with TL1A gene expression because TL1A is a subfamily from TNFα gene, so that as known that TNF gene has affected level TL1A in MS patients. The opinion of Richard *et al*. (3) may be useful to confirm this result who believe similar to the widespread success of TNF-α therapeutic targeting, blocking TL1A signaling may be beneficial in its associated diseases. However, the causal role of TL1A in ongoing disease pathology has not been established, and this remains a key question that needs to be answered if TL1A modulating therapies are to be considered, study of Darweesh *et al* (25) showed that TL1A was particularly elevated in Rheumatoid Arthritis which is also considered auto-immune disease and from disease progression onset and its risk. Moreover, Song *et al* (26) observed that decrease in TL1A level followed TNF-α blocker, believed that TL1A levels may be a useful biomarker of TNF-α activity in Rheumatoid Arthritis.

Conclusion

The current study concluded that the incidence of the disease is more in females than in males, and that the family history of autoimmune diseases is not related to incidence of disease, while the TL1A and TNF-α genes expression was high folding when compared patients with control, and this rise folding was a direct positive relationship between both of patients and control.

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