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Evaluation of IL27 Gene Expression Related with Treatments in Samples of Multiple Sclerosis Iraqi Patients

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Abstract: Background: Multiple sclerosis (MS) is a neurological and demyelinating illness that causes persistent nerves inflammation. The causes of this illness are varied and complex. The goal of the current research was to evaluate Interleukin-27 (IL27) gene expression in a sample of Iraqi patients with MS. **Methods**: Blood samples were collected from 75 Iraqi patents suffered from MS, who taken various treatments as Interferon-ß, Fingolimod, Retuxan and Natalizumab. Also, the study included 75 apparently healthy volunteers as a control group, all subjects with age ranged between (22-58 years). IL27 gene expression was estimated by using Real-time Polymerase Chain Reaction (RT-PCR). The statistical analysis was done by Statistical Analysis System (SAS) to identify the impact of different factors on research parameters **Results**: The results found that the most commonly medication used in treatment MS Iraqi patients was Betaferon 30 (40%) followed by Gilenya 23(32%) with highly statistical significant ($P \leq 0.01$).The results of estimation IL27 gene expression showed noteworthy decrease in patients' group (1.25 ± 0.08 fold) when compared with control group (3.24 ± 0.09 fold) at highly significant difference ($P \leq 0.01$), also the outcome of treatment effect on IL27 gene expression in MS patients revealed that no significant differences between medication types in spite of the patients who taken Betaferon had the highest level of IL27 gene expression (1.40 ± 0.09 fold) among other patients . **Conclusion**: It was concluded that a significant decrease of IL27 gene expression levels in MS patients as compared with control, and these levels were not affected by the different of treatments types.

Keywords: Interleukin-27, Multiple sclerosis, gene expression, Real-time PCR, treatments.

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Introduction

The pathogenesis of MS involves an autoimmune response targeting the myelin sheath, which protective layer surrounding nerve cells (1). The disease's susceptibility is influenced by both hereditary and environmental factors (2). Interleukin-27 is a newly discovered member of the IL12 family, which is recognized for its dual pro- and anti-inflammatory properties. It plays specific roles in modulating the functioning of T-cells. Interleukin-27 is a heterodimeric protein consisting of two subunits, namely Epstein-Barr-induced gene 3 product (EBI3) and IL27p28 (3). The immune response is regulated by IL27, which acts via its receptor IL27Ra/GP130 in a heterodimeric form (4). The presence of IL27 receptors on various cell types, including uterine natural killer (NK) cells, placental trophoblasts, microglia, endothelial cells, and plasma cells, underscores the significant role of IL27 in preserving a harmonious immune state within vulnerable immune environments like the brain and uterus.

Various studies have examined autoimmune disorders, such as MS, and have found evidence suggesting the involvement of IL27 in the immunopathogenesis of these conditions (5). The crucial significance of IL27 in MS has been proven. This cytokine has pleiotropic effects, exerting influence on a range of physiological and pathological processes inside both the immune system and the central nervous system (CNS). A reduction in IL-27 levels is often found in individuals affected by MS (6). Hence and because of the consequence of MS health problem, the present research was carried out to evaluate IL27 gene expression in a sample of Iraqi patients with MS.

Material and methods

The study was conducted in accordance with the guidelines and regulations set out by an ethical committee responsible for overseeing human research. Blood samples were collected from 75 Iraqi patents suffered from MS, who taken various treatments as Interferon-ß, Fingolimod, Retuxan and Natalizumab. Some patients initiated treatment shortly after the onset of their disease, some patients switched treatments over time based on the severity of their disease and the effectiveness of the treatment for their individual conditions. Also, the study included 75 apparently healthy volunteers as a control group, all subjects with age ranged between (22-58 years). Amount 250 µl of blood were obtained from each subject put into 750 µl of Trizol preservation for RNA extraction using RNA purification kit (Promega/USA). Estimation RNA concentration and purity was done according to Mohammed (7) by using Nanodrop (Bioneer/Korea). Expression of IL127 gene was estimated by using a two-step RT-qPCR approach. In the first step, RNA was converted to cDNA utilizing the AddScript Reverse Transcriptase kit (addbio, Korea) according to program are shown in (Table1). Subsequently, in the second step, the RT-qPCR was carried out following the method outlined by Mohammed (8), employing the specific primers provided by Macrogen (Korea) as depicted in (Table 2)., relying on information from NCBI. The PCR amplification was done by using RT-qPCR (Molecular System / Australia) according to program which clarified in (Table 3).

Table (1): PCR program for cDNA conversion

Step	Temp °C
Priming	25 for 10 min
Reverse transcription	50 for 60 min
RT inactivation	80 for 5 min
Hold	12 ∞

Table (2): Primers of IL27 and GADPH genes with their sequences

Primer Name	Sequence 5`- 3`	Reference
IL-27-F	CCCTTTGCTCCCCTGGCGAG	(9)
IL-27-R	AGACTGCAGCAGGTGGCAGC	
GADPH-F	TGCACCACCAACTGCTTAGC	(10)
GADPH-R	GGCATGGACTGTGGTCATGAG	

Table (3): Thermocycler protocol for IL27 gene

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	5min	1
Denaturation	95 °C	20sec	45
Annealing IL27	60 °C	30sec	
Extention	72	20sec	

Statistical analysis

The Statistical Analysis System (SAS) tool was used to identify the impact of various variables on research parameters (11). Furthermore, in order to establish meaningful comparisons between means, the T-test was implemented. Similarly, to determine significant differences between percentages at the 0.05 and 0.01 probability levels, the Chi-square test was employed. Furthermore, the present research used the estimate of the odds ratio as a means to ascertain the risk factor.

Results and discussion

The result of comparison of IL27 gene expression in patients and control groups revealed a substantial decreased in IL27 average folding in patients' group (1.25±0.08-fold) compared to control group (3.24±0.09-fold) with significant differences ($P \leq 0.01$) as elucidated in (Table 4) and (Figures 1,2). The results of treatment effect on IL27 gene expression in MS patients revealed that no significant differences between treatment types related with IL27 gene expression in spite of the (Interferon-ß) drug had the highest level among other drugs groups as illustrated in (Table 5).

Table (4): Comparison between GADPH and *IL27* genes expression in MS patients and control groups

Group	Mean ± SE				
	GADPH	*IL27*	Delta CT	Delta Delta CT	*IL27* Folding
Control	26.18	24.67	-1.51	-1.31	3.24±0.09
Patient	25.71	25.84	0.14	0.34	1.25±0.08
P-value					0.0001**
T-test	--	--	--	--	14.93
** (P≤0.01)					

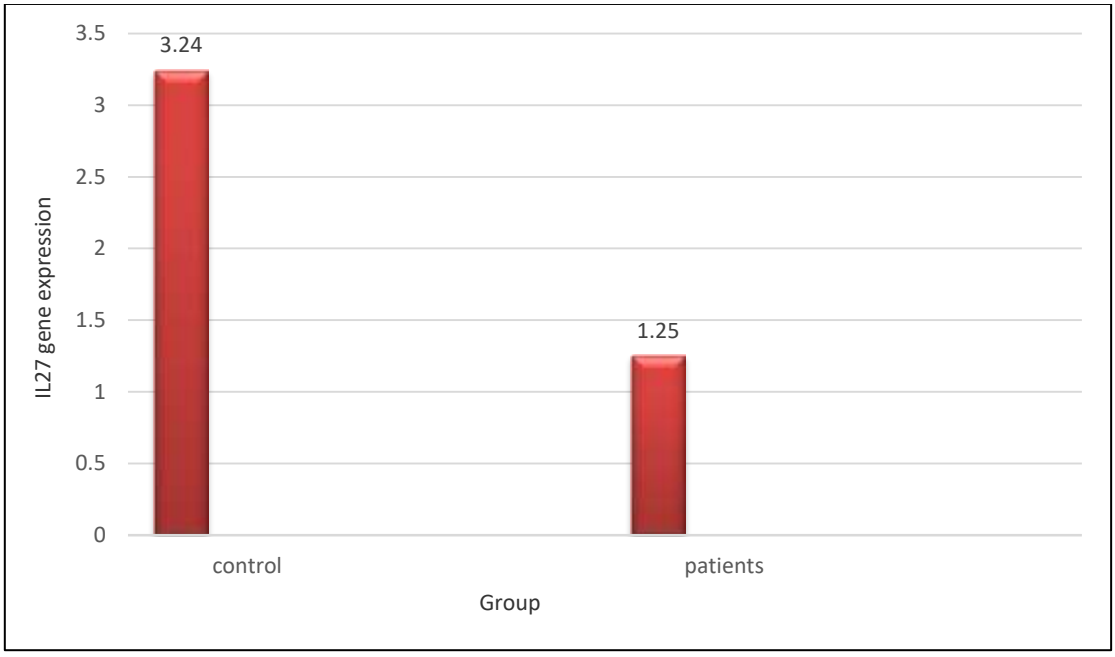

Figure (1): Comparison of IL27 gene expression in patients and control groups

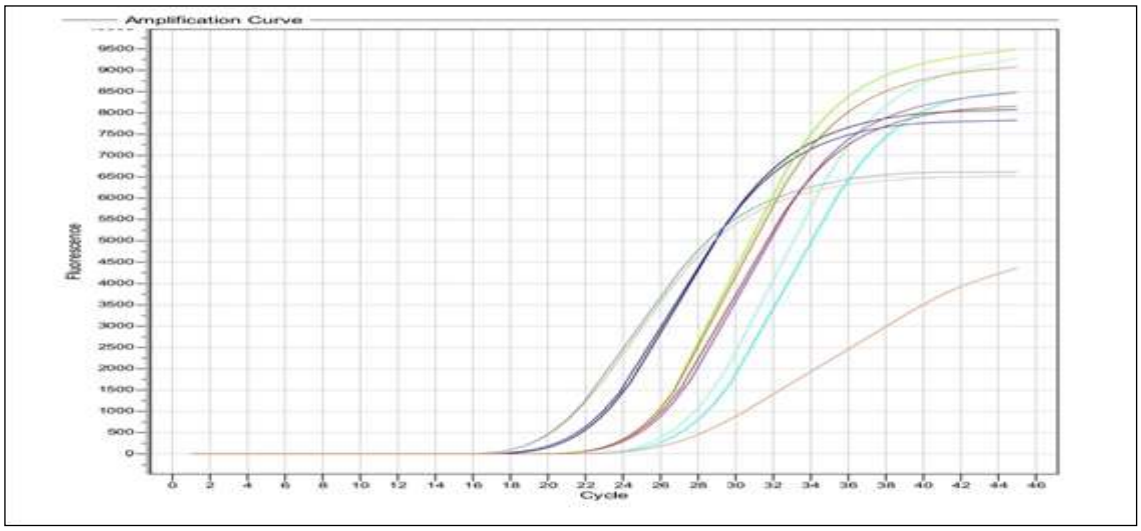

Figure (2): GADPH and *IL27* genes expression in sample MS patients and control groups

Table (5): relationship between treatment and *IL27* genes expression in MS patients

Treatment	NO.	Il27 folding	P-value
Interferon-ß (Beta Feron)	30	1.40± 0.09	0.161 NS
Fingolimod (Gilenya)	24	1.18±0.08	
Retuxan (Rituximab)	15	1.13±0.08	
Natalizumab (Tysabri)	6	1.10±0.07	

Interleukin-27 increases neuronal survival in the CNS in various diseases and injuries. Interleukin-27 antagonizes differentiation and function of type 1 effector cells (Th1), type 2 effector cells (Th2), and IL-17 producing helper T-cells (Th17), inhibits Th9 cell differentiation and function, and regulates B cell responses (12). As a result, a pivotal role is played by IL27 in connecting innate immune responses to adaptive immune responses, and decrease of IL27may be related to a significant amount of inflammation in the CNS and because that the development of multiple autoimmune and inflammatory diseases has been linked to the decrease expression of IL27 (13). The expression of cytokines is exhibited by immune cells under the influence of various factors, including hormonal conditions, inflammation, infections, disease activity, genetic variations in cytokines and therapeutic interventions because some disease-modifying therapies used in the treatment of MS can affect the levels of various cytokines, including IL-27(14). This inflammation is driven by immune cells infiltrating the CNS and releasing pro-inflammatory cytokines. It's possible that this chronic inflammation disrupts the normal production and regulation of IL27. Due to these connections, a direct impact on the progression of MS is made by IL27(15). This outcome is consistent with previous research that demonstrated markedly low levels of IL27 in MS patients during the acute phase of the disease as opposed to those in the stable phase (16). Furthermore, decreased IL27 levels in patients with relapsing-remitting MS (RRMS) were shown in investigations and it was indicated that IL27 is particularly elevated in MS patients with active MRI lesions when compared to those without such lesions (17). The result of not effected IL-27 levels by the different of treatments types that's may due to the fact that treating this disease requires a

long time, and the results may be intangible or a slight improvement may give the patient a chance to continue life, but does not give a chance for complete recovery and the return of IL27 to its normal level. The findings indicated that Interferon-ß medication which commercially known as Betaferon, exhibited the highest percentage 30 (40%) in the treatment of MS patients that involved in current study, followed by Fingolimod that marketed as Gilenya, accounted for 24 (32%) of patients and Retuxan, which known as Rituximab represented at 15 (20%), while Natalizumab which marketed as Tysabri, had the lowest percentage at 6 (8%),with highly statistical significant (P≤0.01), as depicted in (Table 6).

Table (6): Distribution of MS patients according to treatments

Treatment	NO.	Percentage (%)
Interferon-ß (Beta Feron)	30	40%
Fingolimod (Gilenya)	24	32%
Retuxan (Rituximab)	15	20%
Natalizumab (Tysabri)	6	8%
Total	75	100%
P-value		0.0026 **
**** (P≤0.01).**		

Based on the findings derived from the present investigation, it was seen that the pharmaceutical substances enumerated in (Table 6) were used with the highest frequency among the sample population under consideration. Numerous prior studies have corroborated the aforementioned findings, such as the research conducted by Hammood and Mohammed (18). Their study demonstrated that Beta-Feron, Rituximab, and Gilenya exhibited a favorable therapeutic response in comparison to alternative therapies, hence influencing the autoimmunity of patients. The study demonstrated that Interferon-ß (Beta-Feron) exhibited a greater response compared to fingolimod (Gilenya) therapy, with the extent of response being dependent on the length of treatment and the Expanded Disability Status Scale (EDSS) score. Jakimovski *et al*. (19) proposed that Interferon-ß (Beta-Feron) has immune-modulatory and anti-proliferative capabilities, making it the medicine most significantly impacted in MS patients. In their study, Willis and Cohen (20) examined the effects of Fingolimod (Gilenya) on the regulation of sphingosine1-phosphate receptors. They found that this medication slows the egress of lymphocytes from lymph nodes and may possibly have direct effects on the central nervous system. Chisari *et al*. (21) discussed the significance of Retuxan (Rituximab) as an initial oral treatment option for MS. They also highlighted another treatment approach involving a chimeric monoclonal antibody targeting CD20, which has shown efficacy in reducing inflammatory activity among patients with relapsing-remitting MS (RRMS). The most recent findings indicate that Natalizumab (Tysabri) is a treatment option that should not be overlooked. This medication, as supported by Aref *et al* (22), is a highly targeted α4-integrin antagonist specifically designed for severe relapsing-remitting MS cases that have not responded to other treatments. It has been observed that

Natalizumab can have an impact on brain MRI or lead to a significant increase in T2 burden. However, caution should be exercised when considering its use due to the potential risks associated with inadequately treating MS or the progression of the disease. Therefore, it is essential to carefully evaluate the relative benefit-risk profiles of alternative treatment options.

Conclusion

Interleukin-27 gene expression plays critical role in immune responses, subsequently, in the susceptibility to MS. Additionally, a higher risk of MS was observed in patients that have low level of IL27.

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