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G protein coupled membrane receptor expression and IL-17 A in multiple sclerosis

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Identification of molecular biomarkers in blood or cerebrospinal fluid (CSF) is a challenge to preferable observation of disease progression. The objective of study was to detect serum IL-17A in MS patients and detection of G protein-coupled membrane receptor (GPR3) as a prognostic marker of Multiple sclerosis (MS) in correlation with clinical assessment and radiological study. This study was conducted on 87 Egyptian subjects divided into two groups: Group I: control group and Group II: MS patients. Group II was divided into two subgroups (A): MS patients under treatment by interferon beta. (B): MS patients under treatment by soluomedrol. MRI brain and spinal cord, serum IL17A and GPR3 were measured in both groups. It was observed that patients with MS had significant higher serum IL17 A and the very poor responding group has the highest value of IL-17A and the poor sub groups is lower and the lowest value is for the good responding sub groups. In addition the good responding group has the highest value of GPR3 and the poor sub groups is lower in GPR3 and the lowest value is for the very poor sub groups. It was concluded that serum IL-17A is a biomarker for treatment response, GPR3 may indicate a higher inherent disease activity.

Keywords: IL-17A, Multiple sclerosis, GPR3, Interferon beta, Soluomedrol.

INTRODUCTION

Multiple sclerosis (MS) is Known as a complex autoimmune and neurodegenerative disease. The etiology of MS remains largely unknown and therefore the research into the immune pathogenesis of MS is an important area of research. The heterogeneity of MS in terms of pathogenic, immunologic and clinical response to disease modifying therapy (DMT) leads to an intensive search for biomarkers to measure pathological processes that are predictive of MS therapy response (Comabella and Montalban, 2014). (Saher et al. 2010) estimated the prevalence and percentage of MS to other neurological disorders in 10 centers in Egypt. It was found that the percentage of MS patients in relation to the rest of the neurological patients ranged from 0.4% to 1.78%. So the total prevalence was 1.41% in 1000 neurological patients (Saher et al. 2010).The variation in clinical manifestations correlates with the dissemination of lesion sites of pathology within the CNS. These lesions are a hallmark of multiple sclerosis and are caused by immune cell infiltration across the blood-brain barrier (BBB) that promotes inflammation, demyelination, gliosis and neuroaxonal degeneration, leading to disruption of neuronal signaling (Kearney, 2015). Conventional MRI has become the main imaging tool in the MS diagnostic work up as well as in monitoring treatment response to disease-modifying drugs (Polman et al. 2010). Interleukin 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in

various tissues to recruit monocytes and neutrophils to the site of inflammation (Song et al. 2013).The focus on IL-17 as a potential biomarker followed the discovery of IL-17 producing T cells (Th17) as key players in the pathogenesis of MS (Bushnell et al. 2012).
Fredriksson et al. defined GPR3 as members of trans membrane proteins that intermediate the transmission of a loose set of extracellular signals into the intracellular space, and establish signaling mechanisms that control many cellular processes (Fredriksson et al. 2003). GPR3 has a high potential to be a biomarker for predicting future disease activity (Hecker et al. 2011).

2. Aim of the work

This study aimed to detect serum level of IL-17A in MS patients treated with interferon-Beta (IFN-β) and finds out if it plays a role in response to IFN-β as well as detection of G protein-coupled membrane receptor (GPR3) as a prognostic marker of Multiple sclerosis in correlation with clinical assessment and radiological study.

MATERIALS AND METHODS

3.1. Subjects:

This study was conducted on 87 Egyptian subjects divided into two groups: Group I: control group includes 41 normal healthy individuals and Group II: multiple sclerosis patients include 46 patients. Patients were subjected to thorough history taking, detailed neurological examination and clinical assessment of the severity of the disease using Expanded Disability Status Scale (EDSS), MRI brain and spinal cord under ethical conditions and ethical approval from Faculty of Medicine for Girls, Al Azhar University, Cairo, Egypt. Patients in the study groups were recruited from the multiple sclerosis unit, Ain Shams university hospital and Al Zhraa university hospital over the period from January 2014 until July 2015. According to treatment, patients of group II were divided into two sub groups.

Subgroup (A):

Multiple sclerosis patients under treatment by interferon beta:

Twenty three patients (18 females, 5 males) were included. Fifteen of them were treated subcutaneously by interferon beta 1A (Rebif 44 µg) three times a week and 8 of them by interferon beta 1B (Betaferon 250 µg) every other day.

Subgroup (B):

Multiple sclerosis patients under treatment by Soluomedrol 1g per month.

Twenty three patients (14 females, 9 males) treated by Soluomedrol 1g per month.

Inclusion criteria

Patients with multiple sclerosis (RRMS) type (according to revised McDonald's criteria, 2010) from both sexes whose age ranged 20-40 years.

Exclusion criteria

Patients have any other medical conditions that may affect the serum level of IL-17A (such as air way inflammation, rheumatoid arthritis, inflammatory bowel disease, graft rejection and cancer.

3.2. Methods;

All patients were subjected to:

3.2.1. Clinical assessment:

Full history taking, general medical examination and through neurological examination.

3.2.2. Expanded disability status scale:

The degree of disability for all patients was rated according to the EDSS, that provides overall rating of disability based on a (0) (normal neurological examination) to (10) death due to MS.(11).

3.2.3. Magnetic resonance imaging (MRI) brain and spinal cord.

3.2.4. Immunological studies

Venous blood samples from all patients and control groups were collected aseptically from each subject in this study. Each sample was divided into 2 parts: the first part was dispensed in a sterile tube containing EDTA for gene expression of GPR3. The other part was dispensed in a sterile serum separator tube (SST) and serum was separated then stored at −80° C until processing.

3.2.5. Determination of serum level of IL- 17A:

Serum level of IL- 17A was determined by using human IL-17A solid-phase, sandwich-type enzyme-linked immunosorbent assay (ELISA) according to (Strzepa et al. 2011) using Thermo Scientific kit.

3.2.6. Detection of GPR3gene expression by real time PCR.

Briefly Total RNA was extracted from blood samples using QIAamp spin columns which represent a technology for total RNA preparation that combines the selective binding properties of a silica-based membrane with the speed and convenience of microspin technology.

Immediately after blood drawing with the RNEASY Blood RNA system (Quiagen, Dusseldorf, Germany). The mRNA was transcribed into cDNA, using the Omniscript reverse transcription kit (Quiagen, Dusseldorf, Germany).

4. Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage *P*-value <0.05 was considered significant

RESULTS AND DISCUSSION

Characteristics of the study population are shown in table (1).

Table (1): Demographic data for the studied groups

Groups		Control(n=41)	Patients(n=46)	
			Subgroup (A)(n=23)	Subgroup (B)(n=23)
Age(years) Mean ± SD		29.5 ± 6.9	32.5±7.3	32.8±6.0
Sex	Male N%	9 (21.9%)	5 (21.7%)	9 (39.1%)
	Female N%	32 (78.1)	18 (78.3%)	14 (60.9%)

In this study, a patient group has a highly significant serum level of IL-17A than control group, patients treated with interferon beta (subgroup A) was slightly lower than those treated with soluomedrol (subgroup B) as shown in figure (1).

Figure (2) shows that, the very poor responding patients have the highest value of serum level of IL-17A and the poor responding patients are lower and the lowest value is for the good responding patients.

Data obtained from this study revealed that there were a significant positive correlation between serum level of IL-17 A and both EDSS and the number of lesions in MRI in patient group as shown in figure (3, 4) respectively.

Also there was insignificant positive correlation between serum level of IL-17 A and duration of illness figure (5).

In this study, there was no expression of GPR3 in control group, while Patients treated by interferon beta have a slightly higher expression of GPR3 than patients treated by soluomedrol figure (6).

Our data showed that in patients treated by interferon beta (Subgroup A) the good responding patients have the highest value of GPR3 expression and the poor responding patients are lower in GPR3 expression and the very poor responding patients have the lowest value fig. (7).

Figure (8) shows a significant negative correlation between the GPR3 expression and the serum level of IL-17A in the patient group

Discussion

Results showed that patients group has a significantly higher serum level of IL-17A than control group. These results were in agreement with the study done by Babaloo et al. (2015) and Zohreh et al. 2015 who founded a significant increase in the serum levels of IL-17A in MS patients compared to the controls, there was a significant positive correlation of IL-17A serum levels with the number of relapses.

Also these results were in agreement with Rodica et al., (2015) who assumed that the significant increase in the IL17A in MS patients is related to the relapsing remitting phase of MS which results in triggering proinflammatory cells Th1 and Th17 that infiltrate the nervous system which as a result provoking a clinical attack. The percentage of Th17 cells was found to be increased about seven fold in active MS compared to healthy subjects.

Bălaşa et al. indicated that serum level of IL-17A of the patients treated with Soluomedrol are higher than those treated by Interferon beta Balasa et al. (2015).

Rodica et al. (2015) found that a 37.5% were non-responder to beta interferon in patients with RRMS and had significantly higher serum IL17A level when compared to responders group. In addition, a 26.4% were IL17A+ and had a significantly higher number of relapses in previous year and higher EDSS. The majority of IL17A+ patients was non-responders to interferon beta which was in agreement to our results. In addition, it is consistent in part with the results of a study was done by Axtell et al.(2010) who found that, a subset of non-responders to IFNβ had high pretreatment serum levels of IL-17A and

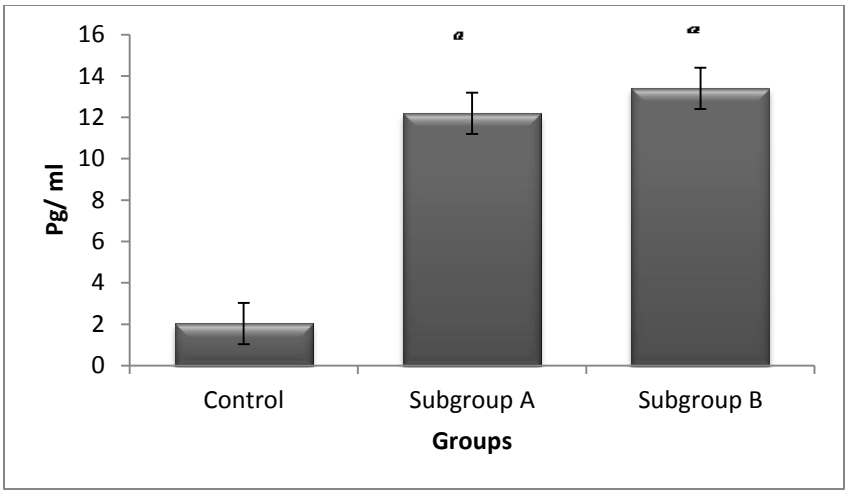

Figure (1): Serum IL-17A in different studied groups.
*a:*significant *p* value compared to the control group.

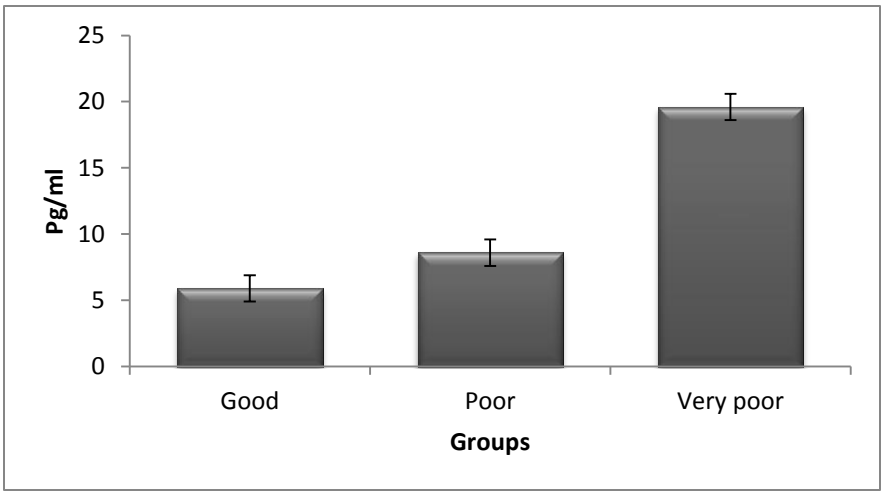

Figure (2): Serum IL-17A in good, poor and very poor responding patients treated by interferon beta (Subgroup A).

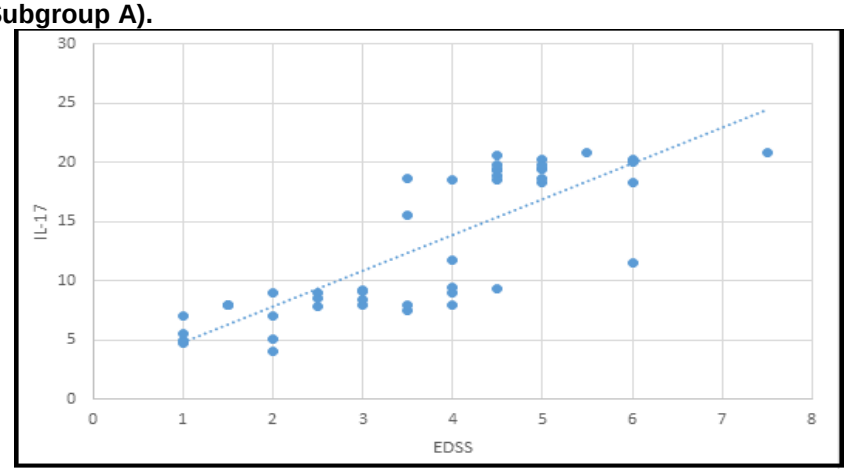

Figure (3): The correlation between EDSS and serum level of IL-17A in patients group. Where *r*=0.816114762 and *p*-value < 0.05*).

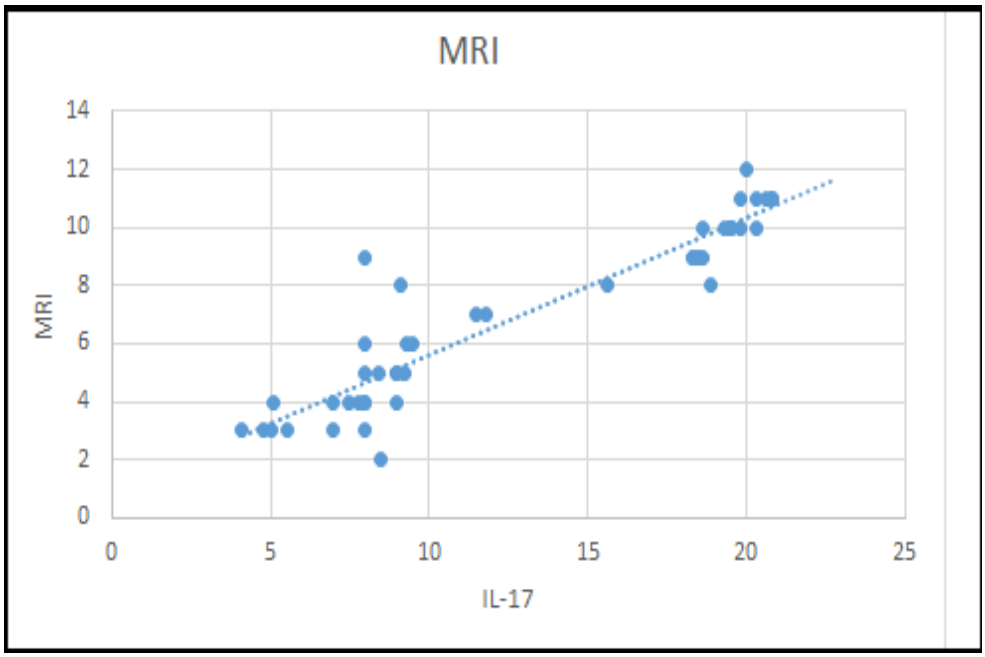

Figure (4): Correlation between number of lesions in MRI and serum level of IL-17A in patients group.
Where *r*=0.927619635 and *p*-value <0.05*.

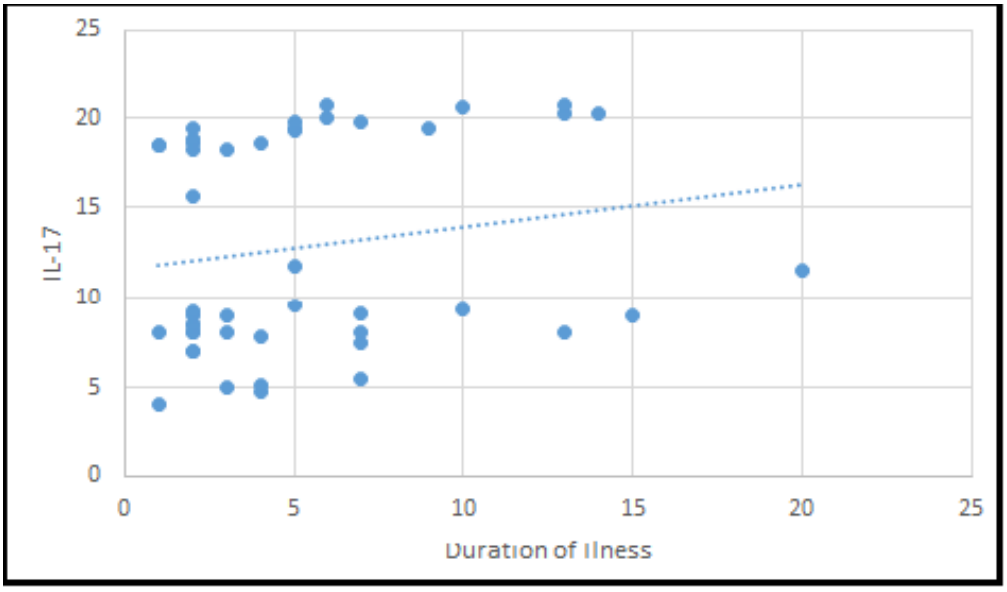

Figure (5): Correlation between the serum level of IL-17A and the duration of disease in patients group.
Where *r*=0.175264466 and *p*-value > 0.05.

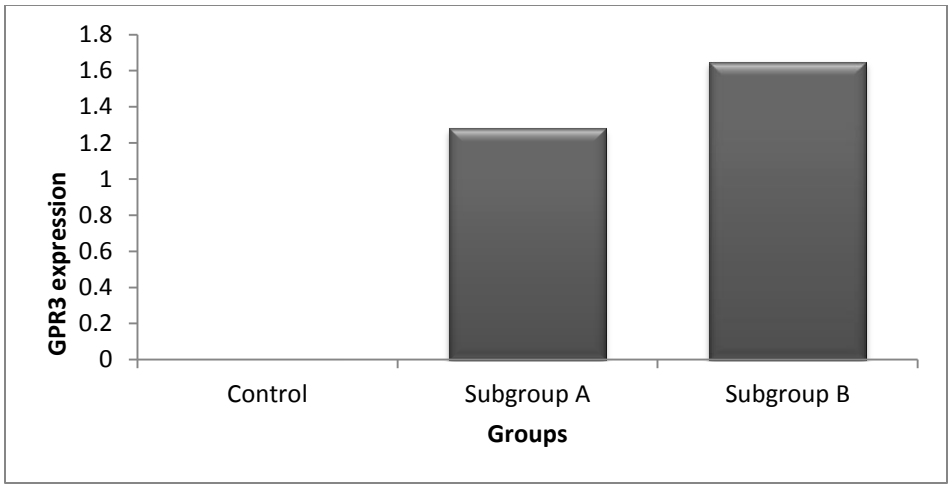

Figure (6): GPR3 expression in control, patients treated by interferon beta (subgroup A) and patients treated by soluomedrol (subgroup B).

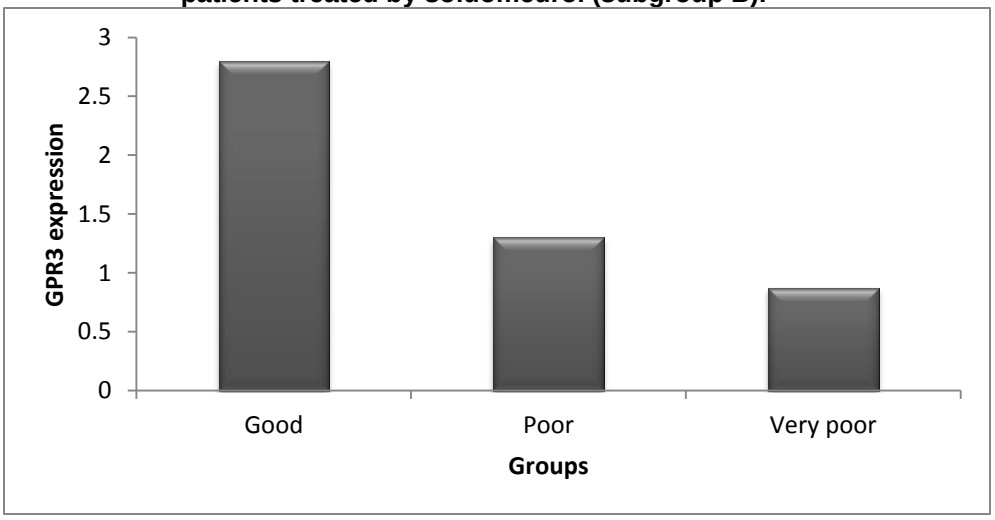

Figure (7): GPR3 expression in good, poor and very poor responding patients treated by interferon beta (Subgroup A).

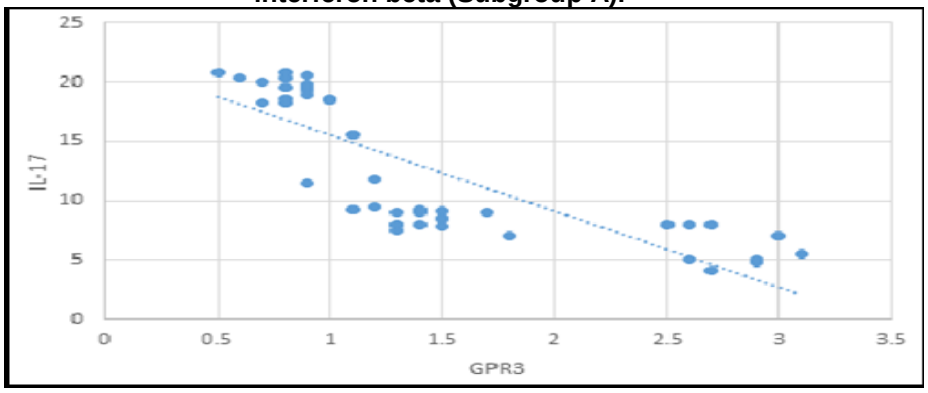

Figure (8): Correlation between GPR3 expression and the serum level of IL-17A in patients group.

endogenous IFNβ, compared to responders which could be explained by the fact that the subset of patients had aggressive Th17 disease, in which the immune system tried to counteract by up regulation of endogenous IFNβ thus, the addition of exogenous IFNβ could not be effective. Interestingly, they reported that in Th17-induced disease, IFN-β treatment still reduced IL-17A production, but without benefit. In addition, IFN-β non-responders showed higher IL-17A concentration in serum compared with responders. A second hypothesis is that IFN-β is a proinflammatory agent during Th17 induced disease. Not only IFN-β treatment be ineffective, but has worsen symptoms. Non-responders had worse disease evolution with more steroid usage and more relapses than did responders.

RRMS patients with elevated level of IL-17A were uunresponsive to the treatment with IFN-β in contrast to patients with decreased level of IL-17A and this response is not influenced by the existence of neutralizing antibodies (Nabs). The novelty of Balasa et al. (2015) based on the fact that they tested patients under IFN-β treatment for 18 months. The reason for choosing this minimum time for treatment is that the immunogenicity of IFN-β expressed by the development of NAbs is settled within first 12-18 months of treatment and their study showed no difference between patients irrespective the type of IFN-β used, while NAbs are much more frequent in patients treated with IFN-β 1b.

In contrast, our results are inconsistent with the study of Hartung et al., who found that serum concentrations of IL-17A alone did not predict response to interferon beta-1b therapy in patients with RRMS Hartung et al. (2013).

Nikolaos et al. (2014) found that serum IL-17A as the main cytokine that featured good responders (GR) from poor responders (PR). GR had significantly higher serum IL-17A levels than PR and significantly higher serum levels of IL-17A, than untreated. GR patients had significantly higher IL-17A/IL-10 and Th17/Th2 ratios and a lower Th1/Th17 ratio of serum cytokines than the PR patients, reflecting their higher serum levels of IL-17A.Significant positive correlation between serum level of IL-17A and the EDSS score in patients sub groups has been observed in our study. Rodica et al. also found significant positive correlation between the level of IL-17A and the aggravation of EDSS score (Rodica et al. 2015).The present results are in agreement with the study of (Bălaşa et al. 2011). They found the RRMS patients with high serum IL-17A levels have higher EDSS. In the present study significant positive correlation between the number of lesions in MRI and EDSS score in patients sub groups and a significant positive correlation in the patients sub groups between IL-17A titer and EDSS which may be explained by the fact that decreased level of serum IL-17A in the periphery may play a critical role in the CNS as IL-17A can disrupt the BBB and let Th17 cells penetrate the BBB. In accordance with our results Durelli et al. found that in patients with prolonged MS (a relapse within ten days) peripheral Th17 cells were significantly increased when compared to patients in the remission phase [18]. In the same line an increase of EDSS can occur due to neurodegeneration of axons and the role of IL-17 is very prominent in the inflammatory phase of MS.

MS is known to be variable because of the variable disease course. MS can respond differently to the same treatment as a result of change in disease duration, disease category, or age of the patient (Dick and Polman, 2009).

Studies have suggested that expression of gene markers in blood may decelerate neurologists to prophesy the long dated evolution of disability and relapses in MS patients.In RRMS patients who have a poor disease course, GPR3 expression is downregulated, and has therefore been privileged for the portentous of MS foretelling (Achiron et al. 2007).

In this study, it was suggested that genes expressed in blood as predictive biomarkers and validated the prognostic value of this gene. However, the GPR3 was expressed at significantly lower levels in patients with poor disease progression in all data sets. GPR3 has therefore a high potential to be a biomarker for predicting future disease activity. In our results patients with low expression of GPR3 were suffered from worse clinical outcomes. Signal of mRNA of GPR3 was minimized in the non-responder than in the responders. These results were in agreement with the study of (Hecker et al. 2011), who indicated that low expression of GPR3 refer to increasing activity of inherent disease, which may be described as a suboptimal therapy response “very poor” group if they showed a rapid impairment of disability with an increase of more than one point in the EDSS within two years after measuring the peripheral blood mononuclear cells (PBMC) gene expression. In addition, patients were described as a “poor” group, which prolonged the “very poor” group with patients who had relapse during the two-year pursue

examination. The “good” groups of patients have no symptoms of relapses and were neurologically relatively settled. In patients ranking it was not important whether they were still treated with IFN-beta after two years. If so, the disease advancement relies on both individual course of the disease and individual benefit of the therapy.

These results were also in concomitant with Gurevich et al. who found that gene expression analysis is a leading method that can be utilized in clinical pursuit to assume MS progression and other autoimmune diseases that is similar to the relapsing-remitting nature (Gurevich et al. 2009)..

CONCLUSION

We concluded that gene expression analysis is a valuable tool that can be used in clinical practice to predict future MS disease activity. Similar approach can be also useful for dealing with other autoimmune diseases that characterized by relapsing-remitting nature

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

HM suggesting the point, designed and reviewed the manuscript. DM performed the practical part and statistical analysis. ESE reviewed the manuscript. ZEK performed the practical part, designed and reviewed the manuscript. MAO collect samples, performed data analysis and wrote the manuscript. All authors read and approved the final version.

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