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Correlation between IL 2, IL21 and IFNAR1 in Patients with Multiple Sclerosis and Healthy Controls

Entedhar Kadhem Hameed[1] and Anwar A. Abdulla[2]*
[1,2]Department of Biology, College of Sciences, Babylon University, Iraq
* Corresponding author: alhussainybio@yahoo.com

ABSTRACT

Regarding the duration of multiple sclerosis and patients divided into two groups ≤ 5 years and > 5 years, the largest number of diseases was (≤ 5years), high significance differences at (P ≤ 0.01). The MS patients were distributed according to EDSS which ranged between (≤ 3) and (> 3) high significance differences at (P ≤ 0.01), the patients were 61 (87.14%). The current study reported that serum level of IL-2 revealed high significant differences at (P ≤ 0.01), (1013.14 ± 391.95 vs. 267.2318 ± 207.76) pg/ml) for patients and controls respectively. Measurement of IFNAR serum level show high significant decrease (791.613 ± 544.066 vs. 1352.995 ± 491.284) pg/ml for patients and controls respectively. While the serum level of IL-21 noted that no significant deference at (P ≤ 0.05) of 70 MS patients (104.851± 268.660 pg/ml) compared with healthy controls of 50 were (68.214± 9.054pg/ml). Regarding, correlation between all study markers WBC types and Interleukins (IL-2, IL-21 and IFNAR) of patients group were explained, positive correlation is observed between Neutrophil and IL21(0.285) at (0.05) level, and positive correlation between Eosinophil and IL-21 and IFNAR (0.241* and 0.270*), respectively. While there was a strong negative relationship between IL-2 and IFNAR which high significant deference correlation (-0.366) at (0.01) level.

KEY WORD: Multiple Sclerosis, IL 2, IL21, IFNAR1

Introduction

Multiple sclerosis (MS) is a progressive complex neurodegenerative disease that affects the central nervous system (CNS). It is caused by autoreactive lymphocytes that cross the blood-brain barrier (BBB) and join the CNS, causing local inflammation that causes demyelination, gliotic scarring, and axonal loss [1,2]. Cytokines are small proteins that enable immune cells to interact with one another and with the tissue in which they are located [3]. In the pathogenesis of MS, IL2 played an important role [4]. The immunomodulatory cytokine IL-21 has pleiotropic effects on both innate and adaptive immune responses. This cytokine promotes lymphoid cell proliferation, increases CD8+ T cell and natural killer cell cytotoxicity, and promotes B cell differentiation into plasma cells [5]. The type I interferon system has been postulated to play a key role in autoimmunity [6]. The aim of this study is to see if there is a correlation between IL 2, IL21, and IFNAR1 in Multiple Sclerosis patients in Iraq.

Material and Methods

Study Subjects

Whole blood samples were taken from 50 healthy people as a control group (21 male and 29 female) and 70 MS patients (44 females and 26 males) from various Iraqi provinces. The patients

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were clinically diagnosed, according to the revised McDonald criteria of 2010 [7]. The age of the case ranges from (14-59) years old.

Analysis of IL 2, IL21 and IFNAR1in sera

Human IL 2, IL21 and IFNAR1 levels were measured in sera of MS patients and controls by the specific kit (ELISA) supplied by Bioassay Technology Laboratory / China.

Biostatistical Analysis

The SPSS statistical package for the Social Sciences was used to analyze the results (version 20.0 for windows, SPSS, Chicago, IL, USA). Pearson correlation analysis was used to examine the degrees of interaction between variables. It was considered meaningful if the two-tailed p-value was less than 0.05 ($p<0.05$) [8].

Results and Discussion

Demographic Study

The present study from table (1) shows 44 (63%) females and 26(37 %) males at patients' group, while healthy controls group included 29(58 %) and 21(42 %) for females and males respectively. Iraqi studies [9, 10] and in United States [11] they reported that more females were represented among male. Due to the female sex hormone (estrogen), which stimulates inflammatory response, and consequently causes autoimmune diseases. Furthermore, women can protect themselves against infection more than men and also produce more CDW lymphocytes and a large number of pro-inflammatory cytokines. [12]. Another significant feature of the gender distribution of multiple sclerosis is that females have a lower frequency of MS patients than males, suggesting that males are more similarly affected [13].

The distribution of the patients according duration and EDSS of the disease as shown in table (1). Regarding the duration of multiple sclerosis and patients divided into two groups ≤ 5 years and > 5 years, with the present result that the largest number of diseases in the first group was identified (≤ 5years), which means high significance differences at ($P \leq 0.01$), the number of cases was 54 (77.14 %) at ($P \leq 0.01$).The MS patients were distributed according to EDSS whichranged between (≤ 3) and (> 3) that mean high significance differences at ($P \leq 0.01$), the number of patients was 61 (87.14%).These patients were distributed according to EDSS which the risk of death ranged from the normal neurological case. Scores from 0 to 3 (≤ 3) mean the patient is completely ambulant, with a score above 3 (>3) for moderately to high disability. As shown in figure (1) marital status was studied for both MS patients and controls. It was found that high significance differences ($P \leq 0.05$) for MS patients and controls where single (20%) and married (80%) P value (0.0001) for MS cases. While marital status for controls single (8%) and married (92%) P value (0.0001) at ($P \leq 0.01$).The educational level majority were uneducated, primary, Secondary and Collage for MS patients and controls. The education of MS patients involved Uneducated (11.4 %), Primary (24.3%), Secondary (22.9%) and Collage (41.4%). It was found that significance differences ($P \leq 0.05$) about these level at P value (0.0049). However, controls high significant differences P value (0.0001) at ($P \leq 0.01$), fig (2). The distribution of the patients according to the age at onset (onset of symptoms) of the disease as shown in fig (3) involved the age at onset groups range from (11-50) years old and they were divided into four groups, involved (11-20), (21-30), (31-40) and (41-50) year.Which found that high significantly frequent cases with multiple sclerosis in the age at onset range from (21-30) year, Mean age ±

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standard deviation of cases was (28.04 ± 2.38) years old, ($P \leq 0.01$). Total mean ± stander deviation of age at onset for MS of 70 patients were 33.285 ± 9.146.The onset age has a unimodal distribution, peaking between 20 and 30 years, and symptoms rarely begin before 10 or after 60 years [14].It typically presents in young adults from 20 -50 years of age, with a peak occurring at 30 years of age, although childhood or older age cases occasionally occur [15]. However, gender distribution of patients with MS seems to be affected by the age of onset, varied geographically, and might be reversed in certain clinical types of MS, considered that the female to male ratio seems to increase with onset prepuberty [16].

Serum Level of Interleukin2,21 and IFNAR

The results in current study reported that serum level of IL-2 revealed high significant differences at ($P \leq 0.01$) between patents and controls, (1013.14 ± 391.95 vs. 267.2318 ± 207.76) pg/ml) for patients and controls respectively. Measurement of IFNAR serum level show high significant decrease (791.613 ± 544.066 vs. 1352.995 ± 491.284) pg/ml for patients and controls respectively, While the serum level of IL-21 noted that no significant deference at ($P \leq 0.05$) of 70 MS patients (104.851± 268.660 pg/ml) compared with healthy controls of 50 were (68.214± 9.054pg/ml), table (4-5).

Cytokines have a complex but crucial role to play in MS, and some cytokines illustrated here have a role that can be both harmful and protective at the same time [17]. Many researchers reported that IL-2 played a significant part in the pathogenesis of MS [18, 19, 20 ,21]. A further important cytokine of the present study is IL-2, which is a kind of cytokine involved in the function and adjustment of immune system, known as pro and anti-inflammatory factor. Which identified as an autocrine immune regulatory cytokine secreted from activated T-cells and has many functions such as regulation of T-cell proliferation, self-tolerance and Treg cell survival.MS patients possess either a lower frequency of Tregs or impairment in their suppressor function, which promotes disease development [22].[23] provided further confirmation of such a position, stating that there was a significant increase in the IL-2 serum level in MS patients compared with controls, and they also indicated because such an increase may lead to inflammation of the CNS, it was therefore suggested that monitoring of IL-2 levels could help diagnose the course of the disease and determine the necessary immunotherapy. IL-2 levels have also been reported to enhance a significant increase in MS patients in both serum and CSF; a finding that indicates its role in pathogenesis of the disease [24 ,25].IL-21 is an immune modulatory cytokine and has multiple effects on innate and adaptive immune responses [26].IL-21 plays important roles in inflammatory, antiviral, and antitumor responses [27]. IL-21 levels of have been observed during the progression of several auto-immune diseases; this implies a pathologic function for this cytokine in autoimmunity was increased [28]. [29, 30] given the therapeutic use of IFN-β in MS, the association of decreased type IFNAR activation in B cells and T cells carrying the MS-protective class I alleles may at first sound counter-intuitive.

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Table 1. Demographic distribution among controls and multiple sclerosis patients

Group	No.	Gender		P value
		Female NO%	Male NO%	
Patients	70	44(62.85) %	26(37.14) %	
Control	50	29 (58) %	21(42) %	
Disease Duration (Years)				
≤ 5 54 (77.14) %		> 5 16 (22.85) %		0.00001 **
Mean ± SD (4.2 ± 3.786)				
EDSS				
EDSS ≤3 61(87.14) %		EDSS>3 9(12.85) %		0.001 **
** means significance differences (P ≤ 0.01).				

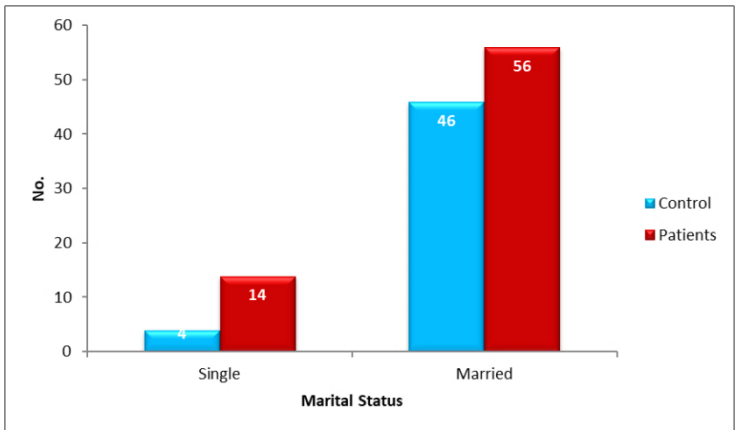

Figure 1. Distribution according martial statues between MS patients and control

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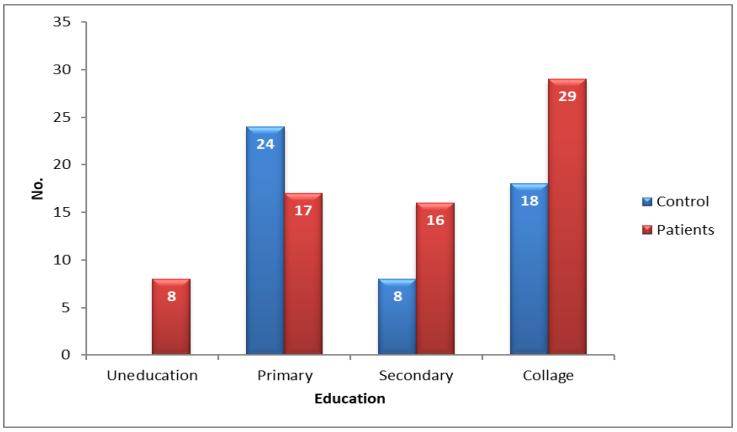

Figure 2. Distribution according education status between MS patients and control

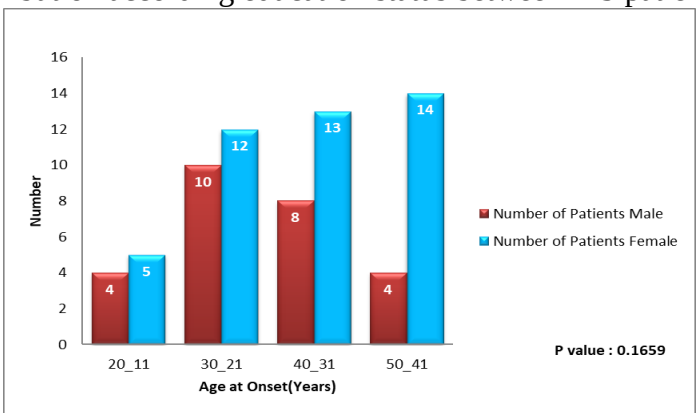

Figure 3. Distribution according age at onset between MS patients and control

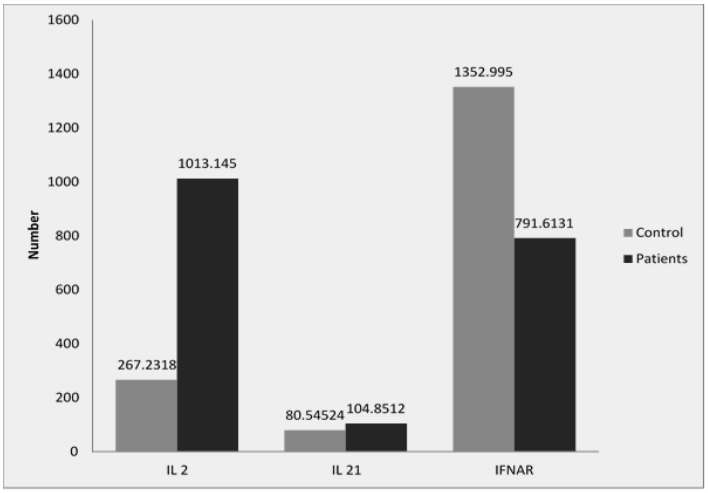

Figure 4. Serum levels mean of IL 2, IL21 and IFNAR1 in multiple sclerosis patients and controls.

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The Pearson correlation analysis of MS patients and controls group

As shown in table (2) correlation between all study markers (WBC types (Monocyte, Lymphocyte, Neutrophil, Basophil and Eosinophil) and Interleukins (IL-2, IL-21 and IFNAR) of patients group were explained, an important highly significant direct correlation is found that there was a strong positivecorrelation between WBC and Neutrophil (0.588) at (0.05) levels. Also, positive correlation is observed between Neutrophil and IL21(0.285) at (0.05) level, and positive correlation between Eosinophil and IL-21 and IFNAR (0.241* and 0.270*), respectively which is significant at (0.05) levels, also shown there was a positive correlation between Basophil and IL-21 were (0.318) at (0.01) levels. While there was a strong negative relationship between IL-2 and IFNAR which high significant deference correlation (-0.366) at (0.01) level.

As shown in table (3) correlation between all study markers WBC types (Monocyte, Lymphocyte, Neutrophil, Basophil and Eosinophil) and Interleukins (IL-2, IL-21 and IFNAR) of controls group were explained. There was a strong positive correlation between WBC and Monocyte, lymphocyte and Neutrophil were (0.375), (0.510) and (0.553) respectively at (0.01) levels. Also, there was a strong positive correlation between Monocyte and Neutrophil, IL-2 and IFNAR were (0.489), (0.425) and (0.361) respectively at (0.01) levels. correlations between Neutrophil and IL-2 (0.423) were noted at 0.01) level. The correlation between Eosinophil and Basophil (0.686) also was strong positive relationship noted at (0.01) level among of the controls group.

In MS patients with an increased Neutrophil and lymphocyte ratio (NLR) the number of neutrophils appears to be higher than in healthy controls [31, 32].[33] shows that the increase in neutrophil counts in RRMS is more likely due to a reduction in apoptosis, and that neutrophils have altered surface molecules expression, likely increasing induction to inflammatory sites.

Table 2. Correlation of immunological parameters in patients

	WBC	**Mon**	**Lym**	**Neu**	**Eo**	**Ba**	**IL 2**	**IL 21**	**IFNAR**
WBC	1	0.15697	0.09553	0.58835**	-0.0565	0.03208	0.05211	-0.11966	-0.04306
Mon	-	1	-0.0258	0.13186	-0.0222	-0.0555	0.06241	-0.0553	-0.0637
Lym	-	-	1	-0.00792	0.038466	-0.00834	-0.1413	-0.02429	-0.12685
Neu	-	-	-	1	-	0.1125	0.0274	0.285341	-

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					0.00777	26	63	*	0.13894
Eo	-	-	-	-	1	0.05732	-0.13868	0.241239*	0.270722*
Ba	-	-	-	-	-	1	0.023156	0.318578**	-0.13167
IL 2	-	-	-	-	-	-	1	-0.13011	-0.36603**
Il 21	-	-	-	-	-	-	-	1	0.13006
IFNAR	-	-	-	-	-	-	-	-	1

Table 3. Correlation of immunological parameters in control

	WBC	**Mon**	**Lym**	**Neu**	**Eo**	**Ba**	**IL 2**	**IL 21**	**IFNAR**
WBC	1	0.375**	0.5105**	0.5539**	-0.1095	-0.2079	0.0353	-0.1009	0.0534
Mon	-	1	0.2612	0.4893**	-0.0486	0.0473	0.4255**	0.0652	0.3612**
Lym	-	-	1	0.27777	-	0.0470	-0.0288	-	0.0776

					0.0449			0.0519	
Neu	-	-	-	1	0.0001	-0.1859	0.4236**	-0.0482	0.2488
Eo	-	-	-	-	1	0.68626**	-0.0708	-0.0815	-0.1171
Ba	-	-	-	-	-	1	-0.11826	0.06023	-0.2687
IL 2	-	-	-	-	-	-	1	0.0444	0.0449
Il 21	-	-	-	-	-	-	-	1	0.0174
IFNAR	-	-	-	-	-	-	-	-	1

Conclusion

The present results showed there was a strong negative relationship between IL-2 and IFNAR which high significant deference correlation (-0.366) at (0.01) level. It's possible that this has a predisposing impact on the development of MS.

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