Original Article

Estimation of Concentration Levels For Both IL-23 and IL-1β in Multiple Sclerosis Patients

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Abstract

Background: Multiple sclerosis (MS) is an inflammatory condition that affects the central nervous system, leading to dysfunction of neural function. Clinically shown with different temporal and pathological patterns and is distinguished by immunity cells. Invasion of the nervous system, removal of the myelin cortex, nerve axis damage, and relapse. Many of the cytokines that played an important role in the pathogenesis of MS were interleukin-1 beta and interleukin-23. **Objectives:** This study aims to assess the concentration levels of interleukin-1 beta and interleukin-23 by measuring them in the serum of MS patients, studying the effect of these variants in the disease, predicting their development, and determining the degree to which these cytokines influence one another. **Materials and methods:** Samples of 50 MS patients were collected after being diagnosed by doctors and 50 healthy people as a control group at Imam Sadiq Hospital in Babylon Governorate between September and December 2024, and the enzyme-related immunoabsorption (ELISA) method was used to measure the concentration level of cytokines in all patients' serum and the control group. To explore their potential role in MS pathogenesis. **Results:** showed that the concentration levels of the interleukin-1 beta and interleukin-23 in MS were higher at the probability level ($P < 0.05$), while the levels of these cytokines decreased in the control group. **Conclusion:** According to the results of the current study, MS patients have a higher concentration level for both interleukin-1 beta and interleukin-23 in their serum compared to the control group.

Keyword: inflammatory, cytokines, ELISA, IL-23, IL-1β

Introduction

Multiple sclerosis (MS) is a neuroimmune disease defined by the breakdown of the myelin sheath that encircles the nerve fibers in the central nervous system as a result of immune cells attacking myelin [1]. This damage leads to permanent inflammation that causes recurrent relapses that affect nerve functions. Multiple sclerosis is one of the inflammatory diseases that affect the brain, cerebellum, brain stem and spinal cord, which is one of the main autoimmune disorders that cause disability at a young age [2]. The disease often appears between the ages of 20 and 40. With a higher prevalence among females compared to males, although it is likely to occur at other ages but at a lower frequency [3]. The disease is caused by a complex interaction between immune and environmental genetic factors, leading to an autoimmune response that attacks myelin and causes devastating neuroinflammation. The exact mechanism of the onset of the disease is still not fully understood [4,5], but studies indicate the role of activated T cells in the onset of the disease. When these cells recognize the myelin antigen, it is presented through antigen-presentation cells (APC) through a toll-like receptor (TLRS), which stimulates T cell activation and fails to achieve self-tolerance. Activated T cells are differentified into subgroup

including Th1 and Th17 cells [6], which produce inflammatory cytokines such as IFN-gamma and other interleukins. These cytokines stimulate other immune cells, such as B and phagocytes, which exacerbate the inflammatory response. These cytokines also affect the endothelial cells of the blood vessels, Increase the expression of adhesion molecules that allow T lymphocytes to infiltrate through the blood-brain barrier (BBB) into the central nervous system [7,8]. Once self-reactive T cells enter the central nervous system, they continue to secrete pro-inflammatory cytokines, increasing the permeability of the blood-brain barrier and causing extensive nerve damage [9], appearing in the form of plaques or sclerosis in nerve fibers. This damage is attributed to the interaction between cellular and mixed immune responses, where different types of immune cells such as B cells and macrophages leak into the central nervous system [10], exacerbating neuroinflammation and the destruction of nerve tissue. Inflammatory-stimulating cytokines play an essential role in the development of multiple sclerosis, most notably interleukin-23 (IL-23) [11], which promotes the survival and activation of Th17 cells, leading to increased production of interleukin-17, interferon-gamma, and interleukin-18, thus promoting neuroinflammation. Interleukin-23 is secreted mainly by dene and monocytes, and is part of the interleukin-12 family, and has a key role in supporting autoimmune responses and promoting inflammation in many autoimmune diseases. When it binds to its own receptor [12], it stimulates 23- to activate Th17 cells and natural killer cells, leading to the release of more cytokines that exacerbate the disease. Studies show that IL-23 promotes the maturation and expansion of Th17 cells, which contributes to the worsening of inflammation and causing greater damage to nerve tissue [13,14]. Multiple sclerosis is also associated with increased levels of interleukin-1 beta , a key cytokine that promotes inflammation by activating the chain of inflammatory reactions [15,16]. IL-18 binds to the latter found on immune cells, especially Th17 cells, which stimulates them to produce IL-17 and IL-22 and increases the expression of IL-23 receptors, amplifying the inflammatory response [17]. Recent research has shown that patients with multiple sclerosis have increased expression of IL-1 receptors on naive cells, Th cells, and memory cells compared to healthy people, indicating an enhanced immune response to this cytokine and its effect on worsening the disease [18]. Interactions between immune cells and inflammatory cytokines exacerbate multiple sclerosis and increase nerve tissue damage. Both IL-23 and IL-1beta play a pivotal role in promoting the inflammatory response, contributing to the development of the disease. Understanding these mechanisms is key to developing treatment strategies that aim to modify the immune response and reduce the severity of the disease

Materials and Methods

Study design and participants

This study was conducted on samples that included fifty patients aged between 14 and 62 years from multiple sclerosis who visit the Department of Neurology at Imam Al-Sadiq Hospital in Babylon Governorate who were diagnosed with the disease by specialized neurologists and fifty healthy people who were examined and outwardly free of diseases as a control group. The samples were divided on the basis of age and sex; the number of male patients was 13 and female patients were 37. Five ml of venous blood was withdrawn [19] and the serums of both groups were examined to

estimate cytokine levels of IL-1 beta and IL-23 by enzyme-linked immunosorbent assay (Abcam Limited), and the data was analyzed using SPSS version 11.5 using the t test.

Ethical approval:

The study was conducted in conformity with ethical guidelines. Before taking the sample, the patients provided verbal and analytical approval. The University of Babylon and Iraq's Ministries of Higher Education and Scientific Research evaluated and approved the study protocol, subject information, and permission form.

Results

According to the results that appeared in the current study and as shown in Table 1, there was no moral difference in the concentration of inflammatory cytokines Interlukin-23 and Interleukin 1-beta between patients and the healthy group about age and sex. Table 2 shows that serum interlukin-23 and interleukin-1 beta were considerably higher in patients with multiple sclerosis than in healthy individuals (all P<0.001). The results of the associations recorded in Table 3 for the serums of multiple sclerosis patients showed the presence of positive moral associations at a probability level (P<0.05) between interleukin-1 beta and interleukin-23, and the link value was 0.838.

Table 1: Comparison of Interleukin-23 and Interleukin -1 Beta Serum Levels in MS Patients and Healthy Controls according to sex and age.

Groups	Factor	Means+sd	P.value
IL-23 (pg/ml) Case	Gender Male (no=13) Female (no=37)	 96.67 ± 578.47 113.48 ± 619.72	0.17
	Age (years) 14-26 (no=19) 26-38 (no=17) 38-50 (no=8) 50-62 (no=6)	 101.92 ± 617.13 105.43 ± 580.19 117.71 ± 130 98.70 ± 98.38	0.23
Control	Gender Male (no=13) Female (no=37)	 24.11 ± 11.21 30.65 ± 11.6	0.24
	Age (years) 14-26 (no=17) 26-38 (no=18) 38-50 (no=8) 50-62 (no=7)	 24.39 ± 9.97 31.43 ± 12.04 29.9 ± 13.25 25.35 ± 9.25	0.17
IL-1beta (pg/ml) Case	Gender Male (no=13) Female (no=37)	 77.03 ± 24.60 64.87 ± 5.635	0.88
	Age (years) 14-26 (no=19) 26-38 (no=17) 38-50 (no=8) 50-62 (no=6)	 78.36 ± 101.75 60.43 ± 1.233 69.77 ± 11.623 67.0 ± 9.363	0.47
Control	Gender Male (no=22) Female (no=28)	 27.06 ± 2.64 25.9 ±2.33	0.29
	Age (years) 14-26 (no=17) 26-38 (no=18) 38-50 (no=8) 50-62 (no=7)	 35 ± 2.82 34.93 ± 3.16 45.56 ± 2.86 45.57 ± 3.23	0.12

Table 2: Comparaison Serum levels of interleukin-23 and interleukin-1 beta for patients and controls.

Cytokines	Case (no.50) Mean ± SD	Control (no.50) Mean ± SD	*P.* value
IL-23 (pg/ml)	158.19 ± 314.06	34.48 ± 744.82	0.001**
IL-1beta (pg/ml)	90.49±660.49	55.75±765.89	0.001**

** The results show statistically significant differences from the control group (p < 0.001).

Table 3: Correlations between interleukin-23 and interleukin-1 beta.

Sample	Links	IL-1beta (pg/ml)
IL-23 (pg/ml)	Correlation	0.838**
	Sig.	0.001

** The results show statistically significant differences from the control group (p < 0.001).

Discussion

The nervous system and the immune system cooperate in the creation and worsening of multiple sclerosis, and neurotransmitters play an

important role in the release of many cytokines, including inflammatory cytokines that exacerbate and develop disease [20]. The mechanism that constitutes the disease and its development is unclear, and recent studies have indicated that the most important cells that form the axis of primates in attacking the nerve-encased layer and aggravating inflammation in the affected area are Th17 cells, these cells and those produced by cytokines that help worsen and damage the nerve tissues to which they migrate. Interleukin-23 plays a crucial role in the differentiation and maturation of Th17 cells, while interleukin-1 beta activates them [21]. Our study no statistically significant difference in immunological criteria within gender patients in the concentrations of interleukin-23 and interleukin-1 beta. On the contrary, our results were inconsistent with previous studies showing a significant male to female difference in the concentration of interleukin-23, as already indicated by [22] showed that women are more susceptible to the disease without males for reasons related to hormones or sexual factors that play a crucial part in balancing the release of cytokines by the cells that produce them [23], Other studies have shown the role of androgens and estradiol, which enhance the function of some immune cells involved in multiple sclerosis [24]. The study of so and so and so and so shows that sex has nothing to do with the disease, and this is what the results of our study agreed with. In terms of the concentration of interleukin-1 beta, there was no moral difference in terms of sex, unlike a study conducted before [25] which showed that females have a high concentration of interleukin-1 beta as a result of sex hormones such as progesterone synthesis and induction of immune responses, including harmful degenerative inflammatory responses in connection with disease progress [26] In our study, no other studies related to the concentration of interleukin-1 beta in terms of sex were shown or consistent, which may be the result of multiple factors related to sample size, which included a small number of participants. Regarding age, we not founds a moral difference for interleukin-23 and interleukin-1 beta, and this is not what our study agrees with studies that showed that the ages in which the disease appears in high rates are from 15-30 years, because these ages are more susceptible to autoimmune diseases, including multiple sclerosis [27] and this is what the results of our study agree with, and perhaps it is related to the preparation and variation of the sample. Although we did not find a moral difference between sex and age. We recorded a rise in the concentrations of interleukin-23, which was measured in the serum of patients In terms of sex and their average concentrations (105 pg/ml), which may be high compared to Chen et al., which was conducted on Chinese patients and the concentration of cytokines in their serums was (47 pg/ml) [28], While our measured concentration is low compared to what was shown by the results of the study conducted by wen et al., which measured the concentration of cytokine in the cerebrospinal fluid of MS patients record high cytokine was measured (540 pg/ml) [29], while in our study we did not measure it in the cerebrospinal fluid. The recent study findings revealed increase of concentration in the cytokines inflammation interleukin-1 beta and interleukin-23 in the sera of multiple sclerosis patients when compared to the healthy group, which reflects the relationship between these two cytokines in the exacerbation and disorder of the disease. Our study agreed with the fact that Mufazalov et al., studies indicated that sclerosis is characteristic of a rise in inflammatory cytokines like tumor necrosis

factor, IL-6, IL-1beta, and interleukin-23 [30].The inflammatory environment available within the affected area is characterized by the infiltration and invasion by both the innate and adaptive immune cells and the availability of inflammatory cytokines secreted by the permanence of the attack on the myelin layer damage in the nervous tissue and causing its relapse, while our study did not agree with the study of Maimone et al. [31], whose study showed that the interleukins that cause the main causes of the disease cannot be measured in the serum of patients accurately because these cytokines, including interleukine-23 and interleukin-1 beta, are cytokines whose sited in the affected area and can be better measured by cerebral spinal fluid. Our study found a positive moral association between interleukin-23 and interleukin-1 beta, a relationship that may be directed between interleukins, as their association shows a pivotal role in the contribution mechanism In the symptoms and aggravation of the disease, and it plays a crucial part in the progress the disease pathway. Ronchi et al., [32] showed increasing concentration of interleukin-23 in the serum of multiple sclerosis patients has an effect on immune cells And for being of inflammatory cytokines works on several mechanisms, including a cell related to the maturation and differentiation of T cells and urging them towards their differentiation to Th17 cells including what is related to its effect on binding to receptors that are qualitative for a currency, and research conducted for sclerosis patients found an increase of expression of IL-1beta receptor in their serum more than normal people, and this may reflect the combined effect of these two inflammatory cytokines [33].

Conclusion

Our study did not find a statistical difference in immunological criteria with gender and age, while it found significant increase in the immune standards in patients' serum compared to the control group serum, and we found a positive correlation between interleukin-1 beta and interleukin-23. The positive association between them may be indicative of a common pathway for them, and studies must be intensified to find more deeply to determine this pathway and inhibit or blocked it because they play a vital crucial part in progression of the disease and that targeting their path may be promising therapeutic signs and goals for Multiple sclerosis.

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Interest Conflicts

None**.**

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