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Evaluate the Levels of Serum Eotaxin-1, Myelin Basic Protein, and Some Immunological and Biochemical Markers in Iraqi Patients with Multiple Sclerosis

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Abstract

The central nervous system (CNS) disease known as multiple sclerosis (MS) is essentially an inflammatory demyelinating condition with a variety of clinical manifestations and variable histological findings. A number of immunological and biochemical markers may alter MS, which is also characterized as an autoimmune illness. MS patients (n = 100) were divided into two groups: newly diagnosed (n = 42) and patients with ongoing treatments (n = 58). These groups were compared to healthy subjects (n = 55); the mean age ±SD was (30±8.46 years), (37±8.06 years), and (31±8.73 years) for MS newly diagnosed patients, patients with ongoing treatments, and healthy subjects, respectively. Studies for serum levels of eotaxin-1, myelin basic protein (MBP), IL-23, 27, 9, and 37 were measured by the ELISA technique. Eotaxin-1, MBP, IL-23, 27, and alkaline phosphate were significantly higher in all MS patient groups, but for IL-37, there was no significant difference between newly diagnosed patients when compared with patients with ongoing treatment. Weak positive correlations were found between IL-9 and myelin (r = 0.282, $p \leq 0.05$) and a weak positive correlation between IL-23 and ALP (r = 0.209, $p \leq 0.05$) in MS patients. A receiver operating characteristic (ROC) curve analysis was applied for the parameters eotaxin-1, MBP, IL-23, 27, and alkaline phosphate, which showed a high sensitivity according to the area under the curve. The present results conclude that eotaxin-1, MBP, IL-23, 27, and alkaline phosphate can be used as diagnostic markers for multiple sclerosis.

Keywords: Multiple sclerosis, eotaxin-1, Myelin basic protein, Interleukins, D3, Liver function enzymes.

تقييم مستويات الايوتوكسين-1 و بروتين المايلين الأساسي و بعض المتغيرات البيوكيميائية في المرضى العراقيين المصابين بالتصلب المتعدد

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الخلاصة

يعد مرض الجهاز العصبي المركزي في الأساس حالة التهابية مزيلة للمايلين مع مجموعة متنوعة من المظاهر السريرية والنتائج النسيجية المتغيرة. قد تغير العديد من الواسمات المناعية والكيميائية الحيوية مرض التصلب العصبي المتعدد ، والذي يوصف أيضًا بأنه أحد أمراض المناعة الذاتية. تم تقسيم مرضى التصلب المتعدد (العدد = 100) إلى مجموعتين حيث تم تشخيصهم حديثًا (العدد = 42) ، والمرضى الذين تحت مجموعة من العلاجات المستمرة (العدد = 58). تمت مقارنة هذه المجموعات مع الأشخاص الأصحاء (العدد = 55) ؛ كان متوسط العمر SD ± (30 ± 8.46 سنة) ، (37 ± 8.06 سنة) ، (31 ± 8.73 سنة) لمرضى التصلب العصبي المتعدد حديثًا ، والمرضى الذين تحت مجموعة من العلاجات مستمرة ، والأشخاص الأصحاء ، على التوالي. تم قياس مستويات الايوتوكسين −1 و بروتين المايلين الأساسي و IL−23 و 27 و 9 و 37 في الدم بواسطة تقنية الاليزا. لقد كان مستوى كل من الايوتوكسين −1 و بروتين المايلين الأساسي و IL−23 و IL−27 والفوسفاتاز القلوي أعلى و بشكل ملحوظ في جميع مجموعات مرضى التصلب المتعدد بينما لم يكن هناك فرق واضح في IL−37 بين المرضى الذين تم تشخيصهم حديثًا عند مقارنته بالمرضى الذين تحت مجموعة من العلاجات المستمرة. تم العثور على ارتباطات إيجابية ضعيفة بين IL−9 مع المايلين ($r = 0.282$ $p\leq0.05$)، وارتباط إيجابي ضعيف بين IL−23 والفوسفاتاز القلوي ($r = 0.209$ $p\leq0.05$) ، في مرضى التصلب المتعدد. تم تطبيق منحنى (ROC curve) لمعلمات Eotaxin−1 وبروتين المايلين الأساسي و IL−23و 27 و الفوسفاتاز القلوي وإظهار حساسية عالية وفقًا للمنطقة الواقعة تحت المنحنى .استنتجت نتائج الدراسة الحالية إلى أنه يمكن استخدام الايوتوكسين وبروتين المايلين الأساسي و IL−23 و 27 كوا سمات تشخيصيه لمرض التصلب المتعدد.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune demyelinating disease of the central nervous system. It has a variety of clinical manifestations and variable histological characteristics [1]. MS is marked by inflammation of the central nervous system (CNS), demyelination, axonal loss, and oligodendrocytes trying to re-myelinate [2]. One of the prevailing explanations is that individuals with a genetic predisposition to MS develop MS as a result of an abnormal immune response. Consequently, MS is typically thought of as an autoimmune disease [3].

Myelin basic protein, an abundant protein found in CNS myelin, has been the subject of some studies on the pathophysiology of MS (MBP). With 30% of the total CNS myelin protein, MBP is the second-most prevalent protein in myelin. Early in the 1960s, it was initially isolated as being the most widely studied myelin protein concerning MS [4,5]. Both MBP and its potential function as a source of autoantigenic epitopes in MS have been extensively studied. It is generally accepted that during the pathogenesis of MS, changes occur in compact myelin as well as the isoform composition and structure of MBP.

Other MS studies recorded that the immune system has a critical contribution to the development of lesions, especially in the acute early stages of the disease [6, 7]. It has been shown that the generation of cytokines by inflammatory cells in the brain plays a role in orchestrating and controlling the immune response as well as mediating tissue damage. The immune system's key players in the pathological characteristics of MS include cytokines and their receptors. Increased Th1 cytokine levels are particularly noticeable during MS relapse, whereas elevated Th2 cytokines are detected in MS patients throughout remission [8].

When people with relapsing-remitting MS get IFN, their clinical and hematological symptoms get worse. This is also true for people with other Th1-type diseases, but it's less obvious in Th2 diseases [9, 10]. In other words, Th2 cells were supposed to have an anti-

inflammatory capacity and act as protective T cells in MS, whereas Th1 cells were previously assumed to be harmful T cells [11, 12]. The goal of this study is to find out how much myelin basic protein (MBP), eotaxin-1, IL-9, IL-23, IL-27, and IL-37 are in the blood of Iraqi people with multiple sclerosis.

2. Materials and methods

Kits used for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), alkaline phosphate (ALP), direct bilirubin (D-bill), total bilirubin (T-bill), and D3 were provided by Siemens Healthcare Diagnostics Inc. Laboratory Diagnostics, 511 Benedict Avenue, Tarrytown, NY 10591-5005, USA. The enzyme-linked immunosorbent assay technique (type sandwich ELISA) was used to assay myelin basic protein (MBP, MyBioSource, China, Cat. No. MBS031329), eotaxin-1 (MyBioSource, China, Cat. No. MBS728700), IL-23 (MyBioSource, China, Cat. No. CSE-E08461h), IL-27 (MyBioSource, China, Cat. No. CSB-E08464h), IL-9 (MyBioSource, China, Cat. No. CSB-E04642h), and IL-37 (MyBioSource, China, Cat. No. CSB-E16185h).

2.1. Study population

The present study was achieved at the Medical City Complex from November 2021 to February 2022. Fifty-five healthy subjects and 100 patients were divided into groups of newly diagnosed (42) and patients with ongoing treatments (58). The mean age ±SD was 30±8.46, 37±8.06, and 31±8.73 for MS newly diagnosed patients, patients with ongoing treatments, and healthy subjects, respectively. Healthy subjects are very close to the patients in the gender ratio. (42.86%) male, (57.14%) female for MS patients newly diagnosed, (41.37%) male, (58.62%) female patients with ongoing treatments, (43.63%) male, and (56.36%) female for healthy subjects. Permissions were obtained from the medical city hospitals in Baghdad, Iraq, and approved by the University of Technology's institutional ethical committee, Baghdad, Iraq (Ref. No. AS 1974-17-10-2021) in accordance with the Helsinki Declaration of 1975, revised in 2000. All participants were informed about the study design and objectives and signed informed consent before the collection of any data or samples.

2.2. Laboratory assessment

Both MS patients n (100) and healthy n (55) volunteers provided specimens. Blood (10 mL) was drawn from each person, and the serum was centrifuged and kept at -20 ℃. Liver function enzymes such as ALT, AST, ALP, D. BILL, T. BILL, and D3 were assayed on an automated biochemical analyzer from Siemens Healthineers, Germany. The tests for serum eotaxin-1, myelin basic protein, and interleukins (9, 23, 27, 37) depended on the sandwich ELISA technique. A specific antibody (pre-coated in the wells) is combined with a sample after addition. An immune complex is created by combining avidin-horseradish peroxidase (HRP) conjugate with biotinylated detecting antibodies. The substrate addition makes it colored. In the end, the blue color is changed into a yellow color by the stop solution. The optical density (O.D.) was measured at 450 nm by using an ELISA reader within 15 minutes after adding the stop solution.

2.3. Statistical data analysis

The analysis was submitted using MedCalc Statistical Software version 16.4.3. Parameters were normally distributed, and the results were shown as mean ±SD. Differences between the groups were tested by using a one-way ANOVA test and an independent t-test. The receiver operating characteristic curve (ROC curve) was used to identify the validity of markers as an indicator of disease. The markers were compared according to the area under the curve. The data were considered significant at $p \leq 0.05$.

3. Results and discussion

For all patient groups, age, BMI, duration of disease, signs and symptoms, and treatments were recorded. Liver function tests and vitamin D3 were also measured, as shown in Table (1).

Table 1: Subject characteristics

Parameters	Control (n=55)	Newly diagnosed MS patients (n= 42)	MS patients ongoing on treatments (n=58)
Gender Male	24 (43.63%)	18 (42.86%)	24(41.37%)
Female	31 (56.36%)	24 (57.14%)	34 (58.62%)
Age (year)	31±8.73a	30±8.46a	37±8.06b
Education Below high school	0(0%)	12(28.57%)	9(15.5%)
High school	4(7.27%)	13(30.95%)	21(36.20%)
University	51(92.72%)	17(40.47%)	28(48.27%)
Marital status Married	45 (81.82%)	23 (54.76%)	42(72.41%)
Unmarried	10 (18.18%)	19 (45.24%)	16(27.59%)
Sign fatigue		37(88.09%)	41(70%)
vision problems		5(11.9%)	10(17.24%)
muscle spasms		35(83%)	46(79.3%)
pain		40(95%)	55(94.8%)
depression and anxiety		8(19%)	13(22.4%)
bladder problems		0	21(36.2%)
speech difficulties		0	4(6.89%)
Total bilirubin $mgdL^{-1}$	0.49±0.03a	0.49±0.03a	0.48±0.03a
Direct bilirubin $mgdL^{-1}$	0.19±0.01c	0.57±0.07b	0.76±0.05a
AST UL^{-1}	19.89±0.71b	22.59±0.99b	28.07±2.51a
ALT UL^{-1}	21.35±1.59b	25.43±5.09ab	32.19±3.45a
ALP UL^{-1}	70.36±2.62b	92.97±4.57a	98.45±3.45a
Vitamin D3 $ng\ mL^{-1}$	29.04±0.84a	13.20±0.93b	28.58±1.32a

Mean with a different letter in the same row is significantly different ($p \leq 0.05$).
AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase

According to this study, the effects of sex on the clinical expression of MS and response to therapy will have implications for the follow-up and treatment of patients with MS. Thus, the effects of sex on MS need to be taken into consideration, and MS is now universally found to be more prevalent in women than men [13].

There was no significant difference in the mean serum ALT levels in newly diagnosed patients (25.43±5.09 UL^{-1}) when compared to the control group (21.35±1.59 UL^{-1}) ($p \leq 0.05$). There was a significant increase in MS patients on treatment (32.19±3.45 UL^{-1}) when compared to the control group ($p \leq 0.05$). As well, there was a significant difference in the newly diagnosed group when compared to patients with ongoing treatments ($p \leq 0.05$), as shown in Table 1.

There was no significant difference in the mean serum AST levels in newly diagnosed patients (22.59±0.99 UL^{-1}) when compared to the control group (19.89±0.71 UL^{-1}) ($p \leq 0.05$). There was a significant increase in MS patients on treatment (28.07±2.51 UL^{-1}) when compared to the control group ($p \leq 0.05$). As well, there was a significant difference in the newly diagnosed group when compared to the patients with ongoing treatments group ($p \leq 0.05$), as shown in Table 1.

There was a significant difference in the mean serum ALP levels in newly diagnosed patients

(92.97±4.57 UL^{-1}) when compared to the control group (70.36±2.62 UL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients on treatment (98.45±3.45 UL^{-1}) when compared to the control group ($p\leq0.05$). No significant difference was observed between the newly diagnosed groups when compared to the patients with ongoing treatments group ($p\leq0.05$), as shown in Table 1.

There was no significant difference in the mean of total bilirubin levels in newly diagnosed patients (0.49±0.03 $mgdL^{-1}$) when compared to the control group (0.49±0.03 $mgdL^{-1}$) ($p\leq0.05$). There was no significant increase in MS patients on treatment (0.48±0.03 $mgdL^{-1}$) when compared to the control group ($p\leq0.05$). No significant difference was observed when comparing the diagnosed groups and the treated group ($p\leq0.05$), as shown in Table 1.

There was a significant difference in the mean direct bilirubin $mgdL^{-1}$ levels in newly diagnosed patients (0.57±0.07 $mgdL^{-1}$) when compared to the control group (0.19±0.01 $mgdL^{-1}$) ($p\leq0.05$). There was a significant increase in MS patients in the ongoing treatment group (0.76±0.05 $mgdL^{-1}$) when compared to the control group ($p\leq0.05$). As well, there was a significant difference when comparing newly diagnosed groups when compared to the patients with ongoing treatments group ($p\leq0.05$), as shown in Table 1.

Some studies have indicated that autoimmune diseases often coexist. For example, some cases have multiple sclerosis, and another disease is a chronic liver disease caused by autoimmune diseases, and studies that demonstrate this are limited and few. While other studies have linked elevated liver enzymes in patients with MS, it is associated with the treatment used, for example, interferon beta. Additional research in MS patients revealed that the variant was substantially linked to elevated levels of aspartate aminotransferase and alkaline phosphatase. These enzymes are indicators of liver injury when they are elevated. According to the researchers' hypothesis, this variant may cause alterations in the expression of the interferon regulatory factor-6 gene (IRF6), which responds to interferon beta and encourages cell death [14].

The final byproduct of heme catabolism, bilirubin, is a potent antioxidant. According to reports, patients with neuromyelitis optica (NMO) and multiple sclerosis (MS) have lower serum bilirubin levels. Although the pathogenesis of optic neuritis (ON) and multiple sclerosis (MS) is similar, it is unknown how endogenous bilirubin contributes to ON [15].

Through the demise of the oligodendrocyte, these cells mediate demyelination and neurodegeneration. They also oxidize essential biological components like DNA, lipids, and proteins. Due to defective redox homeostatic systems and decreased amounts of endogenous antioxidants such as bilirubin, this pathological state manifests. As an endogenous antioxidant, bilirubin is crucial in the regulation of oxidative stress and generation of reactive oxygen species (ROS). It results from the breakdown of heme. Heme oxygenase in the spleen's macrophages breaks down senescent heme, converting it to biliverdin, carbon monoxide, and iron. Biliverdin reductase then turns biliverdin into bilirubin [16].

There was a significant difference in the mean serum D3 levels in newly diagnosed patients (13.20±0.93 ng mL^{-1}) when compared to the control group (29.04±0.84 ng mL^{-1}) ($p\leq0.05$). But there was no significant increase in MS patients in the ongoing treatment group (28.58±1.32 ng mL^{-1}) when compared to the control group ($p\leq0.05$). There was a significant difference when comparing the newly diagnosed group to the patients with ongoing treatments ($p\leq0.05$), as shown in Table 1.

Calcitriol (1α, 25-dihydroxy vitamin D3) can be classified as a seco-steroid hormone. Vitamin D3 in humans is converted to calcitriol as an active final vitamin D3 metabolism product [17]. Research shows that the amount of vitamin D serum in MS patients influences the likelihood of getting MS and modulates disease activity. Most of the evidence indicating higher levels of vitamin D are linked to lower MS risk and lower MS activity came from observational studies. Because of the limitations of this research, it is difficult to state with confidence if vitamin D impacts MS. Patients with multiple sclerosis (MS) may benefit from taking vitamin D supplements, according to previous studies. However, large clinical trials are needed to confirm vitamin D supplements as the best therapeutic option for MS patients. A low serum level of vitamin D has been associated with an increased risk of multiple sclerosis (MS), although the effect of vitamin D treatment on MS activity has not been well studied. No one agrees on the adequate amounts of vitamin D needed for these patients. According to the Institute of Medicine (IOM), more than 50 nmol/L of vitamin D levels suggest adequate vitamin D status [18, 19].

3.1. Serum eotaxin-1 levels

There was a significant difference in the mean of serum eotaxin-1 levels in newly diagnosed patients (45.77±1.09 pgmL^{-1}) when compared to the control group (36.84±0.65 pg mL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients on treatment (48.14±0.73 pg mL^{-1}) when compared to the control group ($p\leq0.05$). As well, there was a significant difference when comparing the newly diagnosed group to patients with ongoing treatments ($p\leq0.05$), as shown in Table 2 (left column).

Table 2: Levels of eotaxin-1 and myelin basic protein in studied groups

Groups	Serum eotaxin-1 Mean ±SD (pg mL^{-1})	Serum myelin basic protein Mean ±SD (ng mL-1)
Control group N=55	36.84±0.65c	2.71±0.22c
Newly diagnosed MS patients group N= 42	45.77±1.09b	6.34±0.47b
MS patients on treatment N=58	48.14±0.73a	7.81±0.22a

Means with a different letter in the same column are significantly different ($p\leq0.05$).

This outcome is consistent with that which Teixeira and her associates have noted about CCL-11, or eosinophil chemotactic protein (eotaxin-1). Numerous neuro-inflammatory diseases, including multiple sclerosis, have been linked to elevated levels [20]. Fernandez-Paredes et al. said that higher levels of eotaxin-1 may be important for eosinophil recruitment during NMOSD remission [21]. They also said that higher levels of CCL-11 and CCL-13 may be important for eosinophil restoration during NMOSD remission. Autoimmunity encephalomyelitis (EAE), an experimental model of multiple sclerosis, is linked to higher levels of eotaxin-1 in the CSF, a tighter blood-brain barrier, less antigen-specific responses, and a predominance of the anti-inflammatory Th-2 phenotype. This suggests that eotaxin-1 may stop neuro-inflammation in this model [22, 23].

3.2. Serum myelin basic protein levels

There was a significant difference in the mean serum myelin basic protein levels in newly diagnosed patients (6.34±0.47 ng mL^{-1}) when compared to the control group (2.71±0.22 ng mL^{-}

^{1}) ($p\leq0.05$). There was a significant increase in MS patients with ongoing treatments (7.81±0.22 ng mL^{-1}) when compared to the control group ($p\leq0.05$). As well, there was a significant difference when comparing newly diagnosed groups when compared to patients with ongoing treatments ($p\leq0.05$), as shown in table 2 (right column).

These results are compatible with Polis and his colleagues found that myelin basic protein (MBP) has been proposed as a marker of neuronal injury in a variety of contexts, and MBP levels may be used to assess the severity of neuronal damage [24].

3.3. Interleukins (IL-9, IL-23, IL-27 and IL-37)

There was a significant difference in the mean serum IL-9 level in newly diagnosed patients (199.02±9.29 pg mL^{-1}) when compared to the control group (170.74±5.22 pg mL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients with ongoing treatments (225.46±8.46 pg mL^{-1}) when compared to the control group ($p\leq0.05$). As well, there was a significant difference when comparing the newly diagnosed group to patients with ongoing treatments ($p\leq0.05$), as shown in Table 3.

There was a significant difference in the mean serum IL-23 levels in newly diagnosed patients (56.37±2.66 pg mL^{-1}) when compared to the control group (40.47±0.41 pg mL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients with ongoing treatments (68.40±3.53 pg mL^{-1}) when compared to the control group ($p\leq0.05$). As well, there was a significant difference when comparing newly diagnosed groups to patients with ongoing treatments ($p\leq0.05$), as shown in Table 3. There was a significant difference in the mean serum IL-27 level in newly diagnosed patients (17.15±0.91 pg mL^{-1}) when compared to the control group (11.55±0.38 pg mL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients with ongoing treatments (15.65±0.47 pg mL^{-1}) when compared to the control group ($p\leq0.05$). But there was no significant difference when compared between the newly diagnosed groups and patients with ongoing treatments ($p\leq0.05$), as shown in Table 3.

There was a significant difference in the mean serum IL-37 levels in newly diagnosed patients (239.16±13.52 pg mL^{-1}) when compared to the control group (165.67±7.78 pg mL^{-1}) ($p\leq0.05$). There was a significant increase in MS patients with ongoing treatments (255.28±9.60 pg mL^{-1}) when compared to the control group ($p\leq0.05$). But there was no significant difference when compared with newly diagnosed groups to the patients with ongoing treatments ($p\leq0.05$), as shown in Table 3.

Table 3: The mean ±SD for serum interleukin levels in all studied groups

Groups	IL-9 pg mL^{-1}	IL-23 pg mL^{-1}	IL-27 pg mL^{-1}	IL-37 pg mL^{-1}
Control group N=55	170.74±5.22c	40.47±0.41c	11.55±0.38b	165.67±7.78b
Newly diagnosed MS patients group N= 42	199.02±9.29b	56.37±2.66b	17.15±0.91a	239.16±13.52a
MS patients on treatment N=58	225.46±8.46a	68.40±3.53a	15.65±0.47a	255.28±9.60a

Means with a different letter in the same column are significantly different ($p\leq0.05$).

An interleukin is a group of cytokines that are activated as a result of the direct interaction between autophagy proteins and immunological signaling molecules (i.e., cytokines), which has also been identified [25]. The cytokines have a crucial role in controlling the immune response and inflammation processes in health and disease. They work synchronously in a

complex network of signaling mediators to govern the pro-inflammatory and anti-inflammatory processes [26]. It has been found that interleukins have an effect on many aspects of the human body, such as metabolic activity, the cardiovascular system, and the neuroendocrine system, which affects homeostasis. Due to their complex behaviors and effects on other cells, patients may develop excessive depression. This applies to many diseases, such as diabetes, autoimmune diseases, tumors, and other neurological diseases, such as MS. Cytokines and chemokines are small-secreted proteins involved in many aspects of cell development, differentiation, and activation functions. A prominent characteristic of these molecules is their effect on the immune system in relation to the development of cell trafficking and immune tissues and organs. Furthermore, they play an important role in initiating and coordinating the organized and sequential recruitment and activation of cells into Mycobacterium tuberculosis-infected lungs [27]. Some of these parameters are deemed to be key elements in allergic asthma pathogenesis. They also help in the diagnosis and management of the disease [28].

Gloria Donninelli and her coworkers discovered that the CNS expresses both IL-9 and its receptor. Furthermore, it was found that human macrophages' anti-inflammatory capabilities are promoted and their activation state is reduced by IL-9. By regulating IL-9 expression in MS, this pathway may contribute to the positive effects of IL-9 shown in MS and may be therapeutically potentiated [29]. Interleukin-23 is a crucial cytokine in the linkage of both innate and adaptive immune responses. According to research conducted in 2018 by Michael and his coworkers, they examined the plasma levels of IL-23 in MS patients and found a significant relationship between IL-23 plasma levels and MS patients in comparison with healthy subjects [30].

There was a study that concurred with ours and discovered that patients with multiple sclerosis had higher levels of the cytokine interleukin-27 in their central nervous systems. This suggests that IL-27 is secreted locally in the CNS at higher levels during autoimmune processes. The current findings suggest that the local synthesis of IL-27 may signal the start of a regulatory response that encourages the resolution of inflammation. Understanding the biology of IL-27 in MS disease could be done by observing how novel immunomodulatory treatments affect cerebral IL-27 production [31]. According to a study conducted in 2015 by Mehrdad Farrokhi and his colleagues, the role of IL-37 in MS has not yet been researched. In this investigation, they compared the blood levels of IL-37 in MS and NMO patients to those in healthy controls. When compared to healthy controls, elevated serum levels of IL-37 in MS and NMO patients were recorded, indicating that these patients may be activated by pro-inflammatory cytokines or other unidentified mechanisms [32].

Receiver Operator Curve (ROC)

The ROC analysis was used to distinguish MS patient groups and control groups to allow the parameters to be organized based on the ROC area they can occupy and whether or not this occupancy is significant. The ROC analysis revealed the descending order (IL-23 = 0.937; Myelin Basic Protein = 0.934; Eotaxin-1 = 0.923; IL-27 = 0.857; direct bilirubin = 0.805; serum ALP = 0.762) of parameters that showed a significant variation. Figure 1 shows the Receiver Operator Curve. According to ROC analysis, IL-23, MBP, eotaxin-1, IL-27, direct bilirubin, and serum ALP show a good area under curve (AUC), so these parameters are considered sensitive parameters for MS (Table 4).

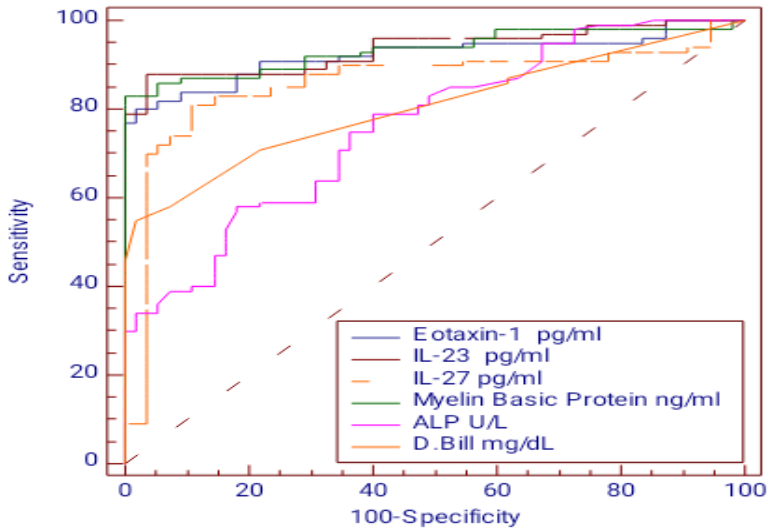

Figure 1: Receiver Operator Curve

Table 4: Area under carve and SE

Variable	AUC	SE [a]	95% CI [b]
Eotaxin-1 pg mL^{-1}	0.923	0.0219	0.869 to 0.959
Myelin Basic Protein ng mL^{-1}	0.934	0.0200	0.882 to 0.967
IL-23 pg mL^{-1}	0.937	0.0190	0.886 to 0.970
IL-27 pg mL^{-1}	0.857	0.0336	0.792 to 0.908
ALP U/L	0.762	0.0385	0.687 to 0.827
D.bill mgdL^{-1}	0.805	0.0339	0.734 to 0.864

IL-23: Interleukin23, IL-27: Interleukin27, ALP: alkaline phosphatase. D.bill: direct bilirubin

Correlation study

There is a significant weak positive correlation between myelin and IL-9 ($r = 0.282$, $p \leq 0.05$), as well as between myelin and AST ($r = 0.204$, $p \leq 0.05$). There is a significant, weak positive correlation between IL-23 and ALP ($r = 0.209$, $p \leq 0.05$). There is a significant weak positive correlation between T-bill and ALP ($r = 0.230$, $p \leq 0.05$). The correlation is shown in Table 5. The data labeled the strength of the association as follows: values of r = 0-0.19 are regarded as very weak, values of r = 0.2-0.39 as weak, values of r = 0.40-0.59 as moderate, values of r = 0.6-0.79 as strong, and values of r = 0.8-1 as very strong correlation.

Table 5: Correlation of parameters

		IL9	IL37	Myelin	AST	ALP	T-bill
IL23	r	.076	.010	.109	.152	.209*	-.098
	p	.451	.923	.281	.130	.037	.332
	N	100	100	100	100	100	100
IL9	r		.256*	.282**	.167	.050	.152
	p		.010	.005	.096	.619	.132
	N		100	100	100	100	100
Myelin	r				.204*	.062	-.100
	p				.042	.541	.324
	N				100	100	100

AST	r				.014	.223*
	p				.893	.026
	N				100	100
ALP	r					-.230*
	p					.021
	N					100
T-bill	r					
	p					
	N					

r: correlation coefficients ; P: p. value ; N; number of patients

Conclusion

The current data indicate that eotaxin-1 and MBP levels are both significantly higher in MS patients and rise with the course of the disease; hence, they may be used as MS prediction markers as well as for tracking the progression of the disease. When compared to control groups, serum IL-9, IL-23, IL-27, and IL-37 levels were considerably higher in both patient groups (newly diagnosed and receiving therapy). ROC analysis shows that IL-23, MBP, eotaxin-1, IL-27, direct bilirubin, and serum ALP have a good area under the curve (AUC). This means that these parameters are sensitive to MS.

Conflicts of interest

There are no conflicts of interest.

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