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Research Article

Multiple sclerosis patients CCL5 levels

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Abstract: Aim of study : multiple sclerosis (MS) is an autoimmune disorder affecting the central nervous system (CNS). This study aims to examine the CCL5 values in patients with Multiple Sclerosis.
Patients and methods: A retrospective study was undertaken on 70 individuals diagnosed with multiple sclerosis utilising archived material. The RNA sequence of CCL5 was analysed using the real-time polymerase chain reaction (qPCR) technique. The CCL5 level was determined using quantitative polymerase chain reaction (RT-PCR) methodology.
Results In total, a collection of 70 samples was obtained. The concentration of CCL5 was identified in all seventy patients and subsequently compared with a set of 30 control samples. There is an observed increase in the levels of CCL5 in patients diagnosed with Multiple Sclerosis (M.S) compared to individuals in good condition.
Conclusion These findings provide support for the elevated levels of CCL5 observed in patients with Multiple Sclerosis. Moreover, compared to control cases, it appears that CCL5 correlates with a more unfavourable outcome in patients.

Keywords: Multiple sclerosis, CCL5.

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1. Introduction

Multiple Sclerosis (MS) is a neurodegenerative disorder that impacts the central nervous system (CNS). The central nervous system (CNS) is most affected by this disease, with a specific focus on the myelin sheath that envelops the axons of neurons. Given that the disease is classified as an autoimmune syndrome, it is widely believed that brain function demyelination and disturbance mainly arise due to the pathological effects of inflammatory reactions. multiple sclerosis (MS), it is possible to see disruptions in the inflammatory response at various stages [1]. The attack on myelin in the brain by T cells and subsequent cytokine activation occurs as a result of immune response pathology and Destruction of the blood-brain barrier. These cytokines exert their effects by augmenting the blood-brain barrier's permeation [1]. This phenomenon enhances the likelihood of a central nervous system (CNS) assault by means of alternative immune cells, specifically B lymphocytes, which will secrete antibodies to designate myelin and oligodendrocytes for phagocytic uptake by macrophages. To some extent, myelin degradation leads to the development of lesions, which serve as the primary diagnostic indicators for Multiple Sclerosis (MS) [1].

Smoking tobacco cigarettes increases the risk of developing Multiple Sclerosis (MS). This association has been observed to coincide with a specific genetic variant that plays a role in the metabolism of inhaled smoke. Evidence indicates a potential association between dietary sodium intake and the Experimentally-induced activation of pathogenic Th17 T cells in mouse models of multiple sclerosis (MS) [3]. This suggests that a diet high in salt may contribute to the rising prevalence of multiple sclerosis (MS) [3]. The prevailing manifestation of multiple sclerosis (MS) is relapsing and remitting MS (RRMS), wherein relapses are instigated by periods of inflammation. The manifestation of disease progression in patients leads to the development of significant neurological impairments, which can be attributed to the occurrence of demyelination and axonal

degeneration. Approximately 15% of patients manifest a steady, primary progressive trajectory of illness, characterised by the gradual accumulation of neurological impairments [3]. Positive long-term outcomes have been observed in individuals undergoing high-intensity immunosuppressive regimens. However, this treatment's efficacy diminishes when it begins at a later stage, probably when the underlying process is characterised by degeneration rather than inflammation. The prevailing view posits that the successful management of early multiple sclerosis (MS) is vital in averting the progression of the disease. However, it must be noted that this notion lacks clinical evidence to substantiate its claims [3]. The occurrence of progressive multifocal leukoencephalopathy (PML) has been observed in individuals harboring latent John Cunningham virus (JCV) infections [4]. Mixantrone, also known as Novantrone, is a cytotoxic agent with immunosuppressive qualities and is utilised in treating several malignancies. The compound is believed to exert its effects through various pathways, including preventing macrophage-mediated demyelination, decreasing pro-inflammatory cytokines, inhibiting includes T-cell activation, suppressing T-cell, B-cell, and macrophage proliferation, and having a deficiency in antigen presentation. [5].

Cytokines have become increasingly significant in identifying and surveilling various diseases as biomarkers for early detection and ongoing monitoring. Chemotactic cytokines, also known as chemokines, facilitate crossing of the blood-brain barrier (BBB) by various cell types at sites of tissue injury [6]. For example, -chemokine ligand 5 (CCL5), also known as RANTES (regulated on activation of normal T cell generated and released), plays a role in immune cell migration by interacting with chemokine receptors (CCRs) present on these cells. It was proposed that there is a link between CCL5 expression and cellular activity in diseases. It has been suggested that this chemokine's blood levels could serve as a biomarker for a number of inflammatory diseases like rheumatoid arthritis. [6], atopic dermatitis, and asthma [7]. Multiple sclerosis is a neurological disorder [8]. A study revealed an elevated concentration of CCL5 in both the serum and cerebrospinal fluid (CSF) of individuals belonging to multiple sclerosis (MS) groups, as well as those without signs of inflammatory or non-inflammatory neurological illness (NIND) following an MS relapse [9]. Increased Th1 cell activity and cytokine network dysregulation have been linked to the observed rise in CCL5 levels during the attack, which is thought to result from the activation of monocytes and macrophages. [10]. The delays in treating multiple sclerosis are mainly attributed to the absence of diagnostic diagnostics despite the disease's high frequency and significant impact. To date, no immune-sensor has been described that enables the specific differentiation between healthy individuals and those with multiple sclerosis based on the quantification of CCL5 in the serum of patients at the predetermined threshold. [11].

2. Material and methods

Patients The study sample consisted of 70 individuals diagnosed with definite multiple sclerosis (MS), while 30 participants without neurological disorders were recruited as a healthy control group. Based on the clinical manifestations of multiple sclerosis, Relapsing-remitting MS (RR-MS), relapse stage MS (RELAPSE-MS), and progressive MS (PROG-MS) (type 1 and 2) are the three subgroups into which people with MS were divided. The initial cohort comprised 25 individuals in a stable phase of the illness, as evidenced by their lack of relapse in the three months leading up to the trial. The relapse group consisted of 23 patients exhibiting relapse symptoms. The samples in this cohort were collected before the initiation of steroid medication.

There were 13 people in the PROG-MS cohort with a diagnosis of secondary progressive MS and 9 with an OPMS diagnosis. All MS patients had never had immunosuppressive or immunomodulatory therapy prior to their diagnosis. Neither the sample collection process nor the preceding months' time period revealed any evidence of concomitant infections. In good health people provided 30 control serum samples.

Serum analysis The serum samples were obtained from individuals diagnosed with Multiple Sclerosis (M.S.).The specimens were placed into appropriate receptacles, adequately identified, and stored in a refrigerated container. The samples were sent to the laboratory within two hours of the collection. The serum samples collected in the laboratory were preserved in a deep freeze at a temperature of -80 °C until they were utilised for molecular analysis and the identification of CCL5 by reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Real-time polymerase chain reaction (qPCR) Using a primer set, we amplified a 203 bp segment of the CCL5 genome using quantitative polymerase chain

reaction(qPCR).Forward:5-AGATTCCTCGGACACCACAC-3;Reverse:5-TGGAGGATAGGTGGAAGTGG -3).

Using a real-time PCR kit (Cat, Lot 71301, Addbio, Korea; Cat, Lot 2001A, ABM, Canada), we amplified the human RNA encoding the chemokine chemokine ligand 5 (CCL5) and detected it in all serum samples. Using a (Analytik JenaQtower3G) instrument at Alamin Centre for Advanced Research and Biotechnology, a mixture with a final volume of 20 l was prepared by mixing all ingredients specified in (Table., year, 1), and the PCR conditions described in (Table., year,2).

Table 1. The Contents of (qPCR) mixture for CCL5

Volume	Item
1 µl	Nuclease-Free H20
10 µl	2x Add RT-PCR SYBR Maste
2 µl	Forward primer (10 µM)
2 µl	Reverse primer (10 µM)
5 µl	RNA template
20 µl	Total reaction volume

Table 2. The optimal CCL5 amplification thermo cycler settings.

Step	Condition	No. of Cycle
Pre-Denaturation	95 °C / 2 min	1
Denaturation	95 °C/30sec	40
Annealing	57.2°C / 30sec	
Extension	72 °C /50.0sec	
Final extension	72 °C / 5 min	1
Hold	Four °C	Forever

3. Results

One hundred distinct clinical cases were gathered, with 70 cases identified as Multiple Sclerosis and 30 as controls. The subject groups in this study were separated into four population groups based on the type of multiple sclerosis (MS) they had, namely RR-MS, Relapse-MS, PROGE M.S, and PROGE || M.S. Within these groups, further divisions were made based on gender, age, disease duration, and number of attacks, Table 3shows that.

Table 3. Baseline characteristics of M.S

Type of M.S	N0. of samples	Gender(F/M)	Age	Disease Duration	Number of attacks
RR-M.S	25(35.71%)	17(68%) /8(32%)	24-65	2-7	1-5
RELAPSE-M.S	23(32.86%)	14(60.86%)/9(39.14%)	21-75	5-12	4-9
PAGE M.S	13(18.57%)	8(61.53%)/5(38.47%)	45-80	7-18	5-11
PROGE || M.S	9(12.86%)	6(66.67%) /3(33.33%)	45-80	7-18	6-13

Four patients with relapse (32.86%), 13 patients with primary progressive MS (18.57%), and nine patients with secondary progressive MS (12.86%) were found to have measurable serum levels of CCL5. All patients had CCL5 in their blood, and as shown in Figure 1, the average patient had a greater CCL5 serum level than the healthy controls.

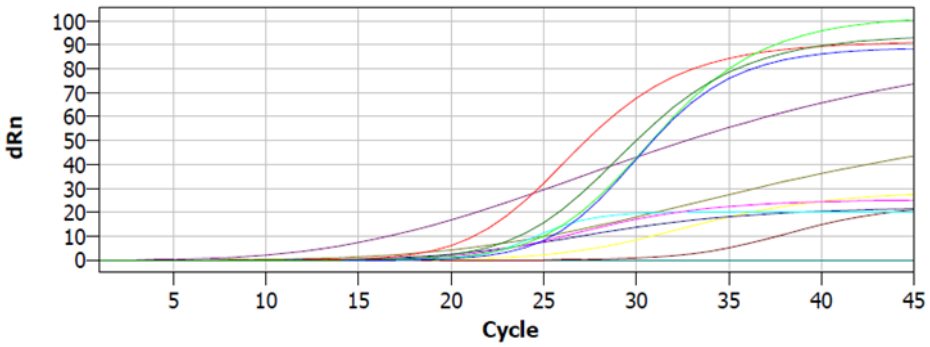

Figure 1. qPCR assay for detecting the increment in CCL5 level of Multiple sclerosis patients

4. Discussion

Chemokines' ability to establish a chemotactic gradient between the walls of blood vessels and the cerebrospinal fluid (CSF) compartment is essential for the transfer of cells from the bloodstream to the central nervous system (CNS). This process either triggers or enhances the inflammatory response. Understanding the correlation between disease activity and the function of chemokines is of utmost importance in multiple sclerosis (MS), a condition characterised by inflammation linked to demyelination and axonal degradation, resulting in irreparable damage to the central nervous system.

Our investigation has demonstrated the concentration of CCL5 in the serum of patients across all groups. In the context of a multiple sclerosis (MS) relapse, it was observed that the serum concentration of CCL5 was up among individuals with MS. These findings align with previous studies that have similarly reported an increase in CCL5 levels in the serum [12]. Furthermore, it has been observed that this phenomenon occurs in blood plasma and other bodily fluids such as cerebrospinal fluid (CNS) [13]. During a relapse, it is probable that this alternate response occurs due to the altered interaction among the cells immediately implicated in the recurrence. An abundance of Th1 cytokines initiates the occurrence of multiple sclerosis relapse, while the functioning of Th2 cytokines is hindered [14]. During an attack, CCL5 levels rise, which is linked to an increase in Th1 cell functioning and the disruption of the cytokine network, both of which contribute to a Th1 shift. Interleukin-4, a type of Th2 cytokine, and transforming growth factor b, an immunomodulatory cytokine, are responsible for the aforementioned expression [15].

Increased chemokine levels are associated with the activation of monocyte/macrophage cells, although no significant correlation between relapse and mononuclear cell count has been found. The potential impact of cytokines on the outcome of chronic relapsing experimental autoimmune encephalomyelitis has been observed in recent studies, as evidenced by the regulation of chemokine production by specific proinflammatory cytokines [16].

It has been found that the ratio of TH1 to TH2 activity in lymphocytes correlates with the concentration of chemokines during a relapse. It has also been noted that the number of cells synthesizing chemokines increases in direct proportion to the severity of a viral infection in the central nervous system (CUN). In patients with inflammatory neurological diseases, there is a statistically significant correlation between the number of cells in the cerebrospinal fluid (CSF) and the concentration of CCL5 [17].

There has only been a little amount of study done to compare chemokine concentrations between MS subtypes. There is little evidence that RR-MS, PP-MS, and SP-MS have significantly different chemokine levels or expression levels [18]. Chemokine levels did not differ significantly between individuals diagnosed with relapsing-remitting MS (RR-MS) and those diagnosed with progressive MS (PROGMS) [19].

The findings point to the existence of an inflammatory process in the brains of those with the advanced form of the disease. The higher levels of the remaining chemokines in MS patients, as well as the results of other studies that have found no significant differences across MS subtypes (18), lend further credence to this conclusion. There appears to be a significant discrepancy between the disease's clinical manifestation and its true manifestation, as revealed by MRI scans. The detection rate of lesions using MRI has been shown to be higher than using clinical examination alone. Demyelination lesions can be detected in healthy brain regions using the MTR histogram method (20).

5. Conclusion

These findings provide evidence for the elevated levels of CCL5 in patients with Multiple Sclerosis in Iraq. Moreover, compared to control patients, it appears that CCL5 correlates with a more unfavourable patient prognosis. It is advisable to conduct advanced molecular investigations in order to validate these findings and gain additional insights into the probable involvement of CCL5 in Multiple sclerosis.

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