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Abstract

Multiple sclerosis (MS) is a chronic neurological disorder. It is an autoimmune disorder, meaning that in MS the immune system which normally protects us from viruses, bacteria, and other threats mistakenly attacks healthy cells. predominantly affects young adults and is characterized by chronic autoimmune activity.

**Evaluation of Interferon Gamma and
Osteopontin levels in Relapsing-
Remitting Multiple Sclerosis Patients**

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This study explores the crucial role of cytokines, like interferon-gamma (IFN- γ) and Osteopontin which promoting inflammation and CNS tissue injury. The current study comprised fifty patients with relapse remitting MS. Blood was drawn from 50 MS patients and 40 healthy people who served as controls. The serum levels of IFN- γ and OPN were determined using an enzyme-linked immunosorbent assay (ELISA).

Our results indicate non-significant differences in IFN- γ level between MS patients and controls group of the study's sample ($P > 0.05$), whereas the level OPN cytokines was significantly higher in patients with RRMS compared with healthy individuals ($P \leq 0.001^{**}$).

The current study shown on significant differences between MS patients and controls in serum levels of IFN- γ . Higher OPN serum level in studied patients suggests that OPN increased in RRMS patients who were in remission phase.

Keywords: Multiple Sclerosis, Relapsing-remitting MS, Interferon Gamma, Osteopontin.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), which can lead to a variety of clinical disabilities such as motor, sensory and cognitive symptoms (1). Inflammation, neurodegeneration, and gliosis are hallmarks of MS. Perivascular lymphocytic infiltrate and macrophages destroy the myelin sheaths that pathologically wrap neurons (2).



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Although the etiology of MS remains unknown, both genetic and environmental factors are involved in breaking self-tolerance (3). MS is divided into the most frequent relapsing–remitting form (RRMS), which may turn into a secondary progressive form (SPMS), and the conversion usually occurs 10 years after disease onset. In addition, the primary progressive form (PPMS) is distinguished. The clinical classification depends on the presence of relapses in the disease course and the formation of lesions in white matter (active or non-active) (4).

The diagnosis of multiple sclerosis is based on thorough symptom analysis, neurological examination and additional investigations, which include magnetic resonance imaging performed accordingly to the revised McDonald criteria (2017) and cerebrospinal fluid examination of the presence of oligoclonal bands (5). Proinflammatory cytokines and T cells reactive against myelin proteins play an important role in the pathogenesis of multiple sclerosis (MS) and its experimental model, experimental autoimmune encephalomyelitis (EAE) (6).

IFN- γ is an essential cytokine that plays an important role in both innate and adaptive immune response. Its receptor (IFN- γ R) is ubiquitously expressed on all nucleated cells in the body, primarily signaling through the transcription factor signal transducers and activators of transcription (STAT)-1, to induce expression of multiple interferon-stimulated genes (ISGs). Historically, IFN- γ is thought to have a pro-inflammatory role in MS and EAE. However, several studies have paradoxically shown that IFN- γ can also mediate protective functions in EAE and MS (3).

In MS, induction of endogenous IFN production in progressive-MS patients showed that some patients with improving symptoms had high levels of serum IFN- γ , while worsening clinical symptoms was related to low serum IFN- γ levels (7).

Osteopontin is an extracellular matrix protein involved in bone remodeling, tissue repair and inflammation. Osteopontin is expressed by various cell types including osteoblasts, fibroblasts, epithelial cells and immune cells such as T lymphocytes and macrophages (8). OPN plays an important role in the pathogenesis of MS by modulating the T helper1 (Th1) and Th17 responses (9). Osteopontin is involved in the pathogenesis of different inflammatory and neurodegenerative diseases, including MS. Osteopontin is released by both resident and infiltrating immune cells, promotes the activation and survival of autoreactive T lymphocytes and the production of inflammatory mediators (10,11). The study aims to assess the concentration of IFN- γ and OPN in RRMS patients by using ELISA technique.

Subjects and methods:

The individuals with RRMS were all in remission phase, which is characterized as a period of recovery with no relapse episodes. A total of 50 MS patients (13 men and 37 women) were enrolled in the study after being referred to the complex MS clinic. Expert neurologists confirmed the diagnosis of MS based on valid diagnostic evidence such as magnetic resonance imaging, the presence of oligoclonal bands in the cerebrospinal fluid (CSF), and evoked potentials according to the McDonald's criteria. The MS patients were classified as previously diagnosed (treated) patients.



A control group of 40 healthy people, including 18 men and 22 women, was also enlisted in the study. All of the people in the control group were in good health, with no acute or chronic illnesses.

Cytokine measurement

At the time of patient enrolment, blood samples for cytokine analysis were taken. MS patients' serum samples were isolated right away, divided into aliquots, and stored in a -80°C freezer for later examination. IFN- γ and OPN levels in the serum are measured using a sandwich enzyme-linked immunosorbent assay.

Results

Levels of interferon gamma (IFN- γ) in MS patients in Comparison with healthy controls

The results indicated non-significant differences between MS patients and controls group of the study's sample (99.2892 ± 56.40892 and 95.2855 ± 27.30319) respectively ($P > 0.05$). (Table 1) (Figure 1).

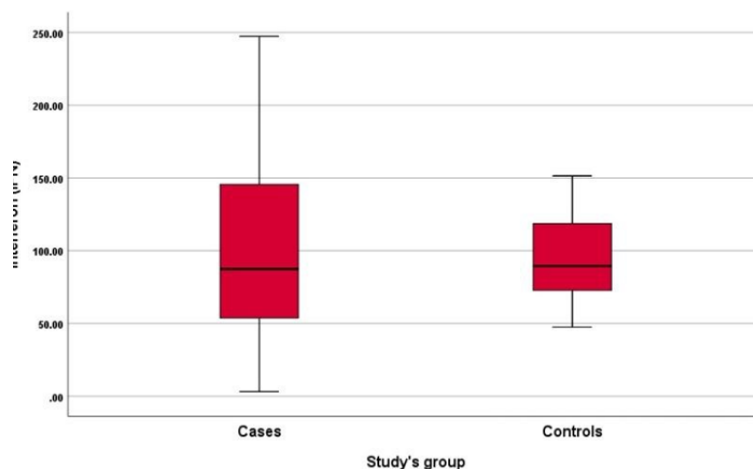
Levels of osteopontin (OPN) in MS patients in Comparison with healthy controls

The results indicated a significant difference in the mean value of osteopontin (OPN) in patients with MS in comparison with healthy controls (112.8146 ± 82.57450 vs. 34.7060 ± 12.13485) with significant differences of 82.6086 ($t = 5.925$, $df = 88$, $P \leq 0.001^{**}$). (Table 2) (Figure 2)

Table 1: Levels of interferon gamma (IFN- γ) in MS patients in Comparison with healthy controls

Study's groups	Interferon gamma(IFN- γ)				
	No.	Mean $\pm$ SD	Std. error	Mean difference	Significance ^c
Cases	50	99.2892 ± 56.40892	7.97743	4.00370	$t = 0.412$, $df = 88$, $P = 0.682$
Controls	40	95.2855 ± 27.30319	4.31701		

a: Unpaired t-Test.



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Figure 1: Levels of interferon gamma (IFN- γ) in MS patients in Comparison with healthy controls

Table 2: Levels of osteopontin (OPN) in MS patients in Comparison with healthy controls.

Study's groups	Osteopontin value (ng/ ml)				
	No.	Mean $\pm$ SD	Std. error	Mean difference	Significancy ^c
Cases	50	112.8146 $\pm$ 82.57450	11.67780	78.10860	$t= 5.925$, df: 88, $P \leq 0.001^{**}$
Controls	40	34.7060 $\pm$ 12.13485	1.91869		

a: Unpaired T-Test.

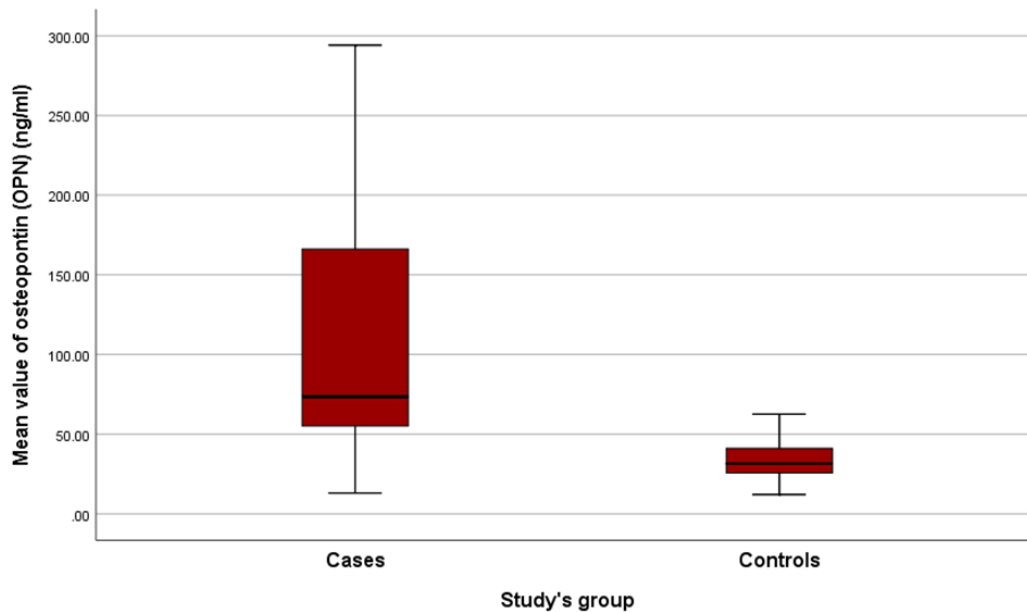


Figure 2: Levels of Osteopontin (OPN) in MS patients in Comparison with healthy controls

Discussion

Multiple sclerosis is a complex disease with various interactions between genetic and environmental factors in patients. Different cytokines may play a greater or lesser role in

disease pathogenesis depending on which interactions are at play within an individual, and these roles may change over time. The aims of the present study to evaluate the



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pro-inflammatory (Interferon (IFN)- γ , and OPN) cytokine serum levels in relapsing remitting-MS patients. Changes in a large number of cytokines, a soluble biomarker of inflammation and leukocyte activation, in serum were demonstrated.

Interferon gamma is a key cytokine that promotes Th1 clonal expansion while suppressing Th 2 clonal expansion. IFN- γ is a major cytokine found in MS lesions, and its levels rise dramatically as the disease progresses. IFN- γ expression rises immediately before attacks of MS. The activation of mononuclear cells, induction of the histocompatibility complex (MHC I and MHC II), and differentiation and apoptosis of T cells are all ways in which IFN- contributes to inflammation and demyelinating processes. IFN- γ could act on astrocytes by releasing a novel subset of chemokines, facilitating an inflammatory milieu and promoting migration of autoreactive encephalitic T lymphocytes, which is a central role for IFN- γ in MS brain inflammation (12).

Results of present study indicate that non-significant differences between IFN- γ concentrations in multiple sclerosis patients who are remission phase and controls. In agreement with these results previous study that shown decrease in IFN- γ levels. As well as IFN- γ expression increases during disease flare-ups and decreases during remissions (13). In contrast with current results, previous studies reported by (14, and 15) that demonstrate elevated IFN- γ concentrations in patients with MS and high levels of IFN- γ associated with disease activity. Along with these findings, IFN- γ concentrations were elevated in MS group compared the healthy subjects, but the difference was not significantly (16). Studies

have reported an increased frequency of T cells producing IFN- γ in response to myelin antigen stimulation of peripheral blood monocytes from patients with RRMS versus healthy controls (17). In immune-mediated demyelinating diseases, IFN- γ promotes myelin degradation and oligodendrocyte mortality by increasing inflammation, which includes activation of macrophages/microglia, overexpression of MHC molecules, and production of inflammatory mediators (18).

IFN- γ may also contribute to MS through its expression in T reg cells. Untreated MS patients have higher frequency of IFN- γ secreting Foxp3+ T reg cells than healthy individuals, and these T reg cells exhibit impaired suppressive efficacy in vitro compared to T reg cells that are not IFN γ + (19). These findings support that IFN- γ plays a pathogenic role in MS. Neuroinflammation and leukocyte infiltration can be influenced by the interaction between IFN- γ and the CNS (20). IFN- γ enhanced CCL2-mediated infiltration of monocytes and macrophages into the spinal cord in these mice (21). These findings show that IFN- γ may play both a protective and a pathogenic role in CNS autoimmunity differential modulation of chemokine production in the brain versus the spinal cord (22).

Osteopontin is a proinflammatory marker produced by systemic immune and central nervous system resident cells that plays a critical role in inflammation and immunity to infection. T helper 1 responses are linked to osteopontin. OPN increases proinflammatory cytokines IFN- γ and IL-12, which are implicated in MS, while decreasing IL-10, which play a protective role in MS and other inflammatory diseases. OPN improves the survival of myelin-reactive T cells. In the brains of MS patients, OPN is highly



upregulated. Other studies shown that OPN levels have also been associated with disease progression and recurrent relapse. In agreement with previous results reported by (23,24).

In this recent study in Iraq, the findings indicate that serum levels of this biomarker are increased among MS patients in comparison with healthy individuals. OPN is highly expressed in activated T cells and can regulate cytokine production to influence the activation pathway (25). Finding higher levels of OPN in samples from MS patients is in agreement with the T cell mediated nature of the disease. OPN is highly expressed in activated T cells and can modulate the activation pathway by cytokine regulation. Osteopontin increased in patients with RR-MS who were in active phase. It could be hypothesized that serum OPN level may be elevated as part of the proinflammatory cytokine milieu taking place in MS patients. In the previous study, when compared the levels of osteopontin in the peripheral blood and CSF among the subtypes of MS patients, the results demonstrates that lower CSF and peripheral blood levels of OPN in clinically isolated syndrome patients compared to patients with progressive subtypes of MS. This finding supports the possibility for coexist of neurodegeneration and neuroinflammation in progressive MS (26). Furthermore, previous analysis demonstrated that significantly higher levels of OPN in peripheral blood of MS patients compared to controls could be an interesting finding of the present study which emphasizes the clinical applicability of this biomarker even more. These findings suggest that higher levels of OPN are associated with more active inflammation and highlight the potential of OPN as a prognostic biomarker for patients diagnosed with MS (27).

The levels of osteopontin in serum and CSF are correlate significantly with inflammation, disease activity and clinical severity (28). However, studies have showed that higher OPN serum level in MS patients is not associated with disease severity (29). These findings were agreement with current study. The levels of osteopontin in serum of MS patients are higher during relapse than during remission phase; however, no differences in OPN levels between patients with relapsing remitting MS and control group participants, as shown by (6,30). OPN may play a role in lymphocyte recruitment into the MS lesion at this site, which includes $\alpha 4\beta 1$ integrin, the target of natalizumab. OPN induces relapses, whereas treatment with anti-OPN antibodies ameliorates the disease. AutoAb levels elevated in the RRMS patients than in the primary and secondary-progressive MS and healthy control groups, and they were highest in the early stages of the disease, and they influence MS evolution and prognosis in association with DMTs (31).

Conclusion:

The study's findings suggested that there is non-significant differences in INF- γ levels between MS patients and controls during remission phase. This study confirms that increased levels of OPN exist in serum of MS patients compared to the control individuals. OPN is a highly promising therapeutic target for multiple sclerosis due to its role in promoting inflammation and neurodegeneration primarily by modulating its proinflammatory effects. IFN- γ promote inflammation and disease exacerbation, it also exhibits protective effects by inducing regulatory T cells and other anti-inflammatory mechanisms.



Conflict of Interest: Non

Funding: Nil

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