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*RESEARCH ARTICLE*

# Detection of opioid growth factor in Multiple Sclerosis Patients

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**Abstract**

Multiple sclerosis (MS) is a recent prevalent central nervous system disease that affects the brain and spinal cord. It is assumed that it is an intermediate immune disorder in the central nervous system and is complex with incomplete etiology. Some research have confirmed that the Opioid growth factor(OGF), which was called chemically Enkephalin [Met5], is considered as a biomarker for the onset of MS. The purpose of this study was to look into the biomarker level of OGF and its correlation with body mass index(BMI) among patients with MS. A total number of 100 patients were diagnosed in two groups (54 early diagnosis of MS without treatment, 46 early diagnosis with treatment) according to McDonald criteria and 50 healthy control groups were included. All demographic data of the study samples were recorded (age, gender), as well as clinical data such as (BMI),and level OGF(serum and saliva) estimated by ELISA method. The current study revealed that the mean±SD of serum OGF in patients with multiple sclerosis for both groups(947.167 ± 134.262 ng/ml) was less concentrated than the control group (1046.642 ± 63.605 ng/ml) with a very large difference (P <0.01). While the mean concentration of OGF in saliva was (960.158 ± 91.684 ng/ml) for patients with multiple sclerosis (early diagnosis group without treatment and the treatment group), its concentration was higher than the control group (880.059 ± 87.700 ng/ml) with a large statistical significance (P <0.01). The current study showed an important correlation between the body mass index and serum OGF level at (p = 0.022) in the early diagnosis group for multiple sclerosis patients with treatment, , while in the early diagnosis group for multiple sclerosis patients without treatment, a very significant association was found between BMI and serum OGF level at (p = 0.009). Interestingly, the current study showed that saliva biomarker (OGF) would be a good predictor for diagnosing MS.



**Keywords**: Multiple sclerosis( MS) ; Opioid growth factor(OGF) ; body mass index(BMI)

## 1. Introduction

Multiple sclerosis is the commonest chronic inflammatory, demyelinating and neurodegenerative disorder in the central nervous system (CNS) of the brain and spinal cord[1]. The disease commonly starts in young adults with onset in the age of 20-50years, with women being at increased risk than men [2], which is described by multifocal inflammation that leads to plaques in myelin degeneration, axonal injury and by a higher degree of individual variability in the severity and progression for symptoms [3]. Though its aetiology remains occult it is now known that environmental factors and susceptible genes are involved in disorder pathogenesis. Results from immunological and genetic studies of patients with multiple sclerosis support the concept that autoimmunity plays a major role in (MS) pathogenesis. However although, it is also well accepted that multiple sclerosis is not only an inflammatory disorder, but also a neurodegenerative disorder.

The term "biomarker" is defined as "biological marker" is widely used as a diagnostic tool, as well as classification of disease, also an indication of disease diagnosis, and finally forecasting and monitoring of the clinical response to the intervention [4] .There are biomarkers for clinical evaluation of MS, and detection of early diagnosis of MS by studying the disease significantly which depends almost entirely on clinical features and several of reliable biomarkers of disease advancement that are created to help guide treatments [5] . Since Opioid Growth Factor (OGF) is consider one of biomarker, it is a small pentapeptide of the class of neuropeptides termed the endogenous opioids that are produced in the brain and generalizes widespread throughout all organs of the body. This peptide has a diverse role in many diseases including

autoimmune disorders. In the early 1980s, its name was enkephalin [Met5] initially it acted as neurotransmitters, but later it was shown to regulate the growth of the normal and abnormal cells, and, tissues, that is why it was called this name opioid growth factor(OGF)that suppresses cells proliferation, such as T-cell and B-cell associated with autoimmune disorders including multiple sclerosis [6] .as well as OGF also prevent poisoning that may lead to cytokines release and inflammatory markers that may facilitate demyelination and nervous degeneration. The mechanism of opioid growth factor (OGF) action is known. OGF enter cells during clathrin-mediated endocytosis, interact with the opioid growth factor receptor (OGFr), and target a cyclin-dependent inhibitory kinases (p16 and p21) which leads to delay cells in the G1/S phase from the cell cycle. The role of opioid growth factor (OGF) in the cause and therapeutic of autoimmune diseases especially of the multiple sclerosis disease is still at its infancy. Some research confirmed that the OGF was considered a biomarker for the onset of MS [7,8] . Thus, the study focused on giving an impression of the level of OGF in MS patients, as well as studying the relationship with the body mass index (BMI) for patients with multiple sclerosis.

## 2. Materials and Method

The practical side of the study was done during the period from August 2019 to January 2020.This study included a total number of (150 cases ), which were classified into three groups, group I without treatment (54 patients) and group II using treatment (46 patients), i.e. the total number of patients is ( 100 patients) diagnosed with MS according to McDonald criteria for a period not exceeding one full year , and group III about (50 healthy control). This study included its own questionnaire which includes all the information that pertains to each patient, where it includes the following: Name, Gender, Age and BMI(body mass index).

Table 1. Patient groups and control included in the study.

| 150 cases | | |
|---|---|---|
| MS without treatment (54 patients) | MS with treatment (46 patients) | (50) healthy controls |

*2.1 Specimen*

*2.1.1 Blood samples*

The venous blood sample was drawn using a disposable syringe with a capacity of 3 ml from each participant of the study and put in sterile tubes, then left for 15 minutes to clot at room temperature and then separated in the centrifuge for no more than 15 minutes at 3000 rpm to obtain serum.

*2.1.2 Saliva samples*

Saliva samples were taken (1.5 - 2.5 ml) and placed in plastic containers, before that the person must wash his mouth with sterile water and then spit in this container and then put it in the centrifuge for a period ranging between ( 10-15) minutes at 2500-3000 rpm. Then, the supernatant was taken and placed in an Eppendorf tube.

*2.2. Study Protocols:*

Quantitative detection of Opioid Growth Factor(OGF) in serum and saliva human was done by ELISA . According to Manufacture company protocol (MyBioSource, USA),As well as Body Mass Index {(BMI)=Kg/$m^2$}.

*2.3 Statistical Analysis:*

Statistical Analysis was carried out by the SPSS-25 statistical program, then these data were analyzed with simple measures of frequency, mean, standard deviation, percentage and range (minimum and maximum values). then reached a set of data and results using the following statistical methods: Students-t-test the difference between two independent methods or the ANOVA test for the difference between more than two independent methods and Pearson Chi-square test or Fisher test. Where the statistical significance was considered at P equal to or less than (0.05).

## 3. Results

Demographic and clinical data of studied population groups: The demographic distribution data of studied population groups variables , such as (age and gender) were registered. As well as clinical parameter variable such as BMI. The age mean $\pm$SD for patients in the group with multiple sclerosis without treatment was (35.54 ± 8.51 years) and the other group multiple sclerosis with treatment was (34.22 ± 8.24 year) while (36.5 ± 8.93 year) in normal population controls, in terms of number and percentage according to sex of participants, the early diagnosis MS without treatment (females 36, 66.7%; males 18, 33.3%) also showed the early diagnosis MS with treatment (females 30, 65.2%; males 16, 34.8%) consecutively in patients versus (females 25, 50. 0%; males 25, 50.0%) in normal controls. In the current study, the Body Mass Index(BMI) of MS patients with normal weight (no. =20 ,(37.0%) of early diagnosis MS without treated group),while(no. =14,(30.4%)of early treated group), and who suffer over weight (no. =20 ,(37.0%) of early diagnosis MS without treatment group), while(no. =22 ,(47.8%) of treated group), as well as obese(no. =14 ,(25.9%) of early diagnosis MS without treatment group),while(no. =10 ,(21.7%) of early treated group).

Regarding the of concentration ( OGF (ng/ml)), the serum and saliva level scored a very big significant difference at (p=0.0001) for both groups in addition to normal control. The mean (Serum OGF) for early diagnosis without treatment (942.855+132.312) and treatment was

(952.228 ± 137.805) and normal control 1046.642 ± 63.605, while the mean (Saliva OGF) for early diagnosis without treatment (975.081 ± 66.973) vs treatment was(942.641±112.381) and healthy control ( 880.059 ± 87.700).(Table2).

Table 2 Demographic and clinical data of studied population groups

| Variables | | MS without treatment Patients (No.=54) | | MS with treatment patients (No.=46) | | Control (No.=50) | | P-Value |
|---|---|---|---|---|---|---|---|---|
| Age (Yrs.) | Mean±SD | 35.54±8.51 | | 34.22±8.24 | | 36.5±8.93 | | 0.428 |
| Gender | Female | No.36 | 66.7% | No.30 | 65.2% | No.25 | 50.0% | 0.165 |
| | Male | No.18 | 33.3% | No.16 | 34.8% | No.25 | 50.0% | |
| BMI | Normal (18.5-24.9) | No.20 | 37.0% | No.14 | 30.4% | No.50 | 100% | |
| | Over weight (25-29.9) | No.20 | 37.0% | No.22 | 47.8% | No.0 | 0.0% | 0.552 |
| | Obese (=>30) | No.14 | 25.9% | No.10 | 21.7% | No.0 | 0.0% | |
| Serum OGF | Mean±SD | 942.855±132.312 | | 952.228±137.805 | | 1046.642±63.605 | | 0.0001 |
| Saliva OGF | Mean±SD | 975.081±66.973 | | 942.641±112.381 | | 880.059±87.700 | | 0.0001 |

Distribution of parameter between studied groups in multiple sclerosis: The Distribution of parameter's concentrations, such as ( Serum OGF and Saliva OGF) between studied groups in multiple sclerosis ,as well as the control that showed a normal distribution function, since accounted for levels of significance through using students-t-testing at (P>0.05).(Table 3).

Table 3 Distribution of serum and saliva OGF parameter concentration between studied groups in multiple sclerosis

| **Concentration** | **Groups** | **No.** | **Mean** | **SD** | **SE** | **P-value compared to control** |
|---|---|---|---|---|---|---|
| Serum OGF (ng/ml) | MS without treatment | 54 | 942.855 | 132.312 | 0.037 | 0.0001 (HS) |
| | MS treatment | 46 | 952.228 | 137.805 | 0.041 | 0.0001 (HS) |
| | Control | 50 | 1046.642 | 63.605 | 0.031 | |
| Saliva OGF (ng/ml) | MS without treatment | 54 | 975.081 | 66.973 | 0.040 | 0.0001 (HS) |
| | MS treatment | 46 | 942.641 | 112.381 | 0.057 | 0.003 (HS) |
| | Control | 50 | 880.059 | 87.700 | 0.035 | |

Regarding Serum OGF concentration in the (table 3) , a very large difference between both studied groups and control (p=0,001).
As for Saliva OGF, there is also a very big difference between the early diagnosis group without treatment and the healthy control at (p=0.0001). In addition, there is a very big difference between the early treatment group and normal control at (p=0.003 ).

Correlation of BMI with the biomarker of the groups allocated to the study: Table (4) shows Person's correlation coefficients between BMI and the biomarker of the groups allocated to the study about MS disease with comparison of statistical significance.

Table 4 The comparable levels for BMI and biomarker with comparisons of statistical significance

| **MS without treatment (n=54)** | | **OGF (ng/ml)** | |
|---|---|---|---|
| | | **Serum** | **Saliva** |
| BMI (Kg/m2) | Pearson Correlation | 0.351** | -0.259 |
| | P-value | 0.009 | 0.059 |
| MS with treatment (n=46) | | Serum | Saliva |
| BMI ($Kg/m^2$) | Pearson Correlation | -0.336* | -0.048 |
| | P-value | 0.022 | 0.753 |
| ****Correlation is significant at 0.01 level. *Correlation is significant at 0.05 level.** | | | |

Regarding body mass index (BMI) and biomarker of multiple sclerosis patients groups, the current study contained a statistical significance at p <0.05 in the early diagnosis group for multiple sclerosis patients with treatment, where the results showed an important correlation between the body mass index and the serum OGF level (p = 0.022) ,while in the early diagnosis group for multiple sclerosis patients without treatment, a very significant association was found between BMI and serum OGF level (p = 0.009).

**4. Discussion**

This study showed that the number of MS female patients was more than males (36, 66.7%vs. 18, 33.3% in early diagnosis MS without treatment group , consecutively),whereas ( 30, 65.2% vs. 16, 34.8% in the treated group , consecutively). Which were almost similar to a study in 2015 (66.67% vs. 33.33% respectively) [9] and as well as, it was similar to other studies [12] . In 2018 the prevalence of female MS was (64.9%) vs. males(35.1%)[ 9] . It was also compatible with a study (2019) (60.0% vs. 40.0%, respectively) [11] . The majority of patients with

multiple sclerosis in this study were in the age group (30-39) years for both groups, It is compatible with[9]. The Present data reveals that serum OGF in patients with multiple sclerosis in both groups had less concentration than the control group. whereby serum and saliva level showed a very big significant difference at (p=0.0001) for both groups in addition to normal control. This proved that Saliva( OGF) biomarker will be a pointer for diagnosing MS and the disease positively concerning early diagnosis MS without treatment. The role of the OGF is still in infancy , which made it the starting point for new studies in autoimmune diseases, especially in its role in multiple sclerosis. no confirmatory data for the low levels of OGF, which is one of the clear signs of autoimmune diseases, which led to an increasing number of studies and research on this [7].

It is controversial that most research has focused on the relationship of obesity in multiple sclerosis, especially when the role and characteristics of fat tissue in autoimmune diseases were discovered, which constitute 50% of the total percentage of body mass. That is why the relationship between multiple sclerosis and obesity became more concerned. Adipose tissue produces a group of molecules called adipokines. These molecules contribute to important activities such as immunity and inflammation, which makes obesity a risk factor for developing multiple sclerosis. But obesity itself does not impact the severity and development of the disease [13] .Whereby, in 2020 another study reached the conclusion of no correlations between obesity and the level of disability in MS even though these indicators did not indicate accurate evidence on this topic [14].

One of the causes of obesity is the presence of a metabolic defect in energy. Obesity results from a defect in energy metabolism, as the central nervous system has a major role in controlling and sensing the energy of the organism and the balance of the entire body's energy, and the nervous system controls the behavior of nutrition and its metabolism, As obesity is one of the main causes of MS disease, and this in turn stimulates OGF, which are molecules produced by the brain and are neuropeptides, which contain a set of functions related to nerves, that are often called neuroimmune modifiers or neurotransmitters, and this explains the role of OGF in MS disease [15,16] .

## 5. Conclusion

These results Indicates that serum OGF levels decreased in MS patients compared to normal individuals. It may be used as an indicator to prevent toxicity that may lead to the secretion of inflammatory cytokines and markers that may facilitate demyelination and neurodegeneration. It is also considered a biomarker of the onset of MS. Thus, the study focused on giving an impression of the level of OGF for MS patients, as well as studying the relationship with the body mass index (BMI) for patients with multiple sclerosis.

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