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# Demographic Characteristics of Glial Fibrillary Acidic Protein (GFAP) in Patients with Multiple Sclerosis, Karbala Governorate, Iraq

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**Annotation:** (MS) is a chronic autoimmune disease that results in damage to the myelin sheath covering nerve cells and the central nervous system.The (GFAP) is uniquely found in astrocytes in the central nervous system and non-myelinating Schwann cells in the peripheral nervous system,and it is assumed to be a biomarker for astrocytic damage in multiple sclerosis patients. The aim of this study was to investigate the demographic characteristics of serum levels of (GFAP) among a group of Iraqi patients with (MS) and a group of healthy individuals.The study included 90 samples,(45) patients with multiple sclerosis in stages (1, 2, 3), and (45) healthy individuals and GFAP levels in serum were studied using the ELISA immunoassay.The results showed that despite the slightly increased levels of GFAP in the patient group with age, the difference was not statistically significant (P=0.408). The average GFAP levels in the male patient group were higher than in females with a value of (P=0.061), which is slightly above the level of statistical significance (P=0.05).

There is a statistically significant increase in GFAP levels, and the data indicated that GFAP levels gradually increase with the duration of the disease, suggesting an exacerbation of multiple sclerosis over time with a value of (P=0.004).Conclusion: These results support the idea that the increase in GFAP is a condition of the disease rather than age in these samples, and that BMI category does not significantly affect GFAP concentrations in the patient group. This supports the idea that the increase in GFAP levels is independent of body weight in this study. Regarding gender, although the difference is not statistically significant, it indicates a trend towards increased GFAP, suggesting that a larger sample size may confirm statistical significance. GFAP may be a biomarker for disease progression, especially in chronic or long-term cases.

## 1. INTRODUCTION

Multiple sclerosis (MS) is a long-term autoimmune disease that occurs due to inflammation in the central nervous system, leading to the removal of the myelin sheath surrounding nerve cells and damage to nerve fibres. It is characterized by multiple lesions in the brain and spinal cord and is more common in women than in men, particularly in younger ages ranging from 20 to 40 years [1].Multiple sclerosis (MS) is known to have several different degrees of severity according to McDonald's diagnostic criteria of 2017, but the two main types are: relapsing-remitting multiple sclerosis (RRMS)[2], where the frequency of relapses often decreases, yet there is deterioration leading to continuous progression of the disease, referred to as secondary progressive multiple sclerosis (SPMS). The latter, where the disease progresses from the onset, is called primary progressive multiple sclerosis (PPMS)[3]. The main environmental risk factors associated with multiple sclerosis in observational studies include obesity, vitamin D deficiency, Epstein-Barr virus infection, and smoking. Because modifying these environmental and lifestyle factors may allow for prevention, it is important to identify the causal links between these factors and multiple sclerosis [4]. Studies conducted on animals and humans have shown that there is increasing evidence that environmental factors and lifestyle factors are not only important in triggering multiple sclerosis but are also involved in the development of multiple sclerosis, such as reduced exposure to sunlight and vitamin D deficiency [5].Studies indicate that the causes of the disease have not been fully identified and remain unknown, but they are likely to involve a combination of genetic predisposition and non-genetic triggers linked to various environmental risk factors that contribute to an increased risk of developing multiple sclerosis [6]. There are many biomarkers that help distinguish between types of multiple sclerosis and other neurological diseases, and the

Glial Fibrillary Acidic Protein (GFAP) is not just a marker of neuronal damage but is an active factor involved in the inflammatory processes of multiple sclerosis. There is a growing body of research on GFAP as a biomarker for the progression of multiple sclerosis.The aim of this study was to investigate the demographic characteristics of glial fibrillary acidic protein (GFAP) levels in serum among a group of Iraqi patients with multiple sclerosis and a group of healthy individuals in the holy province of Karbala. The study included 90 samples, with (45) patients suffering from multiple sclerosis at stages (1, 2, 3) and (45) healthy individuals. One of the significant findings of this study is that GFAP, along with other astrocytic markers, was more strongly associated with disease progression in multiple sclerosis than markers of microglial cells or immune cell activation. This sharply highlights the potential pivotal role of astrocytes in the biology of progressive multiple sclerosis [7].

## 2. METHOD

A descriptive and analytical study was conducted involving 90 samples from the Imam Hussein (PBUH) Medical City in the holy province of Karbala, Iraq. This included 45 patients with multiple sclerosis and 45 healthy individuals from the same geographical area, including the city center and surrounding villages, aged between 18 to 60 years. The criteria for inclusion of multiple sclerosis were determined according to the standard criteria for multiple sclerosis and the recommended diagnostic criteria for multiple sclerosis (McDonald criteria). Three milliliters of blood samples were collected in gel tubes, and after serum separation using a centrifuge, they were stored at a temperature of -20 degrees Celsius. According to the ready kit, a precise quantitative detection of Human glial fibrillary acidic protein was performed using the ELISA Kit.

Samples must be prepared from stored serum at -20°C, and they should reach room temperature before starting. Then, a precise quantitative detection of the glial fibrillary acidic protein (GFAP) should be conducted using the ready-to-use Human glial fibrillary acidic protein ELISA kit. After that, the reagent preparation should begin according to the protocol provided with the kit, and all reagents must reach room temperature before use. After preparing all the detectors, standard solutions, and samples, all components are brought to room temperature before use, as the testing is conducted at room temperature. Subsequently, we determine the number of strips required for the analysis and place them in the designated mold for use.

### 2. 1. STATIC ANALYSIS

The aggregated data in this study were collected from patient records and analyzed using the Statistical Package for Social Sciences (SPSS) version 2. The data were presented as frequencies and percentages or means and standard deviations in appropriate tables and graphs. An independent t-test was used, in addition to ANOVA with post hoc analysis where applicable, to uncover potential relationships between relevant variables in the current study, such as Bonferroni correction. The chi-square ($\chi^2$) test was also used to assess the distribution of reported symptoms among patients. A multiple response test was conducted to evaluate the percentage of responses to symptoms. Multiple linear regression and simple logistic regression were performed to determine the effect of genetic variation on the parameter under study. Statistical correlation was considered significant when the p-value was equal to or less than 0.05 ($p \leq 0.05$).

## 3. RESULTS AND DISCUSSION

In table number (1), the distribution of gender in the control group is as follows: 60% females and 40% males, while in the patient group: 62.2% females and 37.8% males. It is noticeable that the gender distribution is similar in both groups. Regarding age categories, patients tend to be younger: 26.7% of them are in the age category of 20-30 years compared to 15.6% in the control group. Additionally, the majority in both groups are aged between 31-40 years, while there are fewer patients in the age category of 41-57 years (24.4%) compared to the control group (42.2%). As for the categories of the Body Mass Index (BMI), the overweight category is the most common in both groups (about 45%). Meanwhile, obesity is slightly higher in the control group (20%)

compared to the patients (15.6%). Regarding social status, most participants are married, with a slightly higher number of single individuals in the control group (20%) compared to the patients (8.9%). It is worth noting that the biomarker GFAP (glial fibrillary acidic protein), which is divided into two categories: normal (<4) ng/ml, where the table shows that 88.9% of those in the control group have normal levels compared to 57.8% of the patients. In the second category (abnormal, >4 ng/ml), the rate is 11.1% in the control and 42.2% in the patients, indicating that elevated GFAP is more common in patients, suggesting the presence of diseases in the central nervous system. To clarify the other criteria in Table (1), the patient group shows higher rates of hypertension, diabetes, and abnormal GFAP levels, and most patients are in the second stage of the disease, with comorbid clinical conditions present in a significant percentage of.

**Table 1. Descriptive statistics for Socio-Demographic data between patient (n=45) and control group (n=45)**

| | | Control n (%) | Patients n (%) |
|---|---|---|---|
| Sex | Male | 18(40.0) | 17(37.8) |
| | Female | 27(60.0) | 28(62.2) |
| Age (Year) | 20-30 | 7(15.6) | 12(26.7) |
| | 31-40 | 19(42.2) | 22(48.9) |
| | 41-57 | 19(42.2) | 11(24.4) |
| BMI Body Mass Index | Underweight | 1(2.2) | 0(0) |
| | Normal | 14(31.1) | 18(40.0) |
| | Overweight | 21(46.7) | 20(44.4) |
| | Obese | 9(20.0) | 7(15.6) |
| Marital status | Not married | 9(20.0) | 4(8.9) |
| | Married | 36(80.0) | 41(91.1) |
| Duration of disease discovery (months) | 2-24 | - | 18(40.0) |
| | 25-48 | - | 24(53.3) |
| | 49-65 | - | 3(6.7) |
| Residency | City | 31(68.9) | 32(71.1) |
| | Rural | 14(31.1) | 13(28.9) |
| Hypertension | Yes | 0(0.0) | 13(28.9) |
| | No | 45(100.0) | 32(71.1) |
| DM | Yes | 0(0.0) | 11(24.4) |
| | No | 45(100.0) | 34(75.6) |
| Smoking status | Yes | 1(2.2) | 10(22.2) |
| | No | 44(97.8) | 35(77.8) |
| Stage of disease | 1st | - | 10(22.2) |
| | 2nd | - | 27(60.0) |
| | 3rd | - | 8(17.8) |
| GFAP ng/ml | Normal <4 | 40(88.9) | 26(57.8) |
| | Abnormal >4 | 5(11.1) | 19(42.2) |

In Table number (2), There is a statistically significant increase in GFAP levels with an increase in the duration of the disease. Specifically, patients in group C (49-65 months) have significantly higher levels of GFAP (6.65 nanograms/ml) compared to group B (4.60 nanograms/ml), with a p-value of 0.004.

**Table 2. Association between duration of disease discovery and GFAP levels in patients.**

| Parameter | A (2-24) | B (25-48) | C (49-65) | MC | P value |
|---|---|---|---|---|---|
| GFAP Patients | 3.64±0.92 | 4.60±1.66 | 6.65±1.26 | C vs B | 0.004 |

One-way ANOVA test was used with a significant p value of less than 0.05 Results are presented as mean ± SD

The aim of this study was to examine the demographic characteristics of GFAP levels in patients diagnosed based on McDonald criteria for multiple sclerosis and to understand its potential role in early diagnosis of the disease and in differentiating between the various types of multiple sclerosis. This study found that the duration of the disease is the strongest and most statistically significant factor in predicting elevated GFAP, with a value of (P = 0.006), which supports the notion that GFAP increases with disease progression [8]. A odds ratio of 1.070 means that for each additional month since the disease was discovered, the odds of the outcome increase by 1.070 times or 7%. The 95% confidence interval (1.017 – 1.126) indicates statistical significance, with a value of (p = 0.010). It confirms that this is a statistically significant predictive factor ($p < 0.05$).

The duration of disease discovery is a statistically significant predictive factor for the outcome, with an increasing duration of the disease showing a gradual and statistically significant increase in the likelihood of associated events, such as elevated GFAP levels or the severity of clinical symptoms [9]. This supports the idea that a longer duration of the disease is associated with an exacerbation of pathological changes, which is consistent with research conducted by [10],[11]. Moreover, gender is also a statistically significant predictive factor, with females showing significantly lower levels of GFAP compared to males by (-1.046 nanograms/mL), with a value of (P = 0.019). However, age and BMI were not statistically significant predictive factors.

## 4. CONCLUSION

The results support the idea that the increase in GFAP is related to the condition of the disease rather than age, and the BMI category does not significantly affect GFAP concentrations in the patient group, which supports the notion that the increase in GFAP levels is independent of body weight. Regarding sex, although the difference is not statistically significant, it indicates a trend towards elevated GFAP, suggesting that a larger sample may confirm the statistical significance. GFAP could be a biomarker for disease progression, especially in chronic or long-term cases.

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