

Evaluation of the levels of nerve growth factor (NGF), activating transcription factor 3 (ATF3), and some biochemical variables in the blood serum of patients with multiple sclerosis

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Abstract:

This research aimed to evaluate the serum levels of Nerve Growth Factor (NGF), Activating Transcription Factor 3 (ATF3), Vitamin D3, and lipids in individuals diagnosed with multiple sclerosis (MS) as potential biomarkers for the disease. The study involved 90 serum samples obtained from 34 males and 56 females and divided into two groups: a patient group of 60 samples (41 females and 19 males) from individuals with MS, and a control group of 30 samples (15 females and 15 males) from apparently healthy individuals. The concentrations of NGF, ATF3, and Vitamin D3 were measured by ELISA and lipid profile via spectrophotometric methods. All were evaluated in the blood of both patient and control cohorts.

The study's findings indicated that the serum concentrations of NGF, total cholesterol, triglycerides, very low-density lipoprotein, and low-density lipoprotein were significantly elevated ($P < 0.01$) in the patient cohort. No substantial fluctuations were observed in the levels of high-density lipoprotein. The study revealed a significant decrease ($P < 0.01$) in serum levels of ATF3 and vitamin D3 in the MS group relative to the healthy group. The results suggest distinct alterations in the levels of NGF, ATF3, Vitamin D3, and the lipid profile in MS patients, which may highlight novel pathological aspects and potential biomarkers for the disease. This study revealed profound biochemical aberrations in Multiple Sclerosis patients, where elevated NGF and dysregulated Vitamin D3 and lipid profiles signify neuroinflammatory and metabolic disturbances. It confirmed these parameters, particularly Vitamin D3 and ATF3, as potent diagnostic biomarkers of disease activity.

Keywords: Nerve Growth Factor, Activating Transcription Factor 63, Vitamin D3, lipid profile, multiple sclerosis.

تقييم مستويات عامل نمو الأعصاب، وعامل النسخ المنشط 3 ، وبعض المعايير الكيميائية الحيوية في مصل دم مرضى التصلب المتعدد

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مستخلص:

هدف هذا البحث إلى تقييم مستويات المصل لكل من عامل نمو الأعصاب (NGF)، وعامل النسخ المنشط 3 (ATF3)، وفيتامين د3 (Vitamin D3)، والدهون لدى الأفراد الذين تم تشخيصهم بمرض التصلب المتعدد (MS) باعتبارها مؤشرات حيوية محتملة للمرض. شملت الدراسة 90 عينة مصل تم الحصول عليها من 34 ذكراً و56 أنثى، وقُسمت إلى مجموعتين: مجموعة المرضى وتضم 60 عينة (41 إناث و19 ذكور) من المصابين بالتصلب المتعدد، ومجموعة ضابطة (مجموعة سيطرة) تضم 30 عينة (15 إناث و15 ذكور) من الأفراد الأصحاء ظاهرياً.

تم قياس تراكيز كل من NGF، ATF3، وفيتامين د3 باستخدام تقنية الإليزا (ELISA)، بينما تم قياس صورة الدهون (Lipid Profile) باستخدام الطرق الطيفية الضوئية. وقد تم تقييم جميع هذه المعايير في دم كل من مجموعتي المرضى والمجموعة الضابطة.

أشارت نتائج الدراسة إلى أن تراكيز المصل لكل من عامل نمو الأعصاب (NGF)، والكوليسترول الكلي، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة جداً (VLDL)، والبروتين الدهني منخفض الكثافة (LDL) كانت مرتفعة بشكل معنوي ($P < 0.01$) في مجموعة المرضى. ولم تلاحظ تقلبات جوهرية في مستويات البروتين الدهني عالي الكثافة (HDL). كما كشفت الدراسة عن انخفاض معنوي ($P < 0.01$) في مستويات المصل لكل من ATF3 وفيتامين د3 في مجموعة مرضى التصلب المتعدد مقارنة بالمجموعة الصحية.

تشير النتائج إلى وجود تغييرات واضحة في مستويات NGF، ATF3، وفيتامين د3، وصورة الدهون لدى مرضى التصلب المتعدد، مما قد يساهم في الضوء على جوانب مرضية جديدة ومؤشرات حيوية محتملة للمرض. كشفت هذه الدراسة عن اختلالات كيميائية حيوية عميقة لدى مرضى التصلب المتعدد، حيث يشير ارتفاع NGF واضطراب مستويات فيتامين د3 وصورة الدهون إلى اضطرابات التهابية عصبية وأيضية. وقد أكدت الدراسة أن هذه المعايير، وخاصة فيتامين د3 وعامل النسخ المنشط 3 (ATF3)، تُعد مؤشرات حيوية تشخيصية قوية لنشاط المرض.

الكلمات المفتاحية: عامل نمو الأعصاب، عامل النسخ المنشط 3، فيتامين د3، صورة الدهون.

Introduction

Multiple Sclerosis (MS) is a chronic, autoimmune, inflammatory neurological disease that affects the Central Nervous System (CNS). It typically manifests between the ages of 20 and 40 and is more prevalent in women (Talebi et al., 2021). Mechanistically, mononuclear phagocytes are a key pathogenic element, initiating and sustaining inflammation within the CNS (Koliada et al., 2020).

Epidemiologically, the highest prevalence rates are reported in Northern Europe and North America (Khan & Hashim, 2025). Iraq specifically reports a rising incidence, with the relapsing-remitting form (RRMS) being the most common, often presenting initially with visual symptoms (Hassoun et al., 2021). MS is a significant global cause of non-traumatic disability in young adults (Gashi et al., 2024).

The hallmark of MS pathology is the formation of inflammatory lesions within the white matter of the CNS, particularly in periventricular regions, the optic nerve, and the spinal cord (Moise & Friedman, 2020). These lesions are

characterized by inflammation, reactive gliosis, demyelination, and subsequent neuroaxonal degeneration resulting from the loss of myelin-producing oligodendrocytes (Garton et al., 2024).

Nerve Growth Factor (NGF), a quintessential neurotrophin, is a dimeric protein that is crucial for the development, survival, differentiation, and maintenance of specific neuronal populations, notably including peripheral nociceptive sensory neurons and sympathetic neurons, as well as central cholinergic neurons located in the basal forebrain (Capossela et al., 2024; Danos et al., 2025). The biological activities of NGF mainly occur through its strong connection with the Tropomyosin receptor kinase A (TrkA) and are influenced by the weaker p75 neurotrophin receptor (p75NTR) (Franco et al., 2021; Matusica et al., 2013). Besides helping with nerve development, NGF is important for adult nerve changes, healing of nerve fibres, and adjusting how sensitive nerves are, including controlling neuropeptides involved in pain and inflammation (Barker et al., 2020; Zhao et al., 2016). Post-tissue injury, we often observe the upregulation

of NGF (Lee et al., 2021), which may have both reparative (Minnone et al., 2017) and, in some instances, pro-nociceptive effects (Barker et al., 2020).

The activating transcription factor/cAMP response element-binding (ATF/CREB) family, which includes activating transcription factor 3 (ATF3), controls gene activity and helps cells deal with stress (Liu et al., 2024). When cells have problems such as endoplasmic reticulum stress, oxidative stress, or inflammation, the levels of ATF3 exhibit a rapid increase (Kan et al., 2021; Tanaka et al., 2011). ATF3 is a central component in cellular stress response networks that regulate apoptosis, inflammation, metabolism, the cell cycle, and tissue regeneration (Kan et al., 2021; Liu et al., 2024). ATF3 regulates gene function by binding to DNA sequences, such as the cAMP Response Element, and forming partnerships with itself or other bZIP proteins (Fawcett et al., 1999).

Vitamin D3 is considered the active form that regulates calcium and phosphate levels, impacting skeletal health (Charoenngam et al., 2019), the immune system (Durrant et al., 2022),

and the neurological system (Sailike et al., 2024). Individuals with insufficient vitamin D levels, often due to inadequate sunlight exposure, exhibit a heightened susceptibility to MS due to a deficiency that may impair immune system functionality by hindering cerebral defence and regulating inflammation (Ao et al., 2021; Balasooriya et al., 2024).

Lipids are essential for the proper functioning of the nervous system, as they form cellular membranes and myelin sheaths (Benjamins et al., 2012). Cholesterol is essential for the stability of myelin (Saher et al., 2011), while triglycerides (TG) function as a principal energy source for the organism (Sharma et al., 2023). Modifications in lipid metabolism, particularly within pathways linked to arachidonic acid, can initiate inflammation and degradation of myelin in individuals with MS (Broos et al., 2024). The investigation into the influence of total lipid concentrations on MS remains an area of active research (Lőrincz et al., 2024). So, the current study investigates the level of nerve growth factor, activating transcription factor 3, and lipid profile in

the sera of patients with multiple sclerosis.

Materials and methods

Subject: The investigation encompassed a cohort of sixty Iraqi participants (19 males and 41 females), aged between 18 and 40, who had been clinically diagnosed with multiple sclerosis. These subjects sought medical attention at the MS unit within Dr Saad Al-Watari Hospital for neurosciences from August 1st to October 1st, 2024. Furthermore, 30 samples were procured from healthy individuals as a control group (comprising 15 males and 15 females) whose ages ranged from 20 to 35 years.

Methods: This study uses a sandwich-ELISA kit manufactured by Sun Long Biotech to estimate serum NGF, ATF3, and Vitamin D3 levels. Concurrently, lipid profile parameters, including Total Cholesterol (TC), Triglycerides, High-Density Lipoprotein (HDL), were determined using spectrophotometric assay kits supplied by Linear Spainm, while the level of Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were calculated using Friede-

wald's equation (Matas et al., 1994)

Statistical examination SPSS was employed to ascertain the changes in biochemical parameters between the MS patient and control groups, utilising the T-test at a significance level of ($P < 0.05$). The significant parameters were investigated using the receiver operating characteristic (ROC) curve to evaluate their diagnostic accuracy. A P-value of less than 0.05 was considered statistically significant.

Results

The study involved determining the serum levels of NGF, ATF3, Vitamin D3, and lipid profiles of patients with MS and controls. The results obtained are summarized in Table 1.

Table 1: Serum biochemical parameter levels in multiple sclerosis patients compared to the control group

Parameters	Control Mean \pm SD	Patients Mean \pm SD	$p \leq$
NGF pg/ml	501.065 \pm 143.36	584.805 \pm 122.28	0.05
ATF3 pg/ml	773.745 \pm 243.085	540.885 \pm 179.1	0.05
VD3 ng/ml	50.55 \pm 18.73	35.48 \pm 5.55	0.05
TC mg/dl	163.663 \pm 22.052	189.265 \pm 18.378	0.05
TG mg/dl	116.863 \pm 22.792	139.030 \pm 21.821	0.05
HDL mg/dl	47.587 \pm 8.495	44.942 \pm 8.627	NS*
LDL mg/dl	92.704 \pm 23.220	116.517 \pm 19.893	0.05
VLDL mg/dl	23.373 \pm 4.558	27.806 \pm 4.364	0.05

Note: *NS=Non-significant

Table 1 showed that the levels of NGF, TC, TG, LDL, and VLDL ($P \leq 0.05$) were significantly elevated in the sera of the patient group with MS. In contrast, the ATF3 and Vitamin D3 levels significantly ($P \leq 0.05$) decreased with the non-significant difference in

HDL level in the sera of patients compared to the control group.

The study also used ROC curve analysis to evaluate the accuracy of NGF, ATF3, and other biochemical parameters under investigation. The results are summarised in Table 2.

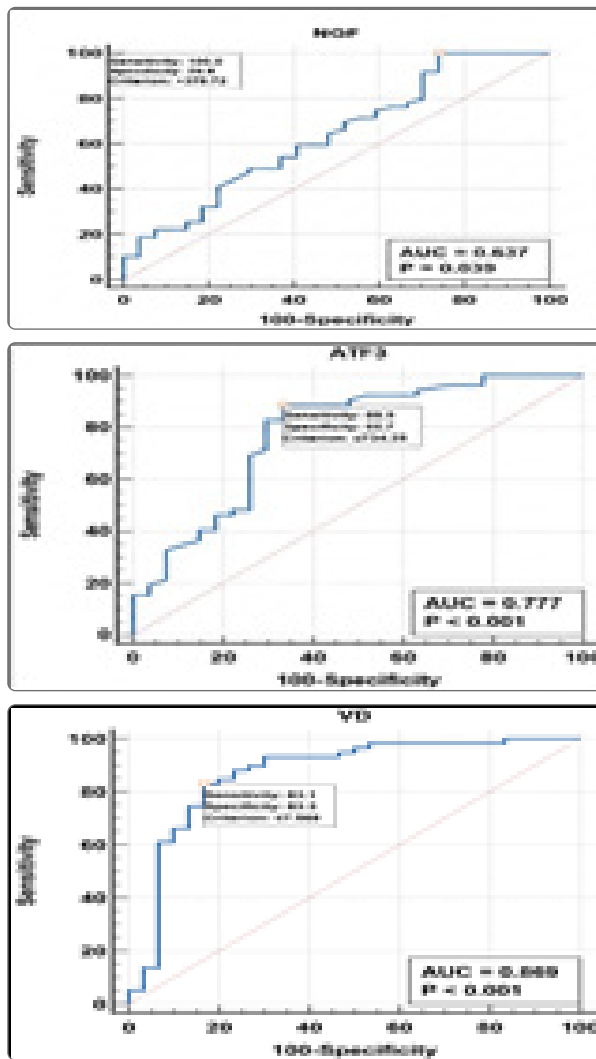
Table 2: AUC, cut-off values, sensitivity, and specificity for biochemical parameters by ROC analysis

Parameters	AUC	Cut-off value	p-value	Sensitivity	Specificity	Variables
NGF	0.637	>378.72	0.039	100%	25.93%	0.637
ATF3	0.777	\leq 734.35	<0.001	88.46%	66.67%	0.777
VD3	0.869	\leq 37.84	<0.001	83.05%	83.33%	0.869
TC	0.822	>166	<0.001	90.00	63.33	0.822
TG	0.756	>113.5	<0.001	90.00	50.00	0.756
HDL	0.581	\leq 37.6	0.2004	25.00	90.00	0.581
LDL	0.794	>104.5	<0.001	76.67	80.00	0.794
VLDL	0.756	>22.7	<0.001	90.00	50.00	0.756

Receiver operator characteristic analysis indicates that the accuracy of ATF3 (AUC 0. 777; $p \leq 0. 001$), VD3 (AUC 0. 869; $p \leq 0. 001$), TC (AUC 0. 822; $p \leq 0. 001$), TG (AUC 0. 756; $p \leq 0. 001$), LDL (AUC 0. 794; $p \leq 0. 001$), and VLDL (AUC 0. 756; $p \leq 0. 001$) were significant difference, with non-diagnostic significant difference

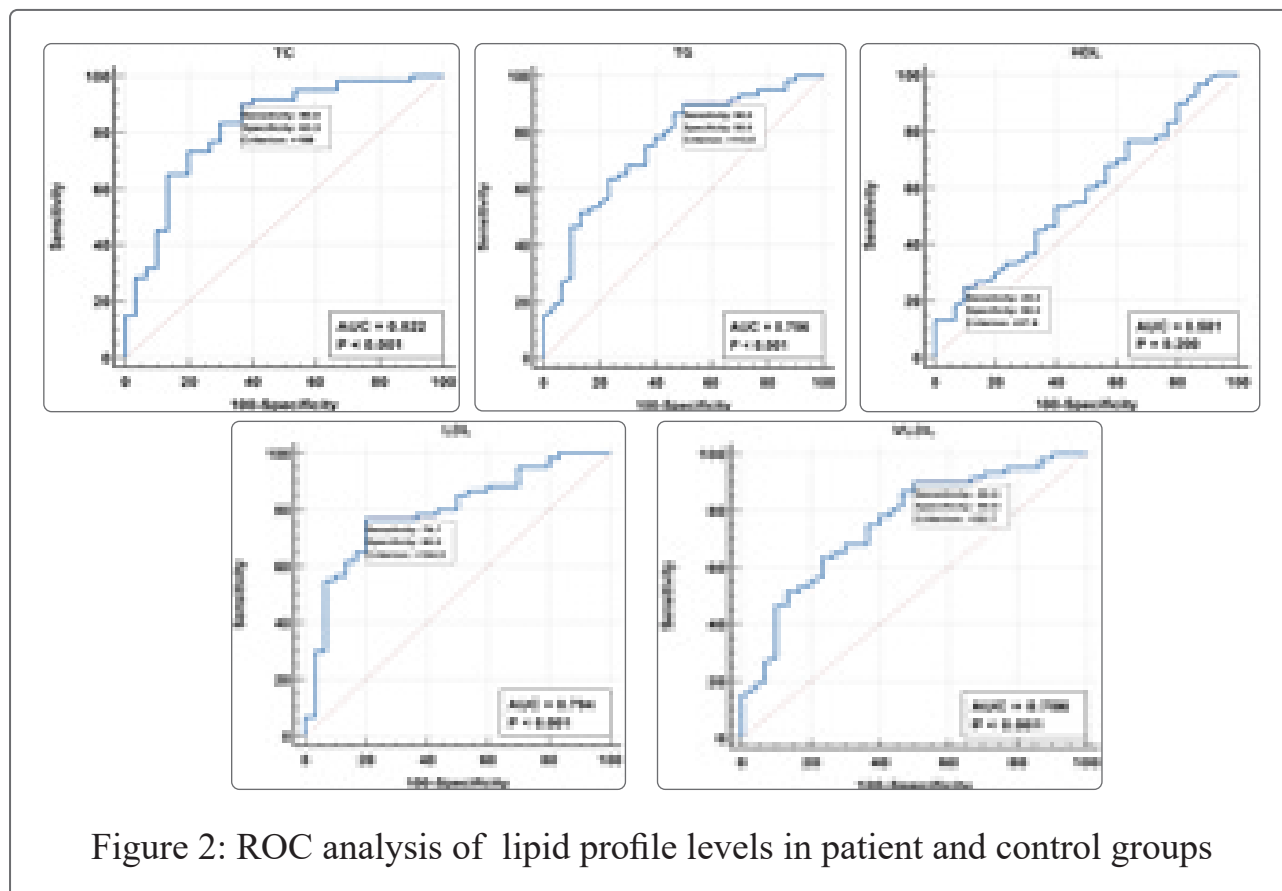
for NGF (AUC 0. 637; $p = 0. 039$), and for HDL (AUC 0. 581; $p = 0. 2004$).

The results from Table 2 and Figures 1 and 2 indicate that higher sensitivity values were reported for NGF (100%), TC (90%), TG (90%), VLDL (90%), ATF3 (88.46%), VD3(83.05%), and LDL (76.67%).



A
B
C

Figure 1: ROC analysis of NGF (A), ATF3 (B), and Vitamin D₃(C) levels in patient and control groups



Discussion

The markedly increased NGF shown in the MS cohort aligns with established literature identifying NGF as a biomarker indicative of neuroaxonal injury and active neuroinflammation. NGF exerts a dual influence in MS pathogenesis, concurrently modulating the immune response and promoting neural repair processes. It actively influences the function of immune cells, such as T and B lymphocytes, while supporting the survival and regeneration of neurons and oligodendrocytes

(Terracina et al., 2023). This observed increase likely stems from the dynamic regulation of NGF expression during neuroinflammation, where pro-inflammatory cytokines and immune cell interactions stimulate its production in glial cells, endothelial cells, and peripheral immune cells (Abe et al., 2007).

The study also revealed a significant reduction in serum levels of ATF3 among individuals diagnosed with MS (540.885 ± 179.1 ng/ml in MS vs. 773.745 ± 243.085 ng/ml in control

subjects; $P < 0.05$). This observed decrease in systemic ATF3 warrants exploration into complex biological and molecular mechanisms. MS susceptibility is heavily influenced by genetic factors, with hundreds of single-nucleotide polymorphisms (SNPs) identified. These SNPs frequently localize to regulatory regions, possessing the capacity to modulate the transcriptional activity of genes within key immune cell populations. For instance, MS-associated SNPs have been shown to regulate gene transcription in CD4⁺ T lymphocytes and monocytes, potentially influencing the expression profile of ATF3 (Gresle et al., 2020).

Another factor contributing to the reduced systemic ATF3 levels may be epigenetic modifications. DNA methylation, a critical regulator of gene expression in immune cells, is frequently altered in the CD4⁺ and CD8⁺ T cells of MS patients. While studies have demonstrated methylation-dependent changes in genes such as SH3YL1 (Fernandes et al., 2019), similar epigenetic mechanisms could contribute to decreased ATF3 expression by modifying its promoter methylation status.

Furthermore, long non-coding RNAs (lncRNAs) are recognised for their capacity to modulate gene expression epigenetically. The aberrant regulation of lncRNAs, including GSTT1-AS1 and IFNG-AS1, has been observed in MS (Hosseini et al., 2019). These lncRNAs impact immune responses and might indirectly affect ATF3 expression; for example, downregulation of GSTT1-AS1 has been linked to increased pro-inflammatory cytokines, which could indirectly modulate ATF3 levels (Ganji et al., 2019; Hosseini et al., 2019).

The concurrent finding of Vitamin D deficiency in this study's MS cohort provides an important context for the lowered ATF3 levels. Vitamin D receptor super-enhancers (VSEs) are known to be hotspots for MS risk variants, and they regulate the expression of various genes. Genes influenced by VSEs, such as GRINA and LRG1, exhibit varied expression in MS patients, suggesting a role for Vitamin D-gene interaction in disease activity (Orton et al., 2022). Although a direct association between ATF3 and VSEs has not been explicitly reported, environ-

mental factors like Vitamin D insufficiency may indirectly influence ATF3 expression through broader regulatory frameworks. Moreover, the chronic elevation of pro-inflammatory cytokines (IL-6, IL-17) characteristic of MS may directly impact the modulation of transcription factors like ATF3. The increased miR-155 observed in MS, which enhances Th17 cell differentiation and stimulates inflammatory responses, is one pathway that could potentially contribute to decreased ATF3 expression (Alipour et al., 2024).

Concerning Vitamin D3 levels, our investigation confirms markedly diminished serum concentrations in MS individuals compared to healthy controls. This observation is highly consistent with the extensive literature linking Vitamin D deficiency to an augmented risk of MS and heightened disease activity (Balasooriya et al., 2024). Vitamin D3 exhibits crucial immunomodulatory capabilities, primarily by suppressing pro-inflammatory Th1/Th17 cell populations and facilitating the proliferation of regulatory T-cells (Sheikh et al., 2018).

The MS patients in this study exhib-

ited a significantly altered lipid profile, characterised by higher serum levels of TC, TG, LDL, and VLDL compared with the control group. Conversely, no significant differences were found in HDL levels. This pattern of systemic dyslipidemia is increasingly recognised as an associated metabolic feature of MS (Noori et al., 2019; Wicks et al., 2024).

The observed increase in cholesterol components is directly relevant to MS pathology, as demyelination—a hallmark of the disease—leads to the breakdown of myelin, a structure inherently rich in cholesterol (Träger et al., 2020). The resulting release of cholesterol from damaged myelin can disrupt central and peripheral lipid homeostasis (Berghoff et al., 2017). Furthermore, the chronic pro-inflammatory state inherent to MS can independently induce dyslipidemia by perturbing hepatic lipid metabolism (Radiková et al., 2020). The high levels of oxidative stress prevalent in MS (Khalaf et al., 2024, and Jiménez-Jiménez et al., 2024) promote the oxidation of LDL, forming oxidised LDL (ox-LDL). This highly pro-inflammatory and atherogenic spe-

cies exacerbates demyelination (Zhang et al., 2020).

The current lipid profile—elevated TC, TG, LDL-C, and VLDL—points towards a systemic environment characterised by “metabolic inflammation.” While the quantity of HDL did not differ significantly, this finding does not exclude an underlying qualitative dysfunction, a phenomenon reported in other MS populations (Radiková et al., 2020). Such functional impairment would diminish HDL capacity to effectively counteract the detrimental effects of the other altered lipid species. Ultimately, this dyslipidemic state suggests that altered lipid species actively contribute to the MS inflammatory milieu, potentially impacting the integrity of the blood-brain barrier (BBB), immune cell activation, and even exerting direct neurotoxic effects.

The ROC analysis established the diagnostic utility of the measured parameters. Vitamin D3 demonstrated exceptional diagnostic capability, achieving the highest AUC value of 0.869 ($p < 0.001$). This result reinforces the widely documented strong inverse correlation between Vitamin D3 defi-

ciency and increased MS risk, establishing VD3 as the strongest biomarker evaluated in this study (Gombash et al, 2022; Giordano et al., 2025). A sensitivity of 83.05% was achieved at a cut-off value of ≤ 37.84 .

Activating Transcription Factor 3 (ATF3) also exhibited good diagnostic capability, with an AUC value of 0.777 ($p < 0.001$), suggesting that serum ATF3 levels could serve as a reliable MS biomarker. The high sensitivity of 88.46% achieved at a cut-off value of ≤ 734.35 ng/ml indicates its reliability as a screening tool for correctly identifying positive cases.

Although NGF showed a statistically significant diagnostic ability (AUC=0.637, $p=0.039$), its overall performance was inferior to ATF3 and VD3. However, the most notable diagnostic aspect of NGF was its ideal sensitivity of 100% at a cut-off value of > 378.72 . This finding suggests that NGF may be a highly valuable tool for ruling out MS (high Negative Predictive Value) when the level is low.

Regarding the lipid profile, TC (AUC=0.822) and TG (AUC=0.756) both showed very good diagnostic

capabilities ($p < 0.001$), with a high sensitivity of 90.00% for both, suggesting that their elevated levels are strong discriminatory indicators linked to MS. LDL-C (AUC=0.794) and VLDL (AUC=0.756) also demonstrated good diagnostic ability ($p < 0.001$). In contrast, HDL-C performed poorly (AUC=0.581, $p = 0.2004$), indicating that HDL quantity alone is not a reliable marker for MS in this population.

Conclusion

The present study demonstrated significant biochemical changes in patients with multiple sclerosis. The elevated levels of nerve growth factor, low levels of vitamin D3, and high levels of atherogenic lipid profile indicate the presence of neuroinflammation, immune dysregulation, and possible metabolic abnormalities in the development of MS. The significant reduction in activating transcription factor-3 suggests its possible role in disrupting stress response pathways and epigenetic dysregulation linked to MS.

These observations underscore the clinical relevance of NGF, ATF3, Vitamin D3, and lipid metabolism as crit-

ical potential biomarkers and factors profoundly influencing disease activity. Among the variables tested, Vitamin D3 emerged as the most powerful indicator, exhibiting the highest discriminative performance (AUC=0.869) between patients and healthy controls, which strongly validates its established crucial role in the disease context. Furthermore, ATF3, TC, TG, and LDL were also identified as highly useful and statistically robust diagnostic instruments.

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Ethical Considerations

This study was performed after receiving full ethical permission from the Scientific Committee in the Department of Chemistry, College of Education for Pure Sciences, University of Samarra. We confirmed that all stages of the research conformed with ethical guidelines, and specimens were collected from patients and healthy individuals only after obtaining informed written permission. We affirm that the participants' privacy and rights were fully maintained throughout the study, and data were collected and processed with the utmost confidentiality in preparation for publishing the research in a reputable scientific journal.

Informed Consent Statement

All subjects involved in the study gave informed consent, and the patients gave written informed consent to publish this paper.

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