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Evaluation of the levels of vimentin, neural cell adhesion molecule, C-X-C motif chemokine ligand 10, toll-like receptor 4, and some minerals in patients with multiple sclerosis

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Multiple sclerosis is a complex autoimmune disorder of the central nervous system and is believed to be caused by dysfunction of the immune system, characterized by inflammatory demyelination with axonal transection. Sixty blood samples were collected from patients (25 females and 35 males) suffering from multiple sclerosis (MS) who visited the MS consultant at Baghdad Teaching Hospital, affiliated to the Medical City. Patients were collected from different age groups ranging from 20–40 years. Forty samples (15 females and 25 males) were collected from healthy individuals as a control group. Several biochemical parameters were tested, such as vimentin, neural cell adhesion molecule-NCAM, C-X-C motif chemokine ligand 10 (CXCL10), toll-like receptor 4 (TLR4), magnesium, zinc in the patients' blood serum. The results of the current study indicated a significant increase in vimentin, NCAM, CXCL10, TLR4 concentration in the blood serum of the G1 group compared to the control group. Also, the result showed a significant low in Mg, Zn concentration in the blood serum of the G1 group compared to the control group. The results also showed a highly significant positive correlation between vimentin concentration and NCAM, TLR4, CXCL10, Mg, Zn in the blood serum of the healthy group, which reached correlation coefficients was 0.557, 0.848, 0.928, 0.633, 0.640, respectively. The results also showed that the area under curve value for the studied variables was excellent, as they can be considered important diagnostic variables for patients with multiple sclerosis.

Keywords: multiple sclerosis; vimentin; neural cell adhesion molecule; C-X-C motif chemokine ligand 10; toll-like receptor 4.

Introduction

Multiple sclerosis (MS) is a complex autoimmune disorder of the central nervous system, with unknown pathogenesis and a poorly understood nature (Mohammed, 2024). The disease usually begins between the ages of 20 and 40 years (Simonsen et al., 2020). Multiple sclerosis is the leading cause of disability in young and middle-aged people, and can cause significant economic and social burdens. This disease causes the body's immune system to attack the membrane that covers the axons of nerve cells in multiple locations, causing inflammation. At the site of inflammation, scars known scientifically as "sclerosis" form. The name multiple sclerosis refers to the presence of multiple spots of these sclerotic scars that appear on the nerve fibers (axons). This attack on nerve cells is an abnormal response, so inflammation of the membrane covering the axons of nerve cells leads to a defect in the transmission of nerve signals, which leads to a loss of the function of nerve axons in the brain, cerebellum, and spinal cord (Barkhof & Koeller, 2024). The causes of multiple sclerosis have not been definitively confirmed, but scientific research is ongoing worldwide to determine the exact reasons that prompt the immune system to attack the myelin sheath. The hypotheses that explain the occurrence of multiple sclerosis are summarized as follows: the immune system attacks the myelin sheath, and the myelin-producing cells fail to produce new sheaths. The disease often affects people with a genetic predisposition to infection and who have been exposed to factors, often environmental, that interact with this genetic predisposition, leading to the immune system being stimulated and attacking the myelin sheath (Oksenberg et al., 2008). Many biochemical and immunological variables are associated with MS patients, as inflammatory cytokines, especially in the upper fluid of mononuclear macrophages, are associated with MS patients (Koliada et al., 2025). Also, vimentin protein, which is one of the filamentous proteins, with a molecular weight of about 57 kDa it is associated with MS (Eriksson et al., 2009). It consists of an alpha-helix structure with a length of 310 acidic and basic amino acids (Perreau et al., 1988). Vimentin is the cellular component responsible for maintaining cell integrity and is the most widespread intermediate filament protein. It is the first to form during cell differentiation and is formed within a wide variety of mesenchymal cells (fibroblasts). In several other cell types derived from the mesoderm, vimentin is expressed. In lymphocytes and macrophages, it is produced in very small quantities. Vimentin is found in cells derived from mesoderm, such as Bowman's capsule in the kidney, endometrium, and ovary, and in the breast, sweat, salivary, and thyroid glands (Ivaska et al., 2007). Multiple sclerosis has also been associated with chemokines. CXCL-10 was discovered as a pro-inflammatory chemokine that regulates leukocyte migration and modulates innate and adaptive immune responses. It was identified in serum, synovial fluid, and synovial tissue and is involved in numerous biological processes and is essential for the inflammatory response (Abdulhakim et al., 2024). So, it was found that chemokines are involved in multiple sclerosis, including CXCL10 (Cui et al., 2020).

Cell adhesion molecules are glycoproteins expressed on the cell surface and have a major role in inflammation and tumors. Cell adhesion molecules and ligands that may play a role in pathological conditions have been discussed as potential therapeutic approaches that require regulating the expression of these molecules, as these molecules are essential for the functioning of the immune system in pathological and healthy conditions (Koh & Park, 2018). In multiple sclerosis, it has been found that many adhesion molecules within the central nervous system participate in inflammatory and neurodegenerative processes associated with progressive disability and increased brain atrophy. It has been found that the neural cell adhesion molecule participates in repair mechanisms and remyelination processes. Since multiple sclerosis is associated with many inflammatory and neurological processes, the current study aimed to evaluate the levels of neurofilament proteins, including vimentin, and the levels of some chemokines, including CXCL10, and adhesion molecules, including NCAM, and minerals in patients with multiple sclerosis. The study

also aimed to determine the correlation between vimentin levels and the biochemical variables studied in patients in addition to studying the area under the curve for the studied variables.

Materials and methods

The study was approved by the Scientific Committee of the Department of Applied Chemistry, College of Applied Sciences, Samarra University. All patients were informed of the study, and their informed consent was obtained; and patient confidentiality was ensured.

The present study involved the collection of sixty blood samples from patients (25 females and 35 males) suffering from multiple sclerosis who visited the MS consultant at Baghdad Teaching Hospital, affiliated to the Medical City. Patients were collected from different age groups ranging from 20–40 years, during the period from 1/12/2024 to 1/5/2025. All information about the patients' participating in the study was recorded in the questionnaire form. Most of the patients suffered from fatigue, weakness, loss of vision, and numbness in the extremities. The disease cases were diagnosed and confirmed by a committee of doctors specializing in multiple sclerosis at Baghdad Hospital. Forty samples (15 females and 25 males) were collected from healthy individuals who did not suffer from any diseases as a control group, and who had no history of infection or clinical evidence of possible infection with the disease or any other medical condition. Venous blood samples were taken from the study samples using a medical syringe with a volume of 5 mL. The drawn blood was placed directly in sealed tubes containing the gel and then left for 10 minutes at room temperature. In order to separate the blood and obtain the serum, it was centrifuged at a speed of 3000 rpm. After completing the separation process, the serum was withdrawn using a fine pipette and then distributed into three small Eppendorf tubes and stored in the refrigerator at –20 °C until biochemical tests were performed. Several biochemical parameters were tested, such as vimentin, NCAM, CXCL10, TLR4, Mg Zn in the two groups.

The study involved the determination of serum concentrations of vimentin, NCAM, CXCL10, TLR4 by using an enzyme-linked immunosorbent assay ELISA kit provided by Fine Test-China. The Mg levels were determined according to the kit prepared by the American company BioVision. Also, the Zn level was determined according to the kit prepared by the United Kingdom company Abcam.

SPSS V27 program was used to calculate the mean ± standard deviation (SD) of the studied biochemical variables between patients and healthy controls at a probability level of $P < 0.05$ using the F-test. The correlation between the vimentin protein and biochemical variables was also studied according to SPSS V27. The area under the curve was calculated using MedCalc V20 for biochemical variables, and the figures were drawn according to Graphpad Prism V.9.

Results

According to the findings of this study, the concentration of vimentin, NCAM, CXCL10, TLR4 was significantly increased in the group of MS compared to healthy people at $P < 0.05$ (Table 1 Fig. 1, 2).

Table 1
Biochemical parameters in all groups (mean ± SD)

Parameters	C	G1	P-value
Vimentin, ng/mL	93.6 ± 20.5	174.1 ± 28.9	<0.0001*
NCAM, pg/mL	82.5 ± 21.5	175.7 ± 32.4	<0.0001*
CXCL10, pg/mL	141.2 ± 21.8	205.6 ± 33.7	<0.0001*
TLR4, ng/mL	4.81 ± 1.11	9.06 ± 1.37	<0.0001*

Note: C – control group, G1 – patients group with multiple sclerosis, NCAM – neural cell adhesion molecule, CXCL10 – C-X-C motif chemokine ligand 10, TLR4 – toll-like receptor 4.

Also, the results indicate that the mineral levels, including Mg, Zn, were significantly decreased in the group of MS compared to healthy people at $P < 0.05$ (Table 2, Fig. 3).

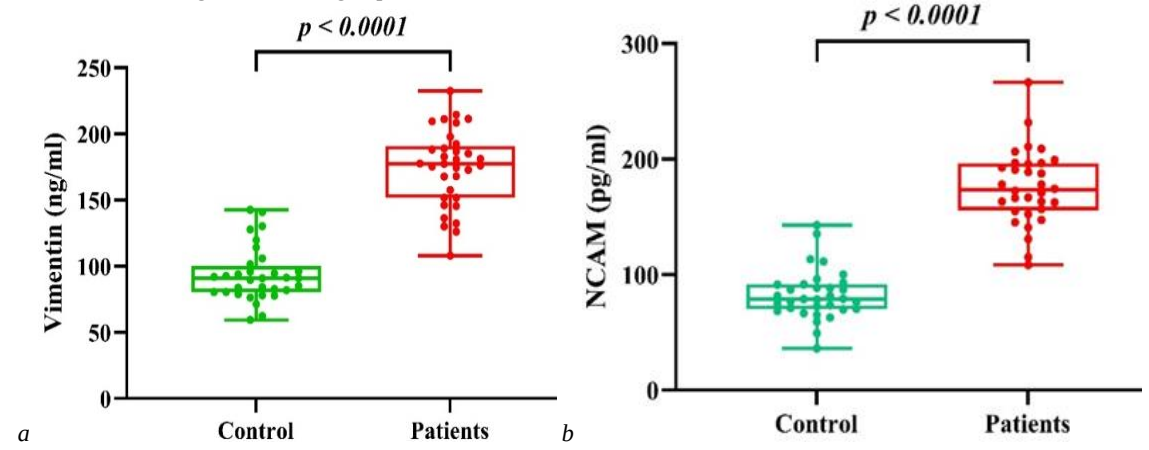


Fig. 1. Concentration of vimentin (*a*) and NCAM (*b*) in patients and healthy people

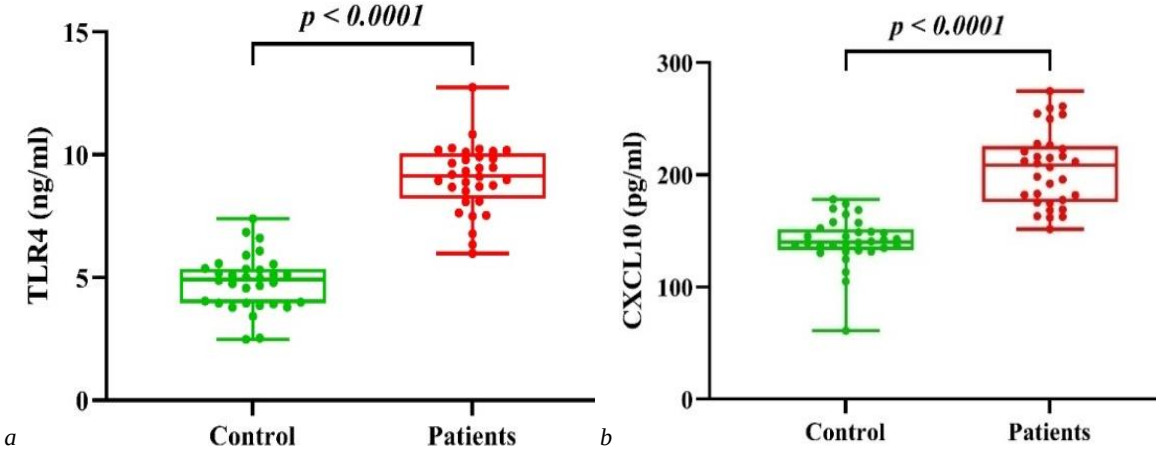
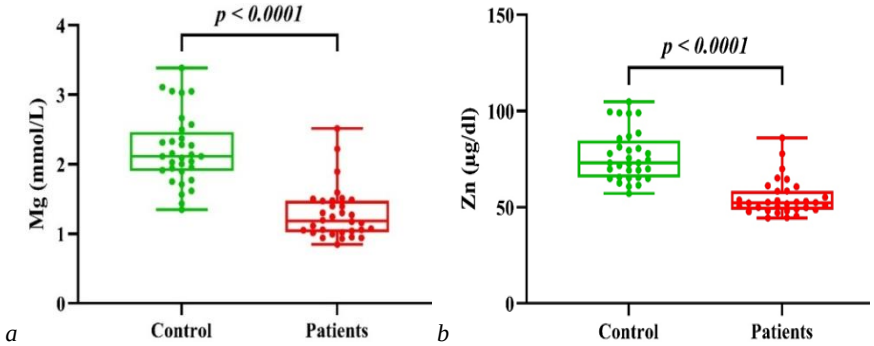


Fig. 2. Concentration of TLR4 (*a*) and CXCL10 (*b*) in patients and healthy people



a *b*

Fig. 3. Concentration of Mg (*a*) and Zn (*b*) in patients and healthy people

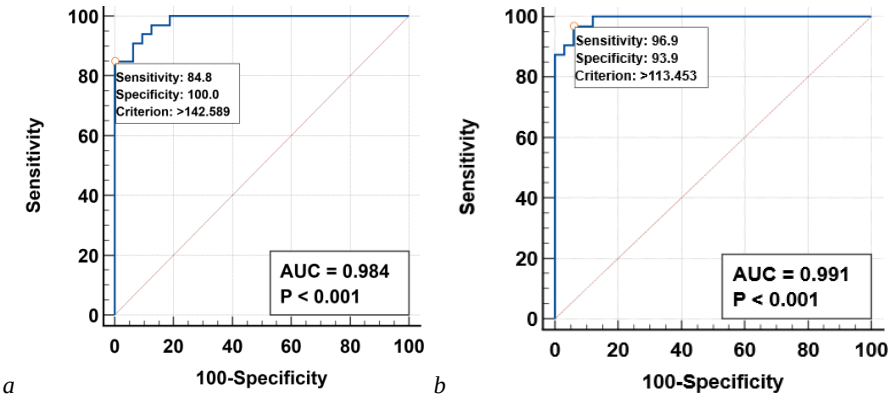
Table 2
Mineral level in all groups (mean ± SD)

Parameters	C	G1	P-value
Mg, mmol/L	2.20 ± 0.51	1.29 ± 0.37	<0.0001
Zn, µg/dL	76.5 ± 12.9	54.8 ± 9.4	<0.0001

Note: C – control group, G1 – patients group with multiple sclerosis, Mg – magnesium, Zn – zinc.

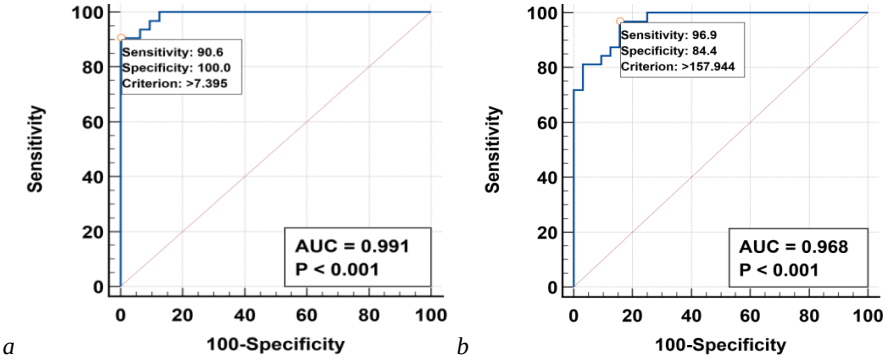
Figures 4 and 5 shows the potential diagnostic significance of serum vimentin, NCAM, CXCL10 and TLR4. The AUC value for vimentin was 0.984 (sensitivity of 84.8%) with specificity of 100.0% for MS (G1) vs. control group (C), while the AUC for NCAM was 0.991 (the sensitivity 96.9% and specificity 93.9%) in the group of patients with MS (G1) and control group (C). Also, the AUS for CXCL10 was 0.968 (sensitivity of 96.9%, specificity 84.4%) for MS (G1) vs control group (C), while the AUC for TLR4 was 0.991 (sensitivity 90.6% and specificity of 100.0%) in the group of patients with MS (G1) and control group (C). So the variables in this study may be considered to have high specificity and be important predictive indicators for diagnosing multiple sclerosis.

Table 3 shows the correlation of the effectiveness of the vimentin protein with the parameter studied, as a high positive significant correlation was found between vimentin level and NCAM, CXCL10, TLR4, Mg, Zn which reached of the correlation coefficient was 0.557, 0.848, 0.928, 0.633 and 0.640, receptivity in the control group, while a negative significant relationship was found between the vimentin and Mg level in the patient group, whose correlation coefficient was –0.453.



a *b*

Fig. 4. ROC of Vimentin (*a*) and NCAM (*b*) in patients and healthy people



a *b*

Fig. 5. ROC of TLR4 (*a*) and CXCL10 (*b*) in patients and healthy people

Table 3
Correlation (r) of vimentin with biochemical parameter

Parameters	Control		MS	
	r	P-value	r	P-value
NCAM	0.557**	0.001	0.172	0.346
CXCL10	0.848**	<0.001	0.197	0.280
TLR4	0.928**	<0.001	0.283	0.116
Mg	0.633**	<0.001	–0.453**	0.009
Zn	0.640**	<0.001	–0.285	0.114

Discussion

Neurofilaments are multicellular intermediate protein filaments found in human neurons that fill the axonal cytoplasm and provide structural support to neurons (Yuan et al., 2017). Therefore, many neurofilament proteins have been used as biomarkers for neurological diseases and axonal damage to the cells of the central nervous system (Petzold, 2005; Khalil et al., 2018). When nerve cells or their axons deteriorate or are damaged, neurofilament proteins are released into the blood and cerebrospinal fluid (Jonsson et al., 2010). Therefore,

these proteins have been used to monitor various neurological diseases, including amyotrophic lateral sclerosis (Rosengren et al., 1996) and multiple sclerosis (Teunissen et al., 2009). Therefore, it was found through the results of the current research that there was an increase in vimentin levels in the blood serum of patients with multiple sclerosis, which reached a value of 174.1 ± 28.9 ng/mL compared to the healthy group, which reached at value 93.6 ± 20.5 ng/mL. It is an intermediate filament protein that plays a role in cell processes, including cell migration, cell shape and elasticity, or organelle stabilization. It can be expressed on the cell surface, and its physiological effects extend far beyond structural and cytoskeletal functions, thus contributing to many pathological conditions, including neurological and inflammatory diseases (Yuan et al., 2025), spinal cord injuries, stroke, bacterial meningitis, glioma, and peripheral nervous system injury. It is known that multiple sclerosis is an autoimmune disease that affects the central nervous system, as T lymphocytes cross the blood-brain barrier, leading to demyelination and axonal degeneration (Lassmann, 2019).

Therefore, some studies have shown that the 14-3-3 protein acts as an adapter for binding vimentin and GFAP in reactive astrocytes at the site of demyelinating lesions in multiple sclerosis. Vimentin is highly expressed in reactive astrocytes found in demyelinating lesions in multiple sclerosis. It is one of the proteins that interacts with the 14-3-3 protein found in cultured human astrocytes, causing its secretion and release into the bloodstream, which is consistent with the results of the current study (Satoh et al., 2004). We also studied the relationship between adhesion cell molecules and multiple sclerosis, including NCAM, was studied. The results of the current study showed an increase in the levels of adhesion cell molecules, including NCAM, in patients with multiple sclerosis, which reached the value 175.7 ± 32.4 pg/mL compared to healthy people, which reached the value 82.5 ± 21.5 pg/mL. In multiple sclerosis, damage is caused to the myelin sheath that surrounds the optic fibers. As a result of this damage, some neurons and helper cells secrete molecules, including NCAM, which participate in the central nervous system in inflammatory and neurodegenerative processes associated with progressive disability and increased brain atrophy. Therefore, these molecules work to repair and remyelinate, and thus promote the growth and guidance of nerve axons (Ziliotto et al., 2019). The NCAM shows intriguing potential for a role in the repair mechanisms and remyelination processes that occur after exacerbation of MS. This is based on its biological properties and the results of cerebrospinal fluid analyses in patients with acute MS, in whom its levels gradually rise after an acute attack and subsequently begin to decline as a result of clinical improvement in treated patients (Massaro et al., 1987, 1997). Therefore, NCAM is important in the development of neuronal products, and low or high levels of it have been linked to brain diseases, suggesting that any imbalance may be the key factor. Therefore, increasing our understanding of the role of NCAM in health and disease could remove some of the mystery surrounding this molecule and even lead to the discovery of new potential therapeutic targets (Gnanapavan & Giovannoni, 2013). Therefore, its levels are a vital indicator of the nervous system's ability to repair itself in multiple sclerosis patients. Its elevation may be temporary as a result of relapses that accompany the disease, indicating the body's attempt to regenerate itself. Its continued decline with the progression of the disease may reflect a decline in the ability to repair itself.

The current study also showed the association of multiple sclerosis with chemokines, including CXCL10, as an increase of CXCL10 concentration in multiple sclerosis patients, which reached the value 205.6 ± 33.6 pg/mL compared to the control group, which reached the value 141.2 ± 21.8 pg/mL. The research results were consistent with the results of many studies, including studies by Comini-Frota et al. (2011). Kürtüncü et al. (2019) which indicated higher CXCL10 values in the patient group compared to the control group, while a study by Moreira et al. (2006) found lower CXCL10 values in multiple sclerosis patients, which is not consistent with the results of the current research.

The elevated levels of CXCL10 in MS patients are considered a marker of immune-mediated inflammatory activity within the central nervous system. Therefore, it was found that patients with a more aggressive metabolic pathway showed a significant positive correlation between the expression of IgG1 and CXCL10, and that increased intrathecal IgG synthesis was positively correlated with CXCL10 in the cerebrospinal fluid of MS patients, indicating a relationship between CXCL10 and IgG production in the pathogenesis of MS (Scolding et al., 2015). Inflammatory conditions also stimulate the secretion of CXCL10 by various cell types, including monocytes, neutrophils, dendritic cells, microglia, and astrocytes (Vazirinejad et al., 2014). Therefore, elevated CXCL10 concentration has been observed in the cerebrospinal fluid in multiple sclerosis (Rotondi et al., 2007).

On the other hand, scientific research indicates that the TLR4 receptor plays an important role in the innate immune response and has a significant relationship with the mechanisms of inflammation in autoimmune diseases, including multiple sclerosis. Therefore, the research results showed a significant increase in TLR4 levels, which reached the value 9.06 ± 1.37 ng/mL in multiple sclerosis patients compared to the control group, which reached 141.2 ± 21.8 ng/mL. A study indicated a relationship between TLR4 levels and multiple sclerosis patients, as many studies have revealed the roles played by TLR4 in multiple sclerosis (Noroozi et al., 2016), as the secretion of type I interferon can increase, to modify immune responses, after innate immune activation through TLR4 (Comabella et al., 2009). TLRs are a family of receptors involved in innate immunity, inducing a wide range of inflammatory responses (Medzhitov, 2001). TLR4 has been linked to several diseases with a significant inflammatory component, including multiple sclerosis (Zhang et al., 2019). TLR4 activation increases the expression and nuclear translocation of the transcription factor nuclear factor kappa-B (NF-kB), leading to the release of pro-inflammatory cytokines such as TNF-α, interleukin-1β (IL-1β), or interleukin-6 (IL-6), as well as the recruitment of chemokines and lymphocytes (Lu et al., 2008; Liu et al., 2017). Therefore, TLR4 has a clear role in enhancing the inflammatory response in multiple sclerosis by stimulating cytokines and immune cells that attack the central nervous system. Therefore, it is a potential target for future treatments aimed at reducing immune inflammation in multiple sclerosis patients.

Some studies have shown that patients with multiple sclerosis tend to have lower levels of magnesium in the blood and cerebrospinal fluid, and that this deficiency may be linked to increased oxidative stress and inflammation in the central nervous system, both of which play a role in myelin deterioration. Therefore, it was found through the results of the current study that magnesium levels decreased in patients with multiple sclerosis, as the average value of its calculation reached 1.29 ± 0.37 mmol/L compared to the control group, which reached 2.20 ± 0.51 mmol/L. The results of the current study agreed with the results of Karpińska et al. (2017), who indicated in their study that magnesium levels decreased and appeared abnormal in patients with multiple sclerosis compared to the healthy group. Therefore, abnormal serum magnesium levels in MS patients should be identified and corrected, as this may improve the health of people with MS. Nutrient minerals in the diet perform important functions in the human body. Magnesium has a significant impact on the nervous system, reducing nerve cell excitability. It also affects autonomic ganglia by increasing their inhibitory effect and weakening their stimulating effect. Therefore, magnesium ions compete with calcium ions for nerve endings, preventing overstimulation (Kim et al., 2012). Therefore, magnesium deficiency may lead to dysfunction in neurons or lymphocytes directly or indirectly (Durlach, 1991), so low magnesium levels are linked to the causes of multiple sclerosis (Masoud & Fakharian, 2007). Neuroinflammation is a hallmark of neurodegenerative disorders, so magnesium is involved in regulating metabolism and maintaining the balance of all tissues, including the brain, as it coordinates the transmission of nerve signals and maintains the integrity of the blood-brain barrier (Maier et al., 2022). Therefore, its deficiency is associated with chronic, low-grade inflammation in the brain (Maier et al., 2021).

Neuroinflammation shares key features with peripheral inflammation, such as the activation of resident macrophages, including microglia, increased inflammatory mediators, recruitment of peripheral immune cells, and local tissue injury (Woodburn et al., 2021).

Therefore, magnesium deficiency induces neuroinflammation (Tsuji et al., 2021). Therefore, it was found that patients with MS who were treated with magnesium experienced significantly fewer exacerbations than those who did not receive treatment (Wasnik et al., 2020). A study reported that patients with normal blood magnesium levels had a better clinical course than patients with low magnesium levels, especially in patients with multiple sclerosis (Karpińska et al., 2017; de Oliveira et al., 2020). However, another study indicated no significant differences in magnesium levels in patients with multiple sclerosis (Nirooei et al., 2021).

Zinc is a trace element essential for the immune system, cellular growth, and nerve balance. Research has shown a relationship between multiple sclerosis patients and zinc levels. It has been found that multiple sclerosis patients often have low levels of zinc in the blood or cerebrospinal fluid. Therefore, it was found through the research results that there was a decrease in zinc levels in patients with multiple sclerosis, as the average value of its calculation reached 54.8 ± 9.4 µg/dL compared to the control group, which reached 76.5 ± 12.9 µg/dL. The research results do not agree with the results of a study by Mahmoud et al. (2023), which indicated that there were no significant differences in zinc levels in patients with multiple sclerosis while the results agreed with Nashmi et al. (2020), Jabbar & Al-Saeed, (2023), Matar & Borjac, (2023), who indicated low zinc levels in patients with multiple sclerosis. Researchers have been interested in measuring zinc levels in patients with multiple sclerosis to investigate the possible role of zinc in causing the disease. It is an essential element for all living organisms, so changes in its level have effects on the nervous and immune systems, as it is found in the brain, especially in the presynaptic vesicles of nerve cells. Therefore, its deficiency or low levels help stimulate the death of nerve cells, and high levels are toxic to the nerves, so maintaining zinc balance in the brain is very important (Clemens, 2021).

We conclude from the research results that the significant positive relationship between vimentin and NCAM in healthy individuals is very important, reflecting an attempt to understand the natural relationship between these two proteins outside the context of disease, which provides a basis for understanding the changes that occur in conditions such as multiple sclerosis. Therefore, the common natural relationship between vimentin and NCAM in healthy individuals can be explained by the common natural functions in neurodevelopment, as both play a role in the growth, differentiation, adhesion, and formation of neural networks (Wang et al., 2023). Therefore, the significant correlation means that the higher the gene or protein expression of one of them, the higher the other, and this reflects an integrative regulatory relationship in the stages of normal neural development. On the other hand, there may be no scientific studies proving the existence of a significant positive relationship between the levels of expression of the protein vimentin, the chemokine CXCL10, and the receptor TLR4 in healthy people (Dhaiban et al., 2020). However, interactions between these proteins may exist in pathological contexts, so the research results may indicate that the association between them is more active in cases of inflammation, making it difficult to observe the association in healthy individuals. It is also inferred from the positive significant relationship between zinc, magnesium, and vimentin in healthy individuals in the present study that zinc may play a vital role in regulating the structure and function of vimentin in healthy cells. This interaction contributes to maintaining the stability and balance of the cellular structure, highlighting the importance of zinc in cellular health. The negative correlation between vimentin and magnesium may be explained by the fact that increased vimentin levels may enhance inflammation, while a magnesium deficiency may exacerbate this condition. Therefore, there is likely an indirect interaction between the two that influences the course of the disease.

In the current study, Receiver Operating Characteristic analysis was used to evaluate the studied variables as biomarkers for differentiating between MS patients and healthy individuals. Therefore, the area under the curve was used to measure the ability of these biomarkers to differentiate between the two groups. The AUC value of vimentin protein was 0.984. The role of vimentin protein in multiple sclerosis patients focuses on inflammation, cell migration, and blood-brain barrier dysfunction. It may thus be concluded from the research that it is considered a diagnostic indicator for multiple sclerosis patients. Therefore, there is a need for more studies on this topic. While the AUC value for the cell adhesion molecule was 0.991 and the chemokine CXCL10 – 0.968, these measurements can be used as promising indicators for the diagnosis of patients with multiple sclerosis. A study also indicated that toll-like receptor 4 shows increased expression in the immune cells of MS patients compared to healthy individuals. Therefore, its effectiveness as a diagnostic biomarker was evaluated using ROC curve analysis and calculating the area under the curve (AUC) to determine its ability to distinguish between the two groups. The AUC value was 0.991. Therefore, it was found that TLR4 has a good discriminating ability between MS patients and healthy individuals, which makes it a promising biomarker in diagnosis (Miranda-Hernandez & Baxter, 2013).

Conclusion

Multiple sclerosis is an autoimmune disease that affects the central nervous system. It is characterized by damage to myelin, which leads to disruption of nerve signal transmission. It has a strong inflammatory and immune basis. Therefore, the protein vimentin may be linked to multiple sclerosis by facilitating cellular movement and interacting with the immune system. Also, elevated levels of NCAM may indicate repair of nerve tissue in MS patients, while elevated levels of the chemokine CXCL10 may be directly related to inflammatory activity in patients. Elevated TLR4 may indicate its involvement in exacerbating inflammation in MS patients, while low levels of minerals, including magnesium and zinc, may contribute to increased neuroinflammation. Zinc and magnesium supplements are therefore essential in reducing disease progression. The high area under the curve values for the studied variables indicate that they are important diagnostic indicators in MS patients.

References

Abdulhakim, M. M., Ibrahim, S. E.-D., Salama, S. M., & Saad Zaghloul, A. S. (2024). Role of CXCL-10 as a biomarker for rheumatoid arthritis. QJM, 117(S1), hcae070.533.

Barkhof, F., & Koeller, K. K. (2024). Demyelinating diseases of the CNS (brain and spine). In: Hodler, J., Kubik-Huch, R. A., & Roos, J. E. (Eds.). Diseases of the brain, head and neck, spine 2024–2027. Springer, Cham. Pp. 189–202.

Clemens, S. (2021). The cell biology of zinc. Journal of Experimental Botany, 73(6), 1688–1698.

Comabella, M., Lünemann, J. D., Río, J., Sánchez, A., López, C., Julià, E., Fernández, M., Nonell, L., Camiña-Tato, M., Deisenhammer, F., Caballero, E., Tortola, M. T., Prinz, M., Montalban, X., & Martin, R. (2009). A type I interferon signature in monocytes is associated with poor response to interferon-β in multiple sclerosis. Brain, 132(12), 3353–3365.

Comini-Frota, E. R., Teixeira, A. L., Angelo, J. P. A., Andrade, M. V., Brum, D. G., Kaimen-Maciel, D. R., Foss, N. T., & Donadi, E. A. (2011). Evaluation of serum levels of chemokines during interferon-β treatment in multiple sclerosis patients. CNS Drugs, 25(11), 971–981.

Cui, L.-Y., Chu, S.-F., & Chen, N.-H. (2020). The role of chemokines and chemokine receptors in multiple sclerosis. International Immunopharmacology, 83, 106314.

de Oliveira, M., Gianeti, T. M. R., da Rocha, F. C. G., Lisboa-Filho, P. N., & Piacenti-Silva, M. (2020). A preliminary study of the concentration of metallic elements in the blood of patients with multiple sclerosis as measured by ICP-MS. Scientific Reports, 10(1), 13112.

Dhaiban, S., Al-Ani, M., Elemam, N. M., & Maghazachi, A. A. (2020). Targeting chemokines and chemokine receptors in multiple sclerosis and experimental autoimmune encephalomyelitis. Journal of Inflammation Research, 13, 619–633.

Durlach, J. (1991). Magnesium: Clinical forms of primary magnesium deficiency. In: Pietrzik, K. (Ed.). Modern lifestyles, lower energy intake and micronutrient status. Springer, London. Pp. 155–167.

Eriksson, J. E., Dechat, T., Grin, B., Helfand, B., Mendez, M., Pallari, H.-M., & Goldman, R. D. (2009). Introducing intermediate filaments: From discovery to disease. Journal of Clinical Investigation, 119(7), 1763–1771.

Gnanapavan, S., & Giovannoni, G. (2013). Neural cell adhesion molecules in brain plasticity and disease. Multiple Sclerosis and Related Disorders, 2(1), 13–20.

Ivaska, J., Pallari, H.-M., Nevo, J., & Eriksson, J. E. (2007). Novel functions of Vimentin in cell adhesion, migration, and signaling. Experimental Cell Research, 313(10), 2050–2062.
Jabbar, A. A., & Al-Saeed, H. H. (2023). Estimation of trace elements (zinc and copper) in serum of multiple sclerosis patients in Baghdad City. Iraqi Journal of Medical Sciences, 21(2), 161–165.
Jonsson, M., Zetterberg, H., Van Straaten, E., Lind, K., Syversen, S., Edman, Å., Blennow, K., Rosengren, L., Pantoni, L., Inzitari, D., & Wallin, A. (2010). Cerebrospinal fluid biomarkers of white matter lesions – cross-sectional results from the LADIS study. European Journal of Neurology, 17(3), 377–382.
Karpińska, E., Karpińska, E., Socha, K., Soroczyńska, J., Jakoniuk, M., Mariak, Z., Borawska, M. H., & Kochanowicz, J. (2017). Concentration of magnesium in the serum and the ability status of patients with relapsing-remitting multiple sclerosis. Journal of Elementology, 22(2), 671–679.
Khalil, M., Teunissen, C. E., Otto, M., Piehl, F., Sormani, M. P., Gattringer, T., Barro, C., Kappos, L., Comabella, M., Fazekas, F., Petzold, A., Blennow, K., Zetterberg, H., & Kuhle, J. (2018). Neurofilaments as biomarkers in neurological disorders. Nature Reviews Neurology, 14(10), 577–589.
Kim, Y.-H., Jung, K.-I., & Song, C.-H. (2012). Effects of serum calcium and magnesium on heart rate variability in adult women. Biological Trace Element Research, 150(1–3), 116–122.
Koh, Y., & Park, J. (2018). Cell adhesion molecules and exercise. Journal of Inflammation Research, 11, 297–306.
Koliada, O. M., Tynynyka, L. M., Koliada, T. I., Minukhin, V. V., Nikolchenko, A. Y., Taran, A. V., & Minukhina, D. V. (2025). Functional state of mononuclear phagocytes in patients with multiple sclerosis carrying disease-associated HLA-DR polymorphism. Regulatory Mechanisms in Biosystems, 16(2), e25085.
Kürtüncü, M., Yılmaz, V., Akçay, H. İ., Türkoğlu, R., Altunrende, B., Çınar, S. A., Ulusoy, C., Gündüz, T., İçöz, S., Kasap, M., Çalışkan, Z., Öttünç, G., Eraksoy, M., & Tüzün, E. (2019). Impact of fingolimod on CD4+ T cell subset and cytokine profile of relapsing remitting multiple sclerosis patients. Journal of Neuroimmunology, 337, 577065.
Lassmann, H. (2019). Pathogenic mechanisms associated with different clinical courses of multiple sclerosis. Frontiers in Immunology, 9, 3116.
Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy, 2, 17023.
Lu, Y.-C., Yeh, W.-C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. Cytokine, 42(2), 145–151.
Mahmoud, H. R., Nasef, A. M., Awad, E. M., & Elhafez, M. A. A. (2023). Role of serum zinc, urinary sodium and urinary uric acid in multiple sclerosis in Egypt. QJM, 116(S1), hcad069.515.
Maier, J. A. M., Locatelli, L., Fedele, G., Cazzaniga, A., & Mazur, A. (2022). Magnesium and the brain: A focus on neuroinflammation and neurodegeneration. International Journal of Molecular Sciences, 24(1), 223.
Maier, J. A., Castiglioni, S., Locatelli, L., Zocchi, M., & Mazur, A. (2021). Magnesium and inflammation: Advances and perspectives. Seminars in Cell and Developmental Biology, 115, 37–44.
Masoud, S. A., & Fakharian, E. (2007). Assessment of serum magnesium, copper, and zinc levels in multiple sclerosis (MS) patients. Iran Journal of Psychiatry and Behavioral Sciences, 1(2), 38–42.
Massaro, A. R., Albrechtsen, M., & Bock, E. (1987). N-CAM in cerebrospinal fluid: A marker of synaptic remodelling after acute phases of multiple sclerosis? Italian Journal of Neurological Sciences, Suppl. 6, 85–88.
Massaro, A. R., Carnevale, A., Tonali, P., & Bock, E. (1997). Glial cell pathobiology in multiple sclerosis detected by CSF markers. In: Teelken, A., & Korf, J. (Eds.). Neurochemistry. Springer, New York. Pp. 451–455.
Matar, A., & Borjac, J. (2023). Iron, zinc, and multiple sclerosis patients. In: Martin, C. R., Patel, V. B., & Preedy, V. R. (Eds.). Vitamins and minerals in neurological disorders. Academic Press. Pp. 341–357.
Medzhitov, R. (2001). Toll-like receptors and innate immunity. Nature Reviews Immunology, 1(2), 135–145.
Miranda-Hernandez, S., & Baxter, A. G. (2013). Role of toll-like receptors in multiple sclerosis. American Journal of Clinical and Experimental Immunology, 2(1), 75–93.
Mohammed, E. M. A. (2024). Understanding multiple sclerosis pathophysiology and current disease-modifying therapies: A review of unaddressed aspects. Frontiers in Bioscience-Landmark, 29(11), 386.
Moreira, M. A., Tilbery, C. P., Monteiro, L. P., Teixeira, M. M., & Teixeira, A. L. (2006). Effect of the treatment with methylprednisolone on the cerebrospinal fluid and serum levels of CCL2 and CXCL10 chemokines in patients with active multiple sclerosis. Acta Neurologica Scandinavica, 114(2), 109–113.
Nashmi, A. D., Hassan, A. F., & Hammady, M. M. (2020). Estimation the level of metals (lead, cadmium, copper and zinc) in multiple sclerosis patients in Basra, Iraq. Indian Journal of Forensic Medicine and Toxicology, 14(3), 1029–1035.
Nirooei, E., Kashani, S. M. A., Owrangi, S., Malekpour, F., Niknam, M., Moazzen, F., Nowrouzi-Sohrabi, P., Farzinmehr, S., & Akbari, H. (2021). Blood trace element status in multiple sclerosis: A systematic review and meta-analysis. Biological Trace Element Research, 200(1), 13–26.
Noroozi, S., Meimand, H. A. E., Arababadi, M. K., Nakhaee, N., & Asadikaram, G. (2016). The effects of IFN-β 1a on the expression of inflammasomes and apoptosis-associated speck-like proteins in multiple sclerosis patients. Molecular Neurobiology, 54(4), 3031–3037.
Oksenberg, J. R., Baranzini, S. E., Sawcer, S., & Hauser, S. L. (2008). The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. Nature Reviews Genetics, 9(7), 516–526.
Perreau, J., Lilienbaum, A., Vasseur, M., & Paulin, D. (1988). Nucleotide sequence of the human vimentin gene and regulation of its transcription in tissues and cultured cells. Gene, 62(1), 7–16.
Petzold, A. (2005). Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. Journal of the Neurological Sciences, 233(1–2), 183–198.
Rosengren, L. E., Karlsson, J., Karlsson, J., Persson, L. I., & Wikkelsø, C. (1996). Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. Journal of Neurochemistry, 67(5), 2013–2018.
Rotondi, M., Chiovato, L., Romagnani, S., Serio, M., & Romagnani, P. (2007). Role of chemokines in endocrine autoimmune diseases. Endocrine Reviews, 28(5), 492–520.
Satoh, J., Yamamura, T., & Arima, K. (2004). The 14-3-3 protein ε isoform expressed in reactive astrocytes in demyelinating lesions of multiple sclerosis binds to Vimentin and glial fibrillary acidic protein in cultured human astrocytes. The American Journal of Pathology, 165(2), 577–592.
Scolding, N., Barnes, D., Cader, S., Chataway, J., Chaudhuri, A., Coles, A., Giovannoni, G., Miller, D., Rashid, W., Schmierer, K., Shehu, A., Silber, E., Young, C., & Zajicek, J. (2015). Association of British Neurologists: Revised (2015) guidelines for prescribing disease-modifying treatments in multiple sclerosis. Practical Neurology, 15(4), 273–279.
Simonsen, C. S., Flemmen, H. Ø., Lauritzen, T., Berg-Hansen, P., Moen, S. M., & Celius, E. G. (2020). The diagnostic value of IgG index versus oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis. Multiple Sclerosis Journal – Experimental, Translational and Clinical, 6(1), 1–6.
Teunissen, C. E., Iacobaeus, E., Khademi, M., Brundin, L., Norgren, N., Koel-Simmelink, M. J. A., Schepens, M., Bouwman, F., Twaalfhoven, H. A. M., Blom, H. J., Jakobs, C., & Dijkstra, C. D. (2009). Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology, 72(15), 1322–1329.
Tsuji, R., Inoue, H., Uehara, M., & Kida, S. (2021). Dietary magnesium deficiency induces the expression of neuroinflammation-related genes in mouse brain. Neuropsychopharmacology Reports, 41(2), 230–236.
Vazirinejad, R., Ahmadi, Z., Kazemi Arababadi, M., Hassanshahi, G., & Kennedy, D. (2014). The biological functions, structure and sources of CXCL10 and its outstanding part in the pathophysiology of multiple sclerosis. Neuroimmunomodulation, 21(6), 322–330.
Wang, T., Qiu, X.-X., Wu, H.-F., Chen, K.-Z., Liu, S.-X., Li, Y.-W., He, T., & Zhao, J. (2023). Vimentin as a potential target for diverse nervous system diseases. Neural Regeneration Research, 18(5), 969–975.
Wasnik, S., Sharma, I., Baylink, D. J., & Tang, X. (2020). Vitamin D as a potential therapy for multiple sclerosis: Where are we? International Journal of Molecular Sciences, 21(9), 3102.
Woodburn, S. C., Bollinger, J. L., & Wohleb, E. S. (2021). The semantics of microglia activation: Neuroinflammation, homeostasis, and stress. Journal of Neuroinflammation, 18(1), 258.
Yuan, A., Rao, M. V., Veeranna, & Nixon, R. A. (2017). Neurofilaments and neurofilament proteins in health and disease. Cold Spring Harbor Perspectives in Biology, 9(4), a018309.
Yuan, Z., Janmey, P. A., & McCulloch, C. A. (2025). Structure and function of Vimentin in the generation and secretion of extracellular Vimentin in response to inflammation. Cell Communication and Signaling, 23, 187.
Zhang, Z., Wang, Y., Chen, L., & Li, Z. (2019). Protective effects of the suppressed NF-κB/TLR4 signaling pathway on oxidative stress of lung tissue in rat with acute lung injury. The Kaohsiung Journal of Medical Sciences, 35(5), 265–276.
Ziliotto, N., Zivadinov, R., Jakimovski, D., Baroni, M., Tisato, V., Secchiero, P., Bergsland, N., Ramasamy, D. P., Weinstock-Guttman, B., Bernardi, F., Ramanathan, M., & Marchetti, G. (2019). Plasma levels of soluble NCAM in multiple sclerosis. Journal of the Neurological Sciences, 396, 36–41.