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Evaluation of some biochemical variables in patients with multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a disease of the central nervous system most often chronic; it is an autoimmune disorder and is neurodegenerative in nature. The impact of dietary inadequacy on MS pathophysiology has been studied with regards to serum levels of key micronutrients such as vitamin D, C, B12, folic acid (B9), magnesium, zinc, copper, calcium and potassium among others. The results indicate that all the above nutrients are far much lower in MS patients than they are in normal people with the exception of iron which was increased at the probability level of ($P \leq 0.05$). Immunomodulatory effects of vitamin D deficiency have been directly linked to both susceptibility and severity of MS and low levels of vitamin B12 and folic acid can potentially lead to ineffective myelin production and high levels of homocysteine that can stimulate neurodegeneration. Magnesium, zinc and calcium deficiency may inhibit the functioning of the neurons and the immune system. Copper, which is essential in treating oxidative stress and mitochondrial dynamics, is usually low in MS, which may exaggerate the destruction of cells. There is also the common reduction in potassium levels, which is an important constituent in neuronal excitability. In another interesting twist, iron deficiency does not exist, but aberrant brain iron deposition is highly common in MS and can stimulate the occurrence of oxidative damage and inflammation. Those conclusions indicate the significance of monitoring and managing the micronutrient deficiencies in patients with MS as one of the components of the overall approach to treatment. Disease progression could be affected and patient outcomes enhanced with the optimization of nutrient status. Additional research is required to prove the existence of causality between the components and the examination of the treatment capabilities of selective supplementation.

Keywords: Vitamin D, Vitamin B12, Folic Acid, Magnesium, Iron.

Introduction

Multiple sclerosis (MS) is a chronic, progressive, immune based neurodegeneration of the central nervous system (CNS), which is characterized by inflammation, demyelination and axonal degeneration. The predilection of MS is extremely multifactorial owing to the complex interplay involving genetic predisposition, exposure to the environmental and increasingly of nutritional status [1-3]. A steady accumulation of evidence

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indicates that the disturbances in micronutrient homeostasis could indeed be related not only to the susceptibility to disease but would also play a role in worsening and symptom load. Of the micronutrients, various vitamins such as vitamin D, vitamin C, vitamin B12, vitamin B 9, or folic acid, magnesium, zinc, copper, calcium, potassium, and iron among others play a critical role in neurological and/or immunological health [4]. They are vital micronutrients in immune moderation, myelin upholding, neurotransmission, oxidative balance, and energy metabolism in the cells. Myelin is a fatty, insulating sheath that encloses and safeguards nerve fiber (axons) in central and peripheral nervous systems to transfer electrical signals fast and effectively between nerve cells. Due to changes in their concentration in the serum, which is common in MS patients, they can aggravate the underlying pathological changes, and this is a factor that leads to clinical deterioration. The most studied one is perhaps vitamin D due to its immunomodulatory effects on T-cells regulation and cytokine suppression. Inadequate vitamin D status has been linked to higher rates of MS, relapses and an augmented MRI disease activity [5]. Methylation, DNA synthesis and myelin are in the center of vitamin B12 and folic acid functions. Poor intakes of such vitamins can cause poor remyelination, excess oxidative stress as well as high levels of homocysteine, which is a neurotoxic metabolite known to cause neuronal damage [6].

Vitamin C is one of the key water-soluble antioxidants that helps to replenish other antioxidants, including vitamin E, and prevents or reduces oxidative damage to neural tissue [7]. This deficiency can predispose the CNS to damage by free radicals [8]. Magnesium participates in more than 300 enzymatic processes, the processes that are needed to carry out the ATP synthesis and respond to the NMDA receptor; the lack of magnesium can worsen synaptic plasticity and inflammation in the brain [9]. Zinc is also an essential cofactor in many enzymes and transcription factors in immune regulation and myelin repair. A zinc deficiency affects the innate and adaptive immune system, which has potential to enhance neuroinflammation in MS [10]. Calcium is instrumental during release of neurotransmitters and intracellular signaling; deficient calcium has potential of causing poor neuromuscular conduction, as well as enhanced neuronal excitotoxicity [11]. Potassium plays a critical role in the maintenance of excitability of the neurons as well as action potential propagation. Decreased potassium is capable of leading to muscle weaknesses, fatigue and disturbance of nerve conduction, which are widespread in MS patients [12].

Copper is essential in trace levels, which plays a major role in mitochondrial respirations and antioxidant protection using enzymes such as cytochrome c oxidase and superoxide dismutase [13]. Lack of copper can exacerbate oxidative stress and undermine cellular metabolism of energy. Iron on the other hand tends to be high in MS as opposed to these deficiencies [14]. Although iron helps transport oxygen and produce myelin, the accumulation of excess iron in the CNS can foster the production of reactive oxygen species further leading to a lipid peroxidation process, mitochondrial dysfunction and prolonged neuroinflammation [15]. Such overloading of iron has been associated with progression of diseases and loss of weight in the brain. On the whole, these micronutrient imbalances could be actively involved in the pathogenesis process of MS instead of being related to the consequence of the disease. It is possible that recognizing and rectifying those deficiencies and surpluses may provide the potentially beneficial adjunctive interventions to enhance the disease and patient quality of life outcomes [16].

Materials and Methods

Data for this study were collected from a sample of 60 patients whose ages ranged between (20-45 years). Additionally, 30 control subjects who's ages ranged between (24-45) were collected. The samples were

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obtained from Baghdad Laboratories. Both patients and controls had 5 mL of venous blood collected. The blood samples were then left to clot for 10 minutes at room temperature. The sample was subjected to centrifugation for ten minutes at a speed of 3000 revolutions per minute (rpm) and it was divided into three equal portions called aliquots. The specimens were stored at a temperature of -20°C in tubes with a capacity of 1.5 mL until they were analysed using ELISA kits and a flameless atomic absorption spectrometer.

Results and Discussions

Table 1: Shows mean ± standard deviation of Vitamin D, Vitamin B12, Folic Acid, Vitamin C and Magnesium

Group	Vitamin D (ng/ml)	Vitamin B12 (ng/ml)	Folic Acid (ng/ml)	Vitamin C (µmol/l)	Magnesium (mg/dl)
Patient	10.05 ± 0.98	9.431 ± 2.791	5.32 ± 1.441	64.721 ± 0.388	1.820 ± 0.031
Control	13.21 ± 1.03	13.761 ± 3.110	8.631 ± 2.851	75.218 ± 1.802	3.440 ± 0.058
P-Value	P≤0.05				

Table 1. Mean ± SD of measured vitamins and magnesium. Vitamin D is already known to play an important immunomodulatory role , affecting T- cell activity and damping autoimmune attack of myelin. This is evidenced in this data set that the level of vitamin D in MS patients (10.05 ± 0.98 ng/ml) is lower than controls (13.21 ± 1.03 ng/ml) and the difference between them is statistically significant at the probability level of P≤0.05 as in illustrated fig.1. The result agree with a study done by *Sintzel et al.* that there is a high association with Vitamin D insufficiency combined with an elevated risk of MS, disease activity and relapse rate. Low levels could compromise the immune regulation to increase inflammation and accelerate the development of the disease. Maximization of vitamin D may form a very important aspect of MS prevention and management [17].

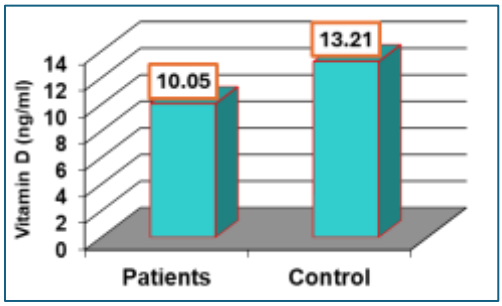


Fig. 1: Comparison between patients and control in Vitamin D

The synthesis and maintenance of myelin and the neuronal functions require vitamin B12, which when deprived, led to demyelination as in MS pathology. The results indicate B12 levels (9.431 ± 2.791 ng/ml) of MS patients to be lower as compared to the controls (13.761 ± 3.110 ng/ml) which has significant difference at the probability level of P≤0.05 as shown in fig.2 . Similar findings by *Cavalcoli et al.* show that the depletion of B12 in MS could be a combination of gastric dysfunctionity due to autoimmune process, and/or malabsorption and/or stimulated utilization during inflammation. The neurological impairment and fatigue caused by insufficient B12 may exacerbate the situation; therefore, monitoring and supplementation are essential: This is done to help personalize the management of MS [18].

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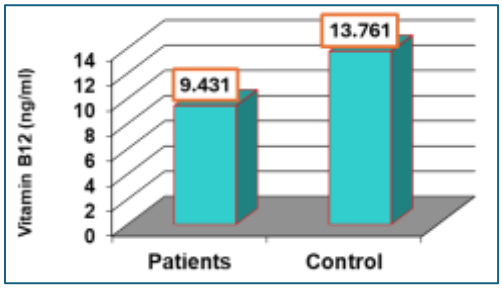


Fig. 2: Comparison between patients and control in Vitamin B12

Folic acid plays a vital role in the synthesis, repair, and methylation reactions, and these are some of the essential processes in the maintenance of myelin. Here, there is a significant difference in the folate levels of patients of MS (5.32 ± 1.441 ng/ml) and those of controls (8.631 ± 2.851 ng/ml) at the probability level of P≤0.05 as presented in fig.3. The results agree with the study done by *Labuschagne et al.* that poor dietary intake, malabsorption, or elevated metabolic demands caused by chronic inflammation are possible causes of folate deficiency in MS. Abnormally low intakes of folate may cause high homocysteine, which is neurotoxic, and it might be neurodegenerative in MS [19].

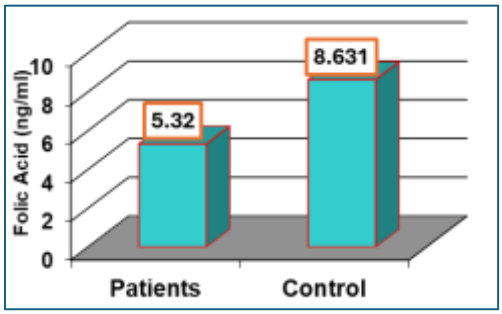
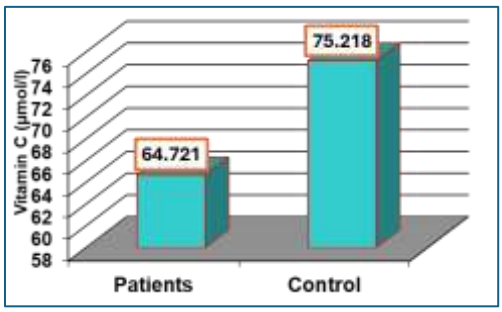


Fig. 3: Comparison between patients and control in Folic Acid

Vitamin C is an antioxidant, neutralizing damaging oxidants such as reactive oxygen species which help in demyelination and also neuronal damage in MS. It is revealed by the numbers that it is lower in patients (64.721 ± 0.388 µmol/l) than in controls (75.218 ± 1.802 µmol/l), and it is found to be statistically significant at the probability level of P≤0.05 as shown in fig.4. A related study by *Carr et al.* proved that this shortage can be one of the reasons why there is increased oxidative stress in MS, as the antioxidant burden gets depleted with the inflammatory processes. Sufficient vitamin C might be able to protect myelin and regulate the immune response, implying that supplementation would be advantageous with regard to lessening disease process [20].



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Fig. 4: Comparison between patients and control in Vitamin C

Magnesium is of utmost importance in nerve conduction, muscle activity and myelin stability, all involved in multiple sclerosis. In this data, the MS patients have very low level of magnesium (1.820 ± 0.031mg/dl) when compared with controls (3.440 ± 0.058 mg/dl) where the difference is statistically significant at the probability level of P≤0.05 as demonstrated in fig.5. These findings which are similar to one by *Enders et al.* which showed that the possible causes of low magnesium in MS are chronic inflammation, an abnormal renal handling mechanism, or dietary deficiency, and it may contribute to muscle cramping, fatigue, and elevated spasticity that could worsen neurological symptoms [21].

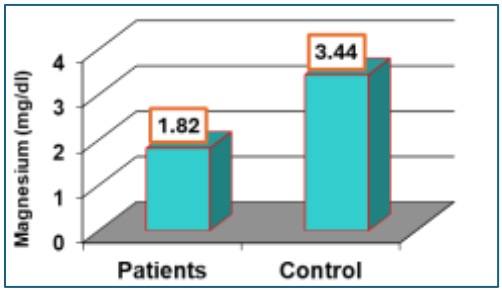


Fig. 5: Comparison between patients and control in Magnesium

Table 2: Shows mean ± standard deviation of Fe, Zinc, Copper, Calcium and Potassium.

Group	Fe (µg/dl)	Zinc (µg/dl)	Copper (µg/dl)	Calcium (mg/dl)	Potassium (mg/dl)
Patient	102.45 ± 16.3	68.04 ± 8.2	105.28 ± 16.2	8.250 ± 0.173	4.122 ± 0.562
Control	62.81 ± 17.7	97.9 ± 4.58	168.83 ± 15.2	11.700 ± 0.197	8.172 ± 0.658
P-Value	P≤0.05				

Table 2. Mean ± SD of measured minerals (Fe, Zn, Cu, Ca, and K). Iron plays an essential role in helping to transport oxygen, activate mitochondria and produce myelin. Interestingly, the iron levels in MS patients (102.45 ± 16.3 0 µg/dl) are higher compared to their controls (62.81 ± 17.7 µg/dl) contrary to the cases of the other nutrients at the probability level of P≤0.05 as shown in fig.6. Excess iron in MS can be the sign of aberrant retention in the brain where it can drive the oxidative damage through production of reactive oxygen species. This result is similar to the study done by *Hofmann et al.* which they showed that the transport and storage abnormalities may also be signified by the peripheral iron overload in the MS pathology [22].

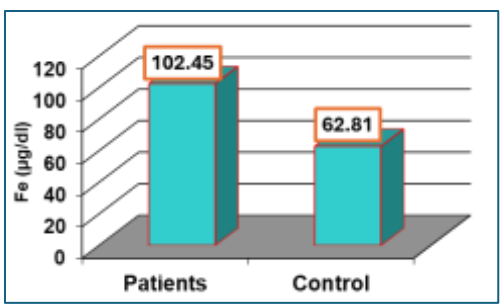


Fig. 6: Comparison between patients and control in Fe (iron)

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Zinc is also involved in the control of immunity, antioxidant response, and neuronal communication. The findings indicate that MS patients have low zinc levels (68.04 ± 8.2 µg/dl) than the controls (97.9 ± 4.58 µg/dl) which are significantly different at the probability level of P≤0.05 as shown in fig.7. Microelement zinc deficiency in MS can play the role of triggering abnormalities in the immune system, increased oxidative stress, and even remyelination problems. Furthermore, *Li et al.* and their similar findings, showed that the functioning of many enzymes covering DNA repair and myelin proteins synthesis requires adequate zinc, so its decrement can thus contribute to the development of diseases [23].

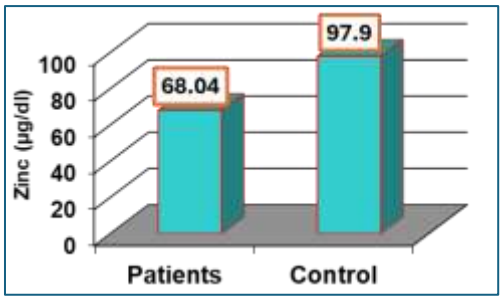


Fig. 7: Comparison between patients and control in Zinc

Copper is also involved in the formation of myelin during its working as enzyme cofactor such as cytochrome c oxidase and superoxide dismutase. In this case, MS patients considerably show lower copper levels (105.28 ± 16.2 and 168.83 ± 15.2 µg/dl) as compared to controls and the difference is highly significant at the probability level of P≤0.05 as shown in fig.8. The result agrees with a study done by *An et al.* which showed that Cu deficiency has the potential to disrupt energy production and antioxidant protection by mitochondria, which is a vital cell component of neurons. Although excessive copper can be toxic through oxidative stress, those deficient in MS could fail to repair myelin, and contribute to neurodegenerative diseases [24].

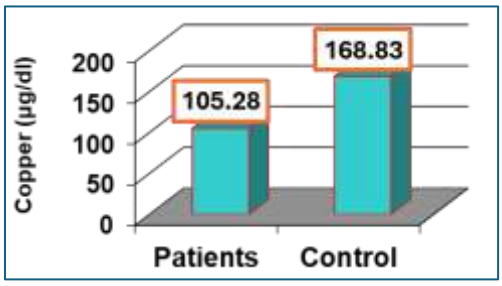


Fig. 8: Comparison between patients and control in copper

Similar study by *Pikor et al.* showed that calcium in the neuronal and immune cells is an essential signaling ion that mediates neurotransmitter release, neuroplasticity, synaptic snapshots, and inflammatory reactions. The results indicate that calcium is considerably low in MS patients (8.250 ± 0.173 mg/dl) as opposed to controls (11.700 ± 0.197 mg/dl), at the probability level of P≤0.05 as shown in fig.9, which is depicted by the data. Lack may be associated with insufficiency in vitamin D and poor absorption or ongoing inflammation. Poor calcium could aggravate neuromuscular dysfunction and enhance the risk of osteoporosis in MS particularly in immobile patients or those using corticosteroid medication [25].

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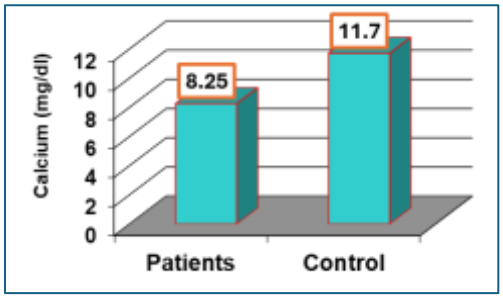


Fig. 9: Comparison between patients and control in calcium

Potassium is involved in resting membrane potential of neurons and normal conduction of nerve impulses, activities that are impaired in MS caused by myelination. The level of potassium in MS patients is significantly lower (4.122 ± 0.562 mg/dl) than in controls (8.172 ± 0.658 mg/dl), in this dataset; this difference is statistically significant at the probability level of $P \leq 0.05$ as shown in fig.10. Similar findings to those by *Mishra et al.* proved that decreased potassium levels may aggravate neuronal excitability, being one of the causes of muscle weakness and exacerbating fatigue which are already key symptoms of MS. Possible causes of such loss include disturbed cellular transport, the taking of diuretics or malnutrition among the affected patients [26].

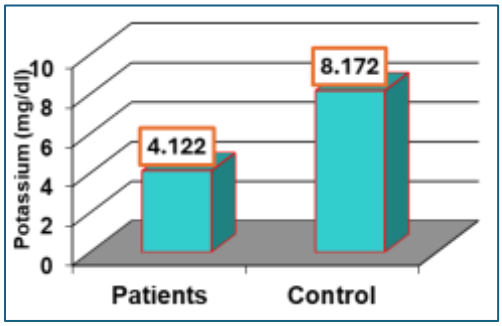


Fig. 10: Comparison between patients and control in potassium

Discussion

Dealing with MS patients, the observed deficiencies of micronutrients could represent both the cause and the effect of the disease development. Neurodegeneration and chronic inflammation in MS can induce metabolic demand and oxidative stress to generate rapid reduction in vitamins and minerals needed to maintain myelination, send signals to the brain, and regulate the immune system. As an example, the lack of vitamin D can negatively affect immune tolerance, and therefore, autoimmune activity can continue to occur, whereas deficiency in B12 and folic acid levels disturbs maintenance methylation processes that are fundamental to myelin restoration. Likewise, lower magnesium, zinc, and calcium may also interfere with the transmission of the synapses and defense cells, promoting the deterioration of the neurological condition even further. The increased iron, detectable, probably not because of increased consumption but abnormal storage, can be caused by the disrupted blood brain barrier integrity, that allows iron accumulation within the neural tissues and initiate oxidative injury. This implies that there is a vicious cycle where imbalance in nutrients and neuroinflammation contribute to each other. By and large, these results show that the monitoring of micronutrients is not only supportive but even essential to disease progression stalling in MS. Specific

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nutritional measures may disrupt the occurrence of certain biochemical processes involved in neurodegeneration.

Conclusion

The reviewed evidence indicates a pattern of micronutrient deficiency in MS, which includes vitamins D, C, B12, folic acid, magnesium, zinc, copper, calcium, and potassium along with abnormal iron metabolism. These dietary disproportions can cause poor myelin synthesis, increased oxidative stress, immune dysregulation, and general development progression of a disease. The fact that vitamin D deficiency is especially closely linked to MS indicates the potential of this metric as a modifiable risk factor, and the fact that the B vitamins, magnesium, zinc, and calcium are also deficient also indicates that nutritional interventions related to this list of vitamins and minerals could help maintain neurological health and a healthy immune system balance. The excessive deposition of iron in MS, on the contrary side, should also be monitored because it may facilitate neuroinflammation and oxidative damage. Overall, these results add to the recent arguments about the necessity of considering and correcting micronutrient status regularly and not just as a means of enhancing overall health but also as a possible modulator of disease progress. Longitudinal, and interventional research with clearer causality and the presence/absence of effective supplementation to curb the severity of disease and improve patient outcomes is required.

Conflict of Interest

The authors declare no conflict of interest.

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