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The Study of Association between Serum NADPH Oxidase Concentrations and Iron Metabolism in Relapsing Remitting Multiple Sclerosis

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Abstract

Background: With an estimated global population of 2.2 million, most of whom are in their twenties or thirties, patients with MS suffer from demyelinating diseases of the brain and spinal cord. Multiple sclerosis is thought to be caused by an overproduction of reactive oxygen species (ROS) and a deficiency in iron metabolism (MS). Regulatory ROS generation is primarily fueled by NADPH oxidases, which are enzymes that break down NADPH into NADPH phosphate. Seven catalytic homologues of the NADPH oxidase (NOX) family, NOX1–5, and two dual oxidases make up the NOX family. Endothelial failure and inflammation are linked to NOX1 and NOX5, but NOX4 is protective of vascular function. Serum endothelial NOXs (NOX5 and NOX 4) and their link to iron metabolism biomarkers in relapsing-remitting MS patients is the primary goal of this research. Also, it is probable that NOXs (NOX5 and NOX 4) and iron metabolism biomarkers have a correlate with disease severity. The following are the steps we took and the results we got: Study participants were divided into two groups: those with RRMS and those who did not have the disease. All of the research individuals had their levels of serum NOX4,5, ferritin, iron, and iron binding capacity, as well as C-reactive protein (CRP), complete blood count (CBC), and erythrocyte sedimentation rate (ESR). Medical history, neurological examination, and the Expanded Disability Status Scale were completed for all subjects (EDSS). Researchers discovered significantly higher levels of NOX5, CRP, and ferritin in patients, as well as significantly lower levels of NOX4 and iron in controls (all p 0.0001). Patients' NOXs, CRP, WBCs, ESR, and iron metabolism indicators were not associated with each other. MS patients may be more susceptible to oxidative stress-related vascular alterations and BBB breakdown because of increased NOX5 expression and reduced NOX4 levels. We also found that RRMS patients had reduced iron and TIBC concentrations. Serum iron levels in MS patients should be regularly monitored due to iron's role in myelination and oligodendrocyte activity.

Key words: NADPH Oxidase, Iron Metabolism, Relapsing Remitting Multiple Sclerosis.

1. Introduction

Neurodegenerative and inflammatory conditions are at the root of MS. Most people with multiple sclerosis (MS) are diagnosed with RRMS, which is characterised by relapses and exacerbations. Reactive oxygen species (ROS), which are tiny, oxygen-derived molecules, may have a role in the pathogenesis of multiple sclerosis (MS). [1]

One of the primary enzymatic sources of ROS, including superoxide anion and its derivatives, is increased activation and concentration of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [2].

Catalytically, the NOX family consists of seven members. Endothelial cells express four NOX isoforms, NOX1, NOX2, NOX4 and NOX5 [3]

One of the most notable and early aspects of MS pathophysiology is blood BBB breakdown and vascular alterations [4]. Endothelial dysfunction and vascular inflammation have been linked to increased NOX1, 2, and 5 expressions. In contrast, NOX4 protects the vessel wall against oxidative stress Neurodegenerative illnesses such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS) have been linked to the involvement of NOX isoforms [5]. Serum levels of NOX1, NOX4, and NOX5 in RRMS patients are unknown, however. MS and dietary intake have been studied in past research to lessen symptoms such as impaired cognitive, sensory, and physical abilities [6, 7]. Micronutrients, such as trace elements, are a significant part of the diet component [8].

ROS generation has been linked to abnormal iron depositions [9]. Patients with MS have been shown to have altered levels of iron deposition and serum indicators of iron metabolism [10, 11]. However, the link between iron metabolism and oxidative stress in MS remains a matter of debate.

2. Patients and Methods

This is a comparative patient control study conducted on 40 patients presented by relapsing remitting multiple sclerosis and 40 healthy controls with matching age, sex. Recruited patient were selected from two hospitals in Benha (Benha University Hospial, Benha Insurance Hospital).Both genders aged 20-45 years.and diagnosed with relapsing remitting multiple sclerosis (the diagnosis is confirmed by findings of MRI, CSF analysis and Evoked Potential s) were included . while excluding Probable multiple sclerosis or clinically isolated syndrome. ,Pregnancy and breast feeding, Any gastrointestinal or hematologic disease, Severe concomitant medical condition (e.g., metastatic cancer, AIDS, renal failure, liver failure…etc.), Patients consumed iron compounds, nutritional supplements or anti-oxidants, and patients had corticosteroid therapy during the last 3 months.Study subjects were informed of the possibility of using the data obtained for academic purpose.

Tools:

All participants (cases & control) were subjected to the following:

1. Medical history taking.
2. Full general and neurological examination.
3. Expanded Disability Status Scale (EDSS) to measure the outcome for disability progression in MS.
4. **Biological investigations:**NOX4 and NOX5 assay by ELISA,CRP,CBC,ESR,TIBC,Ferritin and Sreum iron

Ethical consideration:

An informed written consent was obtained from patients and control subjects before their participation in the current study. It included data about aim of the study, site of the study, study procedure and their acceptance for publication of anonymous data obtained. It was explained to both groups that they can withdraw from the study at any time without any consequences and it will not affect the type and quality of care they are receiving from the facility. It was also assured to all participants regarding the confidentiality of results

Statistical analysis:

The collected data was revised, coded and tabulated using Statistical package for Social Science [12]. Shapiro test, Mean Standard deviation (± SD), Student T Test, Mann Whitney Test (U test), The Kruskal-Wallis test, Chi-Square test, Fisher's exact test, Correlation analysis: and Regression analysis was used. All reported *p* values were two-tailed and $p < 0.05$ was considered to be significant [13, 14, 15].

3. Results

Table (1) Comparison of demographic data between studied groups.

		Control N=40	Case N=40	*P*
Age (years)	**mean±SD**	29.9±5.5	32.2±7.7	0.118
Males	**N (%)**	5 (12.5%)	6 (15.0%)	0.745
Females	**N (%)**	35 (87.5%)	34 (85.0%)	

Table (2) Comparison of demographic data between relapsing and non relapsing MS cases.

		Non relapsing N=27	Relapsing N=13	*P*
Age (years)	**mean±SD**	31.1±7.2	34.5±8.3	0.189
Males	**N (%)**	2 (7.4%)	4 (30.8%)	0.075
Females	**N (%)**	25 (92.6%)	9 (69.2%)	

Table (3) Comparison of duration between relapsing and non relapsing MS cases.

		Non relapsing N=27	Relapsing N=13	*P*
Duration (years)	**median (range)**	5 (1-9)	2 (1-8)	0.053

Table (4) Comparison of NOX 4 level according to studied parameters in control group.

		NOX-4 median (range)	*p*	NOX-5 median (range)	*p*
Gender	**Males**	0.50 (0.42-0.74)	**0.030**	0.298 (0.04-0.56)	0.951
	Females	0.38 (0.15-0.8)		0.22 (0.02-3.5)	
CRP	**Negative**	0.40 (0.15-0.8)	0.776	0.25 (0.02-3.5)	0.302
	Positive	0.39 (0.26-0.7)		0.13 (0.06-0.64)	

Table (5) Correlation of NOX-4 and NOX-5 with other studied parameters among MS group.

	NOX-4		NOX-5	
	rs	*p*	*Rs*	*P*
Age	0.238	0.140	-0.041	0.804
EDDS	0.071	0.662	-0.303	0.057
Duration	-0.120	0.459	0.140	0.390
WBCs	0.126	0.439	0.034	0.834
ESR	0.028	0.866	-0.049	0.764
CRP	0.003	0.983	0.109	0.504
Iron	0.043	0.790	0.074	0.651
Ferritin	0.020	0.901	0.168	0.299
TIBC	-0.179	0.268	0.107	0.509

Table (1) shows that 15% of the sample were males while 85%were females, with mean age was 32.2 years old.

Table (2) shows that No significant differences were found between relapsing and non-relapsing regarding age and gender ($p>0.05$ for each).

From table (3) it was deducted that No significant differences were found between relapsing and non-relapsing regarding disease duration ($p>0.05$).

Table (4) shows higher Nox-4 level was significantly associated with healthy male gender (median=0.5 versus 0.38, $p=0.030$), No significant differences were found between positive and negative CRP regarding NOX-4 level in control group ($p>0.05$) and moreover, no significant differences were found between males and females, as well as between positive and negative CRP regarding NOX-5 level in control group ($p>0.05$for each).

Table (5) conducted that no significant correlation of NOX-4 and NOX-5 levels were found with age, EDDS, duration, WBCs, ESR, CRP, iron, ferritin, TIBC among MS group.

4. Discussion

With an estimated global population of 2.2 million, most of whom are in their twenties or thirties, patients with MS suffer from demyelinating diseases of the brain and spinal cord. Many people reach a progressive phase of the illness after one to two decades (16). Since its inception, MS has been marked by an unpredictable course of relapsing and remitting (RRMS) episodes, which ultimately progresses to secondary progression (SPMS). MS has long been thought of as a white matter demyelinating inflammatory illness [17]. Deficiencies in the central nervous system (CNS) and peripheral blood can both be caused by inflammation, and both processes are mutually dependent, as demonstrated in other neurodegenerative conditions (i.e. amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease) Inflammation and neuro-degeneration are mutually dependent phenomena [18]. The cause of multiple sclerosis (MS) remains a mystery [19]. Several studies have shown that tiny, oxygen-derived molecules called reactive oxygen species (ROS) may have a role in the pathophysiology of multiple sclerosis [1]. NADPH oxidase activity and concentrations are one of the primary enzymatic producers of ROS, including superoxide anion and its derivatives [2]. Deficiencies in ROS generation and iron metabolism may play a role in MS aetiology. NOX1-5 and two dual oxidases comprise the NADPH oxidase (NOX) family of catalytic homologues. Nox4 protects against oxidative stress-induced vascular alterations in MS patients, while the expression of NOX5 and NOX1 is linked to dysfunctional endothelial cells as well as inflammation. These discoveries open up new avenues for combating MS by targeting certain NOX isoforms one at a time [20]. ROS generation has been linked to abnormal iron depositions [9]. The iron metabolism biomarkers and iron deposition in the blood have both been shown to be altered in MS patients [10,11]. However, the connection between iron metabolism and oxidative stress in MS remains unclear. As a result, this research was designed to evaluate the levels of NOXs (NOx5 and NOx 4) and iron metabolism biomarkers in relapsing-remitting MS patients and the probable association between these biomarkers and disease severity. MS patients had considerably lower levels of NOX4 and significantly greater levels of NOX5 when compared to the control group, according to the present research. These findings were in line with those of earlier research showing that patients had greater levels of blood NOX5, CRP titer, ferritin, and lower levels of serum NOX4 iron than did controls [20, 21]. The increase of iron in the human brain with age, however, has been shown to contribute to the aetiology and development of MS in other studies, which have studied haemoglobin and iron levels as well as transferrin and soluble transferrin receptors (sTfR) in MS patients. MS patients had greater sTfR levels compared to the control group, however there was no difference in iron values between MS subgroups and control as well as haemoglobin values and transferrin levels were within normal limits in all individuals [22]. According to the findings of the present research, there are no significant differences in age or gender between relapsing and non-relapsing patients, nor were there any changes in the length of the condition. When it came to laboratory measurements like NOX-4 and NOX-5 concentrations, there were no significant changes between relapsing and non-relapsing patients. Furthermore, in the MS group, there were no significant variations in the levels of Nox-4 and Nox-5 based on gender, EDDS, therapy, or CRP. The NOX-4 and NOX-5 levels were also not linked to any of the following: age, white blood cell count, ESR, C-reactive protein (CRP), ferritin, or total bilirubin-binding capacity (TIBC). These results are in agreement with the previous research on the subject [20].

5. Conclusion

Conclusion: In MS patients, higher NOX5 expression and reduced NOX4 levels may be associated with oxidative stress-related vascular alterations and disruption of the blood brain barrier (BBB). Iron and TIBC concentrations are decreased in individuals with RRMS. The relevance of iron on myelination and oligodendrocyte activities necessitates thorough monitoring of iron levels in MS patients.

6. Limitations

It may not have been statistically significant because of the limited number of patients included in the study. Participants must meet rigorous inclusion and exclusion criteria.

Only NOX4 and NOX5 were tested, which was insufficient in light of the need of measuring additional NOXs (particularly NOX2).

7. Recommendation

Serum iron levels in MS patients should be routinely examined regardless of whether they are at risk of malnutrition, even after accounting for the study's shortcomings. MS patients may benefit greatly from the addition of antioxidants and NOX 5 inhibitors to conventional immunotherapy because of their ability to reduce oxidative stress. Future studies should be done to evaluate the effects of antioxidant therapies and how these therapies could be integrated with the current conventional approaches for treating MS patients. New regenerative therapeutics aimed at inhibiting or correcting the evolution of MS lesions may target the NOX 4 enzymes. There is evidence that iron chelation can protect neurons in animal models of multiple sclerosis, but additional studies are needed to determine whether or not these pharmacological modifications are safe and beneficial in humans.

MS patients' iron status and nutritional condition should be taken into account in addition to other aspects such as biochemical, genetic, and the existence of related comorbidities.

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Conflicts of interest:
There are no conflicts of interest

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