Benha Journal of Applied Sciences (BJAS) print : ISSN 2356–9751
Vol.( ) Issue( ) Oct.(2022), 1-5 online : ISSN 2356–976x
http:// bjas.bu.edu.eg

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

A.A.Khodeir, O.S.El-Shimi , S.M.kassem and M.A.Mohammed
Neuropsychiatry, Dept., Faculty of Medicine, Benha Univ., Benha, Egypt

Abstract

Background: Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the brain and spinal cord, which affects about 2.2 million people worldwide, primarily young adults from 20 to 40 years of age. Overproduction of reactive oxygen species (ROS) and impaired iron metabolism are considered to be possible factors in the pathogenesis of Multiple sclerosis (MS). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are the primary sources of regulated ROS production. The NADPH oxidase (NOX) family consists of seven catalytic homologues, NOX1–5 and two dual oxidases. NOX1 and NOX5 are associated with endothelial dysfunction and inflammation but NOX4 has a protective effect on vascular function. Aim of study: to investigate the status of serum endothelial NOXs (NOX5 and NOX 4) and its relationship with iron metabolism biomarkers in relapsing-remitting MS patients, also to study the possible correlation between NOXs (NOX5 and NOX 4) and iron metabolism biomarkers with disease severity. Methods and Results: The study included 40 RRMS patients and 40 control subjects of matched age and gender. Serum NOX4,5, ferritin, iron, iron binding capacity, C-reactive protein (CRP), compelet blood count (CBC) and erythrocyte sedimentation rate (ESR) levels were measured in all the study subjects.All the participants were subjected to complete history taking, general and full neurological examination, Expanded Disability Status Scale (EDSS). Results: Higher serum NOX5 ($p < 0.0001$), CRP ($p < 0.0001$), ferritin ($p < 0.0001$) and lower serum NOX4 ($p < 0.0001$) and iron ($p < 0.0001$) concentrations were found in the patients than in controls. No correlation was found between NOXs, CRP, WBCs, ESR and iron metabolism biomarkers in patients. Conclusion: Our data suggest that increased NOX5 expression and decreased levels of NOX4 might be related with oxidative stress related vascular changes and BBB disruption in MS patients. We also demonstrated that lower concentrations of iron and TIBC in RRMS patients. Because of the importance of iron on myelination and oligodendrocytes functions serum iron levels should be closely monitored in MS patients .

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

1.Introduction

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disorder. Relapsing-remitting MS (RRMS) is the most common MS pattern characterized by relapses or exacerbations . Although the etiology of the disease is still unknown, it has been proposed that reactive oxygen species (ROS), which are small, oxygen-derived molecules, contribute to MS pathology**(1)**

Elevated nicotinamide adenine dinucleotide phosphate (NADPH) oxidases activations and concentrations are one of the main enzymatic source of ROS including superoxide anion and its derivatives**(2).**

The NADPH oxidases (NOX) family consists of seven catalytic homologues. Four NOX isoforms including NOX1, NOX2, NOX4 and NOX5 are expressed from endothelial cells**(3)**

Blood BBB disruption and vascular changes are determined as one of the prominent and early components in the pathophysiology of MS**(4).** Increased expressions of NOX1, 2, and 5 have been related with endothelial dysfunction and vascular inflammation However, NOX4 exerts protective effects on the vessel wall **(3)** .There has been growing evidence implicating the role of NOX isoforms in the pathogenesis of several neurodegenerative diseases including Amyotrophic lateral sclerosis, Alzheimer's and Parkinson's disease **(5).** However, little is known about the status of serum NOX1, NOX4 and NOX5 in RRMS patients Several previous studies have focused on the interaction between MS and nutritional intake to reduce the symptoms such as decreased cognitive, sensory and physical functions **(6) ,(7)** .One of the important parts of the diet component are micronutrients such as trace elements(**8).**

Abnormal iron depositions have been associated with the production of ROS**(9).** Alterations in iron deposition and serum biomarkers of iron metabolism have been consistently reported in patients with MS**(10) ,(11)**. However, uncertainty still exists about the relationship between iron metabolism and oxidative stress in MS

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 *rs*	*p*	NOX-5 *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>0.05for 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

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the brain and spinal cord, which affects about 2.2 million people worldwide, primarily young adults from 20 to 40 years of age. After one to two decades, many patients enter a progressive phase of the disease (**16**). Multiple sclerosis (MS) is characterized by a relapsing remitting (RRMS) course eventually followed by secondary progression (SPMS) or gradual progression of disability since the beginning. MS has been traditionally considered a focal inflammatory demyelinating disease of the white matter (**17**).Inflammation and neuro-degeneration are mutually dependent phenomena, as Inflammation induces degeneration, through excitotoxicity mechanisms, while neurodegeneration can induce inflammatory response, both in the central nervous system (CNS) and in peripheral blood as demonstrated also in other neurodegenerative conditions (i.e., amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's disease) (**18**). Although MS is still of unknown etiology (**19**). It has been proposed that reactive oxygen species (ROS), which are small, oxygen- derived molecules, contribute to MS pathology (**1**). Elevated nicotinamide adenine dinucleotide phosphate (NADPH) oxidases activations and concentrations are one of the main enzymatic sources of ROS including superoxide anion and its derivatives **(2).** Overproduction of ROS and impaired iron metabolism are possible factors in the pathogenesis of Multiple sclerosis MS. The NADPH oxidase (NOX) family consists of seven catalytic homologues, NOX1-5 and two dual oxidases. NOX1 and NOX5 are associated with endothelial dysfunction and inflammation but NOX4 has a protective effect on vascular function., increased NOX5 expression and decreased levels of NOX4 might be related with oxidative stress related vascular changes in MS patients. These findings provide future opportunities to combat MS with separately target individual NOX isoforms (**20).** Abnormal iron depositions have been associated with the production of ROS(**9**). Alterations in iron deposition and serum biomarkers of iron metabolism have been consistently reported in patients with MS(**10**,11). However, uncertainty still exists about the relationship between iron metabolism and oxidative stress in MS, So this study aimed to investigate the status of serum endothelial NOXs (NOX5 and NOX 4) and its relationship with iron metabolism biomarkers in relapsing-remitting MS patients, also to study the possible correlation between NOXs (NOX5 and NOX 4) and iron metabolism biomarkers with disease severity. By Comparing level of Nox4 and Nox5 between the studied groups, the current study reported that MS cases showed significantly lower NOX4, significantly higher NOX5 when compared to control group. These finding agreed with other studies that showed that higher serum NOX5, CRP titer, ferritin and lower serum NOX4, iron concentrations were found in the patients than in controls **(20, 21).** However, other studies revealed that iron may contribute to the pathogenesis and progression of MS due to its accumulation in the human brain with age, have analyzed hemoglobin, iron, transferrin, and soluble transferrin receptor (sTfR) levels in MS patients and compared with controls. And found that, the sTfR levels were significantly higher in MS patients compared to the control group but no difference in the iron values between MS subgroups and control as well as hemoglobin values and transferrin levels were within normal limits in all patients (**22).** The current study revealed that there are no significant differences between relapsing and non-relapsing regarding age and gender, as well as, no significant differences were found regarding disease duration. No significant differences were found between relapsing and non-relapsing regarding laboratory parameters as well as no significant differences were found between relapsing and non-relapsing regarding NOX-4 and NOX-5 levels.

Moreover, no significant differences were found in Nox-4 and Nox-5 levels regarding gender, EDDS, treatment and CRP in MS group. Moreover, no significant correlations of NOX-4 and NOX-5 levels were found with age, WBCs, ESR, CRP, iron, ferritin, TIBC. These findings are in consistent with **(20).**

5.Conclusion

In conclusion, this study revealed that increased NOX5 expression and decreased levels of NOX4 might be related with oxidative stress related vascular changes and BBB disruption in MS patients. And that lower concentrations of iron and TIBC in RRMS patients. Because of the importance of iron on myelination and oligodendrocytes functions serum iron levels should be closely monitored in MS patients

6.Limitations

The small sample size of patients included and so may not have been statistically powered to find significant relation. Restricted inclusion criteria and exclusion criteria for the participants.

NOX4 and 5 were measured only, which wasn't enough as other NOXs(especially NOX2) are important to be measured

7.Recommendation

Taking into account, all the limitations of the study, Serum iron levels should be closely monitored in MS patients regardless whether they are the risk of malnutrition. Adding antioxidants and NOX 5 inhibitor to conventional immunotherapy in MS may be reasonable and highly beneficial for MS patients due to their ability to reduce oxidative stress so future studies for evaluating the effects of antioxidant therapies and how these therapies could be integrated with the current conventional approaches for the treatment of MS patients should be done. NOX 4 enzymes can represent potential targets of new regenerative therapies ending and reversing the progression of the lesions in MS. Using iron chelating agents as animal models of MS have shown a neuro protective effect by iron chelation;however in MS patients,the effectiveness of these pharmacologic modification is still debatable and requires further investigations.

Other factors including biochemical, genetic and presence of associated comorbidities should be taken into consideration for iron status of MS patients as well as nutritional status

Financial support and sponsorship
Nil.

Conflicts of interest:
There are no conflicts of interest

9.References

[1] RE. Gonsette. Oxidative stress and excitotoxicity: a therapeutic issue in multiple sclerosis? Mult Scler.vol. 14,pp.22–34,2008.

[2] A.Panday, MK. Sahoo, D. Osorio, S. Batra. NADPH oxidases: an overview from structure to innate immunity-associated pathologies.Cell.Moll.Immuno.vol. l12,pp.5-21. 2015.

[3] GR. Drummond, CG. Sobey, Endothelial NADPH oxidases: which NOX to target in vascular disease? Trends Endocrinol Metab.vol. 25,pp.452–463. 2014.

[4] A. Waschbisch, A. Manzel, RA. Linker, DH. Lee Vascular pathology in multiple sclerosis: mind boosting or myth busting? Exp Transl Stroke Med.vol. 3,pp.7. 2011.

[5] A. Ajayi, X. Yu, AL. Ström, The role of NADPH oxidase (NOX) enzymes in neurodegenerative disease. Front. Biol. (Beijing).vol. 8,pp.175–188. 2013.

[6] MJ. Bagur, MA. Murcia, AM. Jiménez-Monreal, et al. Influence of diet in multiple sclerosis: a systematic review. Adv Nutr.vol. 8,pp.463–472. 2017.

[7] A. Armon-Omer, C. Waldman, N. Simaan, et al. New insights on the nutrition status and antioxidant capacity in multiple sclerosis patients. Nutrients.vol. 11,pp.(2):427. 2019.

[8] M. Bredholt, JL. Frederiksen Zinc in multiple sclerosis: a systematic review and meta-analysis. ASN Neuro.vol. 8,pp.(3). 2016.

[9] D. Galaris, K. Pantopoulos Oxidative Stress and Iron Homeostasis: Mechanistic and Health Aspects, Crit. Rev. Clin Lab Sci.vol. 45,pp.1–23. 2008.

[10] N. Bergsland, S. Agostini, MM. Laganà, et al. Serum iron concentration is associated with subcortical deep gray matter iron levels in multiple sclerosis patients. Neuro Report.vol. 28,pp.645–648. 2017.

[11] KPZ. Ferreira, SR. Oliveira, AP. Kallaur, et al. Disease progression and oxidative stress are associated with higher serum ferritin levels in patients with multiple sclerosis. J Neurol Sci. vol.373,pp.236–241. 2017.

[12] IBM Corp, Released IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. 2011.

[13] RS. Greenberg, RS. Daniels, WD. Flanders, JW. Eley, JR. Boring Diagnostic testing. In Medical

epidemiology 3rd edition. McGraw-Hill, New York, NY.vol. 77,pp.89. 1996.

[14] CR. Khothari, Research Methodology: Methods and Techniques, New Age International, New Delhi. 2004.

[15] JE. Fischer, LM. Bachman, R. Jaeschke, A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. Intensive Care Med.vol. 29,pp.1043– 51. 2003.

[16] YaninaTimasheva,Timur R.Nasibullin,Ilsiyar A.Tuktarova,Vera V.Erdman,Timur R.Galiullin, Oksana V.Zaplakhova. Multilocus evaluation of genetic predictors of multiple sclerosis. Gene ,Volume.vol 809,pp.146008. 2022.

[17] J. Kamińska, OM. Koper, K. Piechal, H. Kemona. Multiple sclerosis - etiology and diagnostic potential. Postepy Hig Med Dosw (Online).vol.30,pp.71(0):551-563.2017.

[18] J. Correale, MI. Gaitán, MC. Ysrraelit, MP. Fiol. Progressive multiple sclerosis: from pathogenic mechanisms to treatment. Brain.vol.1,pp.140(3):527-546. 2017.

[19] M. Förster, C. Nelke, S. Räuber, H. Lassmann, T. Ruck, MP. Sormani. Nitrosative Stress Molecules in Multiple Sclerosis: A Meta-Analysis. Biomedicines.vol.14,pp.9(12):1899 . 2021.

[20] HO. Doğan, ÖK. Yildiz. Serum NADPH oxidase concentrations and the associations with iron metabolism in relapsing remitting multiple sclerosis. J Trace Elem Med Biol.vol.55,pp.39-43. 2019.

[21] M. Siotto, MM. Filippi, I. Simonelli, D. Landi, A. Ghazaryan, S. Vollaro. Oxidative Stress Related to Iron Metabolism in Relapsing Remitting Multiple Sclerosis Patients With Low Disability. Front Neurosci.vol. 11,pp.13:86. 2019.

[22] D. ALgin, O. Özdemir, H. Çuruksulu. The Impact of Iron Metabolism in the Pathogenesis of Multiple Sclerosis. Turkiye Klinikleri Journal of Neurology.vol. 10,pp.(1):1-5.2015.