Role of nitric oxide and malondialdehyde biomarkers in relapsing-remitting multiple sclerosis

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Background Oxidative stress (OS) contributes to several mechanisms underlying the pathogenesis of multiple sclerosis (MS).

Aim To assess the role of OS biomarkers in pathogenesis of MS and the effect of interferon-β (IFN-β) on OS in MS.

Patients and methods A total of 40 patients diagnosed as having relapsing-remitting MS with age ranged from 20 to 40 years participated in the study. Of them, 20 patients were on IFN-β for at least 6 months, and 20 patients were not receiving any disease-modifying therapy. Another 20 apparently healthy participants, age matched with the patients, were considered as a control group. Serum levels of malondialdehyde (MDA) and nitric oxide (NO) were evaluated in both patients and control groups.

Results The serum levels of NO and MDA were significantly higher in patients with relapsing-remitting MS than control group, and in those not taking disease-modifying therapy than patients on IFN-β. Serum levels of both MDA and NO were correlated with degree of disability assessed by expanded disability status scale.

Conclusion NO and MDA are reliable markers of OS and could be used as markers of disease progression and treatment response in patients with MS. IFN-β has a strong effect on OS and it may exhibit its effect in the management of MS by acting as antioxidant in addition to its anti-inflammatory effect.

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system in which inflammation, demyelination, and axonal loss occurs from very early stages of the disease [1].

The ultimate causative factors of these processes remain unknown. However, emerging evidence suggests a significant role for oxidative stress (OS) in MS. An imbalance between the production of free radicals [reactive oxygen species (ROS) and reactive nitrogen species] on one hand, and the antioxidative defence on the other hand, leads to OS and nitrosative stress [2].

OS induces demyelination and neurodegeneration by direct oxidation of lipids, proteins, and DNA as well as by the induction of mitochondrial injury, which results in energy deficiency and further amplification of oxygen radical production [3].

Malondialdehyde (MDA) and nitric oxide (NO) are two of the most reliable OS biomarkers. MDA is regarded as a typical biomarker of OS. As it has high reactivity, MDA is toxic, potentially mutagenic, and atherogenic owing to its reactions with biomolecules such as proteins and nucleic acids. Alteration of MDA level in the body often reflects pathological changes, which have been verified in various types of illnesses such as leukaemia, MS, diabetes, cancer, cardiovascular disease, age-related macular degeneration, asthma, atherosclerosis, and liver disease [4].

The increase of NO production in patients with MS damages myelin and oligodendrocytes. NO also increases cyclic GMP, which consequently leads to the increase of the effect of tumour necrosis factor-α and other cytokines [5].

Immunomodulatory therapies have shown to reduce the rate of relapse significantly, and delay the progression of neurological disability in patients with relapsing-remitting multiple sclerosis (RRMS) [6]. Interferons are considered to be members of the cytokine family of proteins [7]. Interferon-β (IFN-β) is a first-line treatment for MS [8] and a key component of the innate immune system [7]. It can cause complex immunomodulatory effects, but its mechanism of action as an immuno-modulator in the treatment of MS is not fully understood [7,9].

This study aimed to evaluate the role of OS biomarkers (MDA and NO) in RRMS and assess the effect of IFN-β on OS in patients with RRMS.

Patients and methods

A total of 40 (two males and 38 females) patients diagnosed as having RRMS (according to McDonald *et al*. criteria 2010) [10], with age ranged from 20 to 40 years, participated in the study. They were divided into two groups according to treatment: group A included 20 patients on IFN-β 1A (Rebif, MERCK Europe, The Netherlands, 44 μg) subcutaneously three times a week for at least 6 months, and group B included 20 patients with RRMS who were not receiving any disease-modifying therapy (DMT). The patients were selected from the neurology outpatient clinic of Al-Zahraa University Hospital, Multiple Sclerosis Unit of Al-Hussein University Hospital, and Multiple Sclerosis Unit of Ain Shams University Hospital. Patients were free of relapse for at least 30 days before sampling. Another 20 apparently healthy women, age matched with the patients, were considered as a control group.

Individuals receiving trace element, antioxidants, or vitamin B complex; patients with other types of MS; patients with acute or chronic illness other than MS; and patients on other DMT for MS were excluded from the study.

After signing of an informed consent to participate in the study, patients were subjected to the following: full history taking, complete general and neurological examination, assessment of disability by expanded disability status scale (EDSS), routine laboratory investigations, and estimation of two biomarkers of OS:

(1) Estimation of serum level of MDA using competitive-binding enzyme immunoassay technique; the kit was supplied by SAB (single way Antibody Company, Hi-Tech Zone, Nanjing, Jiangsu, China).

(2) Estimation of serum level of NO using Biodiagnostic nitrite assay kits using Griess reagents; the kit was supplied by Biodiagnostic (Rohini, New Delhi, India).

The study was approved by the Ethical Committee of Faculty of Medicine Al Azhar University.

Study design

This was a case–control comparative study.

Statistical analysis

Data were analyzed using statistical package for the social sciences (SPSS) version 18.0 (PEARSONEDUCATION, USA). Quantitative data were expressed as mean±SD. The following tests were used: independent-samples *t*-test of significance was used when comparing between two means, analysis of variance test was used to identify the variation in the different variables, and Pearson correlation coefficient (*r*) test was used for correlating data. *P* value less than 0.05 was considered significant, *P* value less than 0.01 was considered highly significant, and *P* value more than 0.05 was considered insignificant.

Results

Demographic and clinical data of the studied groups

The individuals in the MS and control groups were comparable in terms of age and sex as shown by nonstatistically significant differences between the groups ($P>0.5$; Table 1).

Regarding the clinical data, in group A (on IFN-β), the disease duration ranged from 18 to 60 months with a mean of 34.5±13.18; the number of attacks was from 2 to 6, with a mean of 3.60±1.39; and the EDSS was from 0.5 to 2, with a median of 1.25 (0.5–1.5).

In group B (without treatment), the disease duration ranged from 9 to 48, with a mean of 21.85±11.14; the number of attacks was from 1 to 6, with a mean of 3.60 ±1.39; and the EDSS was from 0.5 to 5, with median of 1.00 (0.5–2.0) (Table 2).

Table 1 Demographic data of the studied groups regarding the age and sex

	Group A (*N*=20)	Group B (*N*=20)	Control group (*N*=20)	*P* value
Age (years)				
Mean±SD	29.45±5.72	29.15±4.38	26.55±4.70	NS
Range	20–38	21–38	21–36	
Sex				
Females	20 (100.0)	18 (90.0)	20 (100.0)	NS
Males	0	2 (10.0)	0	

Results of serum levels of nitric oxide and malondialdehyde in the study groups

The serum levels of NO and MDA were significantly higher in patients group than control group (*P*? 0.01; Table 3).

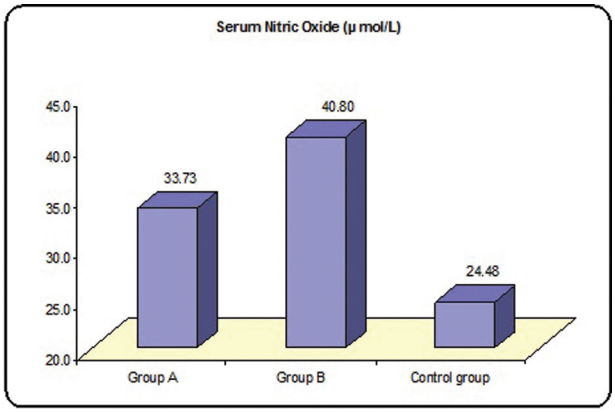
In addition, the serum levels of NO and MDA were significantly higher in group B (on no treatment) than group A (on IFN-β) (*P*? 0.01; Table 4 and Figs 1 and 2).

There was a highly significant positive correlation between the serum level of MDA and NO and the EDSS in group A (on IFN-β) (*P*? 0.01; Table 5).

In addition, there was a significant positive correlation between the serum level of the two biomarkers and the disease duration and number of attacks (*P*? 0.05; Table 5).

There was a highly significant positive correlation between the serum level of MDA and NO and the

Figure 1



Comparison between patient groups and control group regarding the serum level of nitric oxide.

Table 2 Descriptive analysis of group A and group B regarding the disease duration, the number of attacks, and expanded disability status scale

Clinical parameters of the disease	Group A (*N*=20)	Group B (*N*=20)
Disease duration (months)		
Mean±SD	34.5±13.18	21.85±11.14
Range	18–60	9–48
Number of attacks		
Mean±SD	3.75±1.12	3.60±1.39
Range	2–6	1–6
EDSS		
Median (IQR)	1.25 (0.5–1.5)	1.00 (0.5–2.0)
Range	0.5–2	0.5–5

EDSS, expanded disability status scale; IQR, interquartile range.

Table 3 Comparison between patients group and control group regarding the serum level of nitric oxide in µmol/l and malondialdehyde in nmol/ml

	Patient group (*N*=40)	Control group (*N*=20)	Test value[a]	*P* value
Serum nitric oxide (µmol/l)				
Mean±SD	37.26±4.09	24.48±1.76	−13.323	0.000
Range	27.2–43.2	21.4–26.8		
Serum MDA (nmol/ml)				
Mean±SD	2.93±0.59	1.16±0.39	−12.221	0.000
Range	1.90–4.1	0.6–1.7		

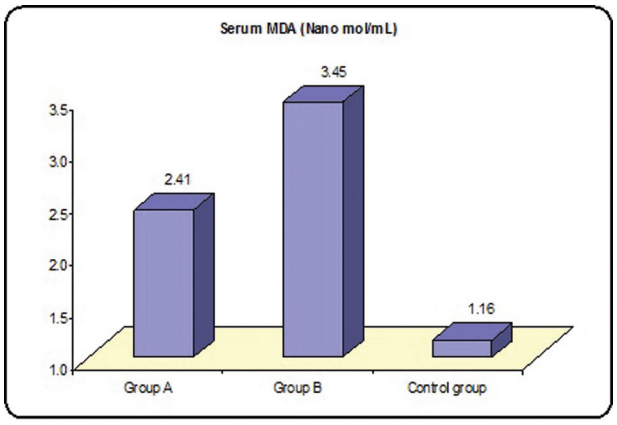
MDA, malondialdehyde. [a]Independent *t*-test.

Table 4 Comparison between the studied groups according to the serum levels of nitric oxide and malondialdehyde

	Group A (*N*=20)	Group B (*N*=20)	Control group (*N*=20)	Test value	*P* value	Post-hoc analysis		
						P_1	P_2	P_3
Serum nitric oxide (µmol/l)								
Mean±SD	33.73±2.20	40.80±1.79	24.48±1.76	360.433	0.000	0.000	0.000	0.000
Range	27.2–35.9	37.1–43.2	21.4–26.8					
Serum MDA (nmol/ml)								
Mean±SD	2.41±0.29	3.45±0.24	1.16±0.39	269.780	0.000	0.000	0.000	0.000
Range	1.90–2.88	3–4.1	0.6–1.7					

MDA, malondialdehyde.

Figure 2



Comparison between patient groups and control group regarding the serum level of malondialdehyde.

Table 5 Correlation between the serum level of nitric oxide and malondialdehyde and disease duration, number of attacks and expanded disability status scale in-group A

	Group A			
	Serum nitric oxide (μmol/l)		Serum MDA (nmol/ml)	
Clinical parameters	*r*	*P* value	*r*	*P* value
Disease duration	0.503*	0.024	0.533*	0.015
Number of attacks	0.510*	0.022	0.522*	0.018
EDSS	0.690**	0.001	0.693**	0.001

EDSS, expanded disability status scale; MDA, malondialdehyde. *Statistically significant. **Highly significant.

Table 6 Correlation between the serum level of nitric oxide and malondialdehyde and disease duration, number of attacks and expanded disability status scale in-group B

	Group B			
	Serum nitric oxide (μmol/l)		Serum MDA (nmol/ml)	
Clinical parameters	*r*	*P* value	*r*	*P* value
Disease duration	0.745**	0.000	0.716**	0.000
Number of attacks	0.706**	0.001	0.711**	0.000
EDSS	0.947**	0.000	0.952**	0.000

EDSS, expanded disability status scale; MDA, malondialdehyde. **Highly significant.

disease duration, number of attacks, and the EDSS in group B (with no treatment) (*P*? 0.01; Table 6).

Discussion

It is suspected that the development of MS can be affected by OS [2]. This study was performed to assess the OS in a group of patients with RRMS by measurement of plasma levels of a byproduct of lipid peroxidation, MDA, and a free radical signaling molecule, NO. Moreover, the effect of interferon (INF-β), as a DMT, on OS in those patients was studied by comparing the two groups, 20 patients with RRMS on INF-β therapy for at least 6 months and 20 patients with RRMS without any DMT, and correlating the results with the clinical parameters of the disease and the degree of disability using EDSS.

In this study, serum MDA levels were found significantly higher in patients with MS of both groups than the healthy control participants. This result is consistent with a study done by Yousefi *et al.* [11] on 38 patients with MS and 38 control, where the serum arsenic and MDA levels were measured during the study to assess the relationship between the serum arsenic level and lipid peroxidation in MS. The study revealed significantly higher MDA level in patients with MS than control.

In addition, Tavassi *et al.* [12] reported that MDA in the serum of patients with MS showed a tremendous, 210-fold, increase in comparison with the concentration measured in serum of controls.

Several studies have demonstrated a significant increase in lipid peroxidation products in the brain, serum, plasma, and cerebrospinal fluid in patients with MS [13], which is a clear evidence that ROS-mediated lipid peroxidation is increased during this pathological condition [12].

Regarding the effect of IFN-β on the serum level of MDA, this study showed that the serum level of MDA was significantly higher in group B (not receiving any specific medications for MS) than group A (on IFN-β). This result is consistent with a study done by Adamczyk *et al.* [2]. The study included 121 patients with RRMS divided into four groups according to the type of DMT. They reported a statistically significant increase in the serum level of MDA in patients not receiving treatment, than in patients on interferon therapy.

In a follow-up study, Noroozi *et al.* [5] found that the serum level of MDA of the same patient group (30 MS) was significantly decreased after 6 months of INF-β therapy.

This indicates that the OS is enhanced in nontreated patients, and lower OS in treated patients may be attributed to the effect of IFN-β therapy.

Abundant evidence points to an important role for NO in the pathogenesis of MS and to its contribution in the various facets of the disorder: inflammation,

oligodendrocyte injury, changes in synaptic transmission, axonal degeneration, and neuronal loss [14].

This study revealed that the serum level of NO was significantly higher in patients group than control group.

This result agrees with a study done by Ibragic *et al*. [15] on 30 patients with MS and 30 control. They found that the serum level of NO in patients with MS was higher than that of the control. Moreover, this result is consistent with the study done by Niedziela *et al*. [16] to assess the serum nitric oxide metabolites (NOx) as one of the reactive nitrogen species and inflammatory parameters in patients with RRMS and to compare the effectiveness of various types of DMTs. They reported that the serum level of NOx was higher in all patients than healthy control.

Similar result was obtained from a study carried out by Ljubisavlievic *et al*. [17] on 57 patients with RRMS and 20 control. They assessed the serum level of NOx in both cerebrospinal fluid (CSF) and plasma of the studied groups and reported that NOx levels, in CSF and plasma, were significantly increased in RRMS compared with control.

Most studies reported significantly higher NOx in patients with RRMS compared with control groups in acute relapse and in remission [18], which, in turn, implies an elevated risk of producing the highly oxidizing radical peroxynitrite (ONOOH–) with serious consequences for the brain tissue integrity [12].

However, this study disagrees with the study done by Acar *et al*. [19] as they found that the serum level of NO was lower in patients with MS than that of the control.

Moreover, Oliveira *et al*. [20] and Kalluar *et al*. [21] reported that the serum level of NO was lower in MS patient group than control group.

They reported that it is likely that the reduction in NOx levels in these studies was associated with redox imbalance and that inflammation elevates ROS levels, and increased NO consumption may occur with high inflammatory activity, resulting in decreased serum NOx bioavailability [20].

This study showed that the serum level of NO in group B (not receiving treatment) was significantly higher than group A (on interferon). This result agrees with a study carried out by Stepien *et al*. [22] on 38 of patients with RRMS. They assessed the serum level of NO at the study entry and at 12, 24, and 36 months of IFN-β therapy. They reported that IFN-β significantly decreased the serum level of nitrite (one of NOx).

These results indicate the beneficial role of IFN-β therapy on decreasing OS activity. Nevertheless, the exact mechanism of action is still unclear. IFN-β appears to directly increase expression and concentration of anti-inflammatory agents whereas downregulate the expression of proinflammatory cytokines, and thus, it decreases the OS. IFN-β also downregulates proinflammatory Th1 cytokines and upregulates anti-inflammatory Th2 cytokines. IFN-β was also reported to decrease induced NO synthesis by the inhibition of inducible NO synthase [22].

This result disagrees with a study done by Noroozi *et al*. [5] which showed that treatment with IFN-β did not decrease the serum level of NO. However, it increased after taking the medication, but the increase was trivial.

The discrepancy in the results could be owing to the complex manifestation of the disorder and the multiplicity of functions that NO serves in the body and the unusually complex biochemistry of NO in normal and inflamed tissues.

In this study, there was a positive significant correlation between serum levels of MDA and duration of the disease, frequency of attacks, and EDSS in both group A (on INF-β therapy) and group B (without DMT).

This result agrees with a study done by Ljubisavljevic *et al*. [23] in which advanced oxidation protein products, MDA, and superoxide dismutase activity were measured and compared with patients' clinical severity (EDSS) and disease duration. They found that there was positive correlation between MDA level and EDSS.

This result is not consistent with Adamczyk *et al*. [2] and Acar *et al*. [19], who reported that no correlation was found between the serum level of MDA and the clinical parameters (disease duration, number of attacks, and EDSS) of patients with MS.

In addition, in this study, there was a positive significant correlation between serum levels of NO and duration of the disease, frequency of attacks, and EDSS in both patient groups.

This result agrees with Stepien *et al*. [22] who reported that the serum level of NO was positively correlated with EDSS of the patients.

However, our results are not consistent with Tavassi *et al*. [12] who reported that changes in serum metabolites (including MDA and NO) in patients with MS failed to correlate with EDSS.

Different outcomes were probably related to the various neurological and clinical status of patients with MS, and different lifestyle, diet, or pharmacological interactions.

Conclusion

The results of this study show that patients with RRMS have high OS activity and that both NO and MDA may be reliable markers for OS, disease progression, and treatment response.

According to the results obtained in this study, a new insight may be drawn that IFN-β may exhibit its effect in management of MS by acting as antioxidant through its anti-inflammatory effect. Ongoing and future studies will increase our understanding of the actions of IFN-β on the immune system and the central nervous system, which will in turn aid advances in the management of MS.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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