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Plasma thiol/disulphide homeostasis changes in patients with relapsing-remitting multiple sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a neuroinflammatory disease and inflammation and oxidative stress play important roles in its pathology. Thiol/disulphide homeostasis (TDH) is a special oxidative stress biomarker that has been found to be affected in several disorders including MS. There is no study demonstrating the effects of attack status of the relapsing-remitting multiple sclerosis (RRMS) patients on TDH levels. Our aim was to determine TDH levels in three different periods of RRMS patients and healthy individuals.

Methods: The study was carried out in 29 patients with RRMS without a prior attack in the last twelve months (MS Control), 21 RRMS patients having a clinical acute attack within the last week (MS relapse), 12 of 21 MS relapse patients one month after the onset of attack and following 1000 mg methylprednisolone for 7 days (MS Remission) and 30 age- and sex-matched healthy individuals. TDH status was determined using an automated spectrophotometric analysis method. TDH levels in all patient groups and control subjects were compared with each other.

Results: The lowest native thiol, total thiol levels and native thiol/total thiol ratio were found in the MS relapse patients in comparison to the MS control, MS remission groups and healthy controls. In contrast, disulphide levels, disulphide/native thiol and disulphide/total thiol ratios were highest in the MS relapse group compared to the other patient groups and healthy subjects.

Conclusion: Our findings indicate that increased oxidative stress in RRMS patients is reflected with decreased native and total thiol and increased disulphide levels. Since the formation of disulphide bonds is reversible, the progression of RRMS involving abnormal TDH may be controlled, converting disulphides to thiols. So, we suggest determining the dynamic TDH status as a novel and special biomarker in the diagnosis and prognosis of the RRMS patients.

What's known

- Multiple sclerosis (MS) is a neuroinflammatory disease and inflammation and oxidative stress play important roles in its pathology.
- Like other inflammatory diseases, oxidative stress is associated with MS playing an important role in the pathogenesis of MS.

What's new

- Our study provides original data on dynamic Thiol/disulphide homeostasis (TDH) status in the relapsing-remitting multiple sclerosis (RRMS) patients at three different periods of the disease and examines whether plasma TDH can be used as a special oxidative stress biomarker in the diagnosis and follow-up of the RRMS patients and their response to the therapy.

1 | INTRODUCTION

Multiple sclerosis (MS) is an autoimmune and demyelinating disease of the central nervous system (CNS).[1] The cause of MS has not been completely understood and is still under investigation, but complex environmental and genetic interactions have been shown to associate with its development. Relapsing-remitting MS (RRMS) is the most common type of MS and often defines the initial disease phase for the majority of patients.[2,3] RRMS is known for clearly defined attacks of new or escalating neurological symptoms. These attacks are mostly referred to as exacerbations or relapses and are followed by a period of partial or complete recovery (remissions). MS is a chronic neuroinflammatory disease with many pathological events such as acute demyelinating attacks, inefficient remyelination, axonal damage, mitochondrial dysfunction and progressive neurodegeneration.[4] The immune system associated with inflammation and oxidative stress plays a role in the pathology of MS.[5,6]

The activation of microglia and astrocytes has been shown to be most frequently associated with the expression and release of oxidative stress-related molecules.[7] In recent studies, it has been shown that in neurodegenerative diseases, these molecules regulate oxidative stress and the synthesis of many antioxidant molecules might increase in response to oxidative stress.[8] Oxidative stress plays an important role in many diseases.[9-16] Similar to other inflammatory diseases, oxidative stress is associated with MS playing an important role in the pathogenesis of MS.[17] Unfortunately, a test in blood and body fluids is not available for the early diagnosis of MS and to follow and assess the course of the disease. Specific biomarkers for the diagnosis and prognosis of MS are currently under investigation, but unfortunately, they are not yet available.

Thiols are essential and potent antioxidant molecules containing functional sulfhydryl groups protecting the organism against harmful effects of oxidative stress damage. Thiol groups in cysteine, homocysteine, glutathione, albumin and other proteins as an antioxidant defence mechanism are oxidised by ROS and form reversible disulphide bonds. The disulphide bonds can be reversibly reduced to thiol groups by antioxidants. This helps to maintain the thiol/disulphide homeostasis. Dynamic thiol-disulphide homeostasis (TDH) has a crucial role in redox states. Disulphide, total and natural thiol levels change to restore deteriorated redox balance. Dynamic TDH is kept within a certain range in healthy humans. Dysregulated dynamic TDH has been found in various disorders.[18] Since the formation of disulphide bonds is reversible, the progression of diseases having abnormal thiol/disulphide homeostasis may be controlled or these diseases may even be prevented.

Interest for studies with thiol/disulphide homeostasis is increasing to determine the state of oxidative stress in various diseases.[19] TDH has been measured usually only in one direction, but the novel method developed by Erel and Neselioglu allows the levels of both variables to be measured separately, as well as jointly.[20] According to the results of our literature search, increased ROS levels were reported in the previous studies performed in the MS patients including even a few studies related to TDH, but there is no information regarding the attack status of the multiple sclerosis patients on TDH levels. TDH has not been studied and measured in the RRMS patients at different periods of the disease. In our study, unlike the previous studies, we recruited three different relapsing-remitting multiple sclerosis patients such as those who had no attacks for 1 year, those who had a new attack within the last week and those one month after the attack and pulse steroid therapy. We measured the TDH levels in three different relapsing-remitting multiple sclerosis patients and healthy controls to clarify the effects of attacks and therapy on TDH status at different periods of the MS patients.

Thus, our study provides original data on this important dynamic TDH status in the RRMS patients at different periods of the disease and examines whether it can be used as a special oxidative stress biomarker in the diagnosis and follow-up of RRMS patients and their response to the therapy.

2 | METHODS AND MATERIALS

2.1 | Study subjects and patient selection

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethical Committee (09.05.2016/2016-123). Written informed consent was received from the MS patients and control subjects before being included into the study.

The patients were recruited at the Neurology Department of Bakirkoy Psychiatry and Neurology Research and Training Hospital. Exclusion criteria for patients and healthy individuals were systemic or other neurological diseases such as cardiovascular disease, cerebrovascular disease, diabetes mellitus, chronic hepatic or renal failure, pregnancy, inflammatory rheumatologic disease, malignancies, Parkinson's disease, Alzheimer disease, polyneuropathy and use of antioxidant substances, lipid-lowering drugs, cigarette, alcohol and

vitamin supplements. Blood was collected for TDH assays from the healthy controls and three different relapsing-remitting multiple sclerosis (RRMS) patients according to the following recruitment criteria as indicated below:

MS Control: 29 patients with RRMS without a prior attack in the last twelve months.

MS Relapse: 21 RRMS patients having a clinical acute attack within the last week.

MS Remission: Only 12 of the 21 MS relapse patients admitted to the hospital one month after the onset of attack and following 1000 mg methylprednisolone for 7 days.

Healthy Controls: 30 age- and sex-matched healthy individuals.

2.2 | Expanded Disability Status Scale (EDSS)

The disease severity was assessed by Expanded Disability Status Scale (EDSS) which ranges from 0 to 10 in 0.5-unit increments that represent higher levels of disability. Scoring is based on neurological examination on measures of impairment in eight functional systems including pyramidal, cerebellar, brainstem, sensory, bowel and bladder function, visual function and cerebral functions.[21]

2.3 | Automated measurements of total/native thiol concentrations

Fasting morning venous blood samples were collected into EDTA-containing tubes from both RRMS patients and healthy individuals for the assessment of dynamic TDH. Plasma samples were separated from cells by centrifugation at 1500× *g* for 10 minutes. The samples were frozen at −80°C until analysis. Dynamic TDH in blood samples was determined by an automatic-spectrophotometric method as described briefly below.[20] The dynamic disulphide bonds (–S–S) in the samples are reduced to free functional thiol groups (–SH) by sodium borohydride (NaBH4). The unused NaBH4 remnants are completely removed by formaldehyde. All thiol groups, including both reduced and native groups were measured after reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). This compound is stoichiometrically reduced by free thiols in an exchange reaction, forming a mixed disulphide and releasing one molecule of 5-thionitrobenzoic acid (TNB), which was measured at 412 nm with a Shimadzu UV-1800 spectrophotometer with a temperature-controlled cuvette holder and a Cobas c501 automated analyser (Roche). The amount of dynamic disulphide was determined by taking half of the difference between total thiol and native thiol groups. Following the determination of native and total thiols, and disulphide amounts, the percentages of disulphide/total thiol ratios, disulphide/native thiol ratios and native thiol/total thiol ratios were calculated.

2-Mercaptoethanol solutions were used as calibrators. For the validation of the method used, 500 μmol/L 2-mercaptoethanol, 480 μmol/L GSH and 430 μmol/L albumin solutions were freshly prepared. These solutions were oxidised with freshly prepared 300 μmol/L H2O2 solution at serially increasing concentrations in order to monitor the thiol/disulphide changes in these solutions for 10 minutes. In addition, Chloramine-T was used as an oxidant instead of hydrogen peroxide for the oxidation of thiol groups, because it contains catalase, which has a high turnover number for hydrogen peroxide. Native thiol, total thiol and disulphide concentrations of the pretreated samples were then determined. The percent recovery of our method was determined via the addition of 200 μmol/L oxidised glutathione to plasma samples. The mean percent recovery was found as 98%-101%.

2.4 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23.0 package program (SPSS Inc Chicago, IL, USA). Quantitative data were given as mean ± SE or medians (interquartile ranges, IQR). Normal distribution and differences between variances were determined using Kolmogorov-Smirnov and Shapiro-Wilk normality test, respectively. For comparisons between the two groups, unpaired *t* test was performed for normally distributed variables. *P* value <.05 was considered statistically significant. Kruskal-Wallis test was used to determine significant differences among groups. If a significant difference was found in Kruskal-Wallis test, Dunn's test was used to assess comparisons between the two groups. For the results of all analyses, $P < .05$ and $P < .001$ were considered as statistically significant. The Spearman correlation coefficient was calculated to analyse the association between two continuous variables.

3 | RESULTS

A total of 29 RRMS patients (age range between 22 and 52; mean age ± SE 37.1 ± 8.0; 16 female/13 male) without prior acute attack within the last 12 months (MS control), 21 RRMS patients (age range between 23 and 43; mean age ± SE 32.5 ± 6.2; 12 female/9 male) with an acute attack within the last week (MS relapse), 12 of 21 MS relapse patients one month after the onset of attack and pulse steroid therapy (age range between 25 and 42; mean age ± SE 34 ± 6.0; 9 female/3male) and 30 healthy controls (age range between 18 and 47; mean age ± SE 42.9 ± 9.3; 20 female/10 male) were enrolled into the study.

Demographical and laboratory results of the three RRMS patient groups and healthy controls were shown in Table 1. No significant difference was observed in terms of age and gender among the patient groups and healthy controls. The disease durations of the MS control and MS relapse groups were similar. Expanded Disability Status Scale (EDSS) was significantly higher ($P < .05$) in the MS relapse group (2.58 ± 0.26) in comparison to the MS control group (1.92 ± 0.16).

We observed statistically significant changes in most of the parameters of dynamic TDH among the MS control, MS relapse MS remission groups and healthy controls (Table 1, Figures 1 and 2).

TABLE 1 Demographical and laboratory findings of healthy control, MS control, MS relapse and MS remission groups

Parameters	Healthy control group (n = 30)	MS control group (n = 29)	MS relapse group (n = 21)	MS remission group (n = 12)	*P* values
Age	42.9 ± 9.3	37.1 ± 8.0	32.5 ± 6.2	34 ± 6.0	NS; .693
Gender (female/male)	20 (%66.7)/10 (%33.4)	16 (%55.2)/13 (%44.8)	12 (%57.2)/9 (%42.8)	9 (%75)/3 (%25)	NS; .703
Expanded Disability Status Scale (EDSS)		1.92 ± 0.16 (1-3.50)	2.58 ± 0.26 (1-3.50)[d]		<.05
Disease duration (mo)		85.5 ± 62.6 (22-273)	78.56 ± 52.01 (24-185)		NS; .823
Total thiol (μmol/L)	452.7 ± 5.2[a,b]	431.5 ± 5.8[b,d]	404.3 ± 8.7[b,d,f]	438.5 ± 8.8[f]	<.0001
Native thiol (μmol/L)	423.7 ± 7.2[a,b,c]	376.4 ± 7.8[a,d]	329.8 ± 7.5[b,d,f]	388.2 ± 9.8[c,f]	<.0001
Disulphide (μmol/L)	14.9 ± 1.4[a,b,c]	28.4 ± 4.0[a,d,e]	37.1 ± 2.8[b,d,f]	24.9 ± 1.8[c,e,f]	<.0001
Disulphide/native thiol ratio (%)	3.5 ± 0.3[a,b,c]	7.5 ± 0.7[a,d,e]	11.3 ± 0.6[b,d,f]	6.4 ± 0.3[c,e,f]	<.0001
Disulphide/total thiol ratio (%)	3.3 ± 0.3[a,b,c]	6.6 ± 0.5[a,d,e]	9.2 ± 0.5[b,d,f]	5.7 ± 0.2[c,e,f]	<.001
Native thiol/total thiol ratio (%)	93.6 ± 0.5[a,b,c]	87.2 ± 1.5[a,d]	81.6 ± 1.5[b,d,f]	88.5 ± 0.9[c,f]	<.001

Note: Thiol/disulphide homeostasis levels were expressed as mean ± SE.

$P < .05$ and $P < .001$ were considered as statistically significant.

Post hoc (Dunn's test) analysis:

[a]Statistically significant difference between healthy control group and MS control group.

[b]Statistically significant difference between healthy control group and MS relapse group.

[c]Statistically significant difference between healthy control group and MS remission group.

[d]Statistically significant difference between MS control group and MS relapse group.

[e]Statistically significant difference between MS control group and MS remission group.

[f]Statistically significant difference between MS relapse group and MS remission group.

The lowest total thiol levels were found in the MS relapse group (404.3 ± 8.7 μmol/L) which was significantly lower in comparison to the MS control, MS remission and healthy control groups (431.5 ± 5.8 μmol/L, 438.5 ± 8.8 μmol/L and 452.7 ± 5.2 μmol/L, respectively, $P < .0001$). There was no significant difference in the total thiol levels between the MS control and MS remission groups (431.5 ± 5.8 μmol/L and 438.5 ± 8.8 μmol/L, respectively). Total thiol levels were significant higher in the healthy controls (452.7 ± 5.2 μmol/L) compared to the MS control and MS relapse groups (431.5 ± 5.8 μmol/L and 404.3 ± 8.7 μmol/L, respectively $P < .0001$). The difference between the total thiol levels between the healthy controls and MS remission group was not significant. Total thiol levels between the MS control group and MS remission group were also not significantly different.

The highest native thiol level was found in the healthy control subjects (423.7 ± 7.2 μmol/L) and the lowest native thiol levels were found in the MS relapse group (329.8 ± 7.5 μmol/L). The native thiol level in the healthy controls (423.7 ± 5.1 μmol/L) was significantly higher in comparison to the native thiol levels in the MS control, MS relapse and MS remission groups (376.4 ± 7.8 μmol/L, 329.8 ± 7.5 μmol/L and 388.2 ± 9.8 μmol/L, respectively, $P < .001$). There was no significant difference in the native thiol levels between the MS control group and MS remission group (376.4 ± 7.8 μmol/L vs 388.2 ± 9.8 μmol/L, respectively). In contrast, the native thiol levels in the MS control and MS remission groups were significantly higher in comparison to the MS relapse group (376.4 ± 7.8 μmol/L, and 388.2 ± 9.8 μmol/L vs 329.8 ± 7.5 μmol/L, respectively, $P < .001$).

We found a significant increase in disulphide levels in the MS relapse (37.1 ± 2.8 μmol/L), MS remission (24.9 ± 1.8 μmol/L) and MS control (28.4 ± 4.0 μmol/L) groups in comparison to the healthy controls (14.9 ± 1.4 μmol/L, $P < .001$). The highest disulphide level was in the MS relapse group which was significantly higher than the disulphide levels in the MS control, MS remission and healthy controls. The lowest disulphide level was found in the healthy controls. There was a significant difference in the disulphide levels between the MS control group and MS remission group (28.4 ± 4.0 μmol/L vs 24.9 ± 1.8 μmol/L, respectively). (Table 1, Figure 1).

If we look at the thiol/disulphide homeostasis ratios in the three MS patient groups and healthy subjects, the disulphide/native thiol ratio and the disulphide/total thiol ratio were significantly higher in the three MS patient groups compared to the healthy control group (Table 1, Figure 2). The statistically highest disulphide/native thiol ratio (% 11.3 ± 0.6) and disulphide/total thiol ratio (% 9.2 ± 0.5) were found in the MS relapse group compared to the MS control group, MS remission group and healthy controls ($P < .001$), whereas the statistically lowest disulphide/native thiol (% 3.5 ± 0.3) and disulphide/total thiol ratios (% 3.3 ± 0.3) were found in the healthy controls in comparison to the three different MS patient groups.

There were significant differences in the disulphide/native thiol ratios among the three MS groups ($P < .001$), the highest significant disulphide/native thiol ratio value in the MS relapse group (% 11.3 ± 0.6) was followed in decreasing order by the disulphide/native thiol ratio values of the MS control (% 7.5 ± 0.7) and MS

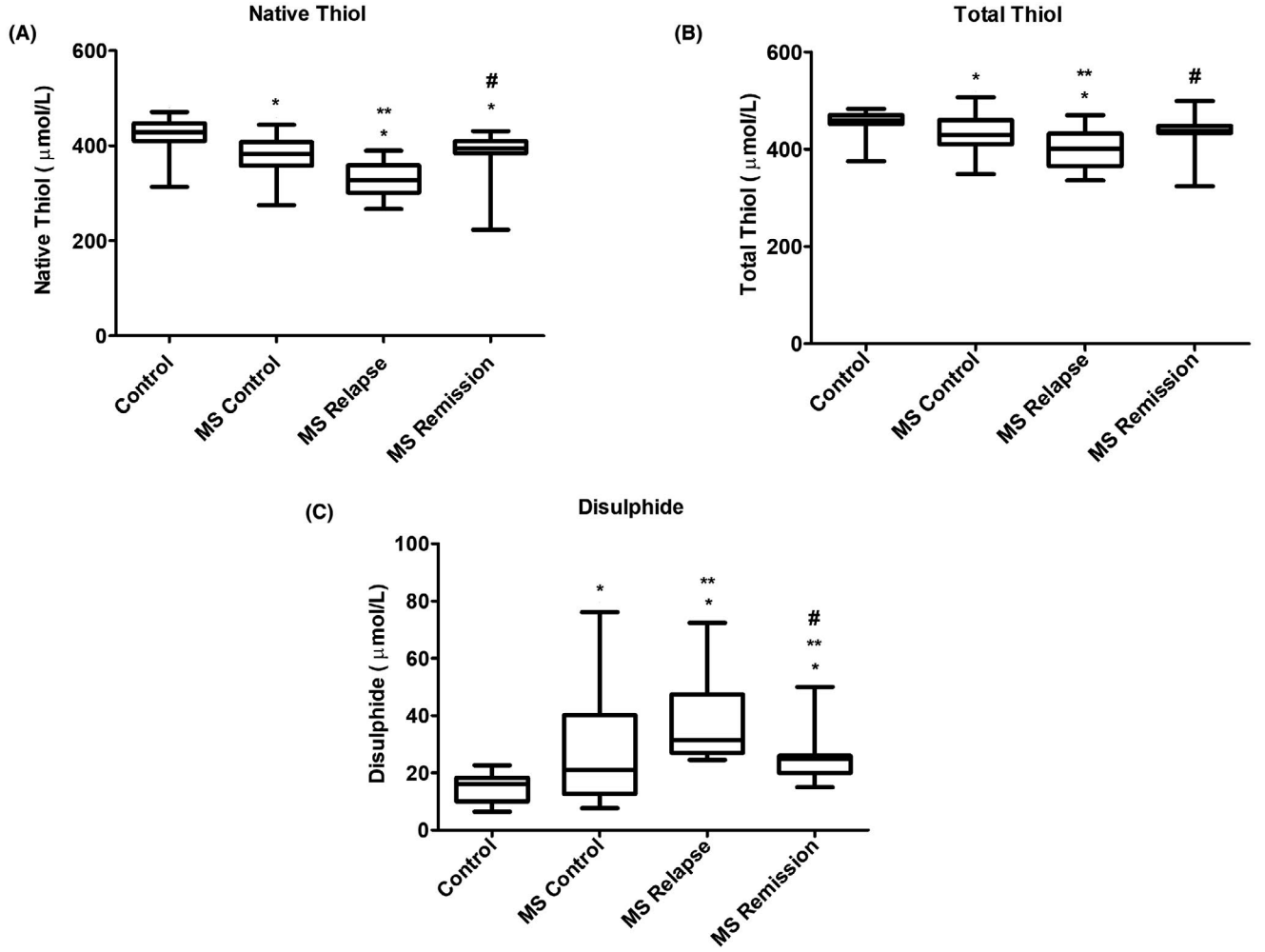

FIGURE 1 The figure shows box-and-whisker plot of dynamic TDH A. The native thiol (Median: 428.5 μmol/L, 383.0 μmol/L, 327.3 μmol/L and 395 μmol/L), B. total thiol (Median: 459 μmol/L, 430 μmol/L, 401.1 μmol/L and 438 μmol/L) and C. disulphide (Median: 16.1 μmol/L, 21.1 μmol/L, 31.4 μmol/L and 25.10 μmol/L) in the Healthy Control, MS Control, MS Relapse and MS Remission groups, respectively (95% confidence interval). The middle lines, upper and lower margin of boxes represent medians. *Values significantly different from the control group ($P < .05$). **Values significantly different from the MS control group ($P < .05$). #Values significantly different from the MS relapse group ($P < .05$)

remission (% 6.4 ± 0.3) groups. The disulphide/native thiol ratio was statistically higher in the MS control group in comparison to the MS remission group (% 7.5 ± 0.7 vs % 6.4 ± 0.3, respectively; $P < .001$).

The disulphide/total thiol ratio in the MS relapse patients (% 9.2 ± 0.5) was significantly higher compared to the MS control (% 6.6 ± 0.5), MS remission (% 5.7 ± 0.2) groups and healthy controls (% 3.3 ± 0.3) ($P < .001$). The disulphide/total thiol ratio was significantly higher ($P < .001$) in the MS control compared to the MS remission groups (% 6.6 ± 0.5 vs % 5.7 ± 0.2) (Table 1, Figure 2).

The lowest significant native thiol/total thiol ratio was found in the MS relapse group (% 81.6 ± 1.5) compared to the MS control (% 87.2 ± 1.5), MS remission (% 88.5 ± 0.9) groups and control subjects (% 93.6 ± 0.5). The highest native thiol/total thiol ratio was found in the healthy controls (% 93.6 ± 0.5) which was significantly higher in comparison to the MS control (% 87.2 ± 1.5) MS relapse (% 81.6 ± 1.5) and MS remission groups (% 88.5 ± 0.9) ($P < .001$). There was no significant difference in the native thiol/total thiol ratios between the MS controls (% 87.2 ± 1.5) ad MS remission groups (% 88.5 ± 0.9).

4 | DISCUSSION

Diagnosis and prognosis of patients with MS are based on symptom patterns consistent with the disease, and brain imaging scans with magnetic resonance imaging (MRI) for confirmation. Although MRI is a reliable method for diagnosing MS, intensive efforts have long been directed towards identifying specific biomarkers in body fluids such as cerebrospinal fluid (CSF) and blood for the early diagnosis of MS.[22,23] The biomarker studies for the diagnosis of MS have increased in the last years. In a recent study performed on a large patient group, serum creatinine and C-reactive protein were investigated as potential diagnostic biomarkers for many neurodegenerative diseases, including MS.[24] However, these two parameters are

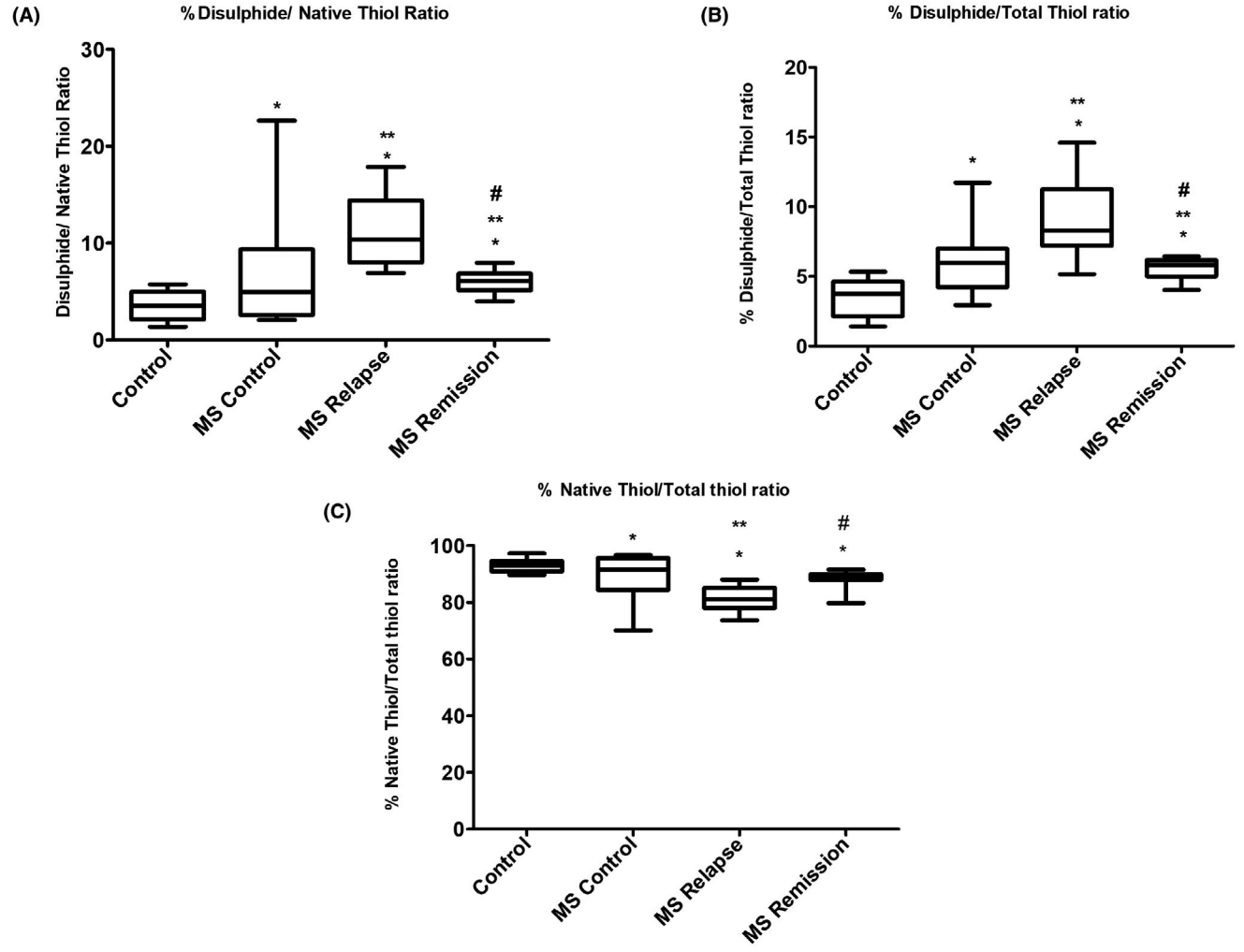

FIGURE 2 The figure shows box-and-whisker plot of percentages of A. disulphide to native thiol (Median: %3.6, %5.0, %10.4 and %6.1), B. disulphide to total thiol (Median %3.7, %6.0, %8.2 and %5.8) and C. native to total thiol (Median %93.0, %91.5, %81.2 and %88.3) in the Healthy Control, MS Control, MS Relapse and MS Remission groups, respectively (95% confidence interval). The middle lines, upper and lower margin of boxes represents medians. *Values significantly different from the control group ($P < .05$). **Values significantly different from the MS control group ($P < .05$). #Values significantly different from the MS relapse group ($P < .05$)

not specific and may reflect other disease processes rather than neurodegenerative and neuroinflammatory diseases.

The pathogenesis of MS is still uncertain, but it is widely recognised that it includes immune system-associated inflammation and oxidative stress in the CNS. So, we focused on dynamic TDH, which might reflect as the common point of these symptoms (inflammation and oxidative stress). Dynamic TDH has been shown to be deteriorated in many neurological diseases such as Alzheimer's, Parkinson's and neurocutaneous diseases.[25-28] Thiol/disulphide homeostasis plays a major role as an important parameter in the evaluation of antioxidant defence and oxidative stress in various diseases.[26,29-31]

Thiols and disulphides in low molecular weight compounds such as cysteine, cystine, cysteinylglycine, homocysteine, g-glutamylcysteine, reduced glutathione (GSH) and oxidised glutathione (GSSG) constitute only a small portion of the body thiol pool. Therefore, thiol and disulphide concentrations of low molecular weight compounds measured in the previous studies may not reflect the total and exact thiol/disulphide status of the body. In contrast, thiols in albumin and other proteins generally constitute the larger portion of the body thiol pool.[32,33] Therefore, we measured total and native thiol concentrations in blood proteins and low molecular weight compounds as the best indicator of thiol/disulphide status and oxidative stress marker.

We have not found any study published in the literature demonstrating dynamic TDH status, which reflects blood total and native thiol concentrations and thiol oxidation product of disulphide levels in the MS patients at different disease periods in comparison to the healthy controls. Therefore, in our study, we investigated the effects of oxidative stress on the pathogenesis of MS with quantification of total thiol, natural thiol and disulphide levels and their ratios. We assessed and compared dynamic TDH changes in three MS patient groups and healthy control subjects. To our knowledge, this is the first study to demonstrate the levels of dynamic TDH in the MS control, MS relapse and MS remission patients. Besides, we determined the disulphide levels formed as a result of thiol oxidation, and

calculated disulphide/native thiol, disulphide/total thiol and native thiol/total thiol ratios to see if the TDH balance might shift to the disulphide or thiol side in the MS patient groups.

In our study, we found depletion of natural thiol and total thiols and increase in disulphides in the RRMS patient groups. In the literature, there is only one study investigating the relation of TDH with visual evoked potentials in the RRMS patients with optic neuritis history. In that study, similar to our findings, no significant correlation between TDH parameters and disease severity and disease duration was found. Only a positive significant correlation was detected between the disulphide/native thiol ratio and P100 latency. The limitations of that study were the inclusion of only one type of MS patient group and the absence of a healthy control group. Since the MS patients were not grouped according to different disease period, and only a single TDH level was given for the whole MS patients, no comparison was made for the TDH levels among the MS patients according to different disease periods. Plus, the data of the MS patients were not compared with the data of healthy control subjects.[34] In contrast to that study, in our present study, MS patients were divided into three different groups according to their attack status as MS control, MS relapse and MS remission groups and the TDH results were compared among the three MS patient groups and also with age- and gender-matched healthy control subjects.

In our study, we found disulphide levels, disulphide/native thiol and disulphide/total thiol ratios in the MS relapse, MS control and MS remission patients higher, whereas native thiol, total thiol levels and native thiol/total thiol ratios in the three MS patient groups lower in comparison to the healthy control subjects. In all three MS patient groups, thiol/disulphide homeostasis was found to shift towards disulphide, the oxidised thiol form. Our data demonstrate that the level of oxidative stress related to the TDH is higher in the three MS patient groups at different disease periods when compared with the healthy subjects. We found that the total thiol and native thiol levels and native thiol/total thiol ratio in the RRMS patients in the remission period one month after the onset of attack and following 1000 mg methylprednisolon for 7 days increased in comparison to the MS relapse group. In contrast, disulphide concentration, disulphide/native thiol and disulphide/total thiol ratios were decreased in the MS remission group in comparison to the MS relapse patients. This indicates that the therapy is efficiently decreases TDH related increases in oxidative stress and helps to restore TDH balance.

In numerous studies, various biomarkers have been examined to clarify the role of several mechanisms in the pathogenesis of MS. In a study, lipid peroxidation products (malondialdehyde and 4-hydroxyalkenals), nitric oxide metabolites (nitrates/nitrites) and glutathione peroxidase activity were found to be significantly increased in the serum of patients with relapsed-remitting multiple sclerosis compared to healthy controls.[35] Oxidative damage triggered by disrupted redox balance reduces glutathione (GSH), the main antioxidant in the brain.[36] In the CSF of MS patients, the GSH levels were significantly lower compared to the healthy controls.[37] It has already been suggested to MS patients having low GSH levels to take GSH supplements to help reducing symptoms.[38] These observations demonstrate that deterioration of redox balances as happens in dynamic TDH homeostasis forms the basis and pathophysiology of neuronal diseases. This hypothesis is supported by our findings showing that native and total thiol levels decrease and disulphide level increase and the dynamic TDH balance is disturbed in the MS patients.

5 | CONCLUSION

In this study, we have investigated the dynamic TDH in MS disease patients at different periods of the disease, using a novel method. Our results demonstrated that measurement of dynamic TDH, as the best indicator of redox status, is important in the diagnosis and follow-up of the multiple sclerosis patients. Since the formation of disulphide bonds, which have detrimental cellular effects, is reversible, the progression of diseases such as MS whose etiopathogenesis involves abnormal thiol/disulphide homeostasis may be controlled, even its progression may be prevented. Therefore, the thiol/disulphide homeostasis is of increasing interest for researchers and clinicians. So, we suggest determining the dynamic TDH as the novel potential biomarker for the follow-up of RRMS patients.

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DISCLOSURE

The authors declare that they have no conflict of interest regarding this study. The authors are responsible for the content and writing of the paper.

AUTHORS' CONTRIBUTIONS

SO, MK, TO and EK conceived of the idea for the study, searched the scientific literature. SO and MK recruited the patients. OE and SN performed the TDH assays. EK did the statistical analysis. SO, EK and TO drafted the manuscript. SO and TO revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Koseoglu M, Ozben S, Gozubatik-Celik G, et al. Plasma copeptin levels in patients with multiple sclerosis. *J Clin Neurosci*. 2020;78:143-146.
2. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*. 2014;83:278-286.
3. Tafti D, Ehsan M, Xixis KL. *Multiple Sclerosis*. Treasure Island, FL: StatPearls Publishing; 2021.

4. Ntranos A, Lublin F. Diagnostic criteria, classification and treatment goals in multiple sclerosis: the chronicles of time and space. *Curr Neurol Neurosci Rep*. 2016;16:90.
5. Groen K, Maltby VE, Scott RJ, Tajouri L, Lechner-Scott J. Concentrations of plasma-borne extracellular particles differ between multiple sclerosis disease courses and compared to healthy controls. *Mult Scler Relat Disord*. 2020;45:102446.
6. Fiedler SE, Spain RI, Kim E, Salinthone S. Lipoic acid modulates inflammatory responses of monocytes and monocyte-derived macrophages from healthy and relapsing-remitting multiple sclerosis patients. *Immunol Cell Biol*. 2020;99:107-115.
7. Ohl K, Tenbrock K, Kipp M. Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp Neurol*. 2016;277:58-67.
8. Sivandzade F, Prasad S, Bhalerao A, Cucullo L. NRF2 and NF-B interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. *Redox Biol*. 2019;21:101059.
9. Hanikoglu A, Kucuksayan E, Akduman RC, Ozben T. A review on Melatonin's effects in cancer: potential mechanisms. *Anticancer Agents Med Chem*. 2018;18:985-992.
10. Aslan M, Ozcan F, Kucuksayan E. Increased small dense LDL and decreased paraoxonase enzyme activity reveals formation of an atherogenic risk in streptozotocin-induced diabetic guinea pigs. *J Diabetes Res*. 2013;2013:860190.
11. Kucuksayan E, Cort A, Timur M, Ozdemir E, Yucel SG, Ozben T. N-acetyl-L-cysteine inhibits bleomycin induced apoptosis in malignant testicular germ cell tumors. *J Cell Biochem*. 2013;114:1685-1694.
12. Kucuksayan E, Konuk EK, Demir N, Mutus B, Aslan M. Neutral sphingomyelinase inhibition decreases ER stress-mediated apoptosis and inducible nitric oxide synthase in retinal pigment epithelial cells. *Free Radic Biol Med*. 2014;72:113-123.
13. Ozekinci M, Kucuksayan E, Erdogan G, et al. Histopathological and biochemical assessment of a novel diagnostic method for ovarian torsion. *Biotech Histochem*. 2020;95:203-209.
14. Dursun E, Timur M, Dursun B, Suleymanlar G, Ozben T. Protein oxidation in Type 2 diabetic patients on hemodialysis. *J Diabetes Complications*. 2005;19:142-146.
15. Aslan M, Ozben T. Reactive oxygen and nitrogen species in Alzheimer's disease. *Curr Alzheimer Res*. 2004;1:111-119.
16. Scoditti E, Massaro M, Garbarino S, Toraldo DM. Role of diet in chronic obstructive pulmonary disease prevention and treatment. *Nutrients*. 2019;11:1357.
17. Ferreira HB, Neves B, Guerra IM, et al. An overview of lipidomic analysis in different human matrices of multiple sclerosis. *Mult Scler and Relat Disord*. 2020;44:102189.
18. Erel O, Erdogan S. Thiol disulfide homeostasis: an integrated approach with biochemical and clinical aspects. *Turk J Medl Sci*. 2020;50:1728-1738.
19. Frijhoff J, Winyard PG, Zarkovic N, et al. Clinical relevance of biomarkers of oxidative stress. *Antioxid Redox Signal*. 2015;23:1144-1170.
20. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47:326-332.
21. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983;33:1444-1452.
22. D'Ambrosio A, Pontecorvo S, Colasanti T, Zamboni S, Francia A, Margutti P. Peripheral blood biomarkers in multiple sclerosis. *Autoimmun Rev*. 2015;14:1097-1110.
23. Guldbrandsen A, Lereim RR, Jacobsen M, et al. Development of robust targeted proteomics assays for cerebrospinal fluid biomarkers in multiple sclerosis. *Clin Proteomics*. 2020;17:33.
24. Cui C, Sun J, Pawitan Y, et al. Creatinine and C-reactive protein in amyotrophic lateral sclerosis, multiple sclerosis, and Parkinson's disease. *Brain Commun*. 2020;2:fcaa152.
25. Vural G, Gumusyayla S, Bektas H, Deniz O, Alisik M, Erel O. Impairment of dynamic thiol-disulphide homeostasis in patients with idiopathic Parkinson's disease and its relationship with clinical stage of disease. *Clin Neurol Neurosurg*. 2017;153:50-55.
26. Gumusyayla S, Vural G, Bektas H, Deniz O, Neselioglu S, Erel O. A novel oxidative stress marker in patients with Alzheimer's disease: dynamic thiol-disulphide homeostasis. *Acta Neuropsychiatr*. 2016;28:315-320.
27. Incecik F, Avcioglu G, Erel O, Neselioglu S, Besen S, Altunbasak S. Dynamic thiol/disulphide homeostasis in children with neurofibromatosis type 1 and tuberous sclerosis. *Acta Neurol Belg*. 2019;119:419-422.
28. Cansever MS, Zubarioglu T, Oruc C, et al. Oxidative stress among L-2-hydroxyglutaric aciduria disease patients: evaluation of dynamic thiol/disulfide homeostasis. *Metab Brain Dis*. 2019;34:283-288.
29. Sonmez MG, Kozanhan B, Deniz CD, et al. Dynamic thiol/disulfide homeostasis as a novel indicator of oxidative stress in patients with urolithiasis. *Invest Clin Urol*. 2019;60:258-266.
30. Gumusyayla S, Vural G, Bektas H, Neselioglu S, Deniz O, Erel O. A novel oxidative stress marker in migraine patients: dynamic thiol-disulphide homeostasis. *J Neurol Sci*. 2016;37:1311-1317.
31. Hanikoglu F, Hanikoglu A, Kucuksayan E, et al. Dynamic thiol/disulphide homeostasis before and after radical prostatectomy in patients with prostate cancer. *Free Radic Res*. 2016;50:S79-S84.
32. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Rad Biol Med*. 2013;65:244-253.
33. Ates I, Kaplan M, Inan B, et al. How does thiol/disulfide homeostasis change in prediabetic patients? *Diabetes Res Clin Pract*. 2015;110:166-171.
34. Vural G, Gumusyayla S, Deniz O, Neselioglu S, Erel O. Relationship between thiol-disulphide homeostasis and visual evoked potentials in patients with multiple sclerosis. *J Neurol Sci*. 2019;40:385-391.
35. Ortiz GG, Macias-Islas MA, Pacheco-Moises FP, et al. Oxidative stress is increased in serum from Mexican patients with relapsing-remitting multiple sclerosis. *Dis Markers*. 2009;26:35-39.
36. Baskol G, Korkmaz S, Erdem F, Canikliogl A, Kocyigit M, Aksu M. Assessment of nitric oxide, advanced oxidation protein products, malondialdehyde, and thiol levels in patients with restless legs syndrome. *Sleep Med*. 2012;13:414-418.
37. Calabrese V, Scapagnini G, Ravagna A, et al. Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and S-nitrosothiols and with changes in glutathione levels. *J Neurosci Res*. 2002;70:580-587.
38. Carvalho AN, Lim JL, Nijland PG, Witte ME, Van Horssen J. Glutathione in multiple sclerosis: more than just an antioxidant? *Mult Scler*. 2014;20:1425-1431.

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