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Periodontal Health and Salivary Thiol-Disulphide Homeostasis in Multiple Sclerosis Patients

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Abstract

Objective
Multiple sclerosis (MS) is a chronic autoimmune disease in which neuroinflammation and oxidative stress play important roles in its pathology. Thiol-disulphide homeostasis is considered a marker of oxidative stress and shown to be affected in several disorders including MS. The aim of this study was to compare salivary disulfide and thiol levels in MS patients with systemically healthy controls and to evaluate whether periodontal status had an effect on thiol-disulfide homeostasis in saliva.

Material and Method
This descriptive study included a total of 184 volunteers, 92 with MS and 92 systemically healthy volunteers. Each person underwent medical, neurological and oral examinations. In saliva samples, native thiol (NT), total thiol (TT), disulphide levels were measured. The ratios of NT/TT, disulphide/NT, D/TT were calculated and compared between the patient and control groups.

Results
There was not any difference in the periodontal parameters between the MS and healthy volunteers ($p>0.05$), however, the biomarkers of thiol-disulphide homeostasis in saliva were significantly different between the groups ($p<0.002$), except for TT. When grouped according to periodontal status, although salivary parameters did not differ in both the MS and control groups ($p>0.05$), MS patients showed decreased NT/TT and increased disulphide/NT ratios compared to the healthy volunteers ($p<0.05$).

Conclusion
Our results have shown that salivary thiol-disulphide balance was shifted to the oxidative side in MS patients.

Keywords: Disulphide, multiple sclerosis, periodontitis, saliva, thiol

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Introduction

Multiple sclerosis (MS) is a heterogeneous, multi-factorial, immune-mediated disease of the central nervous system. It is one of the most frequent neurological diseases in young adults. Though the precise etiology of the disease is unknown, a complex interplay between genetic, epigenetic factors and abnormal immune responses leads to inflammation, demyelination, axonal loss, and gliosis which are the pathological hallmarks of the disease (1). Besides inflammation oxidative stress is also suggested as a preponderant key driver in the pathogenesis (2, 3). In both, inital and chronic stages of MS reactive oxgen species (ROS) and nitrogen species plays a pivotal role. ROS contribute to the formation of many pathological changes such as loss of blood-brain barrier integrity, demyelination, oligodendrocyte death and axonal degeneration in the central nervous system (2). The presence of an imbalance between oxidants and antioxidants, with increased concentrations of ROS in cerebrospinal fluid and blood of MS patients have consistently been reported as one of the common features in persons with MS (2-6).

Periodontitis causing destruction of the periodontium and subsequent teeth loss is a common chronic disorder. Studies report evidence of the relationship between periodontal disease and systemic diseases which are diabetes and cardiovascular disease etc (7).

MS patients may confront with many symptoms during their disease course. Loss of dexterity in MS patients due to sensory, motor or coordination problems and mobility restriction due to increasing disability may affect their ability to perform routine self-oral care. It has been reported that people with MS may be at higher risk of periodontal disease and present with poorer oral hygiene (8). Inflammation and oxidative stress are implicated as the common shared pathogenetic factor in these associations and support the link between the two disease (9-11).

Oxidative stress refers to the proportional imbalance between oxidants and antioxidants, characterized with increased ROS generation and relative deficiency of antioxidants. Generation of ROS, an evolutionarily conserved process, plays an important role in the cell signaling and defense mechanisms. Under normal physiological conditions, low concentrations of ROS have a critical role in various cellular processes such as proliferation, differentiation, and apoptosis whereas higher concentrations leads to tissue damage by triggering many pathophysiological processes such as autophagy, apoptosis, and necrosis (12). The antioxidant system, maintain cellular redox homeostasis and cellular integrity by modulating gene expression and various signaling pathways. Recently, dynamic thiol-disulfide homeostasis stands out as a new oxidative stress indicator in protection from oxidative stress and related mechanisms (13, 14). It has been suggested that dysregulated thiol-disulfide homeostasis has an important role in many diseases in which chronic inflammation is involved in the pathogenesis, such as MS and periodontitis (15-17).

The study aim was determined as salivary disulphide and thiol levels in MS patients and compared to systemically healthy individuals and evaluated whether periodontal status had an effect on thiol-disulphide homeostasis in saliva.

Material and Method

This study was carried out in the Department of Periodontology, Faculty of Dentistry, and the Department of Neurology, Faculty of Medicine at Süleyman Demirel University, Isparta, Türkiye, after obtaining approval from the Clinical Research Ethics Committee (13.12.2018/234). The volunteers were informed in accordance with the Declaration of Helsinki (2002 revision), and then written informed consent was obtained.

MS patients satisfying the criteria for definite MS according to McDonald criteria (18), aged over 18 years, had no neurological attack and corticosteroid use in the last 3 months were included in the study. Exclusion criteria for all participants were as follows: significant cognitive impairment, presence of any comorbid disease (cardiovascular disease, hematological disease, thyroid dysfunction, diabetes, obesity, menopause), pregnancy, breast-feeding, the medication causing gingival enlargement, periodontal treatment in the last 6 months and use of antibiotics and anti-inflammatory drugs in the last 3 months and 1month respectively.

A total of 184 volunteers, including MS patients (n=92) and systemically healthy controls (n=92), were included in the study. Attention was paid to gender and age matching between the groups. Each participant answered a questionnaire regarding the socio-demographics and habits. All participants underwent general medical and neurological examinations. The severity of MS was assessed with the Expanded Disability Status Scale (EDSS) (19). Current medications of MS patients were recorded.

Periodontal records [Plaque index (PI) (20), Gingival index (GI) (21), percentage of bleeding on probing (%BOP) (22), probing depth (PD), clinical attachment level (CAL)] of each participant were obtained after intraoral and radiological examinations. Periodontal status of each individual was classified (23). Intra-examiner analysis showed an intraclass correlation coefficient of 0.96 for PD and 0.94 for CAL measurement. Intra-examiner weighted k(-1 mm) values ranged from 0.84 to 0.93 for PD and 0.84 to 0.92 for CAL, respectively.

Salivary Sampling and Analysis

Unstimulated total saliva samples were taken before the intraoral examination. The salivary flow rate (SFR) of each participant was calculated (24). Samples were stored at –80 °C until laboratory analysis.

Total thiol (TT), native thiol (NT) and disulphide levels in saliva were determined by spectrophotometric method described by Erel and Neselioglu, (25). In this method, the reduction of dynamic disulfide bonds to free thiol groups with sodium borohydride (NaBH4) and the reaction of all thiol groups with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) are evaluated. NT (-SH), TT(-SH+-S-S) and disulfide [(TT-NT)/2] levels were determined as µmol/L. Disulphide/total thiol (D/TT) [-S-S-/(-SH + -S-S-)], disulphide/native thiol (D/NT) (-S-S-/-SH), and native thiol/total thiol (NT/TT) [-SH/(-SH + -S-S-)] ratios were calculated.

Statistics

The data were evaluated using the SPSS v. 20.0 package program (IBM, Chicago, IL, USA). Whether the variables met the parametric assumptions for the normal distribution was evaluated using the Kolmogorov-Smirnov test. Independent samples t-test and analysis of variance was used to compare salivary parameters according to MS with control groups and periodontal status factors for normally distributed continuous variables. Periodontal and salivary parameters were compared between MS subgroups also analyzed with generalized linear models, with age taken as the covariate variable. Chi-square test was performed to evaluate the relationships between categorical data. The significance level was determined as p= 0.05.

Results

Socio-demographics of the study groups are shown in Table 1. The individuals with MS and healthy controls had similar features in terms of age, gender, education level, income, smoking and tooth brushing frequency (p>0.05). Majority of the individuals with MS had relapsing-remitting MS (RRMS) (89%), the remaining 11% had progressive MS (2 primary progressive and 8 secondary progressive). The individuals with progressive MS (PMS) were older (43.70±7.26 years versus 35.78±9.66 years), had longer disease duration (8.30±4.83 years versus 5.81±4.39 years) and more disabled (EDSS score 4.5±1.08 versus 2.81±1.18) in comparison to RRMS group. PMS cases smoked more heavily and had poor oral hygiene practices than RRMS cases (Table1).

The clinical periodontal findings did not revealed any significant differences between healthy volunteers and patients with MS (p>0.05) (Table 2). Likewise, the percentages of periodontal status of individuals in both groups were similar (Table 2). However, PD, CAL and PI were significantly elevated in the individuals with PMS in comparison to the individuals with RRMS and also there was no periodontally healthy and gingivitis person in PMS group (Table 2).

TT levels were not different between MS patients and healthy controls or between people with RRMS and PMS. However, there was a significantly elevated concentration of NT and decreased concentration of disulphide in healthy controls in comparison to MS group (Table 3). Parallel to this, healthy control group had lower D/NT and D/TT ratios and higher NT/TT ratio than MS patients group. A reverse pattern was observed between RRMS and PMS. RRMS cases had lower native thiol and higher disulphide concentrations and also higher D/NT and D/TT ratios than those in PMS cases. NT/TT ratio was not different between RRMS and PMS groups but was higher in controls than those groups.

Oxidative stress parameters did not differed according to periodontal status neither in control nor in MS group. However, comparison exactly according to the periodontal status revealed higher NT/TT and lower D/NT ratios in healthy control group than MS patient group, in spite of similar NT, TT and disulphide concentrations (Table 4).

Discussion

Inflammation and oxidative stress are implicated as the common shared pathogenetic mechanism in the relationship between periodontal disease and systemic diseases. Dynamic thiol-disulphide homeostasis stands out as a novel oxidative stress indicator with its crucial role in antioxidant protection, detoxification, signal transduction, apoptosis, enzymatic activation, and regulation of cellular signaling mechanisms (13,14). Dysregulated thiol-disulphide homeostasis

Table 1 Sociodemographics

Groups / Parameters	Control (92) n(%)	MS (92) n(%)	p*	RRMS (82) n (%)	PMS (10) n (%)	p**
Gender [Female (%)]	60 (65.2)	60 (65.2)	1.000	57(69.5)	3(30)	**0.013**
Age (yrs)	36.8±11.5[a]	34.16±8.8	0.111	35.78±9.66[a]	43.70±7.26[b]	0.083
Education level Primary	28 (30.4)	29 (31.5)		24(29.3)	5 (50)	0.727
High school	20 (21.7)	22 (23.9)	0.896	20 (24.4)	2 (20)	
University	44 (47.8)	41 (44.6)		38 (46.3)	3 (30)	
Income (TL/month) < 2000	13 (14.1)[a]	17 (18.5)		14 (17.1)[a]	3 (30)[b]	0.686
2000-5000	71 (77.2)[a]	69 (75.0)	0.655	63 (76.8)[a]	6 (60)[a]	
> 5000	8 (8.7)[a]	6 (6.5)		5 (6.1)[a]	1 (10)[b]	
Smoking (cigarette/day) None	72 (78.3)[a]	64 (69.6)		61 (74.3)[a]	3 (30)[b]	**0.009**
<10	2 (2.2)[a]	3 (3.3)	0.405	3 (3.7)[a]	0 (0)[a]	
10-20	18 (19.6)[a]	25(27.2)		18 (22.0)[a]	7 (70)[b]	
Tooth brushing frequency < 1/day	13 (14.1)	17 (18.5)		10 (12.2)	7 (70)	**0.000**
1/day	36 (39.1)	36 (39.1)	0.695	33 (40.2)	3 (30)	
≥ 2/day	43 (46.7)	39 (42.4)		39 (47.6)	0 (0)	
EDSS		2.99±1.28		2.81±1.18	4.5±1.08	**0.000**
MS duration (yrs)	-	6.08±4.48		5.81±4.39	8.30±4.83	0.096
Drugs	-			INF: 27 (32.9) GA: 18 (22.0) TRF: 5 (6.1) DMF:16 (19.5) FNG:14(17.1) NTZ: 1(1.2) OCR: 1(1.2)	FAM:3(30) OCR:7(70)	

Sociodemographics are presented as n (%), except age, EDSS, MS duration (mean±standard deviation). MS: Multiple sclerosis, PMS: Progressive MS, RRMS: Relapse-remitting MS, DMF: dimethyl fumarate, FAM: fampridine, FNG: fingolimod; INF: interferon beta1a/b; GA: glatiramer acetate, NTZ: natalizumab; OCR: ocrelizumab; TRF: teriflunomide.

* Comparison between the MS and control groups, ** Comparison among MS subgroups and control (The PMS group consisted of 8 secondary progressive and 2 primary progressive MS cases). Bold denotes significance at $p<0.05$. Letters indicate differences among the MS subgroups and control.

Table 2 Periodontal data and the distribution of individuals according to their periodontal status

Groups	Control	MS	p*	RRMS	PMS	p**
Parameters						
PD	2.80±0.84[b]	2.76±0.93	0.780	2.67±0.85[b]	3.58±1.18[a]	**0.008**
CAL	2.83±2.23[b]	2.78±2.41	0.883	2.51±2.22[b]	5.03±2.81[a]	**0.050**
PI	1.39±0.31[b]	1.39±0.35	0.922	1.36±0.32[b]	1.66±0.47[a]	0.064
GI	1.30±0.24	1.28±0.26	0.706	1.27±0.25	1.42±0.33	0.386
BOP%	20.19±16.07	19.21±18.43	0.701	18.08±17.80	28.50±21.76	0.391
Teeth number	25.96±2.97	26.10±2.55	0.729	26.24±2.32	24.90±3.93	0.919
Periodontal status [n (%)]						
Healthy	26 (28.3)	28 (30.4)	0.428	28(34.1)	-	**0.033**
Gingivitis	4 (4.3%)	9 (9.8%)		9(11.0)	-	
P-S1	16 (17.4%)	18 (19.6%)		15(18.3)	3 (30)	
P-S2	28 (30.4%)	19 (20.7%)		17(20.7)	2(20)	
P-S3	18 (19.6%)	18 (19.6%)		13(15.9)	5(50)	

PD: Probing depth, CAL: Clinical attachment level, PI: Plaque index, GI: Gingival index, BOP %: Percentage of bleeding on probing, P-S1,2,3: Periodontitis - Stage 1,2,3, Bold denotes statistically significance at p<0.05. * Comparison between MS and control groups, ** Comparison among MS subgroups and control. Letters indicate differences among the MS subgroups and control.

Table 3 Salivary parameters in MS and control groups

Groups Parameters	Control	MS	p*	RRMS	PMS	p**
SFR (mL/min)	0.26±0.06	0.27±0.05	0.377	0.27±0.05	0.22±0.06	0.219
TT (µmol/L)	12.56 ±2.03	12.52±2.80	0.908	12.51±2.79	12.57±3.02	0.991
NT (µmol/L)	9.74 ±2.04 [a]	8.59±2.08	0.000	8.58 ±2.15 [b]	8.71 ±1.46 [ab]	0.001
Disulphide (µmol/L)	1.41 ±0.86 [b]	1.96±1.44	0.002	1.97 ±1.46 [a]	1.93 ±1.31 [ab]	0.008
NT/TT	0.78 ±0.12 [a]	0.71±0.17	0.001	0.71 ±0.17 [b]	0.71 ±0.14 [b]	0.004
Disulphide /NT	0.16 ±0.12 [b]	0.27±0.32	0.002	0.28 ±0.34 [a]	0.23 ±0.16 [ab]	0.006
Disulphide /TT	0.11 ±0.06 [b]	0.15±0.09	0.001	0.15 ±0.09 [a]	0.14 ±0.07 [ab]	0.004

SFR: Saliva flow rate, TT: Total thiol, NT: Native thiol. * Comparison between MS and control groups, ** Comparison among MS subgroups and control. Bold denotes statistical significance at p<0.05. Letters indicate differences among the MS subgroups and control.

has been suggested to have a pivotal role in the pathogenesis of many diseases, especially diseases characterized by chronic inflammation including MS and periodontitis (15-17).

In this study, we determined salivary disulphide and thiol levels in MS patients and compared to systemically healthy controls and evaluated whether periodontal status had an effect on thiol-disulphide homeostasis in saliva. Previous studies have reported conflicting results on periodontal health in MS (8,26,27). We did not found any difference between MS patients and healthy controls in terms of periodontal parameters.

Table 4 The salivary parameters according to the periodontal status.

Groups:		MS		Control		
Parameters	**Periodontal status**	**Mean±SD**	**p***	**Mean±SD**	**p***	**p****
TT(µmol/L)	Healthy	13,22±2,67	0.267	12,79±2,29	0,450	0,528
	Gingivitis	12,09±3,27		10,89±1,14		0,283
	P-S1	13,03±3,43		12,93±1,38		0,137
	P-S2	12,19±2,60		12,40±2,32		**0,025**
	P-S3	11,49±2,09		12,53±1,73		0,194
NT(µmol/L)	Healthy	9,43±2,21	0,070	10,08±2,19	0,279	0,179
	Gingivitis	8,90±2,10		8,90±1,40		0,194
	P-S1	8,03±1,89		10,02±2,16		0,496
	P-S2	7,86±2,20		9,12±2,02		1,000
	P-S3	8,46±1,56		10,17±1,76		0,423
Disulphide (µmol/L)	Healthy	1,89±1,65	0,260	1,35±0,80	0,359	0,662
	Gingivitis	1,59±1,35		0,99±0,70		0,510
	P-S1	2,50±1,44		1,46±0,85		0,474
	P-S2	2,16±1,52		1,64±0,95		0,510
	P-S3	1,51±0,84		1,18±0,83		0,914
NT/TT	Healthy	0,74±0,18	0,142	0,79±0,11	0,284	**0,009**
	Gingivitis	0,76±0,16		0,82±0,11		0,767
	P-S1	0,64±0,15		0,77±0,14		**0,050**
	P-S2	0,66±0,20		0,74±0,12		0,157
	P-S3	0,74±0,12		0,82±0,12		**0,022**
Disulphide /NT	Healthy	0,27±0,47	0,547	0,15±0,10	0,348	0,102
	Gingivitis	0,19±0,19		0,12±0,09		**0,033**
	P-S1	0,33±0,22		0,17±0,15		0,102
	P-S2	0,34±0,32		0,19±0,12		0,113
	P-S3	0,19±0,12		0,13±0,11		**0,004**
Disulphide /TT	Healthy	0,13±0,09	0,142	0,10±0,06	0,284	0,241
	Gingivitis	0,12±0,08		0,09±0,06		0,067
	P-S1	0,18±0,07		0,11±0,07		0,079
	P-S2	0,17±0,10		0,13±0,06		0,120
	P-S3	0,13±0,06		0,09±0,06		0,079

TT: Total thiol, NT: Native thiol, P-S1,2,3: Periodontitis - Stage 1,2,3, p*: Within group differences, p**: Between groups differences, Bold denotes significance at p<0.05.

MS often occurs in young adults and affects women more than men. The MS group consisted of young adults and mostly female individuals (27-29). In this study, the sociodemographic features were not different between MS patients and systemic healthy controls, however, the individuals with PMS were older and mostly male, smoked more, had longer disease duration and more disabled in comparison to RRMS group. Also, they had worse EDDS scores and periodontal parameters than the patients with RRMS (28,29). The individuals with PMS had more disability, smoked more cigarettes and brushed their teeth less frequently, which may contribute to deterioration of periodontal health. Smoking is a risk factor for the development and progression of both periodontal disease and MS; in MS the risk increases with duration and intensity and also more robust in males (1,27).

Karim et al (30) have reported higher salivary thiol levels in periodontally healthy controls compared to gingivitis and periodontitis patients and found a negative relation between the severity of periodontitis and thiol level. Tayman et al. (17) have detected lower plasma NT and TT and higher disulphide levels in periodontitis patients than periodontally healthy controls. Unlike the results of these studies, when individuals were compared within the group according to their periodontal status, thiols and disulphide levels in saliva did not differ in either the control group or the MS group. The majority of individuals in the study had localized periodontal disease which may have led to the lack of differences in thiol homeostasis among the periodontal disease subgroups.

Demiröğen et al (31) have reported lower plasma NT and TT levels in patients with SPMS than the naïve MS and healthy controls but disulfide, D/NT, D/TT, NT/TT ratios were similar between the study groups. Arslan et al (32) have found that serum TT and NT levels in relapsed patients were significantly lower than those in remission. In this study, salivary TT levels were not different either between MS patients and healthy controls or between patients with RRMS and PMS. However, there were significantly decreased levels of the NT, NT/TT ratios and increased levels of the disulphide, D/NT, D/TT ratios in MS group in comparison to healthy controls. These significant differences were actually in the comparisons between the RRMS group and the control group data, except NT/TT. These findings may be due to an immune milieu shifted into a more inflammatory one in RRMS (2-6).

That lower NT/TT and higher D/NT ratios were observed in MS patients than systemically healthy controls when compared by periodontal status was though that thiol-disulphide balance in saliva could shifted to the oxidative side in MS patients. In this cross-sectional study, age and sex were matched between MS and systemically healthy volunteers. However, there was not naïve MS patient and the percentage of PMS was relatively few in the study group which may be thought as limitations of the study. Long-term studies with naïve MS patients undergoing periodic periodontal examination may shed light on the shared pathophysiological mechanisms between MS and periodontal disease. Immunomodulatory drugs the MS patients are on may cause immune deviations towards an anti-inflammatory phenotype in targeted brain tissue and also in peripheral immune system (33). In spite anti-inflammatory effect of immunomodulator drugs, the presence of more significant oxidative stress in RRMS supports further the underlying inflammation in the pathogenesis of the disease.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
This study was conducted after the approval of the Clinical Research Ethics Committee of the Faculty of Medicine (approval no: 13.12.2018/234). The volunteers participating in the study were informed in accordance with the Declaration of Helsinki (2002 revision).

Consent to Participate and Publish (If applicable)
Written informed consent was obtained from the volunteers participating in the study.

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Availability of Data and Materials
Authors can confirm that all relevant data are included in the article.

Authors Contributions
FYK: Conceptualization; Project administration; Methodology; Data curation; Validation; Visualization; Supervision; Writing-original draft; Writing-review and editing.

SD: Conceptualization; Project administration; Methodology; Data curation; Validation; Visualization; Supervision; Writing-original draft; Writing-review and editing.

ÇV: Project administration; Formal analysis; Investigation; Validation; Writing-original draft.

Table 4 The salivary parameters according to the periodontal status.

Groups:		MS		Control		
Parameters	**Periodontal status**	**Mean±SD**	**p***	**Mean±SD**	**p***	**p****
TT(µmol/L)	Healthy	13,22±2,67	0.267	12,79±2,29	0,450	0,528
	Gingivitis	12,09±3,27		10,89±1,14		0,283
	P-S1	13,03±3,43		12,93±1,38		0,137
	P-S2	12,19±2,60		12,40±2,32		**0,025**
	P-S3	11,49±2,09		12,53±1,73		0,194
NT(µmol/L)	Healthy	9,43±2,21	0,070	10,08±2,19	0,279	0,179
	Gingivitis	8,90±2,10		8,90±1,40		0,194
	P-S1	8,03±1,89		10,02±2,16		0,496
	P-S2	7,86±2,20		9,12±2,02		1,000
	P-S3	8,46±1,56		10,17±1,76		0,423
Disulphide (µmol/L)	Healthy	1,89±1,65	0,260	1,35±0,80	0,359	0,662
	Gingivitis	1,59±1,35		0,99±0,70		0,510
	P-S1	2,50±1,44		1,46±0,85		0,474
	P-S2	2,16±1,52		1,64±0,95		0,510
	P-S3	1,51±0,84		1,18±0,83		0,914
NT/TT	Healthy	0,74±0,18	0,142	0,79±0,11	0,284	**0,009**
	Gingivitis	0,76±0,16		0,82±0,11		0,767
	P-S1	0,64±0,15		0,77±0,14		**0,050**
	P-S2	0,66±0,20		0,74±0,12		0,157
	P-S3	0,74±0,12		0,82±0,12		**0,022**
Disulphide /NT	Healthy	0,27±0,47	0,547	0,15±0,10	0,348	0,102
	Gingivitis	0,19±0,19		0,12±0,09		**0,033**
	P-S1	0,33±0,22		0,17±0,15		0,102
	P-S2	0,34±0,32		0,19±0,12		0,113
	P-S3	0,19±0,12		0,13±0,11		**0,004**
Disulphide /TT	Healthy	0,13±0,09	0,142	0,10±0,06	0,284	0,241
	Gingivitis	0,12±0,08		0,09±0,06		0,067
	P-S1	0,18±0,07		0,11±0,07		0,079
	P-S2	0,17±0,10		0,13±0,06		0,120
	P-S3	0,13±0,06		0,09±0,06		0,079

TT: Total thiol, NT: Native thiol, P-S1,2,3: Periodontitis - Stage 1,2,3, p*: Within group differences, p**: Between groups differences, Bold denotes significance at p<0.05.

MDÜ: Project administration; Formal analysis; Investigation; Validation; Writing-original draft.

MC: Formal analysis; Writing-original draft.

HO: Formal analysis; Writing-original draft.

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