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Salivary Oxidative Status and the Neutrophil/Lymphocyte Ratio in Multiple Sclerosis

Multipl Sklerozda Tükrükteki Oksidatif Durum ve Nötrofil/Lenfosit Oranı

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Abstract

Objective: To compare the oxidative status in saliva between patients with multiple sclerosis (MS) and systemically healthy controls in the context of periodontal health and to evaluate whether salivary oxidative status correlates with the neutrophil/lymphocyte ratio (NLR).

Materials and Methods: A total of 184 volunteers, 92 with MS and 92 systemically healthy volunteers, participated in the study. Each person underwent medical, neurological, and oral examinations. Saliva samples were taken, and myeloperoxidase (MPO), lactoferrin (LF), total antioxidant capacity (TAOC), total oxidant status (TOS), and oxidative stress index (OSI) levels were determined.

Results: There were no differences in the periodontal parameters between the patients with MS and the healthy volunteers ($P > 0.05$). The NLR was higher in the patients with MS than in the controls ($P = 0.000$). However, patients with MS had non-significantly lower MPO levels and higher LF, TOS, and OSI levels than the controls ($P > 0.050$). There was a significant decrease in TAOC levels in the MS group ($P = 0.016$). There were higher TOS levels in the periodontally healthy patients with MS and higher OSI levels in the periodontitis - stage 2 patients with MS than those in the matched controls. There were also higher TAOC levels in the periodontitis - stage 3 MS group ($P < 0.050$). There were positive correlations between MPO, TAOC levels and the probing depths, the clinical attachment levels (CALs) in the MS and control groups. While higher periodontal parameters and MPO levels were associated with increased disability factors, the CALs and the TAOC and MPO levels were elevated in those with longer disease durations ($P < 0.050$).

Conclusion: The periodontal findings in the patients with MS are not different from those in healthy controls; however, increased MPO and decreased TAOC levels in saliva and higher NLRs in patients with MS indicate a prominent ongoing systemic inflammation despite altered immune surveillance.

Keywords: Multiple sclerosis, myeloperoxidase, lactoferrin, oxidative stress, periodontitis

Öz

Amaç: Multipl sklerozlu (MS) hastalar ile sistemik olarak sağlıklı kontroller arasında tükürükteki oksidatif durumu periodontal sağlık bağlamında karşılaştırmak ve tükürük oksidatif durumunun nötrofil/lenfosit oranı (NLR) ile ilişkili olup olmadığını değerlendirmektir.

Gereç ve Yöntem: Çalışmaya 92 MS hastası ve 92 sistemik sağlıklı gönüllü olmak üzere toplam 184 gönüllü katıldı. Her bireyde tıbbi, nörolojik ve oral muayene yapıldı. Tükürük örneklerinde miyeloperoksidaz (MPO), laktoferrin (LF), total antioksidan kapasite (TAOC), total oksidan durum ve oksidatif stres indeksi (OSI) seviyeleri belirlendi.

Bulgular: MS ve sağlıklı gönüllüler arasında periodontal parametrelerde herhangi bir fark yoktu ($P > 0,05$). NLR, MS'de kontrollerden daha yüksekti ($P = 0,000$). Ancak MS hastalarında kontrollere göre anlamlı derecede düşük MPO, daha yüksek LF, TOS ve OSI seviyeleri vardı ($P > 0,05$). MS grubunda TAOC düzeylerinde anlamlı bir düşüş vardı ($P = 0,016$). Periodontal olarak sağlıklı MS hastalarında TOS ve periodontitis - evre 2 MS hastalarında OSI eşleştirilmiş

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kontrollere kıyasla daha yüksekti ve MS grubunda periodontitis - evre 3'te TAOC daha yüksek bulundu ($P < 0,05$). MS ve kontrol gruplarında MPO, TAOC ve cep derinliği, klinik ataçman seviyesi (CAL) arasında pozitif korelasyon vardı. Daha yüksek periodontal parametre ve MPO seviyeleri artan özürlülük ile ilişkiliyken, daha uzun hastalık süresinde CAL, TAOC ve MPO düzeyleri arttı ($P < 0,05$).

Sonuç: MS hastalarındaki periodontal bulgular sağlıklı kontrollerdekinden farklı değildir, ancak tükürükte artmış MPO ve azalmış TAOC seviyeleri ve MS'de yüksek NLR, değişmiş immün sürveyansa rağmen devam eden artmış bir sistemik enflamasyona işaret etmektedir.

Anahtar Kelimeler: Multipl skleroz, miyeloperoksidaz, laktoferrin, oksidatif stres, periodontitis

Introduction

Oxidative stress, where there is an imbalance between excessive reactive oxygen species (ROS) and nitrogen species production and a relative deficiency of antioxidants, is an important element in the pathogenesis of many diseases, including neurodegenerative and neuroinflammatory ones such as multiple sclerosis (MS) (1). MS is a chronic, inflammatory disease of the central nervous system characterized by immune-mediated demyelination and axonal loss (2). Though its etiology remains unknown, a complex interplay between environmental factors, genetic predisposition, and aberrant immune responses is believed to contribute to the development of the disease. In addition to inflammation, axonal damage, excitotoxicity, demyelination, and remyelination, several studies underline oxidative stress as the key aspect in the pathogenesis of MS (3,4). ROS and nitric oxide synthase play a pivotal role in the initial and chronic stages of MS. ROS released by immune cells contribute to the loss of blood–brain barrier integrity, tight junction gap formation, cytoskeletal changes, demyelination, oligodendrocyte death, and axonal degeneration (3). Studies have shown that, in MS, oxidative stress markers increase, and antioxidant levels decrease (4,5,6).

Periodontitis is a common chronic disorder leading to the destruction of tooth-supporting structures and, subsequently, tooth loss (7). The decrease in the total antioxidant capacity (TAOC) and increase in the total oxidant status (TOS) of saliva in individuals with periodontitis supports the notion that oxidative stress contributes to the disease's onset and progression (1). Many studies have demonstrated the association of periodontal diseases with systemic diseases, such as diabetes, cardiovascular disease, and metabolic syndrome. Oxidative stress is also implied to be the common shared pathogenetic factor in these associations (1,8).

Myeloperoxidase (MPO) is an important oxidative enzyme present in innate immune cells and microglia, which takes part in the host defense system by producing hypochlorous acid, a powerful oxidant (9). MPO levels increase with inflammation and have been reported to increase in periodontitis cases when compared with healthy controls (10). In MS, significantly elevated MPO activity has been shown to associate with demyelination in cortical and white matter homogenates (11). Moreover, along with other oxidative stress markers, MPO elevation has been detected in the sera and saliva of patients with relapsing-remitting MS (RRMS) (4). Lactoferrin (LF) is a unique first-line defense protein controlling and ameliorating oxidative cell injury and bridging innate and adaptive immune function, thereby protecting against microbial infections and preventing systemic inflammation (12). Chronic inflammatory and neurodegenerative diseases have been reported to demonstrate elevated salivary LF levels and negative correlations with disease severity (13). Also, increased LF levels in saliva have been reported to result in higher dental caries (14). There is no study in the literature evaluating salivary LF levels in MS. Oral LF treatments for patients with MS demonstrated an improvement of their clinical status with a profound interferon-gamma decrease and an interleukin-10 secretion increase (12).

Neutrophils are the first defensive cells of the immune system and are crucial factors in scavenging microorganisms and cellular debris in septic and aseptic processes. By engulfing pathogens/debris and releasing ROS and proteases, they contribute to host defense and the maintenance of tissue homeostasis (15). Lymphocytes are active in maintaining effective immune surveillance. The neutrophil/lymphocyte ratio (NLR), suggested as a surrogate marker of systemic inflammation, has been reported to increase in the presence of MS (16,17) and periodontitis (18).

Saliva is an easily and non-invasively accessible biofluid that can be used for detecting biomarkers in different pathological conditions to predict the risk, activity, and prognosis of certain diseases. Elevated salivary oxidative stress markers concentrations have been reported in oral and systemic diseases (1). Saliva is a useful and practical biofluid to measure TAOC and TOS levels (19). One of the oxidative enzymes, MPO, has been evaluated in the brain tissue homogenates and sera of patients with MS (4,5,11). However, there is no study evaluating salivary MPO and LF in patients with MS. The present study aims to compare MPO, LF, TAOC, and TOS levels in saliva between the patients with MS and systemically healthy controls in the context of periodontal health and evaluate whether salivary oxidative status correlates with systemic inflammation degree as determined by NLR.

Materials and Methods

Study Population

This cross-sectional study was carried out in the Department of Periodontology, Faculty of Dentistry and Department of Neurology, Faculty of Medicine at Süleyman Demirel University, Isparta, Türkiye, after the approval decision of the Clinical Research Ethics Committee of Süleyman Demirel University Faculty of Medicine (approval no: 12.13.2018/234). The individuals who participated in the study were informed in accordance with the Helsinki Declaration (2002 revision), and their written consents were obtained.

Patients with MS satisfying the criteria for definite MS according to the McDonald criteria (20) were included in the study. These patients were over 18 years old and had not had a neurological attack or used corticosteroids in the last 3 months. Patients diagnosed with a clinically isolated syndrome and radiologically isolated MS were excluded in the study. Participants who had significant cognitive impairment, presence of any comorbid disease that could result in possible inferences (thyroid dysfunction, hypertension, diabetes, cardiovascular disease, obesity, anemia, or menopause), were pregnant or breast-feeding, used any drugs causing gingival enlargement, used antibiotics

and anti-inflammatory drugs in the last 3 months and 1 month, respectively, or received periodontal treatment in the last 6 months were excluded.

A total of 184 volunteers, 92 with MS and 92 systemically healthy volunteers, were included in the study. Gender and age were considered when creating the groups. All the participants answered a questionnaire regarding their sociodemographic characteristics and habits (oral care, smoking, etc.). Individuals underwent general medical and neurological examinations. MS severity was evaluated by the Expanded Disability Status Scale (EDSS) (21).

Oral Examination

Intra-oral and radiological examinations were given to each participant. Each patient's the plaque index (PI) (22), the gingival index (GI) (23), percentage of bleeding on probing (BOP%) (24), probing depth (PD), and clinical attachment level (CAL) were evaluated by a certified periodontist by using a periodontal probe (William's Periodontal Probe, Hu-Friedy, Chicago, IL, USA). Intra-examiner analysis showed an intraclass correlation coefficient of 0.96 for the PD measurement and 0.94 for the CAL measurement. Intra-examiner weighted κ (1 mm) values ranged from 0.84 to 0.93 for the PD measurement and 0.84 to 0.92 for the CAL measurement. Periodontal disease classification was made according to the 2018 American Periodontology Academy and European Periodontology Federation World Workshop Classification (25). Finally, the decayed, missing, and filled teeth index (DMFT) score was calculated (26).

Blood Samples

A peripheral blood sample was obtained for each enrolled patient during the oral examination and saliva sampling. Participants' blood samples were drawn from the antecubital vein into tubes containing ethylenediaminetetraacetic acid as an anticoagulant after overnight fasting between 08:00 and 10:00 am. The complete blood cell analysis was performed using the flow cytometry method (Beckman Coulter LH 780 Analyzer, Beckman Coulter Inc., Miami, FL, USA). The analyzer was calibrated twice a day by establishing low and high parameters with the control blood samples. Hematological parameters were determined and recorded. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

Saliva Samples and Laboratory Analysis

Before the periodontal examination, unstimulated total saliva samples were collected from each person between 08:00 and 10:00 am, and saliva flow rates (SFRs) were calculated (27). The samples, taken into Eppendorf tubes, were kept at -80°C. For the laboratory analysis, saliva samples were dissolved at 4°C and homogenized by vortexing. Supernatants were collected and centrifuged at 4°C for 6 minutes at 9.000 g (Eppendorf MR 5415, Hamburg, Germany).

MPO and LF levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemicals, Ann Arbor, MI, USA; Elabscience Biotecnology Inc., Houston, TX, USA, respectively). Saliva samples were diluted 1:100 with an ELISA buffer for MPO and 1:1,000 with a diluted sample diluent for LF. The MPO and LF results of the calibration and sampling were determined by reading in the 450 nm ELISA plate reader (Epoch™, BioTek Instruments, Inc., Winooski, VT, USA). MPO and LF concentrations were calculated according to standard curve data with four-parameter logistic fit analysis using MyAssay Readerfit ELISA software (www.MyAssay.com). MPO and LF results are expressed in ng/ml.

TOS and TAOC levels were determined by commercial kits using the colorimetric method (TOS assay kit, REL Assay Diagnostics, Gaziantep, Türkiye; total antioxidant status assay kit, REL Assay Diagnostics, Gaziantep, Türkiye, respectively) (19,28). TAOC levels were measured based on the decolorization of the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) radical cation by antioxidants. The change in absorbance at 660 nm indicated the sample's TAOC level. TOS levels were determined by measuring the color intensity of the oxidation reaction of the ferrous ion-chelator complex to ferric ions produced by the oxidants in the saliva. Hydrogen peroxide (H_2O_2) was used as the standard for preparing the calibration curve. The results were expressed as millimolar Trolox equivalent per liter (mmol Trolox Eq/l) for the TAOC and micromolar H_2O_2 equivalent per liter (μmol H_2O_2 Eq/l) for the TOS. The oxidative stress index (OSI) was calculated as the percentage ratio of TOS:TAOC (19).

Statistical Analysis

The SPSS v. 20.0 for Windows (IBM, Chicago, IL, USA) package program was used to evaluate the study's data. The Kolmogorov–Smirnov test was used to check whether variables provided parametric assumptions for normal distribution. The homogeneity of the variances was evaluated with Levene's test. Independent sample t-tests and Chi-square tests were used to evaluate the relationships between the categorical data and continuous variables. An analysis of variance was used to evaluate salivary parameters and NLRs according to periodontal status. Periodontal and salivary parameters were compared between MS subgroups for multivariance (generalized linear models), with age taken as the covariate variable. The Pearson correlation test was used to evaluate the significance of linear relationships between periodontal, salivary, and systemic parameters and sociodemographics. A logistic regression analysis was used to investigate the relationship between periodontal, salivary, and systemic parameters and MS risk. The significance level was set at $P = 0.050$.

Results

The socio-demographic features of patients with MS and healthy controls are given in Table 1. Age, gender, education level, income, smoking, tooth brushing frequency, and body mass index did not differ between patients with MS and the healthy controls ($P > 0.050$). Most patients with MS had RRMS (89%). Ten patients had progressive MS (PMS). Two of these had primary PMS, and the other eight had secondary PMS. As expected, patients with progressive cases were older and had longer disease durations and more disability factors than those with RRMS. Their smoking and oral hygiene habits also differed from patients with RMMS (Table 1).

The clinical periodontal examination findings in patients with MS were comparable with those in healthy volunteers ($P > 0.005$). Likewise, there was no significant difference in the number of periodontally healthy and unhealthy individuals in both groups (Table 2). When comparing periodontal statuses, only the periodontally healthy patients with MS had a lower BOP%, and

the patients with MS with gingivitis had a lower SFR than those in the matched controls (5.23 ± 2.53 vs. 6.51 ± 2.05, $P = 0.048$; 0.26 ± 0.56 vs. 0.27 ± 0.50, $P = 0.030$, respectively). However, when comparing patients with MS according to MS subtypes, the results revealed that those with a progressive disease status had worse oral health, higher PDs, CALs, and GIs, and lower SFRs (Table 2).

The NLR was significantly higher in the MS group than in the control group ($P = 0.000$) (Table 2). Despite exhibiting a remarkably high systemic inflammation, as defined by their NLRs, patients with MS had lower MPO and higher LF levels in their saliva than the healthy volunteers, though not significantly ($P > 0.050$). Nevertheless, patients with MS had a statistically non-significant increase in TOS and OSI levels ($P > 0.050$) and a significant decrease in TAOC levels ($P = 0.016$) (Table 2).

The intragroup and intergroup comparisons of salivary findings and NLRs according to the participants' periodontal statuses are shown in Table 3. The NLR tended to increase in both groups as the severity of periodontal disease increased, though not significantly. There were no observed significant differences in salivary parameters in terms of periodontal status within or between the groups, except that there were higher TAOC levels in the periodontitis - stage 3 patients in the MS group ($P = 0.007$), higher TOS levels in the periodontally healthy volunteers ($P = 0.049$) and higher OSI scores in the periodontitis - stage 2 patients in the MS group than those in the matched controls ($P = 0.037$).

The significant correlations between the parameters in the MS and control groups are presented in Table 4. In the patients with MS, TOS levels increased as the TAOC and MPO levels increased ($P = 0.002$ and $P = 0.038$, respectively). Also, there was a negative correlation between TAOC and OSI levels ($P = 0.047$). In healthy volunteers, TAOC levels correlated negatively with LF ($P = 0.037$) and OSI ($P = 0.000$) levels, and LF levels correlated positively with OSI levels ($P = 0.002$).

In the MS group, MPO levels yielded positive relationships with disease duration ($P = 0.001$) and EDSS scores ($P = 0.041$)

Table 1. Socio-demographic group features

Parameters	RRMS (82) n (%)	*PMS (10) n (%)	**P	MS (92) n (%)	Control (92) n (%)	P
Gender [female (%)]	57 (69.5)	3 (30)	0.013	60 (65.2)	60 (65.2)	1.000
Age	35.78 ± 9.66	43.70 ± 7.26	0.007	34.16 ± 8.8	36.8 ± 11.5	0.111
Education level						
Primary	24 (29.3)	5 (50)	0.402	29 (31.5)	28 (30.4)	0.896
High school	20 (24.4)	2 (20)		22 (23.9)	20 (21.7)	
University	38 (46.3)	3 (30)		41 (44.6)	44 (47.8)	
Income (TL/month)						
<2000	14 (17.1)	3 (30)	0.509	17 (18.5)	13 (14.1)	0.655
2000–5000	63 (76.8)	6 (60)		69 (75.0)	71 (77.2)	
>5000	5 (6.1)	1 (10)		6 (6.5)	8 (8.7)	
Smoking (cigarettes/day)						
None	61 (74.3)	3 (30)	0.005	64 (69.6)	72 (78.3)	0.405
<10	3 (3.7)	0		3 (3.3)	2 (2.2)	
10–20	18 (22.0)	7 (70)		25 (27.2)	18 (19.6)	
Tooth brushing frequency						
<1/day	10 (12.2)	7 (70)	0.000	17 (18.5)	13 (14.1)	0.695
1/day	33 (40.2)	3 (30)		36 (39.1)	36 (39.1)	
≥2/day	39 (47.6)	0		39 (42.4)	43 (46.7)	
EDSS	2.81 ± 1.18	4.5 ± 1.08	0.000	2.99 ± 1.28	-	-
Disease duration (yrs)	5.81 ± 4.39	8.30 ± 4.83	0.096	6.08 ± 4.48	-	-
Medications	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)	-	-	-	-

Socio-demographics are presented as n (%), except age (mean ± standard deviation). The *P* values were computed with the χ^2 independence test. *PMS group consists of eight secondary progressive and two primary progressive MS cases, **Comparison of MS subgroups. EDSS: Expanded Disability Status Scale, 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

and a negative relationship with the number of teeth (P = 0.003). In healthy volunteers, the higher their teeth number, the lower the TOS (P = 0.003) and OSI (P = 0.016) levels. Likewise, MPO decreased as SFR increased (P = 0.017) (Table 4).

When the relationships between salivary parameters, NLRs, and periodontal parameters were evaluated, there were positive relationships between NLR and GI levels (P = 0.045) and the BOP% (P = 0.044) in healthy controls. However, no relationships existed between NLR and periodontal parameters in the MS group. MPO showed a weak but significant relationship with PD (P = 0.024) and CAL (P = 0.027). While there were positive correlations between TOS and PD and CAL (P = 0.036 and P = 0.035, respectively), TOS levels negatively correlated with teeth numbers (P = 0.003). DMFT index scores increased as LF levels increased (P = 0.028). In the MS group, higher MPO levels correlated with higher PD (P = 0.032) and CAL (P = 0.014). MPO and TAOC levels significantly correlated with teeth numbers (P = 0.003 and P = 0.002, respectively). Also, TAOC showed a weak relationship with PD (P = 0.040) and a moderate relationship with CAL (P = 0.000) (Table 4).

The loss of dexterity in patients with MS due to sensory, motor, or coordination problems and increasing disability may present challenges in their ability to perform effective oral hygiene. To evaluate the effect of disability on periodontal status, patients with MS were divided into two groups according to their EDSS scores (cut-off value of 3.5) (29) and disease duration (≤5 years and >5 years). Socio-demographic group features were the same, except that the group with an EDSS score of ≥3.5 had a higher average age and were both brushing their teeth and smoking less (P < 0.050) (Table 5). Though the distribution of periodontal stages was not different between groups, periodontal examination findings were more severe in patients with MS with high disability factors (PI, GI, BOP%, CAL, PD, number of teeth, and DMFT). CALs were significantly higher in patients with MS with longer disease duration (P = 0.019). In salivary parameters, MPO levels were significantly higher in those with high EDSS scores and longer disease durations, while TAOC levels were higher in patients with MS that had longer disease durations (P < 0.050) (Table 5). Neither clinical dental nor laboratory parameters changed according to the disease-modifying drugs the patients with MS were using.

As a result of the logistic regression analysis performed to determine the periodontal parameters affecting MS, the effects of TAOC, TOS, and NLR on the participants' probability of having MS were found to be statistically significant. TAOC values were found to negatively affect MS, while TOS and NLR were found to

Table 2. Periodontal and salivary parameters and serum NLR in the groups*

Groups	RRMS (n = 82)	PMS (n = 10)	*P	MS (n = 92)	Control (n = 92)	**P
Periodontal parameters						
PD (mm)	2.67 ± 0.85	3.58 ± 1.18	**0.039**	2.76 ± 0.93	2.80 ± 0.84	0.780
CAL (mm)	2.51 ± 2.22	5.03 ± 2.81	**0.034**	2.78 ± 2.41	2.83 ± 2.23	0.883
PI	1.36 ± 0.32	1.66 ± 0.47	**0.044**	1.39 ± 0.35	1.39 ± 0.31	0.922
GI	1.27 ± 0.25	1.42 ± 0.33	0.301	1.28 ± 0.26	1.30 ± 0.24	0.706
BOP%	18.08 ± 17.80	24.50 ± 21.76	0.310	19.21 ± 18.43	20.19 ± 16.07	0.701
Number of teeth	26.24 ± 2.32	24.90 ± 3.93	0.915	26.10 ± 2.55	25.96 ± 2.97	0.729
DMFT	5.81 ± 3.25	7.60 ± 3.03	0.100	6.00 ± 3.26	6.26 ± 3.74	0.614
Periodontal status [n (%)]						
Healthy	28 (34.1)	-		28 (30.4)	26 (28.3)	
Gingivitis	9 (11.0)	-		9 (9.8%)	4 (4.3%)	
P-S1	15 (18.3)	3 (30)		18 (19.6%)	16 (17.4%)	
P-S2	17 (20.7)	2 (20)	**0.033**	19 (20.7%)	28 (30.4%)	0.428
P-S3	13 (15.9)	5 (50)		18 (19.6%)	18 (19.6%)	
Salivary parameters						
SFR (ml/min)	0.27 ± 0.05	0.22 ± 0.06	**0.034**	0.27 ± 0.05	0.26 ± 0.06	0.377
MPO (ng/ml)	897.55 ± 778.32	929.49 ± 826.79	0.979	901.02 ± 779.05	1036.96 ± 962.46	0.294
LF (ng/ml)	1221.31 ± 1319.43	1691.68 ± 1430.44	0.771	1231.97 ± 1658.41	1054.48 ± 1385.87	0.432
TAOC (mmol/l)	0.61 ± 0.36	0.65 ± 0.24	0.883	0.62 ± 0.34	0.72 ± 0.21	**0.016**
TOS (µmol/l)	22.23 ± 20.30	17.20 ± 12.57	0.240	21.68 ± 19.62	17.69 ± 14.74	0.120
OSI	0.50 ± 1.30	0.35 ± 0.13	0.498	0.48 ± 1.28	0.28 ± 0.26	0.147
NLR	2.63 ± 1.26	2.51 ± 0.87	0.671	2.62 ± 1.23	1.80 ± 0.74	**0.000**

Bold denotes statistical significance at P < 0.050. *After the exclusion of age as a possible confounding factor between MS subgroups. **Values are presented as mean±standard deviation except for periodontal status [n (%)]. MS: Multiple sclerosis, PMS: Progressive MS, RRMS: Relapse-remitting MS, PD: Probing depth, CAL: Clinical attachment level, PI: Plaque index, GI: Gingival index, BOP%: Percentage of bleeding on probing, DMFT: The decayed, missing and filled teeth index, P-S1,2,3: Periodontitis - stage 1, 2, 3, SFR: The salivary flow rate, MPO: Myeloperoxidase, LF: Lactoferrin, TAOC: Total antioxidant capacity, TOS: Total oxidant status, OSI: The oxidative stress index, NLR: The neutrophil/lymphocyte ratio

be positively effective. A one-unit decrease in TAOC was 4.870 times more effective in affecting MS. A one-unit increase in TOS was 1.024-fold more effective in affecting MS, and a one-unit increase in NLR was 2.698-fold more effective in affecting MS. Estimated parameters and odds ratios (OR) were as follows: TAOC [β= -1.582 ± 0.611, *P* = 0.010, OR: 0.206 (1/0.206=4.87), 95% confidence interval (CI): 0.062–0.681]; TOS (β= 0.024 ± 0.010, *P* = 0.016, OR: 1.024, 95% CI: 1.004–1.044) and NLR (β= 0.993 ± 0.211, *P* = 0.000, OR: 2.698, 95% CI: 1.784–4.081). TAOC and NLR stood out as strong predictive factors.

Discussion

MS is one of the most disabling diseases in adults. The loss of dexterity due to motor, sensory, and coordination problems may hinder patients when carrying out effective oral hygiene, possibly increasing the probability of oral health problems. Despite the findings being controversial, some studies reported a high proportion of chronic periodontitis in patients with MS (30,31). This study did not observe any differences between patients with MS and healthy controls regarding periodontal parameters (PD, CAL, GI, PI, DMFT, and teeth number). However, those with high

Table 3. The intragroup and intergroup comparisons of MPO, LF, TAOC, TOS, OSI, and NLR according to the periodontal status

Groups		**MS**		**Control**		
Parameters	**Periodontal status**	**Mean ± SD**	***F*** ****P***	**Mean ± SD**	***F*** ****P***	****P***
MPO (ng/mL)	Healthy	892.03 ± 749.91	0.928 0.452	738.15 ± 905.99	1.225 0.306	0.498
	Gingivitis	804.03 ± 518.61		663.08 ± 432.27		0.646
	P-S1	638.56 ± 443.73		1100.14 ± 1024.78		0.092
	P-S2	1044.18 ± 1037.85		1247.11 ± 973.70		0.498
	P-S3	1074.84 ± 871.07		1168.61 ± 1010.84		0.767
LF (ng/mL)	Healthy	995.65 ± 1393.34	0.327 0.859	771.97 ± 893.13	1.783 0.140	0.489
	Gingivitis	1075.64 ± 1293.50		589.33 ± 244.22		0.481
	P-S1	1545.11 ± 1915.89		1815.48 ± 2243.29		0.707
	P-S2	1288.88 ± 2194.62		881.52 ± 772.59		0.369
	P-S3	1304.55 ± 1365.01		1158.53 ± 1711.83		0.779
TAOC (mmol/L)	Healthy	0.60 ± 0.36	**3.736 0.007**	0.76 ± 0.20	2.109 0.086	0.059
	Gingivitis	0.38 ± 0.26		0.45 ± 0.11		0.602
	P-S1	0.54 ± 0.35		0.69 ± 0.22		0.162
	P-S2	0.60 ± 0.34		0.75 ± 0.21		0.079
	P-S3	0.85 ± 0.25[a)]		0.71 ± 0.22		0.086
TOS (µmol/L)	Healthy	25.96 ± 23.66	0.859 0.492	15.84 ± 10.18	1.650 0.169	**0.049**
	Gingivitis	17.23 ± 8.89		14.66 ± 4.74		0.602
	P-S1	15.93 ± 8.97		15.44 ± 9.77		0.879
	P-S2	21.47 ± 15.07		16.07 ± 14.09		0.216
	P-S3	23.22 ± 27.12		25.55 ± 22.90		0.782
OSI	Healthy	0.20 ± 1.68	1.006 0.409	0.23 ± 0.17	1.520 0.203	0.914
	Gingivitis	0.72 ± 0.61		0.33 ± 0.08		0.235
	P-S1	0.89 ± 1.75		0.28 ± 0.28		0.177
	P-S2	0.56 ± 0.76		0.23 ± 0.22		**0.037**
	P-S3	0.28 ± 0.30		0.40 ± 0.37		0.300
NLR	Healthy	2.36 ± 1.20	0.879 0.480	1.63 ± 0.70	0.804 0.526	**0.009**
	Gingivitis	2.76 ± 1.89		1.75 ± 1.19		0.354
	P-S1	2.91 ± 0.98		2.01 ± 0.77		**0.006**
	P-S2	2.42 ± 0.95		1.89 ± 0.73		**0.038**
	P-S3	2.86 ± 1.37		1.73 ± 0.68		**0.004**

**P*: Within-group differences, ***P*: Between-group differences, Bold denotes significance at $P < 0.050$. MS: Multiple sclerosis, SD: Standard deviation, P-S1, 2, 3: Periodontitis - stage 1, 2, 3, MPO: Myeloperoxidase, LF: Lactoferrin, TAOC: Total antioxidant capacity, TOS: Total oxidant status, OSI: The oxidative stress index, NLR: The neutrophil/lymphocyte ratio

EDSS scores had fewer teeth, lower tooth brushing frequencies, and worse periodontal examination findings, supporting the postulate that as the disability factors increase, oral hygiene practices falter. Also, those with PMS had worse oral health. Dulamea et al. (32) showed no relationship between EDSS scores and periodontal parameters. However, Hatipoglu et al. (29) reported higher PI, GI, and PD measurements in patients with MS with an EDSS score >3.5. This study's findings, consistent with Hatipoglu et al. (29), also revealed worse periodontal health in those with higher disability factors. Likewise, DMFT scores varied in different studies (29,33,34). Kovac et al. (34) and Hatipoglu et al. (29) observed no differences in DMFT scores between the MS and control groups and in patients with MS with lower or higher disability factors. Similar to their results, this study found no differences between MS and healthy controls, but those with higher disability factors had significantly higher DMFT scores.

Oral health problems confronted in MS might be multifaceted. In addition to altered immunological responses and limitations due to physical findings, medications used to control disease symptoms might contribute to oral health problems, many of which cause a change in salivary profiles and increase the risk of oral diseases. Saliva, with various molecules in its content, including immunoglobulins and proinflammatory cytokines, forms the first line of defense against microorganisms and is useful for detecting certain biomarkers that reflect periodontal health status and some neurodegenerative and inflammatory diseases (1). Although many studies extensively evaluated saliva content, they omitted the SFR. Only one study reported a decreased SFR in MS (35). This study did not detect any difference in the SFR between MS and healthy volunteers; moreover, the SFR in patients with MS did not change with disability and disease duration. However, though weak, the negative correlation between the SFR and MPO level in healthy volunteers supported the idea that saliva has a protective role in oral health.

Neutrophils, which constitute the first line of defense against bacterial infection, are the most common inflammatory cells in the periodontal tissue and gingival pocket in the presence of periodontitis and are responsible for ROS production (15). Their roles in MS have also been noted (3). In previous studies, the association between NLRs and inflammatory diseases, including MS and periodontitis, has been described, and different cut-off values have been reported (16,17,18,36). In this study's sample, though not significant, the NLR tended to increase in both groups as the severity of periodontitis increased. However, NLRs were significantly higher in the MS group, which might reflect the inflammation underlying MS pathogenesis. The tendency of the NLR to rise in healthy volunteers might imply that periodontal inflammation contributes to the systemic one or vice versa. Nevertheless, NLRs being lower in the periodontally healthy individuals than in those with gingivitis and periodontitis in both the MS and control groups might imply that periodontitis contributes to systemic inflammation.

MPO, a neutrophil activation marker that increases in the presence of inflammation, is considered to contribute to disease pathogenesis by tuning adaptive immune responses and/or inducing vascular permeability (9). Elevated MPO levels in serum and increased MPO activity in active demyelination areas have been found in patients with MS (5,11). Higher salivary MPO activity in periodontitis has also been reported in these patients (10,37). This study did not observe any significant differences in MPO levels regarding periodontal status between the patients with MS and healthy controls. MPO levels tended to increase with the severity of periodontitis, and the significant correlations between MPO levels and PDs and CALs in both groups supported the concept of the role of MPO activity in periodontitis. Minohara et al. (5) reported no significant relationship between high serum MPO levels and MS disease duration. In this study, the patients with

Table 4. Significant correlations

Groups	MS			Control	
Correlations	**r**	***P***		**r**	***P***
MPO-PD	0.224	0.032	MPO-PD	0.236	0.024
MPO-CAL	0.255	0.014	MPO-CAL	0.202	0.027
TAOC-PD	0.214	0.040	TOS-PD	0.219	0.036
TAOC-CAL	0.399	0.000	TOS-CAL	0.190	0.035
MPO-number of teeth	–0.303	0.003	TOS-number of teeth	–0.309	0.003
TAOC-number of teeth	–0.317	0.002	OSI-number of teeth	–0.251	0.016
MPO-TOS	0.217	0.038	LF-DMFT	0.229	0.028
TAOC-TOS	0.318	0.002	MPO-SFR	–0.248	0.017
TAOC-OSI	–0.176	0.047	TAOC-LF	–0.217	0.037
MPO-disease duration	0.333	0.001	LF-OSI	0.312	0.002
MPO-EDSS	0.213	0.041	TAOC-OSI	–0.421	0.000
			NLR-GI	0.209	0.045
			NLR-BOP%	0.210	0.044

Statistically significant at $P < 0.050$. MS: Multiple sclerosis, PD: Probing depth, CAL: Clinical attachment level, GI: Gingival index, BOP%: Percentage of bleeding on probing, DMFT: The decayed, missing, and filled teeth index, SFR: Salivary flow rate, MPO: Myeloperoxidase, LF: Lactoferrin, TAOC: Total antioxidant capacity, TOS: Total oxidant status, OSI: The oxidative stress index, NLR: The neutrophil/lymphocyte ratio

Table 5. Socio-demographic, periodontal, and salivary parameters and NLR according to EDSS score and MS age						
	EDSS			**Disease duration (yrs)**		
Groups Parameters	≤3.5 (n = 70)	>3.5 (n = 22)	*P*	≤5 (n = 53)	>5 (n = 39)	*P*
Socio-demographics						
Age	34.16 ± 8.77	44.55 ± 8.39	**0.000**	34.19 ± 9.70	35.27 ± 8.81	0.074
Gender (n) (male/female)	23/47	9/13	0.489	20/33	12/27	0.488
Education (n)						
Primary school	19	10		14	15	
High school	19	3	0.208	16	6	0.209
University	32	9		23	18	
Income (TL/month) (n)						
<2,000	10	7		8	9	
2,000–5,000	55	14	0.178	41	28	0.586
>5,000	5	1		4	2	
Smoking (cigarette/day) (n)						
None	53	11		37	27	
<10	3	0	**0.018**	3	0	0.282
10–20	14	11		13	12	
Tooth brushing (n)						
<1/day	8	9		8	9	
1/day	29	7	**0.007**	19	17	0.297
≥2/day	33	6		26	13	
Periodontal parameters						
Periodontal status (n)						
Healthy	23	5		16	12	
Gingivitis	8	1		8	1	
P-S1	14	4	0.226	11	7	0.270
P-S2	15	4		10	9	
P-S3	10	8		8	10	
PD (mm)	2.58 ± 0.74	3.33 ± 1.23	**0.001**	2.74 ± 0.96	2.81 ± 0.89	0.720
CAL (mm)	2.18 ± 2.03	4.68 ± 2.58	**0.000**	2.28 ± 2.33	3.47 ± 2.38	**0.019**
PI	1.32 ± 0.25	1.62 ± 0.50	**0.000**	1.40 ± 0.37	1.39 ± 0.32	0.900
GI	1.24 ± 0.22	1.43 ± 0.34	**0.002**	1.29 ± 0.27	1.27 ± 0.26	0.630
BOP%	15.78 ± 14.43	30.12 ± 24.97	**0.001**	19.95 ± 19.35	22.20 ± 17.30	0.655
Number of teeth	26.63 ± 1.96	24.41 ± 3.42	**0.000**	26.55 ± 2.01	25.49 ± 3.07	0.082
DMFT	5.29 ± 3.08	8.27 ± 2.76	**0.000**	5.82 ± 2.86	6.92 ± 3.56	0.079
Salivary parameters						
SFR (ml/min)	0.27 ± 0.05	0.26 ± 0.05	0.492	0.27 ± 0.05	0.26 ± 0.05	0.643
MPO (ng/ml)	808.46 ± 705.28	1195.51 ± 936.06	**0.041**	730.63 ± 457.48	1132.58 ± 1035.14	**0.014**
LF (ng/ml)	1245.91 ± 1744.26	1187.62 ± 1385.18	0.887	1171.30 ± 1632.83	1314.42 ± 1710.49	0.685
TAOC (mmol/l)	0.58 ± 0.34	0.73 ± 0.33	0.078	0.55 ± 0.33	0.71 ± 0.34	**0.024**
TOS (µmol/l)	20.98 ± 17.53	23.92 ± 25.54	0.543	19.77 ± 15.12	24.28 ± 24.43	0.279
OSI	0.51 ± 1.46	0.37 ± 0.35	0.666	0.51 ± 1.64	0.44 ± 0.49	0.809
NLR	2.65 ± 1.32	2.50 ± 0.88	0.628	2.56 ± 1.25	2.69 ± 1.21	0.624

Bold denotes significance at $P < 0.050$. Socio-demographics are presented as n, except age. Age, periodontal and salivary parameters, and NLRs are shown as mean ± standard deviation. EDSS: Expanded Disability Status Scale, MS: Multiple sclerosis, P-S1, 2, 3: Periodontitis - stage 1, 2, 3, PD: Probing depth, CAL: Clinical attachment level, PI: Plaque index, GI: Gingival index, BOP %: Percentage of bleeding in probing, DMFT: The decayed, missing, and filled teeth index, SFR: Saliva flow rate, MPO: Myeloperoxidase, LF: Lactoferrin, TAOC: Total antioxidant capacity, TOS: Total oxidant status, OSI: The oxidative stress index, NLR: The neutrophil/lymphocyte ratio

MS with longer disease durations and greater disability factors had significantly higher salivary MPO levels. Increased MPO levels might have been a contributing factor to worse oral health in this group of patients.

LF, one of the major antimicrobial peptides in saliva, is a unique glycoprotein controlling and ameliorating oxidative cell injury and bridging innate and adaptive immune function, thereby protecting against microbial infections and preventing systemic inflammation (12). A possible protective role of LF has been implied, where it contributes to the pathogenesis and severity of chronic inflammatory and neurodegenerative diseases (13). It has been demonstrated to attenuate inflammatory changes by regulating cytokine profiles with an immune deviation to Thelper2 lymphocytes and provide clinical improvement in experimental autoimmune encephalomyelitis models and patients with MS (12). A rise in LF levels in saliva has been reported in chronic periodontitis (38). In terms of periodontal status, this study observed higher LF levels in the patients with MS than the controls, though not significantly. A positive relationship between LF levels and DMFT scores in healthy volunteers is consistent with Sikorska et al.'s findings (14). In healthy volunteers, the LF levels correlated positively with OSI levels and negatively with TAOC levels. These relationships between LF levels and TAOC and OSI levels suggest LF increases are secondary to inflammation. The high LF levels in patients with MS might also reflect a more inflammatory milieu in MS. To date, there has been no study evaluating salivary LF levels in MS, except one demonstrating the improvement of clinical status with a profound decrease of interferon-gamma and increase of interleukin-10 secretion after oral LF treatment, supporting the theory of LF's anti-inflammatory properties (12).

Oxidant and antioxidant markers play an important role in the pathophysiology of MS (6). Compared with healthy subjects, patients with MS have lower TAOC levels (4) and higher TOS and OSI levels (6). Studies have reported a negative relationship between salivary TAOC levels and periodontitis severity, and TOS and OSI levels increase in saliva in the presence of periodontitis (1,39). However, Zhang et al. (39) noted no significant difference in TOS levels between individuals with periodontitis and periodontally healthy controls. In this study, when the MS and control groups were compared, the mean TAOC level was significantly lower in patients with MS. Though patients with MS had higher mean TOS and OSI levels than the control group, the findings were not statistically significant. There were no significant differences between the groups in the same periodontal status. However, the positive correlations between TAOC levels and PDs and CALs might suggest that oxidative stress associated with increasing severities of periodontal disease indicates an attempt to balance using antioxidants.

All the patients with MS in this sample were taking immunomodulatory drugs. In addition to their *sui generis* efficacy and safety profiles, these drugs induce unique constellations of immune deviations towards an anti-inflammatory phenotype in targeted brain tissue and the peripheral immune system (40). The detection of elevated salivary MPO levels, decreased salivary TAOC levels, and high NLRs in patients with MS point to a salient ongoing systemic inflammation despite changed immune surveillance.

Conclusion

This was a cross-sectional case-control study, and strict criteria were applied for case selection. Moreover, the control group was selected among systemically healthy subjects and strictly matched for age and gender with patients with MS. All comparisons were made according to periodontal status, in conformity with the recent periodontal disease classification (25). All these were strengths of this study. However, it is noteworthy to state some limitations. Most patients with MS had RRMS, and those with PMS only comprised about ten percent of the MS population. However, this is an issue in all MS studies. Another confounding issue is that most patients were taking disease-modifying drugs with anti-inflammatory properties, some of which might have immunomodulatory effects on oral and periodontal tissues (40). Longitudinal studies with larger populations of naive patients with MS will help to understand the mutual effects MS and periodontal disease have on each other. Thus, maintaining oral and periodontal health in patients with MS could help increase their life quality and control MS progression by preventing systemic inflammatory burdens and oxidative stresses that oral diseases may cause.

Ethics

Ethics Committee Approval: Ethics Committee of Süleyman Demirel University Faculty of Medicine (approval no: 12.13.2018/234).

Informed Consent: Written consents were obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ç.V., F.Y.K., S.D., M.D.Ü., Design: Ç.V., F.Y.K., S.D., Data Collection or Processing: Ç.V., F.Y.K., S.D., M.D.Ü., Analysis or Interpretation: Ç.V., F.Y.K., S.D., M.C., H.O., Literature Search: Ç.V., F.Y.K., S.D., Writing: F.Y.K., S.D.

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References

1. Wang J, Schipper HM, Velly AM, Mohit S, Gornitsky M. Salivary biomarkers of oxidative stress: a critical review. Free Radic Biol Med 2015;85:95-104.
2. Wootla B, Eriguchi M, Rodriguez M. Is multiple sclerosis an autoimmune disease? Autoimmune Dis 2012;2012:969657.
3. van Horssen J, Witte ME, Schreibelt G, de Vries HE. Radical changes in multiple sclerosis pathogenesis. Biochim Biophys Acta 2011;1812:141-150.
4. Karlík M, Valkovič P, Hančinová V, et al. Markers of oxidative stress in plasma and saliva in patients with multiple sclerosis. Clin Biochem 2015;48:24-28.
5. Minohara M, Matsuoka T, Li W, et al. Upregulation of myeloperoxidase in patients with opticospinal multiple sclerosis: positive correlation with disease severity. J Neuroimmunol 2006;178:156-160.
6. Acar A, Ugur Cevik M, Evliyaoglu O, et al. Evaluation of serum oxidant/antioxidant balance in multiple sclerosis. Acta Neurol Belg 2012;112:275-280.
7. Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. Periodontol 2000 2012;58:37-68.
8. D'Aiuto F, Nibali L, Parkar M, et al. Oxidative stress, systemic inflammation, and severe periodontitis. J Dent Res 2010;89:1241-1246.
9. Strzepa A, Pritchard KA, Dittel BN. Myeloperoxidase: a new player in autoimmunity. Cell Immunol 2017;317:1-8.

10. Nizam N, Gümüş P, Pitkänen J, et al. Serum and salivary matrix metalloproteinases, neutrophil elastase, myeloperoxidase in patients with chronic or aggressive periodontitis. Inflammation 2014;37:1771-1778.
11. Gray E, Thomas TL, Betmouni S, Scolding N, Love S. Elevated activity and microglial expression of myeloperoxidase in demyelinated cerebral cortex in multiple sclerosis. Brain Pathol 2008;18:86-95.
12. Kruzel ML, Zimecki M, Actor JK. Lactoferrin in a context of inflammation-induced pathology. Front Immunol 2017;8:1438.
13. Carro E, Bartolomé F, Bermejo-Pareja F, et al. Early diagnosis of mild cognitive impairment and Alzheimer's disease based on salivary lactoferrin. Alzheimers Dement (Amst) 2017;8:131-138.
14. Sikorska MH, Mielnik-Blaszczak M, Kapeć E. The relationship between the levels of SigA, lactoferrin and alpha(1) proteinase inhibitor in saliva and permanent dentition caries in 15-year-olds. Oral Microbiol Immunol 2002;17:272-276.
15. Wang Y, Andrukhov O, Rausch-Fan X. Oxidative stress and antioxidant system in periodontitis. Front Physiol 2017;8:910.
16. Hasselbalch IC, Søndergaard HB, Koch-Henriksen N, et al. The neutrophil-to-lymphocyte ratio is associated with multiple sclerosis. Mult Scler J Exp Transl Clin 2018;4:2055217318813183.
17. Demirci S, Demirci S, Kutluhan S, Koyuncuoglu HR, Yurekli VA. The clinical significance of the neutrophil-to-lymphocyte ratio in multiple sclerosis. Int J Neurosci 2016;126:700-706.
18. Doğan B, Fentoğlu Ö, Kırzıoğlu FY, et al. Lipoxin A4 and neutrophil/lymphocyte ratio: a possible indicator in achieved systemic risk factors for periodontitis. Med Sci Monit 2015;21:2485-2493.
19. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103-1011.
20. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17:162-173.
21. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444-1452.
22. Sılness J, Loe H. Periodontal disease ın pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand 1964;22:121-135.
23. Loe H, Sılness J. Periodontal disease ın pregnancy. I. Prevalence and severıty. Acta Odontol Scand 1963;21:533-551.
24. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-235.
25. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018;(89 Suppl 1):S173-S182.
26. Do LG, Roberts-Thomson KF. Dental caries experience in the Australian adult population. Aust Dent J 2007;52:249-251.
27. Gümüş P, Nizam N, Lappin DF, Buduneli N. Saliva and serum levels of B-cell activating factors and tumor necrosis factor-α in patients with periodontitis. J Periodontol 2014;85:270-280.
28. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-119.
29. Hatipoglu H, Canbaz Kabay S, Gungor Hatipoglu M, Ozden H. Expanded Disability Status Scale-Based Disability and Dental-Periodontal Conditions in Patients with Multiple Sclerosis. Med Princ Pract 2016;25:49-55.
30. Sheu JJ, Lin HC. Association between multiple sclerosis and chronic periodontitis: a population-based pilot study. Eur J Neurol 2013;20:1053-1059.
31. Gustavsen MW, Celius EG, Moen SM, et al. No association between multiple sclerosis and periodontitis after adjusting for smoking habits. Eur J Neurol 2015;22:588-590.
32. Dulamea AO, Boscaiu V, Sava MM. Disability status and dental pathology in multiple sclerosis patients. Mult Scler Relat Disord 2015;4:567-571.
33. McGrother CW, Dugmore C, Phillips MJ, et al. Multiple sclerosis, dental caries and fillings: a case-control study. Br Dent J 1999;187:261-264.
34. Kovac Z, Uhac I, Buković D, et al. Oral health status and temporomandibular disorders in multiple sclerosis patients. Coll Antropol 2005;29:441-444.
35. Mortazavi H, Akbari M, Sahraian MA, Jahromi AA, Shafiei S. Salivary profile and dental status of patients with multiple sclerosis. Dent Med Probl 2020;57:25-29.
36. Acharya AB, Shetty IP, Jain S, et al. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in chronic periodontitis before and after nonsurgical therapy. J Indian Soc Periodontol 2019;23:419-423.
37. Liukkonen J, Gürsoy UK, Könönen E, et al. Salivary biomarkers in association with periodontal parameters and the periodontitis risk haplotype. Innate Immun 2018;24:439-447.
38. Wu YC, Ning L, Tu YK, et al. Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population. J Formos Med Assoc 2018;117:841-848.
39. Zhang T, Andrukhov O, Haririan H, et al. Total antioxidant capacity and total oxidant status in saliva of periodontitis patients in relation to bacterial load. Front Cell Infect Microbiol 2016;5:97.
40. Hauser SL, Cree BAC. Treatment of multiple sclerosis: a review. Am J Med 2020;133:1380-1390.