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Research Article

THIOL/DISULPHIDE HOMEOSTASIS AND VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN SALIVA OF TYPE 1 DIABETIC CHILDREN WITH GINGIVITIS

Sultan KELEŞ [1], Özge ÇEVİK [2], Ahmet ANIK [3], Ayşe ANIK [4], Burçin İrem ABAS [2]

[1] 1Department of Pediatric Dentistry, Faculty of Dentistry, Aydin Adnan Menderes University, Aydin, TURKIYE
2Department of Biochemistry, Faculty of Medicine, Aydin Adnan Menderes University Aydin, TURKIYE
3Department of Pediatric Endocrinology, Faculty of Medicine, Aydin Adnan Menderes University Aydın, TURKIYE
4Department of Pediatrics, Faculty of Medicine, Aydin Adnan Menderes University Aydin, TURKIYE
*Correspondence: dtsultank@gmail.com

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ABSTRACT

Objective: To evaluate the thiol/disulphide homeostasis and level of vascular endothelial growth factor (VEGF) in saliva of patients with type 1 diabetes mellitus and gingivitis.

Materials and Methods: Forty children with type 1 diabetes mellitus (DM) and 40 systemically healthy (H) children were included the study. Based on children' periodontal and systemic health status, they were divided into four subgroups: 1) systemically and periodontally healthy subjects (Hh), 2) systemically healthy subjects with gingivitis (Hg), 3) diabetic subjects with periodontal health (DMh), 4) diabetic subjects with gingivitis (DMg). Probing depth (PD), gingival index (PI) and plaque index (PI) were recorded. An automated technique was used to measure the thiol/disulphide homeostasis parameters, and ELISA was used to measure the VEGF concentrations in unstimulated whole saliva.

Results: DM and H groups had comparable clinical periodontal parameters and salivary VEGF levels ($p>0.05$). GI, PI, PD, and disulphide amounts were significantly higher in the gingivitis subgroups (Hg and DMg) than in the periodontally healthy subgroups (Hh and DMh) ($p<0.001$). The gingivitis subgroups (Hg and DMg) had significantly higher amounts of VEGF compared to the periodontally healthy subgroups (Hh and DMh) ($p<0.001$).

Conclusions: Thiol/disulphide homeostasis shifts towards disulphide direction in diabetic children with gingivitis. Thiol/disulphide homeostasis and VEGF levels in saliva may be diagnostic markers of gingival inflammation.

Keywords: Saliva, gingivitis, diabetes mellitus, oxidative stress

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) occurs from autoimmune destruction of pancreatic beta cells, resulting to the loss of insulin production and hyperglycemia, and it is frequently recognized in children and young adults (1). An increase in cytokine release, impaired antioxidation and damage caused by reactive oxygen species (ROS) in beta cells act in its pathogenesis (2).

Thiols are organic compounds that take part in the elimination of ROS via nonenzymatic pathways. They contain succinyl groups, which are made up of a sulfur atom and a hydrogen atom bonded to a carbon atom. An increase in ROS induces oxidation of sulfur atoms and the constitution of covalent bonds between sulfur atoms, resulting in disulfide conversion. The generated disulphide linkages can be reduced to thiol groups, hence preserving thiol/disulphide homeostasis (3,4). Thiol/disulphide homeostasis is required for detoxification and includes the following parameters: native and total thiol; disulphide; and disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios (3,4). These parameters have been investigated as oxidative stress markers in a variety of degenerative disorders, including obesity, coronary heart disease, respiratory diseases, Alzheimer's disease, slow coronary flow, type 1 diabetes mellitus, and periodontal pathologies (5-7). Durmus et al. (8) found that oxidative damage in pancreatic β-cells caused a shift in thiol/disulphide balance in diabetic children, favoring disulphide.

Gingivitis is a common inflammatory disease that affects solely the soft tissues around the teeth during childhood and adolescence (9). The early detection and treatment of gingivitis are crucial because the degradation of periodontal tissues can result in tooth loss if treatment is not received (10). The inflammatory response and oxidant/antioxidant balance is a critical factor in the development of diabetes and periodontal disease, which are both chronic pathological situations that are related by numerous underlying biological mechanisms (11). Increased production of ROS in periodontal tissues of diabetic individuals leads to development of insulin resistance that inhibits cell proliferation and angiogenesis in the periodontal tissues. There are many studies showed that the diabetic children are more prone to have periondontal diseases compared to healty controls (12,13).

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Vascular endothelial growth factor (VEGF) is a multifunctional biomarker that stimulates microvascular permeability and acts as an endothelial cell mitogen (14). It has been shown to act as an serious role in the pathogenesis of growth of tumors, rheumatoid arthritis, atherosclerosis of coronary arteries, Kawasaki disease, T1DM, and periodontal disease (14-16). There are many oxidative stress indicators such as total antioxidant capacity, nitric oxide, glutathione peroxidase, malondialdehyde, total oxidative status, and 8-hydroxydeoxyguanosine detected in the biological fluids of the individuals with T1DM and/or gingivitis (17). Studies concluded that hyperglycemia related to T1DM and the presence of oxidative stress may induce the expression of VEGF (13). Since thiol/disulfide homeostasis is thought to be a marker of oxidative stress, it can be hypothesized that it may be related to VEGF levels in saliva. Therefore, the objective of the study was to evaluate thiol/disulphide homeostasis parameters and VEGF in saliva of type 1 diabetic children with gingivitis.

MATERIALS AND METHODS

Subjects and Clinical Examinations

40 with type 1 diabetes mellitus (DM) and 40 systemically healthy (H) individuals; aged 9–13 years were consecutively included in this research at the Department of Pediatric Dentistry, Faculty of Dentistry, Aydin Adnan Menderes University, Aydin, Türkiye, between July and October 2020. Ethical approval was obtained from the ethics committee of the University (protocol number 2020/114). The trial was carried out in compliance with the Declaration of Helsinki's rules of 1975, as revised in 2013. The study design was explained to the parents of the children and they signed informed consent before the participation. The study was recorded at http://www.clinicaltrial.gov (Protocol Registration Receipt NCT04470635).

After obtaining each participant's medical and dental history, oral examinations were conducted. Depending on their periodontal condition, the children were split up into four subgroups: 1) systemically and periodontally healthy participants (Hh, n = 20), 2) systemically healthy participants with gingivitis (Hg) (n =

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20), 3) diabetic participants with periodontal health (DMh, n = 20), and 4) diabetic participants with gingivitis (DMg, n = 20).

Inclusion criteria were as follows: 1) aged 9–13 years, 2) the patients confirmed as T1DM with a HbA1c value of <7.5% by a pediatric endocrinologist at least twelve months before the trial (18), and not having any other systemic illnesses 3) not having any disease for systemically healthy children (parental reporting) 4) presence of first molars and maxillary and mandibular incisors that are fully erupted and free of cavities.

Exclusion criteria were as follows: 1) uncontrolled diabetic mellitus, 2) periodontitis or periodontal intervention including antibacterial or anti-inflammatory medications within the preceding six months, 3) needing restorative and endodontic treatment, 4) using immunosuppressive medications within the previous 6 months, 5) using orthodontic appliances, and 6) presence of clinical attachment loss.

Periodontal measurements

Gingival index (GI), plaque index (PI), and probing depth (PD) were among the periodontal measurements that were employed (19). First molars and completely erupted, caries-free permanent maxillary and mandibular incisors were used for clinical measurements, and they were thought to represent the entire mouth (19,20).

Data were collected from buccal, lingual/palatinal, mesial, and distal sites of the teeth. The probing depth was noted as the distance from the gingival margin to the bottom of the probed pocket. Participants were confirmed as gingivitis when GI $\geqslant$ 1 and PD $\leqslant$ 3 mm at all measured sites for 12 teeth. Children were diagnosed as periodontal health if they had GI < 1 and PD < 3 mm (19,20).

A pediatric dentist (SK) conducted all clinical periodontal measurements with a manuel periodontal probe (Williams, Hu-Friedy, Chicago, IL). Prior the study, calibration of the researcher was conducted on ten children with gingivitis for PD. The intra-examiner agreement coefficient was 0.97 for PD.

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Gathering of saliva samples

Samples were gathared in the morning from 9:00 am to 10:00 am to reduce the impact of circadian rhythm on biomarker levels. Unstimulated saliva samples were gathered one day after periodontal clinical measurements. The subjects were told not to engage in oral care activities like flossing, brushing, and mouth rinsing, and to abstain from eating and drinking for 2 hours before providing samples. Before collecting saliva, each patient was instructed to irrigate their mouth with water for 2 minutes, wait 10 minutes, and then spit into sterile 50 - mL polypropylene tubes for 5 minutes. Saliva samples were stored at a temperature of -80° C, until further examination.

Measurement of thiol/disulphide homeostasis parameters

Erel and Neselioglu developed an automated analysis method that had been used in many studies, to assess salivary thiol/disulphide homeostasis (3,4). In the first step, the reducible disulphide bonds were decreased to create available functional thiol groups. Formaldehyde was utilized to eliminate the unreacted and spent sodium borohydride, and following the interaction with DTNB, all sulfhydryl groups, comprising both reduced and original groups, were quantified. The level of dynamic disulphide was calculated by subtracting half of the total thiol groups from the native thiol groups. After determining the levels of native thiols, total thiols, and disulphide in μmol/L, the ratios of Disulphide/total thiol, native thiol/total thiol, and disulphide/native thiol percentages were determined (21).

Measurement of VEGF level in saliva

Thawed saliva samples were warmed to 37°C and thoroughly mixed before being analyzed. VEGF level in saliva were measured by the enzyme-linked immunosorbent assay (ELISA) using commercial kits (Human Vascular endothelial growth factor level ELISA kit, Sunred Biotechnology, Shanghai) consistent with the manufacturer's guidelines. The minimum determination level for VEGF level was 20 pg/ml. Plates were quantified at 450 nm with 650 nm as a reference wavelength via an ELISA reader (DTX 880 Multimode Reader,

Beckman Coulter, Miami, FL). VEGF concentrations were calculated from the standard curve and presented in pg/ml.

Statistical Analysis

Based on a prior study (22), the number of participants required was determined using a power analysis software (G*Power version 3.0.8, Heinrich Heine University, Düsseldorf) with a 95% power, 0.83 effect size, and α set at 0.05. According to these criteria, each group needed a minimum of 39 patients. The sample size was changed to 80 in order to account for the possibility of missing samples or data. The statistical software program SPSS version 22.0 (IBM Inc., Chicago, IL) was used to analyze descriptive statistics and the normality of subgroup data. The Kolmogorov-Smirnov normality test was used to validate the distribution of the biochemical and clinical data. The study parameters were analyzed using the Kruskal Wallis test and the Mann-Whitney U test. The Spearman's Rank Correlation tests was used to assess whether the association between the clinical and biochemical parameters were linear, with a significance level of $\alpha = 0.05$.

RESULTS

Subjects sand salivary samples characteristics

For children in systemic health, the mean age (years ± SD) was 11.25 ± 1.89, while for children with type 1 diabetes, it was 10.95 ± 1.83. For children with diabetes and those in systemic health, the gender distributions were 21/19 and 22/18, respectively. For both gender and age, there were no statistically significant differences between the diabetic children and the systemically healthy group ($p > 0.05$).

Periodontal clinical parameters

Table 1 displays the periodontal clinical indicators for children with DM and systemically healthy children. For the 12 permanent teeth, children with DM had PD, GI, and PI numbers that were comparable to those of

children in systemically healthy group ($p > 0.05$). Compared to the periodontally healthy subgroups, the Hg and DMg subgroups had significantly higher PD, GI, and PI values ($p < 0.001$) (Table 2).

Evaluation of thiol/disulphide homeostasis data of the groups and subgroups

Total thiol and disulphide levels were determined higher in systemically healthy children compared to diabetic childen in Table 1 ($p < 0.001$). Native thiol levels were lower in diabetic children compared to healthy controls, but the difference between the groups was not statistically significant ($p > 0.05$).

Table 1. Periodontal clinical parameters, HbA1c values, thiol/disulphide homeostasis parameters and VEGF levels in saliva of the study groups and their comparisons.

Parameters	**H** (n= 40)	**DM** (n= 40)	P*
Age (year)	11.0(10.0-12.8)	10.0(10.0-12.0)	0.461
Gender			0.499**
Female (%)	24(60)	21(53)	
Male (%)	16(40)	19(47)	
HbA1c (%)	5.30(5.20-5.70)	7.30(5.60-7.40)	<0.001
PI	0.80 (0.30-2.00)	0.70 (0.30-2.00)	0.778
GI	1.20 (0.51-2.00)	1.20 (0.43-2.45)	0.729
PD (mm)	1.45 (0.91-2.02)	1.50 (0.96-2.39)	0.491
VEGF (pg/ml)	41.4 (34.8-55.9)	44.0 (33.0-58.4)	0.722
Native thiol (µmol/L)	12.9 (9.3-23.9)	11.2 (4.80-23.9)	0.405
Total thiol (µmol/L)	98.1(61.9-162.3)	51.4 (21.8-90.4)	**0.001**
Disulphide (µmol/L)	101.6 (64.4-177.3)	52.8 (23.6-85.6)	**<0.001**
Disulphide /native thiol(%)	7.58 (4.73-16.39)	3.94 (1.89-9.32)	**0.015**
Disulphide /total thiol (%)	1.10 (1.03-1.23)	1.07 (0.70-1.26)	0.227
Native thiol/total thiol (%)	0.14 (0.07-0.27)	0.27 (0.11-0.55)	**0.023**

*Mann-Whitney U test, **Chi-square test. Data were given as n (%), median (25p-75p).

Native thiol/total thiol ratio was statistically higher in diabetic children compared to systemically healthy controls. Disulphide/native thiol ratio was statistically significantly higher in systemically healthy children. There was no statistically sifnicant difference in terms of disulphide/total thiol ratio between the groups ($p > 0.05$). Native thiol levels were higher in DMh subgroup compared to DMg subgroup. Total thiol level was higher in Hh subgroup compared to DMh subgroup. Disulphide levels were higher in gingivitis subgroups

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compared to periodontally healthy subgroups. DM subgroup presented the highest native thiol/total thiol ratio and the lowest disulphide/native thiol ratio compared to other subgroups (Table 2).

Comparison of salivary VEGF level among groups and subgroups

VEGF were measured in all specimens. There was no significant difference in the VEGF levels in saliva between individuals with DM and systemically healthy individuals ($p > 0.05$) (Table 1). The Hg subgroup had significantly higher VEGF amount compared to the Hh subgroup ($p < 0.001$) (Table 2).

Tablo 2. Anthropometric data and periodontal clinical parameters of the children in the subgroups.

Parameters	**Hh** (n=20)	**Hg** (n=20)	**DMh**(n=20)	**DMg** (n=20)	**P***
Age (year)	11.0(10.0-12.0)	11.0(10.0-13.8)	12.0(10.0-13.0)	10.0(9.0-12.0)	0.323
Female (%) Male (%)	14(70) 6(30)	10(50) 10(50)	11(55) 9(45)	10(50) 10(50)	0.535**
HbA1c (%)	5.30(5.20-5.70)	5.50(5.32-5.70)	7.35(5.71-7.40)	7.30(6.20-7.40)	**<0.001**[a]
PI	0.30 (0.20-0.34)	2.00 (1.43-2.00)	0.30 (0.20-0.40)	2.00 (1.40-2.00)	**<0.001**[b]
GI	0.53 (0.38-0.68)	2.00 (1.74-2.50)	0.45 (0.39-0.69)	2.40 (1.90-2.62)	**<0.001**[b]
PD (mm)	0.92(0.77-1.20)	2.02(1.80-2.36)	0.97(0.76-1.20)	2.39(1.88-2.67)	**<0.001**[b]
VEGF (pg/ml)	35.42 (30.50-39.38)	55.11 (44.72-64.72)	45.59 (29.46-62.05)	42.49 (34.73-58.32)	**<0.001**[c]
NT(µmol/L)	12.36 (8.99-18.83)	15.74 (11.24-24.73)	20.23 (11.24-31.19)	6.74 (3.65-14.61)	**0.013**[d]
TT (µmol/L)	90.89 (46.47-126.83)	112.46 (64.70-183.06)	28.76 (15.15-86.27)	62.65 (45.70-91.15)	**0.004**[e]
D (µmol/L)	95.50 (55.00-134.57)	112.40 (72.08-208.63)	44.18 (17.28-77.70)	60.75 (40.01-92.95)	**<0.001**[e]
D/NT(%)	7.15 (4.73-13.75)	7.58 (4.78-18.17)	2.15 (1.20-5.01)	9.23 (3.17-15.68)	**<0.001**[f]
D/TT(%)	1.10 (0.98-1.21)	1.11 (1.06-1.27)	1.15 (0.66-1.67)	1.06 (0.78-1.12)	0.326
NT/TT(%)	0.14 (0.08-0.27)	0.14 (0.06-0.27)	0.52 (0.19-0.99)	0.11 (0.07-0.31)	**<0.001**[g]

*Kruskal-Wallis test **chi-square test, Data were given as n (%), median (25p-75p),n [a]Group DMh &Group Hh; Group DMh & Group Hg; Group DMg &Group Hg; Group DMg &Group Hh, p<0.001. [b] Group DMh & Group DMg, Group DMh & Group Hg, Group DMg & Group Hh, Group Hg & Group Hh, p< 0.001, [c] Group Hg & Group Hh, p< 0.001, [d] Group DMh & Group DMg, p< 0.001, [e] Group DMh & Group Hg, p< 0.001, [f] Group DMh & Group DMg, p= 0.003; Group DMh & Group Hg, p=0.001; Group DMh & Group Hh, p= 0.007, [g]Group DMh & Group DMg, p= 0.001; Group DMh & Group Hg, p= 0.002; Group DMh & Group Hh, p= 0.005 **Abbreviations:** NT, Native thiol; TT, Total thiol; D, Disulphide; D/NT, Disulphide/native thiol ; D/TT, Disulphide/ total thiol; NT/TT, Native thiol/ total thiol.

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Correlation between thiol/disulphide homeostasis parameters, VEGF level and clinical parameters

Table 3 displays the correlations between biochemical data and clinical periodontal markers. Significant correlations were found between salivary VEGF levels and PI, GI, and PD ($r = 0.425$, $p < 0.01$; $r = 0.337$, $p < 0.01$; $r = 0.228$, $p < 0.05$, respectively). Furthermore, the total thiol and disulphide and the VEGF level were positively correlated ($r = 0.281$, $p < 0.05$; $r = 0.273$, $p < 0.05$).

Tablo 3. Correlations between clinical parameters and biochemical findings of all study groups.

Parameters	HbA1c	PD	GI	PI	VEGF	NT	TT	D	D/NT	D/ TT	NT/ TT
HbA1c	1,000	0,128	0,065	0,057	-0,026	-0,059	-,386**	-,419**	-,276*	-0,033	,278*
PD	0,128	1,000	,735**	,667**	,228*	-0,136	0,174	0,204	,272*	0,051	-,257*
GI	0,065	,735**	1,000	,777**	,337**	-0,104	0,130	0,118	0,201	-0,023	-0,214
PI	0,057	,667**	,777**	1,000	,425**	-0,120	0,185	0,182	,239*	-0,043	-,255*
VEGF	-0,026	,228*	,337**	,425**	1,000	0,167	,281*	,273*	0,007	-0,104	-0,021
NT	-0,059	-0,136	-0,104	-0,120	0,167	1,000	0,181	0,170	-,679**	0,113	,671**
TT	-,386**	0,174	0,130	0,185	,281*	0,181	1,000	,932**	,494**	-,241*	-,540**
D	-,419**	0,204	0,118	0,182	,273*	0,170	,932**	1,000	,537**	0,079	-,514**
D/NT	-,276*	,272*	0,201	,239*	0,007	-,679**	,494**	,537**	1,000	-0,067	-,976**
D/TT	-0,033	0,051	-0,023	-0,043	-0,104	0,113	-,241*	0,079	-0,067	1,000	,265*
NT/TT	,278*	-,257*	-0,214	-,255*	-0,021	,671**	-,540**	-,514**	-,976**	,265*	1,000

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).
Abbreviations: NT, Native thiol; TT, Total thiol; D, Disulphide; D/NT, Disulphide/native thiol ; D/TT, Disulphide/ total thiol; NT/TT, Native thiol/ total thiol.

There was no association between total thiol, native thiol and disulphide levels and periodontal clinical parameters. There was negative association between native thiol/total thiol ratio and PI and PD ($r = -0.255$, $p < 0.05$; $r = -0.257$, $p < 0.05$, respectively). There was positive relationship between disulphide/native thiol ratio and PI and PD ($r = 0.239$, $p < 0.05$; $r = 0.272$, $p < 0.05$, respectively).

DISCUSSION

This study revealed the shift of dynamic thiol/disulphide homeostasis toward disulphide form in individuals with T1DM and gingivitis by evaluating saliva samples for the first time. VEGF levels in saliva

was similar in children with T1DM and healthy controls. However, the salivary VEGF levels were increased in systemically healthy children with gingivitis.

Hyperglycemia can trigger pathways that increase inflammation, apoptosis, and oxidative stress, and T1DM is linked to increased levels of systemic indicators of inflammation that lead to microvascular and macrovascular problems (23). The imbalance between ROS and antioxidant molecules leads to oxidative stress. Redox modification of radical-based cysteine residues or oxidation between two electrons occurs when ROS levels rise above the physiological threshold. The sulfur atom in the cysteine side chain is oxidized in this redox reaction to produce disulphide (24). Thus, when the cellular level starts, oxidant radicals are linked to the initial stage of oxidative damage, and dynamic thiol/ disulphide homeostasis progresses toward disulphide form. Although underlying mechanisms are unclear, pancreatic β-cell failure is a critical event in the onset of T1DM. Because of their low capacity to withstand oxidative stress, β-cells are more vulnerable to it. ROS are linked to proinflammatory conditions at the onset of T1DM (25). Studies on both adults and children have demonstrated that diabetics have higher levels of thiol oxidation than healthy controls (8,21). In this study, total thiol level was significantly higher in healthy controls compared to diabetic children. This data confirmed that the diabetic children might had higher levels of thiol oxidation.

Gingivitis is commonly seen periodontal disease in children and adolescents and it may be induced by plaque, steroid hormonerelated gingivitis, and drug-influenced gingival enlargement. Plaque microorganisms can initiate periodontal diseases and gingivitis is considered as the earliest stage of periodontitis, the development of which occurs in only long-term untreated gingivitis (25). There are many studies revealed that diabetic children were more prone to periodontal diseases compared to their healthy counterparts (26-28). There are many mechanisms to connect periodontal disease with T1DM. Both T1DM and periodontal disease are categorized as inflammatory disorders that share similar pathogenic mechanisms, including the action of pro-inflammatory mediators. Studies revealed that diabetic patients exhibit heightened levels of pro-inflammatory mediators in their gingival tissues, including IL-1β (interleukin-1beta), tumor necrosis factor-alpha (TNF-α), IL-6, matrix metalloproteinases (MMPs), prostaglandins (PGs), the association between

receptor activator of nuclear factor kappa-B ligand and osteoprotegerin (RANKL/OPG), VEGF, and oxidative stress, all of which significantly contribute to the onset and advancement of periodontal disease (29,30).

VEGF is an angiogenic biomarker of importance in inflammation and wound healing (31). VEGF has been determined in human periodontal tissue, gingival crevicular fluid (GCF) and saliva in various periodontal diseases (32,33). Pradeep et al. (34) determined that the GCF VEGF amounts had an increasing pattern from health to plaque-induced gingivitis in systemically healty adults. Moreover, studies evaluated serum levels of VEGF showed that the serum VEGF level significantly increased in participants with diabetes compared to healthy controls (35). In the present study, salivary VEGF level was higher in diabetic children compared to healthy controls without a statistically significant difference. These data suggests that the the similarity in the salivary VEGF levels from diabetic and systemically healthy children in the present study is due to the good glycemic control in the diabetic individuals included to trial. In subgroups, healthy children with gingivitis showed the highest salivary VEGF levels compared to other groups. Additionally, periodontal clinical parameters were significantly correlated with salivary VEGF levels. In accordance with the present study, Padma et al. (36) determined that VEGF levels in GCF increased progressively from healthy to gingivitis in adults. Studies revaled that, T1DM was a more declared significant risk factor for periodontal disease and vascular complications, and there was an important association between HbA1c and oxidative stress (37). Seçkin et al. (35) stated that It may be appropriate to evaluate the levels of VEGF and oxidative stress markers in diabetic children.

Thiol/disulfide homeostasis has recently been used to evaluate the free radical status in an organism both at physiological and pathological conditions, therefore determination of this homeostasis can provide valuable information related to normal or pathological biochemical processes in many disease in children. In this study we used saliva samples to evaluate the thiol/disulfide homeostasis. Hasan et al. (38) performed a study to evaluate the possibility of using saliva, as an alternative biological fluid instead of blood serum to detect thiol homeostasis, and they concluded that saliva might be an alternative sample that is easy to collect from children (39). Tayman et al. (40) found a significant positive correlation between the severity of periodontal disease and serum total thiol and disulphide levels. As far as we know, there is no study evaluate

the thiol/disulfide homeostasis in diabetic children with gingivitis. Therefore, we could not compare our results to the literature. In the present study, total thiol and native thiol levels were lower in diabetic children compared to healthy individuals. Additionally, Tayman et al. (40) reported that the disulphide level was higher in gingivitis subgroup similar to adult studies. This indicates that antioxidant balance is impaired in diabetic patients and systemically healthy children with gingivitis. In this study, disulphide/native thiol ratios were higher in diabetic children with gingivitis. It may be resulted by more reduction of native thiol level compared to disulphide levels. Taken together, it may be suggested that thiol/disulphide homeostasis has potential to act as a diagnostic tool in detecting periodontal diseases in diabetic children.

Studies have shown that serum and tissue VEGF levels are high in diabetic children (35,41). VEGF expression is induced by hyperglycemia, advanced glycation end products, and oxidative stress (42,43). In this study, the positive correlation between disulfide and VEGF levels in saliva may support the relationship between VEGF and ROS-induced oxidative stress response in T1DM.

This study has some limitations. A study group that includes children who had uncontrolled diabetes mellitus should be added the study design. Children's systemic situation for systemically healthy group were recorded on the basis of their parents's reports. Therefore, it is not certain whether they have no any other systemic diseases. Additionally, the cross-sectional design of present study could not allow to establish causal association between thiol disulphide homeostasis parameters, level of VEGF and periodontal status. Prospective longitudinal studies with larger sample sizes should be performed to explore the potential effects of the thiol/disulphide homeostasis.

CONCLUSION

Based on the limitations of this research, it can be inferred that the balance of thiol/disulphide homeostasis shifts toward the disulphide direction in diabetic children suffering from gingivitis. The elevated levels of VEGF in the saliva among the gingivitis subgroups and their positive association with periodontal clinical measurements indicate that VEGF might serve as a valuable marker for diagnosing gingivitis in

children. This study findings need to be confirmed with longitudinal studies with the aim of determining the effect of thiol/disulphide homeostasis and salivary VEGF levels in children with T1DM and gingivitis.

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Authorship contributions

Concept: SK, AA ; Design: SK, OC, AA ; Data Collection or Processing: SK, AA, BIA; Analysis or Interpretation: SK, OC, AA, BIA, Literature Search: SK, OC, AA, BIA; Writing: SK, OC, AA, BIA.

Data availibity statement

Data can be requested from the authors.

Declaration of competing interest

No conflict of interest was declared by the authors.

Ethics

This study was approved by the ethics committee of the Aydin Adnan Menderes University Faculty of Medicine (protocol number 2020/114).

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