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Assessment of DMFT Indexes, Salivary Flow Rate, pH, and Detections of S.Mutans Salivary Levels by a Quantitative Real-Time PCR in Polycystic Ovary Syndrome

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ABSTRACT

Objectives: PCOS is an endocrine disorder that is common in women. However, PCOS effects on oral and dental health have not been stated clearly. The aim of this study is to examine the effects of Polycystic Ovary Syndrome (PCOS), which is common in women of reproductive age, on saliva and dental tissues in these women.
Materials and Methods: One-hundred individuals who were / were not diagnosed with PCOS and insulin resistance were included in this study (n=100). Subsequently, individuals, with PCOS and insulin resistance (PCOSID +), with PCOS and non-insulin resistance (PCOSID-), without PCOS and insulin resistance (ControlID +) and without PCOS and non-insulin resistance (ControlID-) were divided into 4 groups (n=25). DMFT (Decayed, Missing, Filled Teeth) index was used for dental health evaluation, while pH meter was used for saliva pH measurement. Also, Streptococcus Mutans (S. Mutans) numbers were analyzed by the real-time Polymerase Chain Reaction (PCR) method. In statistical analysis p<0.05 was considered significant.
Results: In comparison among the groups, significant differences were found in terms of DMFT index, S. Mutans, and salivary pH values. Among the compared groups, the highest DMFT index, S. Mutans values were found in the PCOSID(+) group, the lowest in ControlID(-) group, while the lowest saliva pH value was found in the PCOSID(+) group.
Conclusions: S. Mutans and DMFT index values were found highly in the saliva of PCOS patients, which is a multifactorial syndrome, and it is determined that salivary parameters have an effect on this situation.

Keywords: PCOS, Real-Time PCR, S. Mutans, Salivary pH

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Introduction

Polycystic Ovary Syndrome (PCOS) is an endocrine disorder of unknown etiology, which is common among women of reproductive age. With a prevalence of approximately 6-8%, PCOS is a clinical picture characterized by chronic anovulation, clinical and/or biochemical hyperandrogenism and polycystic appearance in the ovaries.[1] Several systems are affected by this syndrome and it is presented by menstrual irregularities (oligo-amenorrhea, dysfunctional uterine bleeding), signs of hyperandrogenism (hirsutism, acne, sebaceous skin), obesity and metabolic syndrome.[2]

Insulin resistance (IR) plays a key role in the pathogenesis of PCOS. Insulin resistance is the failure to induce adequate biological response although the insulin is at normal concentration. IR and hyperinsulinemia are some of the common conditions in PCOS patients.[3] Although IR is not a diagnostic criterion of PCOS, it is detected in 50-80% of women with PCOS, regardless of their Body Mass Indices (BMI), and it accelerates the development of diabetes by making patients more susceptible to prediabetes.[4]

In recent years, the relationship between PCOS, which has different complications, and periodontal diseases caused by chronic low-grade inflammation has become a noteworthy issue for reproductive endocrinology. The relationship between PCOS and inflammation, similar to periodontal diseases, and its long-term metabolic consequences has led to the hypothesis that it may be associated with periodontal parameters.[5] Although the underlying biological connections have not been clarified yet, the hyperestrogenic and hyperandrogenic states that occur in PCOS are thought to cause structural changes in the gums and gingival inflammation, and these changes cause bacteria to colonize the gums more easily, increasing the risk of periodontal disease. Therefore, studies report that the detection of periodontal diseases in their early period in PCOS cases and initiation of treatment are crucial for reducing the metabolic complications that may occur in the long term.[6]

If chronic inflammation in periodontal tissues is not treated, tooth-supporting tissues will be destroyed over time and, as a result, dental cavity and loss will occur.[7] Bacteria in the oral cavity play a significant role in the increase of such dental caries. One of these bacteria is Streptococcus mutans (S. Mutans).[8] S. Mutans damages dental hard tissues due to

its acidogenic properties.[9] Studies report that S. Mutans is generally localized in the superficial layers of caries, and facultative bacteria such as lactobacilli colonize towards dentin. [10] A limited number of studies are available in the literature focusing on its relationship with periodontal tissues, but there are no studies investigating the dental hard tissue or oral microbiology.

In the previous limited number of studies investigating PCOS and periodontal state and carious lesions, it remains elusive exactly when dental health starts to deteriorate in PCOS since patients within a wide range of ages are included in the study. Thus, this study aims to investigate bacteria that cause dental cavities in young adults diagnosed with PCOS in their early reproductive period, when metabolic problems are observed to a lesser extent, using the real-time Polymerase Chain Reaction (PCR) method.

Materials and Methods

This clinical study was carried out in Gaziantep University, Faculty of Dentistry, Department of Restorative Dentistry as a multidisciplinary study in collaboration with Gaziantep University Medical Faculty Hospital Gynecology and Obstetrics Department, Microbiology Department and Endocrinology Department. Approval was obtained from Gazianatep University Ethics Committee (2019/311). In the study, serum findings of patients and individuals referred to us were evaluated retrospectively to form groups, and the clinical findings of a total of 100 patients/individuals, 50 of whom were diagnosed with PCOS and 50 were healthy individuals, were evaluated prospectively. All participants were given information about the research, and oral and written informed consent was obtained from all participants.

Experimental Groups

Using 3.1.9 G*Power software analysis, a significance level of 5% ($\alpha = 0.05$) at 0.80 ($1-\beta$) for an effect size of 0.41 was calculated as the sample size. The minimum number of patients was determined as 25 for each group. The results of biochemical parameters were obtained from the patient files, and the groups were standardized according to serum levels of FSH, LH, LH/FSH, HOMA and estrogen.

A total of 100 female patients (n = 100) were included in the study, who were aged 18-33, did not have any systemic disease, were diagnosed with PCOS and had insulin resistance and were categorized as follows: 25 PCOSID (+) patients diagnosed with PCOS but without insulin resistance; 25 PCOSID (-) patients without PCOS but with insulin resistance; 25 ControlID (+) patients without PCOS but with insulin resistance and, finally, 25 ControlID (-) female individuals without PCOS and insulin resistance.

Study groups were formed as follows:

- PCOSID (+) (G1): 25 female patients (n = 25) with PCOS diagnosis and insulin resistance.
- PCOSID (-) (G2): 25 female patients (n = 25) with PCOS diagnosis but without insulin resistance.
- ControlID (+) (G3): 25 healthy volunteers (n = 25) without PCOS but with insulin resistance.
- ControlID (-) (G4): 25 healthy volunteers (n = 25) without PCOS and insulin resistance.

Measurement of saliva flow rate

Saliva samples were taken at 9.00-10.00 in the morning in order to ensure standardization in our study. Individuals were asked not to eat, drink or brush their teeth for at least 2 hours before stimulated and unstimulated saliva samples were taken. Stimulated saliva flow rate, unstimulated saliva flow rate and saliva pH were evaluated.

Measurement of Unstimulated saliva flow rate

Unstimulated saliva flow rate was measured according to the following scores:

- Score 1: Low saliva flow rate; < 60 sec
- Score 2: Normal saliva flow rate; > 60 sec

Measurement of Stimulated saliva flow rate

In order to stimulate the salivary flow, patients were asked to chew a paraffin gum and swallow the first saliva formed in the mouth and then spit the saliva accumulated in the mouth for 5 minutes into a measured container. Saliva flow rate was scored as follows:

- Score 0: Low saliva flow rate; < 0.7 ml/min
- Score 1: Normal saliva flow rate; 0.7-1.1 ml/min
- Score 2: High saliva flow rate; > 1.1 ml/min

Determining the DMFT Index

According to the DMFT index, the total number of decayed (decay, D), missing (M) and filled (F) teeth is divided by the number of people examined, and thus filled, missing & decayed teeth per person are calculated (D+M+F/N = DMFT). Congenital missing teeth, unerupted teeth and supernumerary teeth were not included in this index calculation.

The DMFT index evaluation was performed prior to the study by two physicians and calibrated on 100 patients. Cohen's Kappa coefficient was used to make the clinical evaluation among the physicians. As a result of the examination, the Kappa coefficient for reliability was 0.74 for all variables and the Kappa coefficient for repeatability was 0.80.

Real-time PCR method

Stimulated saliva samples were collected with the help of a pipette and transferred to Eppendorf tubes. They were stored in the freezer (-80ºC) until the study day. Subsequently, the samples were immediately transferred to the microbiology laboratory with an ice battery and kept frozen at -20°C until the PCR analysis.

After the study samples were collected, DNA isolation was performed for S. Mutans with the Genesig S. Mutans detection kit (United Kingdom) according to the manufacturer's instructions.

To investigate the presence of S. mutans bacteria and determine the bacterial load in the isolated eluates, the forward and reverse (forward: 5'-CCGGTGACGGCAAGCTAA-3', reverse; 5' TCATGGAGGCGAGTTGCA-3') primers (Genesig Primer Design, United Kingdom) were designed and

supplied. In our study, S. Mutans strains ATCC 25175 and ATCC 35668 were used as positive controls. Standards were optimized for use in the real-time PCR. First, the master mix was prepared for the real-time PCR. 525 µl of re-suspension buffer solution was added to the lyophilized 2X qPCR master mix in a glass vial and diluted. 165 µl of distilled water from the kit was added to dilute S. mutans primer/probe mix. 500 µl of the buffer solution from the kit was added to dilute the lyophilized S. Mutans positive controls. Six standards were established separately for each strain. An amount of the diluted positive control was put into the first tube of 6 sterile 250 µl Eppendorf tubes.

90 µl of buffer solution was added to the tubes numbered 2, 3, 4, 5 and 6. 10 µl of positive control was taken from the Tube No. 1 and added to the Tube No. 2 and diluted, and similarly, 10 µl of positive control was taken from the Tube No. 2 to be added to the Tube No. 3 and dilute it and thus, standards were established sequentially, including the Tube No. 6. The master mix for PCR was prepared by mixing in a sterile Eppendorf tube, taking into consideration the negative control and standards, and 15 µl of this mixture was added to each tube. 5 µl of sample, negative control and standards were each added to the tubes, the tubes were capped and amplified in Rotor Gene (Qiagen, Germany) to obtain the quantitative analysis result.

Statistical Analysis

Normally distributed numerical variables were expressed as mean ± standard deviation, and non-normally distributed numerical variables were presented as mean values. Frequency analysis was applied to determine the percentage distribution of categorical variables in the groups.

ANOVA - LSD tests and Kruskal-Wallis pairwise tests were used for the statistical analysis. Pearson's Chi-square test/Fisher's exact tests were used to compare the inter-group categorical variables. Pearson's/Spearman's Rho tests were used for correlation calculation. r>0.06 was considered high correlation; r=0.3-0.6 was considered as medium correlation and r < 0.3 was considered low correlation. p<0.05 was considered significant for all values.

Results

Findings related to saliva parameters

No statistically significant difference in unstimulated and stimulated saliva flow rate was found between the groups (p > 0.05).

In inter-group comparison of saliva pH values, the statistical differences between PCOSID (+) and ControlID (+), PCOSID (+) and ControlID (-), PCOSID (-) and ControlID (+) and PCOSID (-) and ControlID (-) groups were found to be statistically significant (p<0.05).

The highest mean pH value of saliva was observed in the ControlID (-) group and the lowest value was observed in the PCOSID (+) group.

Unstimulated and stimulated saliva flow rate distributions of the groups are shown in Table 1, saliva pH mean and standard deviation (±SD) values are given in Table 2, and p values in the inter-group comparison are shown in Table 3.

Findings of S. Mutans value

S. Mutans counts of the saliva samples taken from individuals in all groups were determined by PCR method. In the inter-group comparison, a statistically significant difference was found between PCOSID (+) and ControlID (+), PCOSID (+) and ControlID (-), PCOSID (-) and ControlID (+) and PCOSID (-) and ControlID (-) groups (p<0.05).

The highest mean saliva S. Mutans value was observed in the PCOSID (+) group and the lowest value was observed in the ControlID (-) group.

The mean and standard deviation (±SD) values of S. Mutans in the saliva of the groups are shown in Table 4 and the p values in the inter-group comparison are shown in Table 5.

Findings regarding the DMFT index value

Based on the clinical examination of all individuals in the groups, the DMFT values were recorded.

In the inter-group comparison, a statistically significant difference was found between PCOSID (+) and ControlID (-) groups and PCOSID (-) and ControlID (-) groups (p<0.05).

The highest mean DMFT index value was observed in the PCOSID (+) group and the lowest value in the ControlID (-) group.

The mean and standard deviation values (±SD) of DMFT index values of the groups are shown in Table 6 and the p values for the inter-group comparison are shown in Table 7.

The relationship between saliva parameters and DMFT index values

In the PCOSID (+) group, a negative, moderate and statistically significant correlation was observed between unstimulated saliva flow rate and the DMFT index values (p<0.05).

No significant relationship was observed between stimulated saliva flow rate and the DMFT index values in all groups (p>0.05).

No significant relationship was observed between saliva pH value and the DMFT index values in all groups (p>0.05).

The correlation analysis of the DMFT index values with S. Mutans and saliva parameters is shown in Table 8.

The relationship between the S. mutans count and the DMFT index values

In the PCOSID (+) group, a positive, high and statistically significant relationship was observed between the S. Mutans count in saliva and the DMFT index values (p<0.05).

The correlation analysis between the S. Mutans count in saliva and the DMFT index values is shown in Table 8.

The relationship between saliva parameters and S. Mutans count

In the PCOSID (+) and ControlID (-) groups, a negative, low and statistically significant correlation was observed between the unstimulated saliva flow rate and the S. Mutans count in saliva and a negative, moderate and statistically significant correlation was observed between the PCOSID (-) and ControlID (+) groups (p<0.05).

Table 1. Frequency (n) and Percentage (%) values of unstimulated and stimulated salivary flow rate for all groups

	PCOSID(+)		PCOSID(-)		Control ID(+)		Control ID(-)		p
	n	%	n	%	n	%	n	%	
Unstimulated Salivary Flow Rate									0.934
Low	12	48	11	44	10	40	11	44	
Normal	13	52	14	56	15	60	14	56	
Total	25	100	25	100	25	100	25	100	
Stimulated Salivary Flow Rate									0.631
Low	14	56	12	48	9	36	10	40	
Normal	11	44	13	52	16	64	15	60	
Total	25	100	25	100	25	100	25	100	

* p <0.05 is significant

Table 2. Mean Saliva pH values of groups (±SD)

	PCOSID(+)	PCOSID(-)	CONTROLID(+)	CONTROLID(-)	P
SALIVARY PH	6.82±0.09	6.96±0.09	7.34±0.08	7.42±0.06	**0.001***

* p <0.05 is significant

Table 3: P values of Saliva parameters in the inter-group comparison

	STIMULATED SALIVARY FLOW RATE	UNSTİMULATED SALIVARY FLOW RATE	SALIVARY PH
PCOSID(+) - PCOSID(-)	1.000	0.777	0.160
PCOSID(+) - CONTROLID(+)	0.569	0.569	**0.001***
PCOSID(+) - CONTROLID(-)	0.258	1.000	**0.001***
PCOSID(-) - CONTROLID(+)	0.569	0.774	**0,007***
PCOSID(-) - CONTROLID(-)	0.258	0.777	**0.001***
CONTROLID(+) - CONTROLID(-)	0.569	0.569	0.493

* p <0.05 is significant

Table 4. Mean S. Mutans values of groups (±SD)

	PCOSID(+)	PCOSID(-)	CONTROLID(+)	CONTROLID(-)	P
S. MUTANS	121176.68±78698.41	119189.12±50476.39	13544.57±6036.52	4774.06±3046.02	**0.001***

p <0.05 is significant

Table 5. P values of S. mutans in the inter-group comparison

	S. MUTANS
PCOSID(+) - PCOSID(-)	0.432
PCOSID(+) - CONTROLID(+)	**0.042***
PCOSID(+) - CONTROLID(-)	**0.001***
PCOSID(-) - CONTROLID(+)	**0.013***
PCOSID(-) - CONTROLID(-)	**0.001***
CONTROLID(+) - CONTROLID(-)	0.393

* p <0.05 is significant

Table 6. Mean DMFT index values of groups (±SD)

	PCOSID(+)	PCOSID(-)	CONTROLID(+)	CONTROLID(-)	P
DMFT	5.72±3.84	5.64±3.32	4.60±2.82	3.72±2.47	**0.046***

* p <0.05 is significant

Table 7. p values of DMFT index in the inter-groups comparison

	DMFT
PCOSID(+) - PCOSID(-)	0.929
PCOSID(+) - CONTROLID(+)	0.214
PCOSID(+) - CONTROLID(-)	**0.028***
PCOSID(-) - CONTROLID(+)	0.248
PCOSID(-) - CONTROLID(-)	**0.034***
CONTROLID(+) - CONTROLID(-)	0.328

* p <0.05 is significant

Table 8. DMFT index correlation values (r) between S.mutans and saliva parameters values among the groups

	DMFT							
	PCOSID(+)		PCOSID(-)		ControlID(+)		ControlID(-)	
	p	r	p	r	p	r	p	r
S. MUTANS	**0.001***	0.798	0.077	0.360	0.539	0.129	0.562	0.122
STİMULATED SALIVARY FLOW RATE	0.194	-0.269	0.382	-0.183	0.770	-0.062	0.748	-0.068
UNSTİMULATED SALIVARY FLOW RATE	**0.025***	-0.448	0.267	-0.231	0.849	-0.040	0.811	-0.050
PH	0.983	-0.004	0.483	-0.147	0.879	-0.032	0.968	-0.009

* p <0.05 is significant, r> 0.6 is high.

Table 9. S.mutans correlation values (r) between DMFT index and saliva parameters values among the groups

	S. MUTANS							
	PCOSID(+)		PCOSID(-)		ControlID(+)		ControlID(-)	
	p	r	p	r	p	r	p	r
DMFT	**0.001***	0.798	0.077	0.360	0.539	0.129	0.562	0.122
STIMULATED SALIVARY FLOW RATE	0.302	-0.215	0.090	-0.246	0.165	-0.644	0.239	-0.510
UNSTİMULATED SALIVARY FLOW RATE	**0.021***	-0.255	**0.015***	-0.481	**0.002***	-0.578	**0.026***	-0.244
PH	0.897	-0.027	0.317	-0.209	0.600	-0.110	0.575	-0.118

* p <0.05 is significant, r> 0.6 is high

No statistically significant relationship was found between the stimulated saliva flow rate and the S. Mutans count in saliva in all groups (p>0.05).

No statistically significant relationship was found between saliva pH value and the S. Mutans count in saliva in all groups (p>0.05).

The correlation analysis of the S. Mutans count in saliva and the DMFT and saliva parameters is shown in Table 9.

Discussion

Symptoms of a large number of systemic, bacterial, viral and genetic diseases emerge primarily in the mouth. With the diagnosis of oral symptoms that develop due to these diseases, severe complications that may occur in the future will be prevented.

One of the most common reasons why patients with PCOS apply to the clinic is menstrual irregularity, and most patients present with oligomenorrhea or amenorrhea.[2] In this patient group, GnRH sensitivity decreases against the negative feedback effect of estradiol and progesterone, and the increasing GnRH release frequency results in increased LH in particular. These changes in the central gonadotropin dynamics observed in PCOS can occur primarily or secondarily (due to peripheral hormonal disorders). [11] In PCOS, an increase in serum LH level and, thus, serum LH/FSH ratio is observed due to the disorder in the hypothalamus-pituitary-ovarian axis. Although the LH/FSH ratio is usually high in PCOS patients by approximately 60-70%, a LH/FSH ratio of > 2 helps in the diagnosis of PCOS.[12] Serum FSH, LH and LH/FSH and estrogen levels of our study groups were standardized.

Insulin resistance occurs in women with PCOS by 50-80%. The presence of insulin resistance in PCOS can cause both metabolic disorders and reproductive disorders. The process that starts at an early age with insulin resistance predisposes to several diseases such as cardiovascular diseases, dyslipidemia, hypertension, obesity and Type II DM.[13] It is important to diagnose insulin resistance, which is common in women with PCOS, at an early stage to control possible metabolic disorders.[14] In the literature, the HOMA-IR method is used to assess insulin resistance, and any value that is higher than 2.5 is associated with insulin resistance.[15] In this context, our study groups are divided into subgroups and standardized based on the presence of insulin resistance.

The primary cariogenic bacteria that cause tooth decay is S. Mutans, which secretes lactic acid and produces intense extracellular polysaccharides. [16] Although it is very difficult to determine the level of S. Mutans in dental plaques, the level of S. Mutans in saliva gives an idea about the level of microorganisms in the plaque. A proportional increase is thought to exist between the S. Mutans count in saliva and plaque and tooth decay.[17] For this reason, various studies utilize stimulated saliva sample to determine the S. Mutans level. [18,19] Several methods have been developed to determine the level of S. Mutans in saliva. Non-practical methods such as microbiological culture and other methods with high contamination risk are used in the detection of S. Mutans.[20] S. Mutans kits that provide practical and quick results are also available on the market.[21] The real-time PCR method, which has been used frequently in recent years, is an accurate and precise method for detecting S. Mutans. Studies conducted on S. Mutans demonstrate that real-time PCR method gives more specific and accurate results than other methods.[22] In our study, real-time PCR technique was used to ensure accurate and precise S. Mutans count in stimulated saliva samples.

Although it is effective on the amount of saliva, risk of caries and caries activity, reduced saliva flow rate adversely affects oral health.[23] Several studies on saliva functions report that stimulated saliva is used more advantageously than unstimulated saliva. This is because the stimulated saliva sample is more resistant to daily pH changes.[24]

No consensus was reached in various studies evaluating patients in terms of saliva flow rate in diabetes, which is one of the systemic diseases.[25,26] Malicka *et al.*[27] found that the

unstimulated saliva flow rate was lower in patients with Type II DM than in the control group. On the other hand, in their study in which stimulated saliva flow rates are examined, Boyce *et al.*[28] report that patients with Type II DM have a lower flow rate than the control group; however, statistically significant results could not be obtained. These conflicting results may result from the differences in age, treatment protocols and medications used.[29]

In our study, the highest unstimulated saliva flow rate value was observed in the ControlID (+) group and the lowest value was observed in the PCOSID (+) group; however, no statistically significant difference was found between all groups ($p > 0.05$). In all groups, a significant correlation was found between unstimulated saliva flow rate values and S. Mutans values in saliva ($p<0.05$).

In our study, the highest stimulated saliva flow rate value was observed in the ControlID (+) group and the lowest value was observed in the PCOSID (+) group, however, no significant difference was found between the groups ($p>0.05$). In all groups, there was no significant relationship between stimulated saliva flow rate values and S. Mutans values in saliva and the DMFT index values ($p>0.05$).

Chronically high levels of unmet free estrogen are present due to hypothalamic-pituitary dysfunction observed in patients with PCOS. Estrogen is effective in maintaining the integrity of the oral cavity as well as regulating reproductive functions.[30] High estrogen levels in patients with PCOS affect the vascular permeability of the oral mucosa, which reduces the immune competence of the oral cavity.[31] Zhang *et al.*[32] found that estrogen was present in the cells in the salivary gland ducts and suggested that the salivary glands were one of the estrogen target organs. Another study reported that serum estrogen level and saliva flow rate decreased during ovulation and increased during menstruation. In addition, unstimulated and stimulated saliva flow rates were found to be lower in women compared to men.[33] This suggests that estrogen plays a crucial role in suppressing the saliva flow rate.[34] Findings about the saliva flow rate can be explained this way.

There are studies in the literature evaluating the relationship between various systemic and autoimmune diseases and saliva pH.[35,36] Mumcu *et al.*[37] reported in their study on patients with Behcet's disease that saliva pH was lower compared to the control group. Additionally, Loyola *et al.*[38] reported in their study on Systemic Lupus Erythematosus (SLE) patients that the stimulated saliva flow rate and saliva pH values were lower than the control group, which caused an increase in the DMFT index values.

In our study, the highest mean pH value of saliva was observed in the ControlID (-) group and the lowest value was observed in the PCOSID (+) group ($p<0.05$). These results provided similar results with the DMFT index values and S. Mutans values. Similarly, no significant relationship was observed between saliva pH and the DMFT index values and S. Mutans values in all PCOS and Control groups ($p>0.05$).

Increased estrogen levels in patients with PCOS have an inhibitory effect on parathyroid hormone (PTH) and cause calcium (Ca^{+2}) retention in salivary glands. Thus, any increase in the estrogen level^{+2} results in decreased concentration.[31] Decrease in Ca^{+2} in saliva results in decreased saliva pH and increased incidence of caries.[39] Likewise, the oral environment becomes more acidic as a result of the decreasing pH level of saliva and the level of S. Mutans, one of the cariogenic microorganisms in saliva, increases.[40]

When it is considered that the dental plaque is the primary factor of periodontal diseases, it is important to evaluate periodontal diseases for preventive dentistry.[41] Although few studies are available on the periodontal health of individuals with PCOS[42,43], there is no study in the literature on the effect of PCOS on oral microflora and its relationship with tooth decay, except the study conducted by Surmelioglu *et al.*[44].

In the literature, several studies focus on the relationship of S. Mutans with a majority of systemic diseases.[45,46] Siudikiene *et al.*[45] report that the number of S. Mutans in saliva was higher and tooth decay was more common in diabetic patients. Moreover, the S. mutans count is closely related to saliva pH, saliva flow rate and the DMFT index values.[46]

In our study, the highest saliva S. Mutans value was found in the PCOSID (+) group and the lowest value in the ControlID (-) group ($p<0.05$).A positive and significant correlation was observed between the number of S. Mutans in saliva and the DMFT index values in the PCOSID (+) group ($p<0.05$). In all groups, a negative and significant correlation was found between the number of S. Mutans in saliva and unstimulated saliva flow rate values ($p<0.05$). These results should be interpreted within the DMFT index values and saliva flow rate values.

The insulin sensitivity of tissues varies in patients with insulin resistance. In insulin resistance, glucose breakdown primarily decreases in the muscle and postprandial hyperglycemia occurs. Later, glucose output from the liver increases with the effect of insulin resistance. Thus, fasting hyperglycemia and all-day hyperglycemia begin to emerge.[47] In these cases, the presence of glucose in the oral cavity increases with hyperglycemia and thus, the amount of acid-producing bacteria increases, pH decreases and hyposalivation occurs.[40] Low saliva flow rate causes changes in the oral microbiota. This change results in a higher number of S. Mutans in the oral cavity.[48] In our study, the effect of insulin resistance on oral flora can be thus explained.

An increase in vascularization and inflammatory reactions are observed with estrogen receptors in the gingiva in patients with PCOS due to the increased level of estrogen.[31] Due to the increased level of estrogen, the oral microflora becomes more acidic, the microorganism count in the saliva and the level of S. Mutans increases.[49] Studies suggest that irritability and depression occurring in women in the premenstrual period due to changes in the estrogen and progesterone levels may be reflected in saliva components.[50] Depression reduces saliva secretion, while

positive mood increases the saliva flow rate.[51] An increase in the S. Mutans count is detected in the oral microflora when saliva flow rate decreases.[48] In our study, the effect of PCOS on the oral flora can be explained based on these reasons.

Although general inter-oral parameters do not give any clear result, more accurate results can be achieved by increasing the number of patients. These results showed that insulin resistance alone did not have any effect on S. mutans values in the oral flora. PCOS, a multi-factorial syndrome, is found to significantly increase the level of S. Mutans regardless of interoral factors.

The large number of S. Mutans in the oral microflora paves the way for the formation of caries.[45] In the literature, a large number of studies revealing the relationship between systemic and autoimmune diseases and the DMFT index value can be found.[36,52] Erdem *et al.*[53] report that patients with Behcet's disease had higher DMFT index values than the control group. Glodny *et al.*[54] report in their study on patients with metabolic syndrome that the patients have a higher number of caries than the control group. This may result both from dental caries and the pathophysiological mechanism of Metabolic Syndrome associated with chronic low-grade inflammation and from insulin resistance.

In our study, the highest DMFT index value was observed in the PCOSID (+) group and the lowest in the ControlID (-) group ($p<0.05$).

Previous studies report that the DMFT index is associated with insulin resistance.[55] The presence of glucose in the oral cavity increases with hyperglycemia caused by insulin resistance, and thus, the amount of acid-producing bacteria increases, pH decreases and hyposalivation occurs.[40] Studies report that a relationship exists between hyperglycemia and the DMFT index.[56,57] An increase in the incidence of caries is observed as hyperglycemia increases the risk of hyposalivation.[39] Nevertheless, the mechanisms that give these results are not clear and various theories have been put forward. These include reduced mineralization of enamel and predisposition to tooth decay due to the co-existing hyperglycemia and hyposalivation, decreased salivary gland function due to the resulting inflammatory response and changes in the neuroendocrine response of the salivary glands due to the immune reaction (Allushi, Bagavant, Papinska, & Deshmukh, 2019). Findings related to insulin resistance from our study results can be thus explained.

The high DMFT index values in patients with PCOS are closely associated with the saliva pH, saliva flow rate and S. Mutans values.[58] When we evaluate our results in general, the data obtained from all groups varied. Nonetheless, PCOS significantly increased the DMFT index values, regardless of inter-oral factors.

There were some limitations in our study. Since the patient groups in the study comprised of women in their reproductive period, their effects on saliva parameters and caries lesions in women with PCOS in older age groups are unclear.

Conclusion

When all results were analyzed, the salivary S. Mutans counts and the DMFT index values of the PCOS groups were higher than the control groups. Hyperglycemia caused by insulin resistance and estrogen levels increased with PCOS syndrome are thought to be primarily effective in obtaining these data from our study. The oral health data obtained in this study we conducted on PCOS patients, which are common in our society, are the initial data in the literature. We think that the data obtained from our study will serve as a reference to future studies, and thus, contribute to the literature.

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