Saliva Analysis and DMFT Indexes in Patients with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Hastalarda Tükürük Analizi ve DMFT İndeksleri

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ABSTRACT

Objective: To determine the relationship of various salivary analysis parameters and the DMF-T index in polycystic ovary syndrome (PCOS).

Material and Methods: Saliva samples were obtained from 20 patients with PCOS and 20 samples from healthy individuals. The pH, flow rate, viscosity of saliva and the level of Streptococcus mutans (St. mutans) were evaluated. The effects of PCOS on these features of saliva and the DMF-T index were observed and recorded.

Results: The viscosity of saliva and DMF-T values were significantly higher for patients with PCOS compared to the control group ($p<0.05$). The resting saliva flow rate, pH and St. mutans levels were not significantly higher than in the control group ($p>0.05$). In the PCOS group, a significant correlation was found between the DMF-T index values and the St. mutans and resting saliva flow rate/pH values ($p<0.05$).

Conclusion: The results of the present study indicate that PCOS increased the risk of dental caries and that this syndrome has adverse effects on saliva.

Key Words: Polycystic ovary syndrome, Salivary, pH, St. Mutans

ÖZ

Amaç: Polikistik over sendromlu (PKOS) hastaların çeşitli parametreler dahilinde yapılan tükürük analizlerinin ve DMF-T indeks değerlerinin bu hastalıkla ilişkisini saptamaktır.

Gereç ve Yöntemler: PKOS tanısı konulmuş 20 hastadan ve sağlıklı 20 bireyden tükürük örnekleri alındı. Tükürük pH'sı, tükürük akış hızı, kıvamı, tükürükteki Streptokokus Mutans (St. Mutans) varlığı aynı zamanda dişlerdeki DMF-T indeksleri değerlendirildi. Elde edilen sonuçların PKOS ile ilişkisi araştırıldı.

Bulgular: PKOS'lu hasta grubunun DMF-T değerleri ve tükürük viskozitesi kontrol grubuna göre anlamlı olarak yüksekti ($p<0.05$). PKOS'lu hasta grubunu istirahat tükürük akış hızı, pH'sı ve St. Mutans seviyeleri kontrol grubuna göre anlamlı olarak yüksek bulunamadı ($p>0.05$). PKOS grubunda DMF-T indeks değerleri ile St. Mutans, istirahat tükürük akış hızı ve pH'sı arasında anlamlı ilişki bulundu ($p<0.05$).

Sonuç: Yaptığımız çalışmadan edinilen bulgular sonucunda PKOS'un diş çürüğü riskini artırdığı, aynı zamanda tükürük üzerine olumsuz etkilerinin olduğu ortaya konulmuştur.

Anahtar Sözcükler: Polikistik Over Sendromu, Tükürük, pH, St. Mutans

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a female endocrine disorder diagnosed during reproductive age (1). The three main findings related to PCOS include menstrual irregularities (oligo-amenorrhea) initiating at the peripubertal period, signs of hyperandrogenism (hirsutism, acne, seborrhea of the skin) and the appearance of polycystic ovaries on ultrasound (2). In the long term, this syndrome may cause significant diseases such as Type 2 diabetes and endometrial carcinoma (3). The insulin resistance in Type 2 diabetes may also be present in patients with PCOS but the pathogenesis related to this situation is not clear (1). The insulin resistance could be a result of the cellular insensitivity to insulin in both diseases (4). Insulin is not capable of fulfilling its function so blood glucose levels increase suddenly. The increased glucose level affects β cells in the pancreas. Salehi et al. (5) reported that type 2 diabetes is likely to be seen in 35-40% of women with PCOS (6). As diabetics are susceptible to oral infections, dental caries and tooth loss are common in this patient group (7).

The daily flow rate of an individual's saliva varies within a range of 800-1500 ml (8). The saliva flow rate is defined by measuring the secretion of saliva in unstimulated or stimulated conditions. Non-stimulated salivation is the accumulation of secretions without external stimulants (chewing and tasting) or pharmacological agents (9). The majority of unstimulated saliva is secreted by the submandibular and sublingual salivary glands. Although the unstimulated saliva flow rate varies from individual to individual and depending upon time and environmental conditions, 0.25-0.35 ml/min (mean 0.3 ml/min) is accepted as the at rest value (10). The saliva secreted following electrical, sensory and mechanical stimuli is called stimulated salivation. A large percentage of stimulated saliva is secreted by the parotid gland (11). Although there are many bacteria in the oral cavity and plaque that can produce acid, Streptococcus mutans (St. mutans) is the main pathogenic microorganism in the oral environment (12). St. mutans strongly encourages the initiation of caries (13).

In the 1920s, many indexes have been put forward for the evaluation of oral health status. The DMF-T index was first defined by Klein in the early 1930s. With this index, the amount and incidence of dental caries in individuals can be determined. DMF-T is a numerical expression of caries incidence and is used to calculate the number of caries (D), decay-related losses (M) and filled teeth (F) (14). Depending on whether the third molar teeth are included, DMF-T values vary between 0-28 and 0-32 (15).

So far, no studies have investigated the relationship between PCOS and tooth decay. The aim of this study was to evaluate level of St. mutans, the pH value of the saliva, flow rate of the saliva, consistency of the saliva, and the DMF-T index of the teeth in the patients with PCOS.

MATERIAL and METHODS

The study was carried out following the approval of the ethics committee of Gaziantep University (2018/382) at the clinic of Restorative Dentistry Department. Written consent was obtained from all participants. The research protocol was organized in accordance with "Helsinki Declaration". Twenty patients with polycystic ovary syndrome and 20 patients with no systemic disease who presented to our clinic were included in the study. Before the study, individuals were given the necessary information about the research, and written consent was obtained from the volunteering individuals. A general anamnesis was obtained from patients diagnosed with polycystic ovary syndrome. The inclusion criteria included regular referral to a dentist, brushing the teeth at least twice a day, absence of parafunctional habits such as grinding or clenching, and absence of any systemic disease and particularly those affecting saliva flow. PCOS patients were also free of type 2 diabetes (blood glucose levels were in the normal range). The exclusion criteria were nutritional habits increasing the risk of tooth decay such as excessive sugar intake and consumption of acidic drinks. None of the participants in either the PCOS or the control group presented as obese. The saliva analysis was initiated, by including the participants who were in compliance with the criteria mentioned above. The Saliva-Check Buffer kit (GC America, USA) was used for measuring the amount, pH, and viscosity of both resting and stimulated saliva. Saliva-Check Mutans (Gc Europe, Belgium) kit was used for detection of the St. mutans level.

Referral of the subjects for the research was arranged between 9:30 am to 10:30 am for standardization. All subjects were instructed not to eat or drink anything, not to brush their teeth and not to use any mouthwash for 1 hour prior to the appointment.

At the first step of analysis, the resting saliva was evaluated. For flow rate calculation, the patient's lower lip was folded out and slightly pressed against the labial mucosa with a small sterile gauze and the salivary flow was observed. If the time for accumulation of visible saliva in this region was less than 60 seconds, resting salivary flow rate was considered as normal. Otherwise (exceeding 60 seconds) was accepted as low resting saliva flow. For pH measurement, resting saliva was collected in cups and subsequently instilled onto strips by using pipettes. Depending on the color change of the strips, the pH of resting saliva was classified as healthy (green) or acidic (yellow or red).

In the second step, the viscosity of saliva in the oral cavity was visually examined. Watery clear saliva was accepted as

normal viscosity while frothy, bubbly saliva (or more dense) was considered as increased viscosity.

In the third step, participants were instructed to chew paraffin gum for 5 minutes and following the first 30 seconds of chewing, the saliva was collected every 15-20 seconds for a total of 5 minutes. If the collected saliva was below 3.5 ml, it was considered as very low, 3.5-5 ml was low, and over 5 ml was normal stimulated saliva flow rate. Then, the stimulated salivary pH was measured in the same manner as applied for resting saliva pH measurement.

In the fourth step, the St. mutans levels were evaluated. For this purpose a Saliva-Check Mutans (Gc Europe, Belgium) kit was used. The stimulated saliva obtained at the third stage was used for St. mutans evaluation. One drop of the reagent 1 in the kit was placed into the measuring cup and the reagent in the cup was stirred for 15 seconds. Next, four drops of reagent 2 were added. After a short time, when the saliva turned a greenish color. Three drops of the mixture were applied to the specimen window of the test carrier by using a pipette and evaluated after 15 minutes. The appearance of a thin red line in the test window (T window) means the St. mutans level is more than 500,000 CFU / ml (increased caries risk) while the opposite is also true.

Finally, oral examinations of the patients were performed. After the teeth were dried, examinations were performed using only a mouth mirror and a probe under the reflector light. The DMF-T index was used to determine the decayed, lost and filled teeth of each subject and the DMF-T value for each patient was recorded.

DMF-T indexes and saliva viscosity were completed by one examiner (NEY) and subsequently calibrated by the other one (DS). Calibration was performed on twenty patients by both examiners (DS, NEY) before the main study. The Kappa statistics value obtained for inter-examiner reliability was 0.78 while the analyzed reproducibility was 0.82 for all the variables analyzed which can be considered acceptable.

Statistical Analysis: A descriptive statistical analysis was performed to identify whether the resulting data is within the normal distribution. Since the measured variables did not have a normal distribution, the data was statistically analyzed with the Mann-Whitney U test and Kruskal-Wallis variance analysis, which are nonparametric tests. The correlation analysis was performed by Spearman Rho Correlation Test. A P value of <0.05 was accepted as the level of significance.

RESULTS

DMF-T values, saliva flow rate, saliva pH and St. mutans levels are represented in Table I. There was a significant difference in the mean DMF-T score between the PCOS and control groups. DMF-T values were significantly higher in the PCOS group than the control group (p=0.046). In the evaluation of the saliva flow rate, there was no significant difference between PCOS and control groups in both resting and stimulated saliva (p=0.052, p=0.057). The saliva viscosity of the PCOS group was significantly higher than the control group (p=0.024). In the PCOS group, the resting saliva pH values were not significantly different from the control group (p=0.059). The stimulated salivary pH values were also similar for both groups (p=1). For the evaluation of St. mutans, the PCOS group was not significantly different from the control group (p=0.056).

In the correlation analysis of the study group, a significant relation was found between the DMF-T and St. mutans levels (Table II).

There was a negative correlation between the DMF-T index values and both the resting saliva flow rate and pH for PCOS group, while for the control group no correlation was present between DMF-T and pH (PCOS; p = 0.032,

Table I: The mean DMF-T index, Resting flow rate, Stimulated flow rate, Viscosity of saliva, pH of resting saliva, pH of stimulated saliva, St. mutans levels of the PCOS and control groups.

	PCOS Group (n=20)		Control Group (n=20)		
	mean±sd	**Min-Max**	**mean±sd**	**Min-Max**	**p**
DMF-T	5.85±2.94	2-12	3.95±2.21	1-10	0.046*
Resting saliva flow rate	0.5±0.5	0-1	0.8±0.41	0-1	0.052
Stimulated saliva flow rate	1.25±0.78	0-2	1.7±0.47	1-2	0.057
Viscosity of saliva	0.55±0.51	0-1	0.2±0.41	0-1	0.024*
pH of resting saliva	0.5±0.51	0-1	0.8±0.41	0-1	0.059
pH of stimulated saliva	0.9±0.32	0-1	0.9±0.35	0-1	1
St. mutans	0.55±0.5	0-1	0.25±0.44	0-1	0.056

DMF-T: decayed,missing and filled permanent teeth. **St.Mutans:** Streptococcus mutans
* Mean values are significantly different.

Table II: DMF-T correlation values in both groups.

	PCOS Group (n=20)		Control Group (n=20)	
	Correlation	**p**	**Correlation**	**p**
Resting saliva flow rate	-0.481	0.032*	-0.662	0.001*
Stimulated saliva flow rate	-0.876	0.001*	-0.443	0.059
Viscosity of saliva	0.474	0.035*	0.448	0.47
pH of resting saliva	-0.533	0.016*	-0.397	0.083
pH of stimulated saliva	-0.378	0.1	-0.221	0.35
St. Mutans	0.851	0.0*	0.481	0.032*

***** either positive or negative correlation is present

p=0.001, control; p=0.016, p=0.083). There was a positive correlation between DMF-T and St. mutans levels for both groups (PCOS; p = 0, control; p=0.032). There was a positive correlation between DMF-T values and saliva viscosity while a negative correlation was present between DMF-T and stimulated saliva flow rate values in the PCOS group (p=0.035, p=0.001) (Table II).

DISCUSSION

PCOS is a progressive syndrome that may lead towards insulin resistance, obesity and type 2 diabetes in the long term. Any patient with PCOS may present with one or all of these stages. The main complication related to PCOS is insulin resistance (16). Insulin is produced by beta cells in the pancreas and activates the absorption of glucose into the cells of the human body. If a cellular resistance to insulin develops, more insulin is required to keep blood glucose levels constant, and subsequently hyperinsulinemia occurs, as the pancreas increases insulin production. Insulin resistance is diagnosed in 50-70% of patients with PCOS, regardless of obesity or type 2 diabetes (17). The etiology of insulin resistance is still unknown and its effects on oral health are still unclear. To the best of the researchers' knowledge, no previous study has evaluated the relation of insulin resistance and oral health status. This study's findings suggest that DMF-T values are significantly increased in PCOS patients, compared to control subjects. This may be related to the increase of the viscosity of saliva. However, any possible correlation of insulin resistance with the oral health status should be further studied. Furthermore, the composition of saliva may be changed in patients with PCOS, and thus DMF-T may increase. This issue should also be clarified with further studies.

Obesity and type 2 diabetes are other significant conditions related with PCOS and may also induce gingival disease, tooth decay and related tooth loss, bad breath, hyposalivation and related xerostomy; bacterial, viral and fungal infections; wound healing delay, and increase in caries, gingivitis, periodontitis and periapical abscess (18-21). Due to being newly diagnosed, none of the participants of the present study were overweight or diabetic. However, DMF-T scores were significantly higher for PCOS patients. It can be assumed that with the progression of PCOS and the subsequent development of obesity and type 2 diabetes, the oral health status of these patients may become worse. In fact, Emekli et al. (10) reported that the content, flow rate and quantity of saliva may change and accelerate caries formation. However, in the present study, only the viscosity of PCOS patients was significantly different from the control individuals (22).

At the same time, there is a higher rate of depression in PCOS patients (28-64%) than in women without PCOS (7.1-8%) (24). Although the prevalence of anxiety was 18% in the general population, it was reported to be 34-57% in PCOS patients. Garad et al. (23) reported that the incidence of eating disorders was 21% for PCOS patients and they concluded that these disorders were consequences of PCOS. Following eating disorders, anxiety, depression, obesity, low self-esteem and body image disorders are triggered. In the advanced stages of psychiatric disorders, changes in the quality and amount of saliva, change in oral flora, endocrine dysfunction and decreased resistance to infections occur and these conditions increase the prevalence of periodontal disease (24).

Although PCOS is associated with many diseases, there are no studies dealing with its effects on oral health in the literature. In this current study, the stimulated and unstimulated salivary flow rate values of PCOS women were compared with the values obtained from the control group, and also dental caries, caries-related loss and filled teeth were detected due to St. mutans levels and the salivary pH values, so the relationship between DMF-T values was investigated.

A statistically significant relationship was found between the low saliva flow rate, pH and high DMF-T values in both groups. Also a negative correlation was found between salivary viscosity and DMF-T values in PCOS group. A high DMF-T may be explained by the low saliva flow rate (25).

In conclusion, PCOS patients should be informed and guided by not only by gynecology and endocrinology physicians, but also dentists in terms of oral and dental health. The results obtained from this clinical study aim to explain the cause-effect relationship between PCOS and dental caries; due to the multifactorial etiology of polycystic ovary syndrome, this relationship should be examined in more detail and with extended patient groups. Further studies are required to determine buffering capacity, pH, microbiology, antibacterial components, the effects of different physicochemical properties, levels of calcium and other ions, on dental caries in individuals with PCOS. Obtaining data regarding DMF-T indexes and the treatment demands of PCOS patients will increase their oral and dental health status, and will help in the development of preventive and curative policies.

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