Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.2023.10
Vol. 30, No. 1, 2023, 58-63

Short Communication

The Effect of Controlled Diabetic and Non-diabetic Saliva on *Candida albicans* Adherence to Heat Polymerized Acrylic Resin Discs

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Abstract

Background: Oral candidiasis is one of the most common infections in diabetic patients that may occur due to a decrease in salivary flow rate, alterations in the salivary composition, or both. This study aimed to investigate the effect of diabetic and non-diabetic saliva on *Candida albicans* adherence to the acrylic resin disc specimens, regardless of saliva volume.

Methods: In this case-control study, the population consisted of 26 subjects in 2 groups (13 diabetic patients and 13 non-diabetic patients). In both groups, unstimulated whole saliva was collected. It was investigated for pH, salivary flow rate, and adhesion of *C. albicans* to polymethylmethacrylate disc. After preparing the polymethylmethacrylate discs, the samples were stored in diabetic saliva, non-diabetic saliva, and distilled water (negative control group) for 60 minutes at 37ºC. Then they were immersed in the yeast suspensions containing *C. albicans* and stained with gram stain. Yeast cells were counted using a light microscope.

Results: The pH of unstimulated whole saliva in diabetic patients was significantly lower than in the control group. There was no significant difference between the flow rates of saliva in the two groups. Findings indicated that the adhesion of *C. albicans* to resin in diabetic saliva was higher than in non-diabetic saliva.

Conclusion: This study showed the same amount of salivary flow rate in diabetic and non-diabetic individuals, lower pH, and higher *Candida* adherence to heat polymerization acrylic resin in the diabetic group.

Keywords: *Candida albicans*, Saliva, Diabetes mellitus, Acrylic resins

Citation: Hajisadeghi S, Fateh R, Jangjoo A, Behrouzirad A. The effect of controlled diabetic and non-diabetic saliva on *candida albicans* adherence to heat polymerized acrylic resin discs. *Journal of Kerman University of Medical Sciences*. 2023;30(1):58-63. doi:10.34172/jkmu.2023.10

Received: April 2, 2022, **Accepted**: September 25, 2022, **ePublished**: February 21, 2023

Introduction

Diabetes mellitus is a metabolic disorder described by abnormally high levels of blood glucose and irregular metabolism of carbohydrates, lipids, and proteins (1). The prevalence of diabetes in the world is constantly increasing (2). Complications of diabetes include cardiovascular disorders, nephropathy, retinopathy, peripheral nerve abnormalities, and oral diseases such as gingivitis, periodontitis, and biofilm-induced oral lesions (3,4). Denture biofilm is a dense microbial layer containing microorganisms. Previous studies have shown an association between biofilm and oral candidiasis, in particular denture stomatitis. These lesions occur more frequently and severely in people with weakened immune systems including diabetic patients (5). About 30% and 58% of oral candidiasis and denture stomatitis cases have been reported due to biofilm infections, respectively. Diabetic patients often use medications such as diuretic and antihypertensive drugs, which might reduce the salivary flow rate and thus facilitate biofilm accumulation. Under these conditions, the biofilm matures and facilitates the development of various diseases (4). Several studies have stated a higher prevalence of *Candida* species in the oral cavity of diabetic patients compared to non-diabetic individuals (6,7). The use of dentures in edentulous patients increases the risk of candidiasis in both diabetic and non-diabetic individuals (8). Acrylic resin is the most common material used in denture fabrication, and the

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adhesions of *Candida* to the oral mucosa and dentures are responsible for candidal stomatitis (9).

Edentulism and denture use is more common in diabetic patients (10). Denture stomatitis is an inflammatory condition of mucosa that occurs in the area under partial or complete removable dentures with multifactorial etiology (11). Among the various etiological and predisposing factors, *Candida* species seem to play an important role in the initiation and development of this infection, and *Candida albicans* is the most common microorganism in the biofilm of denture stomatitis (4,12). Also, the most important systemic factor associated with candidiasis is diabetes mellitus. Although the precise mechanisms of increased *Candida* infection in diabetics have not yet been elucidated, high levels of salivary glucose, low salivary pH, and reduced salivary flow rate are assumed to be some of these mechanisms (11).

Saliva plays an important role in the immunity of the oral mucosa and in maintaining the balance of the flora of the oral cavity (13). Therefore, we expect more candidiasis in diabetics due to hyposalivation and reduction of the washing effect. But in addition to changes in saliva volume, there may be changes in the composition or chemistry of saliva. On the other hand, due to increased life expectancy, the number of older patients requesting dentures is increasing. So, we will encounter diabetic patients with denture stomatitis. The initial assumption is that this condition is caused by decreasing the washing effect, due to reducing the volume of saliva (14,15). However, some studies have shown that salivary compounds in diabetics are different from non-diabetic individuals (16,17), and the effect of these factors on *Candida* growth and adherence can be studied. Therefore, it was decided to remove the salivary volume factor, by transferring saliva to the laboratory environment, to investigate the effect of saliva composition on *Candida* adhesion. Concerning this aspect, this in-vitro study aimed to compare the effect of two diabetic and non-diabetic salvias on the adhesion of *C. albicans* to polymethylmethacrylate discs, regardless of saliva volume. If the adhesion of *C. albicans* to the acrylic surface is higher in diabetic saliva, it makes sense to compare the two salivas in terms of chemical properties and factors affecting the growth and adhesion of *Candida*.

Materials and Methods

Study population

In this analytical case-control study, 26 human subjects were evaluated. The case group consisted of 13 diabetic patients (six males and seven females) with an age range of 30-63 years (the average age was 51) and the control group consisted of 13 non-diabetic patients (seven males and six females) with an age range of 23-57 years (the average age was 33.5). All patients were randomly selected among those referred to Shahid Beheshti Hospital Laboratory at Qom University of Medical Sciences. The inclusion criteria for the case group included having controlled diabetes (confirmed by laboratory tests and an internal medicine physician), having no systemic disease other than diabetes, and not taking medications other than blood sugar control drugs for the last three months. The control group consisted of non-diabetic individuals without systemic diseases or medications. Exclusion criteria for both groups were smoking, alcohol use, pregnancy, history of radiation therapy, salivary gland disease, and use of dentures.

Saliva collection

Saliva samples were collected in both groups. Both groups were asked not to eat any food or drink for eight hours before saliva collection. Unstimulated whole saliva was collected between eight and ten in the morning by spitting method, so participants were asked to accumulate their saliva on the floor of the mouth for five minutes and spit it out into a calibrated test tube every 60 seconds (18).

Measurement of salivary flow rate and pH

The saliva volume was measured by graded tubes, and the salivary flow rate was expressed in mL/5 min (18). The pH of the saliva was measured by a digital pH meter (BOECO, Germany). Then all tubes were placed in ice and immediately transferred to the Cellular and Molecular Research Center of Qom University of Medical Sciences. Saliva samples were centrifuged (Hettich, Germany) at 5000 rpm for five minutes to remove any impurities and contaminants present in the saliva. To ensure the removal of *Candida* from salivary samples, after centrifugation of salivary samples and sterilization using a membrane filter with 0.22-µm pore size, their supernatant was transferred to the microtubule and cultured by sterile swap sample of saliva in Sabouraud dextrose agar culture medium (Merck, Germany) and the plate was kept in the incubator for 72 hours, then the microtubules containing saliva was frozen at -70°C for subsequent fungal experiments. After 72 hours, the plates were examined for colony presence or absence, and the samples that formed the colony were excluded from the study, which included one diabetic saliva (19,20).

Acrylic resin discs preparation

Since the level of surface roughness is effective in *Candida* adhesion, attempts were made to produce disks with maximum similarity in terms of surface roughness (21). To prepare heat-cured acrylic disks, a mold with cylindrical cavities with a diameter of 10 mm and a height of 4 mm was made. According to the manufacturer's instructions (Acropars, Iran), acrylic resins were prepared and then packed inside the mold (Figure 1). At least 3 hours after the completion of polymerization, the acrylic discs were polished with a special sequence of burs

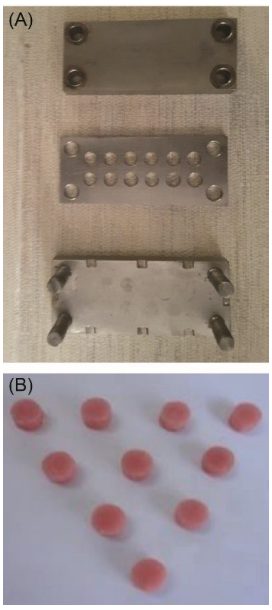

Figure 1. A. Mold; B. Acrylic resin discs

and stones by a single technician, with controlled hand pressure, to obtain almost the same polished surface in all acrylic discs. Then Acrylic resin samples were kept in sterile distilled water at 37°C for 1 week to remove any residual monomer. Immediately before the fungal study, the samples were sterilized in an autoclave for 18 minutes at 15 Psi, 121°C (Pars Mehr, Iran) and immersed in distilled water at 37°C for 24 hours. Then, the resin discs were immersed in diabetic and non-diabetic saliva, and distilled water (as a negative control group) for 60 minutes. After one hour, the resin samples were rinsed with sterile distilled water, then incubated for 24 hours at 37°C (22).

Preparation of suspension of yeast cells

The standard *Candida albicans* strain (ATCC 14053) was used. After culturing *Candida* on Sabouraud-dextrose agar plates (Merck, Germany), the plates were incubated at 37°C for 24 hours to allow the fungi to grow and form isolated colonies. Colonies were suspended in sterile normal saline to obtain turbidity equivalent to 0.5 McFarland standard. Then, the yeast suspension was mixed with 2 mL of Sabouraud Dextrose broth medium (Merck, Germany). Afterward, the resins were immersed in the suspension and transferred to the incubator at 37°C for 3 hours (12,22).

Examination of adhesion of candidate cells to acrylic resin discs

After 3 hours of immersion of the discs inside the suspension in the incubator, they were removed and gently washed with sterile distilled water to eliminate non-adherent cells. After the washing procedure, the specimens were gram-stained and after drying, adherent yeast cells were measured using × 40 magnification under a light microscope. Adherent yeast cells were counted in 10 different fields from each specimen and an average of ten fields were reported as adherent yeast cells for each specimen (22).

Statistical analysis

Statistical analysis of the obtained data was done by SPSS software version 24. Data were summarized using descriptive statistics including mean and standard deviation and median value and the interquartile range was in the form of 25th and 75th percentiles (LQ; UQ). The Shapiro-Wilk test was used to evaluate the normality of data distribution. Independent samples *t* test was used for the comparison of age, salivary flow rate, and *Candida* adhesion amounts between the two groups due to the normality of the data. Mann-Whitney test was used to compare the pH value between the two groups due to the non-normality of the data. A comparison of the groups' gender distribution was done using chi-square test. The significance level was considered 0.05 for all tests.

Results

The results are shown in Table l. In this study, the sample size was 26 persons, 13 in the control group (non-diabetic patients) and 13 in the case group (diabetic patients). In the control group there were seven males (53.8%) and six females (46.2%) and in the case group six males (42.6%) and seven females (53.8%). The chi-square test signed that there was no statistically significant difference between the two groups concerning gender. The age range in the control group was from 23 to 57 years old (mean ± SD = 33.53 ± 12.71) and in the case group was from 20 to 63 years old (mean ± SD = 51.00 ± 10.12). The result of the *t* test showed a significant difference between the two groups regarding age range.

The mean salivary flow rate in the control group was 1.88 ± 0.46 mL/5 min and in the case group was 1.55 ± 0.58 mL/5 min. The *t* test showed no significant difference between the two groups concerning the mean salivary flow rate. The median value of pH measured in the control group was 7.10 and in the case group was 6.50. Mann-Whitney test showed a significant difference between the two groups. The mean number of colonies that indicate the degree of *Candida* adherence to acrylic resin in the control group was 14.10 ± 4.74 and in the case group was 22.38 ± 7.34. T-test showed a significant difference between the two groups in terms of mean adhesion. The number of colonies counted in the distilled water (negative control group) that was set up for the experiment was 3.0. There is a significant difference between the two groups in terms of *Candida* adhesion and salivary pH.

Discussion

The present study aimed to compare the pH, salivary flow rate, and adhesion of *C. albicans* to acrylic resin

Table 1. The comparative evaluation of clinical characteristics, salivary flow rate and pH, and *Candida* adhesion to acrylic resin disk in case and control groups

Variables		Case group (Diabetic patients)	Control group (Non-diabetic patients)	*P* value
Age (y)	Mean ± SD	51.00 ± 10.12	33.53 ± 12.71	0.001 (*t* test)
	Age range	(20-63)	(23-57)	
Gender	Male (n)	6	7	0.69 (Chi-square)
	Female (n)	7	6	
Salivary flow rate (mL/5 min)	Mean ± SD	1.55 ± 0.58	1.88 ± 0.46	0.12 (*t* test)
Salivary pH	Median value Interquartile range (25Q; 75Q)	6.50 (7.05; 6.30)	7.10(7.25; 7.00)	0.03 (Mann-Whitney test)
Candida adhesion (Number of colonies)	Mean ± SD	22.38 ± 7.34	14.10 ± 4.74	0.005 (*t* test)

P value < 0.05 was considered significant.

in diabetic and non-diabetic individuals. According to some studies, because of the reduction of salivary gland function in the forms of diabetic neuropathy and the exertion of large quantities of water in the kidneys and dehydration, the salivary flow rate decreases in diabetic patients (15,23-26). However, studies on salivary gland dysfunction in diabetic patients are controversial. The results of our study showed that there was no significant difference between the salivary flow rate of diabetic and non-diabetic subjects, which confirms the results of previous studies (14,27,28). On the other hand, some studies have shown a significant reduction in salivary flow rate in diabetics compared to non-diabetic individuals (15,23-26). Such contradictions can be attributed to differences in the degree of metabolic control of disease, the technique of saliva collection, the type of saliva collected (stimulated or unstimulated), collection time, condition and position of patient at collection time, the stage of the disease, having other systemic diseases, and age and gender mismatch between study and control groups. The degree of disease control is an important factor in diabetes (24). In our subjects, diabetes was well controlled and proper blood glucose control probably prevented adverse effects on salivary gland volume in patients. In this study, saliva was collected through the standard spitting method (29). In addition, unstimulated saliva was examined in our research. In general, unstimulated saliva is a more appropriate source than stimulated saliva for measuring salivary parameters, since the function of the unstimulated salivary gland is the predominant state, stimulated saliva does not have the stability necessary for such studies, and stimulation with acids such as citric acid could affect the quantity, quality or even the pH of the saliva (24,30). Furthermore, subjects in the study group did not have any systemic disease other than diabetes. The control group also had no systemic disease. Regarding gender, there was no significant difference between the two groups, which increases the confidence in the study. But, in terms of age, the two groups had a significant difference and the mean age of the diabetic group was higher than the control group. Since with aging, autonomic and peripheral neuropathies and age-related vascular changes are more frequent, it is expected that the rate of salivary flow decreases (24), but in this study, although the mean age of the diabetic group was higher, there was no significant difference in flow rate of saliva between the two groups.

In the evaluation of the salivary pH of both groups, diabetic patients had lower salivary pH, which was confirmed by some studies that may be related to microbial activity (26,30,31). Furthermore, in hyperglycemia, salivary acidity increases due to impaired glucose metabolism and the production of large amounts of acetone and beta-hydroxybutyric acid (32).

The primary goal of this investigation was to compare the adhesion of *C. albicans* to acrylic resin in diabetic and non-diabetic saliva, regardless of saliva volume. Physical characteristics of the material surface such as surface free energy, porosity, roughness, and hydrophobicity are important in the adhesion of *Candida* to acrylic surfaces (33). However, when the denture is placed in the oral cavity, its surface is quickly covered with a thin film of saliva. Saliva converges the hydrophobicity of all surfaces of the mouth by eliminating the effect of surface roughness and altering surface free energy. The salivary pellicle decreases the difference in adhesion of *Candida* between the materials, so salivary components are important (22). Saliva is a complex fluid that plays an important role in the health of the oral cavity and provides a natural pH in the mouth. It has antimicrobial and antifungal properties due to innate immune factors (such as lysozyme, lactoferrin, peroxidase, agglutinin, and other immune factors) which prevent the adhesion of microorganisms to the surface of the mouth and the growth and metabolism of microbes (22,34).

In our study, we placed acrylic resins in laboratory environments with the same volume of saliva; therefore, the effect of the volume of saliva and the washing effect was eliminated. The results showed that the number of *C. albicans* colonies attached to the acrylic resin was significantly higher in the saliva of diabetic patients than in non-diabetic individuals. Also, in some studies, the prevalence rate of *Candida* colonization on the denture palatal surface was higher among diabetic patients (35).

A similar trend was seen in which *Candida* carriage was higher in diabetics who used dentures (36,37). Because these studies were in vivo, they attributed the higher rates of *Candida* in diabetics to various factors such as decreased salivary flow rate and pH, salivary buffer capacity, and poor glycemic control. But in this study, due to the equalization of saliva volume and elimination of the washing effect, the reason for the greater adhesion of *C. albicans* to acrylic resin in the saliva of diabetics was the difference in saliva composition between diabetics and non-diabetics. Furthermore, in in-vivo studies, patient-related factors such as oral hygiene, the amount of dietary carbohydrates, dentures conditions, trauma, duration of using dentures, age of dentures, poorly fitting dentures, and smoking habit may affect their salivary *Candida* amount (38-41). In our study, we eliminated these confounding factors by transferring saliva to the laboratory environment.

The effect of salivary components on the adhesion of *C. albicans* to oral surfaces may be very different, some of which, like mucin, may increase adhesion, while some other components, such as lactoferrin, lysozyme, lactoperoxidase, IgA, and histatin may inhibit it (22,42-44). In the present research, due to the difference in *Candida* adhesion to acrylic resin between diabetic and non-diabetic saliva, it seems that these salivary factors are different between diabetic and non-diabetic individuals.

Limitations

The main limitation of this study was the age difference between the two groups. Choosing age-matched controls is suggested for future studies.

Conclusion

This study showed that the salivary flow rate in diabetic and non-diabetic individuals was the same. Also, diabetic patients had lower salivary pH, and above all, the *Candida* adherence to heat polymerization acrylic resin disk is higher in the diabetic group. This phenomenon is probably attributed to the chemical properties of saliva. For later studies, it is recommended that the amount of these salivary components in diabetics and non-diabetic individuals be evaluated and compared.

Acknowledgments

The authors would like to thank Cellular and Molecular Research Center of Qom University of Medical Sciences, which assisted us in conducting this research, and all the patients who participated in the implementation of this project.

Author Contributions

SH: conception and design, revising, final approval; RF: revising, analyzing, and interpreting the data; AJ: revising, methodology; AB: writing, drafting, and data collection.

Conflict of Interests

The authors report no conflict of interest.

Ethical Approval

The present study was approved by the ethics committee of Qom University of Medical Sciences (Ethical Code: IR.MUQ.REC.1395.132) and written informed consent was obtained from all participants before enrolling in the study.

Funding

None.

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