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Original Article

The Relationship Between Salivary *Candida Albicans* Colony Count and Blood Group Antigens in Dentistry Students

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Background and Aims: *Candida albicans* is the most prevalent opportunistic fungal species in the oral cavity. To date, several studies have been investigated the various factors associated with oral candidiasis. On the other hand, it has been proven that blood types antigens lead to some infectious factors. This study aimed to evaluate *Candida albicans* colonies in the saliva of dentistry students based on their blood type to detect a relationship between blood group and incidence of oral candidiasis.

Materials and Methods: In this descriptive cross-sectional study, 200 dentistry students were selected by a simple sampling method, including 100 individuals with blood type O and 100 with other blood types. The unstimulated salivary samples of all the participants were collected by spitting, cultured on Sabouraud medium, and then the isolated *Candida albicans* colonies were enumerated and recorded.

Results: In the present study, samples comprised 77 males and 123 females, of whom 15.5% (31 individuals) carried colony-forming units > 40. The mean of *Candida albicans* colonies in the individuals' saliva with blood type O was 21.55, and it was 10.68 in the other groups. Besides, the differences were statistically significant ($p = 0.024$). There was no significant difference in *Candida albicans* colony count between O positive and O negative blood groups.

Conclusions: The result of this study showed a significant relationship between the number of *Candida albicans* colonies of saliva and the individual's blood type.

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Introduction

Oral candidiasis is the most prevalent human fungal and opportunistic infection, and most cases are produced by *Candida albicans*. Data collection from several reports has identified that the average prevalence of carriers is %17.7 in healthy individuals (range from 1.9 to 62.3%) [1]. Different threshold shaves have been considered for a level of *Candida* colonies number, which can cause the symptomatic lesion. In a study by Zhou, *Candida* colonies count more than 40 colony-forming units (CFU)/ml in the salvia, was regarded as a carrier [2]. Some predisposing factors that shift normal oral flora to candidiasis include salivary gland disorders, consumption of wide-spectrum antibiotics, immunosuppressive drugs, Sjogren's syndrome, acquired immune deficiency syndrome, denture wearers, smoking [3, 4], metabolic diseases like diabetes, hyper-parathyroidism [5], and dry mouth [6]. Blood type is a classification based on the presence of inherited antigenic substances on the surface of red blood cells, which may be proteins, carbohydrates, glycoproteins, or glycolipids [7]. Currently, among 20 blood group systems, ABO and Rh are the most important ones [8].

So far, the relationship of ABO blood types with various infections has been investigated in several studies. Furthermore, studies have investigated the association between blood type antigens and *Candida* colony count. Some studies have concluded a relationship between blood type O and the incidence of oral candidiasis [9]. The presence of blood-type antigens on the surface of mucous membrane cells may increase infection susceptibility because they act as receptors for pathogenic microorganisms [10]. However, several studies also showed the relationship between the genetic inability of blood type antigens secretion and infections susceptibility [11]. Khozimeh et al. carried out a study at Isfahan University. They concluded that *Candida* colony count in blood type O was more than other blood types [12]. A survey by Buford-Mason et al. also showed a strong relationship between *Candida albicans* carriage with blood type O [11]. The controversy in results of previous studies and ever-increasing candidiasis, especially in individuals with underlying disease, necessitates more studies to study the relation between candidiasis and blood groups. So, the present study was conducted to evaluate pertinence between *Candida albicans* colony counts in dentistry students' saliva at Shahid Sadoughi University of Medical Sciences based on their blood type.

Materials and Methods

The current study is a descriptive and analytic cross-sectional research. The samples were obtained from dentistry students whose blood types were determined. Samples were placed in one of two groups as O and non-O blood types because some studies have concluded a relationship between blood type O and incidence of oral candidiasis [9].

The consent form was completed according to the instructions of the ethics committee. This research has been accepted in the ethics

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committee of Shahid Sadoughi University of Medical Sciences with the number IR.SSU.REC.1395.4517.

The questionnaires were filled out about demographic information and individuals' blood type using their driving license. Participants were included was based on determining blood types, having no underlying systemic disease, malignancy or iron deficiency anemia, no use of the oral appliance, no candidiasis, xerostomia, and antibiotics or corticosteroid consumption during the last two weeks, and students did not use any mouthwash, tobacco or alcohol during 2 hours before sampling. Sampling was carried out after at least 2 hours of having breakfast or brushing teeth.

The individuals needed to avoid eating, drinking, and smoking at least 2 hours before sampling, and the collection was conducted during 9-11 am to minimize the saliva composition changes. Unstimulated saliva was collected by spitting, in which the individuals were asked to spit the whole saliva into sterile tubes for 2 minutes and twice each minute. The unstimulated saliva was collected under the abovementioned standard condition [13]. Then, samples were stored on ice, transferred as soon as possible to the laboratory, and cultured immediately.

A 0.1 ml of the saliva of each individual was cultured by micropipette with sterile disposable sampler tip on *Candida* chrome agar medium (prepared by Bio growth company) through a streaking design. Then, the medium was incubated at 30 °C for 48 hours. Special colors of colonies identified the type of *Candida* as *albicans* or non-*albicans Candida* species. *Candida albicans* were counted by colony counter and recorded in designed tables.

Statistical analysis

Data were analyzed using the T-test, Mann-Whitney U test, and chi-squared test using SPSS software, Version 16.

Results

In the current study, 200 participants were evaluated, including two groups of 100 individuals with O and non-O blood types. Of these, 77 were men, and 123 were women. The mean of their saliva *Candida albicans* colony counts in O and non-O groups were 21.55 and 10.68, respectively, and the difference between the two groups was statistically significant (p = 0.024). The results are shown in Table 1.

In this study, the total number of *Candida's* carriers with at least 40 colony count and non-carriers were 28 (14.1%) and 172 (85.9%), respectively. Likewise, the carriers in the blood type O were more than the non-O type, and the difference was statistically significant (p = 0.021) (Table 2).

In the O blood type group, we compared the two Rh groups. There was no significant difference in *Candida albicans* colony count between the O positive and O negative groups (p = 0.581) (Table 3).

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Table 1. The Mean of *Candida albicans* colony counts in O and non-O blood types

Blood group	Mean	Min	Max	SD
Non-O	10.68	0	127	24.24
O	21.559	0	400	40.91

P-value = 0.024

Table 2. The Frequency distribution of carrier and non-carrier in the two groups

Blood group	Non-O	O	Total	P-value
Carrier	8 (8.2%)	20 (19.6%)	28 (14.1%)	0.21
Non carrier	89 (91.8%)	83 (80.4%)	172 (85.9%)	
Total	97 (100%)	103 (100%)	200 (100%)	

Table 3. The mean of *Candida albicans* colony counts in the two Rh groups

Blood group	Rh	Number	Mean	Min	Max	SD
O	Positive	84	22.671	0	400	43.06
	Negative	19	17.00	0	95	31.09

P-value = 0.581

Discussion

In the current study, the number of *Candida albicans* colonies of saliva in O blood type was more than non-O, and the difference was statistically significant. Several studies have been carried out to check the association between blood type and *Candida* colony count. Shin et al. investigated 180 healthy individuals at Seoul University, indicating that ABO blood types and their secretion condition were not associated with the number of *Candida albicans* colonies, which does not agree with the current research. The above study reported that the H antigen, which is on the surface of epithelial cells of individuals with blood type O, acts as a receptor for *Candida albicans* colonization. The collected solution is concentrated and then cultured in the concentrated oral rinse (CRC) method. The research results showed that the accuracy of the CRC method in diagnosing the number of *Candida albicans* colonies was more than other methods and the whole saliva culture (WSC) accuracy was more than the neat oral rinse culture method [10]. In the current study, it was difficult for students to wash their mouths with phosphate buffer, and a higher sample number was found. Therefore, only the WSC method was used.

Khozimeh et al. carried out a study at Isfahan University, in which they checked out 300 participants, including 200 individuals referring to the oral medicine department and 100 students of dentistry, and they were divided into three groups of 100 individuals with A, B, and O blood types. In this study, saliva was collected by spitting technique, and the WSC method was used to culture it on the Sabouraud Dextrose agar medium. The result of the study showed that 43% of participants included in the study had

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Candida albicans fungus in their saliva. In their study, the candidiasis carrier was referred to individuals with even one *Candida albicans* colony on their culture medium. Furthermore, they concluded that *Candida* colony count in blood type O was more than other blood types, which was consistent with our study, but the difference was not statistically significant [12].

Abdollahzadeh et al. calculated the frequency of different blood groups in individuals with denture stomatitis. They showed that the prevalence of the lesion was higher in blood group O [14].

The survey by Buford-Mason et al. on 100 healthy individuals showed that 32% were *Candida* carriers. *Candida albicans* carriage had a strong relationship with blood type O and the non-secretion condition of blood types antigens. These results confirm those observed in our study. In this study, the saliva was collected by mouthwash method, and it seems that this method leads to loss of *Candida* carriers with fewer colony numbers. The result of the study demonstrated that non-secretion blood types and blood type O could be considered a risk factor for *Candida albicans* carriage [11].

Jain et al. investigated 210 individuals who needed to use dentures, of whom % 37.14 were oral *Candida* carriers. In Jain's study, sampling was conducted with a concentrated mouthwash method. According to the result, the number of oral *Candida* carriers was more among individuals with blood type O, and blood type O had more susceptibility to oral candidiasis, which seems to be consistent with our research [9].

Ben-Aryeh et al. surveyed 93 healthy individuals and showed that *Candida albicans* carriage is associated with the non-secretion condition of blood type. In this study, 66% of individuals were *Candida* carriers, but there was no definition of "*Candida* carriers." The study also indicated that the number of *Candia albicans* carriers in individuals with blood type O was more than A and B, confirming our study's result [15].

Everest-Dass et al., in their study, showed that *Candida albicans* had more significant interaction with buccal epithelial cells of individuals with blood type O than other types [16]. Nikawa et al., in their research, stated that individuals with O blood type were more prone to denture stomatitis, and this result is consistent with ours [17]. In the study carried out by Zhou et al., the *Candida albicans* carriage threshold was 40 CFU/ml; however, the different threshold was regarded as an initial point for *Candida* infection in various studies [2]. In the studies conducted by Epstein et al., the 400 CFU/ml and 200 CFU/ml threshold were considered respectively as the initial point of *Candida* infection [18]. In the current study, a similar threshold to Zhou's study was determined for carriers, which seems more logical because these microorganisms may grow in low-density mediums even in the range from 200 or 500 CFU/ml [13].

Our study and similar results likely arise from the special connections that link *Candida* to the host cells, and some types of hexoses and hexosamines have been identified as a receptor of *Candida albicans*. Additionally, carbohydrates in the saliva or blood fluid may cause the increase or decrease of *Candida* connection to the surface of host cells [9]. Carmon and Douglas found that the antigens are

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suitable places for *Candida albicans*. Of those, H antigens on individuals' epithelial cells with blood type O tend to join with the adhesion receptor of *Candida albicans* because L-fucose glycolipid is at the end of H antigen and is appropriate for the adhesion receptor of *Candida albicans* [19].

In the current study, *Candida albicans* colony count in Rh-positive individuals was Rh-negative but the difference was insignificant. In a study by Agbakoba et al., candidiasis in individuals with the O-positive blood group was more than in other blood groups. They believed that *Candida* species tends to D-mannose, L-fructose, and N-acetyl D-glucosamine in the Rh blood group [20].

Conclusion

This study showed a relation between *Candida albicans* colony count and blood type. Besides, *Candida* pathogenicity is basically related to the connection between *Candida* species and the host cells. Surface antigens like blood groups might have ruled in this area.

Conflict of Interest

The authors declare no conflict of interest.

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