

## Original Article

# A comparative study of *Candida albicans* mean colony counts and blood group antigens in the saliva of healthy subjects

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## ABSTRACT

**Background:** *Candida albicans* is the most common opportunistic fungal species in the oral cavity. Various factors associated with *C. albicans* infection have been evaluated so far. In some studies, the relationship between the blood group antigens and *C. albicans* has been discussed. The aim of this study was to assess mean *C. albicans* colony counts in the saliva of healthy subjects and its relationship with ABO blood groups.

**Materials and Methods:** This cross-sectional/analytical study was performed in the Oral Medicine Department, School of Dentistry, Isfahan University of Medical Sciences. Unstimulated whole saliva samples were obtained from 300 healthy subjects, including 100 individuals with blood group O, 100 with blood group A and 100 with blood group B. The samples were cultured on Sabouraud's dextrose agar media to determine the means of *C. albicans* colonies. Data were analyzed by Kruskal-Wallis and Mann-Whitney statistical tests and SPSS 16. Statistical significance was defined at  $P < 0.05$ .

**Results:** The samples included 156 males and 144 females with a mean age of 27.52 years. The mean colony counts in the saliva of individuals with blood groups O, A, and B were 26.4, 19.84, and 21.23, respectively. There were no significant differences between the three groups ( $P = 0.280$ ).

**Conclusion:** Although the mean *C. albicans* colony counts in individuals with blood group O were more than those with other blood groups, the differences were not statistically significant. More research studies are needed in order to prove the role of blood groups in susceptibility to candidiasis.

**Key Words:** ABO blood groups, blood group, *Candida albicans*, saliva

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## INTRODUCTION

Oral candidiasis is the most common opportunistic infection of the oral mucosa.<sup>[1]</sup> In most cases, the lesions are caused by the *Candida albicans* fungal species.<sup>[2]</sup> Previous studies have shown that the oral prevalence of this organism in healthy individuals is between 1.9% and 62.3%.<sup>[3]</sup> This wide variation in prevalence is due to differences in sampling

and laboratory procedures used in the diagnosis of *Candida*.<sup>[4]</sup>

*C. albicans* might be a part of the permanent microflora of the oral cavity. However, when the ecosystem changes, it grows strongly and infection can occur.<sup>[5]</sup> This change works mainly in two directions: relative change or reduction in the oral microbial flora or a significant decrease in tissue resistance.<sup>[6]</sup> Aging, malnutrition, iron, and folic acid deficiencies, long-term use of antibiotics or steroids, frequent infections, radiation, organ transplantation, and long-term treatment with drugs that inhibit immunity, metabolic conditions including diabetes and hypo-parathyroidism,<sup>[5]</sup> hospitalization,<sup>[7]</sup> congenital immunodeficiency,<sup>[8]</sup> and xerostomia<sup>[9]</sup> are among the predisposing factors for candidiasis.

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In some studies, smoking has been considered as a risk factor for candidiasis.<sup>[10,11]</sup> In addition, wearing removable dentures is another risk factor.<sup>[8,12]</sup>

Blood group antigens were first described by Karl Landsteiner.<sup>[13]</sup> Antigens A, B, H and Lewis blood group antigen are sugar molecules that are found at the end of the carbohydrate chains of some glycolipids and glycoproteins. A, B, and H molecules can be seen in the body fluids (such as saliva, tears, and digestive mucus) of 80% of the population. These individuals are called secretors.<sup>[13]</sup>

An association between ABO blood group antigens and susceptibility to certain infections, including candidiasis, has been shown in several studies;<sup>[14-17]</sup> however, contradictory results have been reported by other investigators<sup>[4]</sup> and the relationship between ABO blood group antigens and oral candidiasis is still inconclusive. Therefore, the aim of this study was to assess *C. albicans* colony counts and its relationship with ABO blood groups.

## MATERIALS AND METHODS

In this cross-sectional/analytical study, the study population consisted of patients referred to the Oral Medicine Department, School of Dentistry, Isfahan University of Medical Sciences and a number of dental students who were randomly selected. Individuals with previous candidiasis, long-term use of broad-spectrum antibiotics and steroids, iron deficiency anemia, diabetes, a history of xerostomia, a history of alcohol use or smoking, individuals with blood group AB and those wearing full or partial dentures were excluded. Blood groups were determined using A and B antibodies after collecting two drops of their finger blood and placing the blood samples on a glass slide. Finally, 300 individuals were divided into three groups of 100 subjects with blood groups A, B, and O.

In order to collect saliva samples, after rinsing the mouth with water, 2-3 mL of unstimulated saliva was evacuated by each subject into sterile tubes using spitting method within 10 minutes and at certain hours of the day (8 -10 a.m.). The samples were kept at room temperature and immediately sent to the laboratory (Al Zahra Hospital Clinical Laboratory, Isfahan, Iran); 0.1 mL of saliva, from each sample, was cultured by a relevant expert on a Sabouraud's Dextrose Agar with chloramphenicol medium (Himedia, India) in the lab. The culture media were

incubated for 48 hours at 37°C. Then the number of grown colonies was counted and reported based on Colony Forming Unit (CFU) . Salivating status of the test subjects (secretor, non-secretor) was not evaluated in this study due to the financial constraints and the high cost of the test.

In the last stage, data were analyzed using SPSS 16 (SPSS Inc.,Chicago, IL, USA). Data were analyzed using the Kruskal-Wallis and Mann-Whitney statistical tests. Statistical significance was defined at  $P < 0.05$ .

This study was approved by the Ethics and Research Committee of the School of Dentistry, Isfahan University of Medical Sciences (390141).

## RESULTS

The mean age of the participants in the study was 27.52 years and 65% of the subjects were in the age range of 20-29 years; 43% of participants in the study had *C. albicans* species in their saliva.

Mean *C. albicans* colony counts in females were slightly more than those in males but the difference was not statistically significant ( $P = 0.612$ ).

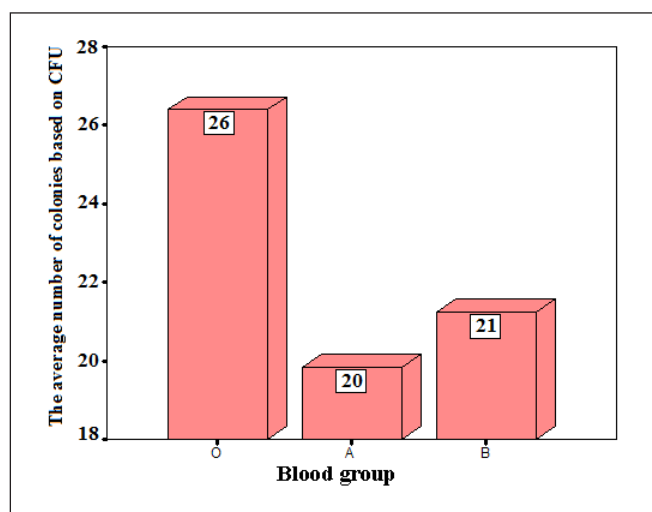
With advancing age, the number of colonies in the saliva of individuals remained roughly the same. Based on Pearson's correlation coefficient there was no relationship between age and the number of *C. albicans* colonies in the saliva of the subjects ( $r = 0.001$ ,  $P = 0.982$ ).

As can be seen in Table 1 and Figure 1, the mean number of colonies in the saliva of subjects with blood group O was more than those with the blood group B; individuals with blood group B had more colonies than those with blood group A, but the difference was not statistically significant ( $P = 0.280$ ).

The rates of oral *Candida* carrier states were 39%, 43%, and 48% in blood groups A, B, and O, respectively [Table 2].

**Table 1: Comparison of the number of *Candida albicans* colonies in the cultured saliva of subjects for each blood group (A, B, O) separately**

Blood group	The mean number of colonies	P
O	26.4	
A	19.84	0.280
B	21.23	



**Figure 1:** Mean number of *Candida albicans* colonies in the cultured saliva of subjects for each blood group (A, B, O), separately.

**Table 2: The percentage of oral *Candida* carrier state and ABO blood groups**

<i>Candida</i> carriage	B	A	O	Total
Carriers	43 (43%)	39 (39%)	48 (48%)	130 (43%)
Non-carriers	57 (57%)	61 (61%)	52 (52%)	160 (57%)
Total	100	100	100	300

## DISCUSSION

In this study, the mean *C. albicans* colony counts in the saliva of 300 patients with the blood groups O, A, and B were studied using WSC technique (Whole Saliva Culture); 43% of participants had *C. albicans* colonies in their saliva and the mean *C. albicans* colony counts in the saliva of individuals with blood group O were more than those with other blood groups, but the difference was not statistically significant ( $P = 0.280$ ).

In 2003 Shin *et al.*, showed that most carriers of oral *Candida* had blood group A but the difference was not statistically significant ( $P > 0.05$ ). These results confirm the idea that there is no relationship between A, B, and O blood groups and their secretory forms with the number of *Candida* colonies. In this study, three methods were used to collect saliva: neat oral rinse culture (NRC), concentrated oral rinse (CRC), and whole saliva culture (WSC). The results showed that the accuracy of CRC in the identification of *C. albicans* colonies was higher than the accuracy of other methods, such as WSC and NRC.<sup>[4]</sup> Smoking has also been identified as a risk factor for oral

candidiasis in several studies.<sup>[4,11]</sup> Therefore, WSC method and non-smokers were used in this study.

Burford-Mason *et al.*, showed that the *C. albicans* carrier state has a relationship with the blood group O ( $P < 0.001$ ) and non-secreting forms of blood group antigens ( $P < 0.001$ ).<sup>[16]</sup>

Ben-aryeh *et al.*, showed that oral *Candida* carrier state has a relationship with the non-secretory form of the blood group ( $P < 0.05$ ). In addition, the number of carriers of *C. albicans* among people with the blood group O was more than those with blood groups A and B, with no significant differences. In their study, the number of colonies of *C. albicans* in the saliva was reported based on the CFU/mL, but no definition of “*Candida* carriers” was provided based on the number of colonies.<sup>[17]</sup>

In none of the previous studies, the mean colony counts have been analyzed based on the age and gender of subjects. In the present study, the mean colony counts in female subjects were slightly more than those in male subjects. As far as age is concerned, there were no significant differences in the mean colony counts between the blood groups.

Binding of *C. albicans* to oral epithelial cells is mediated through a lectin link between a protein on the fungus referred to as mannoprotein adhesin and glycosid receptor on the host epithelial cells.<sup>[18]</sup> After purification of adhesin receptors in different subspecies of *C. albicans* and evaluation of the interaction of these receptors with L-fucos glycolipid antigens related to the blood groups on the surface of epithelial cells, Cameron and Douglas concluded that these antigens can be suitable binding sites for *C. albicans* and among these antigens, H antigen on the epithelial cells of blood group O has higher affinity for binding to the adhesion receptor of *C. albicans* because L-fucos sugar is located at the end of H antigen chain and is properly available to the adhesion receptor of *C. albicans*.<sup>[19]</sup>

In addition, free H antigen found in the saliva and the blood of secretors can be a factor in clearing the mouth from *C. albicans* fungal species.<sup>[4]</sup>

According to the molecular studies conducted on the interactions between adhesin receptor and oral epithelial cell-surface antigens, we should expect that most of the *C. albicans* fungi in blood group O are attached to the oral epithelial cells and fewer

free fungal cells are found in the saliva. Therefore, it seems that saliva collection methods alone are not sufficient to calculate the amount of fungal cells in the oral cavity. As a result, it is recommended that other methods for the study of fungi attached to the epithelial cells of the mouth (e.g., swab from different regions of the mouth) be used in future studies, in addition to saliva collection methods, in order to obtain more precise results in this field. It is also suggested that other more accurate methods be used, including the CRC method, to collect saliva in future studies and also to examine the role of other factors such as smoking and gender. Due to the small number of individuals with blood group AB in the community, subjects with AB blood group were not included in this study but it will be better to examine these individuals in future studies.

## CONCLUSION

Based on the results of this study, the mean *C. albicans* colony counts in the oral cavity of individuals with blood group O was more than those with other two blood groups but the difference was not statistically significant. With advancing age, there was no increase in the mean colony counts in the oral cavity of the subjects in this study. The mean number of colonies of *C. albicans* was not significantly different between males and females. More research studies are needed in order to prove the role of blood groups in susceptibility to candidiasis.

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