ORIGINAL ARTICLE

Estimation of Salivary Candida Count Among Pregnant and Non-Pregnant Women

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ABSTRACT

Objective: Comparison of *Candida* count in the saliva of pregnant and non-pregnant women to determine how much salivary *Candida* count increases in pregnancy as compared to non-pregnant women.

Study Design: Cross-sectional study.

Place and Duration of Study: Pathology Dental Department, HITEC-IMS Hospital Taxila Cantt from 2 December 2021-30 May 2022.

Materials and Methods: The study included 60 pregnant and 60 non-pregnant women of age group ranging from 18 to 40 years. The unstimulated whole saliva of the subjects was collected in sterile plastic containers. The sample was inoculated on Sabouraud dextrose agar. The grown colonies were counted after 48 to 72 hours. Gram staining was performed to confirm the presence of Gram-positive oval budding *Candida.*

Results: The *Candida* count from the saliva of pregnant women was two times greater in number than saliva of non-pregnant women. The mean value in pregnant women was 237.7 CFU/ml while in non-pregnant, it was 103 CFU/ml.

Conclusion: The changes during pregnancy make the oral cavity of pregnant women more vulnerable leading to increased growth of Candida and that increase is twice more than that of non-pregnant women.

Key Words: *Candida, Non-Pregnant Women, Oral Pathology, Pregnant Women, Saliva.*

Introduction

In an oral cavity, saliva plays a very important role in the conservation of the oral mucosa.[1] In saliva, many proteins are antimicrobial in nature that help the oral cavity in protection from infectious organisms.[2] Also, saliva helps in the diagnosis of various diseases not only related to the salivary gland but also many other oral and systemic pathologies.[3]

There are a variety of microorganisms inhabiting the oral cavity. Although *Streptococcus mutans* has shown the major involvement in the formation of dental caries, it's still not the only agent responsible for dental caries formation. Other frequently observed microorganisms involved in dental caries are Gram positive cocci and bacilli mainly the *non-mutans Streptococcus* and *Lactobacilli.* Consumption of sugar containing items facilitates the growth of cariogenic microorganisms.[4]

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Dental caries occurs mainly due to demineralization of enamel and dentine by the acids formed by these cariogenic bacteria. The cariogenic microorganisms produce acids by doing metabolism of dietary sugars.[5] These acids are responsible for the acidic oral environment which favors the further growth of cariogenic microorganisms contributing in the progress of dental caries formation.[6]

Also, along with bacterial components of oral flora, fungi also constitute normal flora. Among fungi, *Candida* speciesare the most dominantly distributed fungi observed in the oral cavity.[7] Caries formation involves a drop in pH, so when the dental plaque develops low pH, it gets prone to caries formation.[8] *Candida* have the ability to produce acids, these features allow the fungus to participate in the formation of dental caries.[9]

C.albicans interacts with *S.mutans* through various biochemical mechanisms to form biofilms which are highly cariogenic. This interaction between *C.albicans and S.mutans* enhances each other's role in the development and progress of dental caries.[10] This favors the evidence of *Candida* species association with dental caries, with special involvement in early childhood caries and root caries. *C.albicans* has been especially identified as a crucial root caries agent.[11] Studies showed that

Candida, along with other bacteria, act as an indicator for dental caries activity and also may lead to transfer of infection from mother to fetus.[12] Apart from involvement in dental caries, *candida* has also been involved in oral candidiasis.[13]

During pregnancy, the female body goes through significant changes in hormonal, metabolic, and immunological systems, which also have an impact on the microbiological composition of the oral cavity.[14] These changes in the microbiological environment of the oral cavity during pregnancy, may favor the growth of *candida* species that are linked with the development of acidic oral environment.[15] This imbalanced environment of microflora in oral cavity may lead to overgrowth of bacteria and other opportunistic pathogens, causing oral diseases, such as dental caries and periodontal diseases.[16]

Keeping in view the involvement of both bacteria and fungi in caries formation, our study aimed on the number and count of *Candida albicans* in saliva of pregnant and non-pregnant women to estimate how much *candida* increases in number during pregnancy. In Pakistan, very few studies have been done to estimate the *candida* count in saliva during pregnancy. As a considerable association has been found between *Candida* and oral health diseases and their relationship with pregnancy,[7,10,11] our study aimed to estimate how much *Candida* count increases in saliva during pregnancy which may affect the overall oral health of pregnant women.

Materials and Methods

This cross-sectional study was conducted in Pathology Dental department HITEC-IMS Hospital Taxila Cantt for a duration of six months from 2nd December 2021-30th May 2022. The study was approved by the ethical review committee ERC/38 of HITEC-IMS Taxila Cantt. Sample size was calculated by WHO criteria.[17,20,23] The samples were collected from 60 outpatient pregnant women and 60 non-pregnant women. Inclusion criteria was healthy women from 18 to 40 years. Exclusion criteria include high-risk pregnancy, smoking, any drug addiction, associated debilitating disease, oral and/or systemic antifungal therapy within 2 months of the study. The consent from all the participants was taken before taking the sample.

The unstimulated whole saliva was collected from pregnant and non-pregnant women in graduated sterile plastic containers. About 1ml of saliva was collected from each participant. As a fresh sample was taken each time and incubated immediately, no storage of the sample was required. From each 1ml salivary sample, 0.2 ml of saliva was inoculated on Sabouraud dextrose agar (SDA) and incubated for 48 to 72 hours at 37°C.

After an incubation period, the creamy pasty colonies appeared on plates. The colony was then Gram stained. The presence of *Candida* was confirmed when Gram-positive budding ovoid yeast cells were seen under the microscope. The colonies of *Candida* were then counted in colony forming unit (CFU/ml). The number of colonies formed in the 0.2ml saliva sample was calculated in 1ml by dividing the number of colonies by 0.2. The serial dilution method was not employed as no dilution of the original sample was done.[18] Data was analyzed on excel sheets followed by descriptive statistics and percentages were calculated.

Results

As shown in Table-I, the mean value in pregnant women was 237.7 CFU/ml while in non-pregnant, it was 103 CFU/ml. The result showed *Candida* count in the saliva of pregnant women was two times greater than the saliva of non-pregnant women.

The results showed *Candida* colony growth potential is more in the saliva of pregnant women than in non-pregnant women.

Discussion

This study was conducted to determine the *Candida* count, the most common oral yeast, in saliva during

Table I: Mean Values Of CFU/Ml in Pregnant and Non-Pregnant Women

Total number of participants	Number of pregnant women (60)	Number of non-pregnant women (60)
120	Mean CFU/ml	Mean CFU/ml
	237.7	103

Table-II: Percentage Values Of CFU/Ml in Pregnant and Non-Pregnant Women

Pregnant women		Non-pregnant women	
Samples with Positive growth of *candida*	Samples with Negative growth of *candida*	Samples with Positive growth of *candida*	Samples with Negative growth of *candida*
83%	17%	72%	28%

pregnancy. Our study gave 237.7 CFU/ml mean value of *Candida* in the saliva of pregnant women showing 83% of samples giving positive growth on agar. In one of the previous studies, the value of 270 CFU/ml was a threshold to distinguish oral Candidiasis from carriage state.[18] Another study showed the same 270 CFU/mL to be the threshold value when measured with the whole saliva sample to distinguish oral Candidiasis from carrier state. The threshold value is important for the diagnosis and prevention of diseases. By controlling contributory host factors, *Candida* counts can be maintained at levels lower than the threshold which might help in preventing *Candida* infections. So, by knowing the threshold amount of *Candida* species (>270 CFU/mL) helps to distinguish oral Candidiasis from oral carriage.[19]

Our mean value of 237.7 CFU/ml during pregnancy suggested that the increase is not significant enough to lead to oral Candidiasis, but it showed considerable number that it may be a contributory factor to the development of oral Candidiasis or dental caries during pregnancy. During pregnancy, the whole-body changes including the oral cavity making it vulnerable to the development of dental caries.[20]

Generally, pregnant women are considered at an increased risk for dental caries probably due to hormonal changes and their associated effects on the oral cavity. Also, during pregnancy, delay in treatment increases the risk.[21] Several studies showed an increase in *Candida* count in saliva during pregnancy.[16,22] So our study showed that how much increase in *Candida* count happens during pregnancy in saliva. Another study provides evidence favoring the point that pregnancy may promote oral yeast growth.[23] One study augmented the fact that in addition to the bacteria, *Candida* count also increased during the pregnancy especially during second and third trimesters of pregnancy.[24] So the increase in *Candida* count may augment the increasing chances of developing dental caries in pregnant women.

In another study, it has been mentioned that chances of yeast infection increase in pregnancy due to hormonal changes, and simple oral hygiene measures may sometimes become less effective in preventing *Candida* growth which renders employing more vigilant oral hygiene measures sometimes involving antifungal drugs. So pregnant women should employ professional tooth cleaning during pregnancy for maintaining a healthy oral environment and reducing oral and systemic problems during pregnancy. Also, there is reduction in *C. albicans* in saliva during pregnancy if proper oral hygiene measures are employed.[25] Meanwhile, the mean value of 103 CFU/ml in non-pregnant women suggests that *Candida* may not be much significant in saliva in healthy non-pregnant women.

As our study only determined salivary *Candida* count but not its clinical correlations, more research is needed in this field to correlate its significance with a clinical perspective. More studies are needed to find methods to imply these results for early detection and diagnosis to prevent future oral pathologies pertaining to salivary *candida*. Also, this emphasizes the importance of vigilant oral hygiene practices during pregnancy.

Conclusion

Our study concludes that salivary *Candida* count increases two times during pregnancy than non-pregnant women depicting their vulnerability towards dental caries and oral *candida* infections. This emphasizes the importance of effective and diligent oral hygiene measures during pregnancy to reduce the possibilities towards dental caries and oral candidiasis formation.

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CONFLICT OF INTEREST
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DATA SHARING STATMENT
The data that support the findings of this study are available from the corresponding author upon request.

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