Original Article

A comparative study to assess risk of oral candidiasis in pregnant and nonpregnant women

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Abstract

Background: The major hormonal changes observed in pregnant women lead to an imbalance in the oral environment. Hence, recent studies suggest that the placenta may harbor a unique microbiome that may have originated in the maternal oral microbiome.

Aim: The present study aimed to assess the risk factor of oral candidiasis in pregnancy and to evaluate the prevalence of *Candida* species in the oral cavity of pregnant women in all three trimesters. The comparison was also done between pregnant and nonpregnant women to evaluate the cause of the prevalence of candidal species.

Materials and Methods: Thirty pregnant and thirty nonpregnant women aged between 20 and 30 years were included in the study that were healthy and who did not have any obvious lesion in the oral cavity. The sterile swabs were used to collect samples from the oral cavity from both the groups by brushing the dorsum of the tongue and buccal mucosa. The pregnant women were followed throughout the pregnancy, i.e., in every trimester, for the sample collection. The samples were then cultured on Sabouraud Dextrose Agar media. The positive growth on culture plates was then inoculated on HiCrome agar differential agar media for speciation.

Results: Statistical analysis was done by comparing the positive growth in pregnant and nonpregnant women using Fisher's exact test. The pregnant women were compared in three trimesters using the McNemar Chi-square test.

Conclusion: The study concludes that there was no significant presence of Candidal species when compared between pregnant and nonpregnant groups. The prevalence of *Candida* species also remained the same.

Keywords: *Candida*, culture, media, microbiology, oral cavity, pregnancy, prevalence

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INTRODUCTION

Candida is a normal inhabitant in the skin, oral cavity, gastrointestinal tract, respiratory tract and genitourinary tract. Many changes in the internal and external factors induce the harmless saprophyte to become a true pathogen. These predisposing factors are aging, pregnancy, AIDS,

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diabetes, steroidal therapy, secondary to bacterial infections and widespread use of certain medical and surgical practices.[1]

The *Candida* species are the 4th most common organisms causing bloodstream infection and constitute 8% of all nosocomial infections.[2] *Candida* infections are the 2nd most frequently diagnosed opportunistic infections in patients.[3] Candidiasis is mainly caused by *Candida albicans*, while there has been striking increase in the frequency with non-*albicans Candida* species in the last few years.[3] The species which are considered pathogenic to humans are *C. albicans, Candida tropicalis, Candida krusei, Candida glabrata, Candida lusitaniae* and *Candida viswanathii*.[4]

Pregnant women are more prone to opportunistic infections and various diseases which may have implications on their health as well as the developing fetus. In pregnancy, thrush is usually caused by the overproduction of glycogen which leads to mucosa becoming more alkaline than acidic, an environment in which most of the microorganisms can thrive.[5] Early diagnosis of candidal infection or prevention of such infections in pregnant women is necessary for the healthy well-being of the mother and the child. This study is undertaken to isolate, identify and evaluate the risk of candidiasis in pregnancy and the role of Candidal species in pregnant and nonpregnant women without oral lesions.

MATERIALS AND METHODS

The sample comprised of 30 pregnant women and 30 nonpregnant women aged between 20 and 30 years attending routine examination to the outpatient department of a private clinic of obstetrics and gynecology. All participants received information concerning the purpose of the study and provided informed consent which was made in three different languages, i.e., English, Urdu and Kannada, before participation. The subjects had not received any oral treatment or antibiotic therapy in the past 3 months.

The pregnant women were examined three times during pregnancy. The first visit was at 7–16 weeks' gestation (early pregnancy), next at 17–28 weeks (middle pregnancy) and the final at 29–39 weeks (late pregnancy). Among the pregnant women group, three dropped out of the study in the 2nd trimester.

Recording of maternal characteristics

Sufficient details regarding the health condition of the subjects were assessed such as blood reports and any complications before the pregnancy. Oral examination was done and oral swabs were taken by brushing the sterile cotton swab on the surface of buccal mucosa and dorsum of the tongue.

Culture procedures

The swabs collected were immediately taken to the laboratory where it was inoculated on plates in triplicate on Sabouraud Dextrose Agar (SDA) under sterile conditions. The inoculated media was incubated aerobically for 48 h at 37°C and checked for any positive growth as shown in Figure 1. If growth was positive, then speciation was done with HiCrome differential agar (Himedia batch no. M1297A-100G) as shown in Figure 2.

RESULTS AND OBSERVATION

The prevalence of *Candida* species was assessed in thirty pregnant women in three trimesters and thirty nonpregnant women were the controls from whom the swab was taken only once, none of the women presented any clinical signs of candidiasis. The comparison was done between the pregnant and nonpregnant women based on the positive growth on SDA with the first swab as illustrated in Table 1.

Among pregnant women, the cases which showed positive growth on SDA in the first trimester were 16.7% from 100% of samples, i.e., only 5 cases out of 30 and 83.3% from 100% of samples showed negative growth on SDA, i.e., 25 cases out of 30.

Among nonpregnant women, the cases which showed positive growth on SDA were 6.7% from 100% of samples, i.e., only 2 controls out of 30 controls and 93.3% from 100% of samples showed negative growth on SDA, i.e., 28 controls out of 30.

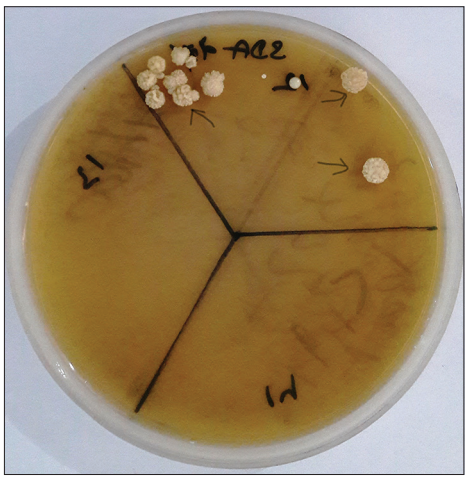

Figure 1: Collected sample swabs were inoculated on plates in triplicate on Sabouraud Dextrose Agar under sterile conditions and incubated aerobically for 48 h at 37°C. Arrows indicate *Candida* colonies

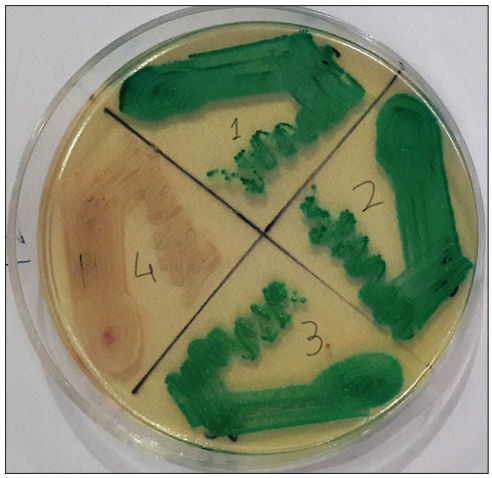

Figure 2: Positive growths were subjected to speciation using HiCrome *Candida* differential agar. 1, 2 and 3 green colony indicates *Candida albicans* 4 cream to white colony indicates *Candida glabrata*

Table 1: Comparison of presence of Candida in Pregnant and non-pregnant study participants by Fisher's exact test

Variable		First trimester		Total	Fisher's Exact Test *P* and significance
		Candida Absent	Candida present		
Pregnancy: No	No	28	2	30	0.424, Not significant
	%	93.3%	6.7%	100.0%	
Pregnancy: Yes	No	25	5	30	
	%	83.3%	16.7%	100.0%	
Total	No	53	7	60	
	%	88.3%	11.7%	100.0%	

Interpretation: The presence of candida was higher in the Pregnant group (16.7%) compared to the non-pregnant group (11.7%). However, the difference was not statistically significant when analyzed by Fisher's exact test

Table 2: Comparison of number and percentage of study participants with or without candida in three terms of pregnancy

Category	First trimester		Second trimester		Third trimester	
	n	%	*n*	%	*n*	%
Candida Absent	25	83.3%	24	80.0%	24	80.0%
Candida present	5	16.7%	3	10.0%	3	10.0%
Drop out (fetal death + miscarriage)	0	0%	1+2	10%	1+2	10%
Total	30	100.0%	30	100.0%	30	100.0%

Interpretation: There were 5 cases with candida present (16.7%) in the first trimester. Two amongst those one dropped out of the study in the second trimester and another suffered a miscarriage. The remaining three continued to harbor the candida in the second trimester as well. When analyzed by Mc-Nemer Chi square test, there was no significant change in prevalence of candida across the trimesters ($P>0.05$)

According to Fisher's exact test, the prevalence of oral yeast was higher in pregnant than in nonpregnant women [Table 1], although no statistical significance was found ($P = 0.424$).

The comparison was also done between three trimesters during pregnancy among the cases as illustrated in Table 2.

Thirty pregnant women were followed in every trimester for swab collection. In the first trimester, 16.7% showed positive growth, i.e., only 5 swabs out of 30 and 83.3% were negative i.e., 25 swabs out of 30.

In the second trimester, 10.0% showed positive growth, i.e., only 3 swabs out of 30 (two positive samples in the first trimester dropped out in the second trimester as there was one fetal death recorded and one miscarriage) and 80.0% were negative, i.e., 24 out of 30 (one miscarriage case resulted in a drop out).

In the third trimester, 10.0% showed positive growth, i.e., only 3 swabs out of 30 and 80.0% were negative, i.e., 24 out of 30 (the same number of cases and controls were followed from the second trimester to the third).

McNemar Chi-square test was applied and it was analyzed that there was no statistically significant change in the prevalence of *Candida* in all the three trimesters. The samples which showed positive growth in the first trimester showed positive growth in the second and third trimesters as well.

DISCUSSION

Pregnancy involves physical and hormonal changes that have a significant impact on every organ system, including the oral cavity. An oral problem associated with pregnancy primarily includes gingivitis and periodontal infection.[6] Estrogens have been described to promote *Candida* infection, enhancing the risk of pregnancy complications and preterm birth. Oral microorganisms are capable of passing through oral mucous membranes, spreading to different body sites and causing systemic or focal infections.[7] Recent studies have described that the placenta may harbor a unique microbiome, and apparently, this microbiome is more similar to the oral cavity than to the vagina microbiome. In fact, it is known that microorganisms may reach the uterus through a hematogenous route and those maternal microbiomes from different body sites, including oral, vaginal, gut, cervical and even the placenta itself, may influence pregnancy outcomes.[8]

Studies have also proved that the presence of periodontal pathogenic microorganisms or their by-products in the intrauterine environment stimulate a fetal immune and inflammatory response that may be responsible for the increased risk of preterm birth and low birth weight.[9] Both estrogen and progesterone hormones lead to increased gingival vascularization and decreased immune

response. In pregnancy, there is an increase in some types of microorganisms which tend to utilize the steroidal hormones of pregnancy for their growth.[8]

Studies done in this field identified maternal periodontitis as a potential risk factor for preterm birth and low birth weight. This potential association between maternal periodontitis and adverse pregnancy outcomes becomes an important concern because preterm birth and low birth weight are a major cause of infant mortality.[10]

Dental and medical practitioners should recognize oral health care as an integral part of the overall prenatal care in order to deliver adequate prenatal care to any pregnant woman. A pregnant woman is first seen by a medical health professional and she may only come to a dentist if advised to do so by her doctor. Hence, it becomes important to evaluate the knowledge of medical health professionals about complete oral health and its association with adverse pregnancy outcomes.[11]

The assessment of Candidal species in pregnancy was done in this study. It was demonstrated that the number of individuals colonized with yeast in the oral habitat during early pregnancy was 16.7% which was higher compared to that of the nonpregnant women who were around 6.7%, but statistically, it was not significant ($P = 0.424$). Various factors in pregnancy, such as a change in the physiological condition, may affect bacterial and fungal numbers, and female hormones have been described as growth factors to promote the proliferation of multiple bacterial species.

Taking in consideration that maternal oral microbiome may represent the major contributor for the intrauterine microbiome, and that yeast may impact fetus development and pregnancy outcomes, we explore the changes of oral yeast colonization throughout pregnancy. Our data suggest that pregnant women may be more prone to oral yeast colonization than nonpregnant women. This result corroborates with a previous study from Fujiwara *et al.* that shows higher *Candida* species prevalence in saliva from women in the middle and late pregnancy in comparison to the nonpregnant group.[12]

The oral yeast levels vary between individuals, and the scientific literature shows that a small percentage of individuals (~20%) present elevated yeast loads, without clinical signs of candidiasis.[13] In a study from 1980, higher values of oral *Candida* were suggested to be indicative of oral candidiasis, but an overall assessment of each individual should be performed.[2] None of the high yeast carriers observed in our study presented clinical signs of candidiasis. The percentage of nonpregnant women with yeast isolates is the evidence that *Candida* sp. is the most frequent yeast colonizing the oral cavity.[14]

The promotion of yeast growth in the oral environment during pregnancy may be associated with the reduced oral pH according to the study done by Rio *et al.* In agreement with this suggestion are studies showing that *Candida*, the most prevalent yeast in the oral cavity, among the pregnant women. Opportunistic microorganisms were present at low levels in the normal healthy oral cavity, whereas these opportunists increase in number during compromised conditions such as immune-compromised or immune-modulated subjects.[9] These findings suggest that pregnancy may promote the colonization of *Candida* species in the oral environment.

In our study, the results of the differential culture media revealed that out of five positive cases in the first trimester four cases were positive for *C. albicans* and only one positive growth showed the presence of *Candida glabrata*. *C. albicans* infection occurs in the vast majority (80% to 90%) of diagnosed vulvovaginal candidiasis cases. *C. glabrata* is the second most frequently occurring fungus species and the dominant non-*albicans* species. It implied that the frequency of vaginal candidiasis cases caused by *C. glabrata* was increasing. This new trend might be related to the widespread and inappropriate use of antimycotic treatments (self-medication, long-term maintenance treatments and repeated treatments for candidiasis episodes).[15]

In the following study, the growth on the SDA culture plates among the positive cases both in pregnant and nonpregnant women ranged from mild to moderate. This indicates that the *Candida* species are trying to be opportunistic in the oral microbiome. It can also be an indication of ascending infections, i.e., the organisms generally ascend from the vagina into the uterus during pregnancy, but many have low virulence and may reside in the uterus before pregnancy.[16]

During the course of study among the positive pregnant cases, there was one case of stillbirth and one case of miscarriage. In pregnancy, studies have suggested that maternal or fetal infection may result from *Candida* spp. with a variety of mechanisms, including direct infection, placental damage and severe maternal illness. First, the fetus may be directly infected through the placenta or membranes, with the organisms damaging a vital organ such as the lung or heart.[16] Second, the placenta may be directly infected without fetal involvement, resulting

in reduced blood flow to the fetus. When early infection occurs, the fetus may develop a congenital anomaly with a subsequent fetal death due to the anomaly. Third, maternal infection may lead to a severe maternal illness. Due to high maternal fever, poor oxygenation or systemic reaction to the illness, the fetus may die without transmission of organisms to the placenta or fetus. Finally, maternal infection may precipitate preterm labor, with the fetus unable to tolerate delivery resulting in stillbirth.[16]

There was one miscarriage case among the negative cases of pregnant women in our study, about whom we were acknowledged through hospital records. The reason for miscarriage is unknown, but a key question arises why some women with infection accomplish a normal pregnancy outcome while noninfected women who are healthy in all aspects have to face the trauma of miscarriage or stillbirth.

As it was observed in the study, that most of the pregnant females were having hemoglobin ranging from 8 to 12 g%, there was a benefit of doubt regarding iron supplement or iron deficiency being the cause for normal commensal becoming opportunistic. One study published in September 2006 issue of the journal "Antimicrobial Agents and Chemotherapy," which proves that iron improves the immune function and increases the susceptibility of *Candida* to antifungal medications in the laboratory. According to the authors of the study, iron also improves the virulence of the *Candida* species, by which it is able to invade the host or human cells. Another study in the March 2011 issue of the journal "PLOS Pathogens" also points out that individuals taking iron supplements to treat anemia and related disorders have an increased risk of getting *Candida* infections. Cheryl Garrison, her book "The Iron Disorders Institute Guide to Anemia," explains that pathogens such as *Candida* in order to invade human cells, it needs free iron. A protein called lactoferrin found in saliva, tears and the vagina binds to the free iron and thereby prevents yeast infections. However, individuals with certain types of anemia have low levels of lactoferrin and may be more susceptible to *Candida* infections.[17]

When the statistical analysis was done to compare between the three trimesters, the prevalence of *Candida* which was observed as positive growth in culture, was present in all the three trimesters and the species identified also remained the same in all the trimesters. By this study, it is observed that the opportunistic yeast will remain the same.

Timely and appropriate treatment should be done. Moreover, we should not ignore even the mild form of fungal infection like *Candida* vaginitis as it also has the potential to spread systemically in the presence of other predisposing factors and the patient can develop obstetrical emergencies. American guidelines on oral and general health during pregnancy and early childhood states that preventive services should be provided as early in pregnancy as possible and that primary prevention including measures to avoid infection and colonization of *Candida* species is an important strategy as preventive care during pregnancy.

CONCLUSION

The study provides evidence favoring the view that pregnancy may promote oral yeast growth. Our results indicated that the early stage of pregnancy is a critical period in which it is important to intervene to improve oral health. Physiological changes of pregnancy might be responsible for the quantitative microbiological increase in early pregnancy and accentuates primary prevention during pregnancy.

Infections early in pregnancy leading to congenital anomalies or other fetal conditions later associated with stillbirth are also considered an infectious cause. Demonstration of maternal infection at an appropriate time in pregnancy with a specific organism known to cause the fetal condition is required. First trimester maternal rubella infection is the prime example of this type of infection; parvovirus infection with fetal hydrops is another example.

The difficult question by the expectant mother, which a gynecologist faces after miscarriage or stillbirth, is "what can be the cause for this mishap." Therefore, careful routine prenatal laboratory tests for the assessment of *Candida* species are also recommended so that the treatment aspect can be assessed based on the type of species.

The following study recommends adequate oral hygiene including professional tooth cleaning in the early stage of pregnancy for establishing a healthy oral environment and preventing oral and systemic health problems of woman during pregnancy. The data suggest that *Candida* species remains the same in all three trimesters and may promote the possible risk factor for the health of pregnant women and fetus.

Studies in the future can be conducted to assess the correlation between miscarriage and stillbirth with oral candidiasis in pregnancy. However, how adverse would be the condition of yeast colonization in pregnancy should

also be assessed in detail to combat Candidal infections. By this study, it can also be recommended to have a larger study group for further analysis of which specific species is predominant in the oral microbiota and their outcomes in pregnancy.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rippon JW. Medical Mycology. Philadelphia: WB Saunders; 1998.
2. Jarwis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. Clin Infect Dis 1995;20:1526-30.
3. Murray MP, Zinchuk R, Larone DH. CHROMagar *Candida* as the sole primary medium for isolation of yeasts and as a source medium for the rapid-assimilation-of-trehalose test. J Clin Microbiol 2005;43:1210-2.
4. Chander J. Textbook of Medical Mycology. 4nd ed. 2018.
5. Cotch MF, Hillier SL, Gibbs RS, Eschenbach DA. Epidemiology and outcomes associated with moderate to heavy *Candida* colonization during pregnancy. Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1998;178:374-80.
6. Han YW. Oral health and adverse pregnancy outcomes – What's next? J Dent Res 2011;90:289-93.
7. Zabor EC, Klebanoff M, Yu K, Zhang J, Nansel T, Andrews W, *et al*. Association between periodontal disease, bacterial vaginosis, and sexual risk behaviours. J Clin Periodontol 2010;37:888-93.
8. Sharma M, Solanki A. Prevalence of *Candida* infection in pregnant women with and without diabetes. Int J Curr Microbiol App Sci 2014;3:605-10.
9. Odds FC. Candida and Candidosis. Baltimore: University Park Press; 1979. p. 104-10.
10. Babic M, Hukic M. *Candida albicans* and non-albicans species as etiological agent of vaginitis in pregnant and non-pregnant women. Bosn J Basic Med Sci 2010;10:89-97.
11. Hashim R, Akbar M. Gynecologists' knowledge and attitudes regarding oral health and periodontal disease leading to adverse pregnancy outcomes. J Int Soc Prev Community Dent 2014;4:S166-72.
12. Fujiwara N, Tsuruda K, Iwamoto Y, Kato F, Odaki T, Yamane N, *et al*. Significant increase of oral bacteria in the early pregnancy period in Japanese women. J Invest Clin Dent 2015;8:e12189. doi. 10.1111/jicd.12189.
13. Monteiro-da-Silva F, Araujo R, Sampaio-Maia B. Interindividual variability and intraindividual stability of oral fungal microbiota over time. Med Mycol 2014;52:498-505.
14. Epstein JB, Pearsall NN, Truelove EL. Quantitative relationships between *Candida albicans* in saliva and the clinical status of human subjects. J Clin Microbiol 1980;12:475-6.
15. Fidel PL Jr., Vazquez JA, Sobel JD. Candida glabrata: Review of epidemiology, pathogenesis, and clinical disease with comparison to *Candida albicans*. Clin Microbiol Rev 1999;12:80-96.
16. McClure EM, Dudley DJ, Reddy UM, Goldenberg RL. Infectious causes of stillbirth: a clinical perspective. Clin Obstet Gynecol 2010;53:635-45.
17. Available from: https://www.livestrong.com/article/549477-chia-seeds-l-glutamine-for-candida. [Last accessed on 2017 Aug 18].