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Frequency of Candidiasis and Colonization of *Candida albicans* in Relation to Oral Contraceptive Pills

Atousa Aminzadeh,[1] Ali Sabeti Sanat,[1,*] and Saeed Nik Akhtar[1,*]

[1]Oral and Maxillofacial Pathology Department, School of Dentistry, Islamic Azad University, Khorasgan, Isfahan, IR Iran

[*]*Corresponding authors*: Ali Sabeti Sanat, School of Dentistry, Islamic Azad University, Khorasgan (Isfahan) Campus, Arghavaniyeh Blvd., Isfahan, IR Iran. Tel: +98-9126494618, Fax: +98-2122912546, E-mail: Alisanat@ymail.com; Saeed Nik Akhtar, Oral and Maxillofacial Pathology Department, School of Dentistry, Islamic Azad University, Khorasgan (Isfahan) Campus, Isfahan, IR Iran. E-mail: s.nikakhtar1982@gmail.com

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Abstract

Background: Candidiasis, the infection caused by *Candida albicans*, is one of the most common infections of the oral cavity in humans. Candidiasis causes irritation and is known for its carcinogenic effects. Thus, it is important to recognize the predisposing factors for this opportunistic infection. Several previous studies have demonstrated an increased frequency of vaginal candidiasis in relation to oral contraceptive consumption.

Objectives: Only a few studies on the relation between oral contraceptives and oral candidiasis have been previously conducted. This study aims to evaluate the possible relation between oral contraceptive pills and oral candidiasis.

Methods: This analytic, case-control study included 40 non-pregnant women divided into two groups: 20 who used oral contraceptive pills and 20 who did not. The groups were matched according to age, oral health, and past and present medical history. Samples were collected from the tongue's dorsum using a cotton swab and inoculated on CHROMagar culture plates. The frequency of positive cultures and the number of *Candida* colonies were compared between the two groups using independent t-tests and Mann-Whitney statistical tests with SPSS18 software.

Results: The frequency of positive cultures of *Candida albicans* was higher (P value = 0.03) for the case group. Also, the number of *C. albicans* and *C. krusei* was significantly higher for the case group compared to the control group (P value = 0.04, P value = 0.03).

Conclusions: The results of the present study demonstrate that oral contraceptives containing estradiol can lead to *Candida* colonization in the oral cavity. It is recommended that further studies comparing the influence of oral contraceptives on *Candida's* adherence to the epithelium is highly recommended.

Keywords: *Candida albicans*, Oral Contraceptives, Fungi Colonization

1. Background

Candidiasis, the infection caused by the fungus *Candida albicans*, is the most common oral infection in humans. Candidiasis has a variety of different clinical manifestations which causes difficulties in the clinical diagnosis. Other Candida species such as *C. tropicalis*, *C. parapsilosis*, and *C. krusei* may also be found in the oral cavity; however, these species rarely cause oral lesions (1, 2).

Both acute and chronic mucocutaneous candidiasis can exhibit different patterns of infection. The most recognizable form is pseudomembranous candidiasis or thrush, which is an adherent white plaque in the oral cavity resembling milk curds. The plaque mass is made of hyphae, yeast, and shed epithelial cells (1, 2).

Common symptoms of candidiasis in patients include a very mild burning sensation or an unpleasant taste (salty or bitter). Sometimes patients complain of oral blisters, which are actually prominent plaques of thick *Candida* granules on the oral mucosa rather than vesicles or blisters (3, 4).

C. albicans is considered a normal oral microflora. Between 30 and 50 percent of the global population have *C. albicans* present in their oral cavity without exhibiting infection. The carrier, increases with age. As much as 60% of the population over 60 years of age carries *C. albicans* without showing evidence of mucosal lesions in the oral cavity (1).

Oral contraceptive pills (OCPs) are a birth control method that uses a combination of an estrogen (estradiol) and a progestogen (progestin). When taken orally every day, these pills inhibit female fertility. They were first approved for contraceptive use in the United States in 1960, and are a very popular form of birth control. They are currently used by more than a 100 million women worldwide and are becoming ever more popular with newer generations both due to their preventive effect and the ease of ad-

ministration (3, 5, 6).

Many methods, such as cytology, biopsy, and observation of *Candida* hyphae, can be used for the laboratorial diagnosis of candidiasis. However, certainty can only be obtained through culture (1).

CHROMagar microbiology relies on proprietary enzyme technology to detect pathogens based on form speed, accuracy, ease of use, and cost benefits (4).

There is less evidence of an etiological association between fungal infection and cancer, although for many years *Candida* subspecies have been implicated in various epithelial cancers. *Candida* infection does not appear to be a risk factor for dysplastic cervical lesions or cervical carcinoma; however, further study is needed regarding *Candida* and its carcinogenesis in relation to oral and esophageal carcinoma. There have been a number of reports of oral or esophageal carcinoma developing in immunocompromised patients with chronic mucocutaneous candidiasis (7, 8).

Lesions infected with *Candida* have shown more progressively severe dysplasia in subsequent biopsies compared to initial biopsies obtained from the same patients who initially did not exhibit candidiasis (9, 10).

Chronic hyperplastic candidiasis (CHC) has a propensity to undergo malignant transformation. Cawson found that 6 out of 10 tissue biopsies initially diagnosed as CHC progressed to oral squamous cell carcinoma (OSCC) while Eyre and Nally (11) reported that 2 out of 3 CHC cases underwent malignant transformation. In contrast, some CHC lesions resolve when treated with antifungal drugs. Lamey et al. reported a case of candidal leukoplakia with epithelial dysplasia that resolved within 11 days of systemic treatment with a triazole antifungal drug (12).

Candida might induce OSCC by directly producing carcinogenic compounds such as nitrosamines. Such a carcinogen will bind with DNA to form adducts with bases, phosphate residues, and hydrogen bonding sites that could cause miscoding or irregularities with DNA replication. Point mutations thus induced may activate specific oncogenes and initiate the development of oral cancer (13).

The risk factors of candidiasis, its specific symptoms, and its impact on the quality of life of patients suffering from it, as well as the role *C. albicans* plays as an etiologic agent or promoter in squamous cell carcinomas, makes understanding candidiasis necessary. Various studies on the increasing rate of vaginal candidiasis after use of OCPs have been carried out, while the oral counterpart of the infection has not been discussed significantly (14). In fact, no relevant study could be found, nor any guidelines for patients regarding how to handle complications from taking these medications. Most other articles on the subject matter are either only vaguely relevant or date back decades ago.

2. Objectives

The present study is intended to establish the role of oral contraceptives in the prevalence of oral candidiasis due to its profound impact on the patient's lifestyle, oral mucosal health, and oral hygiene. Candidiasis can cause oral mucosal hypersensitivity. It can also have a prominent effect on oral epithelial dysplasia that can develop into a more severe problem such as OSCC. Thus, understanding the relationship between oral contraceptives and oral candidiasis may persuade physicians to prescribe adjunct prophylactic medication like nystatin whenever dispensing OCPs.

3. Methods

3.1. Sample Population and Size

The population was all non-pregnant female patients between the ages of 18 - 45 who visited the school of dentistry infirmary on the Isfahan campus of Islamic Azad University to seek daily treatment or periodic oral examination during the period of 2011 to 2012.

3.2. Inclusion and Exclusion Criteria

Volunteers were selected from non-pregnant women who were either taking OCPs or had no history of taking OCPs.

Volunteers who were pregnant, borderline menopausal, younger than 25 or older than 45 years of age, had systemic diseases such as diabetes, smoked, used antibiotics within the past six months, had a recent history of corticosteroid use, had prior treatment of fungal diseases, or were diagnosed or treated for cancer were not included in the study.

Following this set of criteria, 40 subjects were selected out of a total of 118 applicants: 20 of the 75 women with no prior history of OCP consumption and 20 of the 43 women who were currently taking OCPs.

3.3. Sample Size

The statistical equation with a confidence level of 99.9% in the honest declaration of subjects and a confidence interval of 11.09% indicated that at least 17 subjects were needed for each case group. Therefore, 40 women were selected for the study. The patients were divided into two groups of 20 participants each. The case group contained OCP consumers and the control group contained non-consumers.

This study not only selected subjects who met the study criteria, but also took advantage of the participating center's admittance policy. The dental infirmary's patients come from not only the city of Isfahan but also neighboring towns and provinces, thus allowing this study to include a wider and more diverse population in regards to individual lifestyle and socioeconomic standing.

3.4. Data Collection Instruments and Method

A questionnaire, sterile swab, sterile gauze, and culture medium were provided for each patient. The study was conducted in a clinical manner in which the stages of the study were described for each patient. Volunteers who were willing to participate in this study were enrolled by their verbal approval.

The study was carried out under the approval of the ethic committee which is a division of the department of dental research of the school of dentistry. The patients' anonymity was ensured through full nondisclosure. The permission letter to undertake the study was delivered in February of 2011.

3.5. Implementation of the Study

The study was conducted in the oral diagnosis department of the dental infirmary of Islamic Azad University in the city of Isfahan. Verbal consent was obtained from patients before taking samples. In order to assess the oral health status of patients, their plaque indexes were recorded. Patients with similar plaque indexes were selected by the clinician who conducted the examination in order to ensure greater consistency in the study's collected data. The questionnaire was completed to gather information such as age, type of OCP used, days of consumption and duration of therapy, previous history of fungal infection, systemic disease, smoking habits, and recent use of antibiotics or corticosteroid medication. Next, a thorough examination of the oral cavity for the presence of red and white candidiasis lesions was performed. The patient was questioned for symptoms such as a burning sensation or an unpleasant taste.

Since the main repository of *C. albicans* in the oral cavity is the tongue, samples were taken from the posterior part of the tongue with a sterile swab and transferred to a specific culture medium of CHROMagar Candida (CHROMagar 4, Paris, France) in linear streaks. The cultures were then placed in a laboratory incubator for 48 hours at a temperature of 30 - 37°C. The CHROMagar culture medium was prepared according to the manufacturer's instructions: the culture medium powder was added to distilled or deionized water in the ratio of 7.47 grams per liter. The mixture was stirred over a gentle flame until the powder completely dissolved. The solution was then heated to 45 - 50°C and dispensed into sterile plates.

The survey results are based on the color of the microorganism colonies. *C. albicans* turns green, *C. tropicalis* turns metallic blue, *C. krusei* turns pink velvet, and other species are detected in white to purple colors. The colonies were counted and examined by microbiology specialist.

3.6. Methods of Data Analysis

To compare the prevalence of candidiasis between the two groups, a chi-square test and a Fisher's exact test were used. To compare the number of *C. albicans* colonies an independent t-test and the Mann-Whitney test were used.

4. Results

The study included two groups of twenty patients: Group A was a case group of OCP consumers and Group B was a control group of OCP non-consumers.

The purpose was to compare and evaluate positive cultures as well as the number of colonies in the two groups. Patients were mostly asymptomatic; however, within the group taking OCPs, a number of oral candidiasis symptoms such as irritation were reported.

As shown in Table 1 below, the frequency of *C. albicans* and *C. krusei* fungi in Group A was higher than in Group B. The following graph demonstrates the same result.

Table 1. Distribution of the Three Species of *Candida* Between the Two Groups[a]

Type of Fungi	Group A	Group B	P Value
C. albicans	14 (70)	8 (40)	0.03
C. tropicalis	4 (20)	4 (20)	1
C. krusei	6 (30)	3 (15)	0.13

[a]Values are expressed as No. (%).

As shown in Table 1, the frequency of *C. albicans* among OCP consumers was significantly higher than among non-consumers. The chi-square test showed that this difference was not statistically significant (P value = 0.03). The frequency of *C. tropicalis* was similar between the two groups (P value = 1). The frequency of *C. krusei* was higher among OCP consumers than among non-consumers, but the Fisher's exact test showed no significant difference (P value = 0.13).

Due to the abnormal distribution of colony numbers between the two groups, the Mann-Whitney test was used to better compare the results. As shown in Table 2, Figure 2, the number of *C. albicans* (p-value = 0.04) and *C. krusei* (P value = 0.03) among the OCP consumers were significantly

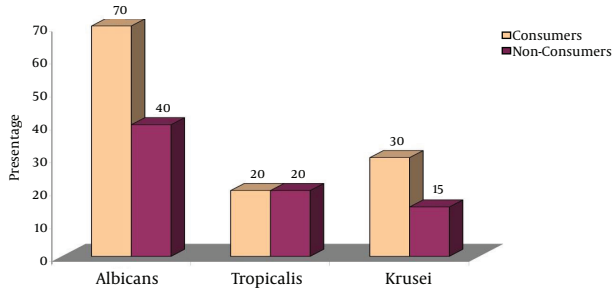

Figure 1. The Relative Frequency of Different Types of Fungi in the Two Groups

higher than among the non-consumers. However, there was no significant difference between the two groups regarding the number of *C. tropicalis* colonies (P value = 0.43).

Table 2. The Mean Number of *Candida* Colonies in each of the Two Groups[a]

Type of fungi	Group A	Group B	P Value
C. albicans	2.6 ± 1	3.5 ± 0.97	0.04
C. tropicalis	2.2 ± 1.8	0.9 ± 0.6	0.43
C. krusei	0.1 ± 0.16	2.2 ± 1.2	0.03

[a]Values are expressed as Mean ± SD.

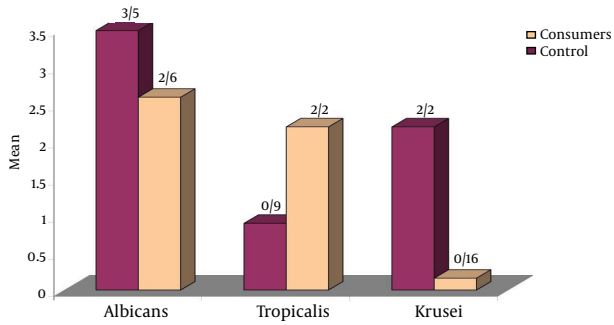

Figure 2. The Mean Number of *Candida* Colonies

5. Discussion

Fungi are eukaryotes that reproduce via budding or the production of filamentous hyphae. *Candida* species are dimorphic fungi that are usually observed in their yeast form at the temperature of the human body and in their mold form at room temperature. *Candida* species are not pathogenic in their yeast form. However, they may become pathogenic when in their hyphae form. *Candida* species are a normal microflora of the skin, thighs, gastrointestinal tract, and vagina. In healthy individuals, different species of *Candida* are benign cohabitants and do not cause disease. However, *Candida*, especially *C. albicans*, is the most common cause of fungal infection in humans. This infection varies from superficial lesions in healthy individuals to fatal disseminated infection in patients with immune disorders (15-17).

Candida species have evolved mechanisms to adapt quickly to changes in the host environment which can be generated by antibiotic therapy, a change in host physiology, or the immune response. In altered conditions, *C. albicans* rapidly shows modified species of its kind, some of which produce a large number of functionally distinct adhesive secretions that play a role in connecting *Candida* to the host's epithelial cells (15, 18). This connection plays a decisive role in the pathogenicity of *Candida* and research indicates that strains that have a low adherence to the epithelial cells in experimental samples are non-pathogenic (19).

C. albicans is the focus of many research papers in oral cancer because among the different types, *C. albicans* is more capable than nitrating the N-methylbenzylamine. This new N-nitrous-methylbenzylamine is a known etiologic agent of esophageal and oral cancer (19, 20).

Candida species usually show adhesion to the superficial layers of the epithelium but in some cases have also been seen in deeper layers. Therefore, *C. albicans* can be seen as a cause of N-nitrous-methylbenzylamine (20).

The estrogen member of the sex hormones group that includes estriol, estradiol, and estrone is mainly created in the ovaries. Many articles have demonstrated the direct correlation between estrogen and vaginal *C. albicans* growth (21, 22). It is believed that estrogen is involved in the growth and increased adhesion of *Candida* species to vaginal epithelial cells and can cause an increase in glycogen levels in vaginal epithelial cells. Glycogen is a nutritional supply for *Candida*. The vaginal lactobacilli also produce glucose by breaking down glycogen and convert it to lactic acid which promotes an acidic environment suitable for *Candida's* growth. Such a correlation has not been seen with progesterone (15, 19, 23-25).

Despite numerous studies on the relationship between OCP, estrogen, and progesterone and vaginal *Candida*, studies of the relationship between OCPs (estrogen and progestin) and oral *Candida* are very limited. Only one article from a French researcher performed in 1974 was found to investigate the direct correlation between OCPs and oral *Candida* (26).

A 1993 study established the role of estrogen in the cell adhesion of vaginal Candida, and then sought to examine the relationship in buccal epithelial cells. The study

showed that during the first half of the menstrual cycle, when estrogen levels are higher, *Candida* adhesion to epithelial cells is greater in comparison to the second half of the cycle during which estrogen levels decrease and progesterone levels increase (27).

Contraceptive pills can have different combinations of estradiol and progestin or progestin alone. All tablets available in the Iranian market contain estradiol. Results of the study showed that the frequency of *Candida*-positive cultures among OCP consumers was higher than that among non-consumers and the number of colonies of *Candida* among OCP consumers was significantly higher than that among non-consumers (28, 29).

Filler and Sheppard (30) also showed that the number of colonies of *C. albicans*, *C. krusei*, and *C. dublins* in the gingival sulcus of OCP consumers was higher than among non-consumers. Their study examined *Candida* species in the periodontal pocket and indicated that the species colonized in the periodontal pocket were *C. albicans* and *C. dublins*. The significant differences between the number of colonies in the periodontal pocket of the two groups OCP consumer and OCP non-consumer could be an indication of the medication's effect. Lyon also states that, although Candida colonization can be seen in the periodontal pocket, the colonization of *Candida* in the periodontal pocket of women is higher than in those of men.

At the beginning of this study the intent was to examine the association between OCP and *Candida* in addition to investigate other factors such as type of medication, time and day of drug consumption, and number of colonies. Due to the lack of cooperation from patients in answering these questions, investigation of some factors was not possible.

5.1. Conclusions

The use of OCPs can increase the likelihood of colonization and growth of *C. albicans* and *C. krusei* in women.

5.2. Study Limitations

One limitation of this study was the difficulty in procuring and maintaining the cultures. Another limitation was the lack of patient cooperation with the researchers.

5.3. Suggestions for Future Studies

We advise conducting a similar study in which mucosal biopsy is done simultaneously, so that the adhesion strength of *Candida* species in both groups can be compared.

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Footnotes

Conflict of Interest: The authors declare no conflict of interest.

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