

Prevalence of *Candida* Species and Oral Candidiasis during Menstrual Cycle in a Sample of Women in Baghdad City

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ABSTRACT:

BACKGROUND:

Menstrual cycle define and reflect the women internal endocrine environment. Ovarian hormones, estrogens and progesterone, are not secreted in constant amounts throughout the cycle. Estrogen and progesterone have been shown to inhibit aspects of both innate and acquired immunity at the systemic or local level furthermore they have been shown to influence on maturation and keratinization of oral mucosa. So there may be possible influence of the menstrual cycle on the adherence of *Candida* to human oral epithelial cells, and may implicate hormonal factors in the aetiology of oral Candidiasis.

OBJECTIVE:

The purpose of this study was to estimate the prevalence of *Candida albicans* and other different *Candida* species in the oral cavity during different periods of menstrual cycle.

METHODS:

One hundred and seventy six oral swabs were taken from 44 females' patients attending dental clinic during the period from May to September 2007 with age range 14-49 years old at different periods of menstrual cycle on days 5, 13, 22 and 28, which represent menstrual phase, ovulatory phase, mid-luteal phase and premenstrual phase respectively.

Swabs were taken from the tongue for isolation of *Candida* species. The swabs were inoculated on Sabouraud's glucose agar incubated at 37° for 72 hours; *Candida* species were identified by gram stain method, germ tube method and fermentation of sugar set.

RESULTS:

The prevalence of *Candida* in the oral cavity at 5th, 13th, 22nd and 28th days of menstrual cycle were 31.8%, 22.7%, 40.9% and 25% respectively. The study shows that the prevalence of *Candida* in the oral cavity was non-significantly higher at 22nd day of menstrual cycle.

CONCLUSION:

There was no significant influence of menstrual cycle on the prevalence of *Candida* in the oral cavity during different periods.

The prevalence of *Candida albicans* was higher during different periods of menstrual cycle in comparison to *Candida tropicalis* and *Candida parapsilosis*.

KEY WORDS: candida, candidiasis, menstrual cycle.

INTRODUCTION:

The reproductive system of the female shows regular cyclic changes may be regarded as periodic preparations for fertilization and pregnancy; the cycle is a menstrual cycle⁽¹⁾. The menstrual cycle define and reflect the women internal endocrine environment. Ovarian hormones, estrogens and progesterone, are not secreted in constant amounts throughout the female monthly sexual cycle⁽²⁾. There are two peaks of estradiol secretion; one just before ovulation and one during the mid-luteal phase.

During the luteal phase, the corpus luteum produce large quantities of progesterone and ovarian secretion increase about 20 folds⁽¹⁾. *Candida albicans* adheres to vaginal and buccal epithelial cells to a significantly greater degree than the other species tested⁽³⁾. Few carriers develop clinical signs of Candidiasis. It is the host's immune competence that ultimately determines whether clearance, colonization, or Candidiasis occurs⁽⁴⁾. The relative influence of progesterone and estrogen on oral mucosal development was investigated. Increased estrogen levels were associated with maturation and keratinization of oral mucosa while high levels of progesterone were associated with retarded

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maturation to intermediate epithelial cells⁽⁵⁾. From the other hand, estrogen and progesterone have been shown to inhibit aspects of both innate and acquired immunity at the systemic or local level^(6,7,8). There may be possible influence of the menstrual cycle on the adherence of Candida to human oral epithelial cells, and may implicate hormonal factors in the aetiology of oral Candidiasis.

PATIENTS AND METHODS:

One hundred and seventy six oral swabs were taken from 44 healthy females at childbearing age from 15 to 49 years old at different periods of menstrual cycle on days 5, 13, 22 and 28, which represent menstrual phase, ovulatory phase, mid-luteal phase and premenstrual phase respectively. The females were non-smokers, had no past or present medical history, under no medication and had regular menses. Specimen collection was done using cotton tipped swab. The swab was taken by gentle rubbing the mucosa of the posterior half of the dorsal surface of the tongue and inoculated directly on Sabouraud's glucose agar for isolation of Candida. The colonies appear medium size, moist, creamy, having a yeasty like odor after four to five days' incubation on the media. Small inoculum from an isolated colony was taken for gram stain film preparation for detection of microorganisms. Candida appears as gram-

positive small oval or budding yeast cells. A very small inoculum from an isolated colony was suspended in 0.5 ml of human serum. The inoculated tubes were incubated at 37°C for 3 hours. After incubation, a drop of the yeast suspension was placed on clean microscopic slide, covered with a cover slip and examined under low power magnification for the presence of germ tube. Production of germ tubes is characteristic of *Candida albicans*. Using the basal fermentation media in a set of test tubes contain glucose, maltose, sucrose and lactose for the differentiation between Candidal species. Take part of colony and inoculate into the tubes containing the set of the sugar broth, emulsify, then incubate at 37°C for 3 days. The result of the test is detected by the change in the color of the broth, with or without the appearance of air bubble in the Durham tube.

RESULTS:

Statistical analysis using Chi-square test revealed that the prevalence of Candida at 22nd day of menstrual cycle was non-significantly higher than the other days of the cycle (P value >0.05) and non-significant relationship was found between the prevalence of Candida in the oral cavity and the menstrual cycle during the different periods (P>0.05) (Figure1). There was no influence of menstrual cycle on the prevalence of Candida in the oral cavity during different periods.

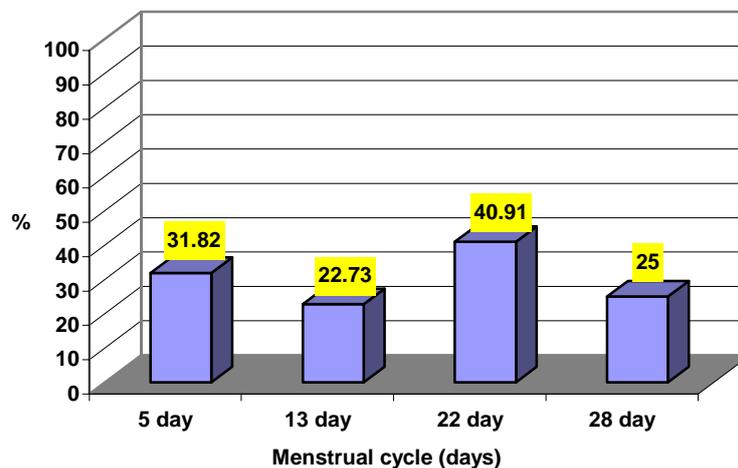


Figure (1): Prevalence of Candida species during different periods of menstrual cycle

Regarding *Candida* species during different periods of menstrual cycle, the prevalence of *Candida albicans* was higher during different periods of menstrual cycle and a high significant difference was found between *Candida* species for each selected day of the cycle (P<0.05). A non-

significant relationship was found between each species of *Candida* and the menstrual cycle during the different periods (P>0.05). So there was no influence of menstrual cycle on the prevalence of each *Candida* species during different periods (Figure2).

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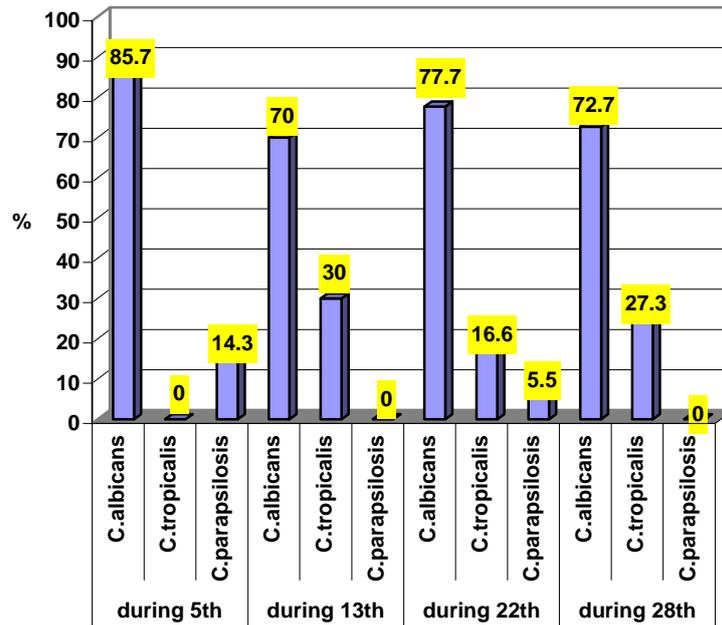


Figure (2) :Prevalence of different species of Candida in the oral cavity during different periods of menstrual cycle

Regarding density of growth and oral Candidiasis during menstrual cycle, the prevalence of scanty Candidal growth was higher during different periods of menstrual cycle and a high significant difference was found between types of growth for each selected day of the cycle ($P < 0.05$). A non-

significant relationship was found between each type of Candidal growth and the menstrual cycle during the different periods ($P > 0.05$). So there was no influence of menstrual cycle on the density of Candidal growth during different periods (Figure3).

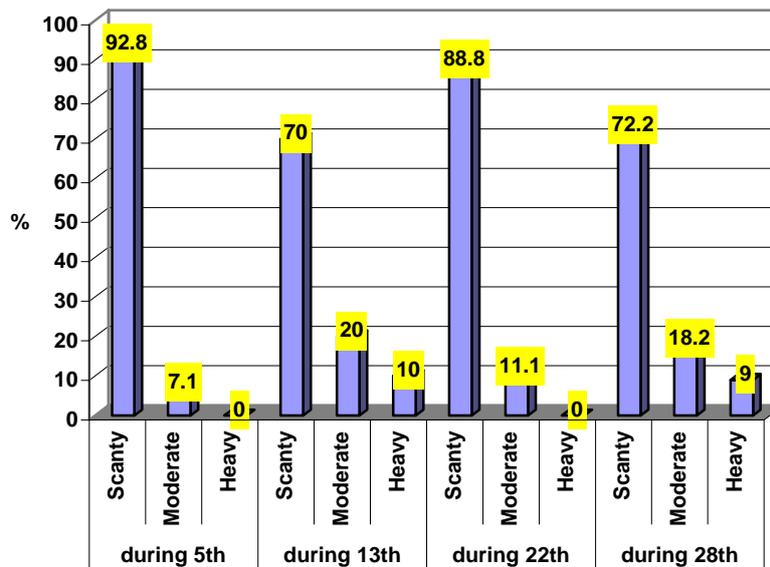


Figure (3) :Types of growth during different periods of menstrual cycle

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Concerning the effect of Age, marital status and time of specimens' collection and oral Candidal flora during menstrual cycle, the relationship between these three factors and positive Candidal carriage during different periods of menstrual cycle was non-significant (Figure4&5).

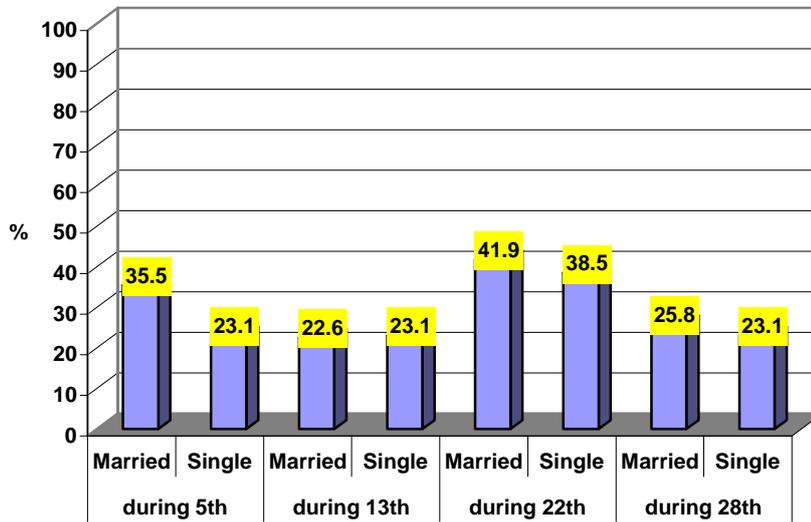


Figure (4) :Relationship between positive culture of Candida species and marital status during different periods of menstrual cycle

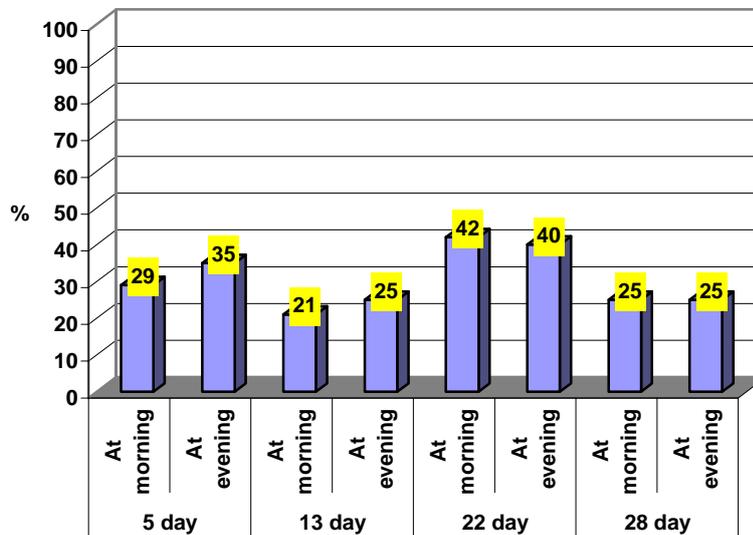


Figure (5) :Relationship between positive culture of Candida species and time of specimens' collection during different periods of menstrual cycle

DISCUSSION:

During the first 5 days of menstrual cycle, oral smear mainly composed of intermediate cells and very few superficial cells. From 6th or 7th day on, the predominant cells are the intermediate cells. Gradually, these cells are replaced by mature superficial cells and the proportion of superficial cells becomes greater. At time of ovulation, the pattern consists predominantly of the superficial cell type. When ovulation has occurred progesterone is being secreted by a corpus luteum, oral smear mainly composed of intermediate cells⁽⁹⁾. There are distinct subpopulations of epithelial cells with high and low affinity for attachment by *Candida albicans* in vitro. Cells of an intermediate size had a greater affinity for yeasts than did cells of other sizes⁽¹⁰⁾. From the other hand, Estrogen and progesterone have been shown to inhibit aspects of both innate and acquired immunity at the systemic or local level⁽⁸⁾ including neutrophil anti-Candida activity⁽¹¹⁾. Both macrophages and polymorphonuclear leukocytes can inhibit hyphal forms of *Candida*⁽¹²⁾. A strong correlation was shown between germination and increased adherence of *Candida albicans* to human buccal epithelial cells⁽¹³⁾. Challacombe (1994) reported that salivary antibodies have been shown to inhibit adherence of *Candida* to buccal epithelial cells⁽¹²⁾. Gomez *et al*, (1993) show significantly higher levels of salivary sIgA in the follicular phase as compared to the luteal phase⁽¹⁴⁾. According to this review, adherence to the epithelial mucus membrane enhance during the luteal phase of menstrual cycle, when estrogen and progesterone levels are elevated. This may explain the high prevalence of *Candida* in the oral cavity on 22nd day of the cycle.

Production of germ tubes is characteristic of *Candida albicans*⁽¹⁵⁾. A strong correlation was shown between germination and increased adherence of *Candida albicans* to human oral epithelial cells⁽¹³⁾. This may explain that the prevalence of *Candida albicans* was higher during different periods of menstrual cycle. The present study was in constant with previous studies, which revealed that *Candida albicans* cells adhered more strongly to epithelial cells than fungal cells of other *Candida* species.

Oral Candidiasis results from yeast overgrowth and penetration of the oral tissues when the host's physical and immunological defenses have been undermined; it is the host's immune competence that ultimately determines whether clearance, colonization, or Candidiasis occurs⁽⁴⁾. The

present study showed that the Candidal growth was scanty in high percentage during different periods of menstrual cycle and there were no signs and symptoms of oral Candidiasis, which mean that the clearance mechanisms still efficient in spite what the previous studies revealed about the inhibitory effect of both estrogen and progesterone on immune system.

Regarding the presence of *Candida* and its relation with age, no precise explanation can be given for this finding, but possibly the menstrual cycle, which reflect the women internal endocrine environment, not affected by age in women during childbearing age. In contrast, *Candida* colonization⁽¹⁶⁾ and oral Candidiasis⁽¹⁷⁾ increase in the elderly.

Regarding the presence of *Candida* and its relation with the time of specimens' collection, no precise explanation can be given for this finding.

Concerning the effect of marital status and the presence of *Candida*, *Candida* can be spread by repeated close contact with the some one who has it. If one person in household has it, there is a possibility everyone else in the house can often get it. A person can also pass *Candida* to another person through certain sexual activities. This can explain what the present study revealed that there was non-significant increased in the prevalence of *Candida* in the married group in comparison with non-married.

CONCLUSION:

1. No significant influence of menstrual cycle on the prevalence of *Candida* in the oral cavity during different periods.
2. The prevalence of *Candida albicans* was higher during different periods of menstrual cycle in comparison to *Candida tropicalis* and *Candida parapsilosis*. High significant difference was found between *Candida* species for each selected day of menstrual cycle, but the menstrual cycle appear to have no significant influence on the prevalence of each *Candidal* species during different periods.
3. Scanty growth of *Candida* in the oral cavity was recorded during different periods of menstrual cycle. But the menstrual cycle appear to have no significant influence on density of *Candidal* growth during different periods.
4. The menstrual cycle appear to have no influence on the etiology of oral Candidiasis.
5. Age, marital status and time of specimens' collection did not exert a significant influence on the prevalence of *Candida* in the oral cavity during different periods of menstrual cycle.

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