Short Communication

Menstrual Cycle Related Changes In Human Whole Salivary pH And Salivary Leukocyte Count

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Abstract

Introduction: Steroid sex hormones influence every system of the body including the oral cavity. It influences the composition and flow rate of saliva. The aim was to estimate the changes in human whole salivary pH and salivary leukocyte count during the various phases of menstrual cycle.

Materials and method: 30 female patients between 18-35 years were included in the study. They were free from any systemic illness or medication. Unstimulated saliva was collected between 10AM and 11AM during the follicular (7th day), ovulatory (12th-14th days), and luteal (21st day) phases and evaluated for changes in salivary pH and leukocyte count.

Result: The salivary pH showed significant difference in value between ovulatory and luteal phase while the salivary leukocyte count showed significant difference in values during all the three phases.

Conclusion: Steroid sex hormones do influence the composition of saliva and thus influence the health of periodontal tissues indirectly.

Key Words: salivary pH, salivary leukocyte count, menstrual cycle

Introduction

Steroid sex hormones play a role on different organ systems of the body. For example oestrogen exerts influence on bones, electrolyte balance and metabolism (1). Within the oral cavity they exert their influence on periodontal tissues, vasculature, bone turnover rate, and wound healing (2, 3, 4).

Variation in the composition and flow rate of saliva occurs due to variation in the levels of these hormones. This influences the integrity of the hard and soft tissue of the oral cavity as saliva though not essential, is required for maintenance of tissues (5, 6).
Thus, aim of the study is to estimate menstrual cycle related changes in human whole salivary pH and salivary leukocyte count.

Materials and Methods

Subjects

Thirty females between 18 years to 35 years of age were a part for this research after obtaining a written consent from them. All the selected females were free from systemic disease or use of medication. Questionnaires were distributed to them to find out the average duration of their menstruation and their menstrual cycle, age, and their diet. The saliva samples were collected from the subjects between 10 AM and 11 AM, at least one hour after breakfast, with the patient being seated, corresponding to the follicular phase (7th day), midcycle or ovulatory phase (12th-14th days), and luteal phase (24th day). The day for collection of saliva for ovulatory and luteal phase was determined for individuals based on their average menstrual cycle duration.

Method for estimation of salivary pH

About 1 ml of unstimulated saliva was collected in a test tube. The pH was measured with pH meter and recorded for each person.

Method for estimation of number of leukocytes in saliva

In a test tube 1 ml of saliva was mixed with 1 ml of Giemsa stain. Using the leukocyte-pipette (as used for normal blood leukocyte count), the salivary cellular elements were counted in a Thoma haemocytometer immediately, in order to avoid further disintegration of the cellular elements. Only clear forms of white blood cells were counted. No disintegration products of these cellular elements were counted.

Statistical analysis

Data was presented as mean±S.E.D. Results collected at 3 phases of menstrual cycle were analyzed using ANOVA test and p-value was obtained. The inter-phase comparison was carried out by Bonferroni test. The p-values less than <0.05 were considered statistically significant. The results were statistically represented using multiple bar charts.

Results

Phases 1, 2, and 3 correspond to follicular, ovulatory and luteal phase respectively.

Salivary pH

The variation in the mean of salivary pH is seen. The p-value obtained after the statistical analysis is 0.012 (Fig. 1, Table I). It is significant. On inter-phase
comparison, only the salivary pH change between ovulatory and luteal phase is significant (Fig. 1, Table II).

**Salivary Leukocyte count**

The variation in the mean of salivary leukocyte count is seen. The p-value obtained is <0.0001, which is highly significant (Table I). Inter-phase comparison shows highly significant difference in the leukocyte count between follicular and ovulatory, ovulatory and luteal, and luteal and follicular phases (Table II).

**Discussion**

The oral cavity is moistened by a film of fluid called saliva, which is produced by the salivary glands. It is a complex fluid and coats the surfaces of teeth and the oral mucosa. Saliva has a number of functions including buffering and antimicrobial activity.

Oestrogen has been shown to influence oral tissues and salivary gland. Studies have shown a decrease in the salivary flow rate following menopause (7, 8). Hence blood levels of oestrogen and salivary flow rate are directly proportional to each other. This may thus influence the properties of saliva including the salivary pH, buffering capacity and leukocyte count. The buffering action helps maintain the pH within the normal range i.e. 6.5-7.5. Bicarbonates are the most important buffer and, to some extent, phosphate ions (6). Christie et al showed carbonic anhydrase may also be one of the contributors to the buffering activity in the oral cavity and thus, maintain salivary pH (9). Composition of saliva has also shown a change in the IgA levels, sialic acid, and the buffer capacity due to variation in levels of oestrogen and progesterone (10). Yalçın et al have showed that salivary pH increases in women on hormone replacement therapy (11). Laine M et al (1988) have shown that salivary pH and buffering capacity decrease during pregnancy due to elevated levels of oestrogen in the plasma (10). Maria I Rockenbach et al (2006) observed the mean salivary pH in non-pregnant women of Brazil was 7.5 (5). The mean salivary pH recorded in our study was lower than reported. Significant difference was found between the ovulatory and luteal phases, which indicates that lower levels of oestrogen during the luteal phase has had an influence on it.

Calouius PEB (1958) stated the normal salivary leukocyte count in healthy individuals with teeth to range between 110 to 1,364 cells per 1 c.mm and during inflammatory periodontal conditions to range between 770 to 11,896 cells per 1 c.mm (12). In the present study, the leukocyte counts fall within the range for healthy individuals. The steroid sex hormones may also alter immunologic factors and responses, including antigen expression and presentation, and cytokine production, as well as the expression of apoptotic factors and cell death (13). Morley has suggested a variation in the neutrophil count during the menstrual cycle owing to variation in the level of oestrogen (14). Barbara and England have also shown two peaks in the neutrophil count coinciding with the peaks of oestrogen in the body with a delay of one or two days. They said it could be due to the influence of oestrogen for the...
first peak and probably a combined action of oestrogen and progesterone for the second peak. Ito et al have quoted oestrogen inhibits the chemotaxis (15). Maywood (1934) found no correlation between the salivary leukocyte and blood leukocyte counts (16). Gingival inflammation increases as the levels of oestrogen increase in the blood as oestrogen creates a favourable environment for the oral microorganism (17). The present study observed highest leukocyte count in saliva during the ovulatory phase. This may be due to an overall increase in leukocyte count predominantly the neutrophils, as the levels of oestrogen are at its peak just before ovulation. The overall salivary leukocyte count fell in the range for healthy individuals with teeth as the subjects had clinically healthy gingival.

Conclusion

Sexual hormones play an important role in influencing organ systems of the body. In the oral cavity it influences the health of periodontium indirectly by affecting the salivary pH and the leukocyte count.

References