The Effect of Menstrual Cycle on Periodontal Health – A Clinical and Microbiological Study

Varsha Shourie[a]/Chini Doraswamy Dwarakanath[b]/Gujjar Vittalrao Prashanth[c]/Ramesh Vishwanathmurthy Alampalli[d]/Shyam Padmanabhan[e]/Shweta Bali[f]

Purpose: Fluctuations in female sex hormones result in changes in the gingival and periodontal tissues. The purpose of this study was to compare the periodontal status of premenopausal women at different time points during their menstrual cycle and to find the associated subgingival microbiota.

Materials and Methods: One hundred premenopausal women participated in the study and were divided into two groups: group I consisted of 50 subjects with clinically healthy gingival, and group II consisted of 50 subjects with chronic gingivitis. Group II was further divided into group IIa and group IIb. Group IIa consisted of 25 subjects who did not receive any periodontal therapy during the study period. All the examinations were performed at three points during the menstrual cycle: ovulation (OV), pre-menstruation (PM) and menstruation (M). Plaque Index (PI), Gingival Index (GI), Papillary Bleeding Index (PBI), probing depth (PD), subgingival temperature (ST) recording, gingival crevicular fluid (GCF) collection, and estimation and microbiological examination using the benzoyl-DL-arginine-naphthylamide (BANA) test was carried out. For group IIb subjects, all the examinations were performed again during the next menstrual cycle, which followed 4 weeks after periodontal therapy.

Results: Women with clinically healthy gingiva exhibited negligible changes throughout the menstrual cycle, whereas women with gingivitis showed aggravated inflammation during ovulation and pre-menstruation as compared to menstruation. However, there was no alteration in subgingival microbiota. After treating gingivitis, the next menstrual cycle following 4 weeks after periodontal therapy was monitored, and no periodontal changes were detected.

Conclusion: Ovarian hormones have a negligible effect on clinically healthy periodontium. However, these hormones may exaggerate pre-existing inflammation in gingival tissues, but the clinical significance of these changes remains uncertain.

Key words: gingival crevicular fluid, gingivitis, inflammation, menstrual cycle, periodontium, sex steroid hormones

Oral Health Prev Dent 2012; 10: 185-192 *Submitted for publication: 06.05.11; accepted for publication: 11.11.11*

[a] *Senior Lecturer, Department of Periodontology and Implantology, M.A.Rangoonwala College of Dental Sciences and Research Centre, Pune, Maharashtra, India.*

[b] *Professor and Head, Department of Periodontology and Implantology, Director of Post- graduate Studies, Oxford Dental College and Hospital, Bangalore, India.*

[c] *Professor and Head, Department of Periodontology and Implantology, Al-Ameen Dental College and Hospital, Bijapur, Karnataka, India.*

[d] *Professor, Department of Periodontology and Implantology, Oxford Dental College and Hospital, Bangalore, India.*

[e] *Professor, Department of Periodontology and Implantology, Vydehi Institute of Dental Sciences, Bangalore, Karnataka, India.*

[f] *Professor, Department of Periodontology and Implantology, Santosh Dental college and Hospital, Ghaziabad, UP, India.*

Correspondence: *Dr V. Shourie, Department of Periodontology and Implantology, M.A. Rangoonwala College of Dental Sciences and Research Centre, PUNE-411001, Maharashtra, India. Tel: +91-9823624656. Email: drvarshashourie@yahoo.com*

Hormones are specific regulatory molecules that modulate reproduction, growth and development, maintenance of the internal environment, as well as energy production, utilisation and storage (Mariotti, 1994). Hormonal effects reflect physiological/pathological changes in almost all types of tissues of the body. Targets for a number of hormones such as androgens, estrogen and progesterone have also been localised in periodontal tissues (Gornstein et al, 1999). Consequently, systemic endocrine imbalances may have an important impact in periodontal pathogenesis.

Researchers have shown that changes in periodontal conditions might be associated with variations in sex hormone levels. This association is evident in periodontal disease classification (Armitage, 1999), which includes the following hormone-

related disease categories: puberty-associated gingivitis, menstrual cycle-associated gingivitis and pregnancy-associated gingivitis. Gingival changes have also been reported to occur in relation to the different phases of the menstrual cycle. Over 60 years ago, Mühlemann (Mealey and Moritz, 2003) described a case of 'gingivitis intermenstrualis', which he observed as consisting of bright red haemorrahagic lesions of the interproximal papillae identified prior to menstruation. Holm-Pedersen and Loe (1967) found that the hormonal variation of the menstrual cycle has no effect on clinically healthy gingiva, but pre-existing gingivitis seems to become aggravated during menstruation. Lindhe and Attstrom (1967) found the gingival fluid flow to be greater on the day of ovulation than during the menstrual phase.

Hence, it is possible that hormonal changes during the menstrual cycle may alter the vulnerability of the female gingiva; however, very few studies have been carried out to substantiate this phenomenon and the available literature is inconclusive. In addition, there are hardly any reports in the literature in which microbiological examinations have been carried out to detect possible alterations in the subgingival microbiota during different phases of the menstrual cycle (Fisher et al, 2008). Moreover, the effect of the menstrual cycle on oral tissues is not only of interest to dental researchers, but also to clinical dentists, since many of the tissue changes encountered during different phases of the menstrual cycle may influence the response to various types and phases of dental treatment. Thus, this study was conducted to evaluate the effect of the menstrual cycle on periodontal health.

MATERIALS AND METHODS

A total of 100 pre-menopausal women (age range 18–40 years, mean 25.2 ± 4.5 years), fulfilling the criteria determined for the study, were recruited using stratified random sampling from a pool of patients from the outpatient department of Oxford Dental College, Hospital and Research Centre, Bangalore, India. Informed written consent was obtained from all subjects prior to their enrollment. The study was approved by the ethics committee of Oxford Dental College.

The inclusion criteria were:

1. Pre-menopausal women in the age group 18–40 years.
2. Normal and regular menstrual cycle, 25 to 35 days long (mean 28.2 ± 0.06). The subjects were asked to record the dates of their menstruation for a period of 6 months to determine the regularity and length of their menstrual cycle.
3. Available for clinical examination in the upcoming 3 months. The exclusion criteria were:
   a. Pregnancy or intention to become pregnant in the coming year.
   b. Metabolic or systemic condition that might affect the periodontium.
   c. Use of oral contraceptives.
4. Women undergoing immunosuppressive therapy.
5. History of antibiotic therapy in the last 6 months.
6. Women requiring prophylactic antibiotics before treatment.
7. Smokers and/or tobacco chewers.

The first examination was carried out on the second day of their menstruation and the second examination was scheduled during ovulation.

Ultrasound monitoring was performed to detect ovulation. The third examination was carried out during pre-menstruation, i.e. following ovulation and prior to the onset of the next menstruation. Hence, all the subjects were examined at three different time points: ovulation (OV), pre-menstruation (PM) and menstruation (M). The group IIb subjects were examined again during the next menstrual cycle which followed 4 weeks after periodontal therapy. The subjects were divided into two groups: group I consisted of 50 subjects with clinically healthy gingiva, based on clinical findings (Armitage, 1996); group II consisted of 50 subjects whose gingiva showed clinical signs of inflammation but there was no evidence of attachment loss (Armitage, 1996). Group II was further subdivided into groups IIa and IIb. Group IIa consisted of 25 subjects belonging to group II who did not receive any periodontal therapy during the course of the study. Group IIb consisted of 25 subjects belonging to group II who received periodontal therapy, which included oral hygiene instructions and scaling during the course of the study.

A detailed case history was recorded on a specially prepared case sheet at the first examination time point.

The following measurements were performed for all patients at each of the three time points:

1. Plaque Index (PI) (Silness and Loe, 1964) measured at six sites around each tooth; 2. Gingival Index (GI) (Loe and Silness, 1963) measured at six sites around each tooth; 3. Papillary Bleeding Index (PBI) (Saxer and Mühlemann, 1971) around each tooth; 4. probing depth (PD): measured at six sites around each tooth.

Gingival crevicular fluid (GCF) was collected from the selected site/tooth at all three examination time points. Microbiological examination was carried out at all three examination time points, using subgingival plaque samples obtained from the selected site/tooth.

Selection of site/tooth for sampling plaque and GCF and recording subgingival temperature

For group I subjects, only the maxillary buccal or mandibular lingual first molar surfaces were selected for collection of plaque and GCF samples and recording of subgingival temperature (Wojcicki et al, 1987). Only one site was selected for each patient to carry out all the above-mentioned procedures, and the selected site remained the same for the subsequent examination time points.

For subjects belonging to groups IIa and IIb, before receiving treatment, the tooth with the highest gingival index score at the first examination time point was selected for collection of plaque (Yanover and Ellen, 1986), GCF and recording of subgingival temperature. The sample collections and subgingival temperature recordings were performed on this same tooth at each subsequent examination time point during the menstrual cycle. In group IIb subjects, after receiving treatment during the next menstrual cycle four weeks after periodontal therapy, the same tooth was used for sampling, which was selected prior treatment.

Microbiological procedure

The commercially available diagnostic test kit based on the hydrolysis of benzoyl-DL-arginine-naphthylamide (BANA) was used to assess the presence of *Treponema denticola*, *Porphyromonous gingivalis*, and *Tanerella forsythia* along with *Capnocytophaga* species (Kazor et al, 1999; Morita 2001). After removal of supragingival plaque, subgingival plaque of the representative site or tooth was collected using a sterile curette. The samples were placed on a BANA-impregnated strip (OralB Laboratories; Redwood City, CA, USA) along the lower border of the test card. An upper reagent strip containing Evan's black dye was then activated by moistening with distilled water, and the two strips were folded over so they contacted one another. After folding, the card was incubated at 35°C for 5 min (Feitosa, 1993; Loesche, 1997). Results that produced a definite blue and a pale blue were recorded as positive and weak positive, respectively. No reaction was recorded as negative.

Gingival crevicular fluid sampling

GCF collection was performed in the afternoon hours. Any supragingival plaque was carefully removed prior to sampling. Strips of Whatman filter paper cut to 2 x 10 mm were used. The tooth/site and the surrounding tissues were dried by a blast of air and kept dry by means of cotton rolls placed in the vestibule. The strips were placed at the entrance of the orifice of the gingival crevice and parallel to the long axes of the tooth (Lindhe, 1967). Extreme care was taken to avoid any physical irritation of the crevicular epithelium. The strips were left in place for 3 min (Lindhe, 1967). After removal, the strips were stained in a 0.2% solution of ninhydrin (Lindhe, 1967).

Recording of subgingival temperature

A sensor was designed and fabricated for this study, and the instrument used to measure the subgingival temperature was a thermistor (Kung et al, 1990) connected to a battery-powered digital thermometer (micro care thermometer, GE thermometrics; Bangalore, Karnataka, India).

The sensor or thermistor is a disk made of a ceramic-based semiconductive metal oxide that has a protective covering made of an epoxy resin known as thysol. The sensor was passed through a commercially available 20-gauge (0.812 mm), 3-cm-long stainless steel needle for easy access and insertion into the gingival sulci. The sensor present at the terminal end of the thermometer was placed gently in the sulcus and designed such that a beep sound would be heard upon completion of the temperature recording, which can be viewed in the display window.

Sequence of sampling

The clinical parameters, including plaque index, gingival index, papillary bleeding index and probing depths, were recorded in the morning hours. Following the recording and assessment of the above-mentioned clinical parameters, microbiological examination was carried out. GCF collection and estimation and subgingival temperature recording were performed in the afternoon hours.

Statistical analysis

Frequency tables were generated using SPSS (version 13 for MS Windows; Chicago, IL, USA). To determine whether the mean values of different indices at different time points during the menstrual cycle – ovulation, pre-menstruation and menstruation – differ statistically significantly, one-way ANOVA using Bonferroni's correction for post-hoc multiple group comparisons was applied. $P < 0.05$ was considered to be statistically significant, and $P < 0.01$ was considered highly significant.

RESULTS

The plaque index values were basically identical throughout the study, i.e. for ovulation, pre-menstruation and menstruation measurements, in all the groups (Fig 1).

The mean gingival index was extremely low in group I, so for ease of calculation, the value was rounded off to 0. Hence, the mean gingival index for group I was considered 0 at all three time points during the menstrual cycle.

For Group IIa, the mean gingival index during ovulation, pre-menstruation and menstruation was 1.35 ± 0.25, 1.18 ± 0.2 and 0.95 ± 0.30, respectively (Fig 2). Despite the similarity in the plaque index at the three points, the gingival index was significantly higher during ovulation (OV vs M, $P < 0.01$; OV vs PM, $P < 0.01$) and pre-menstruation (PM vs M, $P < 0.01$) as compared to menstruation (0.95 ± 0.30) (Fig 2).

For group IIb subjects, the mean pre-treatment gingival index during ovulation, pre-menstruation and menstruation was 1.35 ± 0.21, 1.23 ± 0.22 and 1.05 ± 0.26, respectively (Fig 2). Similar to group IIa subjects, group IIb subjects exhibited significantly higher pre-treatment gingival index values at the ovulation (OV vs M, $P < 0.01$; OV vs PM, $P < 0.01$) and pre-menstruation time points (PM vs M, $P < 0.01$) as compared to menstruation, despite the similarity in the plaque index throughout the cycle. In group IIb, the mean post-treatment gingival index values decreased significantly to 0, and exhibited no change throughout the cycle (Fig 2).

The papillary bleeding index scores showed patterns identical to those of the gingival index scores in the present study. The mean papillary bleeding index values at all three time points during the menstrual cycle were very small decimals and were rounded off to 0 for group I subjects (Fig 3).

For group IIa subjects, the mean papillary bleeding index during ovulation, pre-menstruation and menstruation was 1.17 ± 0.27, 1.02 ± 0.26 and

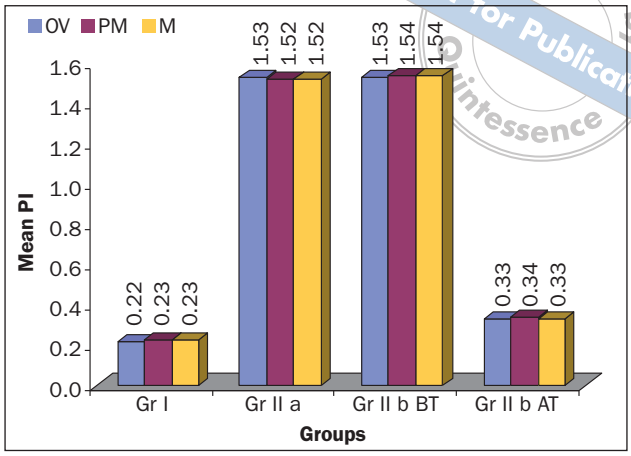

Fig 1 Intra- and intergroup comparison of plaque index. OV = ovulation; PM = pre-menstruation; M = menstruation.

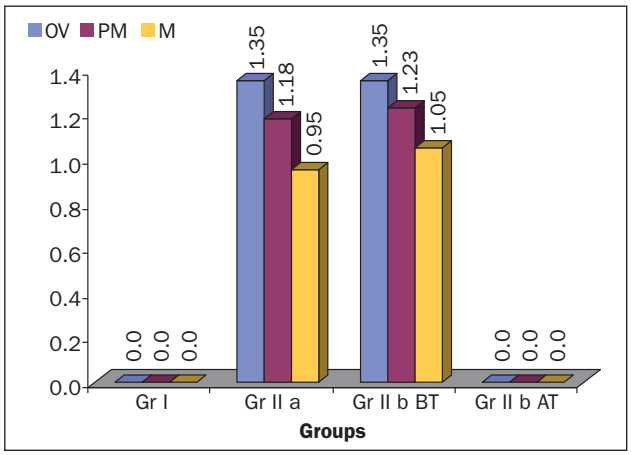

Fig 2 Intra- and intergroup comparison of gingival index. OV = ovulation; PM = pre-menstruation; M = menstruation.

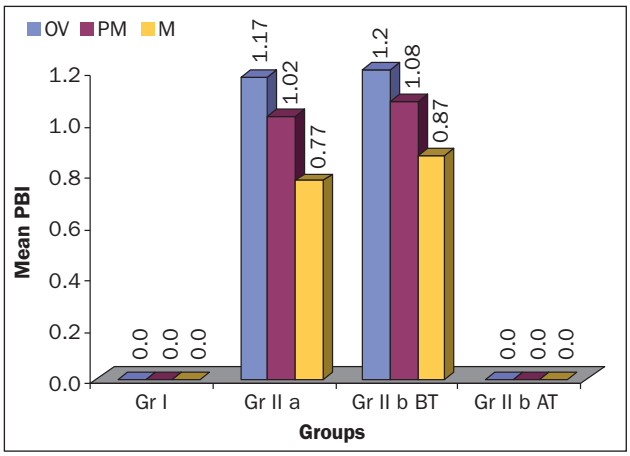

Fig 3 Intra- and intergroup comparison of papillary bleeding index. OV = ovulation; PM = pre-menstruation; M = menstruation.

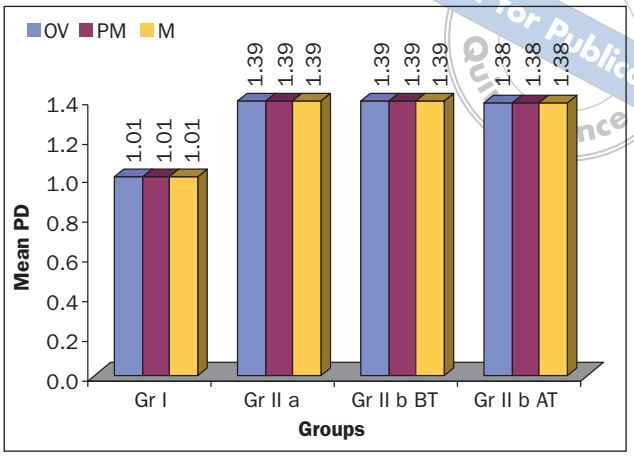

Fig 4 Intra- and intergroup comparison of probing depth. OV = ovulation; PM = pre-menstruation; M = menstruation.

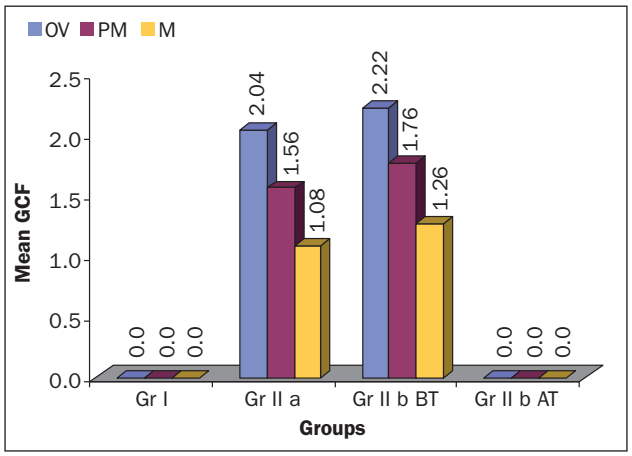

Fig 5 Intra- and intergroup comparison of gingival crevicular fluid. OV = ovulation; PM = pre-menstruation; M = menstruation.

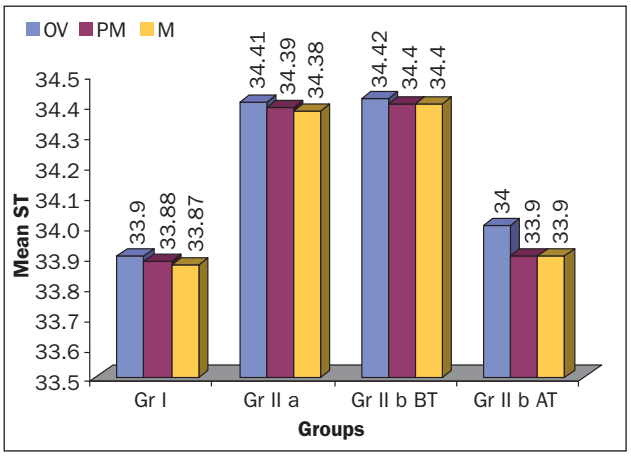

Fig 6 Intra- and intergroup comparison of subgingival temperature. OV = ovulation; PM = pre-menstruation; M = menstruation.

0.77± 0.27, respectively. Despite there being no change in the plaque index throughout the cycle, the papillary bleeding index was higher at the ovulation (OV vs M, $P < 0.01$; OV vs PM, $P < 0.01$) and pre-menstruation time points (PM vs M, $P < 0.01$) than during menstruation (Fig 3).

In group IIb subjects, the mean pre-treatment papillary bleeding index during ovulation, pre-menstruation and menstruation was 1.20 ± 0.22, 1.08 ± 0.25 and 0.07 ± 0.27, respectively (Fig 3). Similar to group IIa, group IIb exhibited higher papillary bleeding index values at the ovulation (OV vs M, $P < 0.01$; OV vs PM, $P < 0.01$) and pre-menstruation time points (PM vs M, $P < 0.01$) than during menstruation, in spite of the plaque index remaining the same throughout the cycle. In group IIb, the mean post-treatment papillary bleeding index values decreased significantly to 0 and did not change throughout the menstrual cycle (Fig 3).

Probing depths

The overall mean probing depth in groups I, IIa and IIb (before treatment) was 1.01 ± 0.7 mm, 1.39 ± 0.14 mm, 1.39 ± 0.12 mm respectively. The probing depths remained similar throughout the menstrual cycle in each of the groups (Fig 4). There was no significant difference in probing depths in group IIb before and after treatment (Fig 4).

Microbiological examination using BANA

Results of BANA were assessed as positive or negative, depending on the colour change (as given by the manufacturer) on the test strip used. None of the patients in any of the groups exhibited a positive BANA result at any time during the study.

GCF volume

The mean GCF volume collected in group I (throughout the menstrual cycle) was a very small decimal value and was rounded off to 0 for ease of calculation. In group IIa, the mean GCF volume collected was 2.04 ± 0.54 ml, 1.56 ± 0.55 ml and 1.08 ± 0.64 ml during ovulation, pre-menstruation and menstruation, respectively (Fig 5).

There was a statistically significant increase in GCF collected at ovulation (OV vs M, $P < 0.01$; OV vs PM, $P < 0.01$) and pre-menstruation time points (PM vs M $P < 0.01$) as compared to menstruation. Similar to group IIa, in group IIb, the mean volume of GCF collected before treatment was greater at ovulation (OV vs M, $P < 0.01$, OV vs PM, $P < 0.01$) and pre-menstruation time points (PM vs M, $P < 0.01$) than during menstruation: 2.22 ± 0.54 ml, 1.76 ± 0.54 ml and 1.26 ± 0.54 ml, respectively (Fig 5).

Compared to the pre-treatment volume, the post-treatment GCF volume collected in group IIb decreased significantly to almost 0.

Subgingival temperature

The subgingival temperature recorded in group I was 33.9 ± 0.17°C, 33.83 ± 0.17°C and 33.87 ± 0.17°C at ovulation, pre-menstruation and menstruation time points, respectively (Fig 6). The difference in subgingival temperature at the three time points of the menstrual cycle was not statistically significant. In group IIa, the temperature recorded at ovulation, pre-menstruation and menstruation was 34.41 ± 0.10°C, 34.39 ± 0.13°C and 34.38 ± 0.13°C, respectively. These differences were not statistically significant. Similarly, in group IIb, pre-treatment subgingival temperature during ovulation, pre-menstruation and menstruation was 34.42 ± 0.13°C, 34.40 ± 0.09°C and 34.40 ± 0.11°C, respectively (Fig 6); the differences were not statistically significant. After treatment, the respective subgingival temperatures dropped significantly to values resembling those of group I (Fig 6).

DISCUSSION

Microbial dental plaque initiates periodontal disease, but the form and severity of the disease is dependent on the environmental, genetic and host defenses to this challenge (Kinane et al, 2006). Systemic factors and exposure to different environmental conditions may modify the normal defenses and influence the resultant periodontal disease (Kinane et al, 2006). Among the systemic factors, sex hormones have been suggested as important modifying factors that may influence the pathogenesis of periodontal disease (Kinane et al, 2006).

Researchers in the field of periodontology have shown that changes in periodontal conditions might be associated with variations in sex hormone levels. This association is evident in the classification of periodontal disease. The majority of clinical observations were based on women at various times in their lives. There is, however, very little available in the literature concerning the relationship of menstruation to conditions in the oral cavity. This lack of definitive information is probably due to the transient nature of the menstrual period and its short-term effects on the oral structures.

Moreover, there has been only one report in the literature investigating the subgingival microflora throughout the menstrual cycle (Fisher et al, 2008). The results of the present study highlight two important points: first, women with a clinically healthy periodontium did not exhibit any significant periodontal changes during their menstrual cycles, whereas those having pre-existing gingivitis showed aggravation of inflammation at ovulation and pre-menstrual time points as compared to menstruation. Second, there were no changes in the subgingival microbiota during the menstrual cycle. These findings are in agreement with earlier reports.

In terms of the Plaque Index and the gingival index of the subjects with clinically healthy periodontium, the results of the present study were comparable with the findings in Holm-Pederson and Loe's study (1967), i.e. no statistically significant differences were found in the above-mentioned parameters throughout the menstrual cycle. The variation in the flow of GCF (from clinically healthy gingival crevices) throughout the cycle was not statistically significant, both in the present study

and in that of Holm-Pederson and Loe (1967). Holm-Pederson and Loe (1967) concluded that the menstrual cycle had no significant influence on the clinical state of the gingiva when the investigations were confined to surfaces which were subjected to controlled oral hygiene.

In another study, Lindhe and Attstrom (1967) examined the variation in gingival fluid flow during the menstrual cycle in a group of young females suffering from mild gingivitis. They concluded that fluid flow was greater during ovulation than during menstruation, which is in agreement with the findings of the present investigation.

In the present study, the women who suffered from gingivitis exhibited an increased GCF flow during ovulation as compared to the time of menstruation.

Macheti et al (2004) carried out a study to determine the effect of menstrual cycle on periodontal health, finding that despite the similarity in plaque index throughout the menstrual cycle, the gingival index was significantly higher at the ovulation and pre-menstruation time points as compared to menstruation. The mean gingival index value of their study group suggests that the subjects had some pre-existing gingival inflammation. In the present study, the subjects with gingivitis exhibited higher gingival index scores during ovulation and pre-menstruation than during menstruation, which is in accordance with Macheti et al (2004).

In the present study, the subjects exhibited higher papillary bleeding index scores during ovulation and pre-menstruation as compared to menstruation. The use of the papillary bleeding index gave a more objective measure of gingival inflammation.

The most interesting result of the present study was that, when the subjects with gingivitis were treated and there was resolution of inflammation in the gingival tissues, there were no significant periodontal changes observed through the next menstrual cycle which followed four weeks after periodontal therapy. This finding further substantiates the observation that if the tissues are free of inflammation, the effect of sex hormones is negligible. It is suggested that the phenomenon of increased gingival inflammation (given pre-existing gingivitis) may be attributed to the increased levels of the female sex hormones during ovulation (when estradiol is at its peak and progesterone comes into play) and a second peak of these hormones just before menstruation (when progesterone is at its peak and estradiol also reaches high levels).

It is noteworthy that despite the similarity in plaque index scores, the gingival index and papillary bleeding index scores as well as GCF volume were significantly different throughout the cycle in women suffering from gingivitis, which suggests that this phenomenon was not related to a shift in plaque index.

A direct effect of female sex hormones on periopathogenic micro-organisms in promoting gingival inflammation can be hypothesised. The fluctuations in sex hormone levels during the menstrual cycle could be accompanied by similar fluctuations in bacterial flora. Hence, it was relevant to carry out microbiological examinations at the different time points through the menstrual cycle in the present study.

The BANA test has been used to detect the most common periopathogens, i.e. *Porphyromonas gingivalis, Treponema denticola* and *Tanerella forsythia* along with *Capnocytophaga* species during different phases of the menstrual cycle (Feitosa et al, 1993). In the present study, none of the subjects showed a positive result using BANA in any of the groups, suggesting that there was no alteration of the subgingival microflora during the menstrual cycle. At the time of attainment of puberty and during pregnancy, there is a much higher production of female sex hormones compared to different phases of the menstrual cycle, which might be the reason for altered subgingival microflora in pregnant females and in individuals attaining puberty.

Basal core body temperature is known to be elevated during ovulation (Guyton, 2000). Although the subgingival temperatures recorded in this study were slightly higher at ovulation than during menstruation, particularly for groups IIa and IIb (before treatment), these differences were not statistically significant in any of the groups. Whether the higher temperatures observed were due to increased inflammation (Maiden et al, 1998) during ovulation or because of the above-mentioned phenomenon is uncertain.

The present study did not include subjects suffering from periodontitis nor those having irregular menstrual cycles. Future studies are needed to determine if the fluctuating levels of female sex hormones together with other factors produce a cumulative effect and ultimately lead to periodontal breakdown in patients having periodontitis. In addition, whether subjects with irregular menstrual cycles are more prone to pathological changes in the periodontium is a question that needs to be addressed in future investigations.

The clinical significance of the periodontal changes discussed above in subjects with gingival inflammation remains uncertain. There have been no reports in the literature of cases where chronic gingivitis has progressed to periodontitis because of the effect of the female sex hormones per se. However, one cannot ignore the observed effect of these hormones on inflamed gingival tissues. Thus, it is the duty of the clinician to reinforce the importance of oral hygiene maintenance, especially in female patients, while scientists need to focus more on sex-specific research.

CONCLUSION

The results of the present study indicate that the ovarian hormones may exaggerate pre-existing inflammation in the gingival tissues, although the subgingival microbiota do not change. However, if the tissues are clinically healthy and free of inflammation, these hormones do not have any significant effect on the periodontal tissues during a woman's menstrual cycle.

REFERENCES

1. Armitage GC. Periodontal disease diagnosis. Ann Periodontal 1996;1:37.
2. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1–6.
3. Feitosa AC, Amalfitano J, Loesche W.J. The effect of incubation temperature on the specificity of BANA (Benzoyl DL-arginine-napthylamide) test. Oral Microbiol Immunol. 1993;8:57–61.
4. Fisher CC, Persson RE, Persson GR. Influence of the menstrual cycle on the oral microbiota in women – A case control study including men as a control subject. J Periodontol 2008;79:1996–1973.
5. Gornstein RA, Lapp CA, Bustos-Vald, Zamorano P. Androgens modulate interleukin-6 production by gingival fibroblasts *in vitro*. J Periodontol 1999;70:604–609.
6. Guyton AC. Female physiology before pregnancy 535 and the female hormones. In: Guyton AC, Hall JE (eds). Textbook of medical physiology, ed 10. Philadelphia: WB Saunders, 2000.
7. Holm-Pederson P, Löe H. Flow of gingival exudate as related to menstruation and pregnancy. J Periodontal Res 1967;2:13–20.
8. Kazor C, Taylor WG, Loesche WJ. Prevalence of BANA hydrolyzing periodontopathic bacteria in smokers. J Clin Periodontal 1999;26:814–821.
9. Kinane DF, Peterson ML, Stathopoulou PG. Environmental and other modifying factors of the periodontal diseases. Periodontol 2000 2006;40:107–119.
10. Kung RJV, Ochs B, Goodson JM.Temperature as a periodontal diagnostic. J Clin Periodontal 1990;17:557–563.
11. Lindhe J, Attstrom R. Gingival exudation during the menstrual cycle. J Periodontal Res 1967;2:194–198.
12. Loesche WJ, Kazor CE, Taylor GW. The Optimization of BANA test as a screening instrument for Gingivitis among subjects seeking dental treatment. J Clin Periodontal 1997;24:110–114.
13. Loe H, Silness J. Periodontal disease in pregnancy.I.Prevalence and severity. Acta Odontol Scand 1963;21:533–551.
14. Machtei EE, Mahler D, Sanduri H, Peled M.The effect of menstrual cycle on periodontal health. J Periodontol 2004;75:408–412.
15. Maiden MFT, Janner ACR, Macuch PJ, Murray Z, Kent RL Jr. Sublingual temperature and microbiota in initial periodontitis. J Clin Periodontal 1998;25:789–793.
16. Mariotti A. Sex steroid hormones and cell dynamics in periodontium. Crit Rev Oral Biol Med 1994;5:27–53.
17. Mealey BL, Moritz AJ. Hormonal Influences: effect of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. Periodontol 2000 2003;32:59–81.
18. Morita M, Wang H. Relationship of sulcular sulfide level to severity of periodontal disease and BANA Test. J Periodontol 2001;72:74–78.
19. Mühlemann HR, Son S. Gingival sulcus bleeding – a 512 leading symptom in initial gingivitis. Helvetica Odontologica Acta 1971;15:107–113.
20. Silness J, Loe H. Periodontal disease in pregnancy II– Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–135.
21. Wojcicki CJ, Harper DS, Robinson PJ. Differences in periodontal disease-associated microorganisms of subgingival plaque in prepubertal, pubertal and postpubertal children. J Periodontol 1987;58:219–223.
22. Yanover L, Ellen RP. A Clinical and Microbiologic Examination of gingival disease in parapubescent females. J Periodontol 1986;57:562–567.