The Saudi Dental Journal (2023) **35**, 251–254

King Saud University

The Saudi Dental Journal

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ORIGINAL ARTICLE

Comparing gingival inflammation and salivary acidity to hormonal variation during menstruation

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Received 2 October 2022; revised 12 February 2023; accepted 14 February 2023
Available online 20 February 2023

KEYWORDS

Gingivitis;
Gingival inflammation;
Menstrual cycle;
Female sex hormones;
Salivary pH

Abstract *Introduction:* Hormonal changes are associated with healthy female in different stages of her life, such as menarche, pregnancy, and menopause. The main female hormones (i.e., Estrogen and progesterone) act as modulators and effectors during different phases of menstrual cycle. Additionally, these hormones were shown to affect the body inflammatory status. Few studies addressed gingivitis and female hormones. Although gingivitis main cause is dental biofilm, female hormones might modulate this inflammation. If no treatment provided, gingivitis might proceed to periodontitis and alveolar bone destruction may appear.

The study aim: was to assess the changes in gingival inflammation, and salivary pH in relation to changes in hormonal levels during different menstrual phases in young females.

Method: 25 healthy young females with regular menstrual cycles were included. The volunteers were invited to visit the clinic during their 2nd day of menstrual cycle (menses phase (MP)). During the visit, plaque index (PLI) and gingival index (GI) were scored. Additionally, salivary pH was calculated. Follow-up readings were taken on the 20th day of menstruation (pre-menstrual phase (PMP)). The difference in PLI, GI and salivary pH was analysed using *t*-test and chi-square test.

Results: The study showed that PLI and GI increased statistically significantly with increasing levels of female hormones during PMP and decreased during MP (P-value = 0.012 for PLI and 0.0003 for GI). Salivary pH decreases slightly during PMP and saliva becomes more alkaline during MP (P-value = 0.015).

Conclusion: The study showed increased gingival inflammation and plaque accumulation during the premenstrual period. It is recommended to raise awareness of gingival inflammation among

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Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2023.02.001

adult females to prevent progressive changes of periodontitis. However, due to the limited sample size of this study, a comprehensive population-based study is needed to support the findings.

1. Introduction

Female sex hormones are not always at the same level. During female life, there are different phases that she may go through: Menstruation, pregnancy, use of contraceptives, menopause, and the use of replacement sex hormones. The menstrual cycle represent the main sign of puberty in females. The ovary, pituitary gland, hypothalamus, and endometrium work together harmoniously to provide the menstrual cycle. It can be modulated and influenced by different levels of sex hormones (Silberstein and Merriam, 2000). The four main sex hormones that show variation in their levels during the menstrual cycle, are estrogen, progesterone, follicular stimulating hormone (FSH), and luteinizing hormone (LH). From the first day of menstruation, the first week is characterized by low levels of all female sex hormones. In the second week of menstruation, all female sex hormones, with the exception of progesterone, rise to their highest level and prepare for ovulation. In the third week of the menstruation, there is a sudden decrease in these hormones and a gradual increase in progesterone. Then, from the middle of the third week estrogens and progesterone rise steadily. Toward the end of the fourth week, estrogen and progesterone hormone levels decrease (Allen et al., 2016; Groome et al., 1996; Welt et al., 2003). During the fourth week of menstruation, women experience exacerbation of many chronic diseases, such as diabetes mellitus (Gugapriya et al., 2014). In addition, changes in metabolism might occur during this period (Wallace et al., 2010). It has also been shown that gingival inflammation increases during the premenstrual phase (Setijanto et al., 2019).

Gingivitis is a form of periodontal disease characterised by inflammation of the gingival tissue without destruction of the underlying alveolar bone. The main cause of gingivitis is the accumulation of bacterial biofilm on adjacent teeth (Lang & Bartold, 2018). Gingivitis can be diagnosed clinically by inspection. The inflamed gingiva is characterised by redness, oedema, and bleeding. Steroid hormones may act directly on the gingiva. However, pre-existing plaque-induced gingivitis may be an important factor in detecting hormone-related changes during menstrual cycle (Holm-Pedersen & Loe, 1967).

Studies have conflicting results regarding gingivitis during premenstrual period. Some have shown that clinical changes in the periodontal tissue have been noted during periods of hormonal fluctuations (Amar & Chung, 1994). Many women reported an increase in gingival inflammation and discomfort associated with their menstrual cycle (Machtei et al., 2004). Other studies have shown that changes in the sex steroid hormones during the menstrual cycles might have a limited effect on the gingival inflammatory status (Becerik et al., 2010), and female patients are not aware of any changes in their gingivae during the menstrual cycle (Amar & Chung, 1994). Salivary flow rate could also be influenced by hormonal changes in females. Women have a lower flow rate and seem to have greater fluctuations in salivary pH than men (Lukacs & Largaespada, 2006).

1.1. Aim of the study

The aim of this study was to assess the changes in gingival inflammation and the amount of plaque accumulation between the second and 20th day of the female menstrual cycle. These are the two days when female sex hormones are at their lowest and highest level of them, respectively.

A second objective was to determine the difference in salivary acidity between the second and 20th day of the menstrual cycle.

2. Materials and methods:

25 female dental students volunteered to participate in this study. The volunteers signed an informed consent form and a copy of the form and information sheet was kept with them. The Department of Periodontology, College of Dentistry, University of Tikrit (Iraq) was informed through the annual planning of research activity report 2013. The age ranged from 18 to 20 years. All subjects had a regular menstrual cycle (approximately 28–30 days) with no previous hormonal problems.

On the 2nd day of the menstrual cycle, when the estrogen and progesterone hormones were low, a saliva sample was taken after being stimulated by raw chewing gum (sugar and flavour free). The salivary pH was measured with WTW series Inolab machine. On the same day, the subjects' teeth and gingivae were examined using a dental mirror and a UNC-15 periodontal probe. The Loe and Silness gingival index (GI) was recorded (Loe & Silness, 1963). This index is composed of 4 scores ranging from 0 (no inflammation) to 3 (severe gingivitis). The Silness and Loe plaque index (PLI) was also recorded (Silness & Loe, 1964).

Salivary pH, GI and PLI were recorded again on day 20 of the menstrual cycle when levels of the hormones estrogen and progesterone were too high. Volunteers who used mouthwashes only at the second visit were excluded.

The collected data were analysed using IBM SPSS statistics 25. Means and percentages were calculated. A paired *t*-test was used to determine the difference of GI and PLI between the 2nd and 20th visit readings. Salivary acidity readings were divided into 4 groups: Group 1 included salivary pH between 6 and 6.5; Group 2 included salivary pH between 6.6 and 7.1; Group 3 included salivary pH between 7.2 and 7.6; and Group 4 included salivary pH between 7.7 and 8.2. Chi-square test was used to analyse the difference between the four groups of salivary acidity and the two readings of the subjects. A p-value of less than 0.05 was considered statistically significant.

3. Results:

On the 2nd day of menstruation, the mean PLI was 0.25 (range 0–1.29). It increased to the double on the 20th day of menstruation (The mean PLI was 0.58 (range 0–1.71)). Statistically, this increase was highly significant (p-value = 0.012).

An increase was also seen in the mean GI between the 2 visits (i.e., the 2nd and 20th day of menstruation). The mean GI was 0.251 (range 0–1) on the 2nd day of menstruation, and 0.89 (range 0–3) on the 20th day. Statistical analysis revealed a highly statistically significant change (p-value = 0.0003). Please refer to Table 1.

Regarding the acidity of the saliva, 68 % of the subjects had moderately alkaline saliva (pH 7.7–8.2) on the 2nd day of menstruation, 28 % had a slightly alkaline pH between 7.2 and 7.6, and only 4 % were between 6 and 6.5. None of the subjects, however, had a neutral salivary pH. On the 20th day of menstruation, salivary pH was slightly alkaline (pH 7.2–7.6) in 64 % of the volunteers. 28 % of the volunteers had moderate alkaline saliva (pH 7.7–8.2). 4 % of the volunteers had a salivary pH between 6.6 and 7.1, and the remaining 4 % had a salivary pH between 6 and 6.5. The chi-square test showed a statistically significant difference (P = 0.015). Please see Table 2.

4. Discussion

This study investigated the changes in gingival inflammation and plaque accumulation in relation to physiological hormonal fluctuations during the female menstrual cycle. Changes in salivary pH were also investigated. An increase in the gingival inflammation and plaque accumulation was demonstrated with the increase in sex steroids hormones levels. In addition, the acidity of saliva increased slightly with increasing sex steroid hormones.

Although the results of the study were significant, it has limitations. The main limitation in this study is the short follow-up period and the lack of controls. Subjects were only examined at two visits, without being re-examined at their next menstrual cycle. The lack of follow-up over more than one menstrual cycle could increase the likelihood of confounding the results. In addition, evaluating oral indices at more than two visits per month (i.e., One visit per week) would provide more detail on the relationship between changes in plaque accumulation and gingivitis in relation to varying hormone levels during the menstrual cycle. The sample size of the study is small, which has an impact on the generalisability of the study. In addition, the volunteers were dental students. This population may have greater intention on their oral hygiene and may take care of their oral health if they feel they need more care. This could influence the results of the study. In particular, our study looks at plaque accumulation and gingival inflammation at two different periods of the months. This means that they might be aware of the changes of their gingival inflammation and increase their efforts to reduce it. However, during data collection and dental examination we excluded those who made extra efforts to reduce dental plaque and control inflammation by using mouthwashes in the second visit.

Table 1 Mean PLI and mean GI in relation to menstrual days.

	2nd day of menstruation	20th day of menstruation	*t*-test	p- value
Mean PLI	0.248	0.581	−2.62	0.012*
Mean GI	0.251	0.890	−4.02	0.03*

* Statistically significant.

Table 2 Salivary pH in relation to menstrual days.

	Group1 pH 6–6.5	Group2 pH 6.6–7.1	Group3 pH 7.2–7.6	Group4 pH 7.7–8.2
2nd day of menstruation	1	0	7	17
	4 %	0 %	28 %	68 %
20th day of menstruation	1	1	16	7
	4 %	4 %	64 %	28 %
X^2 = 8.688				
p- value = 0.0147*				

* Statistically significant.

This study showed an increase in the gingival inflammation with increasing sex steroid hormones during the 20th day of menstruation. Other studies showed similar results (Machetei et al., 2004; Shourie et al., 2012; Koreeda et al., 2005). However, few studies did not agree with these results (Amar & Chung, 1994; Becerik et al., 2010). The interaction of estrogen and progesterone with inflammatory mediators could help explain the increased levels of inflammation observed during periods of hormonal fluctuation (Markou et al., 2009; Soory, 2000). Others suggested that sex steroid hormones exacerbate pre-existing gingivitis (Holm-Pedersen & Loe, 1967). They show that gingivitis cannot be initiated by hormonal changes alone. The normal pH range of saliva is between 6.2 and 7.6, with 6.7 being the average pH. In the oral cavity, saliva maintains the acidity of the environment close to neutrality (6.7–7.3) by two mechanisms. First, saliva washes away carbohydrates that could be metabolised and fermented by bacteria producing acids as by-products. Second, the buffering activity of saliva neutralises the acidity of beverages and foods, as well as bacterial activity (Baliga et al., 2013).

Based on the statistical analysis we can see that the pH of the saliva decreases during the second visit (i.e., in the days preceding the menstrual cycle), while the pH of the saliva increased on the second day of menstruation. Studies have shown female steroid hormones can be detected in saliva during ovulation (Chatterton et al., 2005; Lu et al., 1999) which could have an influence on the acidity of saliva.

From the results of this study, it is important to raise the awareness of gingival inflammation among women and the possibility of developing gingivitis in the post-ovulation period. In addition, the drop in the salivary acidity during this period might have an adverse effect on dental in general, such as tooth decay.

5. Conclusion

Within the limitations of this study, our study showed that plaque accumulation and gingival inflammation increased significantly in the post-ovulation period and decreased in the pre-ovulation period. In addition, the saliva pH decreases slightly when the level of female hormones increases, and saliva becomes more alkaline when the level of hormones decreases. However, we propose a large population-based cohort study in which periodontal parameters are examined during the different phases of menstruation, and followed for more than one

menstrual cycle. It is recommended to determine female steroid hormones in blood and saliva, and to determine salivary pH, to understand the changes in these parameters during the different phases of the female menstrual cycle.

6. Declaration

The authors declare there is no conflict of interest to be mentioned in this study. All authors contributed to this study. Both authors agreed to the final version of the manuscript.

This research project was conducted in the College of Dentistry, Tikrit University, Iraq.

7. Authors statement

Nahla Kamal Asaad (NKA) and Hadeel Mohammed Abbood (HMA) recruited the volunteers, obtained consent forms and informed the subjects about the procedures. HMA examined the periodontal health and recorded plaque index and gingival index. NKA collected saliva samples and analysed the salivary p., HMA ran the statistical analysis and wrote the first version of the manuscript. Both authors reviewed the final version of the manuscript and agreed to be published. No funding was received for this study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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