

Original Article**Influence of menstrual cycle and salivary  $\beta$ -estradiol on volatile sulfur compound**

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**Abstract**

**BACKGROUND:**  $\beta$ -estradiol is a steroid sex hormone that plays important role in oral physiology. The aim of this study was to determine the association of the menstrual cycle and salivary  $\beta$ -estradiol with the production of volatile sulfur compounds (VSC).

**METHODS:** In this cross-sectional study, twenty female dental students in good oral and general health were recruited for evaluation of VSC, salivary flow,  $\beta$ -estradiol concentration, oral dryness feeling and dysmenorrhea. Data were analyzed by marginal homogeneity test and xtgee model.

**RESULTS:** The only covariates that significantly related to VSC were being at the follicular phase ( $B = -0.21$ ,  $P = 0.02$ ), being at the pre-menstrual phase ( $B = -0.25$ ,  $P = 0.01$ ), stressfulness ( $B = 0.02$ ,  $P = 0.01$ ) and oral dryness feeling ( $B = 0.34$ ,  $P = 0.02$ ). Salivary  $\beta$ -estradiol was at the level of  $59.7 \pm 31.2$  in those having halitosis ( $VSC \geq 75$ ) while in the others ( $VSC < 75$ ) it was about  $40.8 \pm 18.0$  ( $P < 0.009$ ). This difference was not statistically significant in multivariate analysis.

**CONCLUSIONS:** The menstrual cycle, stress and oral dryness were the most important determinants of halitosis. The salivary  $\beta$ -estradiol level could not explain such relationship as its effect was eliminated considering the other factors.

**KEY WORDS:** Halitosis, menstrual cycle,  $\beta$ -estradiol, volatile sulfur compound.

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Halitosis is a term defined as offensive mouth odor that can have intra or extra-oral sources.<sup>1</sup> Volatile sulfur compounds (VSC) caused by activation of anaerobic gram negative micro-organisms on sulfur containing substrates in oral cavity such as food, saliva, blood and epithelial cells can have effect on patient's social health<sup>2</sup>. This activation mainly happens with gingival bleeding, periodontitis, tongue coating, dental caries, periodontitis around the implantation, dental necrosis, oral ulcers, food debris and other factors.<sup>3</sup>

There are many extra-oral factors that cause

oral mouth odor such as respiratory disturbances like sinusitis, nasal discharge, infection, lung carcinoma and other systemic disease including digestion disease, diabetes mellitus and uremia.<sup>3,4</sup> Endocrine system affects oral homeostasis. Previous studies showed that sex hormones have influence on periodontal tissue.<sup>5,6</sup> For instance, some reports indicated that women in menopause phase feel more oral dryness and discomfort.<sup>7</sup>

$\beta$ -estradiol is a steroid sex hormone that plays important role in oral physiology. It seems that the decrease of this hormone during

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menopause influences on oral epithelium and causes discomfort during this period.<sup>7</sup> To the best of our knowledge, there are few articles about the association between steroid sex hormones and halitosis.<sup>2</sup> The aim of this study was to evaluate the influence of menstrual cycle and estradiol hormone on production of VSC in healthy young women volunteers.

## Methods

This study was approved by the ethic committee of Kerman University of Medical Sciences (No. K/89/10). In this cross-sectional survey we recruited the study subjects using convenience sampling method. Based on the findings from Calil et al.<sup>2</sup> that recruited 14 women in their study, a power analysis was done and it was found that with a sample of 20, we would have a power of 98% (considering Alpha as the level of 5%). Therefore, twenty undergraduate female dental students between 20 to 26 years old were voluntarily participated in this study. Participants were signed a consent form after that the aim and method of the study were fully explained for them.

### *Inclusion and exclusion criteria*

All participants should have been healthy and free of systemic disease, had at least 20 teeth, no dental caries, calculus and no defective restoration. All subjects who had either smoking habit or were on medicine consumption were excluded from the study. All subjects completed an information form including demographic data, history of smoking and habits.

All subject received periodontal examination by a periodontist for approving periodontal status of the subjects. They could be included in this study if they had plaque index and bleeding index under 10%, probing depth equal or less than 3 mm and without tongue coating.<sup>8</sup> Women could participate in this study if they had regular menstruation cycle, did not have pregnancy and did not use contraceptive or hormonal therapy during previous 6 months.

### *Procedure*

The subjects were asked to detect the different

times of their menstrual cycles for scheduling. These times were including 1/ days 1-4 for menstrual phase, 2/ days 7-11 for follicular phase, 3/ days 24-27 for the pre-menstrual phase.<sup>2</sup> Some rules for the day of sampling were explained for subjects. They were prohibited using any spicy foods like onion and garlic and smelling cosmetics for 24 hours before the experiment. They should avoid using breath freshener or mouth rinse for 1 week and eating or drinking at least 8 hours before the experiment but they were allowed to drink water up to 3 hours before it.<sup>2</sup>

Participants were asked to complete the Beck Anxiety Inventory (BAI) questionnaire. BAI is a 21-item questionnaire that the degree of severity of anxiety is scored using Likert scale (0-7 indicating no anxiety, 8-15 mild, 16-25 moderate and 26-63 sever anxiety). To prevent possible anxiety, we avoided doing experiment during examination time or other stressful situations.<sup>2,9</sup>

Saliva was collected by spitting method for 5 minutes in the plates that were weighted before. Salivary flow rates were assayed in ml/min after weighting by digital scale (AND-GF). The mean salivary flow less than 0.1 ml/min was defined as dry mouth.<sup>10</sup> Then, the VSC measurements were done by sulfide monitor (Halimeter, Interscan, Ca, USA). The subjects were requested to avoid opening mouth one minute before measurement and deep breathing during that time. A plastic straw (sampler) were kept on the posterior dorsal of the tongue by subjects while not touching the oral mucosa. They were asked to keep opening their mouth about 1.5 cm during sampling. The device were recorded the peak of VSC after 3 times measurements. The mean of VSC were evaluated as without halitosis for values less than 75 ppb, physiologic or mild halitosis for 75-110 ppb and sever halitosis for values equal or more than 110 ppb.<sup>11</sup>

Saliva samples were kept in freezer at -20°C. The mean of  $\beta$ -estradiol hormone was evaluated by radioimmunoassay (RIA) method using commercial kit (Diasorin, Italy) after centrifuging 5000 rpm for 5 minutes and filtering

by Elisa method.

### Statistical analysis

The relation of halitosis and different phases of menstruation cycle was determined by the marginal homogeneity test. The VSC score (outcome variable) has been transferred to normal distribution by square root transformation. Xtgee model (normal distribution for the dependent variable and the log as the link function) was applied for estimating the effects of menstrual cycle considering different covariates on the VSC score. P value < 0.05 was used as the level of significance. Stata (version 10 for windows, TX, USA) was the statistical software used for the analysis.

### Results

Finally, the data of 20 subjects were analyzed. Overall, the percentage of severe and mild halitosis was about 16.7% and 43.3%, respectively. At the menstrual phase, about 50% of the subjects had severe halitosis while it was reduced to less than 1% in both follicular phase and pre-menstrual phases ( $P < 0.001$ ) (Figure 1).

Sever Halitosis was reported among 58.3% of those with dysmenorrhea, and it was about 27.5% among the others ( $P = 0.8$ ). Almost all with oral dryness feeling presented halitosis (VSC  $\geq 75$ ) compared to others with only 55.5% halitosis ( $P = 0.035$ ) (Table 1).

Multivariate analysis (xtgee model) showed

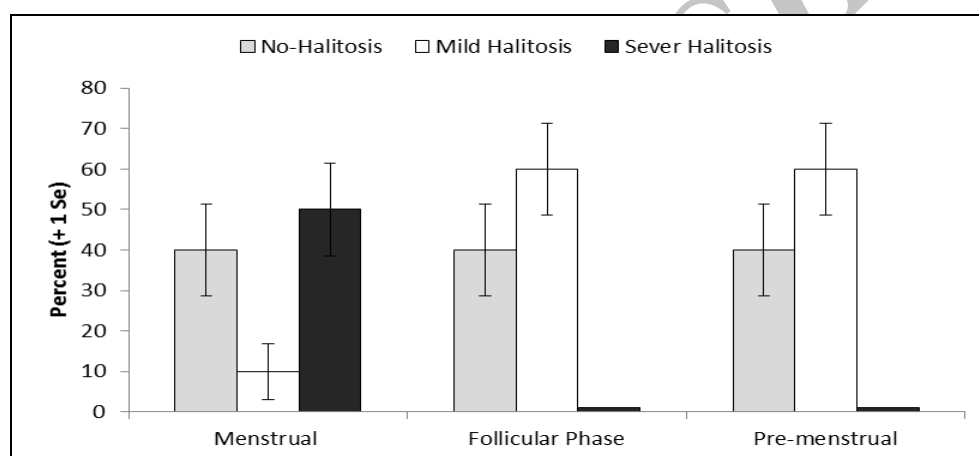


Figure 1. The percentage of halitosis by menstrual cycle

Table 1. Halitosis in relation to oral dryness feeling and dysmenorrhea

Covariates	VSC $\geq 75$	VSC < 75	P-value
Oral Dryness feeling (%)	6 (100%)	0	0.035
Dysmenorrhea at menstrual phase (%)	8 (66.7%)	4 (33.3%)	0.81
Dysmenorrhea at Pre-menstrual phase (%)	7 (100%)	0	0.015
Saliva $\beta$ - estradiol pg/ml (Mean + SD)	59.7 + 31.2	40.8 + 18.0	0.009
Saliva Weight gr/5min (Mean + SD)	1.68 + 1.3	1.9 + 0.6	0.32

Table 2. Multiple xtgee modeled the relationship of different covariates against square root of volatile sulfur compounds

Covariates	Beta	Se	P-value
Follicular phase	-0.21	0.09	0.02
Pre-menstrual phase	-0.25	0.08	0.001
Stress	0.02	0.003	0.001
Oral dryness feeling	0.34	0.15	0.02
Dysmenorrhea	0.04	0.09	0.64
Saliva $\beta$ -estradiol (pg/ml)	-0.003	0.001	0.06
Age	-0.01	0.03	0.88

the covariates that were significantly related to square root of VSC were follicular phase, pre-menstrual phase, stress and oral dryness (Table 2).

## Discussion

The results of this study showed that mouth odor was influenced by different phases of menstruation. It was associated with other covariates like stress and oral dryness feeling. The salivary  $\beta$ -estradiol Level could not explain the relationship between menstrual cycle and the halitosis and its effect was ruled out considering the other factors. The present study showed that the level of VSC was higher in menstruation in comparison with follicular and pre-menstruation phases. This result is in accordance with previous studies.<sup>2,9,12</sup>

In the unadjusted analysis, it was shown that there was significant relationship between  $\beta$ -estradiol and halitosis. It seems that steroid hormones have effect on oral mucosa in women. There are few studies about the effect of sex hormones on oral mucosa in women.<sup>13</sup> Study of Agha-Hosseini et al. showed that there was a negative correlation between the concentration of  $\beta$ -estradiol hormone and oral dryness feeling in menopause women.<sup>7</sup> Although  $\beta$ -estradiol level has fluctuation in menstrual cycle and is reduced in pre-menstrual and menstrual phases, we found that the different level of this hormone did not have significant effect on halitosis when the association was adjusted. In addition, it has been shown that estradiol hormone can cause increase in the number of anaerobes such as *Prevotella intermedia*,<sup>14</sup> but Calil et al. did not see this effect in their study.<sup>2</sup> Fischer et al. could not find any cyclic variation of microbial flora during menstruation cycle too.<sup>15</sup>

A decreased salivary flow was not observed significantly during different phases of the menstrual cycle. Calil et al. had the same result in their research.<sup>2</sup> There was significant corre-

lation between sensation of oral dryness feeling with halitosis but not salivary flow rate. Although the exact mechanism of influence of oral dryness feeling on production of VSC has been not understood, it seems that the sensation of oral dryness is associated with the psychological aspects and anxiety on mouth odor.

All women who suffered from pre-menstrual syndrome had higher VSC. Previous studies had similar results.<sup>9,16</sup> This might be caused by the pain and anxiety in this special period of time in women life. In adjusted analysis this was not statistically significant. Anxiety and stress could be influenced by fluctuation of hormonal changes during different phases of menstrual cycle and affect psychiatric symptoms such as oral dryness feeling and eventually can increase the VSC. Anxiety and stress could have effect on sympathetic system activation and then the composition of saliva,<sup>17</sup> but its mechanism has not been fully understood so far.

## Conclusion

In conclusion, this study showed the possibility of influence of sex hormones on production of VSC. Halitosis is becoming one major problem for society and more research on it can pave the way for its solution. It seems that different sex hormones are involved on mouth odor and this subject needs more research for discovering the possible causes.

## Conflict of Interest

Authors have no Conflict of Interest.

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## References

1. Rayman S, Almas K. Halitosis among racially diverse populations: an update. *Int J Dent Hyg* 2008; 6(1): 2-7.
2. Calil CM, Lima PO, Bernardes CF, Groppo FC, Bado F, Marcondes FK. Influence of gender and menstrual cycle on volatile sulphur compounds production. *Arch Oral Biol* 2008; 53(12): 1107-12.

3. Van den Broek AM, Feenstra L, de BC. A review of the current literature on aetiology and measurement methods of halitosis. *J Dent* 2007; 35(8): 627-35.
4. Cortelli JR, Barbosa MD, Westphal MA. Halitosis: a review of associated factors and therapeutic approach. *Braz Oral Res* 2008; 22(Suppl 1): 44-54.
5. Guncu GN, Tozum TF, Caglayan F. Effects of endogenous sex hormones on the periodontium--review of literature. *Aust Dent J* 2005; 50(3): 138-45.
6. Machtei EE, Mahler D, Sanduri H, Peled M. The effect of menstrual cycle on periodontal health. *J Periodontol* 2004; 75(3): 408-12.
7. Agha-Hosseini F, Mirzaei-Dizgah I, Mansourian A, Khayamzadeh M. Relationship of stimulated saliva 17 $\beta$ -estradiol and oral dryness feeling in menopause. *Maturitas* 2009; 62(2): 197-9.
8. Muhlemann HR, Son S. Gingival sulcus bleeding--a leading symptom in initial gingivitis. *Helv Odontol Acta* 1971; 15(2): 107-13.
9. Queiroz CS, Hayacibara MF, Tabchoury CP, Marcondes FK, Cury JA. Relationship between stressful situations, salivary flow rate and oral volatile sulfur-containing compounds. *Eur J Oral Sci* 2002; 110(5): 337-40.
10. Liu XN, Shinada K, Chen XC, Zhang BX, Yaegaki K, Kawaguchi Y. Oral malodor-related parameters in the Chinese general population. *J Clin Periodontol* 2006; 33(1): 31-6.
11. Murata T, Yamaga T, Iida T, Miyazaki H, Yaegaki K. Classification and examination of halitosis. *Int Dent J* 2002; 52(Suppl 3): 181-6.
12. Tonzetich J, Preti G, Huggins GR. Changes in concentration of volatile sulphur compounds of mouth air during the menstrual cycle. *J Int Med Res* 1978; 6(3): 245-54.
13. Meurman JH, Tarkkila L, Tiitinen A. The menopause and oral health. *Maturitas* 2009; 63(1): 56-62.
14. Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. *J Periodontal Res* 1980; 15(2): 111-22.
15. Fischer CC, Persson RE, Persson GR. Influence of the menstrual cycle on the oral microbial flora in women: a case-control study including men as control subjects. *J Periodontol* 2008; 79(10): 1966-73.
16. Calil CM, Marcondes FK. Influence of anxiety on the production of oral volatile sulfur compounds. *Life Sci* 2006; 79(7): 660-4.
17. Garrett JR, Ekström J, Anderson LC. *Glandular Mechanisms of Salivary Secretion*. 1<sup>st</sup> ed. Basle: S. Karger Publishing; 1998.