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# مجلة ميسان للدراسات الاكاديمية

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# The relationship of salivary cortisol and Volatile Sulfur Compounds with Halitosis among pregnant woman

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**Abstract:**

Halitosis, commonly known as bad breath, significantly impacts an individual's social life, self-esteem, and overall quality of life. While local oral factors predominantly cause halitosis, systemic conditions and hormonal changes, especially during pregnancy, can also influence its occurrence. Conducted at Al-Sader Teaching Hospital in Missan, Iraq, from October 2023 to February 2024, this cross-sectional study involved ninety female participants, divided equally into a patient group of pregnant women aged 20 to 40 years suffering from halitosis and a healthy control group of non-pregnant women. The study meticulously adhered to inclusion and exclusion criteria to ensure a focused and relevant participant pool. Measurements of VSCs and salivary cortisol levels were undertaken using standardized methods and advanced technology. The study found a significant elevation in the levels of cortisol and VSCs among the pregnant group compared to the control group, suggesting that pregnancy-induced hormonal changes and stress response could contribute to or exacerbate conditions leading to halitosis. Elevated cortisol levels and VSCs in pregnant women underscore the multifaceted relationship between pregnancy, hormonal changes, stress, and oral health, particularly concerning halitosis.

**Keywords**: Halitosis, Pregnancy, Volatile Sulfur Compounds, Salivary Cortisol, Oral Health, Hormonal Changes.

## Introduction:

Halitosis, also known as bad breath, is a complex condition that can significantly impact person's social life, self-esteem, and overall quality of life. Recent research suggests that local oral factors do not always cause halitosis but can also be influenced by systemic conditions and hormonal changes. In this context, that led to increased interest in the relationship between halitosis and various physiological and microbial factors in pregnant women (Alzoman et al., 2022).

Pregnancy is a remarkable physiological state characterized by a cascade of hormonal fluctuations and adaptations that extend beyond the reproductive system(Mohammed, 2024), which; that dynamics are known to exert profound effects on various bodily systems, including the oral cavity (Fakheran et al., 2020). The oral environment, a complex ecosystem of microorganisms and biochemical processes, can profoundly affect hormonal shifts during pregnancy (Alzoman et al., 2022).

The interplay between these hormonal changes, the presence of volatile sulfur compounds (VSCs), salivary cortisol levels, salivary flow rate, and the colonization of bacteria, particularly *Porphyromonas gingivalis*, on the tongue of pregnant women, presents an intriguing avenue for scientific exploration (Giannella et al., 2023; Ye et al., 2019a) .

Volatile sulfur compounds, primarily hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, are recognized as key players in the etiology of halitosis since malodorous compounds are generated through the microbial metabolism of sulfur-containing amino acids in the oral cavity (Milella, 2015). While the relationship between VSCs and halitosis is well-established, recent research suggests that other factors, such as hormonal changes and systemic health conditions, could influence their production and subsequent impact on breath odor (Smith et al., 2023). Consequently, investigating the association between halitosis and VSCs in pregnant women may shed light on the complex interplay between oral microbiota and systemic influences.

Salivary cortisol, a steroid hormone secreted by the adrenal glands in response to stress, has been implicated in various physiological processes, including immune modulation, metabolism, and maintenance of homeostasis (Yahya et al., 2019). In light of this, during pregnancy, salivary cortisol levels undergo dynamic changes levels and fluctuate due to hormonal fluctuations, potentially affecting the oral environment (Yassin & Al-Mizraqchi, 2023). Moreover, a previous study showed that stress can alter oral homeostasis and is associated with gingivitis, periodontal diseases, development of halitosis too (Batista et al., 2017).

**Aim:** The study aims to investigate the association between halitosis in pregnant women and the levels of volatile sulfur compounds and salivary cortisol, emphasizing the role of hormonal changes and stress on oral health during pregnancy.

**Martial and methods:**

In this cross-sectional study conducted at Al-Sader Teaching Hospital in Missan, Iraq, from October 2023 to February 2024, a total of 123 participants were initially tested. However, 33 individuals who did not meet the inclusion criteria were excluded, resulting in the recruitment of ninety female participants. These participants were then divided into two distinct groups: the patient group (P), which consisted of forty-five pregnant women aged between 20 and 40 years, and the healthy control group (C), comprising forty-five non-pregnant, healthy women. To ensure informed participation, all participants were provided with an information sheet and a consent form, along with a brief explanation of the study's objectives and its significance.

**Ethical approval:**

The study protocol was approved by the scientific committee at the Basic Science Department/College of Dentistry/University of Baghdad, on 15/10/2023, (Project No. 845823)

The study's inclusion criteria targeted pregnant women within the age range of 20 to 40 years, specifically those in their third trimester and experiencing their first pregnancy. Conversely, the exclusion criteria were designed to omit participants showing signs and symptoms of any systemic diseases, those with a history of chronic conditions such as diabetes mellitus, hypertension, heart diseases, tonsillitis, sinusitis, and pharyngitis, as well as individuals with a history of smoking or alcohol consumption.

**Data Collection:**

**1- Halitosis Status Assessment:**

Trained examiners conducted clinical assessments of halitosis using standardized methods, including odor scoring (Halimeter).

**2- Volatile Sulfur Compounds (VSCs) Measurement:**

Breath samples were collected from participants using a Halimeter device which quantitatively measured VSC levels in parts per billion (ppb), providing an objective assessment of halitosis (Gufran et al., 2016).

The Halimeter device is designed for the measurement of Volatile Sulfur Compounds (VSCs) in exhaled breath, which are often indicators of oral health and can be associated with bad breath. The user were positioned 1 cm away from the sensor and exhale steadily through the mouth after inhaling through the nose. This breath was maintained for 5 seconds, and the test can be performed up to once per hour.

**Samples Collection:**

Five ml of unstimulated saliva sample were collected in the morning between 9-12 am, by drooling passively for five minutes into saliva collection tube according to the University of South California School of Dentistry Guidelines for saliva collection. Furthermore, it was emphasized that participants were instructed to maintain a state of relaxation in their posture for the whole of the data collection procedure. In addition, any samples verified to have blood contamination had to be discarded. Salivary samples were collected throughout the time interval from 9:00 AM to 12:00 PM (Qasim, 2013). After the subject's oral cavity was rinsed with sterilized water one time, a period of 1-2 minutes was observed to allow for the clearing of water. The saliva samples were centrifuged at a rotational speed of 3000 revolutions per minute (rpm) for a period of 10 minutes(Abdulqader & Mahmood, 2024; Hassan & Athab, 2024). The liquid fraction that remained after centrifugation, referred to as the supernatant, was afterwards stored in Eppendorf tubes at a temperature of -20˚C until for determined analyzed for salivary levels of Salivary Cortisol using Electrochemiluminescence immunoassay (ECLIA) techniques kits.

**Statistical Analysis:**

The Statistical Package for Social Sciences (SPSS) version 26 and Microsoft Excel 2013 were employed for data processing. Differences between group means were studied, Mann-Whitney U, and Spearman correlation were also used.

**Results:**

This study contained ninety Iraqi women between 20-40 years who met the inclusion criteria and were divided into two groups. The patient group included 45 pregnant subjects experiencing halitosis, while the Control Group included 45 healthy, non-pregnant women.

The demographic characteristics of 90 subjects participated in this study are illustrated in (Table 1), which shown that the mean age was (26.38±5.824) years for P group, and (29.13±6.937) years for healthy controls. There were non-significant differences (P>0.05) in the age between the two study groups.

**Table 1: Demographic Characteristic of age in the Study Groups.**

| Characteristics | | P group n= 45 | Control group n= 45 | P-value |
|---|---|---|---|---|
| Age | Range | 20 - 39 | 20 – 39 | 0.099 |
| | Mean | 26.38 | 28.44 | |
| | SD | ±5.824 | ±5.937 | |
| | SE | 0.868 | 0.885 | |
| Significant, p<0.05, non-signifcant, (p>0.05).,SD: Standard Deviation, SE: Standard Erarr | | | | |

The results of this study revealed a significant elevation in the median levels of Cortisol hormone among P group (16.48 pg/ml) compared to the control group (6.72 pg/ml), (P=0.000), as observed in Table (2).

**Table 2: Descriptive and Analytic Statistics of Median values of Cortisol hormone for the P & Control groups**

| Cortisol hormone | P group n= 45 | Control group n= 45 | Mann-Whitney U | p- value |
|---|---|---|---|---|
| Median | 16.48 pg/ml | 6.72 pg/ml | 312.01 | 0.000* |
| Minimum | 3.19 pg/ml | 1.50 pg/ml | | |
| Maximum | 43.73 pg/ml | 30.59 pg/ml | | |
| SD | 9.47 | 8.16 | | |
| SE | 1.41 | 0.88 | | |
| Significant, p<0.05, non-signifcant, (p>0.05).,SD: Standard Deviation, SE: Standard Erarr | | | | |

The characteristics of the level of V.S.C in this study are illustrated in Table (3), which shows that the mean value was (0.46±0.12) for P group, and (0.32±0.15) for healthy controls. There were significant differences (P<0.05) in the levels of V.S.C between the two study groups.

**Table 3: Statistical and Characteristic of level of V.S.C in the Study Groups.**

| Characteristics | | P group n= 45 | Control group n= 45 | P-value |
|---|---|---|---|---|
| V.S.C | Mean | 0.46 | 0.32 | 0.000 |
| | SD | ±0.12 | ±0.15 | |
| | SE | 0.02 | 0.02 | |
| Significant, p<0.05, non-signifcant, (p>0.05).,SD: Standard Deviation, SE: Standard Erarr | | | | |

The relation between Age and V.S.C demonstrated in table 4. The result revealed a statistical non-significant different between Age (year) and V.S.C in both groups.

**Table 4: mean value of V.S.C among Age years**

| Age (year) | P group | Control group |
|---|---|---|
| | V.S.C (Mean±SD) | V.S.C (Mean±SD) |
| 20-25 | 0.458±0.123 | 0.318±0.159 |
| 26-30 | 0.470±0.116 | 0.329±0.154 |
| 31-35 | 0.450±0.057 | 0.367±0.057 |
| 36-39 | 0.457±0.135 | 0.291±0.170 |
| p-value | 0.947 NS | 0.877 NS |
| non-signifcant at p>0.05.,SD: Standard Deviation | | |

Table 5 illustrated the correlation between cortisol levels and various variables in both patient and control groups, revealing no significant correlations (p > 0.05) in control group. While in patient group the result observed a weak positive significant correlation between cortisol levels and variables (Age, VSC) in the patient group (r=0.329, p=0.014), (r = 0.214, p = 0.010), respectively.

Regarding the correlation between volatile sulfur compounds (VSC) and age, within both the patient and control groups. The findings indicate that weak positive correlation non-significant correlations observed (p >0.05) in either group. As recording in Table 5.

**Table 5: Correlation analysis between variable**

| | Correlation coefficient-r | | | |
|---|---|---|---|---|
| | P group | | Control group | |
| | r | P-value | r | P-value |
| Age X cortisol hormone | 0.329** | 0.014 | -0.228 | 0.131 |
| V.S.C X cortisol hormone | 0.214** | 0.010 | 0.000 | 0.998 |
| Age X V.S.C | 0.068 | 0.656 | 0.014 | 0.928 |
| ** (P<0.05), NS: Non-Significant. | | | | |

**Discussion:**

Pregnancy is a unique and sensitive phase for dental health due to physical, hormonal, and emotional changes (Nicolau et al., 2007). These changes make pregnant women more prone to oral

health disorders, including gingival inflammation, owing to oestrogen and progesterone swings. Hormonal changes might worsen tooth plaque inflammation, perhaps causing severe gingivitis (Carrillo-de-Albornoz et al., 2010).

The reversibility of gingival inflammation clinical measures such bleeding on probing at the end of pregnancy or after parturition shows their temporary character. Periodontal therapy during pregnancy improves quality of life (Musskopf et al., 2018), emphasizing the necessity of early dental health care. Greater gingival inflammation is linked to poorer sociodemographic status, particularly low educational attainment (Payal et al., 2017), suggesting that dental care knowledge and accessibility are crucial. Poor oral hygiene knowledge, perceived need, and inaccessibility to dental clinics contribute to this relationship (Lubon et al., 2018). Stress, anxiety, poor self-efficacy (Jamieson et al., 2014), and multigravida may also lead to oral hygiene neglect, worsening periodontal health.

Periodontitis, gingivitis, and poor dental health, especially in pregnant women, produce oral malodor, which is caused by oral bacteria producing volatile sulphur compounds (VSC) (van den Broek et al., 2008).

This study compares pregnant and non-pregnant women's halitosis status using clinical evaluation and examines volatile sulphur compounds (VSCs), salivary cortisol.

Regarding age, this study found that there is non-significant difference between the pregnant women group and control group. The mean age of the patients was (26.38±5.824) years. This could be because halitosis is a common problem that affects people of all ages, including pregnant women.

A recent study did not find a significant difference in the mean age between the pregnant women with halitosis and the control group (Fakheran et al., 2020). This is consistent with other studies that have found that halitosis is a common problem that affects people of all ages, including pregnant women.

A study by Fakheran et al. (2020) suggests that age and hormonal changes during pregnancy can significantly affect oral health, potentially influencing halitosis. While specific studies directly correlating age with halitosis in pregnant women are limited, it's known that hormonal fluctuations can impact oral microbiota and gum sensitivity, contributing to conditions like gingivitis, which are associated with bad breath (Fakheran et al., 2020).

The result of cortisol in this study revealed a significant elevation in cortisol levels among the pregnant group (16.48 pg/ml) compared to the control group (6.72 pg/ml), The association appeared to be closely related with the perceived severity of the stress. However, cortisol, known as the "stress hormone," plays a significant role in the body's response to stress (Ali & Diab, 2013). It affects various bodily functions, including metabolism, immune response, and inflammation (Noushad et al., 2021). Elevated cortisol levels suggest that pregnancy induces a stress response that could contribute to or exacerbate health conditions, including oral health issues (Macrì et al., 2023).

These findings are consistent with Rowińska et al. (2021) who conclude that stress and high cortisol levels can affect saliva production and composition, leading to dry mouth (xerostomia), which exacerbates bad breath as saliva plays a crucial role in cleaning the mouth and controlling bacterial growth (Rowińska et al., 2021). Furthermore, high cortisol levels can suppress the immune system,

making individuals more susceptible to infections, including periodontal disease, a leading cause of halitosis.

It has been suggested that salivary cortisol better assess the *hypothalamic–pituitary–adrenal (HPA)* function than serum cortisol since cortisol in saliva is present only in the unbound form. Binding proteins in plasma increases markedly during pregnancy and cortisol present in saliva gives a more accurate measure of the biologically active hormone (Perogamvros et al., 2010).

According to a study by Obel et al., (2005) levels of salivary cortisol significantly increased from early to late pregnancy (Obel et al., 2005). In addition to previous studies also found that the pregnancy-related increases in cortisol occurs as an increase in the unbound circulating cortisol (Smith et al., 1999). According to McEwen, (McEwen, 2000) who conclude that the hormonal stress response is required to adapt to challenging external stimuli but prolonged secretion of cortisol can have negative consequences on the human organism. During pregnancy stress hormones are necessary for normal fetal organ maturation and for preparing the fetus for extra uterine life, but excessive stress may have negative influences on fetal development (Valsamakis et al., 2019).

Regarding Volatile Sulfur Compounds (VSCs), the result of the present study found significant differences in the levels of Volatile Sulfur Compounds (VSCs) between the pregnant group and control group. The increased VSC levels in pregnant women may be attributed to hormonal changes during pregnancy, which can alter oral homeostasis and contribute to the production of VSCs (Lima et al., 2013). The hormonal fluctuations during pregnancy may be lead to an increased inflammatory response to dental plaque, contributing to conditions like gingivitis, which create an environment conducive to the proliferation of VSC-producing bacteria (Kleinberg & Codipilly, 2008). VSCs, primarily hydrogen sulfide, methyl mercaptan, and dimethyl sulfide, are the major contributors to halitosis, produced by anaerobic bacteria in the mouth that break down proteins into sulfur-containing amino acids (Lee et al., 2023).

Actually, the tongue coating comprises a wide range of bacteria and secretions from the postnasal area, gingiva, saliva, and dead epithelial cells. The microorganisms present in tongue coating produce various metabolites, including volatile sulfur compounds (VSCs) such as hydrogen sulfide (H2S), methyl mercaptan (CH3SH), and dimethyl sulfide (CH3SCH3). The production of VSC metabolites has been considered the leading cause of intraoral halitosis. The consistent presence of tongue coating suggests that the bacterial composition, rather than the amount of tongue coating, may be responsible for oral halitosis. Therefore, characterizing the bacterial composition of the tongue microbiome may provide insight into the key organisms involved in the development of oral halitosis (Seerangaiyan et al., 2018; Ye et al., 2019).

These findings are in accord with Lima, et al. (Lima et al., 2013) Gingival and periodontal tissues have receptors for sex hormones, whose concentration might change, leading to an increase in the vascular permeability and a decrease in the keratinization of the gingival epithelium. On the other hand, a study by Calil and Marcondes (Calil & Marcondes, 2006), the subjects had good systemic and oral health, excluding the possibility that the effect of stress on VSC production might be related to negligence in oral hygiene, as it has been reported to occur in depressive patients. Another study

reported that periodontal pockets were positively correlated with VSC concentrations in the mouth (Calil & Marcondes, 2006). Snel et al, (Snel et al., 2011). However, other studies reported no correlation between the periodontal pocket and VSCs. Moreover, no significant periodontal pathogens were observed in the groups of patients with halitosis (Riggio et al., 2008). Based on the threshold for oral malodour detection patients was at higher risk for halitosis detection than healthy controls by Halimeter. This finding is in line with previous reports by Kozlovsky et al. (Kozlovsky et al., 1994) and in partial agreement with Bosy et al. (Bosy et al., 1994), who failed to associate oral malodour with periodontitis, despite the fact that the intensity of malodour was greater in periodontitis patients than in healthy subjects in their study.

**Conclusion:**

Elevated cortisol levels in pregnant women indicate a stress response that may exacerbate oral health conditions, including halitosis. The study highlights a significant elevation of cortisol among the pregnant group compared to the control group, suggesting a link between stress and oral health during pregnancy. The study found significant differences in the levels of VSCs between pregnant and non-pregnant women, with pregnant women showing higher levels. VSCs are major contributors to halitosis, produced by anaerobic bacteria in the mouth.

**Data Availability:**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Funding:**

The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

**Conflict Of Interest Statement:**

The authors declare no conflict of interest.

**Patient consent for publication:**

Written informed consent was obtained from all patients in the present study for the publication of their data and any related images.

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# علاقة الكورتيزول اللعابي والمركبات الكبريتية المتطايرة برائحة الفم الكريهة لدى المرأة الحامل

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**المستخلص**

رائحة الفم الكريهة، المعروفة باسم رائحة الفم الكريهة، تؤثر بشكل كبير على الحياة الاجتماعية للفرد، واحترام الذات، ونوعية الحياة بشكل عام. في حين أن العوامل الفموية المحلية تسبب رائحة الفم الكريهة في الغالب، إلا أن الظروف الجهازية والتغيرات الهرمونية، خاصة أثناء الحمل، يمكن أن تؤثر أيضًا على حدوثها. أجريت هذه الدراسة المقطعية في مستشفى الصدر التعليمي في ميسان، العراق، في الفترة من أكتوبر 2023 إلى فبراير 2024، وشملت تسعين مشاركة من الإناث، مقسمات بالتساوي إلى مجموعة مريضة من النساء الحوامل الذين تتراوح أعمارهم بين 20 إلى 40 عامًا ويعانون من رائحة الفم الكريهة ومجموعة مراقبة صحية. مجموعة من النساء غير الحوامل. التزمت الدراسة بدقة بمعايير التضمين والاستبعاد لضمان وجود مجموعة مشاركين مركزة وذات صلة. تم إجراء قياسات VSCs ومستويات الكورتيزول اللعابية باستخدام طرق موحدة وتكنولوجيا متقدمة. وجدت الدراسة ارتفاعًا كبيرًا في مستويات الكورتيزول و VSCsبين المجموعة الحامل مقارنة بالمجموعة الضابطة، مما يشير إلى أن التغيرات الهرمونية الناجمة عن الحمل والاستجابة للضغط النفسي يمكن أن تساهم في أو تؤدي إلى تفاقم الحالات التي تؤدي إلى رائحة الفم الكريهة. تؤكد مستويات الكورتيزول المرتفعة و VSCsلدى النساء الحوامل على العلاقة المتعددة الأوجه بين الحمل والتغيرات الهرمونية والإجهاد وصحة الفم، وخاصة فيما يتعلق برائحة الفم الكريهة.

**الكلمات المفتاحية**: رائحة الفم الكريهة، الحمل، المركبات الكبريتية المتطايرة، الكورتيزول اللعابي، صحة الفم، التغيرات الهرمونية.