The Impact of Pregnancy and Menopause on the Correlation Between Salivary Calcium Levels, Calcium Intake, and Bleeding on Probing (BOP)

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Aims: Evaluate the impact of pregnancy and menopause on the correlation between salivary calcium levels, calcium intake, and bleeding on probing (BOP), so it can be used as indicators to determine the oral health status of pregnant and menopausal women.

Materials and Methods: This was a descriptive study using a cross-sectional approach. Participants included 26 menopausal women, 24 pregnant women, and 35 control subjects. Salivary calcium levels were measured using an atomic absorption spectrophotometer, and calcium intake was assessed using a semi-quantitative food frequency questionnaire. Other supporting data included height, weight, blood pressure, blood glucose level, salivary pH, and volume.

Results: The mean salivary calcium levels of pregnant women (0.72 ± 0.61 mmol/L) were lower than those of control subjects (1.69 ± 0.81 mmol/L), but the mean salivary calcium levels of menopausal women were higher (1.99 ± 1.24 mmol/L). Most of the subjects in all three groups had inadequate calcium intake. The mean BOP values of pregnant and menopausal women were higher than those of the control subjects. This study found the only variable that correlated with the salivary calcium level was the menopausal group’s blood glucose level (P = 0.009).

Conclusions: Pregnancy and menopause did not have an impact on the relationship between salivary calcium levels and BOP but had an impact on the relationship between salivary calcium levels and calcium intake. Calcium intake did not affect salivary calcium levels in both conditions when compared with the control group.

Keywords: Bleeding on probing, calcium intake, menopause, pregnant, salivary calcium

INTRODUCTION

Women experience events involving hormonal changes throughout their lives, including pregnancy and menopause.[1] Hormonal changes cause not only systemic but also local effects.[1,2] Salivary calcium levels decrease when estrogen production increases and vice versa.[3] Optimal calcium intake can be obtained by eating foods abundant in calcium or taking calcium supplements.[4]

The exact changes in pregnant women’s salivary calcium levels are still unclear.[5] Due to a decrease in estrogen, menopausal women experience a 20–25% reduction in calcium absorption, thus increasing their salivary calcium levels.[6,7] Low levels of salivary calcium can make teeth susceptible to caries because of the demineralization process. Conversely, high levels of salivary calcium escalate calculus formation.

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thus increasing the risk of periodontal disease; high salivary calcium levels are a risk factor for the severity of the periodontal disease.\textsuperscript{[8]} Hormonal activity during pregnancy also affects the state of tissues that support teeth. One study stated that 10–27\% of pregnant women have experienced gingival swelling and bleeding on probing (BOP).\textsuperscript{[9]}

Studies on salivary composition—especially calcium—are still limited. Additionally, there has not been much discussion of calcium intake and gingivitis in pregnant and menopausal women. We conducted this study to evaluate and find the correlation between salivary calcium levels, calcium intake, BOP in pregnant and menopausal women. The results of this study can be used as indicator to determine the oral health status of pregnant and menopausal women, thereby helping to ensure they consume sufficient calcium to avoid the impacts of hormonal changes on the oral cavity.

**Materials and Methods**

This was an analytic study using a cross-sectional approach. Data were collected from October through November 2019 in the primary clinic St. Laurentius Sukajadi, primary health care (PHC) center Sekeloa, PHC outpost Merpati VI, Sukawening Jatinangor, PHC outpost Sukanegla Jatinangor, PHC outpost Cikeruh Jatinangor, and Bandung Mariya Midwife Clinic. The population in this study included pregnant and menopausal women around Jatinangor and Bandung, Indonesia. The sampling method used was nonrandom consecutive sampling. We enrolled 26 menopausal women, 24 pregnant women, and 35 control subjects after obtaining informed consent.

Our study included pregnant women aged 18–35 years in every trimester confirmed by obstetrical specialist doctors and midwives as well as other pregnant women who were willing to participate. We excluded pregnant women with accompanying systemic diseases such as diabetes mellitus, hypertension, Bell’s palsy, Sjogren’s syndrome, heart disease, obesity, tuberculosis, and HIV; pregnant women who smoked or drank alcohol; pregnant women who took drugs that can affect salivary secretions; pregnant women who were undergoing radiation therapy; and pregnant women who used dentures or fixed orthodontic devices.

The data collected included salivary calcium levels, calcium intake, and BOP. Salivary calcium levels were measured using an atomic absorption spectrophotometer (AAS), and calcium intake was assessed using a semi-quantitative food frequency questionnaire (FFQ) with a list of ingredients taken from previous studies that had modified portions or measures of food quantities. BOP was measured with a WHO probe using the Bleeding Points Index (Ainamo and Bay, 1975). Other supporting data included height, weight, blood pressure, blood glucose level, salivary pH, and volume. This study has been conducted in accordance with the ethical principles mentioned in the Declaration of Helsinki and was approved by the Health Study Ethical Committee number 1386/UN6. KEP/EC/2019.

**Statistical analysis**

The correlation between salivary calcium levels and various study variables was analyzed using the Spearman correlation.

**Results**

Table 1 shows the subjects’ general and systemic characteristics. The mean ages of pregnant and menopausal women in the study were 27 ± 6.36 years and 57.5 ± 5.23 years, respectively. The pregnant women’s mean height and weight were higher than those of the menopausal and control groups. Among the three groups, the menopausal women had the highest mean systolic and diastolic blood pressure and blood glucose levels.

| Table 1: General and systemic characteristics of subjects | Pregnant \( (n = 24) \) | Menopause \( (n = 26) \) | Control \( (n = 35) \) |
|---|---|---|---|
| **General characteristics** | | | |
| Age (years) | 27.54 ± 6.36 | 57.5 ± 5.23 | 29.11 ± 8.12 |
| Height (cm) | 155 ± 4.4 | 149 ± 4.98 | 152.7 ± 11.03 |
| Weight (kg) | 64.5 ± 14.2 | 61 ± 12 | 55.28 ± 10.4 |
| **Systemic condition** | | | |
| Systolic blood pressure (mm/Hg) | 110.83 ± 11.50 | 129.12 ± 20.09 | 104.2 ± 11.03 |
| Diastolic blood pressure (mm/Hg) | 74.60 ± 9.40 | 84.2 ± 8.55 | 70.34 ± 9.71 |
| Blood glucose level (mg/dL) | 90.80 ± 8.88 | 111 ± 26.37 | 93.71 ± 18.47 |

SD = standard deviation
Table 2 shows the subjects’ demographic characteristics. Of the 24 pregnant women, only 11 (45.8%) continued their education up to junior high school. Only 1 (4.2%) pregnant woman completed a diploma or undergraduate education program. Most pregnant women were housewives. Overall, most of the pregnant women in this study were poorly educated. Of the 26 menopausal women, 13 (50%) were elementary school graduates and the majority (84.6%) were housewives (22 of 26). Of the 35 control subjects, 14 (40%) were senior high school graduates.

Table 3 shows the results of the subjects’ salivary examination. Pregnant women had the lowest mean pH of saliva among all the groups. Both the pregnant and menopausal women had a lower salivary volume than the control subjects. The pregnant women’s mean salivary calcium levels were lower than those of the control subjects. On the contrary, the menopausal women’s mean salivary calcium levels were higher.

Table 4 shows the subjects’ mean calcium intake values and whether or not their intake was adequate. The calcium intake values were determined by interviewing the subjects using a semiquantitative FFQ. This study’s limitations included the fact that calcium intake values could not be precisely measured but instead were determined by relying on the respondents’ ability to respond accurately to the questionnaire. To minimize inaccuracies, aids were used to help respondents estimate the portion sizes of food consumed. The results showed calcium intake levels were highest in the control subjects (541.87 mg). Most of the subjects in all three groups had inadequate calcium intake, including 96.16% of menopausal women, 96% of pregnant women, and 88.6% of control subjects.

Table 5 shows the subjects’ BOP examination results. The mean BOP value was highest in the pregnant group and lowest in the control group. There was a large standard deviation in BOP for each group, which implied the results were highly variable.

Table 6 shows the correlation between salivary calcium levels and various study variables, including generic and systemic characteristics, BOP, and calcium intake. In the study subjects, the only variable that significantly correlated with the salivary calcium level

### Table 2: Demographic characteristics of subjects

|            | Pregnant (n = 24) | Menopause (n = 26) | Control (n = 35) |
|------------|------------------|-------------------|-----------------|
|            | Frequency (n)    | Percentage (%)    | Frequency (n)   | Percentage (%)    | Frequency (n)   | Percentage (%)    |
| Education  |                  |                   |                 |                   |                 |                   |
| Elementary | 3                | 12.8              | 13              | 50                | 1               | 2.85              |
| Junior high| 11               | 45                | 7               | 27                | 5               | 14.3              |
| Senior high| 8                | 45                | 4               | 15.3              | 14              | 40                |
| Undergraduate| 1           | 4.1               | 0               | 0                 | 12              | 34.3              |
| Post-graduate| 1            | 4.1               | 0               | 0                 | 1               | 2.85              |
| Other      | 0                | 0                 | 2               | 7.7               | 2               | 5.7               |
| Occupation |                  |                   |                 |                   |                 |                   |
| Retired    | 0                | 0                 | 0               | 0                 | 0               | 0                 |
| Unemployed | 0                | 0                 | 0               | 0                 | 0               | 0                 |
| Civil servant| 0           | 0                 | 1               | 3.85              | 1               | 2.85              |
| Housewife  | 18               | 75                | 22              | 84.6              | 11              | 31.4              |
| Private employee| 3         | 12.8              | 1               | 3.85              | 2               | 5.7               |
| Entrepreneur| 2               | 8.1               | 2               | 7.7               | 2               | 5.7               |
| TNI/POLRI  | 0                | 0                 | 0               | 0                 | 0               | 0                 |
| Other      | 1                | 4.1               | 0               | 0                 | 19              | 54.3              |

### Table 3: Results of subjects’ salivary examination

|            | Pregnant (n = 24) | Menopause (n = 26) | Control (n = 35) |
|------------|------------------|-------------------|-----------------|
| pH         | Mean 5.6 SD 0.57 | Mean 6 SD 1       | Mean 6.45 SD 0.57 |
| Salivary volume (mL/5 min) | 1.7 SD 0.98 | 1.54 SD 1.11 | 2.32 SD 1.43 |
| Salivary calcium levels (mmol/L) | 0.72 SD 0.61 | 1.99 SD 1.24 | 1.69 SD 0.81 |

SD = standard deviation
was the menopausal group's blood glucose level. In the control subjects, salivary calcium level was significantly correlated with blood glucose level and calcium intake.

**Discussion**

The mean systolic and diastolic blood pressure of pregnant women was higher than that of the control subjects, although still within normal limits. Pregnancy can affect changes in the cardiovascular system and kidney function. During pregnancy, the volume of blood plasma increases, which results in higher cardiac output and blood pressure as the heart pumps blood with greater force. Additionally, compared with nonpregnant women, the rate of blood flow through the kidney and the glomerular filtration rate in pregnant women both increase by 40–65% and 50–58%, respectively. Blood pressure rises if the kidney parenchyma is burdened beyond its capacity by an increase in cardiac output, causing pregnant women to be more susceptible to hypertension.[10]

The menopausal women’s mean weight, systolic and diastolic blood pressure, and blood sugar levels were higher than those in the control subjects. Menopause negatively impacts many risk factors for cardiovascular disease and metabolic disorders. As women age, their resting metabolic rate decreases, causing an increase in body fat and a reduction in fat-free mass. The energy imbalance from menopausal women's decreased resting metabolic rate can lead to increased body weight and obesity unless energy intake is proportionally reduced.[11,12] Increased body weight and obesity are associated with decreased insulin sensitivity. Insulin resistance causes high levels of insulin circulation as well as sodium and fluid retention, which causes high blood pressure.[13]

In our study, pregnant women's salivary calcium levels were not only lower than the control subjects' levels but also lower than normal levels (1.32 ± 0.24 mmol/L). These results are consistent with several studies that suggested pregnant women's salivary calcium levels are lower than those of nonpregnant women. Estrogen is known to play a role in salivary composition changes; salivary calcium levels decrease when estrogen increases. A study showed that β subtype estrogen receptors are present in both mucosal and serous acinar and duct cells in the minor salivary glands, parotid glands, and submandibular glands. It has been proven that estrogen receptors modulate the maintenance and function of the salivary glands. Although the distribution of β estrogen receptors may explain how estrogen mediates changes in the saliva's inorganic composition, the exact mechanism by which this occurs is unclear.[5,14,15]

The menopausal women's mean salivary calcium levels were higher than normal, with a mean of 1.99 ± 1.24 mmol/L. Our results are consistent with those of other studies, which showed menopausal women had decreased estrogen levels and increased salivary calcium levels.[3,16,17] This may occur because a decrease in estrogen suppresses the absorption of calcium by reducing the

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**Table 4: Calcium intake and adequacy of subjects**

|                  | Pregnant (n = 24) | Menopause (n = 26) | Control (n = 35) |
|------------------|-------------------|--------------------|-----------------|
| Calcium intake (mg) |                   |                    |                 |
| Mean             | 510.9             | 447.05             | 541.87          |
| SD               | 533.77            | 292.96             | 456.04          |
| Calcium intake category (%) | | | |
| Adequate         | 4                 | 3.84               | 11.40           |
| Inadequate       | 96                | 96.16              | 88.60           |

**Table 5: BOP examination of subjects**

|                  | Pregnant (n = 24) | Menopause (n = 26) | Control (n = 35) |
|------------------|-------------------|--------------------|-----------------|
| BOP (%)          |                   |                    |                 |
| Mean             | 8.8               | 6                  | 5.73            |
| SD               | 7.51              | 7                  | 4.87            |

**Table 6: Correlation between salivary calcium levels and various study variables**

| Level with | Menopause | Pregnant | Control |
|------------|-----------|----------|---------|
|            | Correlation coefficient (r) | P value | Correlation coefficient (r) | P value | Correlation coefficient (r) | P value |
| Age        | 0.256     | 0.18     | 0.116   | 0.54     | 0.014   | 0.933   |
| BMI        | −0.005    | 0.978    | 0.266   | 0.155    | 0.090   | 0.579   |
| Systolic   | −0.117    | 0.545    | −0.039  | 0.837    | 0.175   | 0.279   |
| Diastolic  | −0.094    | 0.629    | −0.128  | 0.501    | −0.038  | 0.815   |
| Blood glucose level | **−0.478** | **0.009** | −0.263 | 0.176 | **−0.617** | **0.001** |
| BOP (%)    | −0.286    | 0.133    | −0.191  | 0.311    | 0.136   | 0.403   |
| Calcium intake | −0.307    | 0.105    | 0.012   | 0.949    | **−0.468** | **0.002** |

r = Spearman rank correlation coefficient

Bold entries in this table show statistically significant correlations (P value < 0.05) according to the Spearman's Rank correlation coefficient.
increasing the release of calcium from the bones. Serum calcium levels are regulated by many factors that prevent a significant increase. An excess of serum calcium is excreted in the saliva or urine through the process of homeostasis. Saliva can be thought of as a filtrate of serum blood derivatives; the process of saliva production is associated with the balance of bodily fluids; and the flow of blood through the network of salivary nodes.

The salivary calcium level is dependent upon the serum calcium level because all salivary electrolytes come from the serum, which is actively transferred to acini and secreted through striated and excretory ducts.

In our study, inadequate calcium intake in the pregnant, menopausal, and control groups may have been related to the subjects’ demographic conditions, which were fairly similar between the three groups. Subjects largely resided in rural areas and had low levels of education and employment. Lack of education, including insufficient knowledge of the importance of calcium intake, could have led to the subjects’ inability to choose calcium-rich, healthy foods. Nutritional knowledge was associated with the subjects’ level of formal education, even though low levels of formal education do not necessarily imply low levels of knowledge attainment; knowledge is also gained through nonformal education and experience. Although one study implied menopausal women had low levels of calcium intake because they lacked knowledge of the importance of consuming calcium to prevent osteoporosis, the researchers did not assess the women’s knowledge of osteoporosis.

Occupation may have also played a role; low economic status may influence a person’s or family’s ability to purchase calcium-rich foods. Low levels of calcium intake cause an increase in bone resorption to meet fast-changing extracellular calcium needs.

In our study, the pregnant and menopausal women had higher mean levels of BOP than the control group. On the basis of the calculation method established by Ainamo and Bay in 1975, BOP ratings were not divided into a category of low or high but were expressed only as a percentage of the amount of bleeding compared with the surface area inspected. Our study had a large standard deviation for BOP, demonstrating a wide variation in the data. This could have occurred because a different number of teeth were inspected on each subject, leading to a large variation in the surface area.

Hormonal changes can cause a worsening in gingival tissue health. Pregnancy does not cause inflammation of the gums directly; instead, bacterial plaque causes gingival bleeding during pregnancy. Hormonal changes lead to an excessive gingival response to plaque, which modifies the resulting clinical picture. Hurrahmi et al. found that 60% of pregnant women experienced pregnancy-related oral health effects and 10%–27% had swelling and bleeding, including gingival BOP. This occurs because estrogen and progesterone stimulate the formation of prostaglandins in the gingiva, suppress the production of T lymphocytes, and increase the amount of Prevotella intermedia, all of which may cause an excessive inflammatory response in pregnant women’s gingiva, causing it to bleed easily.

Menopausal women experience a decrease in estrogen, which cause changes in gingival inflammation. Estrogen deficiency causes decreases in cell proliferation, differentiated cells, and keratinizing gingival epithelium and leads to a reduction in collagen tissue formation. These conditions lead to the reduction and atrophy of gingival epithelium thickness, inflammation that occurs easily, irritation, changes in color from pale to erythematous and bleeding that occurs easily upon probing and teeth-brushing. Menopausal gingivostomatitis refers to menopause-related changes to gingival conditions. Our study’s results align with the theory that BOP is one of the clinical signs of gingivitis at menopause. Gingival BOP indicates inflammatory lesions in both the epithelium and connective tissue. The fact that gingival tissues bleed when their margins are touched by blunt instruments shows that epithelial and vascular changes have occurred.

Our study also aligns with the work of Setyohadi et al. which stated menopausal and postmenopausal women experience gingivitis because of decreased estrogen. Hormonal changes at menopause are a supporting factor for gingivitis. Another study by Hidayati et al. showed most postmenopausal women experience moderate gingivitis with easily bleeding gums, which suggests that gingivitis in menopausal women is influenced not only by changes in hormones but also by oral hygiene.

BOP in postmenopausal women is associated with higher calcium levels. Salivary calcium levels are a risk indicator for the severity of the periodontal disease. High levels of salivary calcium cause plaque mineralization to form a calculus. The calculus becomes a place for more plaque and bacteria to accumulate, resulting in gingival inflammation. A calculus that is not cleaned can cause further inflammation, including changes in vascular dilation, increased blood flow, and
more sensitive gingiva, which results in BOP. Poor oral hygiene can cause an increased accumulation of plaque and bacteria that aggravates gingivitis in menopause. The salivary flow rate of menopausal women declines due to estrogen deficiency, which causes hypofunction of the salivary gland. A low salivary flow rate causes food and bacteria to easily attach to the surface of teeth, thus increasing plaque retention. We found that menopausal women's salivary volume per 5 min and pH were both lower than those of control subjects. These results align with studies by Bhat et al. and Mahesh et al. which stated the salivary flow rate of postmenopausal women was lower than that of control subjects. However, a study by Pulin et al. showed no significant difference in the salivary flow rate between menopausal women and control subjects. Many studies have demonstrated a decreased salivary pH in menopausal women. It should be noted that the severity of periodontal disease varies depending on an individual's response to various irritants and oral hygiene habits. This could possibly explain why control subjects also experienced BOP.

This study found a negative correlation between salivary calcium levels and blood glucose levels in menopausal women. Changes in hormones and body composition in menopausal women are suspected as the causes of impaired glucose metabolism, thus triggering fluctuations in blood glucose levels and increasing the risk of type 2 diabetes. Several studies have suggested that diabetes is associated with decreased levels of salivary calcium through the disruption of salivary gland function, which causes changes in the salivary composition.

The results of this study show a negative correlation between salivary calcium levels and calcium intake in the control subjects. It indicates that low calcium intake is associated with increased salivary calcium levels. Adequate calcium intake has been associated with a lower risk of periodontal disease because it influences alveolar bone density. Several studies have shown that periodontal diseases are correlated with high salivary calcium levels. This may explain the pathway of the relationship between calcium intake and salivary calcium levels in this study. The control subjects in this study were not examined for periodontal disease. Future studies that include the subject's periodontal tissue conditions are needed to further explain these findings.

In menopausal and pregnant subjects, the correlation between salivary calcium levels and calcium intake was not significant. This means that pregnancy and menopause are confounding factors in the relationship between the two variables. Calcium intake might not be the major factor for the alterations of salivary calcium levels in menopausal and pregnant women.

**CONCLUSION**

Pregnant women's salivary calcium levels were lower than those of control subjects; however, menopausal women's salivary calcium levels were higher. Both pregnant and menopausal women had inadequate calcium intake, possibly caused by their low educational and economic status. BOP occurs more often in pregnant and menopausal women. Pregnancy and menopause did not have an impact on the relationship between salivary calcium levels and BOP, but had an impact on the relationship between salivary calcium levels and calcium intake. Calcium intake did not affect salivary calcium levels in both conditions when compared with the control group.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**AUTHORS CONTRIBUTION**

Sri Tjahajawati: conceptualization, methodology, investigation, resources, data curation, validation, writing - review and editing, supervision and funding acquisition. Anggun Rafisa: conceptualization, methodology, validation, writing - original draft, writing - review and editing, supervision. Kintan Nurpratiwi Gumilar: formal analysis, investigation, resources, data curation, writing - original draft. Fitri Nurzanah: formal analysis, investigation, resources, data curation, writing - original draft. Rasmi Rikmasari: methodology, supervision, funding acquisition.

**ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT**

This study was approved on May 18, 2020, by the Health Study Ethical Committee of Faculty of Medicine, Padjadjaran University, number 1386/UN6. KEP/EC/2019.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available on request from the corresponding author.
The data are not publicly available due to ethical restrictions.

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