Original Article

Salivary Calcium Level and Its Correlation with Salivary pH, Salivary Volume, and Calcium Intake in Hypertensive Female Patients with Different Blood Sugar Levels

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ABSTRACT

Aims: This study aimed to evaluate the salivary calcium level and its correlation with salivary pH, salivary volume, and calcium intake in hypertensive female patients with different blood sugar levels. **Materials and Methods:** This cross-sectional study included 49 female subjects with hypertension. Subjects were divided into three groups based on the HbA1c test: normal, prediabetes, and diabetes. Unstimulated whole saliva was collected using the spitting method. Salivary calcium levels were evaluated using an Atomic Absorption Spectrophotometer. Salivary pH was obtained using a universal indicator pH paper test. Calcium intake was assessed by a semi-quantitative food frequency questionnaire. The Kruskal–Wallis test was used to compare salivary parameters and total calcium intake within study groups. Spearman rank correlation and multiple regression analysis were used to evaluate the correlation between salivary calcium levels and all variables in the study. **Results:** No significant difference in salivary calcium, volume, pH, and total calcium intake was observed within the study groups. However, a significant correlation was found between salivary calcium levels and salivary pH in hypertensive females with normal blood glucose levels. Moreover, salivary calcium levels have a significant correlation with systolic blood pressure in prediabetes and diabetes groups. **Conclusions:** This study found a decrease in all salivary parameters (calcium, pH, and volume) as well as a low calcium intake in hypertensive females, despite no significant difference found in groups with different blood glucose levels. Blood glucose levels appeared to be a confounder in the relationship between salivary calcium with salivary pH and systolic blood pressure.

Keywords: *Calcium, diabetes, hypertension, intake, saliva*

Introduction

Systemic conditions could affect the normal function of the saliva, altering its electrolytes components such as calcium ions.[1,2] The composition of saliva is affected by the salivary flow rate, volume, and pH. Salivary glands control these three salivary parameters. Therefore, any factors that affect the salivary glands, such as food intake, could also affect these salivary parameters.[3,4]

Many studies have explored salivary calcium levels in patients with hypertension or diabetes but showed conflicting results. Research on hypertensive subjects

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found an increase in salivary calcium,[5] but other studies showed a decrease.[6] A study found higher salivary calcium levels in patients with type 1 and type 2 diabetes compared with age-matched control.[7] Other studies have found similar and lower salivary calcium levels in patients with diabetes.[6,8-10] Shirzaiy *et al.* found that sex is a confounder for salivary calcium levels. The study showed that the salivary calcium levels were significantly different between diabetic and non-diabetic female subjects but not in male subjects.[10]

Hypertension and diabetes are closely interlinked bidirectionally and often presented together. However, many studies investigating the calcium concentration in individuals with hypertension exclude subjects with other systemic diseases, such as diabetes and vice versa. Therefore, this study aimed to evaluate the effect of different blood glucose levels (normal, prediabetes, and diabetes) on salivary calcium levels in hypertensive female patients. This study also explored the correlation between salivary calcium levels and other parameters that affect the composition of the saliva, such as salivary pH, volume, and calcium intake.

Materials and Methods

This cross-sectional study included females with a principal diagnosis of hypertension in accordance with the ACC/AHA hypertension guidelines 2017, which is defined as having systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥ 80 mm Hg.[11] This study was conducted in 2019 in Bandung and Jatinangor, Indonesia. The sampling technique was consecutive sampling. Subjects were selected from outpatients in St. Laurentius Sukajadi Clinic, Puskesmas Sekeloa, Posyandu Merpati VI Dusun Sukawening Jatinangor, Posyandu Dusun Sukanegla Jatinangor, and Posyandu Desa Cikeruh Jatinangor. The ethical approval was obtained from Research Ethics Committee No. 1386/UN6.KEP/EC/2019. All the procedures were performed as per the ethical guidelines laid down by the Declaration of Helsinki (2013).

This study included female subjects aged between 18 and 65 years old, who were diagnosed with hypertension for more than a year and a maximum of 10 years based on patients' medical records, and willing to participate until study completion by signing an informed consent. Subjects were interviewed if they had a history of alcohol drinking and smoking, Bell's palsy, Sjogren's syndrome, HIV, or tuberculosis, and acute dental pain; took insulin injections; and used orthodontic appliances and if they were undergoing radiotherapy, pregnant, or menstruating. The medical record was checked to confirm subject's response. Subjects with these conditions were excluded from the study because these conditions might affect the salivary gland function and alter the composition of saliva.[12]

A total of 49 subjects were collected. All the subjects underwent a hemoglobin A1C (HbA1c) test and they were interviewed regarding their experience of diabetes symptoms. The HbA1c test is suitable for diagnosing diabetes because of its ability to evaluate the average blood glucose level in the previous 2 or 3 months.[13] The subjects were divided into three groups based on the results of the HbA1c test as:

- Normal: below 5.7%
- Prediabetes: 5.7%–6.4%
- Diabetes: 6.5% or above

Unstimulated whole saliva was collected using the spitting method. An hour prior to saliva collection, subjects were asked not to consume food, drink, or brush their teeth. Salivary volume was estimated by calculating the amount of saliva spitted in a centrifuge tube every 1 min for five times (5 min total). One mL of the collected saliva was dissolved in 5 molar nitric acid. Calcium levels in dissolved saliva were determined using Atomic Absorption Spectrophotometer (AAS) AAnalyst 400 (Perkin Elmer, Waltham, MA, USA) at the central laboratory of Universitas Padjadjaran. The salivary pH was obtained using a universal indicator pH paper test (pH 0–14 Universal Indicator pH Paper, Merck, Darmstadt, Germany). The pH score was recorded by observing the color change during the pH paper test. Subjects were interviewed using a semi-quantitative food frequency questionnaire (FFQ) to assess total calcium intake. The FFQ consisted of 35 food items, their pictures and portion sizes. Other supporting data collected in the current study included weight, height, level of education and occupation. Subjects' weight and height were measured using a digital scale and microtoise (OneMed, Danderyd, Stockholms Lan, Sweden), respectively. Samples from different groups were always analyzed in the same batch, and laboratory technicians were unable to differentiate between groups.

All measurement tools were calibrated in accordance with industry standards. Each measurement was performed at least two times. Inter-examiner calibration was performed on all examiners by an expert serving as a validator. The validator and examiners each evaluated the same group of 10 subjects and compared their findings.

Statistical analysis

Categorical variables such as age, level of education, occupation, nutritional status, and blood glucose levels were described in absolute counts and percentages. The

Shapiro–Wilk test was used to evaluate the normality of the data. Continuous data were presented as median and range as the Shapiro-Wilk test shows that the data of this study was not normally distributed. The Kruskal–Wallis test was used to compare salivary calcium levels, salivary volume, salivary pH, and total calcium intake within study groups. The correlation between salivary calcium levels and all variables measured in the study including age, BMI, salivary calcium, salivary pH, salivary volume and calcium intake was assessed using Spearman rank correlation and multiple regression analysis. Values of $P < 0.05$ were defined as statistically significant. All analyses were performed using Microsoft Excel 2016 (Microsoft Corp, Version 16.0. Redmond, Washington), Statistical Package for Social Sciences (IBM Corp, Version 26.0. Armonk, New York), and displayed in tabular form.

Table 1: Basic characteristics of study subjects ($n = 49$)

Characteristics	Total	%
Age (years)		
< 40	7	14.29
40–49	12	24.49
50–59	20	40.82
≥ 60	10	20.41
Level of education		
Primary	18	36.73
Middle	15	30.61
Secondary	16	32.65
Occupation		
Housewife	42	85.71
Entrepreneur	5	10.20
Private employee	1	2.04
Civil servant	1	2.04
Nutritional status		
Underweight	4	8.16
Normal	16	32.65
Overweight	18	36.73
Obese	11	22.45
Blood glucose levels		
Normal	17	34.69
Prediabetes	14	28.57
Diabetes	18	36.73

Results

Table 1 shows that most female hypertensive subjects were 50–59 years old (40.82%). Less than one-third (32.65%) of the subjects completed high school. With regard to occupation, nutritional status, and blood glucose levels, most of the subjects were housewives, overweight, and diagnosed with diabetes. The salivary calcium levels were found to increase with the increase in blood glucose levels [Table 2], but the results were not statistically significant. In addition, no significant difference in salivary volume, pH, and total calcium intake per day was found within the study groups.

The findings of this study indicate a significant association between salivary calcium levels and salivary pH in hypertensive females with normal blood glucose levels [Table 3]. This association was not found in prediabetes and diabetes groups. Salivary calcium levels have a significantly negative correlation with systolic blood pressure in the prediabetes group. However, in the diabetes group, the correlation between the two variables has a positive value. Other parameters such as age, body mass index (BMI), salivary volume and pH, calcium intake, and diastolic blood pressure show no significant associations with salivary calcium levels in the three groups.

We used multiple regression analysis to evaluate more factors that correlate with salivary calcium levels [Table 4]. The findings suggest that salivary calcium levels were associated with systolic blood pressure in all groups. Age and diastolic blood pressure affected the salivary calcium levels in normal and diabetes groups. Moreover, BMI and calcium intake were only associated with salivary calcium levels in the normal group.

Discussion

Our findings showed that hypertensive females tended to be older than 40, have a higher BMI and have a lower level of education. Previous studies reported that the increase in blood pressure is associated with race, aging, obesity, lifestyle, level of education, and socioeconomic status.[14,15] An increase in blood pressure is a general

Table 2: Comparison of salivary parameters and calcium intake within study groups

Parameters	Blood glucose levels			*P* Value*
	Normal ($n = 17$)	Prediabetes ($n = 14$)	Diabetes ($n = 18$)	
Salivary calcium level (mmol/L)	0.72 (0.22–4.27)	0.74 (0.43–3.35)	0.84 (0.34–5.47)	0.318
Salivary volume (ml/5 minutes)	1.0 (0.8–7.8)	1.35 (0.8–3.0)	1.0 (0.4–2.0)	0.056
Salivary pH	6 (4–7)	6 (5–7)	5.8 (5–7)	0.514
Calcium intake per day (mg)	367.0 (107.4–890.91)	214.9 (86.3–963.9)	336.1 (48.5–1689.4)	0.323

Values are median (range)

*Statistically significant ($P < 0.05$) according to the Kruskal–Wallis test

Table 3: Correlation between salivary calcium levels and various variables within study groups

Correlation between salivary calcium levels and	Blood glucose levels					
	Normal (n = 17)		Prediabetes (n = 14)		Diabetes (n = 18)	
	r	P	r	P	r	P
Age	0.271	0.293	0.102	0.730	–0.070	0.783
Body mass index (BMI)	0.112	0.670	0.229	0.431	–0.123	0.627
Salivary volume	–0.332	0.193	–0.086	0.770	–0.086	0.734
Salivary pH	0.522	0.032*	–0.083	0.777	–0.242	0.333
Calcium intake per day	0.196	0.450	0.108	0.714	–0.276	0.268
Systolic blood pressure	–0.322	0.207	–0.584	0.028*	0.483	0.043*
Diastolic blood pressure	–0.177	0.498	–0.519	0.057	0.229	0.360

*Statistically significant ($P < 0.05$) according to the Spearman rank correlation.

Table 4: Factors associated with salivary calcium levels based on multiple regression analysis

Blood glucose levels	Regression equation	R^2 multiple
Normal (n = 17)	Salivary calcium levels = –0.665 + 0.080*age-0.081*BMI+0.002*calcium intake-0.045*systolic blood pressure+0.063*diastolic blood pressure	76.3%
Prediabetes (n = 14)	Salivary calcium levels = 4.351 –0.021*systolic blood pressure	34.4%
Diabetic (n = 18)	Salivary calcium levels = 5.845–0.089*age+0.029* systolic blood pressure -0.045* diastolic blood pressure	38.0%

characteristic of aging in both sexes.[16] In age-standardized prevalence, the number of hypertensive women is lower compared with men.[17] However, after menopause, the number of hypertensive women is increasing, which might be due to sex hormones.[18,19] Sedentary lifestyle and an unhealthy diet, which can lead to the accumulation of body fat, are the behavioral risk factors for hypertension. The level of education and occupation are social determinants that affect these behavioral risk factors.[20]

Subjects in this study had a lower value of all salivary parameters than normal or hypertensive subjects in other studies,[6,8,21,22] although no significant difference was observed in groups with different blood glucose levels. Systemic conditions can cause dysfunctions in salivary glands, which affect the quality and quantity of salivary production and secretion.[23] Some studies have found that hypertension and diabetes may cause salivary gland deterioration, which is associated with hyposalivation and xerostomia.[24-26] The decrease in salivary parameters such as calcium levels, pH, and volume is correlated with a more acidic oral cavity that favors demineralization, thereby increasing the risk of caries and tooth loss.[27]

Many factors play a role in the regulation mechanism of calcium homeostasis and membrane transport that can determine calcium concentration. In patients with hypertension, a change in electrolyte balance was observed. Some authors reported higher blood calcium levels in hypertensive patients.[28,29] In most cases, hypertension is often associated with renal function impairment causing hypercalciuria.[30] This damage leads to an increased amount of calcium excreted in the urine, which may cause low calcium levels in saliva. However, further research is needed to confirm this conjecture.

Despite subject's lower calcium intake than the daily recommended value in this study,[31] we found no significant correlation between calcium intake and salivary calcium level in all groups. Previous studies on pregnant, menopausal, and smoking females also demonstrated this association.[12,32] Our current findings are consistent with the prior study results, that is, salivary calcium levels are affected by the secretion of the salivary glands, and they do not reflect the amount of calcium intake.[33] Research by Kim *et al.* suggested that individuals with high levels of plasma calcium and low calcium intake were at a higher risk of developing hypertension.[29] Some authors reported that the regular intake of high calcium-containing food could prevent hypertension.[34,35] However, others found that calcium supplementation in pregnant and menopausal women did not show a significant decrease in blood pressure.[36,37] These findings indicate that dietary supplementation of a single nutrient might not lower the risk of hypertension. It should be followed with a healthy balanced diet.[36]

In this study, blood glucose levels appeared to be a confounder in the relationship of salivary calcium with salivary pH and systolic blood pressure in hypertensive female patients. We discovered a significantly positive correlation between salivary calcium levels and salivary pH in hypertensive females with normal blood glucose levels. Previous studies also found that salivary pH alteration may cause changes in electrolyte concentration

in saliva, including calcium.[38-40] Furthermore, this study found a significantly negative correlation between salivary calcium levels and systolic blood pressure in the prediabetes group. On the contrary, a significantly positive correlation was found in the diabetic group. The contrast direction of the correlation may be due to other electrolyte changes caused by secretory function alteration of salivary glands, which only emerge when individuals have been diagnosed with diabetes. Research by Mata *et al.*[7] found that lower magnesium secretory capacity was associated with higher salivary calcium levels in patients with diabetes, which may be one of the rationales that need further investigation.

The HbA1c test, which used to measure blood glucose levels in this study, is the most suitable tools for diagnosing diabetes because of its ability to evaluate the average blood glucose level in the previous 2 or 3 months.[13] However, the majority of the subjects of this study were outpatients with controlled hypertension; thus, we cannot categorize them by hypertension level because the number of samples at each level was not matched for analysis. The sample for this study was also drawn from the same population and in the same setting in Indonesia. Therefore, the research findings may be generalized to urban settings in lower-middle-income countries.

Conclusion

This study found a decrease in all salivary parameters (pH, volume, and calcium level) as well as a low calcium intake in hypertensive females, despite no significant difference found in groups with different blood glucose levels. Blood glucose levels appeared to be a confounder in the relationship of salivary calcium with salivary pH and systolic blood pressure.

Future scope/clinical significance

This study did not explore plasma calcium levels, urinary calcium levels, and other salivary electrolytes, which may affect calcium homeostasis and calcium concentration in saliva. Future studies regarding calcium levels in plasma and urine, and electrolyte changes caused by secretory function alteration of salivary glands in hypertensive females with diabetes, must be investigated.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contribution

A. Rafisa contributed to conception, design, data acquisition, data analysis and interpretation, drafted and critically revised the manuscript; S. Tjahajawati contributed to conception, design, data acquisition, data analysis and interpretation, and funding acquisition; AR. Friandina, INAP Laksana, C. Zubaedah contributed to design, data acquisition, and data analysis and interpretation.

Ethical policy and institutional review board statement

The ethical approval has been obtained on November 20, 2019 from Research Ethics Committee Universitas Padjadjaran No. 1386/UN6.KEP/EC/2019.

Patient declaration of consent

The informed consent of the participant was obtained prior to data collection. The informed consent included description of clinical investigation, risks and discomforts, benefits, alternative procedures or treatments, confidentiality, compensation and medical treatment in event of injury, contacts and voluntary participation. The informed consent form was approved by Research Ethics Committee Universitas Padjadjaran No. 1386/UN6.KEP/EC/2019 on November 20, 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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