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### Abstract

**Background:** Saliva content is essential in the evaluation of the oral condition. Moreover, it is a simple diagnostic method for monitoring of periodontal diseases. Therefore, the present study aimed to evaluate salivary calcium and magnesium levels in periodontitis patients with gingival recession affected with diabetes and hypertension.

**Materials and Methodology:** The cross sectional analytical study was conducted on 150 Saudi patients aged between 25 - 50 years old (85 males and 65 females). They divided into three equal groups: control group (I), diabetic patients group (II), and hypertensive patients group (III). Plaque index (PLI), Gingival index (GI), clinical attachment loss with gingival recession (CAL) were recorded. Salivary samples were collected to evaluate the salivary calcium and magnesium levels. Statistical analysis by ANOVA test and student t-test were used for clarifying the correlation between the levels of calcium and magnesium in saliva with the values of clinical periodontal parameters of the study groups.

**Results:** The results of the current study show that the mean age in group III was more than the mean of age in group I and group II and, the mean of age in group II was more than group I. There were significant differences in clinical parameters were found in the comparison between group III and group II to control group (p < 0.05). There were significant differences in salivary calcium and magnesium level in the comparison of the study groups (p < 0.005). Salivary calcium and magnesium levels in group II and group III were lower than group I. There were high statistical significance differences in GI and CAL with a decrease in salivary magnesium level in group III compared to group I and II.

**Conclusion:** According to this study, we concluded that the salivary calcium and magnesium levels were decreased in hypertensive and diabetic patients. Moreover, we found that there was a decreased salivary magnesium level with increased severity of periodontal diseases, whereas vice versa in the comparison between the salivary calcium level and severity of periodontal diseases.

Keywords: Diabetes and Hypertension; Salivary Calcium; Salivary Magnesium

### Abbreviations

PLI: Plaque Index; GI: Gingival Index; CAL: Clinical Attachment Loss; CEJ: Cementoenamel Junction; PMNL: Polymorphonuclear Leukocytes; GCF: Gingival Crevicular Fluid; ± SD: Standard Deviation; G: Group; CP: Clinical Parameters; Mg: Magnesium; Ca: Calcium

#### Introduction

Periodontitis is a bacterial inflammatory disease of supporting periodontal tissue. It causes the destruction of hard and soft tissues of periodontium and teeth loss in the end [1,2]. Some evidences may be used for assessment of periodontal status like gingival recession that is defined as the apical migration of marginal gingival away of cementoenamel junction (CEJ) and root surface exposure, which may be the reason of some associated complications like to facilitate plaque accumulation, bleeding of the gingiva, dentine hypersensitivity and addition of that aesthetic problems [3,4].

Some systemic diseases play an important role as modifying factors of periodontal tissue response to bacterial factors through their effect on the immune system by a decrease in the numbers of polymorphonuclear leukocytes (PMNL) and reduction of their functions that may lead to exacerbating of periodontal disease [5].

Diabetes mellitus is a syndrome which effects on tissues due to lack of insulin, thus the rise of glucose levels in gingival crevicular fluid (GCF) and serum of diabetic patients causes alteration of nature and quality of oral microbiota that could impact on periodontal tissue [6]. Uncontrolled diabetes mellitus leading to abnormal changes in the oral mucosa, including burning, sensation, cheilosis, drying and cracking, xerostomia and changes in oral normal flora, especially increased of *Staphylococci*, hemolytic *Streptococci*, and *Candida albicans* furthermore, the defect in the immune system and increased sensibility to secondary infection and aggravation of periodontal disease are the most changes [7].

Hypertension is a chronic systemic disease affects the patient when the blood pressure in the arteries more than normal, there are two types of hypertension, essential or primary (90% - 95%) that affects the patient without medical complications and secondary (5% - 10%) that is associated with medical problems in heart, arteries, endocrine system and kidneys [8]. Over the past 10 years, there were some studies done to assess the correlation between hypertension and, periodontitis. These studies revealed that there were a correlation between hypertension and increased in some periodontal parameters and reduction in periodontal health status [9-13].

Saliva is a biological fluid is released into the oral cavity by major and minor salivary glands; it forms the oral environment and helps as easy diagnosis and monitoring method, the addition of that, it is lubricant fluid for protection of oral and upper gastrointestinal mucosa so the alterations in the contents of saliva and its influx may effect on teeth and oral mucosa safety [14,15]. On the other hand, other studies have displayed that increased incidence of periodontal disease and plaque formation with increased calcium concentration in saliva and other researchers detected that periodontal disease prevalence was more with increased the mineralization in the oral cavity due to the effect of some factors in saliva [16,17].

Salivary magnesium perhaps has a protection character from periodontal disease due to its capacity to decrease the effect of bacterial toxins on periodontal tissue and reduce of the inflammatory response to oral microbiota especially with some systemic disease like diabetes and hypertension [18,19].

#### Aim of the Study

The present study was designed to assess the salivary calcium and magnesium levels in periodontitis patients with gingival recession affected by diabetes and hypertension.

### **Materials and Methods**

#### The patients' samples

The current cross-sectional analytical study included one hundred fifty periodontitis patients aged between 25 - 50 years old (85 males and 65 females) selected from the outpatient clinics, college of dentistry, King Khalid University. The stratified sampling was used

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and according to that, the participants were divided into three categories of subjects: group I: 50 patients without systemic diseases (control group), group II: 50 patients with diabetes mellitus and group III: 50 patients with hypertension. The patients with other systemic diseases and periodontitis patients without gingival recession were excluded.

The proposal of the study was reviewed and approved by the Ethical board, College of Dentistry, King Khalid University for gained the ethical clearance certificate (SRC/ETH/2018-19/081). The participants' consent obtained before the beginning of the study, all steps of ethics during this study done according to the Helsinki Statement Ethical Standards 1975 reviewed in 2008. Detailed patients' case histories recorded.

### **Clinical examination**

The clinical examination included the case history, the assessment of the general health through the medical reports moreover assessment of oral and periodontal health by plaque index (PLI) [20], gingival index (GI) [21] and clinical attachment loss (CAL) measurement.

### Assessment of some salivary minerals

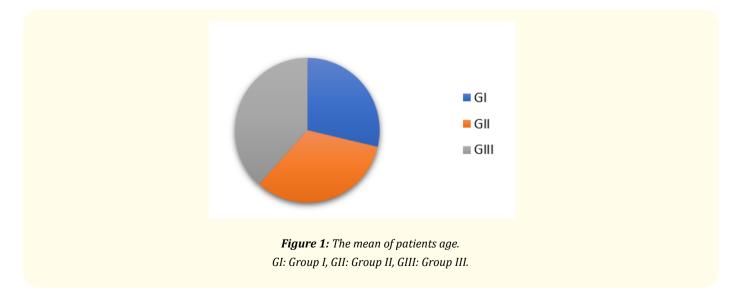
The participants should be not to eat or drink at least one hour before saliva collection and washing their mouth with water 1 - 2 minutes to clean the mouth then the unstimulated saliva was collected from the floor of the mouth into calibrated saliva collecting tubes. The samples were sent to biochemical analysis to spectrophotometry determination of salivary calcium and magnesium.

#### Statistical analysis

ANOVA test and student t-test were performed to analyze the data in the current study. The mean and standard deviation ( $\pm$  SD) of periodontal parameters further the levels of Calcium and Magnesium in saliva was assessed by ANOVA test. The student t-test was used to compare periodontal parameters and the levels of some minerals in saliva between group I, II and III. P-value (0.05) was considered the significance level in this study ( $p \le 0.05$ ).

### Results

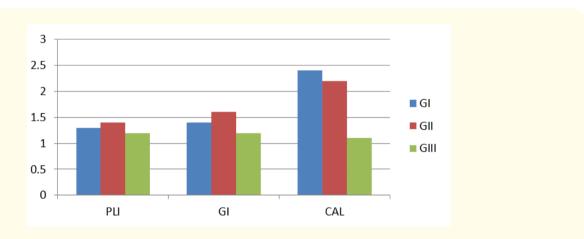
The results of the present study were statistically analyzed by ANOVA test, student t-test and coefficient of correlation (r). The mean and  $\pm$  SD of patients' age in our study recorded in table 1 and figure 1. The mean age in the hypertensive patient group (group III), was more than the men of age in the control group (group I) and diabetic patient group (group II) and the mean of age in group II was more than group I. There were highly significant differences in the mean of age between group III to group I and group II as clarified in table 1 and figure 1 (p < 0.000).



Dt Crouns	Mean of Age ±	ANOVA				
Pt. Groups	SD*	F	p- value			
Healthy patients (GI)	32.4 ± 1.2		0.000**			
Diabetic patients (GII)	37.2 ± 1.7	8.624				
Hypertensive patients (GIII)	43.3 ± 2.6	8.024				
*: Standard deviation. **: P- value high statistical significance						
The age of the patients were illustrated as mean $\pm$ SD; 'n' number of patients' value was assessed; p $\leq$ 0.05 was be a statistical significant.						

Table 1: Mean and ± SD of patients age.

On the other hand, there were significant differences were found in PLI, GI and CAL between group I, II and III are seen in table 2 and figure 2 (p < 0.05). The clinical findings revealed that GI and CAL values of the hypertensive patient group are lower than the diabetic patients' group and a control group, which is considered evidence of reduced severity of periodontal diseases among the hypertensive patient group more than the other groups in the present study (Table 2 and figure 2).

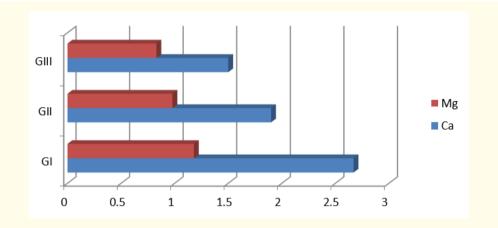


**Figure 2:** Periodontal parameters. PLI: Plaque Index; GI: Gingival Index; CAL: Clinical Attachment Loss.

Ps. Gro	PLI <sup>+</sup>	GI#	CAL##			
Healthy patie	nts (GI)	1.3 ± 0.6	$1.4 \pm 0.3$	$2.4 \pm 0.3$		
Diabetic patients (GII)		$1.4 \pm 0.2$	$1.6 \pm 0.6$	$2.2 \pm 0.2$		
Hypertensive (GIII)	1.5 ± 0.1	$1.2 \pm 0.2$	1.1 ± 0.5			
ANOVA	F	9.019	9.852	7.733		
	p- value	0.017***	0.011***	0.021***		
<ul> <li>*: Plaque index. *: Gingival index. *: Clinical attachment loss. **: P- value significant differences.</li> <li>The correlations between diabetes mellitus, hypertension and periodontal parameters were illustrated as mean ± SD; 'n'</li> </ul>						
number of patients; p value was assessed; p < 0.05 was a statis- tical significant.						

Table 2: Mean of Age ± SD of periodontal parameters.

On the other hand, table 3 and figure 3 displayed statistically significant differences in the mean values of salivary calcium and magnesium (mmol/L) between periodontitis patients without systemic diseases, periodontitis patients with diabetes, and periodontitis patients with hypertension (P < 0.005). The results showed that the levels of salivary calcium and magnesium (mmol/L) in the periodontitis patients with hypertension (GII) were lower than periodontitis patients with diabetes (GII) and periodontitis patients without systemic diseases (GI). Table 3 and figure 3 show increased salivary calcium and magnesium levels (mmol/L) of periodontitis patients without systemic diseases more than periodontitis patients with diabetes and hypertension.



*Figure 3:* Mean of salivary calcium and magnesium. *GI: Group I; GII: Group II; GIII: Group III; Mg: Magnesium; Ca: Calcium.* 

Ps. Grou	р	Calcium (mmol/L)	Magnesium (mmol/L)			
Healthy patients (GI)		2.67 ± 0.57	$1.18 \pm 0.37$			
Diabetic patients (GII)		1.90 ± 0.52	0.98 ± 0.18			
Hypertensive patients (GIII)		1.59 ± 0.36	0.83 ± 0.18			
ANOVA	F	5.421	3.215			
	p- value	0.002 <sup>π</sup>	0.005π			
<sup>#</sup> p value statistical significance.						
The correlations between diabetes mellitus, hypertension and Salivary Calcium and Magnesium were illustrated as mean ± SD; 'n' number of patients; p value was assessed; p < 0.05 was a						
statistical significant.						

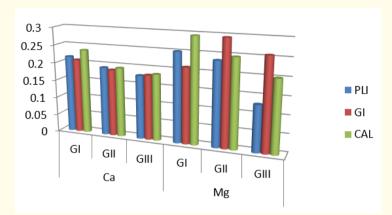
Table 3: Mean and ± SD of salivary calcium and magnesium.

The correlation between salivary calcium levels and magnesium (mmol/L) and clinical parameters of the current study summarized in table 4 and figure 4. There were differences in salivary calcium level (mmol/L) and clinical periodontal parameters of the three groups with high statistically significant differences. There were highly statistically significant differences in the correlation between both gin-

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gival index (GI) and clinical attachment loss (CAL) and a salivary magnesium level (mmol/L) of periodontitis patients without systemic diseases and periodontitis patients with diabetes and periodontitis patients with hypertension (Table 4 and figure 4).



**Figure 4:** Mean and ± SD of salivary calcium and magnesium according to periodontal parameters. GI: Group I; GII: Group II; GIII: Group III; Mg: Magnesium; Ca: Calcium; PLI: Plaque Index; GI: Gingival Index; CAL: Clinical Attachment Loss.

СР	Calcium(mmol/L)		T-test		Magnesium (mmol/L)			T-test		
	GI	GII	GIII	p- value	Т	GI	GII	GIII	p- value	Т
PLI	0.216	0.192	0.178	0.165	-0.270	0.251	0.235	0.129	0.315	-1.492
GI	0.208	0.187	0.181	0.516	-0.392	0.211	0.295	0.257	0.002*	-2.022
CAL	0.237	0.194	0.185	0.142	-0.331	0.259	0.247	0.201	0.007*	-2.490

\*p - value Significance, \*: p- value high statistical significance. CP: Clinical Parameters.

The correlations between clinical attachment loss, and salivary calcium and magnesium were illustrated as mean ± SD; 'n' number of patients; p value was assessed by student t-test; p <0.05 was be a statistical significant.

Table 4: Mean and ± SD of salivary calcium and magnesium according to periodontal parameters.

#### Discussion

The saliva is an obvious diagnostic source to recognize the disease and assess its diagnosis during the treatment; therefore the biomarkers of saliva may be used to evaluate the health and pathology of periodontal tissues [22,24,25].

In the present study there were statistical significance differences in PLI, GI and CAL between periodontitis patients without systemic diseases, periodontitis patients with diabetes and periodontitis patients with hypertension, this is an evidence that the presence of dental plaque is the main reason of initiation and progression of periodontal diseases among the patients of the current study. Similar findings were detected by Armitage in 1999 [26].

In the results of the study that was carried out by Arthur (1991). The mean PLI of diabetics was more than the mean of PLI of the control group, and there were no statistically significant differences in others periodontal parameters [27].

These results were different than the results of Benveniste (1967) and Bernick., *et al.* (1975) studies, who detected no significant difference between the diabetic and non-diabetic patients on PLI value and they found that the mean GI of diabetic patients was more than the mean GI of the control group that is similar the results of our study and the results of Cohen., *et al.* (1970) study [28-30].

To clarify the correlation between hypertension and periodontal disease the epidemiological systematic examinations should be done on hypertensive and healthy samples [31]. It is well recognized that periodontal disease and hypertension have similar causes like stress, socioeconomic causes, smoking and progression of age. Irrespective of these causes and according to the scientific statement issued by the American Heart Association (AHA) that there is a correlation between cardiovascular disease and periodontal disease [32].

In the present study, the oral hygiene of hypertensive patients was evaluated by PLI. The PLI of the control group was lower than the hypertensive patients' group by a specific professional explanation of this index, it was detected that the oral hygiene of hypertensive patients was poor [33].

In our study GI is more in a group (III) than group (I), this results harmonious with the results of Tsakos., *et al.* (2010) study in the United States displayed that there was an association between hypertension and gingival bleeding regardless of the effect of clinical signs of chronic inflammation and behavioral, socio-demographic and socio-demographic causes [34].

On the other hand, the patients in the present study exhibited statistically significant difference when CAL was compared where the mean of CAL of patients without systemic diseases was higher than diabetic and hypertensive patients that may be attributed to the effect of diabetes mellitus and hypertension with some inorganic salivary elements on the pathogenesis of the periodontal disease.

The different organic and inorganic salivary contents help in preserving and safety of oral tissues where the elevation of calcium in saliva leads to increased plaque mineralization which is the main factor of periodontal disease, moreover, there were previous studies that have detected the true relation between increased salivary calcium and increased severity of periodontal disease and there are some systemic factors like hypertension and diabetes mellitus influence on salivary calcium and other minerals and other studies detected that there was a correlation between low salivary magnesium and other minerals levels and reduced severity of periodontal disease [35-37]. In the present study the values of salivary calcium and magnesium levels are more in group I compared to group II and III. Moreover, periodontal parameters and salivary calcium value with all periodontal parameters, whereas there were significant statistical differences in salivary calcium value with all periodontal parameters, whereas there were significant statistical differences with gingival index and clinical attachment loss.

There were some limitations in the current study that should be clarified where there were some factors not included in the design of the study which may impact on the results such as participants' oral hygiene measures assessment, socioeconomic level, asking about the bad oral habits and education level of patients moreover identification of hypertension and diabetes mellitus types. On the other hand, the results of the present study can be useful for identifying the needs of this population regarding periodontal therapy and oral health maintenance and may help as a basis in designing strategies the future studies.

#### Conclusion

From our study results, we concluded that there is an inverse correlation between the level of salivary calcium, magnesium, PLI, GI and CAL in healthy patients and the patients with diabetes mellitus and hypertension. We found that the salivary calcium and magnesium levels were appeared with increasing severity of the periodontal disease among healthy patients and decreasing among diabetic and hypertensive patients. The present study reaffirmed the importance of chemical and physical analysis of saliva to evaluate the periodontitis patients with some systemic disease, but we need further studies with big specimens to induct these results.

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### **Conflict of Interest**

There are no conflicts of interest in this article publication.

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