Original Article

Interactive Effect of Salivary Protein Carbonyl, Total Glutathione, pH, and Flow Rate on Root Caries Severity: A Case–Control Study

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ABSTRACT

Aims and Objectives: Oxidant and antioxidant components in saliva play an essential role in caries development. The purpose of the current study was to disclose the interactive effect of salivary protein carbonyl (PC), total glutathione (GSH), pH, and flow rate on root caries severity. **Materials and Methods:** The control and study groups consisted of 90 older adults of both genders classified into six groups: normal salivary flow rate with no root caries (control), normal salivary flow rate with incipient root caries, normal salivary flow rate with shallow root caries, hyposalivation with no root caries, hyposalivation with incipient root caries, and hyposalivation with shallow root caries. Each group consisted of 15 older adults. The study participants were selected from those patients who attended the teaching hospital at the College of the Dentistry/University of Baghdad and fit the study's criteria. Unstimulated saliva was collected. Both salivary pH and flow rate were determined immediately. After that, saliva was subjected to biochemical analysis to determine PC and total GSH levels colorimetrically. Root surface caries was diagnosed clinically using the Root Caries Index. Data were statistically analyzed using descriptive statistics, two-way univariate analysis of variance, two-way multivariate analysis of variance, and Pearson's correlation coefficient (α = 5%). **Results:** Salivary total glutathione revealed a significant interactive effect with salivary flow rate and root decay severity. Levels of salivary total GSH were significantly higher in subjects with shallow root caries than those with incipient root caries; no root caries levels of salivary PC were significantly high in the hyposalivation group, but no correlation with caries severity was found. High root caries severity was found to be associated with reduced salivary pH and flow rate. **Conclusion:** A significant interactive effect was recorded for salivary flow rate, pH, and total GSH on root caries severity except for salivary PC.

Keywords: *Root caries, salivary flow rate and pH, salivary protein carbonyl, total glutathione*

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Introduction

The major and minor salivary gland secretions and gingival crevicular fluid constitute the whole saliva mixture. Saliva is a heterogeneous liquid mainly composed of water and encompasses organic and inorganic constituents.[1] It has a multipurpose defense mechanism that depends on the amount and makeup of the substance.[2] It functions as a cleaning, lubricating, oral health maintaining, and a buffering agent for the teeth and oral mucosa while also serving

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as a calcium and phosphate storage solution. The ions in saliva remineralize initial carious lesions (DES-RE process).[3,4] Salivary pH and buffering capacity are crucial characteristics in managing the ion exchanges throughout the remineralization and demineralization of enamel. Normal salivary pH ranges from 6.2 to 7.6. The equilibrium of hydrogen bicarbonate in saliva serves as a determinant. It was evident that salivary pH and buffering capacity of the study group were statistically significantly lower than those of the control group.[5,6] The salivary antioxidant system is the first line of defense against harmful reactive oxygen species (ROS).[1] Salivary nonenzymatic antioxidants include glutathione (GSH), ascorbic acid, and uric acid. Also, superoxide dismutase, catalase, and GSH peroxidase are the main salivary antioxidant enzymes.[7] A condition known as oxidative stress occurs when abnormally high levels of ROS/reactive nitrogen species (ROS/RNS) activity or compromised redox signaling cause the oxidation of cellular biomolecules. Although numerous physiological processes (including cell growth and differentiation, mitogenic response, extracellular matrix remodeling, and death) depend on free radicals, excessive generation of ROS affects gene expression, cytokine and chemokine production, and cellular metabolism.[8,9] When proteins are exposed to ROS, modification of amino acid side chains occurs, disturbing cellular metabolism. Carbonyl (CO) groups are produced on protein side chains when they are oxidized and are known as protein carbonyl (PC). These moieties are chemically stable, which is useful for both their detection and storage.[10]

A multifactorial condition known as root caries causes softened, brownish, and uneven tissue to appear on the root surface close to the cement–enamel junction. Two recent systematic reviews of observational longitudinal and cross-sectional studies have found several risk factors for root caries, including age, socioeconomic level, gingival recession, oral hygiene status, and smoking.[11-14]

A systematic review by Zhang *et al.*[13] identified several variables belonging to various categories related to root caries, such as aging, low socioeconomic status, smoking, gingival recessions, and poor oral hygiene. Saliva's antioxidant qualities help to maintain a balance between free radicals, which is crucial for safeguarding the oral mucosa. Free radicals are extremely unstable molecules that can pick up or lose electrons from other molecules to become more stable. They become unstable when they pick up electrons from proteins, lipids, and nucleic acids, setting off a chain of events that damages cells.[3] In systematic reviews and meta-analyses, in addition to studies among children and adolescents, a relation between salivary oxidative stress biomarkers and dental caries clinical indices was recorded.[3,15,16] It is crucial to note that the oral cavity is a dynamic environment with intricate relationships between its parts, rather than a static one. The salivary flow rate and other defensive mechanisms affect salivary clearance or flushing action. Salivary flow rate affects salivary pH buffering and antibacterial effects.[5]

In addition, the activity of salivary antioxidants is a concerted action that involves multiple interactions with antioxidants from other sources. However, no studies could be found that measured levels of salivary total GSH, PC, hyposalivation, or saliva acidity in relation to root caries severity and the interactive effect of these salivary parameters on root caries severity. Therefore, this study was designed and conducted among older adults aged 50–60 years to determine salivary PC and total GSH levels with salivary flow rate and pH measurement and their impact on root caries severity. We consider the null hypothesis that hyposalivation, low pH, and oxidative stress are not associated with higher severity of root caries.

Materials and Methods

This is a case–control, single-blind study. The study participants were selected from those patients who attended the teaching hospital at the College of the Dentistry/University of Baghdad and fit the study's criteria. The control and study groups comprising 90 older adults aged 50–60 years of both genders) were divided into six groups: normal salivary flow rate with no root caries (control), normal salivary flow rate with incipient root caries, normal salivary flow rate with shallow root caries, hyposalivation with no root caries, hyposalivation with incipient root caries, and hyposalivation with shallow root caries. Each group consisted of 15 participants as shown in Figure 1 (study design). The ethical approval had been obtained with the number 233001.

The sample size was calculated Using G power 3.0.10 (Program written by Franz-Faul, Universitatit Kiel, Germany) With power of study = 85%, alpha error of probability = 0.05 two sided, assume effect size of ANOVA as 0.4 , with six groups, with all these condition the sample size is 72 subjects adding 10 % as an error rate 5 thus 80 subjects is sample size with that 90 subjects is enough and more calculated than G power. [17]

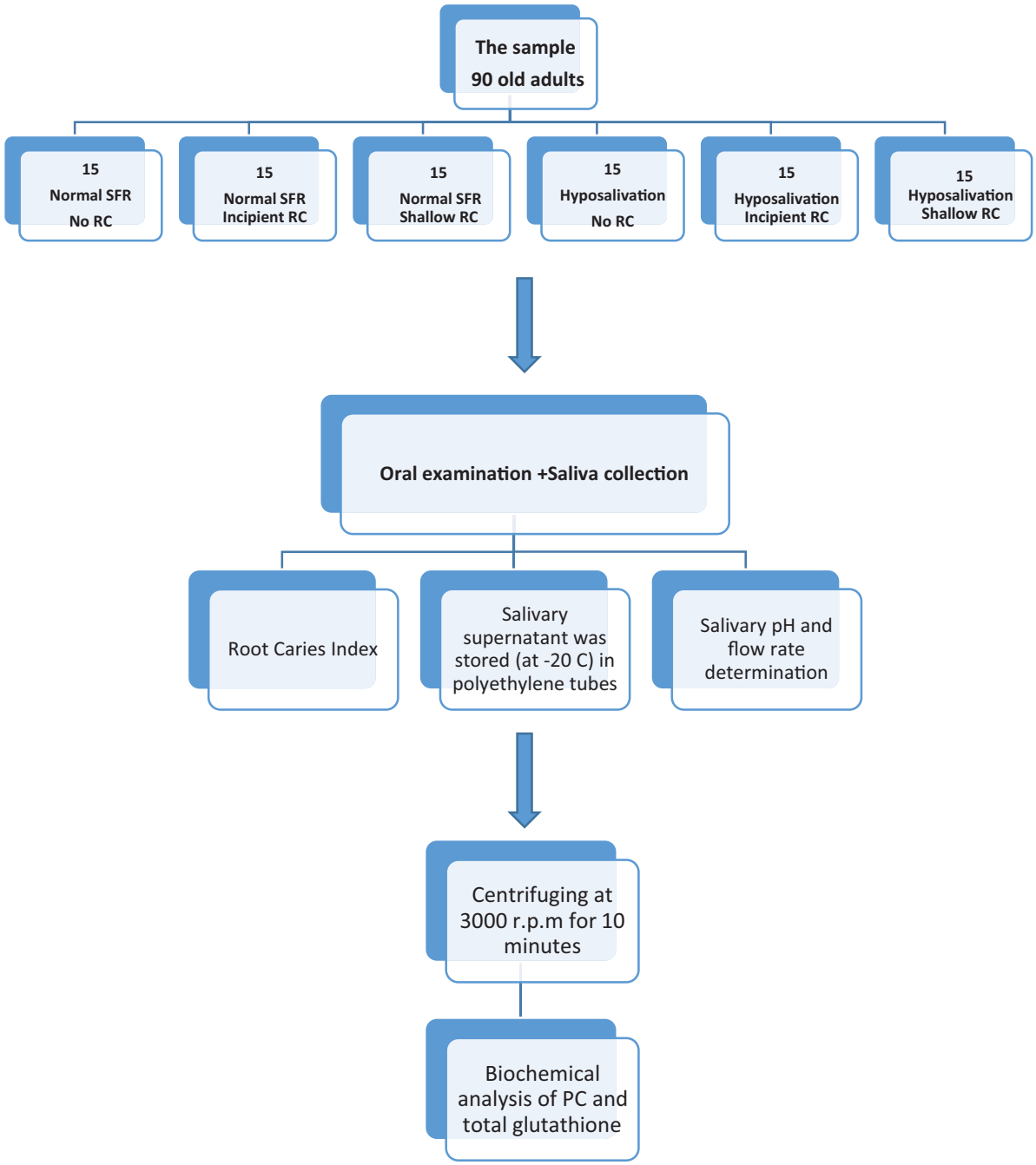

Figure 1: Study Design (SFR: salivary flow rate, root caries, PC: protein carbonyl)

Informed consent was obtained from the patients after explaining the research protocol in detail. The inclusion criteria for subjects were as follows:

- Non-smokers
- Free from any medical conditions
- Free from any other condition that affects the salivary secretory mechanism
- Not taking any medications or nutritional supplements with xerogenic effects
- Not wearing any fixed or removable dental prostheses or orthodontic appliances.

Salivary specimen was collected in a standardized environment, considering the instructions cited by Navazesh and Kumar.[18] Unstimulated salivary samples were gathered. One hour before saliva collection, the subjects abstained from food, chewing gum, and beverages. They were seated in a chair, then their mouths were irrigated with distilled water and relaxed for at

least 5 min. They were then instructed to minimize their movement and tilt their forehead downward, and a test tube was placed below it. After that, the subjects were instructed to keep their mouths open to allow saliva to drain into the tube for 5 min. At the end of the collection time, the subjects were instructed to collect any residual saliva in the mouth and spit it quickly into the test tube. The actual trail continued for 5 min. After all the foam disappeared, saliva was positioned into a cooler box and sent to the laboratory and centrifuged for 10 min at 3000 rpm (revolution per minute); subsequently, the supernatant was seperated using a micropipette and deep freezed (-20ºC) in polyethene tubes for chemical analysis. Salivary pH was immediately measured using an electronic pH meter, and the salivary flow rate was determined using a measuring cylinder (in mL/min). Then salivary specimens were subjected to laboratory biochemical analysis. The flow rate for normal, unstimulated saliva is between 0.25 and 0.4 mL/min. A resting flow rate range of 0.1–0.25 mL/min is regarded as low and that of less than 0.1 is regarded as very low. Both low and very low salivary flow rates were regarded as hyposalivation in the current study. Salivary PC level was analyzed using a PC assay kit (Clementia Biotech, New Delhi, India). The level of salivary total GSH was determined colorimetrically. Biochemical laboratory work was conducted at the Poisoning Consultation Center at Gazi Al-Hariry Hospital in Baghdad city. Root surface caries was diagnosed clinically using the Root Caries Index (RCI) described by Katz[19] as follows:

$$RCI = \frac{(R-D)+(R-F)}{(R-D)+(R-F)+(R-N)} \times 100$$

where R–D is recession combined with decayed root surface, R–F is recession combined with filled root surface, and R–N is recession combined with sound root surface.

Gingival recession was recorded as present if at least 0.5 mm of root surface was visible.

Further classification of decayed root surface according to severity was used[20]:

Grade I: Incipient
Grade II: Shallow
Grade III: Cavitation
Grade IV: Pulpal involvement.

Clinical examination of root caries status was conducted by a single examiner using a No. 05 clinical mirror and CPI community periodontal index probe.[21] The intraexaminer agreement was obtained by repeating the measurements in 10 patients, obtaining a 0.850 Kappa coefficient for RCI.

Statistical Package for Social Sciences (SPSS, IBM Company, Chicago, Illionis) software, version 25, was used for data analyses. Both descriptive statistics (i.e., number, mean, and standard deviation) and interferential statistical tests, such as two-way univariate analysis of variance (two-way ANOVA), two-way multivariate analysis of variance (two-way MANOVA), and Pearson's correlation coefficient, were applied. The 95% confidence level (P 0.05) was accepted.

Results

Levels of salivary total GSH were significantly higher in subjects with shallow root caries than those with incipient and no root caries ($P < 0.01$). Also, total GSH levels were significantly higher in subjects with hyposalivation than those with normal salivary flow rates ($P < 0.01$). Subjects with no root caries and normal salivary flow rates had the lowest total GSH concentration than those with incipient root caries and shallow root caries with normal salivary flow rates. Regarding those with hyposalivation, salivary total GSH levels were higher among those with shallow root caries, followed by those with incipient root caries and caries-free root surfaces. Both salivary flow rate and root caries severity show significant interactions with total GSH ($P < 0.01$).

On the other hand, salivary PC levels in subjects with caries-free root surface and incipient root caries were the same but significantly lower among those with shallow root caries. ($P < 0.01$). PC levels were significantly lower in those with normal salivary flow rates than in those with hyposalivation ($P < 0.01$). PC concentration was nearly the same in all three groups of normal salivary flow rate with different root caries severity. For those with hyposalivation, irrespective of root caries severity (free, incipient, and shallow caries), PC level was the same. Both root caries severity and salivary flow rate categories had no significant interaction with salivary PC level ($P > 0.05$).

A significant association was found between increased root caries severity with hyposalivation ($P < 0.01$) and reduced salivary pH ($P < 0.01$). In subjects with normal salivary flow rate, salivary pH was higher in those with no root caries (pH value = 7.6) than those with incipient root caries (pH value = 7.07) and then those with shallow root caries (pH value = 7.03). In subjects with hyposalivation, salivary pH was higher in the caries-free group than that in the incipient and shallow root caries group. There was a statistically significant ($P < 0.05$) interaction between root caries severity and salivary flow rate in pH, as shown in Tables 1 and 2. A highly significant interaction

Table 1: Descriptive statistics (mean ± SD) of salivary protein carbonyl (nmol/L), total glutathione (ng/mL), and pH among the control and study groups

Study populations	No root caries mean ± SD (*n*)	Incipient root caries mean ± SD (*n*)	Shallow root caries mean ± SD (*n*)	Total (*n*)
Salivary glutathione				
Normal salivary flow rate	0.01 ± 0.06 (15)	0.03 ± 0.08 (15)	0.51 ± 0.74 (15)	0.18 ± 0.48 (45)
Hyposalivation	0.01 ± 0.06 (15)	0.09 ± 0.11 (15)	1.96 ± 1.90 (15)	0.69 ± 1.41 (45)
Total (*n*)	0.01 ± 0.05 (30)	0.06 ± 0.09 (30)	1.24 ± 1.60 (30)	0.44 ± 1.08 (90)
Salivary protein carbonyl				
Normal salivary flow rate	1.12 ± 0.04 (15)	1.13 ± 0.01 (15)	1.11 ± 0.02 (15)	1.12 ± 0.03 (45)
Hyposalivation	1.3 ± 0.02 (15)	1.3 ± 0.03 (15)	1.3 ± 0.002 (15)	1.3 ± 0.2 (45)
Total (*n*)	1.24 ± 0.12 (30)	1.24 ± 0.11 (30)	1.23 ± 1.11 (30)	1.2 ± 1.11 (90)
Salivary pH				
Normal salivary flow rate	7.6 ± 0.23 (15)	7.07 ± 0.169 (15)	7.03 ± 0.22 (15)	7.22 ± 0.32 (45)
Hyposalivation	7.1 ± 0.48 (15)	7.04 ± 0.4 (15)	6.76 ± 0.32 (15)	6.97 ± 0.42 (45)
Total (*n*)	7.33 ± 0.44 (30)	7.05 ± 0.29 (30)	6.89 ± 0.30 (30)	7.09 ± 0.39 (90)

Table 2: Statistical differences and interactions of salivary GSH, PC, and pH among control and study groups

Variables	Two-way ANOVA test		
	F-test	*P*-value	
Salivary glutathione			
Salivary flow rate	8.19	0.005**	
Root caries severity	20.63	0.000**	
Salivary flow rate * root caries severity	7.27	0.000**	
Salivary protein carbonyl			
Salivary flow rate	1520.3	0.000**	
Root caries severity	0.38	0.000**	
Salivary flow rate * root caries severity	1.22	0.299	
Salivary pH			
Salivary flow rate	13.72	0.000**	
Root caries severity	14.38	0.000**	
Salivary flow rate * root caries severity	3.38	0.039*	
Variables	**Two-way MANOVA test**		
	F-test	*P*-value	Wilks
Salivary flow rate	501.09	0.000**	0.052
Root caries severity	9.04	0.000**	0.565
Salivary flow rate * root caries severity	4.25	0.001**	0.749

*significant ($P<0.05$)

**highly significant ($P<0.01$)

was recorded between root caries severity and salivary flow rate categories regarding salivary GSH, PC, and pH ($P < 0.01$), as shown in Table 2.

The profile plot showing pH, GSH, and PC marginal means among the control and study groups is shown in Figures 2–4, respectively.

Pearson's correlation coefficient revealed a positive relation between salivary total GSH and salivary flow rate among those who had hyposalivation with incipient and shallow root caries. The relation was significant only in the case of incipient caries and highly significant in the case of shallow root caries. Also, a significant positive correlation was found between PC and salivary flow rate among those with hyposalivation with shallow root caries. Salivary pH had significant positive correlation with salivary flow rate among those with normal salivary flow rate with incipient and shallow root caries, as well as among those with hyposalivation with incipient root caries. Except for those with hyposalivation and shallow root caries, the correlation of pH with salivary flow rate was significantly inverse, as shown in Table 3. A significant positive correlation was found between salivary total GSH and root caries value among those with normal salivary flow rate and shallow root caries. Also, salivary pH recorded a highly significant positive correlation with root caries value among those with normal salivary flow rate and shallow root caries, as shown in Table 4.

Discussion

A significant interaction was recorded between salivary total GSH with salivary flow rate and root caries severity. Significantly high levels of salivary PC were detected in the hyposalivation group, but no correlation with caries severity was found. High root caries severity was associated with reduced salivary pH and flow rate. A significant positive correlation of salivary GSH with a salivary flow rate was found in subjects with hyposalivation and incipient root caries and hyposalivation and shallow root caries. In addition, GSH had a significant positive correlation with root caries in those with a normal salivary flow rate and shallow root caries.

In 2015, Al-Souz and Al-Obaidi[22] conducted a study to compare lead acid battery factory workers

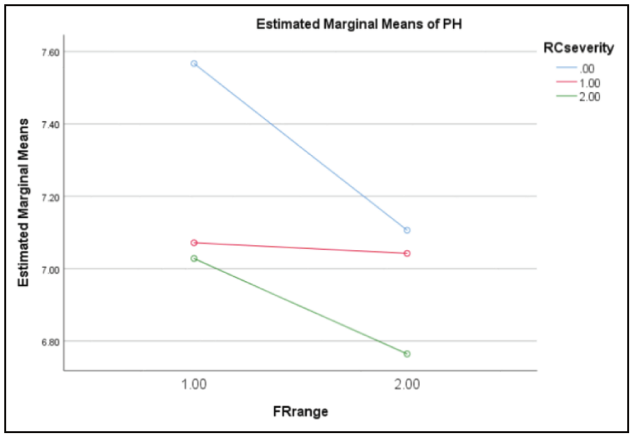

Figure 2: Profile plot of the estimated marginal mean of salivary pH (RC: Root caries, FR: flow rate)

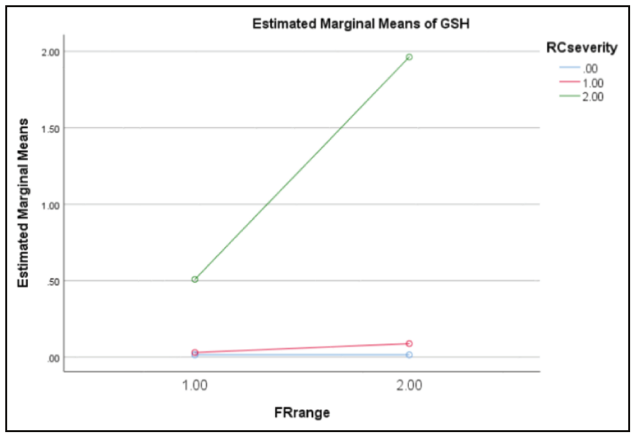

Figure 3: Profile plot of the estimated marginal mean of salivary GSH (RC: Root caries, FR: flow rate)

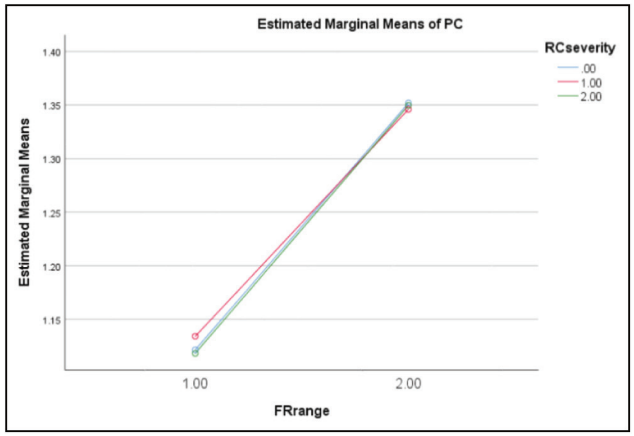

Figure 4: Profile plot of the estimated marginal mean of salivary PC (RC: Root caries, FR: flow rate)

to a nonexposed group to evaluate specific salivary antioxidants and their relationship with dental caries. The antioxidant levels (uric acid, catalase, and GSH peroxidase enzymes) were discovered to be higher in the study group than in the control group, with a nonsignificant difference for uric acid, a highly significant difference for catalase, and a significant difference for GSH peroxidase enzymes. However, total protein levels were found to be significantly lower in the study group than in the control group. Also, dental caries severity was measured by DMFS values, which were considerably greater in the study group than in the control group. The relationships between salivary antioxidants and dental caries for both groups were modest or nonsignificant. These findings are in agreement with those of the present study because the present study found a high salivary total GSH level with severe form of root caries ($P < 0.01$). Also, significantly higher total GSH levels in subjects with hyposalivation than those with normal salivary flow rates were found. ($P < 0.01$). On the other hand, patients with active carious lesions may have lower levels of GSH in their saliva, which could be a sign of a propensity toward decreasing antioxidant status. These contradicting results demonstrate that etiology and disease progression can have an influence on oxidative damage and antioxidant status biomarkers in saliva.[23]

Abd Al Hussain and Hussein[24] discovered in 2020 that dental caries activity was linked to increasing free radical formation, which led to an increase in caries experience over time. The immune cells may be damaged by ROS, which might increase the buildup of microbial plaque on tooth surfaces and increase the severity of caries. On the other hand, antioxidants may be diminished or depleted due to the increased production of free radicals. This explains the favorable relationships between salivary PC and dental caries discovered in the current investigation. These findings are in disagreement with the findings of the present study because the level of salivary PC was the same among those with caries-free root surface and incipient root caries but lower among those with shallow root caries, with a highly significant difference ($P < 0.01$), which indicate a relationship between the severity of root caries and PC. This disagreement may be due to the methodological difference between the two studies.

Due to radiation-induced hypofunction of the salivary glands, dental caries is the main side effect postradiotherapy patients with head and neck cancer are more likely to experience. Clinical hyposalivation has been linked to average radiation doses of more than 20–39 Gray. Additionally, a dose–response connection has been established, with quantitative salivary gland output being demonstrated to be inversely related to radiation exposure.[25] This also supports indirectly the findings of the present study; regarding those with hyposalivation, a higher concentration of the salivary

Table 3: Correlations of salivary total glutathione, protein carbonyl, and pH with salivary flow rate of the study groups

Study populations	Control no root caries mean ± SD (n)		Incipient root caries mean ± SD (n)		Shallow root caries mean ± SD (n)	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Salivary glutathione						
Normal salivary flow rate	-0.294	0.288	0.186	0.507	0.192	0.492
Hyposalivation	-0.486	0.066	0.612	0.015*	0.930	0.000**
Salivary protein carbonyl						
Normal salivary flow rate	0.439	0.101	0.08	0.776	0.502	0.056
Hyposalivation	-0.143	0.612	-0.017	0.952	0.954	0.000**
Salivary pH						
Normal salivary flow rate	0.298	0.280	0.952	0.000**	0.975	0.000**
Hyposalivation	0.187	0.505	0.951	0.000**	-0.875	0.000**

*significant (P<0.05)

**highly significant (P<0.01)

Table 4: Correlations of salivary total glutathione, protein carbonyl, and pH with root caries of the study groups

Study populations	Control No root caries mean ± SD (*n*)		Incipient Root caries mean ± SD (*n*)		Shallow root caries mean ± SD (*n*)	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Salivary glutathione						
Normal salivary flow rate	-0.149	0.595	-0.237	0.396	0.595	0.019*
Hyposalivation	-0.149	0.595	-0.218	0.435	-0.286	0.302
Salivary protein carbonyl						
Normal salivary flow rate	-0.095	0.737	0.249	0.370	-0.501	0.057
Hyposalivation	0.106	0.706	-0.145	0.606	-0.286	0.302
Salivary pH						
Normal salivary flow rate	0.07	0.804	0.419	0.120	0.671	0.006**
Hyposalivation	0.034	0.905	-0.134	0.635	0.286	0.302

*significant (P<0.05)

**highly significant (P<0.01)

total GSH was found among those with shallow root caries, followed by those with incipient and caries-free root surfaces. The salivary flow rate and root caries severity showed highly significant interactions with total GSH ($P < 0.01$).

Low salivary pH was found in subjects with shallow root caries (highly significant, $P < 0.01$). Furthermore, decreased salivary flow rate was associated with a low salivary pH ($P < 0.01$). For those with a normal salivary flow rate, salivary pH was higher (7.6) among those with no root caries (caries free) than those with incipient (7.07) or shallow root caries (7.03). For the hyposalivation category, salivary pH was higher in the no root caries group than in the groups with incipient and shallow root caries. There was a statistically significant ($P < 0.05$) interaction between root caries severity, salivary flow rate, and pH, as shown in Tables 1 and 2. A highly significant interaction was recorded between root caries severity and salivary flow rate categories regarding salivary GSH, PC, and pH ($P < 0.01$), as shown in Table 2.

Multiple studies focused on discussing the relationship between oxidative stresses, however, due to methodological differences in the study design, interventional techniques, and the results tabulation, it seems hard to compare their results and the results of the current study.[26-31] It is worth mentioning that the present study had the following limitations: small sample size; standardization concerning the same dietary pattern was not feasible; and oral hygiene measures and fluoridated measures were required. In addition, it was difficult to determine the source of antioxidants in saliva, whether it was the host, diet, or microbial origin.

Conclusion

A significant interactive effect was recorded for salivary flow rate, pH, and total GSH on root caries severity, except for salivary PC. It is essential to perform further studies that consider other salivary oxidative components and other salivary physical properties, such as viscosity in relation to root caries severity.

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This study was self-funded by the researcher.

Conflicts of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

Authors contributions

Baydaa Ahmed Yas approve the final version.

Ethical policy and institutional review board statement

The study protocol had been approved by the ethical committee at the College of Dentistry/University of Uruk (i.e., the ethical committee authorized this study, No. 233001). Each volunteer was informed of the study's goals and gave their consent after understanding them. The use of human volunteers in this study was authorized by the World Medical Association's Code of Ethics (Declaration of Helsinki).

Patient declaration of consent

The author certify that she have obtained all appropriate patient consent forms. In the form the patients have given their consent for their clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

Data availability statement

Data set available for request please contact the following person Baydaa Ahmed Yas. E-mail: drbaydaaumusama@gmail.com.

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