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The Diagnostic Aids of Salivary Trace Metals for Dental Caries among Systemically Healthy

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Abstract

Objectives of the study: This research aimed to assess levels for salivary trace elements, iron and copper as potential biomarkers for dental caries in Iraqi patients. **Methods:** Stimulated salivary samples were collected from 60 participants aged from (20-27) years divided into two groups, first group (patients with cavitated D2 enamel-dentine caries) and the second group (control patients with initial enamel caries but not cavitated lesions) to determine iron and copper concentrations. **Results:** There had been a significant elevation of salivary iron and copper in caries group ($p<0.001$). There was a positive relationship between iron and DMFT scores ($r = 0.3$, $p = 0.03$). ROC analysis revealed that iron had strong diagnostic performance (AUC=0.9), while copper showed moderate accuracy (AUC=0.7). **Conclusion:** Salivary iron demonstrated high sensitivity and specificity for detecting dental caries and may serve as a reliable biomarker. Copper may also be supportive but less specific. These finding suggested that the diagnostic accuracy of salivary trace elements, though further standardized collection methods of saliva are recommended.

Key words: Dental caries, saliva, biomarkers, iron, copper.

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Introduction

The dental caries is a complex illness which is brought on by means of the host's dental tissue, dental biofilm, and the breakdown of food sugars. It resulted from repeated cycles of demineralization and remineralisation at the interface of the biofilm and the tooth surface (Anil *et al*., 2022). Because dentine and enamel are structurally different, caries develops differently in dentine than in enamel. As dentine has few minerals and tiny tubules that let minerals and bacteria to pass through, the body of the enamel lesion becomes increasingly demineralized. Consequently, the surface enamel weakens and eventually collapses (Duncan *et al*., 2019).

Saliva is a biological fluid that is very appealing for identifying biomarkers. Saliva analysis provides a real-time data, and the current technologies allow detection of various types of biomarkers. simultaneously (Navazesh, 1993; Buzalaf *et al*., 2020). Ionomics, which is the study of the ionome is described as "the mineral nutrient and trace element composition of an organism, representing the inorganic component of cellular and organismal systems." (Salt *et al*., 2008; Baima *et al*., 2022). Elements (such as zinc, iron, copper, selenium, and iodine) are required in very small amounts and, therefore, are known as trace elements (Chellan & Sadler, 2015). Trace elements are minerals found in very small quantities in the body. Some are important for essential body functions in both plants and animals, too much or too little of these elements can lead to serious health issues. They exist in different forms in nature and help the body work properly (Islam *et al*., 2023)

Iron in the human body is the most prevalent necessary trace element. There are two forms of iron that are important to biology: (Fe3+) which is the oxidized ferric form and (Fe2+) which is the reduced ferrous form. Human body content of iron is almost about 4.0 gm. in men and about 3.5 gm. in women (Geissler & Singh, 2011; Anne Marie *et al*., 2023). The functional importance of iron in acting as a cofactor, required for the activity of many enzymes and molecules, lies in its ability to undergo redox cycling between its two predominant oxidation readily states, Fe^{3+} and Fe^{2+} (Wallace, 2016). Additionally, a vast range of biological functions, including cell development and proliferation, depend on iron. For example, Iron-containing proteins are required for oxygen transport, ATP generation, energy metabolism and DNA synthesis and repair (Poprac *et al*., 2017). Additionally, redox-active iron might produce reactive oxidative species (ROS), and iron-mediated ROS harm lipids, protein, & DNA. These damaged molecules can trigger senescence or cell death, leading to various diseases (Ray *et al*., 2012; Nakamura *et al*., 2019).

Copper (Cu) is an essential micronutrient involved in various physiological functions such as connective tissue (Festa & Thiele, 2011; NikhalaShree *et al*., 2023). Because of their redox potential, copper ions are advantageous biological co-factors for several enzymes, such as ceruloplasmin, cytochrome oxidase, superoxide dismutase, and lysyl oxidase (LOX). Cu fights infection and promotes wound healing. Disorders of Cu metabolism have been associated with

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many diseases (Hordyjewska *et al*., 2014; Devi *et al*., 2016).

Materials & Methodology

This study was a case-control study. All the participants in the study were notified about the aims of the investigation, and they were free to agree or refuse to share. An informed consent form was provided for signing before participating in the study. A specially designed case sheet was prepared with the patient's name, age, gender, full medical and dental history, medications and smoking status. Before the collection of saliva samples from the subjects, clinical parameters (DMFS and DMFT) were thoroughly investigated. Sample collection was started from January 2025 to May 2025 in the Periodontics and Operative departments of Babylon University College of Dentistry teaching clinics. The Mustansiriyah University College of Dentistry Research Ethics approved the protocol.

Subjects

All participants with age range (20-27) years, the study's healthy control group (n = 30 subjects) had a healthy, intact gingiva and teeth with initial enamel changes, filled or missing teeth, but without active cavitated dentinal lesions (DMFT > 0 but without active caries). The second group (dental caries group, n = 30) included subjects with at least two cavitated enamel–dentine carious lesions (DMFT > 2, Grade 2 lesions extending into dentine). DMFT & DMFS examined on five surfaces (buccal, mesial, lingual, distal and occlusal) of 28 teeth with exclusion of wisdom teeth (Klein *et al*., 1938).

Inclusion Criteria

Adults aged (20-27) years, with dental caries at least two cavities [cavitated lesions (D2 enamel-dentin caries)], stable health conditions with no medications, no recent oral procedures and non-smokers.

The exclusion criteria

Systemic diseases with medications, included patients who refused to participate for study, cancer or chemotherapy, infections, smoking and alcohol consumption.

Saliva collection

To get 10 mL of stimulated saliva in the morning, patients should be instructed to stimulate salivary secretion by using sugar-free gums at their regular chewing frequency. In order for saliva to flow and collect in the front area of the oral cavity, the participants will be told to tilt their heads forward. A big, sterile container will be used to receive the salivary fluid. The obtained saliva samples should be frozen at -24 °C after being cold-centrifuged for 10 minutes at 2,500 rpm and 0–5°. This method of collecting saliva produces samples that are debris-free and have a lower viscosity, improving accuracy and repeatability. The collected samples will sent and subjected to trace element analysis using atomic absorption spectroscopy (Bagewadi *et al*., 2022).

Ethical approval

Mustansiriyah's University / College of Dentistry Research Ethics approved the protocol. (The Study Number: MUPRV010 and Title: (Use my saliva).

Statistical analysis

Analyzing data was performed through using SPSS [version 26]. The Shapiro–Wilk test confirmed normal

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distribution of variables. Independent t-test was used for group comparisons, Pearson correlation for relationships between continuous variables, ICC for reliability, and ROC for diagnostic accuracy.

Oral examination

Every participant in this research must have at least 20 teeth and be in overall good health; will cooperate and sign a consent form after being informed, non-smokers because smoking effect on the tooth minerals and increase brittleness due to high calcium levels in saliva of smokers than the nonsmokers. This increasing in concentration we attributed to decrease of pH of saliva which retrieving calcium from teeth to circulation (Abed *et al*., 2012). The examination is performed in dental clinic for each subject under the study for, the following parameters.

Indicators of decaying, missing, and filled teeth and surfaces (DMFT & DMFS)

The DMF index is a technique for evaluating dental caries by taking into account cavitated, repaired, and lost teeth to evaluate prevalence. It is approved by the World Health Organization (WHO) and widely used in international research (World Health Organization, 2018; Pauli *et al*., 2021). When third molars are taken into account, the DMF-T index scores range from 0 to 32 and the DMF-S index scores range from 0 to 148 for tooth surfaces. The surfaces of each tooth are examined: anterior teeth have distal, mesial, lingual, and facial surfaces, whereas posterior teeth have lingual, mesial, distal, and occlusal surfaces. Surfaces with carious lesions and fillings are categorized as D (destroyed), M (missing) if they were removed because of caries, and F (filled) if they had a filling; extractions made for non-caries purposes, including orthodontics, are not considered carious (Klein *et al*., 1938; Cappelli & Mobley, 2008; Zinke *et al*., 2018).

Results

The clinical parameters (DMFS and DMFT)

The normality of (DMFS) and (DMFT) scores in the control and dental caries groups was tested by using the Shapiro-Wilk test. Table 1 showed that each group's DMFS and DMFT scores were normally distributed, with p-values > 0.05 signifying no discernible departure from normalcy.

To show comparisons of DMFS & DMFT between control & caries groups, the independent t-test was used. Results had been summarized within (Table 2). Mean of DMFS score within the carious lesion group was significantly higher (21.0 ± 10. 9) compared to the control group (7.8 ± 4. 9) ($p<0.001$). The caries group's mean DMFT score was much higher (8.2±2.8) in caries group when compared to the control group (4.1±2.2). The statistical significance of this difference was also shown ($p<0.001$).

Analysis of iron and copper

In Table 3, the salivary concentrations of iron and copper were analyzed between control and caries. To check for statistical differences, the independent T test was employed. Iron: A significant difference ($p<0.001$) had been seen in the salivary iron levels among groups. At 0.02 ± 0.006 mg/L, the control group's mean iron level was much lower than the caries group's (0.04 ± 0.008 mg/L). Copper: A significant difference was also found in salivary

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copper levels across groups ($p < 0.001$). The caries group had the greatest mean copper level (0.002 ± 0.0007 mg/L), whereas the control group had the lowest (0.001 ± 0.0005 mg/L).

Correlation of caries indices with iron and copper

Pearson's correlation test checked the link between salivary iron, copper, and clinical parameters within the study groups (Table 4). Iron showed a significant moderate positive relation with DMFS and DMFT ($r = 0.6$, $p < 0.001$). A significant weak positive correlation within the study participants groups was assessed between copper and clinical indices ($r = 0.3$, $p = 0.007$ for DMFS and $p = 0.002$ for DMFT).

The diagnostic accuracy of iron and copper

Salivary biomarkers' diagnostic accuracy in distinguishing various oral conditions was assessed using "Receiver Operating Characteristic (ROC) curve analysis". "The Area Under the Curve (AUC) was used to evaluate each biomarker's performance, along with optimal cutoff points, sensitivity, and specificity values for each comparison group" (Table 5). Iron again demonstrated high diagnostic performance with an AUC of 0.9 ($p < 0.001$), and perfect sensitivity (100%) with 90% specificity at a 0.03 cutoff. Copper showed moderate diagnostic performance (AUC = 0.7, $p = 0.001$) with a cutoff of 0.001480% sensitivity and 50% specificity in identifying instances of patients with dental caries.

Discussion

The relationship between salivary iron and copper and dental caries in Iraqi patients was clarified by the current study, which also addressed the accuracy of diagnosis of these metals as biomarkers.

DMFT and DMFS

Both the control and caries groups' DMFS and DMFT scores, as determined by the Shapiro-Wilk test, had a normal distribution, with all p-values more than 0.05. This validates the use of parametric tests for analysis, including the Independent t-test and Pearson correlation, and this normalcy is required to guarantee that the sample accurately represents the general populations.

Similar to the findings of (Frencken *et al*., 2020), which showed that caries prevalence should be based on cavitated dentine carious lesions (d/D-component) rather than the dmf/DMF index because the M- and F-component do not refer to a disease stage, the caries group had significantly higher levels of DMFT and DMFS than the control group. These clinical markers are used to assess caries status (Klein *et al*., 1938).

Salivary iron and copper

In comparison of iron in groups, iron showed a significant elevation in dental caries group rather than control group as iron can accelerate pathological changes as (Salnikow, 2021) According to his research, iron is mostly attached to proteins and enzymes that facilitate electron and redox transport. In cells, there is no free iron, but a tiny amount known as "the labile iron pool (LIP) is loosely present in the cytoplasm, when iron balance is disturbed—like in cancer—cells at different levels including absorption,

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systemic transportation, and cellular uptake and storage and inflammatory cells especially need extra iron for growth". This increases the LIP, which can cause reactive oxygen species (ROS) and other dangerous reactions such the Fenton reaction. These ROS have the ability to harm lipids, proteins, and DNA, which might result in cell death or alterations that could cause cancer. However, the results of (Sharifi *et al*., 2021), which demonstrate that children with caries had considerably lower salivary and serum iron and ferritin levels, contradict the current study. The results have clinical and practical implications: Iron and ferritin supplements may help prevent or reduce dental caries in children at risk. Children who have dental caries may also experience additional health problems connected to iron and ferritin. Additionally, the dental caries group's salivary copper levels were noticeably greater than those of the control group, indicating a connection between elevated salivary copper levels and a higher caries incidence. Because copper in saliva affects the enzyme system involved in carbohydrate breakdown (enolase activity) and dental caries activity, as (Reddy *et al*., 2021) explained in his work, copper levels in saliva can be utilized as a caries risk assessment tool.

Correlation

While the DMFT index mostly reflects prior activity, salivary iron was positively connected with caries indices in the cavitated caries group, suggesting that more decaying teeth are associated with greater iron levels. (Zhou *et al*., 2017) found a considerable correlation between pH and iron levels. Furthermore, a lower pH may make iron more soluble and available for oral microbial growth, which might lead to a microbiota that may have a clinical effect on the development of caries. The results of (Xu *et al*., 2023) contradict this study and explain that the carious score was lower in the high iron group but significantly higher in the iron deficiency anemia group than in the caries group. This is because iron may contribute to the pathological damage caused by childhood caries by influencing enamel mineralization. According to comparable earlier research, salivary concentrations of copper were found to be considerably higher in the group with high caries activity, which explained a strong positive association with caries indices (Hussein *et al*., 2013). This may be explained by the idea that minute quantities of copper in the tooth dissolve into the saliva by demineralization, leading to the elevated levels in the saliva (Duggal *et al*., 1991). A single sample of saliva may not accurately reflect trace element levels or their relationship to DMFT due to natural changes over time, which is why another study of copper revealed no correlation with caries indices (Borella *et al*., 1994) explained the association between trace elements like Cu in saliva and DMFT in adult population. Additionally, employing the supernatant requires a consistent approach to avoid inaccurate findings (Jamal Abbas *et al*., 2020). Furthermore, the lack of statistically significant differences in salivary variables between children with early childhood caries and those without it may indicate the multifactorial nature of early childhood caries, where behavioral, dietary, and microbial factors may be more important than salivary composition alone (Borella *et al*., 1994).

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ROC curve analysis

Salivary iron had a high sensitivity and specificity for diagnosting dental caries, making it a useful biomarker for caries differentiation as (Poletto *et al*., 2021) found a link between trace elements and dental caries, and proposed that these elements may play a role in the metabolism of the bacteria that produce the carious process. The goal was to measure the amounts of Al, Cu, Fe, Mn, and Zn in the saliva of kids taking part in an oral hygiene program and look into any connections to dental caries. In terms of the diagnostic precision of copper as a dental caries indicator, which has a weak specificity to identify healthy persons but a fair sensitivity to identify sick ones, However, (Hegde *et al*., 2014) found that an increase in copper in saliva in the presence of many decayed tooth surfaces may be the result of the carious process breaking down the hydroxyapatite crystal structure of enamel and releasing the copper ions from the enamel into the saliva, allowing copper to be used as a supporting biomarker with iron for caries diagnosis.

Conclusions

Salivary iron and copper levels were found to be significantly higher in individuals with dental caries than in the control group. Iron, particularly showed a significant positive relationship with caries indices that suggesting its high accuracy for diagnosing dental caries, therefore iron can be considered as a valuable biomarker. Copper levels were also higher in caries cases and showed a significant positive correlation with caries indices. These results indicate that the salivary iron and copper support the monitoring and early diagnosis of dental cavities. However, a standardized saliva collection and analysis procedures is necessary to ensure reliability and consistency of results. Furthermore, large-scale studies are required in future to explore their clinical application.

A conflict of interest

According to the authors, this study has no conflicts of interest. There were no institutional, personal, or financial ties that may have influenced the findings or interpretations of this investigation.

Conflict of interest

The authors reported that they have no conflicts of interest.

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Table 1: Normal distribution of DMFS and DMFT scores.

	Groups	**Shapiro Wilk**		
		Statistic	**Df**	**P value**
DMFS	Control	0.8	30	0.8
	Caries	0.9	30	0.07
DMFT	Control	0.7	30	0.4
	Caries	0.8	30	0.4

DF: Degree of freedom (sample size),

DMFS and DMFT: Caries indices (Decayed, missing, filled surfaces and teeth).

Table 2: Differences in DMFS and DMFT indices between study groups.

		N	**Mean**	**SD**	**T**	**Df**	**Sig.***
DMFS	Control	30	7.8	4.9	-5.9	40.4	<0.001
	Caries	30	21.0	10.9			
DMFT	Control	30	4.1	2.2	-6.2	58	<0.001
	Caries	30	8.2	2.8			
***Independent T test.**							

N: Number, SD: Standard deviation, T: Statistics, Df: Degree of freedom, Sig: Level of significance.

Table 3: Salivary iron and copper concentrations among study groups

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Variables	Groups	N	Mean Mg/L	Sd Mg/L	T	df	Mean Difference	P-value*
Iron	Control	30	0.02	0.006	-13.7	58	-0.02	<0.001
	Caries	30	0.04	0.008				
Copper	Control	30	0.001	0.0005	-3.4	58	0.0006	<0.001
	Caries	30	0.002	0.0007				

*Independent T-test.

Table 4: Correlation coefficients (r) and significance levels (p-values) for the relationship between clinical parameters and salivary iron and copper levels

Parameters	Iron		Copper	
	r	p-value	R	p-value
DMFS	0.6	**<0.001**	0.3	0.007
DMFT	0.6	**<0.001**	0.3	0.002

r : Correlation coefficient, p-value: Level of significance.

Table 5: Salivary biomarker performance in diagnosing dental caries

Biomarker	Goups	AUC (Area Under Curve)	P value	Optimal cutoff point	Sensitivity	Specificity
Iron	Control – Caries	0.9	<0.001	0.03	1.0	0.90
Copper	Control – Caries	0.7	0.001	0.0014	0.80	0.50

Figures

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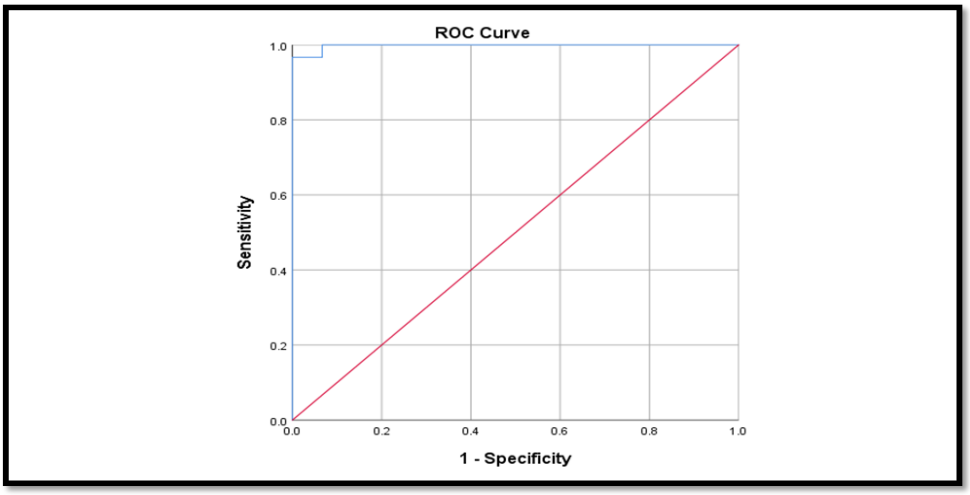

Figure (1): Iron (Caries groups).

ROC: Recceiver operating characterstic curve (blue line), AUC or Area under the curve (red line).

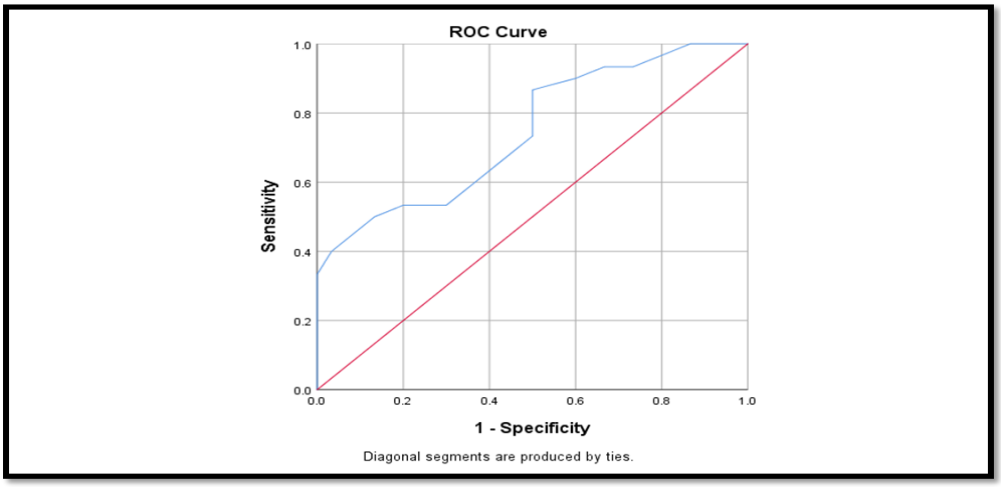

Figure (2): Copper (Caries groups).