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Comparative In-Vitro Study Of Sound And Carious Primary And Permanent Enamel Content Of Selected Trace Elements

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Abstract: Introduction: The crown of the tooth is coated in dental enamel, the toughest substance in the human body and is made up of many trace elements and small inorganic substances in various amounts. It is still early to determine how crucial trace elements are to preventing and reducing tooth caries.

Aim: The present in vitro study aimed to investigate and compare some trace element concentrations (Ba, Sr, Ni, and Ti) in carious and sound enamel of permanent and primary teeth.

Methodology: This study investigates and compares some trace element concentrations (Ba, Sr, Ni, and Ti) in the sound and carious enamel of primary and permanent teeth. A total of 50 primary teeth and 75 permanent teeth. The specimens were allocated into four groups: Group A: 25 sound primary teeth. Group B: 25 carious primary teeth. Group C: 25 sound permanent teeth. Group D: 25 carious permanent teeth. Sample preparation and wet ashing were undertaken, and finally, sample analysis was carried out by three analytical techniques: (XRF), (AAS) and (ICP-MS).

Results: there was a remarkable variance between the groups regarding Ba, Sr, and Ti mass% ($P < 0.01$). There was a substantial difference between the groups regarding Ba, Sr, and Ti concentration ($P < 0.01$).

Conclusion: (Sr) was higher in sound permanent teeth than in sound primary teeth and more in all carious groups. Also (Ba) was higher in carious teeth therefore (Ba) was positively associated with caries. (Ti) are positively associated with caries.

Keywords: *Trace elements, Carious, Primary teeth, Permanent teeth, Enamel*

Introduction

The human body's toughest non-living tissue is enamel. It comprises the portion of the tooth that is exposed to the oral cavity and gives the crown of teeth structure and contour. Human enamel is made up of 4% organic material, water, and 96% inorganic minerals [1].

The main inorganic component of human enamel is hydroxyapatite in crystalline form. Trace amounts of other inorganic elements can be found in the hydroxyapatite crystals. Trace elements are those that are found in less than 0.01% of an object or micrograms per gram. The characteristics of enamel are altered by its inclusion of various trace components [2].

Various investigators reported the presence of 44 trace elements in enamel. These constituents interact to generate complex compounds that have a variety of uses, including resistance to erosion and abrasion pressures, as well as involvement in the pathogenesis of dental caries. Certain elements, including Cu, Ti, Ni, and Cd, promote caries whereas others, like Al, Fe, and Sr, restrict it [3].

The environment is the source of the trace elements found in human dental enamel both during and after the tooth's development and mineralization. Nevertheless, when the level rises too high, all necessary trace metals become poisonous [4].

This study aimed to investigate and compare some trace elements concentrations; Barium, Strontium, Nickel, and Titanium (Ba, Sr, Ni, and Ti) in carious and sound enamel of permanent and primary teeth.

Subjects and methods

Patients:

This experimental in-vitro study was conducted on A total of 50 primary teeth; incisors, canines, and molars from children aged 5 to 12 and 75 permanent teeth from adults aged 12 to 18 were gathered [4].

The teeth were divided into four groups: **Group A:** 25 sound primary teeth. **Group B:** 25 carious primary teeth. **Group C:** 25 sound permanent teeth. **Group D**: 25 carious permanent teeth. In addition, 25 carious permanent teeth were considered as a pilot study. This study was performed after the approval of the Institutional Review Board (IRB).

Samples with the following criteria were included; sound primary incisors that were removed due to over-retention in youngsters or pre-shedding mobility in the 7–12 age range. Primary incisors with caries were excised because of pre-shedding mobility, over-retention, or carious destruction in youngsters (ages 5 to 12). Sound permanent teeth that had been removed for orthodontic reasons or periodontal disease in teens (in the age group of 12-18 years). Premolars and molars with caries that were extracted for periodontal disease, orthodontic or prosthodontic purposes, or for other reasons (in the 12–18 year age range).

Samples with the following characteristics were excluded; removed teeth with fluorosis and developmental abnormalities. Teeth are taken out of individuals with systemic illnesses. Teeth with filling and endodontic treatment.

Methods:

<u>Sample preparation</u>

After the teeth were gathered, saliva, blood, and tissue fragments were properly cleaned with tap water. After soaking in H2O2 to dissolve connective tissue, the teeth were cleaned with deionized water and allowed to air dry for the entire night on a fresh piece of filter paper at room temperature. With the use of a rubber polishing cup, the teeth were cleaned and polished. A sharp spoon excavator was used to eliminate the soft cavities from the carious teeth [5].

Using a cylindrical dental grinding stone and grinding disk attached to a straight hand piece (micro-motor unit Kavo, EWL K-11), enamel samples were mechanically ground while the dentinal region remained intact. The entire grinding process was carried out inside a clear glass box to keep the resulting enamel dust from flying around and being lost. The speed at which the grinding was done was roughly 10,000 rpm. The enamel dust that had formed was gathered onto glass containers marked A, B, C, and D.

Wet ashing

Enamel powder is dissolved in an appropriate acid to create a transparent solution that can be used for further mineral analysis. For instance, to create a transparent solution, 1 gm of enamel dust from enamel sample (A) was obtained and wet-ashed in 2 ml of nitric acid in a volumetric flask. To achieve a volume of 100 millilitres, the solution was further diluted with double-distilled deionized water. Nitric acid was present in the final solution at a concentration of 1 M. Solution A is the prepared solution that is marked. The enamel samples B, C, and D underwent comparable processing to provide the corresponding solutions B, C, and D [4].

The standard solutions were made by a chemist and included roughly 1000 µg/ml of the specific element. For every ingredient that needed to be examined, the standard solutions were diluted appropriately to create the working standard solutions [6].

Sample analysis:

Three analytical techniques were used:

First, we used X-ray fluorescence (XRF) (JMS-01222, JOEL- Japan). Second, we used an inductively coupled plasma mass Spectrometry (ICP-MS) (JMS-PLASMAX2, JOEL- Japan) model. Third, we employed the Varian-240-USA model of the Atomic Absorption spectrophotometer (AAS). After adding standard solutions to the (AAS) containing known concentrations of the components, absorbance-concentration curves were produced. After adding the experimental solution to the (AAS), a calibration graph was produced. The calibration curve was used to determine the elemental concentrations in the test solution. The recorded values were expressed as parts per million (ppm) [7]. All four enamel samples had their concentrations of the four trace elements (Ba, Sr, Ni, and Ti) evaluated. For every element (in the same sample), six measurements were taken and calculated. The results were tallied, and the variations in the means across the four groups were contrasted.

Statistical Analysis:

Using IBM Inc.'s SPSS 22.0 for Windows, all data were gathered, tabulated, and statistically examined (Chicago, IL, USA). The mean and standard deviation (SD) values of the data were displayed. One-way ANOVA was employed to compare the four groups. For pairwise comparisons between the groups when the ANOVA test is significant, Tukey's post-hoc test was employed. A p-value of less than 0.05 was deemed to be significant.

Results

X-Ray Fluorescence (XRF) results

As regards primary teeth and permanent teeth; carious teeth showed a statistically significantly higher mean (Sr) mass % than sound teeth. Regarding sound and carious teeth; permanent teeth showed a statistically significantly higher mean (Sr) mass % than primary teeth. So the concentrations of (Sr) were substantially elevated in the sound enamel of permanent teeth than in the sound enamel of primary teeth.

Carious primary teeth showed substantially the highest mean (Ba) mass %. There was no remarkable variation between sound primary teeth, sound permanent teeth and carious permanent teeth; all showed the substantially lowest mean (Ba) mass %. Concerning primary teeth; carious teeth showed a notably higher mean (Ba) mass % than sound teeth. Respecting carious teeth; primary teeth showed a substantially higher mean (Ba) mass % than permanent teeth.

Sound permanent teeth showed the statistically substantially highest mean (Ti) mass %. As regards primary teeth; there was a non-significant variance between mean (Ti) mass % in sound and carious teeth. For the permanent teeth; sound teeth showed a statistically significantly higher mean (Ti) mass % than carious teeth. Regarding sound teeth; permanent teeth showed a statistically substantially higher mean (Ti) mass % than primary teeth. Respecting carious teeth; there was no remarkable variance between mean (Ti) mass % in primary and permanent teeth.

The concentrations of (Ni) were below the detection limit so it was not clinically significant in all enamel samples.

There was a statistically significant difference between the groups regarding Ba, Sr, and Ti (P-value <0.01).

Table (1): The mean, standard deviation (SD) values and results of comparison between (Ba, Sr, and Ti) mass % in the four groups

	Sound primary		Carious primary		Sound permanent		Carious permanent		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Ba	0.060 ^{b}	0.005	0.112 ^{a}	0.014	0.080 ^{b}	0.007	0.070 ^{b}	0.026	<0.01
Sr	0.052 ^{c}	0.008	0.069 ^{b}	0.007	0.072 ^{b}	0.002	0.084 ^{a}	0.006	<0.001
Ti	0.012 ^{b}	0.006	0.01 ^{b}	0.004	0.088 ^{a}	0.04	0.009 ^{b}	0.004	<0.001

**: Significant at P ≤ 0.05, Different letters are statistically significantly different*

Atomic Absorption Spectrophotometer (AAS) results

Carious permanent teeth showed the statistically substantially highest mean (Sr) concentration. This was followed by sound permanent teeth then carious primary teeth. Sound primary teeth revealed the lowest mean (Sr) concentration. Concerning primary teeth and permanent teeth; carious teeth showed statistically remarkably higher mean (Sr and Ti) concentration than sound teeth. As regards sound and carious teeth; permanent teeth showed statistically notably higher mean (Sr and Ti) concentration than primary teeth.

Carious primary teeth showed the statistically substantially highest mean (Ba) concentration. Sound primary teeth showed the statistically significantly lowest mean (Ba) concentration. As regards primary teeth; carious teeth showed a statistically significantly higher mean (Ba) concentration than sound teeth. As regards sound teeth; permanent teeth showed a statistically significantly higher mean (Ba) concentration than primary teeth. As regards carious teeth; primary teeth showed a statistically remarkably higher mean (Ba) concentration than permanent teeth.

Carious permanent teeth showed the highest mean (Ti) concentration. This was followed by carious primary teeth then sound permanent teeth. Sound primary teeth showed the lowest mean (Ti) concentration.

The concentrations of (Ni) were not clinically significant in all enamel samples.

There was a statistically significant difference between the groups regarding Ba, Sr, and Ti (*P*-value <0.01).

Table (2): The mean, standard deviation (SD) values and results of comparison between (Ba, Sr, Fe, and Ti) concentrations in the four groups

	Sound primary		Carious primary		Sound permanent		Carious permanent		*P*-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Ba	34.1 ^{c}	1.6	48.6 ^{a}	0.7	41.7 ^{b}	5.6	41.7 ^{b}	7.9	0.001*
Sr	137.3 ^{d}	2.5	158.2 ^{c}	2.4	186.9 ^{b}	2.2	235.4 ^{a}	2.8	<0.001*
Ti	2.1 ^{d}	0.5	7.2 ^{b}	0.5	3.7 ^{c}	0.2	9.4 ^{a}	0.3	<0.001*

**: Significant at P ≤ 0.05, Different letters are statistically significantly different*

Discussion

It's a complicated topic how trace elements affect the frequency of caries. Nonetheless, the unequivocal proof of the negative correlation between fluoride consumption and caries prevalence suggests that trace elements may have an impact on caries [8].

This study was designed to investigate and compare some trace elements concentrations (Ba, Sr, Ni, and Ti) in sound and carious enamel of primary and permanent teeth where previous studies strengthen that (Sr and Ba) are caries inhibiting, while (Ti and Ni) are caries promoting. Following this study, **Falla-Sotelo et al.,** [3] investigated elements (Sr, Ba and Fe) which are caries inhibiting, while (Mn, Ti, Cu, Pb, Ni and Cd) are caries promoting.

Kamberi et al., [5] studied (Ti) concentration as it was higher on the outer surface of enamel than in the enamel-dentin border in correlation with the presence of dental caries. In addition **Rao et al**., [9] investigated (Sr) and (Ba) concentrations where (Sr) and (Ba) increase osteoblasts and reduce the number and activity of osteoclasts, they play a vital role in bone development. As a result, the concentrations of (Ba) and (Sr) were depleted in the various areas.

In this study primary teeth were collected from 5 to 12 years old children and permanent teeth from 12 to 18 years old adults the same was done by **Shashikiran et al.,** [4] while **Amr and Helal,** [6] used primary teeth collected from 5 to 12 years old children and permanent teeth from 12 to 40 years old adults which were far from the main interest in pedodontics.

Sample preparation and wet ashing were the same in all studies that measured trace element concentrations in enamel samples [4,8,10,11].

Many studies [4,10,12,13] **Halling et al.** [12]**, (Burguera et al.,** [13] **Tanaka et al.,** [10] **and Shashikiran et al.,** [4] carried out Sample analysis using atomic absorption spectrometry as it was time-consuming for large routine scale analysis and very wide-spread technique, while **Amr and Helal,** [6] and **Hare et al.,** [14] used inductively coupled plasma-mass spectrometry as It is an effective method that enables the multi-element ultra-trace analysis of a large range of samples, others **Gierat-Kucharzewska et al.**, [15] **Falla-Soteloet al.,** [3] and **Gierat-Kucharzewska and Karasiński,** [16] used X-ray fluorescence gaining The advantages that there is no need for sample preparation (the material can simply be placed in the beam), and that it is a non-destructive technique. In the present study, sample analysis were carried out by three analytical techniques for the same samples to gain the benefits and overcome the disadvantages of each technique alone.

This study discusses the results of trace elements of interest by (XRF), (AAS) and (ICP-MS).

In the present study results obtained by (XRF) and (AAS) showed that the concentrations of (Ba) were substantially elevated in ***sound permanent teeth*** (0.090) mass%, (43.7) ppm than that in ***sound primary teeth*** (0.070) mass%, (36.1) ppm, respectively. ***Carious primary teeth*** revealed the highest mean (Ba) concentration (0.123) mass%, (50.6) ppm respectively. ***Carious teeth*** showed a remarkably higher mean (Ba) concentration than ***sound teeth***, ***and primary teeth*** showed a substantially higher mean (Ba) concentration than ***permanent teeth***. The results are the same obtained by both devices except that ***sound primary teeth*** showed the statistically significantly lowest mean (Ba) concentration (36.1) ppm in (AAS) record. On the other hand by (ICP-MS) its concentrations were not significant in all groups.

By the result of (ICP-MS) in this study **Lynch** [8] found that The influence of Ba levels in human enamel on the prevalence of caries was unable to reveal an apparent and significant association.

One possible complicating issue is that (Ba) is a prevalent element in dental restoration, resulting in contamination. **Amr and Helal** [6] noticed a statistically substantial variation in (Ba) levels between permanent and primary teeth. The levels were greater in permanent teeth than in primary teeth which are the result of (AAS) in this study. However, these results contrast those reported by **Gierat-Kucharzewska and Karasiński** [16] as they found lower concentrations of (Ba) in the various primary and permanent teeth than sound teeth. In addition, **Rao et al.** [9] record that The (Ba) levels in various zones are significantly lower than the rest of the tooth specimen. Since (Sr) and (Ba) play an important role in bone formation by enhancing osteoblasts and decreasing the quantity and activity of osteoclasts, the carious areas revealed depletion in (Ba) and (Sr) levels. In this study (Ba) was higher in carious teeth so (Ba) was positively associated with caries.

The results obtained by both (XRF) and (AAS) show that the mean concentrations of (Sr) were remarkably elevated in ***sound permanent teeth*** (0.060) mass%, (184.9) ppm than that in ***sound primary teeth*** (0.043) mass%, (135.3) ppm respectively. ***Carious teeth*** showed statistically substantially elevated mean (Sr) concentrations than ***sound teeth*** and ***permanent teeth*** exhibited notably elevated mean (Sr) concentrations than ***primary teeth***. While by (ICP-MS) its concentrations were over 134 ppm in all teeth.

In this study, **Hare et al.** [14] mentioned that (Sr) was observed at high concentrations exceeding 120 ppm in all teeth samples. **Gierat-Kucharzewska et al.** [15] concluded that the content of (Sr) is higher in caries permanent teeth than in primary ones. In addition, **Shashikiran et al.** [4] reported that The levels of (Sr) were substantially greater in the sound enamel of permanent teeth than the levels of (Sr) in primary teeth. **Amr and Helal** [6] identified a statistically significant variation in (Sr) levels between permanent and primary teeth. The levels were higher in permanent teeth than in primary teeth.

Although the present study indicated that as regards primary teeth and permanent teeth, carious teeth showed statistically significantly higher mean (Sr) mass % than sound teeth **Falla-Sotelo et al.** [3] concluded that (Sr) is caries inhibiting. **Gierat-Kucharzewska and Karasiński** [16] found that lower concentration of (Sr) in the carietic primary and permanent teeth, concerning its concentration in healthy teeth. In addition, **Rao et al.** [9] recorded that Carious regions of teeth have low (Sr) concentrations. It has been established that the (Ca/Sr) ratios are inversely correlated for enamel. This could be because (Ca) is substituted by (Sr) during hydroxyapatite synthesis, so it should be used in combination with another element to discover its effect.

Results obtained by (XRF), (AAS) and (ICP-MS) are the same which reported that ***carious permanent teeth*** showed the highest mean (Ti) concentration(0.08) mass%, (9.3) ppm and (9.9) ppm, This was followed by ***carious primary teeth*** (0.07) mass%, (7.1) ppm and (6.91) ppm then ***sound permanent teeth*** (0.067) mass%, (3.6) ppm and (3.81) ppm and ***sound primary teeth*** showed the lowest mean (Ti) concentration (0.01) mass%, (2) ppm and (1.59) ppm respectively. ***Carious teeth*** revealed a substantially elevated mean (Ti) concentration than ***sound teeth*** and ***permanent teeth*** showed a substantially higher mean (Ti) concentration than ***primary teeth***.

Kamberi et al. [5] showed that (Ti) has an affinity to accumulate in human dental hard tissues as was reported previously, (Ti) concentrations in enamel are very small, while in dentin are higher. (Ti) values in the outer enamel were higher than those reported previously. **Gierat-Kucharzewska et al.** [15] conclude that the content of (Ti) is higher in caries permanent teeth than in primary ones, which agrees with the current study. Additionally, **Falla-Sotelo et al.** [3] concluded that (Ti) is caries-promoting. On the other hand, **Amr and Helal** [6] report that the concentrations of (Ti) were statistically significantly lower in permanent teeth compared to primary teeth. In this study, carious teeth showed statistically significantly higher mean (Ti) concentration so we conclude that the (Ti) are positively correlated with caries.

The concentrations of (Ni) were not clinically significant in all our dental samples by all techniques. Following this study, **Hare et al.** [14] reported that (Ni) was observed at lower levels than those of other elements and was not detected in some tooth samples. While **Amr and Helal** [6] revealed that there was a statistically substantial variation in the levels of (Ni) between permanent and primary teeth. The concentrations were higher in permanent teeth compared to primary teeth. In the present study the concentrations of (Ni) were not clinically significant in all dental samples by all techniques indicate that (Ni) levels in teeth are most likely determined by environmental exposure.

An assessment of trace elements (Ba, Sr, Ti, and Ni) in sound and carious enamel of primary and permanent teeth was done by three analytical techniques for the same samples and statistical analyses were done to compare the obtained results.

Conclusion

(Sr) was higher in sound permanent teeth than that in sound primary teeth and more in all carious groups. Also (Ba) was higher in carious teeth therefore (Ba) was positively associated with caries. This might be due to the fact that (Ba) is a common component of dental restorations leading to (Ba) contamination. (Ti) are positively associated with caries. It was observed that (Ni) were not significant in all dental samples by all techniques suggesting it governed by environmental exposure.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Author contributions:

Experimental design: Somaia Ghobar. Data interpretation: All authors. Statistical analysis: Mohamed H.A. Gadelmawla, Sara Shahin. Writing and revision: All authors. All authors read and approved the final manuscript.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

Declaration

The authors did not use artificial intelligence (AI)– assisted technologies (such as Large Language Models [LLMs], chatbots, or image creators) in the production of submitted work and we assert that there is no plagiarism in our paper, including in text and images produced by the AI.

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