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Effect of Trace Elements on Dental Caries in Human Tooth

Chem-J Yem, Chung-Liang Lin, Chao-Chin Hu
May-Ling Jang, Wen-Kang Chen, Ming-Yung Chou*

A number of trace elements have been studied for their possible roles in dental caries. In this work, we collected 60 human teeth which were assigned to three groups: (1) normal teeth; (2) dental caries I (outer caries); (3) dental caries II (inner caries). Each sample was digested by use of microwave acid-digestion method.

Trace element concentrations were analyzed by atomic absorption spectrophotometer. The concentrations of Zn, Cd, Cu, Fe, Mn, and Pb in normal teeth were lower than those in dental caries with significant difference ($p < 0.01$). The distribution of Ni in normal teeth was significantly higher than that in dental caries ($p < 0.01$).

According to the results, we may say that trace elements of Zn, Cd, Cu, Fe, Mn, and Pb have cariogenic effects, and Ni has inverse association with caries. Serving as the basis for future research and correlation to human teeth are discussed.

Key Words: Trace elements, Dental caries, Cariogenic

It has long been believed that "airs, waters and places" have a direct bearing on human health. Particular attention has been paid to the local geochemical environment including some trace elements with possible influence on teeth(1,2,3,4,5,6).

A number of trace elements have been studied for their possible role in dental caries, many of them provide some the most convincing evidence that trace elements can affect the health of teeth(2,7,8,9). One of the possible mechanisms is by incorporation in teeth enamel during development and mineralization, hence, hydroxyapatite crystals, making up the enamel by alternation of trace elements composition, become more or less resistant to enamel disollution during the initiation of the caries process(1).

Davies and Anderson(2) reported the principal component of enamel was microcrystalline hydroxyapatite set in a protein matrix. The protein represents about 1% of the enamel dry weight and appears to resemble Keratin in expectation of less sulphur content. The apatite fraction approximates to the composition $(Ca_{9.5}\ Mg_{0.2}\ Na_{0.1}\ H_{0.5})\ (PO_4)_{5.7}\ (CO_3)_{0.5}(OH)_2$. But apatite, whether biogenic

Department of Biochemistry and School of Dentistry,|* Chung Shan Medical & Dental College, Taichung, Taiwan, R.O.C.

or geochemical, contains traces of many other elements which occur in the crystal structure through isomorphous replacement. That is, one ion can substitute for the normal ion if it has comparable size and it dose not differ by more than one charge unit[1,2]. The initial phase of teeth destruction is most probably due to the dissolution of the mineral phase of the enamel by the acids, which were produced by the micro-organisms on the enamel surface[10].

As shown previously, low prevalence was associated with raising concentrations of Ca, Mg, Mo and V while concentrations of Cu, Fe, and Mn were higher in the samples with higher prevalence[11]. A strong inverse relationship was found between caries prevalence and the contents of Sr, Ba, K, Mg, Ca, and Li in garden soil and possible direct association Cu and Pb in soil. There was consistent evidence of an inverse association with concentration of V, Mo, Mn, Al, Ti, and P and of direct association with concentration of Pb, Cu, Cr, Zn, and Se in the stape foodstuffs (sago, sweet potato, and Chinese taro)[12,13].

Most of the studies were only considered the simple relationship between caries and individual element, and proposed comparative analysis of soil from some areas, deficiency or excess of trace elements becomes important for teeth. We want to know the various trace elements concentration of normal teeth and caries, it should be possible to identify those elements in teeth and to show their association with caries.

MATERIALS AND METHODS

Preparation of Teeth

A total of 60 human teeth were extracted for orthodontic or caries from Chung Shan Medical and Dental College Hospital. The teeth were taken from individuals 19-53 years of age, the donors included 34 men and 26 women. Samples were assigned to three groups: (A) normal teeth -- there were no any spot lesions on enamel surface (n=25); (B) Dental caries I (outer caries)--teeth which had small lesions into the enamel and dentin (n=25); (C) Dental caries II (inner caries)-- teeth which had extensive lesions into the pulp (n=10). All the teeth were cleaned by deionized water, dried 45 min at 100°C, cooled, and finally ground to a fine powder in a carnelian mortar.

Analysis

About 0.5g of the samples were required for assay to obtain the concentration of trace elements. Each sample include a blank were digested at telflon microwave digestion bomb with 24 ml hydrochloric acid and 8 ml nitric acid (Merck, suprapur grade) by modified microwave acid-digestion method[14,15], an inexpensive efficient way to prepare samples for analysis by atomic absorption spectrocopy [14]. The microwave oven, CEM-MDS 810 was used to dry the samples. Each acid products was diluted with deionzed water ($\geqslant$ 18 Ω cm^{-1} resistance) after digestion to the total volume of 100ml in order to ensure that sample concentration within each element's calibration range.

The quantifications of trace elements were analyed by atomic absorption spectrophotometer [Flame AAS (PERKIN-ELMER 4000) as well as Graphite Furnace AAS (PERKIN-ELMER 5100)], for Pb, Fe, and Zn, they were mersured by Flame AAS only. Graphite Furnace AAS is particularly suitable for the assay of Cd, Cu, Ni, and Mn. Table (1,2) list the PERKIN -ELMER AAS settings for analysis of those elements.

Regression Modelling

The emphasis in this study was on the specification of various trace elements concentration of normal teeth and caries, therefore, two sided t-test of signifiance difference (P

Table 1. Flame AAS. (PERKIN-ELMER 4000) Settings

Element	Wavelength (nm)	Slit Width (nm)	Light Source	Flame Type
Pb	283.3	0.7	Hollow Cathode Lamp	air-acetylene
Fe	248.3	0.2	Hollow Cathode Lamp	air-acetylene
Zn	213.9	0.7	Hollow Cathode Lamp	air-acetylene

References: Techniques in Flame Atomic Absorption Spectrophotometry, by Perkin-Elmer Corp.

Table 2. Graphite Furnace AAS. (PERKIN-ELMER 5100) Settings

Settings \ Element	Cd	Cu	Ni	Mn
Wavelength (nm)	228.8	324.8	232.0	279.5
Low Slit (nm)	0.7	0.7	0.2	0.2
Tube/Site	Pyro/ Platform	Pyro/ Platform	Pyro/ Platform	Pyro/ Platform
Pretreatment Temp. (°C)	900	1000	1400	1400
Atomization Temp. (°C)	1600	2300	2500	2200
Rollover (A)	0.8	0.7	0.7	1.1
Matrix Modifier	0.2mg $NH_4H_2PO_4$ 0.01mg $Mg(NO_3)_2$	—	—	0.05mg $Mg(NO_3)_2$

References: Techniques in Graphite Furnace Atomic Absorption Spectrophotometry. by Perkin-Elmer Corp.

< 0.01) were used. This was in contradistinction to ONEWAY test (0.01 level) for obtained precise estimation.

Determination of statistical significance depends upon comparison to basic residual variability in the system. The software of SPSS/PC$^+$ system (SPSS inc. 444N Michigan Auenue Chicago IL 60611) was utilized for analysis the difference of each elements between the three groups of teeth.

RESULTS

The mean and standard deviation (SD) values of the concentration of the sevent elements Zn, Fe, Ni, Mn, Cd, Pb, and Cu in the teeth were given in Table (3). The values were measured from the whole teeth by atomic

Table 3. Mean Concentration of Zn, Fe, Ni, Mn, Cd, Pb, and Cu in Whole Teeth

Sample	Sample Sizes	Mean Concentration ± SD (ppm)						
		Zn	Fe	Ni	Mn	Cd	Pb	Cu
A: Normal Teeth	25	170.16 ± 20.27	28.32 ± 6.74	4.18 ± 0.60	1.42 ± 0.40	0.07 ± 0.03	2.52 ± 0.58	0.26 ± 0.05
B: Dental Caries I	25	216.32 ± 26.00	52.06 ± 9.66	2.80 ± 0.71	2.21 ± 0.37	0.12 ± 0.05	5.75 ± 1.11	0.44 ± 0.09
C: Dental Caries II	10	247.40 ± 54.74	50.19±12.83	1.86 ± 0.70	1.97 ± 0.75	0.15 ± 0.05	4.95 ± 1.94	0.46 ± 0.20

absorption spectrophotometry.

Fig (1) shows the distribution of individual element in all the samples. From the figures, we could see there were some variation between the different groups of each element. The group A (normal teeth) of Zn, Cd, and Cu were lower than those in B and C (caries I and II); Fe, Mn, and Pb were observed in the group A were lower than those in B which in turn were higher than those in C. This might have somethings associated with the weak correlation between the surface concentration of trace elements with whole enamel values [16]. The distribution of Ni in group A was higher than those B and C, the Ni may reduce the susceptibility of teeth to dental caries in this case.

The values between normal teeth and dental caries (group A vs. group B; group A vs. group C) were significantly different ($P < 0.01$), but dental caries I and II were not different except the Ni case, as listed in Table (4). The results of statistics by t-test were corresponded with ONEWAY test and the results were listed in Table (5).

DISSCUSSION

We know that cavity formation would not improperly be attributed to trace elements, and the age, sex, original of drinking water, and tooth diameter of donors were included as co-variates [1]. Apart from the expected age and sex effects and the persistent beneficial of F [3,17,18], the elements Zn, Fe, Mn, Ni, Cd, Pb, and Cu indicated that there are explicit correlation of trace elements to dental caries (tables 3-5). Probably Ni has a negative association with caries, while Zn, Fe, Mn, Cd, Pb, and Cu are positively associated with dental caries.

In many studies in which the teeth act as cumulative markers of trace-element level

Table 5. Significant Relationships Found by ONEWAY Test (Scheffe Procedure; SPSS/PC$^+$ System)

	Trace Element																											
	Zn				Fe				Ni				Mn				Cd				Pb				Cu			
		A	B	C		A	B	C		A	B	C		A	B	C		A	B	C		A	B	C		A	B	C
Pairs of	A	–	–	–	A	–	–	–	A	–	–	–	A	–	–	–	A	–	–	–	A	–	–	–	A	–	–	–
Groups	B	*	–	–	B	*	–	–	B	*	–	–	B	*	–	–	B	*	–	–	B	*	–	–	B	*	–	–
Variable	C	*	–	–	C	*	–	–	C	*	*	–	C	*	–	–	C	*	–	–	C	*	–	–	C	*	–	–

1. * Denotes pairs of groups significantly different at the 0.01 level.
2. A: Normal Teeth; B: Dental Caries I; C: Dental Caries II

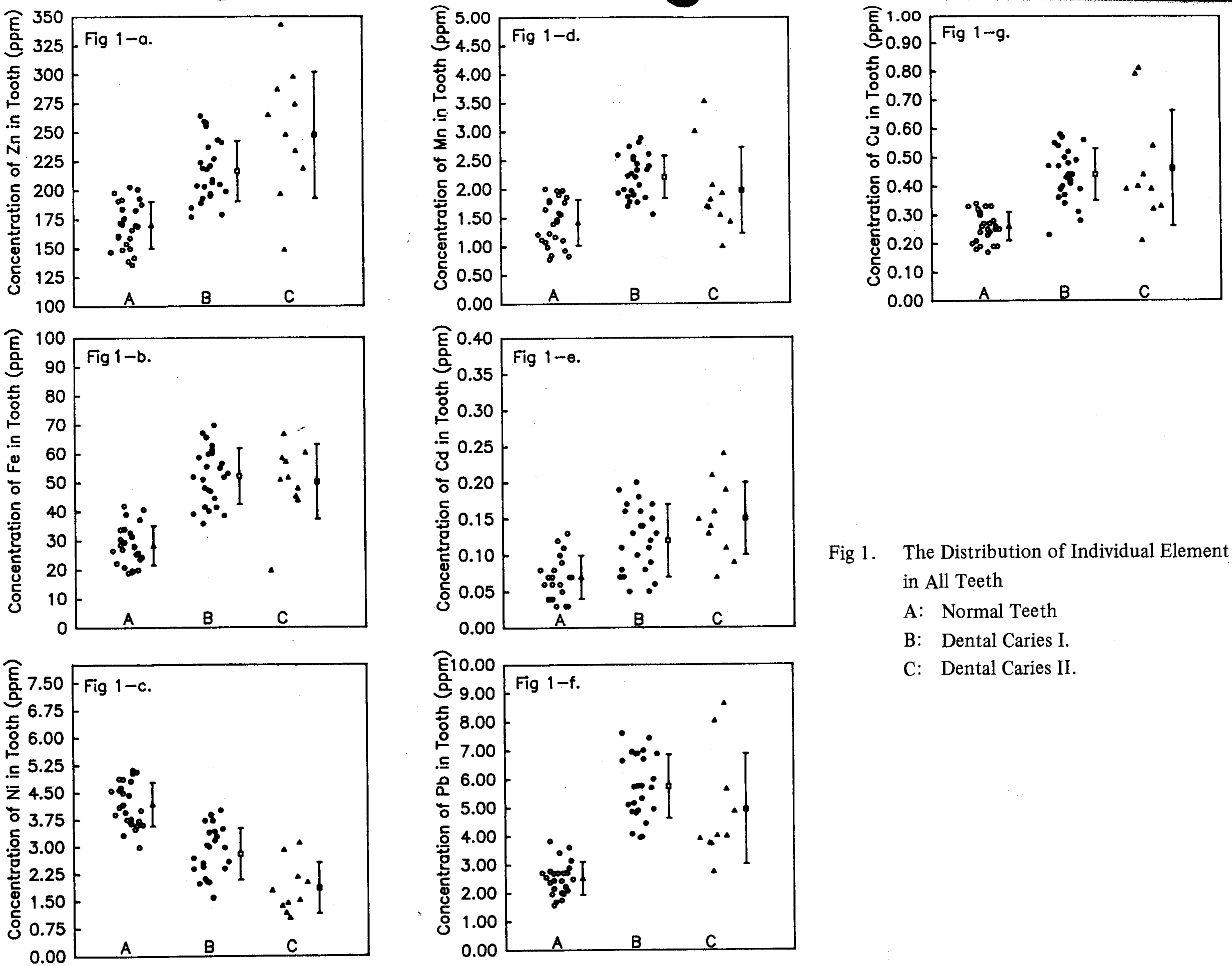

Fig 1. The Distribution of Individual Element in All Teeth

A: Normal Teeth

B: Dental Caries I.

C: Dental Caries II.

Table 4. Significant Relationships Found by t-Test (SPSS / PC+ System)

Trace Element	2-Tail Probability			
Zn	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.001*	–	–
Fe	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.000*	–	–
Ni	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.000*	0.001*	–
Mn	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.008*	–	–
Cd	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.000*	–	–
Pb	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.003*	–	–
Cu	Group	A	B	C
	A	–	–	–
	B	0.000*	–	–
	C	0.009*	–	–

1. * Groups significantly different ($p < 0.01$)
2. A: Normal Teeth; B: Dental Caries I; C: Dental Caries II

[16], some of them are bound to the mineral structure or organic matrix of the enamel at the time of its formation, others are adsorbed on the enamel surface later on in life-span (airs, foods, waters, etc)[16,19]. According to the observations of previous investigators [11,13,20,21,23,24], we believe that trace elements which are bound to enamel or in the environment can play an important role in the caries process.

The concentrations of Cd, Cu, and Mn in normal teeth are significantly lower than caries ($P < 0.01$), these results support the findings on the cariogenic effects of Cu, Cd, and Mn [25,26,31]. In addition, the studies of high prevalence were associated with higher concentration of Cu, Mn, and Fe in water supply, food, soil, and vegetables[11,13,20,25]. Our results about iron are the same as remarked above; however, Emilson and Krasse[27] reported caries reductions in rat by specific iron salts[28]. Brames[12,13] reported that Zn in the staple foodstuffs had a direct association with caries prevalence and Zn had a positive effects to teeth decay. Another study of Fang[29] observed that dietary Zinc may be an important trace mineral in the process of post-eruptive mineralization of the enamel and might reduce the susceptibility of teeth to caries. Zn was considered to be a doubtful element among trace elements that inhibit caries development[30]. This is similar to iron, the effects of them to teeth may dependent on the type of the salts and their concentrations within. The results we got in caries reveal the values of Zn are significantly higher than normal teeth ($P < 0.01$), indicating Zn has cariogenic effects on teeth.

Ni in normal teeth is significantly higher than that in caries, and between caries I and II are significantly different ($P < 0.01$), implying Ni may have inverse association with caries. Lead was of particular concern and had influenced on dental studies in two ways: the possibility that dental health might be affected by environmental factors and the use of teeth as monitors of the body burden of lead[2]. As shown previously, lead in the soil appeared to be directly associated with increased dental caries[12,13], and Buttner[23] indentified lead as having a cariostatic effect. In conclusion, the effects of lead on caries may associate with age[22], and the interaction between elements as well[2]. In our study, lead in normal teeth are lower than that in caries ($P < 0.01$), Pb may be accompanied by poor dental health.

Because the distribution of trace elements in teeth were inhomogeneously, the determination of its reliable average value was difficult. The inhomogeneity would explain partly the ambiguity of many caries-trace-element studies [16]. For this, we analysis the whole teeth to study the accumulation of trace elements. The standard deviation (SD) values of elements are too large (table 3) especially in some caries II groups to which the teeth are more incomplete than the other two groups, due largely to the inhomogeneity of the elements in teeth. In addition, the variation of elemental concentration except Ni between caries I and II are not clear enough to associate with the inhomogeneity. Otherwise, individual variation and interaction between elements should be considered. Due to the different depth-concentration profiling techniques and the different teeth-sampling methods, direct comparison of results between different authors can only be relative[16,19].

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人類齲齒中微量元素之研究

巖正傑　林忠亮　胡超群

詹美玲　陳文貴　周明勇*

本實驗自中山醫學院附設醫院收集年齡層19-35歲之34位男性26位女性之牙齒共60顆，依其蛀蝕程度分為三組(1)正常牙齒——牙釉質表面無任何蛀蝕現象；(2)齲齒 I ——蛀蝕發生在牙釉質及牙本質表層；(3)齲齒 II ——蛀蝕侵犯牙本質內層或牙髓腔，所有牙齒以二次水洗淨乾燥後用瑪瑙研砵磨成細粉，並由微波酸消化法分解，分解後之溶液以原子吸收光譜儀定量微量元素。

實驗數據經由t-test及ONEWAY test比較其差異性，我們發現鋅、鎘、銅、鐵、錳和鉛在正常牙齒低於齲齒之含量，並且有明顯的差異（$p<0.01$），由此結果可以推論上述微量元素含量之提高可能有促進牙齒蛀蝕之作用，此外，鎳的分佈趨勢則為正常牙齒明顯高於齲齒（$p<0.01$），鎳之多寡和蛀牙發生呈反比關係，這些結果有進一步研究的價值，並可作為防止齲齒發生之參考。

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中山醫學院　生化學科　*牙醫學系