Asian Journal of Chemistry; Vol. 28, No. 10 (2016), 2211-2216

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19929

Determination of Strontium in Human Teeth and Fingernails of Healthy and Carious Patients Resident in Karbala, Iraq by Using ICP-OES

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Received: 25 February 2016; *Accepted*: 9 April 2016; *Published online*: 30 June 2016; AJC-17969

In this study, strontium levels in biological (human teeth and fingernails) samples collected from Iraqi individual residents in Karbala city, Iraq were determined by using inductively coupled plasma optical emission spectrometry. The determination of strontium was undertaken for human teeth (2.613 ± 0.450 mg/Kg) and fingernail samples (2.184 ± 0.636 mg/Kg). Significantly higher fingernail levels of strontium were found in carious patients (2.599 ± 0.234 mg/Kg) when compared with healthy individuals (1.363 ± 0.246 mg/Kg) at a probability level, $p < 0.05$. The influence of various factors including gender, smoking activity, health status, individual's age and drinking water on strontium levels was determined using a two tailed t-test. Significantly higher fingernail levels of strontium were found in carious patients and smokers when compared with healthy individuals and non-smokers, respectively at a probability level, $p < 0.05$. On the other hand, no significant effects were found for gender, smoking activity and age on the strontium levels in the human teeth carious samples. Similar results were also reported for fingernail samples in terms of the effect of gender and age. A strongly positive significant correlation was seen between fingernails strontium and drinking water strontium ($r = 0.894$, $p < 0.05$). In contrast, there is not any significant correlation between the level of strontium in teeth and washed fingernails ($r = 0.195$, $p < 0.05$).

Keywords: Teeth, Fingernails, Strontium, ICP-OES.

INTRODUCTION

The concentration of essential trace elements are homeostatically regulated when the health status of individuals is under normal conditions (healthy individuals) [1]. On the other hand, the metabolism of these elements is altered in diseases, and may play significant roles in the pathogenesis and progress of these diseases [2-5]. Strontium has been reported in the literature as a non-essential element [6]. The biological effects of strontium are linked with bone disease due to the strontium being accumulated in bones as a "look-a-like" to calcium. The excess of strontium could cause disturbance in the metabolism of calcium, then it can displace calcium in hard tissue metabolic processes [7-9]. Moreover, the prevalence of rickets in a strontium-rich soil area is increased due to calcium displacement [7]. Adults and children are both exposed to strontium *via* drinking water and food, but young children have more hand-to-mouth activity or may eat soil accidentally and thus consume more strontium. In adults, strontium attaches mainly to the surface of bones, whilst in children the function of strontium can be changed due to the bone growth. Therefore, strontium interferes with normal bone development at high concentrations [8,10]. A previous study has found that the highest levels of strontium in saliva samples were determined in the areas where strontium in drinking waters was highest [11,12]. Strontium in drinking water can enter the bloodstream from the intestines and through the skin during bathing/swimming. In addition, infants may absorb more strontium from their intestines than adults [13]. As a result, strontium can be distributed throughout the body, where it can enter and leave cells quite easily. Furthermore, strontium enters the human body by mouth but it does not pass through the intestinal wall to enter the bloodstream. Animal studies have indicated that the critical target after oral exposure to stable strontium is the skeleton [14]. Previous studies have shown that strontium, could play a significant role in the development of dental caries [15,16], Many studies have previously discussed the relationship between trace elements and dental caries patients by comparing them with healthy individuals [17,18]. One study in Japan has shown that the strontium levels in saliva of dental caries patients were significantly increased when compared with those for healthy individuals [15,19]. In contrast, the concentration of fluoride in toothpaste inhibits strontium dissolution from teeth which leads to the protection of the teeth [19].

Teeth are a part of skeleton, and it is useful to utilize as a bio-indicator of great interest due to the reason that it contains information on exposure to essential or non-essential elements that become deposited in the teeth material [20,21].

Recently, human fingernail tissue has been recognized as an invaluable tissue for the assessment of exposure to various pollutants in an occupational and/or environmental setting. It provides a useful indication of exposure to many toxic, essential and non-essential trace elements over a long period of time, as this material remains isolated from any metabolic activity in the human body [22-25]. Thus, they are considered to be a finger-print of the body's trace element levels over a period of time, which is not possible with materials such as blood [22]. In addition, the levels of trace elements in fingernail tissue were found to be higher than those of body fluids and other accessible tissues [26,27].

The main aim of this study is to determine the levels of strontium in human teeth and fingernails for carious patients and healthy individuals. This can use to evaluate whether there is any relationship between the levels of strontium and the onset of dental caries. Furthermore, the main objectives of this work were to investigate whether human teeth and fingernail can be used as biomarkers for strontium levels in the human body; to evaluate the levels of strontium in relation to smoking activity of an individual; to explore whether environmental samples (water) make any significant contribution to the strontium levels of tissues under investigation and to examine whether factors like gender and age may affect the strontium levels in teeth and fingernails of the individuals under study.

EXPERIMENTAL

Study population: Human teeth (n = 50) and fingernails (n = 75) samples were collected from Iraqi individuals resident in Karbala which is a city in Iraq located about 100 Km south west of Baghdad. The participants were clearly informed of all the study procedures before signing the informed consent form. Generally, volunteers were interviewed at the time of sampling to obtain some general information about their health status, lifestyle, typical diet and smoking habits. The samples were collected from individuals in relation to different forms of smoking (none and active), varying in gender (male and female) and age from eight to sixty years old.

Sample collection and preparation

Human teeth: Teeth samples without filling were collected by dentists to reduce the risk of contamination from mechanical operation [28]. Prior to analyses, each tooth was soaked in H_2O_2 for 2 h to remove connective tissue, and then washed with deionized distilled water in an ultrasonic water bath by using the international atomic energy agency (IAEA) procedure. The same procedure was repeated three times with deionized distilled water. The washed samples were dried in an oven overnight at 60 °C and subsequently stored in a labelled poly-ethylene bag until pre-analysis digestion [29].

Fingernails: Fingernail samples were collected from all 10 fingers using acetone distilled deionized water washed clippers [30]. The main advantages to collect all fingers rather than one big finger are: sufficient sample mass and an estimate of the complete hand of exposure [31]. The determination of elements contents in fingernails can be considered as an indicator of level in other tissues and that reflect mineral metabolism in the body [32,33]. The sequential washing procedure, namely International Atomic Energy Agency (IAEA) procedure [acetone-water-water-water-acetone] has been used [34]. In this method, a sufficient volume of acetone was added to each tube to cover the fingernails sample. All tubes were sonicated for 10 min (35 MHz) at room temperature and then separated by centrifugation (1000 rpm for 5 min). The same procedure was repeated three times with deionized distilled water and finally with acetone. Samples were dried in an oven overnight at 60 °C then stored at room temperature in labelled polyethylene bags. The wet digestion method using a Kjeldahl™ tube was employed by using 1 mL of concentrated nitric acid for human teeth and washed fingernails [35].

The new JY 2000-2 ICP optical emission spectrometer (ICP-OES) Horiba Scientific was used in this study in order to determine the levels of strontium human teeth, fingernails, pooled and certified reference materials).

Precision and accuracy: In this study, precision levels were evaluated for any matrix effects by replicate analysis (n = 10) of a "pooled" sample that was prepared from at least 5 samples of fingernails. The accuracy value was checked based on the triplicate analysis of the human scalp hair CRM GBW 09101, as indicated in Table-1. In general, good levels of precision were obtained for strontium with perfect value of (0.724% RSD). The use of certified reference materials (CRMs) was employed to determine the validity and accuracy of the method. The measured value was highly comparative to certified value, and the recovery value was found to be 98 % for strontium, as described in Table-1 [22]. The value of limit of detection (LOD) for strontium was also determined (0.003 mg/L).

TABLE-1
ACCURACY (R, %) AND PRECISION (RSD, %) LEVELS FOR HUMAN SCALP HAIR CRM GBW 09101 AND POOLED HUMAN FINGERNAIL SAMPLES, RESPECTIVELY

Elemental level (mg/kg)			
Accuracy (n =3)			Precision (n =10)
Measured value (mean ± SD)	Certified value mean	Recovery (%)	RSD (%)
4.11 ± 0.03	4.19	98	0.72

SD is standard deviation, RSD is relative standard deviation (quoted as a % in brackets)

RESULTS AND DISCUSSION

Strontium levels of human teeth and washed fingernails from Karbala, Iraq: The levels of strontium in teeth for carious patients and fingernails for healthy individuals and those with dental caries disease have been compared with other results reported by several researcher [24]. In general, the results are in agreement with values reported by other authors for fingernails but there are some differences in the case of teeth (Table-2). A possible explanation is that the elemental levels in human biological samples vary from one country to another because of geographical differences, nutritional status and the environmental factors [24]. Therefore, it is difficult to establish reference ranges for trace elements in human fluids and tissues

TABLE-2
POPULATION DATA FOR STRONTIUM LEVELS (mg/Kg) IN CARIOUS TEETH (n = 50) AND WASHED FINGERNAILS (n = 75) FROM INDIVIDUALS RESIDENT IN KERBALA (IRAQ), ALONG WITH LITERATURE RANGE

Variable	Teeth	Fingernails	Literature range: Human teeth	Literature range: Fingernails
Mean ± SD	2.613 ± 0.450	2.184 ± 0.636	(106 ± 28) [Ref. 16,29] (3.62 ± 2.02) [Ref. 16,29]	(1.0-7.6) [Ref. 26,36] (0.14-5.54) [Ref. 26,36]
Range	1.350 – 3.730	1.000 – 3.016		
Lower quartile	2.388	1.502		
Upper quartile	2.843	2.636		
Confidence interval at 0.05 %	0.125 (2.488 – 2.737)	0.114 (2.040 – 2.328)		

SD is standard deviation, RSD is relative standard deviation, CI is confidence interval for mean, n is the number of samples

because of the effects of said factors, as they impose restrictions on the interpretation of the results.

Influence of caries-link to human health: In this work, the results of healthy individuals and dental caries patients resident in Kerbala have been compared in order to evaluate whether there are any significant differences in the levels of strontium between the two groups.

This data is used to describe whether dental caries plays any significant role in these differences by increasing or decreasing the strontium levels inside the human body through the effect on the metabolism of strontium. The carious patients have a mean age of 33.180 ± 11.23 years (range 8-60 years) with no other chronic or infectious diseases as reported in the questionnaires of participants in this study. The mean and standard deviation values for strontium levels in fingernails of the healthy and carious populations were compared by using F-test and a two-tailed t-test and the results obtained are listed in Table-3. In general, significantly higher fingernail levels of strontium are found in carious patients (2.599 ± 0.234 mg/Kg) when compared with healthy individuals (1.351 ± 0.246 mg/Kg) at a probability level, $p < 0.05$. It is clear that there is a good agreement between this result and those reported in the literature [19,38], as indicated in Table-3. The box-plots for strontium levels in the populations under investigation are summarized in Fig. 1.

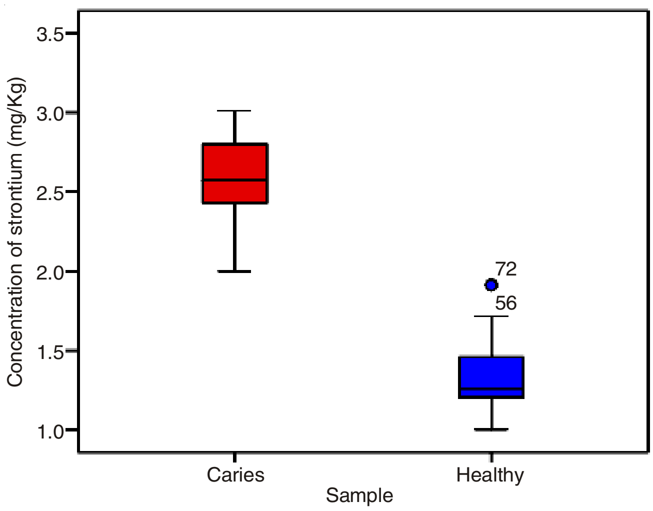

Fig. 1. Strontium levels (mg/Kg) in washed fingernails for different population groups: healthy individuals (n = 25) and dental caries patients (n = 50), middle band, box and whiskers represent the median, 25th and 75th percentile, and 5th and 95th percentile, respectively

Influence of gender: The total population of Kerbala (n = 125) was divided into two gender groups, males and females. The effect of gender on the levels of strontium in teeth and washed fingernails samples was investigated and the mean and standard deviation (± SD) for each gender group. In order to determine whether there is any significant difference that can be attributed to gender, F-test and a two-tailed t-test were undertaken on the teeth and fingernails data from individuals. Although the levels of strontium are slightly higher in females (2.31 ± 0.56 mg/Kg) than males (2.07 ± 0.68 mg/Kg) for fingernail samples, there is no significant effect of gender on the levels of strontium ($t_{(73)} = 1.69$, $t_{crit} = 1.99$, $p < 0.05$), (where the number in brackets is the number of degrees of freedom and the critical value (t_{crit}) is determined at the probability level of $p = 0.05$), as presented in Table-3. Interestingly, similar

TABLE-3
INFLUENCE OF VARIOUS FACTORS UPON THE LEVELS OF STRONTIUM IN HUMAN FINGERNAIL SAMPLES ALONG WITH THE VALUES OF LITERATURE VALUES

Factor	Published value: Mean ± SD (mg/Kg)		Present study: Mean ± SD (mg/Kg)		F-test: Variance	F_{calc}	Sig.	Two-tailed t-test: t_{calc}	df	Sig.	t_{crit}
HS [Ref. 29]	Healthy ($n_1 = 459$)	Caries ($n_2 = 433$)	Healthy ($n_1 = 25$)	Caries ($n_2 = 50$)	EVA	0.019	0.892	21.399	73$^+$	< 0.001	1.993
	82 ± 17	106 ± 28	1.363 ± 0.246	2.599 ± 0.234							
G [Ref. 37]	Male ($n_1 = 42$)	Female ($n_2 = 69$)	Male ($n_1 = 41$)	Female ($n_2 = 34$)	EVA	8.896	0.004	1.699	73$^+$	0.094	1.993
	482 ± 270	445 ± 246	2.07 ± 0.68	2.32 ± 0.56							
SA [Ref. 37]	Smoker ($n_1 = 30$)	Non-smoker ($n_2 = 125$)	Smoker ($n_1 = 13$)	Non-smoker ($n_2 = 62$)	EVA	19.583	< 0.001	2.823	73$^+$	0.006	1.993
	638 ± 364	439 ± 234	2.62 ± 0.34	2.09 ± 0.65							

HS is healthy status, G is gender, SA is smoking activity, SD is standard deviation, n_1, n_2 are the number of samples, df = degrees of freedom at n_1 - 1 and n_2 - 1 for F-test, $^+$degrees of freedom for t-test ($n_1 + n_2 - 2$), F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at $p = 0.05$, Sig. = level of significance, EVA = equal variances assumed.

results were reported in this study for strontium levels in carious teeth samples for females (2.67 ± 0.50 mg/kg, n = 27) and males (2.55 ± 0.38 mg/kg, n = 23) at the same level of probability $t_{(48)} = 0.953$, $t_{crit} = 2.011$, $p < 0.05$. Similar results were also found by other researchers [22,19]. It was found that strontium has similar levels for saliva samples in males (mean ± SD: 7.44 ± 3.54 g/L strontium) and (mean ± SD: 7.97 ± 3.70 g/L strontium) for females.

Influence of smoking activity: Smoking is considered to be a major environmental risk factor associated with many serious systemic diseases, including respiratory diseases, heart diseases, caries and cancers [39-41]. It was found that cigarette tobacco which can cause several health problems and disorders [42,43]. The effect of smoking activity on trace elements in various invasive and non-invasive human fluids and tissues has been studied by several other researchers [8,22,43]. Multi-element analysis of various brands of imported cigarette tobacco collected from Kerbala is determined in the previous study in order to evaluate whether or not any relationship exists between their levels in cigarette tobacco and human health. In this study, the levels of strontium in tobacco are ranged 75 ± 14 (53-102 mg/Kg) [37]. In this study, the population of Kerbala was divided into smokers and non-smokers. The influence of smoking activity on the strontium levels in washed fingernails and teeth was examined by using F-test and a two-tailed t-test. A significant effect of smoking activity for strontium in fingernails ($t_{(73)} = 2.823$, $t_{crit} = 1.993$, $p < 0.05$). In other words, high level of strontium was found in the human fingernails of smokers (2.617 ± 0.341 mg/Kg) when compared with those of non-smokers (2.093 ± 0.648 mg/Kg). In contrast, there is no significant differences observed for strontium between smokers (2.522 ± 0.121, n = 11) and non-smokers (2.642 ± 0.501, n = 39) for carious teeth samples by suing a two tailed t-test ($t_{(48)} = 0.755$, $t_{crit} = 2.011$, $p < 0.05$). Similar results were also reported when the strontium mean value for smokers was compared with non-smokers by using a two tailed t-test [37].

Influence of drinking water: The relationship between the levels of strontium in drinking water and washed fingernails was investigated. The strength and direction of this relationship was evaluated using correlation coefficient (r) analysis. The value of r was calculated and then subjected to a significance test. A strongly positive significant correlation is found for strontium levels between drinking water and fingernails ($r = 0.894$, $t_{(8)} = 8.612$, $p < 0.05$, $t_{crit} = 2.306$), as shown in Fig. 2. In this study, the strontium levels in drinking water (domestic bottled) are higher than those reported in other countries such as Dhaka (127.6 g/L strontium); Karanikong (217.98 g/L strontium); Japan (81.88 g/L strontium) and Saudi-Arabia (376.46 g/L strontium) [44,45]. The last study in Kerbala has

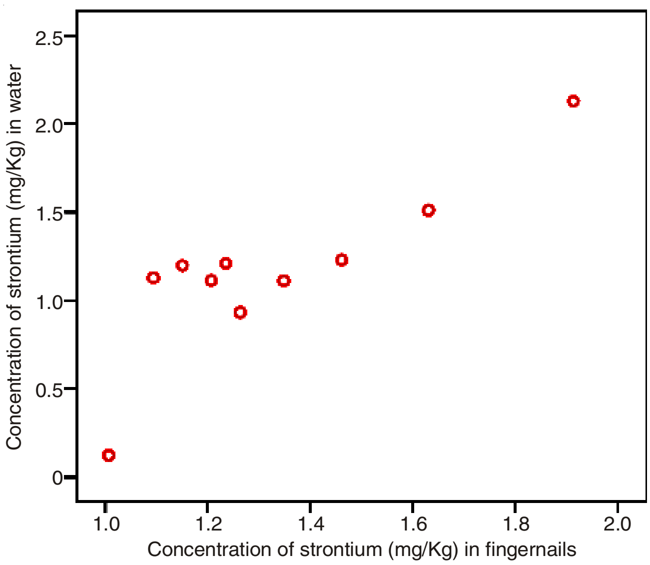

Fig. 2. Correlation between strontium levels in washed fingernails and drinking water

reported that the strontium levels in drinking water, namely well, artesian, river, tap, bottled, and commercial) were found to be in ranges 1.51 14.38, 1.16 8.31, 0.34 2.76, 0.08 2.11, 0.02 1.54, and 0.02 0.12 mg/L strontium, respectively [37]. The highest levels of strontium were found in ground water. The presence of strontium may be due to natural distribution throughout rocks, soil, dust, coal and oil in this region. Eventually, it is moved to the ground water through the natural re-crystallisation or weathering of rocks and soils [14]. The soil in Iraq includes high levels of oil and, therefore, it might be a reasonable source for strontium. In addition, human activities could also increase the levels of strontium in the environment, where strontium is used to produce ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, medicines, colour television picture tubes and a red colour in fireworks [46].

Influence of age: The influence of age was also studied in order to evaluate whether or not this parameter provide any significant effects on the levels of strontium in washed fingernails. The studied population was divided into three age groups: ≤ 20 years (n = 13), 21-39 years (n = 40) and ≥ 40 years (n = 22). One-way ANOVA was used to check whether there were any significant differences exist between the age groups of strontium levels at the probability level of $p < 0.05$. The results show that there is no significant difference for strontium between the age groups, as presented in Table-4. Although the levels of strontium in the age group (≤ 20 year) are different when compared with the two remaining groups, there is no significant difference at the $p < 0.05$, as reported in

TABLE-4
ANALYSIS OF VARIANCE ANOVA FOR STRONTIUM LEVELS IN WASHED FINGERNAILS FOR INDIVIDUALS FROM KERBALA CITY, IRAQ

Source of variance	Sum of squares	df	Mean square	F	Sig.
Between groups	1.692	2	0.846	2.154	0.123
Within groups	28.274	72	0.393		
Total	29.966	74			

df = degrees of freedom, for between-groups (df_B) = number of groups – 1; within-group (df_W) = df_T – df_B; Total number of degrees of freedom (df_T) = number of observations – 1, mean square = (SS/df), F is the calculated value for F-test, $F = MS_B/MS_W$, Sig. is the significance level.

TABLE-5
STRONTIUM MEAN AND STANDARD DEVIATION VALUES IN HUMAN TEETH AND FINGERNAILS FROM KERBALA, IRAQ ALONG WITH THE LITERATURE VALUES

Published value*		Present study								
Mean ± SD (mg/Kg)		Mean ± SD (mg/Kg)		F-test			Two-tailed t-test			
Teeth	Fingernails	Teeth (n_1 = 50)	Fingernails (n_2 = 50)	Variance	F_{calc}	Sig.	t_{calc}	df	Sig.	t_{crit}
3.62±2.02	0.14-5.54	2.612±0.449	2.599±0.234	EVA	7.231	0.008	0.195	98+	0.846	1.985

SD is standard deviation, n_1, n_2 are the number of samples for teeth and fingernails, respectively, df = degrees of freedom at n_1 - 1 and n_2 - 1 for F-test, +degrees of freedom for t-test (n_1 + n_2 - 2), as described in Appendix E, F_{calc} and t_{calc} are the calculated values for F-test and t-test, respectively, t_{crit} is critical value at P = 0.05, Sig. = level of significance, EVA = equal variances assumed.
Source: *Athanassouli *et al.* [15]; Gault *et al.* [48].

Table-4. In the light of these results, the levels of strontium are increased when the age of individuals increase (Fig. 3). The possible explanation for these findings is that the bioaccumulation of strontium in human fingernails is a complicated process influenced by several factors during fingernails growth, namely metabolic changes, age, gender and living environment quality [24,47].

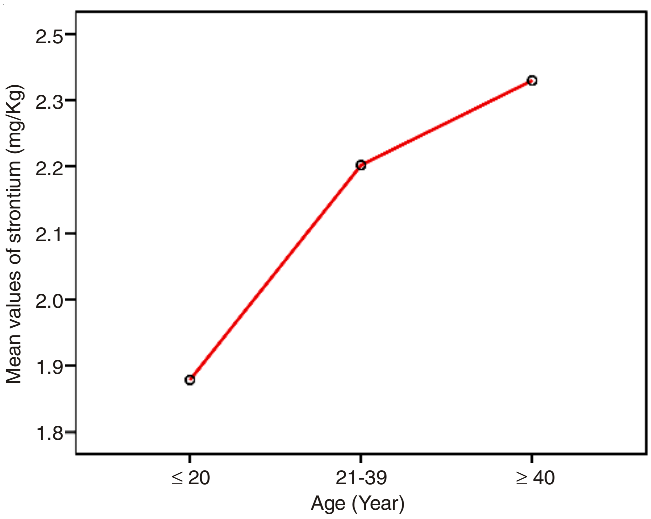

Fig. 3. Concentrations of strontium in washed fingernails for ≤ 20 years (n = 13), 21-39 years (n = 40), ≥ 40 years (n = 22) of age (mg/Kg)

Comparison of strontium levels of teeth and washed fingernails: In total 100 teeth and washed fingernails samples were collected from the same carious patients in order to test whether there are any significant differences between the strontium levels in both media. An F-test and a two-tailed t-test were used to compare the two mean values for strontium in the two media. It was found that there is no significant difference ($t_{(98)}$ = 0.195, t_{crit} = 1.98, p = 0.846) for strontium between teeth (2.612 ± 0.449 mg/Kg) and washed fingernails (2.599 ± 0.234 mg/Kg), as shown in Table-5. The value of r was subjected to a significance t-test to evaluate if there was any significant correlation. The findings show that there is no significant correlation between washed teeth and fingernails for strontium (r = 0.168, p < 0.05) (Fig. 4).

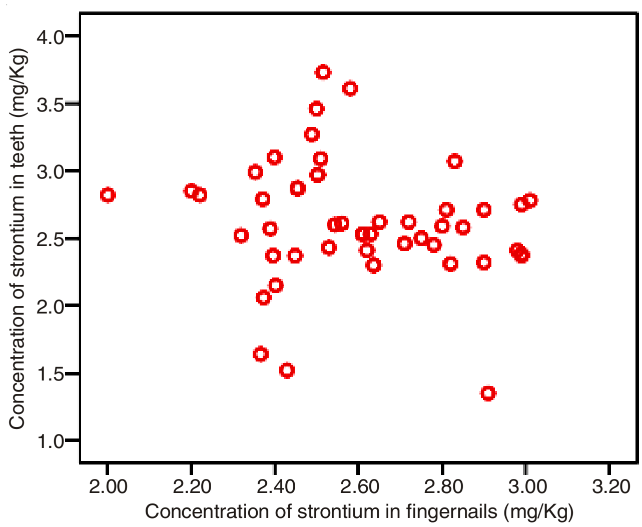

Fig. 4. Concentrations of strontium (mg/Kg) in teeth (n = 50) and washed fingernails (n = 50)

Conclusion

In the light of these results, the use of human teeth and fingernails as a potential biomarker for assessing human health status has been evaluated using several studies, namely the influence of health status, smoking activity, gender, age and drinking water. In conclusion, the present study highlights the use of teeth and fingernails tissue as biomarkers for the strontium level in the human body for healthy and dental caries patients in Iraq. In addition, this study provides a preliminary assessment of the determination of strontium levels in teeth and washed fingernails for Iraqi individuals in Karbala city, Iraq.

ACKNOWLEDGEMENTS

The authors thank Department of Chemistry, University of Kerbala for assistance and financial support. Acknowledgments are also due to the Ministry of Science and Technology for their support throughout the analysis of samples.

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