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Evaluation of elements in human dental tissue in terms of determination of postmortem interval: Experimental study

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Abstract

Despite many methods, there has not been a method yet that exactly determines PMI. This study aims to examine the relationship between the element levels in the dental tissue and the postmortem process in a corpse whose exact time of death is unknown and in the open field and to evaluate its use in determining the time of death by evaluating the increase-decrease-changes. For the study, the healthy and extracted third molars of the patients who applied to the Oral and Maxillofacial Surgery Polyclinic for the extraction of their third molars for various reasons and whose third molars were extracted by the dentists were taken. The samples were divided into 6 groups, with 10 samples in each group. The teeth were left to decay in the open air in a jaw-like plastic model. Na, Mg, P, Ca, Fe, Mn, Co, Zn, Cd, Pb, and Si elements were measured using Inductively Coupled Plasma-Mass Spectrometer analysis method. In our study, it was determined that there were statistically significant changes in the Na, Mg, Fe, Co, Pb, Si elements in the tooth structure. For the Co and Na elements, 2 regression equations were created that can be used to predict the time of death. This work showed that postmortem tooth element concentration changes can be used to predict the time of death. As a result of experiments that will cover longer periods with more elements and be carried out in different environments, a great contribution will be made to the subject of estimation of time of death which is not clear yet.

Keywords: Forensic medicine, postmortem interval, element analysis

Introduction

There has not been a method yet that can be used alone to determine the time of death [1]. It is seen that the methods proposed in the studies do not have a standardized procedure [2]. The environmental conditions and personal factors in which the corpse is found differ from case to case are the possible reasons for this situation [3]. For such reasons, reliability is tried to be increased by using many methods together, and efforts to improve current approaches continue [4].

In studies conducted to determine the postmortem interval (PMI), it is definitively not possible to say the time elapsed after death; however, the opinion which is possible to comment on the time intervals after death is common [5]. There are many methods and new studies to make accurate time estimations for PMI determination. The most common method used in routine forensic medical practices is the examination of external

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physical appearance and changes [6]. Decreased accuracy with prolonged PMI causes limitations in all methods. Regardless of the technique, the decomposition and decay of tissues make it difficult to determine PMI [7]. Especially in cases of skeletal remains, the time of death becomes more difficult to predict as the soft tissues are greatly reduced or completely absent. Methods for estimating the time of death, especially for the late period, are carried out on bone and dental tissues. With its solid anatomy, isolated structure, and more resistance to decay and burning, the tooth stands out as an advantageous tissue in terms of obtaining accurate and long-term results in the determination of PMI compared to other tissues [8].

Our aim in this study is to examine the relationship between the element levels in the dental tissue and the postmortem process in a corpse whose exact time of death is unknown and who is found in the open field; is to research its usability in determining the time of death by evaluating the increase-decrease changes.

Material and Methods

On 25.06.2020, approval numbered 83116987-441 was received from the Tokat Gaziosmanpaşa University Faculty of Medicine Dean's Clinical Research Ethics Committee, stating that there was no ethical or scientific objection to our study.

Within the scope of our study, a total of 60 people were given 60 healthy and whole third molar teeth, which were not impacted or had the highest Class I or II impaction degree. These were obtained from 60 different individuals, who are over the age of 18, without additional systemic disease, not using pills continuously, non-smokers, not working in the industry, not having a filling or another dental treatment, and lastly applied to the same city center. This application was applied in the Tokat Gaziosmanpaşa University Faculty of Dentistry Oral Dental and Maxillofacial Surgery Polyclinic and those individuals were informed of the consent form and their written consent was obtained.

The tooth samples were taken from the Oral, Dental, and Maxillofacial Surgery Polyclinic at the 0th hour (Group 1), 1st month (Group 2), 3rd month (Group 3), 6th month (Group 4), 9th month (Group 5), and 12th month (Group 6), a total of 6 groups were formed. After the teeth of 5 groups other than Group 1 were collected from the volunteers, a Type II model was placed in a mandible-like model made of hard white plaster ($CaSO_4$-$2H_2O$; calcium sulfate dihydrate) with the crown part outside and the neck and root parts in hard plaster, and a jaw model was created (Image). The plaster mold was kept frozen until it hardened. Later, the jaw-like model was reconstructed by forming an intraoral-like structure by covering it with striated muscle tissue of approximately 1 cm thickness, 9-10 cm length, and 50 g weight of animal origin.

To leave the prepared jaw-like models to the decay process, a cage environment woven with fine wire was created in a way that would allow the entry of air and insects and not allow the entry of large animals. First, Group 6 was left to decay in the iron wire. Afterward; Group 5, Group 4, Group 3, and Group 2 were left to decay at three-month intervals (Image).

Image. Jaw model created with teeth placed in hard plaster and groups left to decay at three-month intervals

After group 1 teeth were collected, all teeth were separated from their jaw-like models. The teeth separated from the jaw model were completely cleansed of soft tissues by brushing using pure water and quickly delivered to the chemistry department for chemical analysis.

Chemical Analysis

Since the tooth samples were in separate containers for each group before the analysis procedures were solid, the teeth were brought into solution before the analysis. In the solution process, first of all, the whole teeth were cut into pieces with a manual-armed 25-ton hydraulic press machine. The shredded teeth were thoroughly ground and melted in high-pressure Teflon containers in the microwave system. For this procedure, 500,000 micrograms were taken from the teeth and transferred to microwave tubes. Incineration was applied at 200°C for 50 minutes in a Milestone Ethos D Microwave Labstation brand microwave defrosting device. 5 ml of nitric acid (HNO_3) was used in the incineration process. Suspended solid particles in the samples were passed through a 0.45-micron syringe tip filter (Isolab). Macro and micro elements of dental samples taken from 6 groups of volunteers were analyzed using Agilent 7800 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent technology, Japan). "Mass Hunter 4.4 Workstation Software 7800 ICP-MS Top C.01.04" software was used to calculate the measurements. Each sample was analyzed once, with each reading being the average of 3 parallel readings. Sodium (Na), Magnesium (Mg), Phosphorus (P), Calcium (Ca), Iron (Fe), Manganese (Mn), Cobalt (Co), Zinc (Zn), Cadmium (Cd), Lead (Pb), Silicon (Si) elements were measured in all groups.

Statistical Analysis

Descriptive analyzes were performed to give information about the general characteristics of the study groups. The data of continuous variables were given in the form of the mean. Differences between groups were analyzed by Kruskal Wallis Analysis of Variance considering parametric conditions. The Mann-Whitney U test was used for multiple comparisons, and

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it was considered statistically significant when the p-value was calculated less than 0.05. Ready-made statistical software was used in the calculations (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY). For the variables with a significant difference, the differences between the groups were evaluated with the Mann-Whitney U Test and a value of $p<0.05$ was considered statistically significant. IBM SPSS Statistics 19 and Microsoft Excel 15 programs were used for creating the regression equations.

Results

It was observed that %71.7 of the volunteers were female and %28.3 were male. Each group was formed from the teeth of 10 volunteers. The mean age of the volunteers included in the study was found to be 24.85. It was observed that the youngest age was 19 and the oldest was 52. The mean age of Group 1 was 24.9, Group 2 was 23.1, Group 3 was 26.2, Group 4 was 24.3, Group 5 was 25.3, and Group 6 was 25.3.

Elemental measurements were made with an ICP MS instrument for 6 groups consisting of 60 teeth. As a result of the measurement, the distribution of ppb value averages according to the groups, standard deviation values, and statistical p values are given in Table 1. The ppb values of the statistically significant elements Na, Mg, Fe, Co, Pb, and Si are given in Table 2 separately for each tooth.

It was determined that there was a regular and statistically significant increase between the groups in the measurements made for the Co element ($p<0.001$) (Table 1). The average values of the Co element for each group are shown in Figure 1.

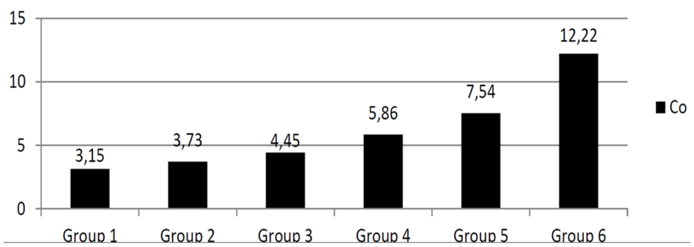

Figure 1. Average ppb values of cobalt (Co) element in each group

Na element increased in 1 month. Afterward, it was observed that it decreased regularly ($p<0.001$) (Table 1). The average ppb values of the Na element for each group are shown in Figure 2.

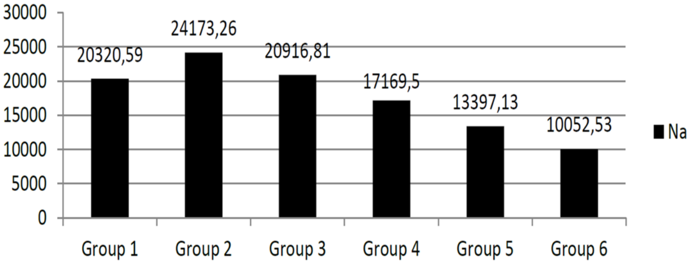

Figure 2. Average ppb values of sodium (Na) element in each group

Table 1. Mean ppb values of the groups, standard deviation value, statistical p-value

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6	p
Co	3.15±0.51	3.73±0.42	4.45±0.35	5.86±0.61	7.54±1.9	12.22±1.83	<0.001
Na	20320.59±5818	24173.26±1096	20916.81±2240	17169.5±991	13397.13±1717	10052.53±2520	<0.001
Mg	341116±55761	299558±40623	391488±250792	307193±54733	267189±46591	382688±136990	0.031
Fe	131984±62736	190384±24327	174929±22583	196414±21041	221737±130587	325596±513471	0.003
Pb	54.43±45.72	246.86±293.7	341.15±256.69	266.47±108.95	359.87±188.63	748.91±416.35	<0.001
Si	6472.38±6019	7094.77±4296	14399.28±6151	11932.79±7316	12204.94±4551	18091.91±6713	0.002
P	8201601.85±1000003	8325802.85±1263838	10387536.09±6547150	8563405.52±1264443	7445632.93±2638499	10331033.11±4064658	0.701
Ca	10182010.98±1204978.24	10173430.6±1320208.54	12992435.91±7615457.13	11264530.74±1655401.45	9753051.92±3446986.83	13334771.39±4870072.81	0.139
Mn	78.72±88.47	68.6±83.37	18.48±14.8	2.34±2.14	8.8±10.73	4.48±0.52	0.101
Zn	8897.49±1660	8231.09±1465	9373.97±4563	8724.69±2008	7637.83±3129	7786.39±1981	0.624
Cd	1.2±1.37	0.42±0.21	0.74±1.14	0.49±0.16	0.39±0.37	0.49±0.51	0.165

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Table 2. Individual ppb values of statistically significant elements for each tooth (Grp1.1=Group 1, 1st tooth)

	Na	Mg	Fe	Co	Pb	Si
Grp 1.1	10587.497	427759.149	5904.835	2.649	19.200	6451.598
Grp 1.2	11420.698	353751.700	59082.772	2.757	45.494	5126.251
Grp 1.3	17344.956	235191.916	99980.919	2.750	8.170	996.596
Grp 1.4	18032.520	348354.514	102918.529	2.845	32.901	5871.937
Grp 1.5	21377.435	271269.969	174139.687	2.861	13.214	4825.681
Grp 1.6	22773.975	352005.512	178139.540	4.013	124.659	7115.366
Grp 1.7	26247.463	388503.208	185924.496	3.185	124.487	16383.408
Grp 1.8	23461.681	314015.549	194894.419	3.964	90.196	1316.313
Grp 1.9	25476.665	347065.571	153847.901	2.987	12.943	3711.084
Grp 1.10	26483.002	373249.783	165008.366	3.523	73.011	6453.219
Grp 2.1	24120.860	287237.498	168220.544	3.231	131.098	2760.041
Grp 2.2	24212.813	352062.034	133824.786	3.124	1076.537	5740.415
Grp 2.3	25730.987	214164.809	205416.808	4.180	135.691	1112.347
Grp 2.4	25722.264	317998.262	194779.165	3.707	128.984	9135.236
Grp 2.5	23879.160	299149.077	212961.466	3.553	193.357	5254.544
Grp 2.6	24587.813	306684.054	195041.790	3.517	118.387	7355.201
Grp 2.7	23719.384	260134.695	210965.005	3.581	213.124	6021.700
Grp 2.8	23948.895	332238.348	190554.961	4.047	143.457	5554.361
Grp 2.9	23968.378	338252.039	209675.989	3.878	205.067	10087.591
Grp 2.10	21842.002	287662.014	182400.179	4.451	122.876	10831.501
Grp 3.1	24235.786	347370.059	166612.577	3.874	695.028	12799.993
Grp 3.2	21961.959	323534.061	195238.255	4.573	237.770	2148.842
Grp 3.3	22486.640	363998.966	200772.137	3.970	172.986	14604.987
Grp 3.4	20944.911	333726.816	213997.210	4.653	280.753	13065.414
Grp 3.5	20980.133	248733.384	136766.413	4.266	167.611	25584.411
Grp 3.6	20654.477	364270.787	161961.559	4.236	257.176	17680.618
Grp 3.7	23162.025	288813.334	173945.317	4.726	217.644	20569.201
Grp 3.8	19612.031	240015.468	160184.198	4.522	247.913	13494.736
Grp 3.9	16748.743	1094457.724	166676.342	4.922	929.094	11860.201
Grp 3.10	18381.365	309965.619	173137.446	4.791	205.544	12184.384
Grp 4.1	16166.929	226863.027	179449.646	4.987	291.047	12595.057
Grp 4.2	17920.142	336810.924	195621.949	5.293	312.649	22534.614
Grp 4.3	16715.321	377027.407	197626.680	5.409	283.590	14695.454
Grp 4.4	19201.840	209517.386	222689.032	5.528	185.960	15390.381
Grp 4.5	16009.604	297545.205	226008.110	5.694	140.450	15068.164
Grp 4.6	17942.914	281967.216	184996.920	5.994	160.330	643.357
Grp 4.7	16545.904	308399.052	186573.187	6.028	231.847	1014.359
Grp 4.8	17647.893	337366.883	176091.921	6.100	391.229	9168.961
Grp 4.9	16951.728	358381.406	224801.643	6.556	482.696	20262.520
Grp 4.10	16592.690	338057.828	170289.315	6.992	184.910	7955.024
Grp 5.1	12962.024	239087.722	176843.956	7.046	339.445	13344.135
Grp 5.2	12638.906	268831.120	178910.550	6.905	271.662	22797.460
Grp 5.3	12826.468	313394.375	591875.561	7.196	238.110	8987.119
Grp 5.4	12387.964	190703.127	180675.925	7.687	354.990	7727.361
Grp 5.5	11308.039	267373.382	186671.311	7.901	300.222	10040.220
Grp 5.6	12378.141	286676.075	177532.825	8.494	659.985	11094.034
Grp 5.7	12448.251	244016.622	194155.251	9.187	579.174	13568.053
Grp 5.8	11461.521	220842.337	201341.712	8.873	287.814	12690.552
Grp 5.9	11542.637	220495.702	170577.360	9.374	546.949	6957.220
Grp 5.10	11620.235	153281.216	158787.151	8.773	20.363	14843.202
Grp 6.1	10224.026	316423.404	165699.063	9.896	1471.901	9734.658
Grp 6.2	10930.984	354760.652	165202.852	10.327	898.433	22408.180
Grp 6.3	11612.794	279634.684	178087.384	10.845	439.733	22451.272
Grp 6.4	10482.578	383533.336	165017.835	11.233	530.380	13036.635
Grp 6.5	11998.928	256343.694	172195.893	11.734	483.391	8368.636
Grp 6.6	10407.614	273427.522	163705.105	12.120	597.537	14365.793
Grp 6.7	9008.546	591630.998	156036.933	12.516	432.464	19639.270
Grp 6.8	9184.518	354925.832	144526.770	13.780	596.820	20066.859
Grp 6.9	8242.255	351066.857	1786735.782	14.070	514.839	20406.077
Grp 6.10	8433.091	665135.048	158761.029	9.711	1523.647	30441.701

Although there were fluctuations between the groups in the measurements made for the Mg element, it was observed that a statistically significant result was obtained (p:0.031) (Table 1). In the measurements made for the Fe element; except that the 1st month group was higher than the 3rd month group, there was a regular and statistically significant increase in the months (p:0.003) (Table 1). Although there was a decrease in the measurements for the Pb element in the 6th-month group, there was a significant increase between the groups statistically (p<0.001) (Table 1). In the measurements made for the element Si, it was statistically determined that there was a significant increase between the groups, except for the 3rd month group (p:0.002) (Table 1). It was determined that the 12th month group increased by %48 compared to the 9th month group. The average ppb values of the Mg, Fe, Pb, and Si elements for each group are shown in Figure 3.

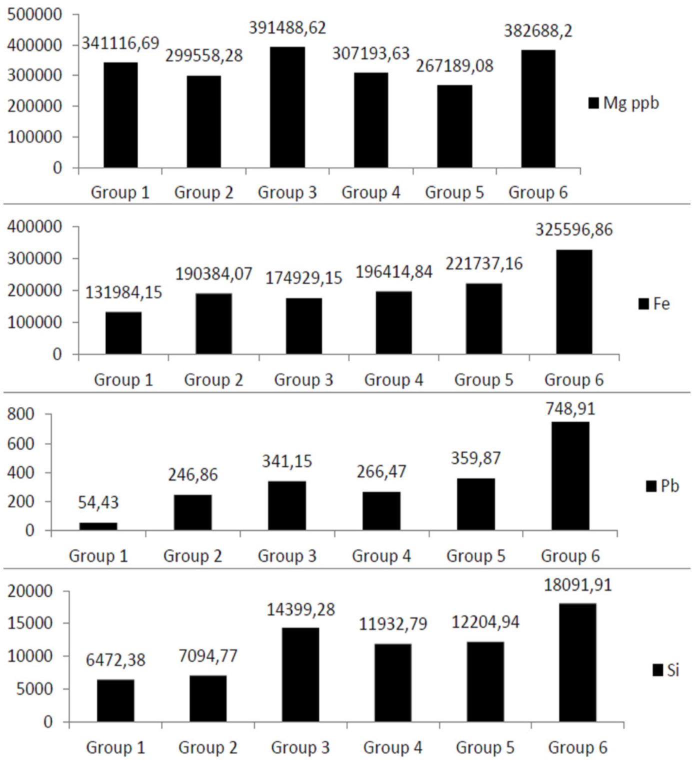

Figure 3. Average ppb values of Mg, Fe, Pb, Si elements in each group

No statistical significance was found in the measurements made for P, Ca, Cd, Zn elements, and there were fluctuations between the groups (p>0.05) (Table 1). In the measurements made for the Mn element; although a continuous decrease was observed between the 0th hour, 1st month, 3rd month, and 9th months, no statistical significance was found (p>0.05) (Table 1). Average ppb values of P, Ca, Cd, Zn, and Mn elements for each group are shown in Figure 4.

Regression equations for Co and Na elements

As a result of the analyzes and statistical studies, we have obtained an equation that will allow us to estimate the time of death from the dental Co element concentration. The regression equation obtained by regression analysis:

t(month)=1.340xCo (conc ppb)–3.085 R^2=%91.6 p=0.03

Concentration is calculated in ppb and the result in time is calculated in months. In this equation, R^2=%91.6, which is the explanation rate of the dependent variable of our independent variable. The p-value was found to be 0.03 and a statistically significant result was obtained.

The regression equation created by regression analysis will allow us to estimate the time from the tooth Na element concentration:

t(month)=23.111–1.052.7xNa (conc ppb) R^2=%90.05 p=0.04

Concentration is calculated in ppb and the result in time is calculated in months. In this equation, R^2=%90.05, which is the rate of explanation of the dependent variable of our independent variable. The p value was found to be 0.04 and a statistically significant result was obtained.

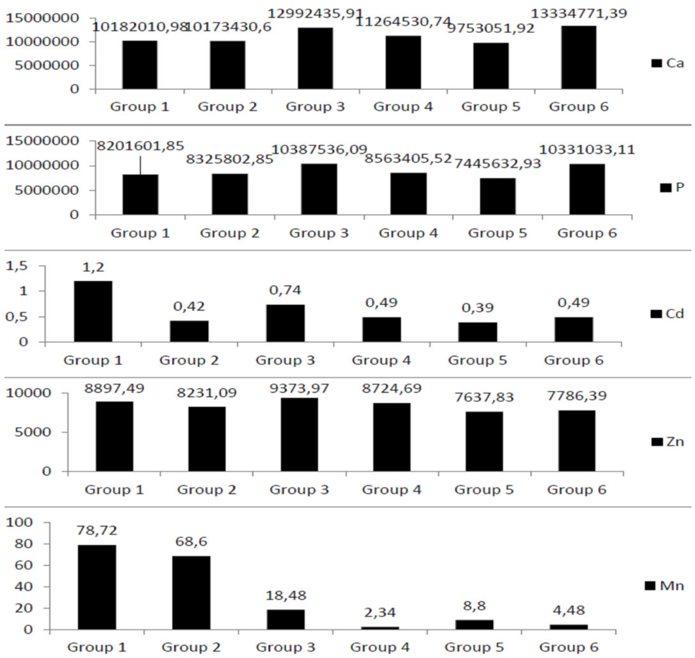

Figure 4. Average ppb values of P, Ca, Cd, Zn, Mn elements in each group

Discussion

In studies that try to predict the time of death by evaluating post-mortem biochemistry, elemental analysis has been performed in tissues such as the brain, myocardium, kidney, liver, bone, and skeletal muscle, as well as body fluids such as vitreous fluid, cerebrospinal fluid, blood, pericardium, synovial fluid [5,9-11]. In the literature, some studies try to predict the time of death by evaluating the postmortem dental tissue, and the subject has generally been discussed in histopathological and molecular dimensions, and no study has been found on experimental or autopsy tissues for estimating the time of death by evaluating the biochemistry of human dental tissue after death [12,13]. In a study using diatoms adhered to the surface of tooth enamel to determine PMI for underwater corpses, it was found that while seawater components detected on the surface of tooth enamel increased, the amount of major dental components such as Ca and phosphorus gradually decreased, and a regression formula was created to estimate the immersion time of the corpse in water [8,14]. In the literature, the number of studies on experimental or autopsy tissues for estimating the time of death by evaluating postmortem human dental tissue biochemistry is limited.

Various personal and environmental factors such as age, gender, genetics, nutrition, drinking water, region of residence, occupation, smoking, diseases, and medications affect tooth element levels [15-17]. In our study, patients over the age of 18, who do not have additional systemic diseases and do not use pills continuously, do not smoke, do not work in the industry, have not had any filling or dental treatment for the tooth to be extracted, and apply from the same city center, are included in the study. The average age between the groups was very close to each other, and it was observed that the smallest 23.1 and the largest 26.2 years of age were observed in the groups.

A tooth consists of enamel, dentin, cementum, and pulp structures. The element content of these layers differs from each other. It is known that there are differences in the amount of major and minor elements even within these structures [14]. It should be kept in mind that the number of elements in the tissues may not be homogeneously distributed in the element analyses to be made for the estimation of the time of death. In this case, taking the sample to be taken in the same area of the tissue will ensure that the analysis result is more reliable. In our study, we decomposed the tooth layers as a whole, crushed the tooth with all its layers, turned it into powder, and subjected it to the melting process. In this way, we treated the teeth as a whole and prevented the differences arising from the layers.

As a result of the measurements, we determined that there were significant changes in the postmortem concentrations of Na, Mg, Fe, Co, Pb, and Si elements. In particular, the variation of the Co element by months was remarkably regular. We determined that the increase in the Pb element and the Fe element and the decrease in the Na element were regular except for one group. We created a regression equation that will allow us to estimate the time of death in months for Co and Na elements.

When we apply the equation for the Co element to the Co concentrations of 60 teeth separately, the calculated postmortem time (pmt) values for each tooth are given in Table 3 in months and the calculated pmt averages of the groups, that is, the t (months) estimation averages, are given in Table 4.

Table 3. Pmt values (in months) calculated according to the Co equation for each tooth (G1.1= Group1, 1st tooth)

G1.1=0.4	G1.2= 0.6	G1.3= 0.6	G1.4=0.7	G1.5=0.7	G1.6=2.2	G1.7=1.1	G1.8=2.2	G1.9=0.9	G1.10=1.6
G2.1=1.24	G2.2=1.1	G2.3=2.5	G2.4=1.8	G2.5=1.6	G2.6=1.6	G2.7=1.7	G2.8=2.3	G2.9=2.1	G2.10=2.8
G3.1=2.1	G3.2=3	G3.3=2.2	G3.4=3.1	G3.5=2.6	G3.6=2.6	G3.7=3.2	G3.8=3	G3.9=3.5	G3.10=3.3
G4.1=3.6	G4.2=4	G4.3=4.1	G4.4=4.3	G4.5=4.5	G4.6=4.9	G4.7=5	G4.8=5	G4.9=5.7	G4.10=6.3
G5.1=6.3	G5.2=6.1	G5.3=6.5	G5.4=7.2	G5.5=7.5	G5.6=8.3	G5.7=9.2	G5.8=8.8	G5.9=9.4	G5.10=8.6
G6.1=10.1	G6.2=10.7	G6.3=11.4	G6.4=12	G6.5=12.6	G6.6=13.2	G6.7=13.7	G6.8=15.4	G6.9=15.8	G6.10=10

When we applied the equation for Co element results in all groups, we obtained results close to real pmt. It was noteworthy that the pmt mean calculated with the equation for the 3rd month group was 2.9 months.

Table 4. Known pmt for element Co and pmt calculated by equation

Known PMI	Calculated pmt (group averages)
0 Hour	1 Month
1 Month	1.9 Month
3 Month	2.9 Month
6 Month	4.8 Month
9 Month	7.7 Month
12 Month	13.2 Month

In the literature review, we could not find a study for the estimation of death time in any tissue for the Co element. Although there is no study evaluating the Co element in the postmortem period, we see that the regular and significant increase seen in our study is promising for the estimation of time of death in human dental tissue in the open field.

The equation for the Na element was applied to the Na concentrations of 60 teeth separately. No significant results were found in groups 1,2 and 3. Calculated postmortem time (pmt) values for each tooth belonging to Groups 4, 5 and 6 are given in Table 5 in months, and the calculated pmt averages of the groups, that is, t (months) estimation averages, are given in Table 6.

Table 5. Pmt values (in months) calculated according to the Na equation for Groups 4, 5 and 6 (G4.1=Group 4, 1st tooth)

G4.1=6.9	G4.2=4.2	G4.3=5.5	G4.4=2.9	G4.5=6.2	G4.6=4.2	G4.7=5.7	G4.8=4.5	G4.9=5.3	G4.10=5.7
G5.1=9.4	G5.2=9.8	G5.3=9.6	G5.4=10	G5.5=11.2	G5.6=10	G5.7=10	G5.8=11	G5.9=10.9	G5.10=10.8
G6.1=12.3	G6.2=11.6	G6.3=10.9	G6.4=12	G6.5=10.5	G6.6=12.1	G6.7=13.6	G6.8=13.4	G6.9=14.4	G6.10=14.2

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Table 6. The known pmt for the element Na and the pmt calculated with the equation

Known PMI	Calculated pmt (group averages)
0 Hour	-
1 Month	-
3 Month	-
6 Month	5 Month
9 Month	8.1 Month
12 Month	12.5 Month

Considering the known PMI values of the Na element concentrations of the teeth and the pmt values calculated with the equation, no significant results were obtained in the 0th hour, 1st month, and 3rd month groups. In the 6th month, 9th month, and 12th month groups, it was observed that the results were close to the real pmt values. We think that consistent results could not be obtained in the 0th hour, 1st month, and 3rd month groups because the mean Na element concentration increased in the 1st month group compared to the 0th hour group and then decreased regularly in all groups. In the 6th month, 9th month, and 12th month groups, consistent results were obtained with the equation.

Walden et al. reported that Na element decreased in bone with decay in pig bones covering 140 days postmortem [18]. In our study, however, the Na element increased in the first month and decreased regularly in the following months. The reason for the increase in the Na element in the first month may be that the organic substances decompose as well as the inorganic substances and increase the Na value. Later, with the progression of decay, a significant decrease in Na element occurred. Nagai et al., in a study on the bovine femur bone, determined that Na element decreased in the 6th and 12th month groups in the freshwater environment compared to the control group, and increased in the 6th and 12th month groups compared to the control group in seawater and underground [18]. This study showed that the concentrations of the elements can be affected by the measurement environment. Our study was carried out in an open area under conditions that do not come into contact with the soil. Tooth element change measurements should be made by conducting experiments in various environments and its relationship with PMI should be evaluated.

In a study evaluating 140 days postmortem in buried pig bones, it was stated that the Fe element increased slightly in the postmortem period due to soft tissue destruction, and decreased in line with the postmortem interval in the following period [19]. In our study, when evaluated as months, although the Fe element increased in 1 month, it was observed that it decreased in the 3rd month, similar to this study. However, it was determined that the Fe element increased significantly in the 12-month period.

In the literature review, no study was found for estimating the time of death in any tissue for the Pb and Si elements. In our study, it was determined that Pb and Si elements increased within months in the postmortem period. The Pb element may differ between the genders, the levels of this element may change as age progresses, and it may be affected by external factors such as environmental pollution [18,19]. Since age and gender equality could not be fully achieved in our study, Pb element measurements may have been affected by this situation. Although all teeth were collected from patients who applied from the same city center, dental Pb concentrations may have been affected depending on the environmental pollution level of the place where the people lived in the past. Since the element Si is a substance found in glass materials, this situation should also be taken into account when evaluating the Si results. Although Pb and Si elements seem to be useful for estimating the time of death, more comprehensive studies are needed.

In a study in which PMI was estimated in pig bones, it was stated that the Mg element showed a fluctuating change in the bone with decay, but did not show a significant increase or decrease. Similarly, in our study, although the change in Mg element within months was statistically significant, it was determined that it fluctuated between groups. In the same study, it was reported that the Ca element fluctuates in the postmortem period and is not an appropriate marker for estimating the time of death [18]. The essential element found at the highest level in the tooth structure is the element Ca [20]. In our study, no statistical significance was found for the Ca element, and fluctuations were observed between the groups. This may have been caused by the presence of Ca in the hard cast plaster we used while creating the chin model and the fact that Ca is an element that can be affected by personal characteristics and dietary habits.

It was noted that the graphs of the Ca element and P element and the graphs of the Cd element and Zn element were in similar formations. The concentration changes of P, Zn, and Cd elements in the post-mortem period did not give statistically significant results and their graphics showed fluctuations. Although the Mn element concentration was not statistically significant, it showed a graphic decrease between the groups except for the 6th-month group. In the literature review, we could not find a study for the Mn element to predict the time of death in any tissue. In our study, we think that the decreasing trend in the Mn element within months can be a guide in determining the time of death and more studies are needed.

Trace elements such as Na, Mg, Mn, Co, Zn, Pb, and Si may have changed depending on the natural decay process of the tooth, as well as diagenesis, that is, contamination from the environment. Elements in the content of rainwater and snow water may have affected the element level in the tooth decay process. Fe, Ca, Mg from the soil, and Fe element from the cage may have passed. There may have been a transition of Ca from the jaw-like model. The biggest difficulty in working on tooth element levels is the uncertainty of the initial value and the possibility of being affected by environmental effects.

Tooth element levels can be affected by many factors such as personal, environmental, and dietary characteristics. One of the limitations of our study is that the amount of dental elements varies from person to person. Tooth element levels can vary depending on age and gender. The inability to provide age and gender equality between groups is another limitation of our study.

Studies are reporting that the Pb element is higher in men than in women [20]. It has been reported that the Co element increases with age [21]. However, in another study, it was reported that the Co element first increased with age and then decreased. It has been reported that Pb and Mg element levels increase with age, regardless of gender [20]. These studies show that; since tooth element levels are affected by age and gender, it would be more accurate to do studies that indicate ranges in element studies.

Conclusion

It is expected that the markers that can be used to predict the time of death will draw a stable and regular increase or decrease curve. In our study, we evaluated the increases and decreases in the postmortem amounts of 11 elements in human dental tissue by months and investigated whether the element concentration changes could contribute to the estimation of the time of death. We obtained significant results in the concentration changes in the post-mortem period for Na, Mg, Fe, Co, Pb, and Si elements. We created two equations for Co and Na elements that are statistically significant and have high predictive performance. With these equations, we think that we can make a great contribution to the subject of estimation of time of death, which is not clear yet. The time change of the elements in the postmortem dental tissue is promising as a new method that can be used in postmortem interval estimation. Postmortem changes in human dental elements should be studied with larger sample groups, different decay environments, longer time, and more element analysis and should be compared with other postmortem interval estimation methods.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

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Ethical approval

On 25.06.2020, approval numbered 83116987-441 was received from the Tokat Gaziosmanpasa University Faculty of Medicine Dean's Clinical Research Ethics Committee, stating that there was no ethical or scientific objection to our study.

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