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The comparison of DNA extraction techniques in human bone and tooth samples exposed to high heat

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Abstract
In the identification of forensic cases, it is critical to obtain information to identify the victim's identity from burnt bone and tooth remains. When bone and tooth are burned, both their physical and chemical properties change significantly, which prevents anthropological evaluations and successful DNA profiling for identification purposes. Heat causes difficulties in the identification techniques in which bones and teeth are used, depending on the degree of heat exposure. For this reason, with the changes occurring in bone and tooth during combustion, it is necessary to evaluate the results of observation and analysis, and to be used the information obtained in a certain order. It is very difficult to extract DNA from the samples obtained from fire, explosion, motor vehicle and aircraft accidents and other traumatic events by traditional methods. The only biological material available to determine the typing and the identification of missing persons or unknown remains in different situations, such as mass fatality events, wars or socio-political events, is often only human remains. Effective DNA extraction procedures are critical steps in successful DNA analysis of skeletal remains. Due to variations in DNA and heterogeneity within the bone, unfortunately, the only reliable method for obtaining DNA from overly degraded specimens such as burnt bones and teeth in the literature, is still not available. In the study, present extraction methods are compared and as a result it was suggested that phenol-chloroform and Qiagen DNA Mini Kit could be used in routine with a newly developed modified procedure in order to increase efficiency and efficiency in forensic genetic identification.

Keywords: Forensic genetics, DNA extraction, fire, degraded bone, tooth

Introduction

It is a known fact that the bone and tooth tissues are resistant to environmental conditions such as high heat, humidity and microorganism activation, and they retain their structural properties for a long time even if the corpse is decayed. However, because they reveal a number of personal characteristics of the victim, these tissues provide important information in forensic sciences [1-3].

Particularly, the tooth tissue is protected against the negative effects of the external environment by the surrounding epithelium, ligament, muscle and bone tissues. First of all pulp, and then cementum, dentin and periodontal ligament are rich for DNA analysis in terms of Short Tandem Repeats known as STR. Bones and teeth, even after many years of putrefaction, are the best preserved tissues of DNA compared to other tissues [4-6].

Bones and teeth, which are important evidence for genetic identification, may be exposed to different environmental conditions. In cases, where the integrity of the body has been severely disrupted by fire, there are many studies in the literature regarding the detection of a profile by extracting the DNA for the purpose of identification from burnt bones and teeth remnants. However, there is no comprehensive study to determine the most appropriate method for feasibility and other factors by comparing the available DNA extraction methods in tooth and bone tissues exposed to high and ultra-high heat [7-13]. On the basis of this information, there can be forensic cases that only burned and severely degraded bone and dental specimens in the crime scene.

Explosive mass death incidents, suicide attempts, accidents by car or aircraft, house fires or fire that high degree heat arises by

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intentionally to conceal of a crime, for the purpose of DNA analysis, hard tissues such as bones and teeth remain in the crime scene. DNA extracted from the burned bone fragments and teeth may be extremely degraded, which makes it difficult and even impossible to make amplification of genetic markers. In addition, burned bones and teeth are also very vulnerable to external DNA contamination.

When a body burns, the skin tissue partially or completely burns or disappears completely. Rupture and decay are frequently seen in the muscle tissue that is exposed to heat. In the same way, the soft tissue in the face also burns and the skull may be exposed. Therefore, the person may not be identified visually. Fingerprint analysis may not be possible because the hands and feet are also burned. Antemortem dental records and fingerprints may not be available. In this case, only the genetic analysis option remains for the identification of the residues.

In the study, it is aimed to compare the different methods of DNA extraction in bones exposed to different degrees of heat; well-preserved, half burnt, black burnt, blue-gray burnt and blue-gray-white burnt bones to create new modified extraction method. DNA extraction and the successful PCR amplification are particularly difficult in burnt bones and teeth. This is vital important for the disclosure of the crime, yet it is more difficult because of the existing methods are inadequate and unsatisfying. Most of the methods are complicated and time consuming, and no method has reached the level that can be used routinely. Bone extraction protocols performed in forensic laboratories are limited in cases where bones are exposed to environmental conditions over a long period of time that they cannot give reproducible results [14]. Since DNA extraction is still difficult, some methods are complicated and time-consuming, yet any method does not reach an acceptable level of routine use, we aimed to determine the most appropriate method among the available methods and to develop a better method with various modifications. According to all these, the aim of this study is to create a fire simulation environment that bone and tooth tissues exposed to low, medium, high, ultra and high heat, compare the current DNA extraction methods in the literature and ultimately develop a better method that can be used routinely.

Material and Methods

In the study, a total of 50 specimens were used, consisting of 25 bone and 25 tooth samples taken from 22 women and 28 men aged between 13 and 71 years. The samples were collected with the permission of the Dean of Istanbul University Cerrahpaşa Medical Faculty Clinical Research Ethics Committee of 26 March 2014, numbered 83045809 / 604-02-7830. DNA extraction and quantification of all collected samples were performed in Forensic Molecular Genetics Student Laboratory of Istanbul University-Cerrahpaşa Institute of Forensic Medicine.

Collection of bone and tooth samples

Twenty-five bone samples; femoral head, femoral diaphyseal parts, tibia medial and lateral condyle fragments and diaphysis pieces, ulna shaft parts, laminectomy and vertebra parts excised in hernia operations, and bone tumor operations excised from the vertebral bodies and costa bones are collected from Bakirkoy Dr. Sadi Konuk Training and Research Hospital Department of Orthopedics and Traumatology, Istanbul Training and Research Hospital Department of Orthopedics and Traumatology and Department of Neurosurgery and Cerrahpasa Medical Faculty Department of Orthopedics and Traumatology and Department of Neurosurgery.Twenty-five dental specimens consist of primary teeth, incisors, premolars and molars which were collected from Okmeydanı Oral and Dental Health Hospital.

Early processing

Following collection of the samples, each bone and tooth sample was first sterilized with 10% bleach prior to the extraction step. After that, mechanical cleaning with brush, surgical scissors, scalpel and bistoury was performed, and all visible soft tissue were removed on the hard tissue. All samples were subjected to sanding and allowed to dry on a hotplate for five days. 25 bone samples were divided into small pieces with Planmed Bone Crusher and Dremel Multipro device. The tooth samples were already small and did not need to be subjected to shredding due to their natural hardness. Subsequently, all samples' surfaces were sterilized with 0.5% sodium hypochlorite, washed with distilled water for five minutes, allowed to dry at room temperature and subjected to UV-C irradiation for one hour. The quality standards and recommendations of the ISFG DNA Commission were taken into account to avoid contamination.

Exposure of samples to different degrees of heat

Bone and tooth samples were exposed to heat between 50°C-1000°C; high, very high and ultra-high heat, starting from 50°C, followed by 10°C increments followed by a 50°C increments and in the last 100°C in the ash furnace. When selecting the heat range, especially in the case of fire in the house or car, the temperature increased to 800-1000°C take into account. Bone and tooth samples exposed to 7 different period (10, 15, 20, 30, 40, 50, 60 minutes) and 25 different temperature (50, 60, 70, 80, 90, 100, 110, 120, 130, 140 , 150, 160, 170, 180, 190, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 °C) in the ash furnace, and a fire scene simulation were performed. Bone and tooth samples were exposed to heat in the ash furnace for 60 minutes at 50 °C, 50 minutes at 60 °C, 40 minutes at 70 °C, 30 minutes at 80 °C, 30 minutes at 90 °C, 30 minutes at 100 °C, 30 minutes at 110 C, 30 minutes at 110 °C, and 30 minutes at 120 ° C, 30 minutes at 130 °C, 30 minutes at 140 °C, 30 minutes at 150 ° C, 30 minutes at 160 °C, 30 minutes at 170 °C, 30 minutes at 180 °C, 30 minutes at 190 °C, 20 minutes at 200 °C, 20 minutes at 250 °C, 15 minutes at 300 °C, 15 minutes at 400 °C, 10 minutes at 500 °C, 10 minutes at 600 °C, 10 minutes at 700 °C, 10 minutes at 800 °C, 10 minutes at 900 °C, 10 minutes at 1000 °C (Figure 1 and Figure 2).

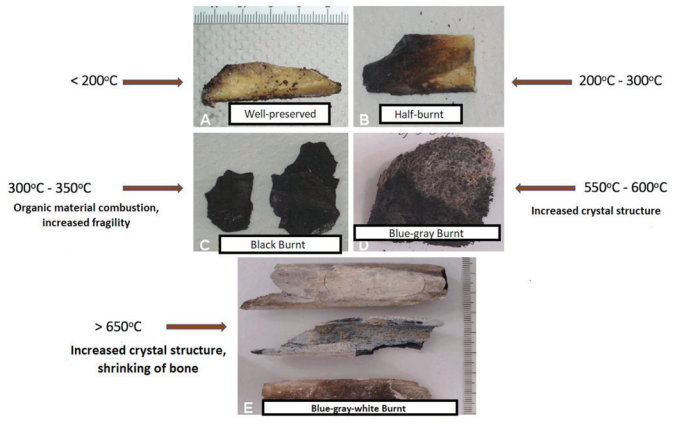

Figure 1. Color changes in bones exposed to high heat

Figure 2. Color change in teeth exposed to high heat

Trituration of Samples and Demineralization-Decalcification Process

After burning, the samples were triturated with Dentreal Bone Grinder Bone Mill and Retsch Cryomill Ultra Centrifugal Bone Grinder. As known, the inorganic section constitutes 70% of the bone and that contains calcium hydroxyapatite crystals. Ca+ 2 ions, whose main role is to participate in bone mineralization, forms large areas of mineralization within the bone tissue. These regions are physical barrier against extraction reagents which make it difficult to reach cells containing DNA. In order to reach the DNA in the most efficient way, Ca+ 2 ions should be removed from the material. For structurally rigid DNA sources, such as bones and teeth, most of today's extraction protocols are based on the principle of incubation of the powdered material in extraction buffer in a high volume of ethylene diamine tetra-acetic acid followed by collection of the supernatant. In the literature, there are differences between DNA extraction methods for burned bone samples. Most publications argue that the powdered bone should be incubated in a lysis buffer, then supernatant should be discarded and rest material should be studied. Some publications argue that a complete demineralization process should be performed to ensure that the bone powder is fully physically dissociated and that all DNA can be obtained. In accordance with this information, for each gram of pulverized samples, 1 g of tooth and 3 g of bone were subjected to incubation overnight in a 56ºC water bath for decalcification in 1.5 mL of 0.5 M EDTA. This process was repeated for two more nights with replacement of the solution. The samples were then centrifuged and the upper rest supernatant was discarded, and the remaining decalcified pellet was subjected to the application of extraction methods.

DNA extraction

The following 10 different procedures were used for extraction purposes:

1) Phenol-Chloroform Organic Extraction Method
2) CTAB + Isoamyl Alcohol-Chloroform Organic Extraction Method
3) Sodium Chloride-Sodium Acetate Extraction Method
4) Silica Extraction Method
5) Chelex® 100 Inorganic Extraction Method
6) QIAamp® DNA Mini Kit Inorganic Silica Based Extraction Method
7) QIAamp® DNA Mini Kit + Phenol-Chloroform Modified Compound Method
8) Qiaquick Based Inorganic DNA Extraction Method (Bosnian Method)
9) Invisorb Spin Forensic Kit Inorganic Based Extraction Method
10) DNA IQ System Inorganic Based Extraction Method

Among the 10 different methods studied, the maximum amount of DNA obtained is the QIAamp® DNA Mini kit + Phenol-Chloroform Modified Compound Method. The procedure of this method is as follows: The entire decalcified pellet was placed in a 1.5 pell microcentrifuge tube. Over 180 µl of ATL buffer, 20 µl of proteinase K were added and vortexed. It was incubated at 56ºC for one hour. 200 µl of AL buffer was added and vortexed. Incubated at 70ºC for 10 min. 200 µl of 96-100% purity ethanol was added and vortexed. The resulting mixture was transferred to the QIAamp® mini spin column and centrifuged at 8000 rpm for 1 min. 500 µl AW1 buffer was added and centrifuged at 8000 rpm for one min. 500 µl of AW2 buffer was added and centrifuged at 14000 rpm for one minute. The QIAamp® mini spin column was placed in a sterile 1.5 µl microcentrifuge tube. 50 µl of AE buffer was added. Incubated at room temperature for 1-2 min and centrifuged at 8000 rpm for one minute. After centrifugation, over the 50 µl pellet, pre-prepared 500 µl of Phenol: Chloroform: Isoamyl alcohol buffer at 25: 24: 1 ratios was added and centrifuged at 3000 rpm for 2 min (Discarding supernatant, this process was repeated on pellet 3 times). 250 µl of 3M sodium acetate and 2.5 ml of cold ethanol were added to the pellet. Incubated at -20ºC for 12 hours. Centrifuge at 15000 rpm for 30 min and rest supernatant discarded. Tris-EDTA buffer was added onto the pellet. Incubated at 56ºC for 20 minutes (wait until the pellet dissolves) and store at -20ºC until DNA measurement.

DNA Determination

The amounts of DNA isolated after each extraction procedure were performed on the Qubit® Fluorometer-Invitrogen ™ device using the Quant-iT ™ dsDNA HS Assay Kit (Invitrogen ™).

Purification Process

After DNA isolation, the isolates in which the maximum amount of DNA were obtained by our modified method, were purified by Amicon Ultra Centrifugal Filter (Merck Millipore). This process is as follows: Each isolate was put into separate Amicon Ultra filters. 200 µl of 0.4M NaOH was added and centrifuged at 4000 rpm for 15 min. 200 µl of 10 nM tris (pH 7.5) was added and centrifuged at 4000 rpm for 15 min. A new sterile tube was placed by replacing the filter's waste tube. 30 µl of TE buffer was added to the filter and centrifuged at 5000 rpm for 15 min and DNA quantification was performed.

Results

In the study, different bones such as femur, humerus, tibia, costa, premolars and molars, incisors and canine teeth were used. In order to verify which types of bone give the utmost and highest quality DNA, we have developed a semi-standardized protocol for

DNA extraction from the bones by evaluating the different temperature at which they were exposed. In addition to the conventional phenol-chloroform organic method for DNA extraction, the efficacy and productivity of DNA extraction from bone and tooth samples were evaluated in various commercial kits including QIAamp® DNA Mini kit (Qiagen, Hilden, Germany), QIAamp® DNA Investigator kit (Qiagen, Hilden, Germany), DNA IQ ™ System (Promega, Milan, Italy), Genorise Bone DNA Extraction Kit (Genorise Scientific, PA, USA), HiPurATM Bone DNA Extraction Kit (HiMedia, India). In addition, in our experimental studies, we performed a modified protocol using phenol-chloroform and removing the DNA from proteins and waste material in order to better purify the samples and compared them with other extraction methods. In the modified protocol, we performed the new step by incubating different kit solutions in lysis buffer. The phenol-chloroform step was found to provide clearance of the samples by preventing the waste material from interacting with columns or magnetic beads in the burned samples.

Accordingly, the best results were obtained by the QIAamp® DNA Mini kit + Phenol-Chloroform Modified Method, which is the 7th procedure. DNA concentrations obtained by the modified procedure are shown in Table 1 as before purification and in Table 2 as after purification. According to the data obtained, the lowest amount of DNA in dental samples was 0.02 ng / µl and the highest was 5.67 ng / µl. The amounts of DNA obtained in the bone samples were 0.041 ng / µl and 13.48 ng / µl respectively.

Table 1. Quantity of DNA of Bone and Dental Specimens According to Results of Qubit Fluorometer obtained from QIAamp DNA Mini Kit + Phenol-Chloroform Modified Method

Different Temperature Levels Exposed in Ash Furnace	DNA Quantities obtained from Bone Samples (ng/µL)	DNA Quantities obtained from Tooth Samples (ng/µL)
50°C…...60 minute	4.53	2.38
60°C…...50 minute	6.02	1.07
70°C…...40 minute	7.12	1.02
80°C…...30 minute	5.61	0.98
90°C…...30 minute	3.23	0.73
100°C….30 minute	2.91	0.55
110°C….30 minute	5.17	0.48
120°C….30 minute	3.89	0.92
130°C….30 minute	2.53	0.35
140°C….30 minute	1.95	0.87
150°C….30 minute	1.13	0.49
160°C….30 minute	2.78	0.59
170°C….30 minute	1.17	0.28
180°C….30 minute	1.05	0.11
190°C….30 minute	0.32	0.24
200°C….20 minute	0.08	0.32
250°C….20 minute	0.07	0.19
300°C….15 minute	0.04	0.13
400°C….15 minute	-	-
500°C….10 minute	-	-
600°C….10 minute	-	-
700°C….10 minute	-	-
800°C….10 minute	-	-
900°C….10 minute	-	-
1000°C...10 minute	-	-

Table 2. Quantity of DNA of Bone and Dental Specimens According to Results of Qubit Fluorometre Obtained from after Purification

Different Temperature Levels Exposed in Ash Furnace	DNA Quantities obtained from Bone Samples (ng/µL)	DNA Quantities obtained from Tooth Samples (ng/µL)
50°C….60 minute	11.68	5.67
60°C….50 minute	13.48	2.95
70°C…..40 minute	12.30	2.71
80°C….30 minute	12.32	2.55
90°C….30 minute	10.79	1.65
100°C….30 minute	9.99	1.47
110°C….30 minute	9.72	1.42
120°C….30 minute	7.56	1.29
130°C….30 minute	7.27	1.09
140°C….30 minute	6.15	1.05
150°C….30 minute	6.01	1.02
160°C….30 minute	5.70	1.011
170°C….30 minute	4.13	0.90
180°C….30 minute	3.32	0.72
190°C….30 minute	1.17	0.68
200°C…..20 minute	0.29	0.53
250°C….20 minute	0.21	0.31
300°C….15 minute	0.21	0.29
400°C….15 minute	0.082	0.11
500°C….10 minute	0.041	0.02
600°C….10 minute	-	-
700°C….10 minute	-	-
800°C….10 minute	-	-
900°C….10 minute	-	-
1000°C....10 minute	-	-

Discussion

Due to the reduced quality and quantity of DNA extraction from bones and teeth that have been burned, soaked in the water or buried for a long time has always been a difficult process in the forensic sciences, and it is still challenging today.

There are different types of events in which burned bones and teeth are delivered to judicial units for identification purposes [9,11,15,16]. These events are seen in particular as vehicle accidents [9,10], mass deaths [9] and domestic fires [11,17]. In addition to such accident incidents, it is possible to encounter cases where the victim was burned in order to cloak a crime [10,11,18-20]. Heat-induced fragmentation and disintegration in burned bones and teeth make identification and anthropological investigations extremely difficult. In addition, in highly burned bones and teeth DNA analysis is also extremely difficult.

When the morphological tests fail due to deformation and fragmentation, DNA profiling is the only option in the identification of highly burned bone and tooth samples. However, incidents of burned bones and teeth and published studies reveal that this practice is essentially difficult. Because the organic matrix in which the DNA exists is partly lost in the early stages of the combustion process.

Several studies have suggested that DNA typing can be used to investigate burned bones [21-25]. In a study conducted by Cattaneo et al., which is one of the pioneer studies in this field, replication of

the human mitochondrial DNA is evaluated in the bones obtained from experimentally incinerated human compact bones (for 20 minutes, 800°C-1200°C) and from the burned and carbonized bones and actual forensic events [25]. They could not obtain reproducible DNA in any of these burned bones and concluded that DNA typing could not be successful in burned bones. As the heat reached a temperature of 800°C to 1200°C in real fire events, the researchers chose this temperature range. For this reason, we also considered to expose the samples to the temperatures in this range. In other studies, more detailed investigations have been performed by varying the temperatures and time of burning and evaluating the amplification product length [21,23,24]. Especially in these studies, bovine compact bones were used and temperature increases ranging from 10°C to 50°C were applied and bones were burned at a temperature of maximum 250°C. Afterwards, the DNA extracted and polymerase chain reaction (PCR) targeting nuclear or mitochondrial DNA was performed. It is founded that DNA is non-reproducible in the bones burned at 200°C for 2 hours and at 210°C for 45 minutes [21]. The relationship between target length and PCR success was also evaluated by these studies. Accordingly, it is concluded that the shorter the PCR target length, the higher the burning temperatures. Tsuchimochi et al. have done a similar study using teeth [26]. In the heated and burned teeth, they tried to amplify the DNA Y- choromosome extracted from the dental pulp, yet they found that the multiplication was not amplified for teeth burned at 400°C for two minutes. Accordingly, it can be said that the temperature of the threshold value for the success of DNA replication in the teeth is higher than that of the bones. Because the dental pulp is partially protected by the surrounding enamel and dentin. However, even at an average temperature of 400°C over a short period of time for two minutes, the DNA is degraded yet it cannot be reproduced, indicating that the DNA is not sufficiently resistant to heat even in this part of the tooth. However, in our study, even though it was very low, at 400°C by the modified extraction method, 0.1 ng/µl DNA was obtained. Particularly when the amount of DNA of 0.5 ng/ µl and below is considered to be highly deformed due to high temperatures although it is taken into consideration that dental specimens are not exposed to the canal treatment, it is presumed that they can be decayed and amalgam filled.

In the study of Schwark et al., it was observed that DNA amplification results obtained from burned bones of real forensic events were better [22]. They classified the degree of combustion according to bone color and were able to successfully amplify DNA at the highest degree of combustion known as blue-gray-white. This discoloration indicates that the combustion temperature is 500°C and above, and contradicts the results of other studies. However, there is a possible logical explanation of this contradiction. Schwark et al. used a commercially available nuclear DNA typing kit with a high PCR cycle in order to increase the sensitivity, as well as a multiplex PCR system, which was optimized for the reproduction of extremely decayed DNA by shortening the target length for PCR [27,28]. Similarly, in our study, a DNA of 0.041 ng/µl was obtained in the burned bone samples at 500°C. In fact, this amount shows that the used and modified extraction method works well. Accordingly, this highly sensitive typing system seems to work well in extremely degraded DNA. However, in a bone sample obtained in the event of fire, different regions may have been exposed to different temperatures due to different and uneven distribution of flames, and the selected bone area for the extraction may be lower than that of the primary region used for the classification of the combustion color. Therefore, in order to clarify this issue, it is necessary to study bone samples that are burned under more stringent temperature controls.

Bone and tooth tissues are resistant to environmental conditions such as high temperature, humidity and microorganism activation. These tissues provide important information in identification because they protect their structural properties for a long time in the decay and reveal some personal characteristics of the victim [1-3].

In our study, necessary precautions have been taken in all laboratory stages in order to prevent contamination by implementing the standards. In addition, in the process of collecting bone specimens from operating room and dental samples from clinics, possible contaminants were tried to be eliminated. When in the laboratory stage, the emphasis was on the removal of the samples from possible external contaminants as much as possible prior to the DNA extraction stage. Although some studies emphasize that in the cleaning stage the use of sodium hypochlorite (NaOCl) may have some disadvantages, NaOCl has been used during the pre-process in our study because of the use of NaOCl in most studies. In our study, samples were kept under UV light for one hour as suggested by Ricaut et al. in a 2005 study [29].

In the study of Tilatta et al., they crushed the dental specimens, pulverized the outer surfaces of these tooth samples, using the endodontic drilling technique pulpas were removed, and compared the DNA amount and profiles [30]. In these powdered tooth samples, they obtained full profile (9%) in 32 specimens, and in 24 samples (75%) no profile was obtained. Using the endodontic drilling technique, pulpas were removed in the series of teeth from 24 samples of the 32 samples and full profile was achieved with 75% success. In the study of Alakoç et al., using "orthrograte entrance" method, amplification products were obtained in 58 of the 72 ancient specimens, namely 80.1% of the nucleus DNA amplification products [31]. These researchers did not harm the morphological structures of ancient tooth samples by the method they used. According to these two studies, it has been shown that the technique applied to the tooth and the environmental conditions to which the tooth samples are exposed is very important in the DNA extraction stage of the dental samples. In addition to these studies, when other studies in the literature are evaluated, it is predicted that the method used to powder the samples before the extraction process affects the success rate of the study [32]. However, endodontic drilling technique could not be applied in our study due to insufficiency of existing devices and instruments.

In addition, it has been assumed that with the preferred bone samples are particularly compact bones and that tooth samples are not endodontically filled, the contamination is minimized during collection, and that the most suitable method for DNA extraction from these samples is effective on the success rate of the study.

Each of the commercial DNA extraction kits used in our study gave good results during DNA extraction from the samples, and provided DNA to be obtained quickly and easily. Determination of the most suitable one among the methods of extraction is the basis of this study. Although Chelex extraction method is inexpensive,

simple, does not contain more stages and not risky in terms of health, pure DNA could not be obtained. The phenol chloroform isoamyl alcohol method of tooth and bone samples was carried out despite long duration, risk of contamination, carcinogenicity and risk of health of those who carried out the analysis. In addition, the phenol chloroform isoamyl alcohol method is combined with the QIAamp® DNA Mini Kit method, which is the most effective and successful method of DNA extraction.

According to our analysis results, the amount of DNA in the dental samples ranged from 0.02 to 5.67 ng / µl (Table 2). It was determined that the result of DNA measurement amount obtained in 6 (24%) of the total 25 burned tooth samples was below 0.05 ng / µl. The results of Silva et al.'s study in 2012 showed similarity to the results of our study [9]. In order to contribute the standardization of DNA extraction and analysis procedures, these researchers exposed tooth samples to high temperatures to create a burned human simulation and evaluated the DNA samples. As a result of their studies, exposure of samples directly to high temperatures such as 600, 800 and 1000°C did not reveal successful DNA amplification. Although the DNA concentration obtained was suitable for subsequent analysis, it was not possible to obtain PCR product from dental samples exposed to high temperatures.

In another study, three different extraction methods compared by exposing dental samples to temperatures of 200, 400, 500 and 600°C for 60 minutes. In half of the samples that exposed to temperatures of 200°C and 400°C, genomic DNA extraction was successful, while at higher temperatures he was able to achieve success in mtDNA extraction only in one of the methods [33].

The application of the DNA extraction protocol based on the use of phenol-chloroform and the QIAamp® DNA Mini-Kit modified extraction method for low amounts of fresh bone and tooth samples exposed to certain temperatures over certain periods of time yielded the expected good results. However, in our study it has been seen that a good purification process contribute greatly to increase the amount of DNA in DNA extraction.

Conclusion

The only biological material available for the identification and genealogy of missing persons or unknown remains in different situations, such as mass death incidents, wars or socio-political events, is often the only human remains. DNA extracted from the bone is often a low amount, due to chemical and physical damage caused by the internal and external properties of the bone, it is in different degradation stages. Effective DNA extraction procedures as well as proper DNA amplification are critical steps in successful DNA analysis of skeletal residues. Due to variations in the heterogeneity of the DNA in the bone, the only reliable method of obtaining DNA from extremely degraded samples, as in burned bones, is unfortunately still not available. In our study, we aimed to compare the different DNA extraction methods in well-preserved, half-burnt, black burnt, blue-gray burnt and blue-gray-white burnt bones exposed to different degrees of heat, and developed a new modified method. For this purpose, a new method of extraction has been developed and proposed for routine use in forensic sciences..

Conflict of interest
The authors declare that there are no conflicts of interest.

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Ethical approval
The study protocol has approved from local ethic committee.

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