

Assessment of the most reliable sites in mandibular bone for the best deoxyribonucleic acid yield for expeditive human identification in forensics

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Abstract

Background: In recent years, the techniques used to identify human remains post accidents, trauma or in case of criminal investigation have been expanded, improved and rendered more complex by the emergence of technologies based on deoxyribonucleic acid (DNA) analysis. In the head and neck area, tooth has been proven to be the best quantitative source for DNA but in certain cases where the mandible specimen is edentulous or the tooth is extensively destroyed with caries, large dental restorations, mobile, or if they show any perimortem or postmortem fractures, sampling of such tooth specimen is usually avoided. In such situations, bone is considered the next best site for DNA analysis. Mandible being the largest, strongest and dense cortical bone is the most prominent facial bone that can be easily disarticulated. It can be analyzed for the best short tandem repeat (STR) segment qualitative amplification using polymerase chain reaction (PCR) technique for forensic analysis which can be used for gender and age determination.

Aim: The aim of this study is to determine the best site for optimum quantitative and qualitative yield of DNA for amplification using specific and standard STR segment by conventional PCR technique.

Methodology: Fifteen mandibular samples exposed to different environmental conditions were collected. Bone pieces of 1 cm × 1 cm were cut from each mandible from three sites, i.e., the ramus, angle and body, wherein the genomic DNA was isolated and was subjected to PCR using restricted number of 25 cycles.

Results: The STR segment D3S1358 from clone RP11-438F9 used for the study showed very good amplification in restricted number of PCR cycles in the ramus region with number of repeats in every 15th genomic region.

Conclusion: This study highlights the use of mandibular bone for the expeditive human identification. As per the study, the ramus of the mandible gave high quantitative and qualitative yield of DNA with thick amplification band of the STR segment as compared to the body and angle of the mandible. Thus ramus of the mandible can be preferred over other sites for molecular forensic investigations.

Keywords: Forensic odontology, genomic deoxyribonucleic acid, mandible bone, polymerase chain reaction, ramus, short tandem repeat segment

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INTRODUCTION

Forensic identification of individuals involves multidisciplinary team efforts relying on positive identification methodologies as well as presumptive or exclusionary methodologies.^[1] It involves the cooperation and coordination of law enforcement officials, forensic pathologists, forensic odontologists and other specialists as deemed necessary.

Since the late 1890s, forensic dentistry has gradually established itself as important, often indispensable, in medicolegal cases, in particular for identification of the dead. Dental hard tissues are extremely resistant to fire and are usually the only remains after cremation and an extended period of burial.^[2]

The cells within the hard tissues (bones and teeth) are embedded within a dense biomineral matrix and are largely protected from the effects of putrefaction and decomposition. The hard tissues can therefore act as a source of deoxyribonucleic acid (DNA).^[3]

DNA preserved in bones can vary greatly from one cadaver to the next due to environmental conditions, leaving the remaining DNA limited in quantity or degraded.^[4] However, the teeth and jaws are very resistant to extreme conditions and become valuable identification tools in situations irrespective of the environmental conditions.^[5]

As there is very limited data on the application of the bones of head and neck regions to forensic analysis, the study involves the use of mandibular jaw bone as best site for DNA analysis in the head and neck region. It is the most commonly found bone in human remains and sometimes the only bone available. The changes in mandible even help in estimating the age of a person.^[6]

Mandible bone can be substantiated to be a rich and reliable source for DNA extraction as follows:

- It is the most prominent and dense cortical bone facial bone^[7]
- Can be easily disarticulated
- A macroskeletal unit with higher surface area than any other facial bone and much higher than any single tooth which till date is considered to have a good DNA yield^[8]
- It has a rich vascular supply from inferior alveolar arteries and nerve supply through divisions of trigeminal nerve; hence, any soft tissue specimen associated with the mandible would result in better DNA yield^[9]
- As the mandible ossifies both through endochondral and intramembranous ossification which starts from

the Meckel's cartilage,^[9] it is estimated to have ample bone marrow content and various other cells which can be taken as a basis for the qualitative analysis of DNA yielding sites which might aid in more reliable and faster identification in forensics.

Aims and objectives

1. To assess if mandibular bone can yield qualitative and quantitative DNA for amplification
2. To assess multiple sites (3 sites, i.e., ramus, body and angle) of mandible and determine the best site for optimum qualitative yield of DNA for amplification
3. To determine the best mandibular site which gives specific and standard short tandem repeat (STR) segment using conventional polymerase chain reaction (PCR) in restricted cycles
4. To establish that jaw bone can be preferably used as prime site in case of remains which have been subjected to extreme conditions for expeditive human identification.

METHODOLOGY

Sample collection

A total number of 15 mandibular specimens were collected from various oral pathology and forensic science departments.

Three sites from each mandible, i.e., ramus, body and angle were marked; 1 cm³ of bone was removed from each site [Figure 1]. Considering three sites from each mandible, a sum total of 45 samples were collected (15 × 3 = 45 samples).

Case details on the approximate age and sex of the specimens along with the source, i.e., how the specimen

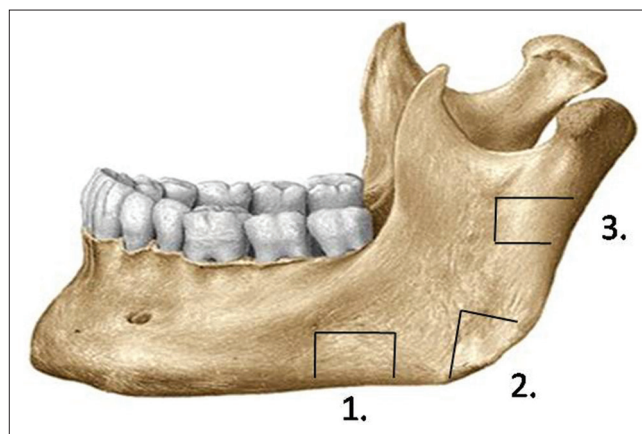


Figure 1: Illustration of the mandible bone with marked sites for deoxyribonucleic acid retrieval. 1. Body 2. Angle 3. Ramus

was retrieved which included either a pathology, trauma, skeletal remains, or unknown was noted.

Procedure

The three sites, i.e., ramus, angle and body were marked on the mandibular bone of the same side which was free of any pathology. Approximately 1 cm³ of the bone was removed from each site using bone cutting burs [Figure 2a-c].

Bone samples from the desired site were stored in 10% neutral buffered formalin solution for approximately 24 h. The samples were transferred to DNA extraction buffer for further processing. Genomic DNA isolation protocol was followed, and quantitative yield of DNA was analyzed using spectrophotometer. For qualitative analysis, restricted PCR cycles were performed. The amplified samples were visualized using 1% agarose gel using the reference ladder [Figure 3].

All the samples (45) were checked for the amplification of STR segment D8S1179 [clone RP11-438F9] on chromosome 13 under restricted PCR cycles (25 cycles) using Thermal Cycler ABI2720.

The reference 1 kb ladder that contains 10 DNA fragments was used to analyze the amplification products.

Primers used for STR amplification shown in Table 1.

OBSERVATION AND RESULTS

All the previous studies used long femur bone and ribs in the whole human body for STR amplification which is still

considered as an important source in forensic identification; followed by teeth in head and neck area. In our study, 15 mandibular specimens were collected from various oral pathology and forensic science departments irrespective of the climatic conditions they were exposed. Forty-two percent of the samples collected were from the fresh dead bodies due to trauma or accident, 36% of the samples were hemimandibulectomy cases of pathology, 21% of the samples collected had no data pertaining to the etiology of the mandible removal and hence was categorized as unknown and 1% of the samples were the old skeletal remains which were sent to the forensic department for investigation. The age range of the known samples varied

Table 1: Primers used for polymerase chain reaction amplification

STR primers	Sequence
Forward primer	5'-GCNGATNACTNTTTTCNTACCAA-3'
Reverse primer	5'-AGNTGGNATATNGTGGGNCCTTNT-3'

STR: Short tandem repeat

Table 2: The quantity of deoxyribonucleic acid isolated using spectrophotometer values

Sample number	Concentration of DNA obtained		
	Ramus (ng/μl)	Body (ng/μl)	Angle (ng/μl)
Sample 1	50	25	25
Sample 2	100	50	50
Sample 3	50	25	25
Sample 4	50	25	25
Sample 5	100	50	50
Sample 6	100	25	50
Sample 7	100	50	100
Sample 8	50	25	50
Sample 9	100	50	100
Sample 10	100	50	50
Sample 11	25	25	25
Sample 12	50	25	50
Sample 13	50	25	25
Sample 14	100	50	25
Sample 15	100	25	100

DNA: Deoxyribonucleic acid

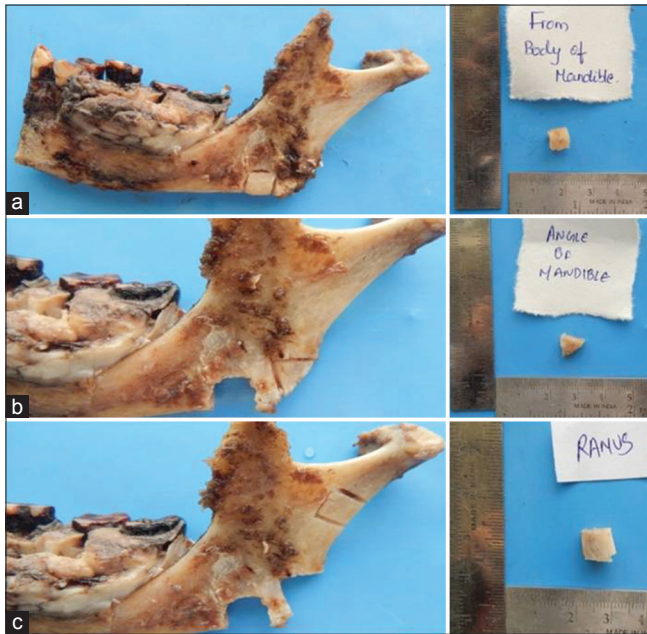


Figure 2: Left hemimandibulectomy specimen (a) body, (b) angle of mandible and (c) Ramus region marked with indentation

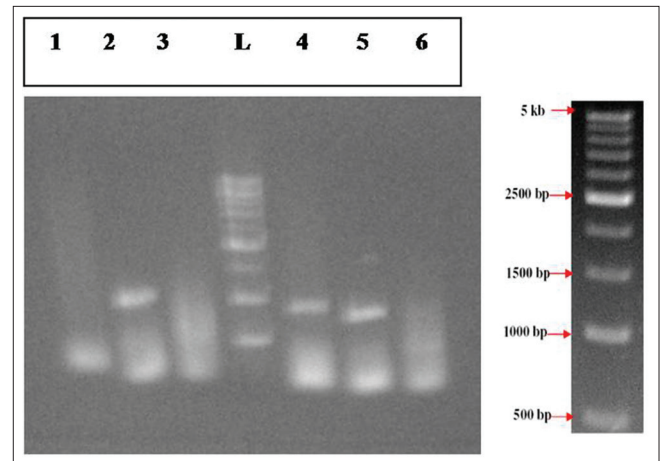


Figure 3: Genomic deoxyribonucleic acid isolated from the mandible sites on 1% agarose gel

between 8 and 50 years and period of retrieval varied from 1 month to 3 years. DNA was obtained from all the three sites of the mandible and the observations of the spectrophotometric analysis were done [Table 2].

For qualitative analysis, amelogenin gene was used initially for the amplification, which expressed maximum amplicon only in ramus region for Taq polymerase than chrome polymerase.

As the amelogenin gene did not show good amplification in body and angle region, STR segment DS1878 (clone RP11-438F9) on chromosome 13 was used for the subsequent samples. The results of the study have been formulated as follows:

As per the spectrophotometric gel band of all the 15 samples, genomic DNA was successfully retrieved from all the samples and quantified irrespective of the environmental conditions, age, time period of retrieval and the side from which the specimens were collected. Same amount of sample was used for genomic DNA isolation from all samples.

As quantified using spectrophotometric analysis approximately 20 ng to 100 ng of genomic DNA was recovered from each given sample where ramus gave the best quantitative yield in the range of 50–100 ng/ul compared to the other sites [Table 2].

In few samples, the ramus and angle yielded the same quantitative yield per microliter which can be attributed to the source from which the sample was collected or could be because of the wastage of the sample during the laboratory procedure. The next best site for the quantitative yield was angle of the mandible. The gel electrophoresis

reading on correlating with the base pair band thickness showed that all the sites were amplified for the DNA STR segment D3S1358 sequence from clone RP11 438F9 on chromosome 13. On comparing the band thickness and base pair spike, it was found to be thickest for the mandibular ramus region in all samples.

The total number of repeat sequence is 15.

Sequence of the repeats-GATAGATAG
ATAGATAGATAGATAGATAGATAGATAGATA
GATAGATAGATAGATAG ATAGATA

CDS:GCAGATGTACTGTTTTTCCTACCAA
TGACAGTCCCTGTGAACCTGTCCTTTACTGAC
TTTACAGAGTCCCTCTTTCTCCTTTCTGCCCCCT
AGGTGAGTAT ATCC TCAAGAATTTACACATCAC
TCTCTGTATTAGTCAGGATTCTCTAGAGGA
ATAGAAATAATATGATGTATAGATGATAGAT
AGATAGATAGATAGATAGATAGATAGATAGC
ACACGATCACAAGGTCCCACAATATACCATCT.

Statistical analysis

DNA retrieval for ramus is 100% whereas in case of body and angle of the mandible retrieval was evident in 93.3% of samples [Table 3, Figures 4 and 5].

Chi-square test was used to check the quantitative yield of DNA among the three sites and the difference was statistically significant at $P < 0.05$. In addition, intergroup comparison was done using Kruskal–Wallis test which is an extension of Mann–Whitney U-test [Table 4 and Figure 6].

Among the fifteen mandibular ramus samples which were tested for amplification was maximum for ramus region [Table 5 and Figure 7].

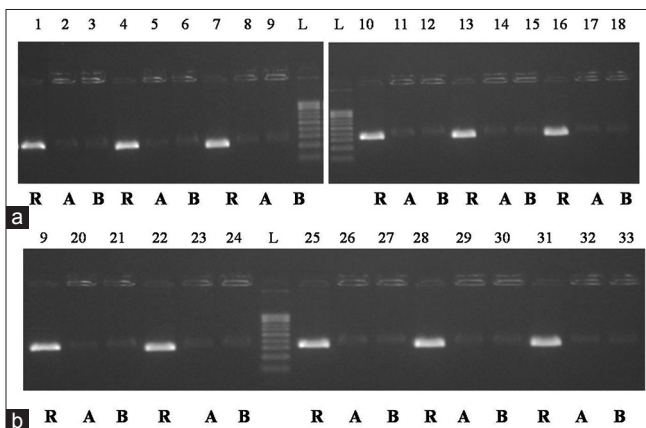


Figure 4: (a and b) Extraction of genomic deoxyribonucleic acid for the amplification of short tandem repeat sequence D3S1358 clone RP11-438F9 on chromosome 13 represented on 1% agarose gel. RAB refers to Ramus, angle and body of each mandible sample

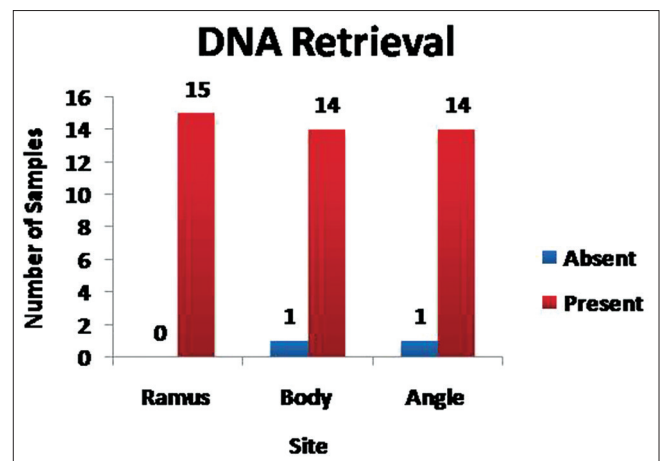


Figure 5: Frequency distribution of deoxyribonucleic acid retrieval

DISCUSSION

Human identification is one of the major fields of study and research in forensic science because it deals with the human body and aims at establishing human identity mainly when there is little remaining material to perform such identification (e.g., in fires, explosions, decomposing bodies, or skeletonized bodies).^[10] Proper identification of the dead is required for legal and humanitarian reasons which include settlement of property, facilitate remarriage of surviving spouse and allow cremation or burial of the body according to relevant religious and cultural customs.^[11]

In recent years, DNA evidence has become the gold standard of forensic testing and is an invaluable tool for the criminal justice community. The high credibility of DNA evidence in court stems from the fact that it uses a statistical approach based on population genetics and empirical testing, in contrast to other types of forensic evidence, such as ballistics, blood-spatter analysis and fiber analysis, which rely on expert judgment and have limited connection to established forensic science.^[12]

The advancement and application of forensic DNA analysis have greatly evolved making personal identification and paternity determination a lot more simpler as compared to the previous craniometric and morphometric techniques. The use of bones and human remains as sources for the detection of DNA polymorphism is a relatively recent advance in forensic identification. A common problem with this kind of analysis is the preservation of DNA.^[12,13]

Earlier studies have shown inconclusive results regarding what type of remains hold a better chance of yielding adequate amounts of good quality DNA. Some studies suggest that DNA is better preserved in bones (Kim *et al.*

2008, Hagelberg *et al.* 1991), others suggest that they get degraded along with the bone (Ginther *et al.* 1992, Rees and Cox. 2010, Alonso *et al.* 2001).

As DNA degradation is obviously a key factor in determining whether a DNA can be recovered, an alternate explanation for variable success rates may be that the observed results do not reflect on the quality of the DNA *per se*, but where and how it is preserved in the bone, and the efficiency of the different extraction methods used. During the past few years, ancient nuclear DNA studies from human remains of up to 8000 years have been described. Cortical and cranial bones, as well as teeth, were found to provide sufficiently preserved DNA for analysis. Molecular studies in the head and neck region have been limited to the teeth specimens.^[7,8,14-18]

This study applies molecular analysis of genomic DNA specifically for mandibular bone, whereas earlier studies in forensic science have mostly concentrated on the craniometric and morphometric analysis of mandibular bone.

STR segment D3S1358 clone RP11-438F9 on chromosome 13 was chosen for our study. All the previous studies exhibited very good amplification on the femur bone.^[8,19-25]

Similarly, in our study, conducted in the jaw bone samples, the STR chosen gave very good amplification for all the three sites in mandibular bone; however, the base pair band

Table 3: Frequency distribution of the deoxyribonucleic acid retrieval at the three sites

DNA retrieval	Group			Total
	Ramus (%)	Body (%)	Angle (%)	
Absent	0	1 (6.7)	1 (6.7)	2 (4.4)
Present	15 (100.0)	14 (93.3)	14 (93.3)	43 (95.6)
Total	15	15	15	45

Fisher's exact test, $P=1.00$ (NS). NS: Not significant, DNA: Deoxyribonucleic acid

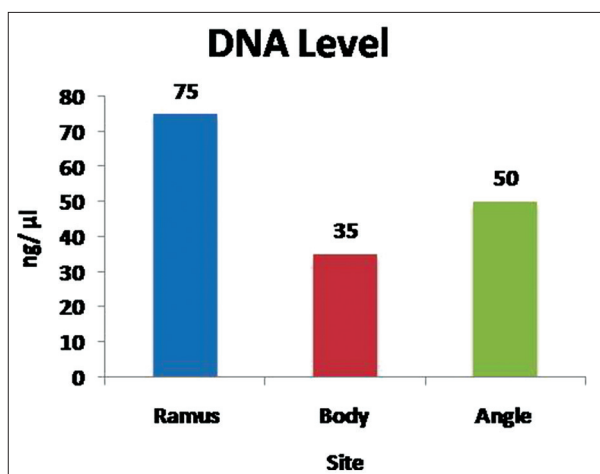


Figure 6: Mean distribution of the quantitative deoxyribonucleic acid yield at the three sites of the mandible

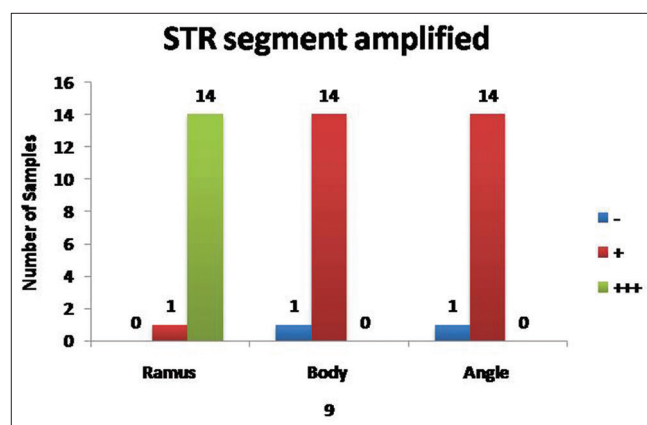


Figure 7: Frequency distribution of short tandem repeat segment amplification

Table 4: Descriptive statistics for the quantitative deoxyribonucleic acid yield from the three sites

Group	n	Mean (SD)	Median (Q1-Q3)	Range
Ramus	15	75 (28.35)	100 (50-100)	25-100
Body	15	35 (12.68)	25 (25-50)	25-50
Angle	15	50 (28.35)	50 (25-50)	25-100

SD: Standard deviation

Table 5: Frequency distribution of qualitative deoxyribo nucleic acid analysis

STR segment amplified	Group			Total
	Ramus (%)	Body (%)	Angle (%)	
No amplification	0	1 (6.7)	1 (6.7)	2 (4.4)
Slight amplification	1 (6.7)	14 (93.3)	14 (93.3)	29 (64.4)
Thick band of amplification	14 (93.3)	0	0	14 (31.1)
Total	15	15	15	45

Fisher's exact test, $P < 0.001$. STR: Short tandem repeat**Table 6: Qualitative analysis using short tandem repeat**

Sample number	Sites	Qualitative yield: Base pair spike	PCR cycles	STR segment (RP11-438F9) on chromosome 13	Comment
2-15	Ramus Body Angle		25		Ramus gave the best amplification

STR: Short tandem repeat, PCR: Polymerase chain reaction

thickness was evenly high in the ramus region. Hence, the ramus region has better quantitative yield as well as very good qualitative DNA amplification compared to the other sites [Tables 2, 6 and Figures 6, 7].

As amelogenin gene has been proved successfully on teeth for identification, it was tested in our study for amplification wherein only the ramus site showed amplification with no amplification in the body and angle region. Multiallelic polymorphic STR markers include an improved power of discrimination and the possibility of information regarding geographic origin which is now being extensively studied.^[26-29]

In our study, the site which gave the maximum amplification was determined using restricted PCR cycles (25 cycles) than conventional 35–40 cycles, so the yield can be determined in less time. Therefore, as per the study results, the ramus region gave high qualitative DNA base pair band in restricted PCR cycles.

This study clearly states that ramus region is the high yielding site for DNA identification; the possible reasons can be correlated with the development of this site of the mandible.

Considering the development of the mandible, ramus develops by a rapid spread of ossification backwards into the mesenchyme of the first branchial arch diverging away from Meckel's cartilage. The mandible ossifies both through intramembranous (in the symphyseal region) and

endochondral ossification (the ramus region). Later in the development, the accessory nuclei of cartilage make their appearance. These accessory nuclei possess no separate ossification centres but are invaded by the surrounding membrane bone and undergo absorption.^[9,30]

As endochondral ossification lead to the formation of dense corticated bone with good amount of cellularity due to chondroblasts and osteoblasts cells involved, this can be attributed to high quantitative and qualitative amplification of DNA from these site.^[31]

This type of ossification pattern is involved in natural growth and lengthening of bone, and bone apposition is seen in the ramus region of the mandible; therefore, making it more dense and corticated area of the jaw extending posteriorly to the lower body region.^[31]

The mandibular bone is the strongest and hardest bone which does not easily decompose after death.^[32-34] The mandible in the head and neck area being the most prominent bone is subjected to trauma more frequently in the facial skeleton, in case of any major accidents or disasters. In majority of cases, trauma occurs in the condyle region (36%), followed by body (21%), angle (20%) and symphysis (14%).^[30] The ramus region is usually spared, and hence, the site can be used as a very important source for investigation for DNA analysis. More than 90% of the jaw pathologies occur in the mandibular posterior region which involves the body and the angle region and the involvement of the ramus region occurs very late, and hence, the site is more available for analysis.

Considering the anatomy of ramus which is quadrilateral in shape and has two large surfaces and four borders offering a single muscle attachment on the lateral border to the masseter muscle, it is less cumbersome a task to harvest a bone from the site due the large surface area as compared to the femur bone which being the only thigh bone is long and slender with cylindrical body and offers attachment point for all the muscles that exert their force over the hip and knee joints, though both femur and mandible undergo similar ossification pattern.^[31]

Therefore, in all the samples of our study, ramus region of the mandible gave very good quantitative DNA yield along with very high qualitative amplification of the chosen STR segment.

CONCLUSION

As molecular genomic analysis has been limited to long bones of the body and teeth in jaws, our study highlights

the use of the STR CODIS to the jaw bone where all the sites gave good amplicon though the best amplification was seen at ramus site. This study highlights that the ramus of the mandible in the head and neck region can be substituted for femur bone^[8,19-25] owing to its easy accessibility and feasibility for bone harvest with its ample nuclear genomic DNA from bone marrow irrespective of the DNA extraction procedure.

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Conflicts of interest

There are no conflicts of interest.

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