

# Estimation and quantification of human DNA in dental calculus: A pilot study

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## Introduction

Human identification is the process of establishing a person's individuality through certain unique features that differentiate one from the others.<sup>[1]</sup> Identification through genetic material has proved to be the most accurate method, but there are cases where analyzing human DNA can be difficult. For example ill-preserved skeletal/dental remains or if family members/legal personnel deny the destruction of remains for the sole aim of identification.<sup>[2]</sup>

Previous studies reported that mitochondrial DNA is present in dental calculus.<sup>[3-5]</sup> However, they used archaeological dental calculus and identified just the maternal ancestry

## Abstract

**Context:** Identification using DNA has proved its accuracy multiple times in the field of forensic investigations. Investigators usually rely on either teeth or bone as the DNA reservoirs. However, there are instances where the skeletal or dental remains are not available or not preserved properly. Moreover, due to religious beliefs, the family members of the dead do not allow the investigating team to damage the remains for the sole purpose of identification. **Aim:** To investigate the presence of human DNA in dental calculus and to quantify the amount, if present. **Materials and Methods:** This prospective single-blinded pilot study included twenty subjects selected from the patients visiting a dental college. The samples of dental calculus were collected from the thickest portion of calculus deposited on the lingual surfaces of mandibular incisors. These samples were decontaminated and subjected to gel electrophoresis for DNA extraction. **Results:** DNA was found in 85% cases. The amount of DNA varied from 21 to 37 µg/ml of dental calculus. **Conclusion:** Dental calculus is a rich reservoir of human DNA.

**Key words:** Dental calculus, DNA, identification, mineralized plaque

of the individual. Herein, we investigated human DNA in nonarchaeological dental calculus.

## Materials and Methods

### Study design

In this single-blinded, prospective, pilot study the aim was to investigate the presence/absence of human DNA in dental calculus. The objective was to quantify the amount of DNA if present in dental calculus. This study was conducted in the Department of Oral Medicine and Radiology, Kothiwal Dental College and Research Centre, Moradabad over a period of 30 days (from July 2016 to August 2016).

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The patients visiting the outpatient department of dental college; patients in whom mandibular incisors are present; patients in whom calculus deposits are present on the lingual surface of the mandibular anterior teeth, and those who are willing to participate were included in this study. The sample size of the study was calculated using the following formula with 85% power of test and 5% error:

Necessary sample size = Z score \* standard deviation \* (1 – standard deviation)/margin of error.

The equipments used were diagnostic instruments (dental probe, mirror, and tweezers), aluminum foils, sterile plastic pouches, and micro centrifuge tubes.

### Study protocol

After taking Institutional Ethical clearance (Ref. No.: KDCRC/IERB/11/2016/05) and informed written consent, the demographic data of the subjects were recorded in the pro forma. Then, the other investigator collected samples of dental calculus.

### Sample collection

Using a sterile probe, pressure was applied on the edge of the thickest portion of dental calculus until it detached from tooth surface free [Figure 1a]. Care was taken to collect a larger sample (measuring more than 2 mm in widest dimensions) rather than multiple small fragments. An aluminum foil was placed alongside the tooth to collect any pieces of calculus that might fall off. Once calculus was removed from the tooth; it was collected gently using tweezers on sterilized gauze pieces and tipped gently in small labeled sterile plastic pouches [Figure 1b]. The samples were stored at – 20°C in nonfreezing refrigerator to prevent any microbial growth. The collected samples were then sent to BioAxis DNA Research Laboratory, Hyderabad, for DNA analysis.

### Decontamination

The dental calculus samples were kept immersed in Petri dish containing molecular grade bleach (4% dilution) for 5 min and then rinsed in ethanol (90%). The samples were then air dried for 5 min on sterilized Petri dishes.



**Figure 1:** (a) Intraoral photograph showing dental calculus on lingual surface of mandibular incisors. (b) Sample of dental calculus

The dried calculus samples were placed in sterile plastic pouches and hammered lightly. The opposing corner of the pouch was cut with a sterile scissor, and powdered calculus was poured in clean, labeled micro centrifuge tubes (measuring 1.5 ml).

### DNA extraction

Blank tubes without calculus were used as a control to monitor DNA content present in laboratory environment. 700 µL lysis buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 50 mM EDTA, pH 8.0, 0.5% SDS pH 8.0 and 20 µL proteinase K [20 mg/ml]) were added to 0.2 g of finely powdered sample, vortexed and incubated at 56°C, overnight. Next, 720 µL of extraction buffer (phenol:chloroform:isoamyl alcohol of 25:24:1 ratio) was added to the sample, vortexed and then centrifuged for 2 min at 15,000 rpm. The upper aqueous layer was transferred to a sterile micro centrifuge tube. This process of extraction buffer was repeated twice. Later, 720 µL of isobutanol was added to the aqueous layer, vortexed, and centrifuged (2 min) at 15,000 rpm. At this stage, the lower aqueous layer was used and transferred to the reservoir column of Centricon-100 concentrator (Millipore).

Then, 1 ml of Tris-EDTA buffer for washing was added into the same reservoir column, followed by centrifugation at 3000 rpm for 20 min or until the sample had spun through. This washing step was repeated twice. Finally, the sample in the collection column which contained the DNA was transferred to a sterile micro centrifuge tube.

### Agarose gel electrophoresis

Forty milliliter of 0.8% agarose gel was prepared and kept in microwave oven at power level 800 v for 2 min for proper dissolving and to get a clear transparent solution. The agarose solution was allowed to cool at room temperature, and 5 µl of ethidium bromide was dissolved. The gel casting tray, chamber and combs were wiped and cleaned with 70% ethanol. The boundaries of the tray were sealed with cello tape carefully. The agarose gel was poured into the tray, comb was placed properly, and the gel was allowed to solidify for about 20–30 min. After solidification, the comb and tape were removed carefully.

The loading samples were prepared by mixing 10 µl of the extracted DNA and 5 µl of loading dye. The samples were loaded in the corresponding wells made by removing the comb. The gel was allowed to run for 45 min to 1 h at 100 volts. After the electrophoretic run, the gel was placed under ultraviolet light and photographed [Figure 2].

### Statistical analysis

The values were tabulated using Microsoft Excel, and the results were summarized as mean ± standard deviation. Descriptive statistical analysis was employed for the quantitative variables in the study.

## Results

This pilot study included twenty subjects (12 males and 8 females) with a mean age of 39 years. Human DNA (genomic) was found in 17 subjects (85%). The amount of DNA varied from 21 µg/ml to 37 µg/ml with mean quantity of 23.5 µg/ml. Table 1 shows quantification of DNA in dental calculus samples. On the basis of the findings, we reject the null hypothesis and accept alternate hypothesis.

## Discussion

Dental calculus or tartar refers to the deposits seen on the

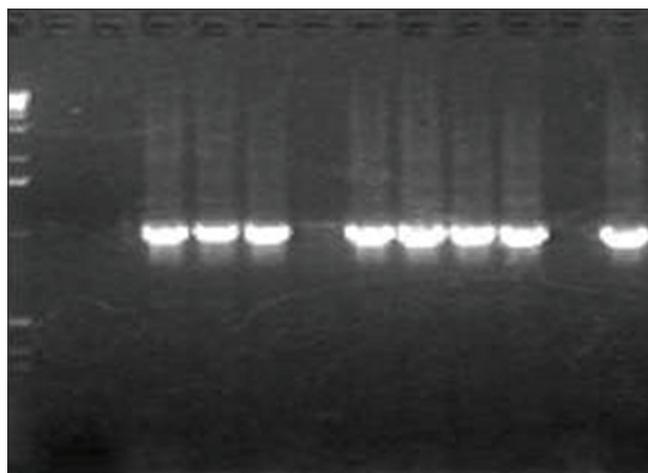


Figure 2: Gel electrophoresis to quantify DNA

teeth or dental prosthesis in the absence of adequate oral hygiene maintenance. It is an adherent crust of chalky material, creamish yellow to brownish black in color, hard stone like in consistency, caused by a buildup of dental plaque [Figure 1a].

Dental calculus is a microbial biofilm consisting of dietary components, oral microbes and host secretions such as saliva and gingival crevicular fluid. It acquires human DNA through host secretions and immunity associated process such as NETosis.<sup>[4]</sup>

Early investigators extracted plant remains or phytoliths from the archaeological dental calculus and reported that dental calculus can provide an insight about the dietary habits of the individuals. These studies formed the foundation of identifying unknown through dental calculus.<sup>[5-7]</sup>

Black *et al.* in 2011 conducted a research where they detected human mitochondrial DNA within archaeological dental calculus using polymerase chain reaction.<sup>[3,5,8]</sup> Later Warinner *et al.* compared the quantity of DNA present in dental calculus and dentine of same tooth. They found that dental calculus and dentin contained 437 ng/mg and 0.6 ng/mg DNA, respectively.<sup>[3,8]</sup>

The results of the present study reveal that genomic DNA was present in 85% cases. The quantity of DNA ranged from 21 to 37 µg/ml of dental calculus. Our findings are in accordance with the results of the previous studies.<sup>[4]</sup>

Table 1: Shows the presence and quantity of human DNA in dental calculus

Sample number	Gender (male/female)	Age (years)	Optical density (OD 260)	Amount of DNA present (µg/ml)
1	Male	30	0	0
2	Female	32	0	0
3	Male	29	0.43	21.5
4	Female	32	0.51	25.5
5	Female	40	0.48	24
6	Female	44	0.46	22.5
7	Male	45	0.61	30
8	Male	43	0.55	27.5
9	Female	40	0.60	29.5
10	Female	32	0.55	27.5
11	Female	43	0.73	37
12	Female	45	0.48	24
13	Male	45	0.65	33
14	Male	42	0.68	34
15	Female	40	0.51	22.5
16	Female	44	0.63	31.5
17	Male	45	0.41	21
18	Female	42	0.71	35.5
19	Male	38	0	0
20	Female	35	0.48	24
Total=20	12 female; 8 male	Mean±SD=39±5.57	DNA present=17; Absent=03	Mean±SD=23.52±11.17

SD: Standard deviation

## Summary

Dental calculus serves as an excellent DNA reservoir because of the following reasons:

- Dental calculus deposits are abundant and very commonly seen in oral cavity
- Calculus can be used as a DNA reservoir in cases where destructive analysis of teeth or bones is not permitted
- Calculus can serve as DNA reservoir in instances of unavailable or ill preserved samples of skeletal/dental remains
- It is densely mineralized, preserving biomolecules and micro-debris over a long period of time
- Dental calculus is the richest known source of DNA (as reported by Warinner *et al.*).<sup>[4]</sup>

### Strength and limitations of the study

According to the best of our Knowledge, this is the first study that has estimated and quantified human DNA in a large number of nonarchaeological dental calculus samples. However, this study reports the amount of total DNA found in samples of dental calculus and does not provide an insight into other types of DNA (such as microbial and extracellular DNA) present in dental calculus. Since DNA other than human can potentially be present in the samples, results of this study might overestimate the quantity of human DNA.

### Future research

The results of the present study proved that dental calculus contains human DNA. Further studies can be focused at creating genetic fingerprint using DNA which can be useful in identifying the unknown. Gender determination and age estimation from DNA analysis of dental calculus can thereby be attempted. As a follow-up of the present study, the authors are working on isolation of human DNA and its utilization for human identification.

## Conclusion

Dental calculus contains human DNA in sufficient quantity. This article is an attempt to create awareness about the latent

potential of dental calculus to serve as an investigative tool in forensic studies.

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### Conflicts of interest

There are no conflicts of interest.

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