QUANTIFICATION OF HUMAN DNA IN DENTAL CALCULUS AND BLOOD:A CROSS-SECTIONAL, DESCRIPTIVESTUDY

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree

Of

MASTER OF DENTAL SURGERY

In

PUBLIC HEALTH DENTISTRY

By

DR. SUKDEB CHAKRABORTY

Enrollment No. 12103302962

Under the guidance of

DR. ANURADHA P.

Professor & Head

Department of Public Health Dentistry

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

BATCH: 2021-2024

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Quantification of human DNA in dental calculus and blood: A Cross-Sectional, Descriptive Study"** is a bonafide, & genuine research work carried out by me under the guidance of **Dr. Anuradha P, Professor and Head,** Department of Public Health Dentistry, BabuBanarasi Das College of Dental sciences, BabuBanarasi Das University, Lucknow, Uttar Pradesh.

Date: 31/01/2024

Place: Lucknow

Sukdeb Chakraborty

Dr. Sukdeb Chakraborty

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Quantification of human DNA in dental calculus and blood: A Cross-Sectional, Descriptive study"** is bonafide work done by Dr.Sukdeb Chakraborty, under my direct supervision & guidance in partial fulfillment of the requirement for the degree of MDS in Public Health Dentistry.

31/1/20

(GUIDE)

Dr. Anuradha P

Professor & Head

Public Health Dentistry

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled " **Quantification of Human DNA in dental Calculus and Blood** " is bonafide work done by **Dr. Sukdeb Chakraborty**, under my direct supervision & guidance in partial fulfillment of the requirement for the degree of MDS in Public Health Dentistry.

(CO- GUIDE)

31/1/24 **Dr. Jiji George**

HOD

Oral and Maxillofacial Pathology

ENDORSEMENT BY THE HOD

This is to certify that this dissertation entitled **"Quantification of human DNA in dental calculus and blood: A Cross-Sectional, Descriptive study"is** a bonafide work done by Dr. Sukdeb Chakraborty, under the direct supervision & guidance of **Dr. Anuradha P**, Professor& Head, Department of Public Health, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

31/11/24

Dr. Anuradha P.

Professor& Head

Public Health Dentistry

ENDORSEMENT BY THE HEAD OF THE INSTITUTION

This is to certify that this dissertation entitled **"Quantification of human DNA in dental calculus and blood: A Cross-Sectional, Descriptive study"is** a bonafide work done by Dr. Sukdeb Chakraborty, under the direct supervision & guidance of **Dr. Anuradha P**, Professor& Head, Department of Public Health, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Dr. Puneet Ahuja

Principal

BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
Babu Banarasi Das University
Ayodhya Road, Lucknow-226028

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Babu Banarasi Das University shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/ research purpose.

Date: 31/01/2024

Place: Lucknow

Dr. Sukdeb Chakraborty

ACKNOWLEDGEMENT

I take the opportunity to express my profound gratitude and deep regards to my guide **Dr. Anuradha P.** Professor and Head, Department of Public Health Dentistry, Babu Banarasi Das College of Dental Sciences, BBDU Lucknow for her exemplary guidance, constant encouragement throughout the course of this dissertation and to bring this dissertation to a successful completion. I am greatful to her for having instilled in me the joy of creative expression and knowledge.

I wish to express my sincere thanks to **Dr.Puneet Ahuja**, Principal Babu Banarasi Das College of Dental Sciences, BBDU Lucknow for providing me the facilities to complete this task.

I am thankful to **Dr.Sugandha Agarwal**, **Dr.Shweta Singh**, **Dr.Piyush Chaudhary,Dr. Sakchhi Tiwari** and **Dr Siddharth David** for their support & encouragement during the preparation of this dissertation.

I am thankful to my post-graduate seniors Dr. Shraddha Mishra, Dr. Archita Agarwal and Dr. Aman kumar for their valuable information and help in this work.

I shall be forever indebted to my parents, my family members and my friends for their constant support and encouragement.

Above all I bow my head in gratitude to ALMIGHTY GOD for bestowing his blessings on me.

Dr. Sukdeb Chakraborty

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF ABBREVIATIONS

- bp - Base Pairs
- DNA - Deoxyribonucleic acid
- gDnA - Genomic DNA
- PCR - Polymerase chain reaction
- RBCs - Red Blood Cells
- SPSS - Statistical Package For Social Sciences
- STR - Short Tandem Repeats
- VNTR - Variable number of Tandem repeats
- WBCs - White Blood Cells

ABSTRACT

Background: whole blood is the most common sample type used for obtaining high purity DNA. Blood has proven a very consistent and reliable source of genetic material for many avenues of testing and research, but it can also be a time consuming, painful, expensive and invasive collection method. Previous studies reported that DNA is present in dental calculus. Herein, we investigated human DNA in nonarchaeological dental calculus as well as we compared the amount of human DNA in dental calculus and blood.

Methodology: The present comparative – study was conducted to evaluate the amount of human DNA in dental calculus and blood. This study was conducted in the 35 subject aged 18-60 years those reporting to the OPD of Department of Public Health Dentistry, Babu Banarasi das college of Dental Sciences, Lucknow and fulfilling the eligibility criteria were included in the study. After taking Institutional Ethical clearance and informed written consent, the demographic data of the subjects was recorded in the proforma.

The samples of dental calculus were collected from the thickest portion of calculus deposited on the lingual surfaces of mandibular incisors and three-milliliters whole blood samples were collected in 3 ml EDTA collection sterile tubes These samples were decontaminated and subjected to gel electrophoresis for DNA extraction.

Results: The mean of concentration of DNA in blood (70.19±14.55µg/ml) was higher than the mean concentration of DNA in dental calculus (54.92± 11.729µg/ml), but the difference in both the means were found to be non-significant ($p<0.218$). On comparing in females, the mean of DNA concentration in blood was (68.27±17.172µg/ml) and in calculus was (57.14±8.565µg/ml) respectively and this difference was also found to be non-significant ($p<0.23$).

Conclusion: Since Dental calculus contains ample amount of human DNA so we conclude that the dental calculus can act as a good substitute and replace collection of DNA from human blood and will be successfully used to serve as an investigative tool for forensic purpose.

Key words- Dental calculus, Blood, human DNA, DNA identification, mineralized plaque.

INTRODUCTION

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA).The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T).DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases.

Every human being is characterized based on the unique DNA sequence, due to hyper variable regions of DNA, which are specific for an individual. The order of the base pairs (bp) in the DNA of every individual is different except in identical twins.[1]

The intron regions of the DNA, which contain sequences that are 20-100 bp in length, and are repeated at different locations along the chromosome, like AGACTAGACATT – AGATTAGGCATT, which are called sequence polymorphisms.[1] The length polymorphism like (AATG) (AATG) (two repeats) and (AATG) (AATG) (three repeats) are termed as 'Short tandem repeats' (STRs), which are used in forensic identification.[1,2]

Friedrich Miescher. In 1869, while investigating the composition of leukocytes (white blood cells) he was able to isolate an unknown Substance that behaved differently to the proteins in solution.[3] Experimenting with alkali and acidic conditions. Miescher had obtained for the first time a crude precipitate of what is now known as deoxyribonucleic acid (DNA).

Blood is an excellent source of human DNA. A spot of blood, approximately 50µl in volume, is enough DNA for analysis. Whole blood is a common biological sample for DNA extraction and is used in forensics, cancer diagnoses, and various other biological tests.[4-6]

The DNA was amplified using the polymerase chain reaction (PCR) and tested by gel electrophoresis or capillary electrophoresis. These repeated sequences are named DNA fingerprints or DNA typing (profiling).

DNA profiling is a standard forensic DNA system used in human identification, criminal case work, as well as paternity testing, worldwide.[7] Initially, the forensic community used Variable number of Tandem repeats (VNTR) testing; but this method required a large amount of material and had low quality results, especially when only small amount of biological material samples were available. Currently. in most forensic samples, the study of DNA is usually performed by STR analysis.[8]

The storage duration and storage temperature, from blood collection to DNA extraction, are critical. As a result, storage duration and storage temperature of blood samples largely influence the correctness of physical test and the success of the extraction of DNA.[9]

Dental calculus is a form of hardened dental plaque. Calculus is derived from Greek word "Calcis," used for various kinds of stones.Dental calculus is formed by precipitation of minerals from saliva and gingival crevicular fluid in plaque on the teeth. This process of precipitation kills the bacterial cells within dental plaque, but the rough and hardened surface that is formed provides an ideal surface for further plaque formation. This leads to calculus build up, which compromises the health of the gingiva. Calculus can form both along the gum line, where it is referred to as supragingival and within the narrow sulcus that exists between the teeth and the gingiva, where it is referred to as subgingival calculus.[10]

Calculus is composed of both inorganic (mineral) and organic (cellular and extracellular matrix) components. The mineral proportion of calculus ranges from approximately 40-60%, depending on its location in the dentition and consists primarily of calcium phosphate crystals organized into four principal mineral phases: Octa calcium phosphate, hydroxyapatite, whitlockite, and brushite. The organic component of calculus is approximately 85% cellular and extracellular matrix.[10]

Several studies have found that saliva, dental calculus, dentine, dental pulp are promising alternative sources of DNA. Previous proteomic analysis of ancient and modern dental calculus identified a high proportion of immune proteins, particularly from neutrophils, suggesting that human DNA enter dental calculus as a result of inflammation-related immunological activity, including the release of neutrophil extracellular traps.[11] Dental calculus has recently attracted a lot of attention because it contains a board range of diagnostically relevant molecules (i.e.DNA).

Once mineralized within dental calculus, human DNA, and proteins can preserve for thousands of years. Dental calculus thus serves as an important non skeletal reservoir of ancient human DNA. Conventional techniques for recovering ancient human DNA typically require the destruction of bone or tooth tissue during analysis, and this has been a cause of concern for many native and indigenous communities. Dental calculus represents an important alternative source of ancient DNA that does not damage or disturb the integrity of skeletal remains. In addition, because dental calculus is the richest known source of DNA in the archaeological record, it presents unique opportunities for investigating archaeological sites with preservation challenges.[11]

Using advanced sequencing technologies, anthropologists demonstrate that human DNA can be significantly enriched from dental calculus (calcified dental plaque) enabling the reconstruction of whole mitochondrial genomes for maternal ancestry analysis – an alternative to skeletal remains in ancient DNA investigations of human ancestry.[12]

Warinner et al stated that "Dental calculus contains more DNA than any other known archaeological material." Archaeological bone typically contains <2 ng of DNA per milligram of tissue. By contrast, dental calculus contains more than 40 ng of DNA per milligram of tissue, and we have even measured samples that contain more than 500 ng/mg of tissue. No other material in the archaeological record contains so much DNA.[11]

Recent studies have suggested that dental calculus may be more resistant to environmental contamination than any other sources of DNA, and higher overall DNA yield have been reported from dental calculus than from any other archaeological source.[11,12]

However, collection of blood samples may not be feasible in large epidemiologic studies where participants are dispersed all over the country or because the method requires venepuncture done by blood collector, making collection of blood samples prohibitively expensive and painful.[13] Furthermore, study subjects may be reluctant to provide blood samples, thereby reducing participation rates.

Therefore, less invasive and more cost-efficient procedures for collecting DNA are needed. Therefore, this study was conducted to compare the amount of human DNA in dental calculus and blood.

AIM
AND
OBJECTIVES

Aim:

To compare the amount of human DNA present in dental calculus & blood.

Objectives:

To evaluate the amount of human DNA in dental calculus.

To evaluate the amount of human DNA in blood.

REVIEW OF LITERATURE

YamadaY et al in 1997[14] Genetic identification can be complicated by long intervals between the time of death and examination of tissues, and sometimes only bone and teeth may be available for analysis. Several investigators have described the isolation of nuclear DNA from these materials, but all have indicated that the DNA is significantly degraded. Recently, the polymerase chain reaction (PCR) and direct DNA sequencing have enabled rapid and reliable characterization of specific highly polymorphic DNA sequences from different individuals. Above all, mitochondrial DNA sequences offer several unique advantages for the identification of human remains. The isolation of mtDNA from a tooth and the symmetrical PCR amplification and direct DNA sequencing of its most polymorphic regions are reported.

Gaytmenn R et al in 2003[15]conducted study in which region(s) of the tooth contained quantifiable DNA, if all regions contain similar yields of DNA and whether there is enough DNA in all regions to justify DNA extraction from a found tooth fragment. Results demonstrated that there is sufficient quantity of DNA in the crown body, root body, and root tip to support DNA extraction. Additionally, the root body is the region with the highest yield of DNA.

Robert A. Philibert et al in 2008[16]evaluated large scale genotyping projects that have used DNA derived from whole-blood or lymphoblast cell lines. But over the past several years, a number of investigators had begun to used DNA prepared from saliva for genotyping studies, particularly for use in behavioural genetic studies. However, the comparability of DNA from these two sources has not been rigorously analysed by unbiased sources. In this communication they compare the single nucleotide polymorphism genotyping results from DNA derived from whole-blood samples obtained from 474 participants from the lowa Adoption Studies with that of saliva samples prepared from 555members of the Strong African-American Families project. They found that DNA prepared from whole-blood preformed significantly better than that prepared from saliva. Genotyping success was significantly associated with the concentration of human DNA in the saliva sample as determined by quantitative PCR, but not with the total amount of DNA as determined by UV spectroscopy. They concluded that investigators contemplating the choice of source materials of DNA for genotyping studies will need to balance the ease and economy of saliva-based DNA collection

Preus HR et al in 2011[17]conducted a study to identify reactive bacterial a DNA in archaeological human dental calculus by transmission electron microscopy (TEM) or gold-labeled antibodyTEM.

TEM showed calcified as well as non-calcified bacteria. Immunogold labeling occurred over the cytoplasmic portions of the sectioned bacteria. The result demonstrated that it is possible to identify a DNA sequences from bacteria in archaeological material of considerable age by this technique.

Reinius LE et al in 2012[18] studied whether such variation might affect the interpretation of methylation studies based on whole blood DNA. They found in healthy male blood donors there is important variation in the methylation profiles of whole blood, mononuclear cells, granulocytes, and cells from seven selected purified lineages. CpG methylation between mononuclear cells and granulocytes differed for 22% of the 8252 probes covering the selected 343 genes implicated in immune-related disorders by genome-wide association studies, and at least one probe was differentially methylated for 85% of the genes, indicating that whole blood methylation results might be unintelligible. For individual genes, even if the overall methylation patterns might appear similar, a few CpG sites in the regulatory regions may have opposite methylation patterns (i.e., hypo/hyper) in the main blood cell types. They conclude that interpretation of whole blood methylation profiles should be performed with great caution and for any differences implicated in a disorder, the differences resulting from varying proportions of white blood cell types should be considered.

AftenieLoredana Mariana et al 2012[19] assessed the blood biospecimens that was present in the logistical and financial challenges. As a result, saliva biospecimen collection has become more frequent because of the ease of collection and lower cost. This article described an assessment of two different methods for collecting samples: whole blood and whole saliva samples used further for DNA extraction and HLA genotyping in immunogenic in immunogenic disease on a group of patients registered at Molecular Genetics Laboratory Faculty of Medicine "Ovidius" University Constanta. Their data showed that only 81% of the requested participants delivered a blood sample, whereas 19% delivered a saliva sample because they refuse the first sampling method. Analysis of purified genomic DNA by Nano Photometer and agarose gel electrophoresis revealed that blood and saliva samples resulted in DNA with the best quality. PCR analysis showed that DNA from 100% of the blood samples and 93% of the saliva samples could be subsequently amplified. This study showed that the response rate of self-collection saliva samples had to be considered for the patients that have a low response rate of blood sampling.

The quality of genomic DNA from saliva samples was comparable with blood samples as assessed by purity, concentration, yield and PCR amplification. They conducted that the use of saliva

samples is a good alternative to blood samples to obtain genomic DNA of high quality and it will considerably increase the participant's response rate for genetic studies.

De La Fuente et al in 2013[20]reported a molecular methodology to obtain and analyse ancient bacterial DNA from archaeological dental calculus. Recent and archaeological DNA samples, as old as 4000 bp, were successfully extracted and amplified with species specific PCR primers. They proposed this approach in order to: detect the presence of specific bacterial species infecting past human populations; compare the composition of ancient oral microbiomes among human population; and analyse the genetic variability and covariation of bacteria and human host populations. Genomic analysis of bacteria from dental calculus is a promising source of evidence for palaeopathological and micro-evolutionary studies, focused either on micro-organisms or their human hosts.

Khare P et al in 2014[21] compared the quantity of DNA samples extracted from saliva with those extracted from blood an order to assess the feasibility of extracting sufficient DNA from saliva for its possible use in forensic identification. Blood and saliva samples were collected from 20 volunteers and DNA extraction was performed through phenol Chloroform technique. Mean quantity of DNA obtained in saliva was 48.4±8.2 µg/ml and in blood was 142.5±45.9µg/ml. Purity of DNA obtained as assessed by the ratio of optical density 260/280, was found to be optimal in 45% salivary samples while remaining showed minor contamination. Despite this positive F13 STR amplification was achieved in 75% of salivary DNA samples, Results of their study showed that saliva may prove to be a useful source of DNA for forensic purpose.

Laura S. Weyrich et al in 2015[22] said that dental calculus (calcified tartar or plaque) was today widespread on modern human teeth around the world. A combination of soft starchy foods, changing acidity of the oral environment, genetic predisposition, and the absence of dental hygiene all lead to the build-up of microorganisms and food debris on the tooth crown, which eventually calcifies through a complex process of mineralization. Millions of oral microbes were trapped and preserved within this mineralized matrix, including pathogens associated with the oral cavity and airways, masticated food debris, and other types of extraneous particles that enter the mouth. As a result, archaeologists and anthropologists are increasingly using ancient human dental calculus to explore broad aspects of past human diet and health. Most recently throughout high DNA sequencing of ancient dental calculus has provided valuable insights into the evolution of the oral micro biome and shed new light on the impacts of some of the major bio cultural transition on human health throughout history and prehistory. Here, they provided a brief historical overview of archaeological dental calculus research, and discussed the current approaches to ancient DNA

sampling and sequencing. Novel applications of ancient DNA from dental calculus are discussed, highlighting the considerable scope of this new research field for evolutionary biology and modern medicine.

Andrew T. Ozga et al in 2016[23] estimated the archaeological dental calculus which was the rich source of host-associated biomolecules. Importantly, however, dental calculus was more accurately described as a calcified microbial biofilm than a host tissue. As such, concerns regarding destructive analysis of human remains may not apply as strongly to dental calculus, opening the possibility of obtaining human health and ancestry information from dental calculus in cases where destructive analysis of conventional skeletal remains is not permitted. Here they investigate the preservation of human mitochondrial DNA (mt DNA) in archaeological dental calculus and its potential for full mitochondrial genome (mitogenome) reconstruction in maternal lineage ancestry analysis. Extracted DNA from six individuals at the 700-year-old Norris Farms #36 cemeteries in Illinois was enriched for mtDNA using in-solution capture techniques, followed by Illumina high-throughput sequencing. Full mitogenomes (7-343) were successfully reconstructed from dental calculus for all six individuals, including three individuals who had previously tested negative for DNA preservation in bone using conventional PCR techniques. Mitochondrial haplogroup assignments were consistent with previously published finding and additional comparative analysis of paired dental calculus and dentine from two individuals yielded equivalent haplotype results. All dental calculus samples exhibited damage patterns consistent with ancient DNA, and mitochondrial sequences were estimated to be 92-100% endogenous. DNA polymerase choice was found to impact error rates in downstream sequence analysis, but these effects can be mitigated by greater sequencing depth. Dental calculus was a viable alternative source of human DNA that can be used to reconstruct full mitogenomes from archaeological remains.

Weyrich LS et al in 2017[24]described the shotgun-sequencing of ancient DNA from five specimens of Neanderthal calcified dental plaque (calculus) and the characterization of regional differences in Neanderthal ecology. At Spy cave, Belgium, Neanderthal diet was heavily meat based and included woolly rhinoceros and wild sheep (mouflon), characteristic of a steppe environment. In contrast, no meat was detected in the diet of Neanderthals from E1 Sidron cave,

Spain, and dietary components of mushrooms, pine, nuts, and moss reflected forest gathering. Differences in diet were also linked to an overall shift in the oral bacterial community (microbiota) and suggested that meat consumption contributed to substantial variation within Neanderthal microbiota. Evidence for self-medication was detected in an E1 Sidron Neanderthal with a dental

abscess and a chronic gastrointestinal pathogen (Enterocytozoonbieneusi). Metagenomic data from this individual also contained a nearly complete genome of the archaeal commensal Methanobrevibacteroralis (10.2x depth of coverage)-the oldest draft microbial genome generated to date, at around 48,000 years old. DNA preserved within dental calculus represents a notable source of information about the behavior and healthy of ancient hominin specimens, as well as a unique system that is useful for the study of long- term microbial evolution.

Mann AE et al in 2018[25] studied Dental calculus is a mineralized form of dental plaque, a sequentially generated microbial biofilm that entraps microbial, dietary, host, and ambient debris during spontaneous calcification events. Unlike body mucosal surfaces that have continual cell turnover, teeth do not remodel. Consequently, they are relatively stable environments for bacterial colonization during biofilm development, making the formation of dental calculus difficult to prevent without mechanical removal. As a result, dental calculus is prevalent in the archaeological record, and due to its excellent morphological preservation, it has long been an attractive target for microscopic analysis. More recently, dental calculus has been explored as a source of ancient DNA (a DNA) and has been shown to retain an excellent record of the human oral microbiome. It may provide insights on ancient diet through isotopic analysis and it serves as an alternative source of endogenous host DNA.

Rubio L et al in 2019[26] correlated colour modifications with the concentration of human DNA in teeth burned at different temperatures, Spectrophotometry was used to measure the colour of 40 teeth heated at temperatures of 100, 200, and 400^0C for 60 min. DNA was extracted by phenol-chloroform extraction and quantified by real-time quantitative PCR using the Quantifier human DNA quantification kit. Preliminary results indicated an association of higher temperature with changes in colorimetric variables and a decrease in DNA concentrations. A significant positive correlation was found between luminosity values and DNA concentration ($r = 0.4727$, $p = 0.0128$) and between chromaticity a* values and DNA concentration ($r = 0.4154$, $p = 0.0250$). Spectrophotometry analysis of the color of burned teeth may predict the feasibility of extracting human DNA for identification purposes.

Ottoni C et al in 2019[27]characterised the oral microbiome of six ancient Egyptian baboons held in captivity during the late Pharaonic era (9^{th} – 6^{th} centuries BC) and of two historical baboons from a zoo via shotgun metagenomics. They demonstrated that these captive baboons possessed a distinctive oral microbiome when compared to ancient and modern humans, Neanderthal and a wild chimpanzee. These results may reflect the omnivorous dietary behaviour of baboons, even

though health, food provisioning and other factors associated with human management, may have changed the baboon's oral microbiome.

Singh U et al in 2019[28]investigated the presence of human DNA in dental calculus and to quantify the amount, if present. 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 get electrophoresis for DNA extraction. DNA was found in 85% cases. The amount of DNA varied from 21 to 37 µg/ml of dental calculus. Dental calculus is a rich reservoir of human DNA.

Ziesemer KA et al in 2019[29] explored whether calculus is also a viable substrate for whole human genome recovery using targeted enrichment techniques. Total DNA extracted from 24 paired archaeological human dentin and calculus samples was subjected to whole human genome enrichment using in-solution hybridization capture and high-throughput sequencing. Total DNA from calculus exceeded that of dentin in all cases, and although the proportion of human DNA was generally lower in calculus, the absolute human DNA content of calculus and dentin was not significantly different. Whole genome enrichment result in up to four-fold enrichment of the human endogenous DNA content for both dentin and dental calculus libraries, albeit with some loss in complexity. Recovering more on-target reads for the same sequencing effort generally improved the quality of downstream analyses, such as sex and ancestry estimation. For nonhuman DNA, comparison of phylum-level microbial community structure revealed few differences between pre capture and post capture libraries, indicating that off target sequences in human genome enriched calculus libraries may still be useful for oral microbiome reconstruction. While ancient human dental calculus does contain endogenous human DNA sequences, their relative proportion is low when compared with other skeletal tissues. Whole genome enrichment can help in increasing the proportion of recovered human reads, but in this instance enrichment efficiency was relatively low when compared with other forms of capture.

Velsko IM et al in 2019[30]compared the microbial profiles of modern dental plaque, modern dental calculus, and historic dental calculus to establish expected differences between these substrates. Metagenomic data was generated from modern and historic calculus samples, and dental plaque metagenomic data was downloaded from the Human microbiome Project. Microbial composition and functional profile were assessed. Metaproteomic data was obtained from a subset on historic calculus sample. Comparisons between microbial, protein, and metabolomic profiles revealed distinct taxonomic and metabolic functional profiles between plaque, modern calculus, and

historic calculus, but not between calculus collected form healthy teeth and periodontal disease-affected teeth. Species co-exclusion was related to bio film environment. Proteomic profiling revealed that healthy tooth samples contain low levels of bacterial virulence proteins and a robust innate immune response. Correlations between proteomic and metabolomic profiles suggest co-preservation of bacterial lipid membranes and membrane-associated proteins. Overall, they found that there were systematic microbial differences between plaque and calculus related to biofilm physiology, and recognizing these differences is important for accurate data interpretation in studies comparing dental plaque and calculus.

Lippolis C et al in 2019[31] assessed the RNA stability over time; we investigated 40 saliva-derived micro RNAs (miRNAs), in archaeological and modern tartar samples by classic PCR followed by quantitative PCR. Three miRNAs were not present in both ancient and modern tartar samples, while 10 out of 37 miRNAs had significant different levels in ancient than modern samples. Nine miRNAs were down-regulated and only one was up-regulated. Since these different levels were not induced by gum inflammation and were independent to guanine –cytosine content in miRNA sequences, used as degradation biomarker, they hypothesis was also supported by recent studies that demonstrate a close relationship between nutrients and changes in miRNA expression in healthy individuals.

Koehn K et al in 2019[32]explored genetic identification of skeletal human remains is often realized by short tandem repeat (STR) genotyping of nuclear DNA. Dental DNA is preferred to DNA from bone for the better protection of the endogenous DNA. Especially if whole tooth grinding is intended to access the DNA, contaminations with exogenous DNA have to be avoided. The immersion of the tooth in sodium hypochlorite (NaOCl, known as bleach) is one common procedure to clean the outer surface from extraneous DNA and PCR inhibitors. To investigate the impact of bleaching on endogenous DNA and the decontamination success, 71 recently extracted teeth were differently treated with sodium hypochlorite (2.5 or 5.0% NaOCl for 30 or 60 s, 5.0% NaOCl for 10 min, and control group) in the beginning of the extraction process, whereas equally handled afterwards. Quantitative and qualitative evaluation of the extracted DNA was performed. There was a great variation for the DNA concentration of the extracts even within a group of the same NaOCl treatment. Complete DNA profiles from single persons with alleles for the 16 ESS (European Standard Set) STR loci were obtained for all regarded teeth. A statistically significant difference between the DNA yields of the treatment groups was not determined. Moreover, a negative effect of NaOCl (2.5% and 5.0%) on the DNA recovery could not be observed. Significant larger amounts of DNA were extracted from anterior teeth in contrast to posterior teeth.

Carrasco P et al in 2020[33]examined the performance of a three-part nuclear DNA analysis workflow for teeth samples based on (1) improved dental tissue recovery using the Dental Forensic Kit (DFKMR) (Universidad de los Andes) and DNA extraction with Quick Extract^{TM} FFPF DNA Extraction Kit (Lucigen®), (2) quantification with Inno Genomics Technologies) for sensitive assessment of total human and male DNA quantity/ quality, and (3) massively parallel sequencing for simultaneous genotyping of 231 short tandern repeat (STR) and single- nucleotide polymorphism (SNP) markers with the ForenSeq®DNA Signature Prep Kit (Verogen, Inc.). Initial evaluation of artificially degraded blood samples (n=10) achieved highly sensitive and informative quantification results with Inno jQuant®HY, enabling successful first pass genotyping with the MiSeqFGx®System. Twenty –three STR alleles (out of 85) and 70 identity informative SNP loci (out of 94) were recovered from two pg total long target DNA input (0.86 ng total short target input) and an InnoQuant degradation index (DI) of 460 (severely degraded). THe three-part workflow was subsequently applied to teeth samples (dental pulp, root cement tissues; n=13) with postmortem intervals (PMI) of the teeth ranging from 8 days to approximately 6 months. Informative SNP and STR DNA profiles were obtained, to include 78 STR alleles and 85 identity informative loci typed (of 94 total SNP targets) in a 1 month four-day PMI root cement samples with one pg. Total long target DNA input and a DI of 76. These data incident successful performance of the propose workflow from degraded DNA from teeth samples.

Sawafuji R et al in 2020[34] applied DNA metabarcoding to dental calculus of premodern Japan for the taxonomic identification of food items. DNA was extracted from 13 human dental calculi from the Unko-in site (18^{th}-19^{th}century) of the Edoperiod, Japan. polymerase chain reaction (PCR) and sequencing were performed using a primer set specific to the genus Oryza because rice(oryzasativa) was a staple food and this was the only member of this genus present in Japan at time. DNA metabarcoding targeting plants, animals (meat and fish), and fungi were also carried out to investigate dietary diversity . They detected amplified products of the genus Oryza from more than half of the samples using PCR and Sanger sequencing. DNA metabarcoding enabled us to identify taxa of plants and fungi, although taxa of animals were not detected, except human. Most of the plant taxonomic groups (family /genus level) are present in Japan and include candidate species consumed as food at that time, as confirmed by historical literature. The other groups featured in the lifestyle of Edo people, such as for medicinal purpose and tobacco. The result indicated plant DNA analysis from calculus provides information about food diversity and lifestyle habits from the past and can complement other analytical methods such as micro- particle analysis isotope analysis.

Forshaw R et al in 2022[35] suggested that Dental calculus is recognized as a secondary etiological factor in periodontal disease, and being a prominent plaque retentive factor, it is routinely removed by the dental team to maintain oral health. Conversely, dental calculus can potentially be useful in forensic studies by supplying data that may be helpful in the identification of human remains and assist in determining the cause of death. During the last few decades, dental calculus has been increasingly recognized as an informative tool to understand ancient diet and health. As an archaeological deposit, it may contain non-dietary debris which permits the exploration of human behavior and activities. While optical and scanning electron microscopy were the original analytical methods utilized to study microparticles entrapped within the calcified matrix, more recently, molecular approaches, including ancient DNA (a DNA) and protein analyses, have been applied. Oral bacteria, a major component of calculus, is the primary target of these a DNA studies. Such analyses can detect changes in the oral microbiota, including those that have reflected the shift from agriculture to industrialization, as well as identifying markers for various systemic diseases.

Samantha H Blatt et al in 2022[36] suggested that Dental calculus may be used to supplement or augment traditional forensic DNA samples as it has been shown to incorporate endogenous host-DNA and microbial DNA and external environmental inclusions. Analysis of calculus may provide important insights into decedents' lifestyle, diet, possible geolocation, and disease experience, resulting in a more holistic antemortem profile of unidentified individuals. Anthropologists have long analysed calculus in investigations of human prehistory, dietary shifts, and disease loads in populations, but the use of calculus in forensic contexts has not been widely adopted and only minimally explored. This article explores the utility and significance of analysing dental calculus as a non-invasive method during the human identification process, offers methodological overviews, and provides a critique and considerations for using endogenous microbial and host-DNA and calculus as forensic evidence.

METHODOLOGY

Study Design: Cross-sectional, Descriptive study.

Study Setting:

The study was conducted at the Department of Public Health Dentistry, BBD College Of Dental Sciences, Lucknow and collection of Blood Sample was carried out in Department of Oral pathology, BBD College Of Dental Sciences, Lucknow.

Study population

Males and females of age group 18-60 years.

Sample Size Estimation

The sample size was calculated based on previous study by Singh U et al (2017)[28]

The sample size of the study was calculated using the following formula with 85% power of test and 5% error:

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

Ethical clearance

This study was reviewed and approved by the ethical committee of **BBD College Of Dental Sciences, Lucknow** (BBDU). Ref.No.- BBDCODS/IEC/09/2022. IEC Code: 23

Schedule of the Study

The study was conducted over a period of three months from November 2022 to January 2023.

Training:

The dental calculus samples were collected by a trained examiner strictly following the guidelines of the collection protocol.

Collection of Blood samples was done by a single expert trained at the Department of Oral pathology, BBD College of Dental Sciences, Lucknow.

Study subject / sample:

The patients aged 18-50 years were selected from the OPD of Public Health Dentistry Unit of BBD College of Dental Sciences, Lucknow. A total of 35 patients, who fulfilled the eligibility criteria were recruited in the study as study samples.

Eligibility Criteria:

Inclusion criteria:

- Subjects with age 18-50 years.
- Subjects with heavy bands of supra-gingival calculus,
- No periodontal therapy in past 6 months.
- Subjects who signed the written informed consent.

Exclusion criteria:

- Subject who are under medication for any systemic disease.
- Lactating females / pregnant women
- subjects undertaking orthodontic treatment / any dental procedure.
- Subjects with any acute oral mucosal lesions or suspected oral malignancies.
- Subjects with adverse oral habits like tobacco/pan chewing.

Sampling method:

- Convenient sampling method was employed.

Armamentarium Required:

1. Plane Mouth Mirror
2. Dental Probe
3. Tweezers
4. Aluminium Foils
5. Sterile Plastic Pouches
6. Betadine
7. Sterile Cotton Rolls
8. Kidney Trays
9. Disposable Gloves
10. Disposable Mouth-mask
11. Disposable Salivary Ejector tips
12. 3ml EDTA sterile tubes
13. Dry-Ice
14. Portable Container

Methodology: The present study was conducted to evaluate the amount of human DNA in dental calculus and blood. This study was conducted in the 35 subject aged 18-50 years, those reporting to the OPD of Department of Public Health Dentistry, BBD College of Dental Sciences, Lucknow. The subjects who were fulfilling the eligibility criteria were included in the study. After taking Institutional Ethical clearance and informed written consent, the demographic data of the subjects was recorded. Using a sterile probe, pressure was applied on the edge of the thickest portion of dental calculus until it detached from tooth surface. Care was taken to collect a larger sample (measuring more than 2mm in widest dimensions) rather than multiple small fragments. A sterile aluminium foil was placed alongside the tooth to collect any pieces of calculus directly into it. After that calculus was collected gently using tweezers on sterilized gauze pieces and tipped gently in small labelled sterile plastic pouches. The samples were stored at 20^0C in nonfrosting refrigerator to prevent any microbial growth. The collected samples were then sent to Bio Axis DNA Research Laboratory, Hyderabad, for human DNA analysis.

Lab Procedure for Decontamination:

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

DNA Extraction from Dental Calculus:

The dental calculus in powdered form of 40-60mg was added to 500µl of EDTA. After that it was incubated at 37^0C for 3 days. After the incubation 1ml of TRIS NaCl EDTA(TAE) buffer with sodium dodecyl sulphate (SDS)and add 10µl of proteinase K was added and mixed by pulse vortexing for 15 seconds. Then, the mix was incubated at 56^0C for 24 hours. Later on transfer the entire lysate into 2 ml eppendorf.1 ml of lysate was added with 1 ml of phenol chloroform isoamy1 alcohol and pulse vortexing is done for 15 seconds and then incubated for 5 minutes at room temperature.

Then, it was Centrifuged at 10,000 rpm for 10 min in refrigerated centrifuge. The supernatant was collected into a 1.5 ml fresh tube and the collection tube was discarded containing the flow through. Then, the equal volume of isopropanol was added and again incubated for 30 minutes to 1 hour at room temperature and again Centrifugation at 10,000 rpm for 15 min in refrigerated

centrifuge was carried out. Supernatant was discarded and DNA pellet was washed with 70% ethanol twice Lastly,

DNA pellet was dissolved in 20 µl nuclease free water (NFW) and stored at -4℃ until the PCR procedure was carried out.

Blood Samples:

The subjects were referred to the Department of Oral pathology, BBD College Of Dental Sciences. Three-millilitre whole blood samples were collected in 3ml EDTA collection sterile tubes and samples were stored at -20℃ until human DNA extraction.

DNA Extraction From blood (Bunce method)

Two solutions were prepared for extraction of DNA from blood.

Solution A contains:

- 0.32g Sucrose
- 10mM Tris buffer
- 5mM $MgCl_2$
- 1% v/v Triton

Solution B contains:

- 10mM Tris
- 400 mM NaCl
- 2mM EDTA

Procedure

1 ml of anticoagulated blood (EDTA to protect DNA) was taken and 3ml of solution A was added in it. The whole blood was shaken slowly for about 10 minutes and then incubated at 37℃ for 5 minutes. Then, blood sample was centrifuged at 5000 rpm for about 10 minutes. Then, the pellets were taken by removing the supernatant and add twice the volume of solution B to the pellet and shake the pellet to dissolve it. Later again incubated at 37^0C for 5 minutes and 650µl of chloroform was added for decolourising the blood sample and then shaken well for 60 minutes. Then, the decolourised sample was centrifuged at 4000rpm for 10minutes. Then we took the supernatant and added equal volume of ice-cold ethanol or isopropanol. Mixture was now incubated a -20 for 30 minutes or 4 overnight. Sample in than again centrifuged at 12000rpm for 5 minutes and supernatant was again discarded and pellet was washed with 70% ethanol. Centrifugation was

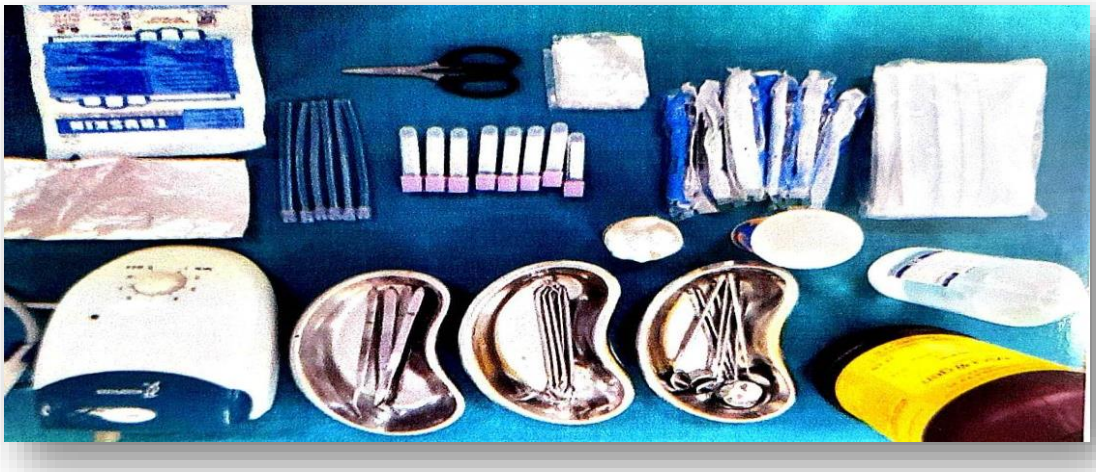
carried out again at 12000rpm for 5 minutes. The supernatant was discarded and the pellet was air dried. Lastly, Dissolve the pellet in 50-100µl of TE buffer.

Agarose Gel Electrophoresis

Forty millilitres of 0.8% agarose gel were 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 5µl 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, combo 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.

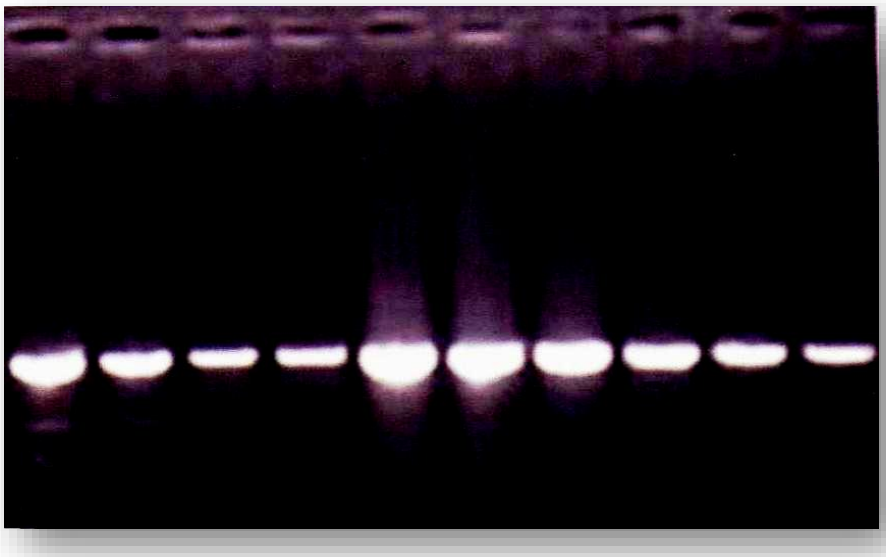
Statistical analysis

Data was entered into Microsoft Excel Spreadsheet and was checked for any discrepancies. Summarized data was presented using Tables and Graphs. The data was analyzed by using paired t-test on SPSS 21.

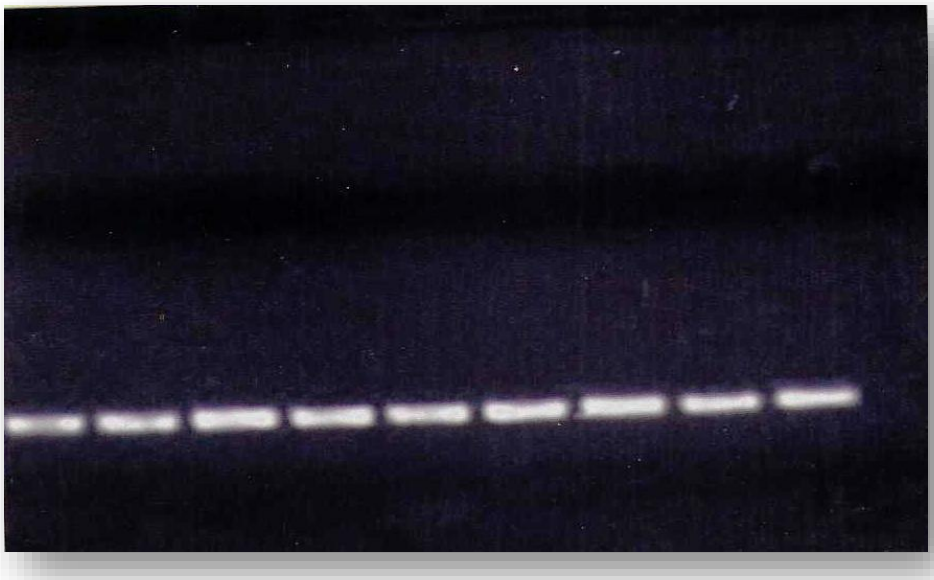


Armamentarium used in the study

Intraoral photograph showing dental calculus on lingual surface of mandibular incisors.



Gel electrophoresis to quantify DNA in blood



Gel electrophoresis to quantify DNA in dental calculus

sample collection by investigator

RESULTS

This study was done to compare the amount of human DNA present in dental calculus and blood among 33 subjects aged 18-50 years. Out of 35 subjects, 20 were female and 15 were male.

Table 1 showed that human DNA in dental calculus was found in all 35 subjects present in the study. The amount of human DNA in dental calculus varied from 40 to 69 µg/ml with mean quantity of 54.92 µg/ml.

S no.	Gender	Quantity of human DNA in dental calculus (µg/ml)
1	Female	62
2	Female	58
3	Female	48
4	Female	68
5	Female	57
6	Female	65
7	Male	53
8	Male	58
9	Female	40
10	Male	61
11	Female	49
12	Female	57
13	Female	49
14	Female	54
15	Female	61
16	Male	65
17	Female	56
18	Male	59
19	Male	68
20	Male	57
21	Male	54
22	Female	56
23	Male	69
24	Male	50
25	Male	57
26	Female	48
27	Female	64
28	Male	65
29	Female	48
30	Female	55
31	Female	43
32	Male	59
33	Male	58
34	Female	48
35	Female	58

Table2 showed that human DNA in blood was present in all the subjects recruited in DNA in blood varied from 54 to 83 µg/ml. with mean quantity of 70.19 µg/ml.

S no.	Gender	Quantity of human DNA in blood (µg/ml)
1	Female	82
2	Female	76
3	Female	58
4	Female	86
5	Female	76
6	Female	73
7	Male	73
8	Male	76
9	Female	54
10	Male	68
11	Female	62
12	Female	73
13	Female	66
14	Female	74
15	Female	78
16	Male	83
17	Female	73
18	Male	76
19	Male	82
20	Male	77
21	Male	69
22	Female	72
23	Male	86
24	Male	68
25	Male	76
26	Female	66
27	Female	83
28	Male	83
29	Female	68
30	Female	64
31	Female	58
32	Male	72
33	Male	73
34	Female	59
35	Female	64

Graph 1 shows the gender wise distribution of the study population in which out of 35 individual 15 (43%) were male and 20 (57%) were females.

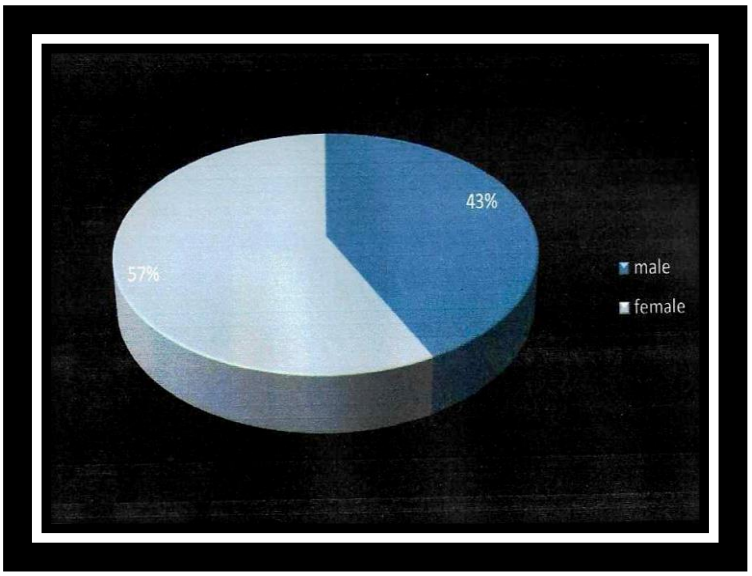


Table 3 and Graph 2 shows the mean of concentration of DNA in blood (70.19 ± 11.729 µg/ml) was higher than the mean concentration of DNA in dental calculus (70.19 ± 11.729 µg/ml) but the difference in both the means were found to be non-significant (p<0.218)

Variable	Mean	N	Std. Deviation	t value	p value
DNA Conc. in blood	70.19	35	14.554	19.656	<0.218
DNA Conc. in Calculus	54.92	35	11.729		

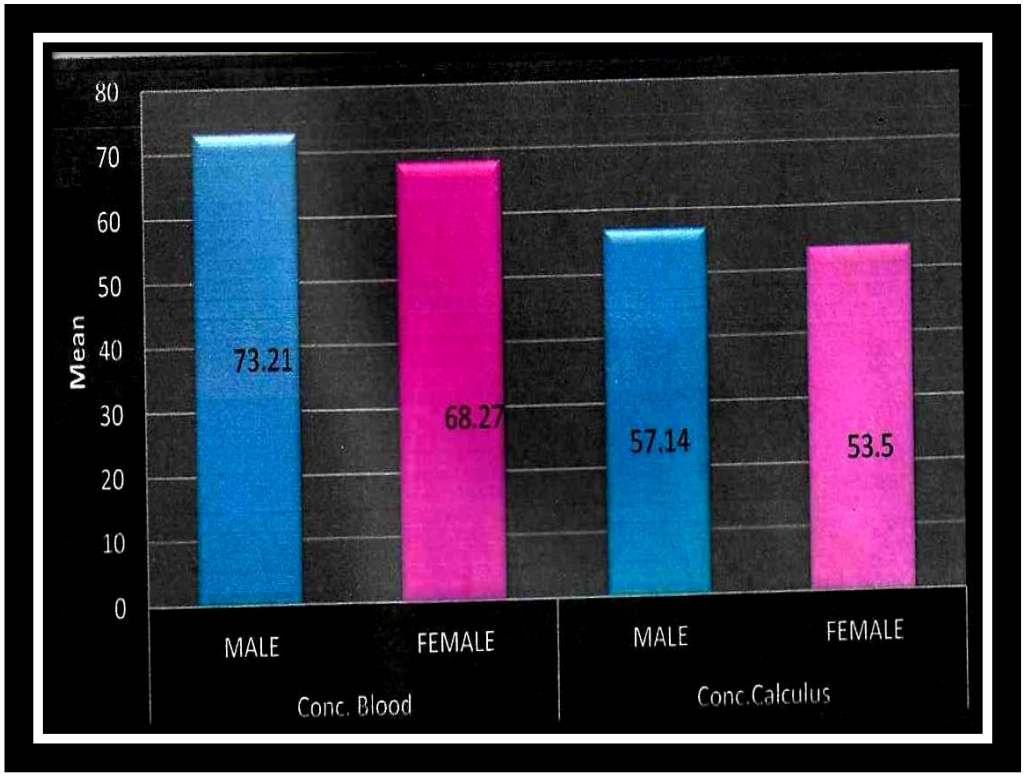


Table 4 and Graph 3 shows the distribution of mean of concentration in blood and calculus among male and female. On comparing in females the mean of DNA concentration in blood was (68.27 ± 17.172 µg/ml) and in calculus was (53.50 ± 13.359 µg/ml) respectively and this difference was found to be statistically non-significant (p<0.128) while on comparison in males the mean of DNA concentration in blood was (73.21 ± 8.825 µg/ml) and in calculus was (57.14 ± 8.565 µg/ml) respectively and this difference was also found to be non-significant (p<0.23)

Gender	Variable	Mean	Std. Deviation	t value	p value
Female	Conc. Blood	68.27	17.172	12.795	
	Conc. Calculus	53.50	13.359		<0.128
Male	Conc. Blood	73.21	8.825	18.948	<0.23
	Conc. Calculus	57.14	8.565		

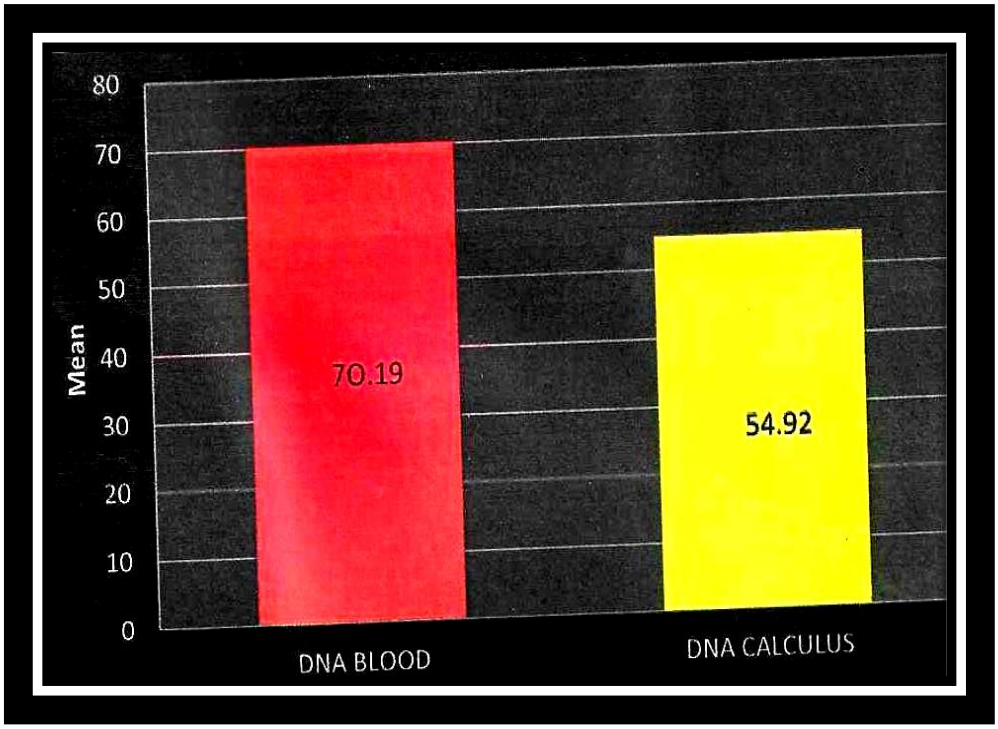
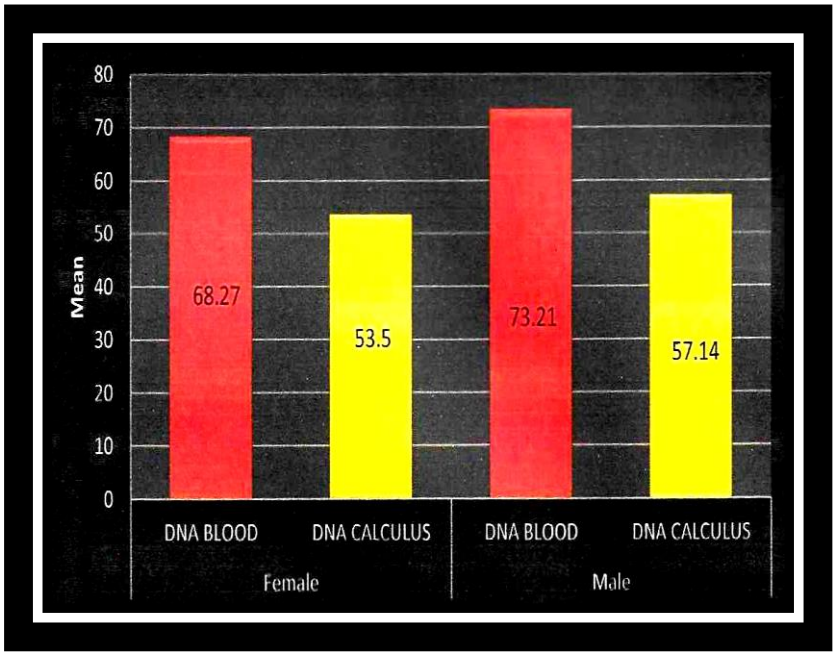


Table 5 and Graph 4 shows comparison of human DNA conc. in blood and dental calculus among male and female. On comparing mean of DNA conc. in blood among male was (73.21 ± 8.825 µg/ml) and in female was (68.27 ± 17.172 µg/ml) and this was found to be statistically non-significant while on comparing the DNA conc. in calculus among male was (57.14 ± 8.565 µg/ml) and in females was (53.5 ± 13.359 µg/ml) and this was found to be statistically non-significant.

Variable	Gender	N	Mean	Std. Deviation	t value	p value
Conc. Blood	Male	15	73.21	17.172	1.135	0.265
	Female	20	68.27	13.359		
Conc. Calculus	Male	15	57.14	8.825	0.997	0.326
	Female	20	53.50	8.565		



DISCUSSION

DNA or deoxyribonucleic acid is the fundamental building block for an individual's entire genetic makeup. It is a component of virtually every cell in the body further, a person's DNA is the same in every cell.[37] DNA contains the instructions needed for an organism to develop. survive and reproduce. To carry out these functions, DNA sequences must be converted into messages that can be used to produce proteins., which are the complex molecules that do most of the work in our bodies. DNA provides the complete genetic code for a person and contains the blueprint for building the proteins that are critical for our bodies to function.[38]

The Swiss biochemist Frederich first observed DNA in the late 1800_s. But nearly a century passed from that discovery until researchers unravelled the structure of the DNA molecule and realized its central importance to biology. For many years, scientists debated which molecule carried life's biological instructions. most thought that DNA as too simple a molecule to play such a critical role. Instead, they argued that proteins were more likely to carry out this vital function because of their greater complexity and wider variety of forms.[38]

The importance of DNA became clear in 1953 thanks to the work of James Watson. Francis Crick, Maurice Wilkins and Rosalind Franklin. By studying X-ray diffraction patterns and building models, the scientists figured out the double helix structure of DNA-a structure that enables it to carry biological information from one generation to the next.[38] In organisms called eukaryotes, DNA is found inside a special area of the cell called the nucleus. Because the cell is very small, and because organisms have many DNA molecules per cell, each DNA molecule must be tightly packaged.

This packaged form of the DNA is called a chromosome. Researchers refer to DNA found in the cell's nucleus as nuclear DNA. An organism's complete set of nuclear DNA is called its genome. Besides the DNA located in the nucleus, human and other complex organisms also have a small amount of DNA in cell structures known as mitochondria. Mitochondria generate the energy the cell needs to function properly.[38]

Forensic DNA analysis has played a crucial role in the investigation and resolution of thousands of crimes since the last 1980s.[39] Forensic scientists can use DNA in blood, semen, skin, dental calculus, saliva or hair found at a crime scene to identify a matching DNA of a an individual.

Characterization, or "typing" of blood, semen, and other body fluids has been used foe forensic purpose for more than 50years. It began with blood groups, such as those of the ABO system, and later was extended to serum proteins and red cell enzymes and in some forensic applications, particularly paternity testing, to human leukocyte antigens (HLA), which are associated with tissue types.[40]

Blood is a fluid that moves through the vessels of a circulatory system. Whole blood is a common biological starting sample for DNA extraction. Whole blood contains red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma, with DNA found in the nuclei of WBCs.[41] DNA found in blood is of high quality and is used in forensics, cancer diagnoses, and various another biological test. More quantitative methods, such as quantitative PCS (qPCR) can be used to observe inhibition, or contamination, in amplified samples.[41]

Several studies have found that exfoliated buccal epithelial cells, saliva, dental calculus, dentine, dental pulp is promising alternative sources of DNA. The use of dental calculus as

a source of DNA allows certain technical advantages over the use of blood. Collection is easier and painless, and does not have the religious implications of using the blood, especially from hepatitis and AIDS patients, due to the use of sharp objects such as needles.[42] Calculus contains cellular material and thus can be typed by DNA analysis. Calculus inside the mouth, give more than enough cells and DNA for the serologist to perform DNA typing. This can be an alternative to gathering controls by venipuncture.[43]

Recent research on ancient dental calculus (calcified dental plaque) has shown that it is the richest known source of ancient DNA in the archaeological record, exceeding the DNA content found in bone and dentin by more than an order of magnitude.[25,12,11] Consequently, dental calculus is potential cell types contributing host DNA include white blood cells (e.g., neutrophils, basophils, eosinophils, monocytes, and lymphocytes)and oral epithelial cells.[25,11] Previous proteomic analysis of ancient and modern dental calculus identified a high proportion of immune proteins, particularly from neutrophils, suggesting that human DNA may enter dental calculus as a result of inflammation-related immunological activity, including the release of neutrophil extracellular traps[11]

In our study it was found that human DNA was present in all cases. The quantity of human DNA in blood ranged from 54 to 83µg/ml (mean 70.19), this value is similar to the values found in the previous study done by Gong, R., & Li, S. (2014)[44] whereas in contrast other study done by Silke

Rosinger et al. (2010)[45] shows 20-25 mg/ml of DNA were extracted from whole blood. The difference observed in the study might be due to the method of extraction, collection and the technique used for analysis.

The quantity of human DNA in dental calculus in our study ranged from 40 to 69µg/ml (mean 54.92), this value is similar to the values found in the previous study done by **Wanier et.1(2019)**[11]which states that dental calculus typically contains more than 40 ng of human DNA per milligram of tissue. In contrast, other study conducted by **Singh, U., & Goel, S. (2017)**[28] showed human DNA ranging from 21 to 37µg/ml of dental calculus it might be due to the collection and decontamination method used in the study.**Samantha H Blatt et al in 2022**[36] suggested that Dental calculus may be used to supplement or augment traditional forensic DNA samples.This article explores the utility and significance of analysing dental calculus as a non-invasive method during the human identification process, as forensic evidence.

In our study of comparison of quantification of human DNA in blood and dental calculus among study subjects, there was no such difference were found in DNA quantification values. The preferred choice of genetic material has been blood samples because they provide large amounts of cells containing not only DNA but also a range of physiologic agents. However, collection of blood samples may not be feasible in large epidemiologic studies where participants are dispersed all over the country or because the method requires venepuncture done by trained staff, making collection of blood samples prohibitively expensive. Furthermore, study subjects may be reluctant to provide blood samples, thereby reducing participation rates. Therefore, less invasive and more cost-efficient procedures for collecting DNA are needed; therefore, we recommend quantification of DNA through dental calculus which is quite easy as compared to blood.

Dental calculus is unique among archaeological substrates in that it is abundant, nearly ubiquitous, preserves over long time periods, and contains both host genomic and acquired health/disease related information.[12] Moreover, because dental calculus is less porous and thus, less susceptible to degradation by environmental microbes than dentine or bone, it offers an alternative source of ancient human DNA that may persist when other skeletal tissues fail to yield DNA.[12]

In addition, because dental calculus is the richest known source of DNA inarchaeological record, it presents unique opportunities for investigating archaeological sites with presentsunique opportunities for investigating archaeological sites with preservation challenges.

DNA concentrations from blood were found to be in the optimal range while DNA obtained from dental calculus was near the optimal range. Because of the case of collection, the convenient

storage of dental calculus samples, the high response rate, and the high DNA quality, we suggest that dental calculus samples are a good alternative to blood samples in epidemiologic studies. So, we conclude that the dental calculus contains human DNA in sufficient quantity and can act as a good substitute and replace collection of DNA from human blood and will be successfully used to serve as an investigative tool for forensic purposes.

According to the result of our study it was found that similar amount of human DNA was present in dental calculus and blood. Further studies can be focused at creating genetic fingerprinting using DNA in dental calculus which can be useful in identifying the unknown. DNA analysis for gender determination from dental calculus thereby further need to be attempted.

LIMITATIONS

The study has following limitations:

1. Storage of dental calculus sample at -20℃ until human DNA extraction.
2. Difficulty in sample transport to Hyderabad in stipulated time.

CONCLUSION

The most popular methods for collecting human DNA are blood samples. The process of collecting blood carries certain disadvantages. As the collection of blood is difficult and painful and collection of blood also has some religious implications especially in countries like India. There are higher risks of transmission of diseases during the collection of blood samples venepuncture is often the main reason for recruitment refusal. Beside this there may be some instances which may cause failure to extract DNA as in Blood clotting (due to poor mixing with EDTA anticoagulant or use of serum tube), use of anticoagulants other than EDTA, or excessive haemolysis following poor sample storage or excessive delays in sample transport. Dental calculus collection in one of the non-invasive methods that yields high quality human DNA. It can be collected in both laboratory or in the fields due to case of collection as per the requirements. Dental calculus may also enable researchers to retrieve DNA from skeletal samples where bone or other biological tissues are too degraded for analysis. It represents an important alternative source of ancient DNA that does not damage or disturb or integrity of skeletal remains. This study demonstrates benefits of using dental calculus as an alternate source for DNA analysis. As removal of calculus from the teeth of skeletal remain is less destructive to the individual remains than the traditional method of DNA analysis and most of the tribes will be willing for DNA analysis through dental calculus as human remains will not be compromised while removing the calculus. Therefore, we conclude that dental calculus is a good source of human DNA thereby, can be used as an alternative of blood and as well as it can serve as an investigative tool in forensic studies.

RECOMMENDATION

The most popular methods for collecting human DNA are blood samples. The process of collecting blood carries certain disadvantages. As the collection of blood is difficult and painful and collection of blood also has some religious implications especially in countries like India. There are higher risks of transmission of diseases during the collection of blood samples.

- Dental calculus collection in one of the non-invasive methods that yields high quality human DNA. It can be collected in both laboratory or in the fields due to case of collection as per the requirements.
- This study demonstrates benefits of using dental calculus as an alternate source for DNA analysis.
- Therefore, we conclude that dental calculus is a good source of human DNA thereby, can be used as an alternative of blood and as well as it can serve as an investigative tool in forensic studies.

REFERENCES

1. Butler JM. San Diego: Academic Press. Forensic DNA typing: Biology, technology and genetics of STR markers: 2005.

2. Dahm R. Friendrich Miescher and the discovery of DNA. Dev Biol. 2005 Feb 15;278 (2): 274 – 88.

3. Duarte GR, Price CW, Littlewood JL, Haverstick DM, Ferrance JP, Carrilho E, Lander JP. Characterization of dynamic solid phase DNA extraction from blood with magnetically controlled silica beads. Analyst. 2010 Mar; 135 (3) : 531-7

4. Manjunath BC, Chandrasekhar BR, Mahesh M, Vatchala Rani RM. DNA profiling and forensic dentistry - A review of the recent concepts and trends. JForensic Leg Med. 2011; 18: 191-7.

5. Gong R, Li S. Extraction of human genomic DNA from whole blood using a magnetic microsphere method. Int J Nanomedicine. 2014 Aug 8; 9:3781-9.

6. Hung, PY., Jiang, PS, Lee, EF. et al. Genomic DNA extraction from whole blood using a digital microfluidic (DMF) platform with magnetic beads. MicrocystTechno 23, 313-320(2017).

7. Buckleton J, Triggs CM, Wash SJ. Washington, D.C: CRC press. Forensic DNA evidence interpretation; 2005

8. Immel UD, Kleiber M, Neerman-Arbez M, Kiltschar M. An isolated exclusion in the FGA system. Int Congress Series. 2004; 1261: 14850.

9. Cushwa WT, Medrano JF Effects of blood storage time and temperature on DNA yield and quality. Biotechniques. 1933 Feb; 14 (2): 204-7

10. Damle, Satyawan. (2016). Genetic determination through dental calculus: promise and hope! Contemporary Clinical Dentistry.

11. Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N.,

Grossmann, J., Cappellini, E. (2014). Pathogens and host immunity in the ancient human oral cavity. Nature Genetics, 46, 336-344.

12. Ozga, A. T. et al. Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. American journal of physical anthropology 160, 220-228 (2016).

13. Hasen TV. Simonsen MK, Nielsen FC, hundup YA. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiologic Biomarkers Prev. 2007 Oct; 16.

14. Yamada Y et al Sequencing mitochondrial DNA from a tooth and application to forensic odontology 1997Jun; 15(1):13-6.

15. Gytmenn R, Sweet D. Quantification of Forensic DNA from Various Regions of Human Teeth. Journal of Forensic 2003; 48(3)

16. Philibert RA, Zadorozhnyaya O, Beach SR, Brody GH. Comparison of the genotyping results using DNA obtained from blood and saliva. Psychiatr Genet. 2008; 18 (6): 275-281.

17. Preus HR, Marvik OJ, Selvig KA, Bennike P. Ancient bacterial DNA (a DNA) in dental calculus from archaeological human remains. Journal of Archaeological Science. 2011; 38(8): 1827 – 1831.

18. Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cell: implications for cell lineage and studies on disease susceptibility. PLoSOne. 2012;(7): e41361.

19. Aftenie, L., Franciuc, I., Martinescu, A., & Honcea, A. Comparison Between Currently Used Blood Samples and New Saliva DNA Collection Method For Quality Of Genomic DNA And Genotyping, ARS Medica Tomitana, 2012, 18 (1), 19-23.

20. De La Fuente, C., S. Flores, and M. Moraga. 2013. DNA from human ancient bacteria: A novel source of genetic evidence from archaeological dental calculus. Archaeometry 55: 767-778.

21. Khare P, Raj V, Chandra S, Agarwal S. Quantitative and qualitative

assessment of DNA extracted from saliva for its use in forensic identification. Journal of Forensic Dental Sciences, 2014 May ;6(2): 81-85.

22. Weyrich, Laura & Dobney, Keith & cooper, Alan. Ancient DNA analysis of dental calculus. Journal of human evolution, 2014;79.

23. Ozga, A. T. et al. Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. American journal of physical anthropology 160, 220-228 (2016).

24. Weyrich LS, Duchene S, Soubrier J, Arriola L, Llamas B, Breen J. Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. Nature. 2017Apr20;544(7650):357-361.

25. Mann AE, Sabin S, Ziesemer K, Vagene AJ, Schroeder H, Ozga AT et al. Differential preservation of endogenous human and microbial DNA in dental calculus and dentin. Scientific Reports. 2018; 8:9822.

26. Rubio L, Sioli JM, Gaitan MJ, Martin-de-las-Heras S. Dental color measurement to predict DNA concentration in incinerated teeth for human identification. PLoS ONE. 2018;13(4): e0196305.

27. Ottoni C, Guellil M, Ozga AT, et al. Metagenomic analysis of dental calculus in ancient Egyptian baboons. Sci Rep. 2019;9(1):19637.

28. Singh U, Goel S. Estimation and quantification of human DNA in dental calculus: A pilot study. J Forensic Dent Sci. 2017;9(3): 149-152.

29. Ziesemer KA, Ramos-MadrigalJ, AE, Brandt BW, sankaranarayanan K, OzgaAT et al. The efficacy of whole human genome capture on ancient dental calculus and dentin. American Journal of physical anthropology. 2019;168(3):496-509.

30. Velsko IM Yates JAF, Aron F, Hagan RW, Frantz LAF, Loe L et al. Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. Microbiome. 2019 ;7(102): 1-20.

31. Lippolis C, Cavallini A, Panzarino G, SandroSaponetti S (2019) Long-term persistence of Saliva-derived micro-RNA in human dental calculus for Forensic Sic Today 5(1):001-007.

32. Koehn K et al Effect of sodium hypochlorite decontamination on the DNA recovery from human teeth. International Journal of Legal Medicine, 06 Nov 2019,134(1):93-99

33. Carrasco P et al. Optimizing DNA recovery and forensic typing of degraded blood and dental remains using a specialized extraction method, comprehensive qPCR sample characterization, and massively parallel sequencing. International Journal of Legal Medicine volume 134, pages 79-91(2020).

34. Sawafuji R, Saso A, Suda W, Hattori M, Ueda S. Ancient DNA analysis of food remains in human dental calculus from the Edo period, Japan. PLoS ONE. 2020.

35. Forshaw, R et al. Dental calculus - oral health, forensic studies and archaeology: a review. *Br Dent J* **233**,961–967(2022)

36. Samantha H. Blatt, Jake R. Shields, and Amy R. Michael. Dental Calculus Reveals Life History of Decedents in Forensic Cases: An Anthropological Perspective on Human Identification. Forensic Genomics. Mar 2022.5-16.

37. A DNA EvidencePrimer.The National Law Enforcement andCorrections TechnologyCentreSystem2000 https://www.ojp.gov/pdffiles1/nij/nlectc / 211825. pdf

38. Deoxyribonucleic Acid (DNA) Fact Sheet. National Human Genome Researchinstitutehttps://www.genome.gov/about-genomics/fact-sheet/Deoxyribonucleic Acid-Fact -Sheet.

39. Doleac, Jennifer L.,The Effects of DNA databases on Crime (August 1, 2016)

40. National Research Council (US) Committee on DNA Technology in Forensic Science. DNA Technology in Forensic Science. Washington (DC): National Academies press (US); 1992. Introduction. Available from :https://www,ncbi.nlm.nih.gov/book/NBK234533/.bbh

41. Lee K and Tripathi A (2020) parallel DNA Extraction From Whole Blood for Rapid Sample Generation in Genetic Epidemiological Studies. Front. Genet. 11:374.

42. Carvalho SP, Sales-peres A, Ribeiro-Bicudo LA, Silva RH. Quality evaluation of DNA obtained from stored human saliva and its applicability to identification in forensic dentistry. Rev odontocienc2010; 25:48-53.

43. Sinha , Parul & Raj, Vineet & Chadra, Shaleen & Agrawal, Suraksha. (2014). Quantitative and qualitative assessment of DNA extracted from saliva for its use in forensic identification. Journal of forensic dental sciences.

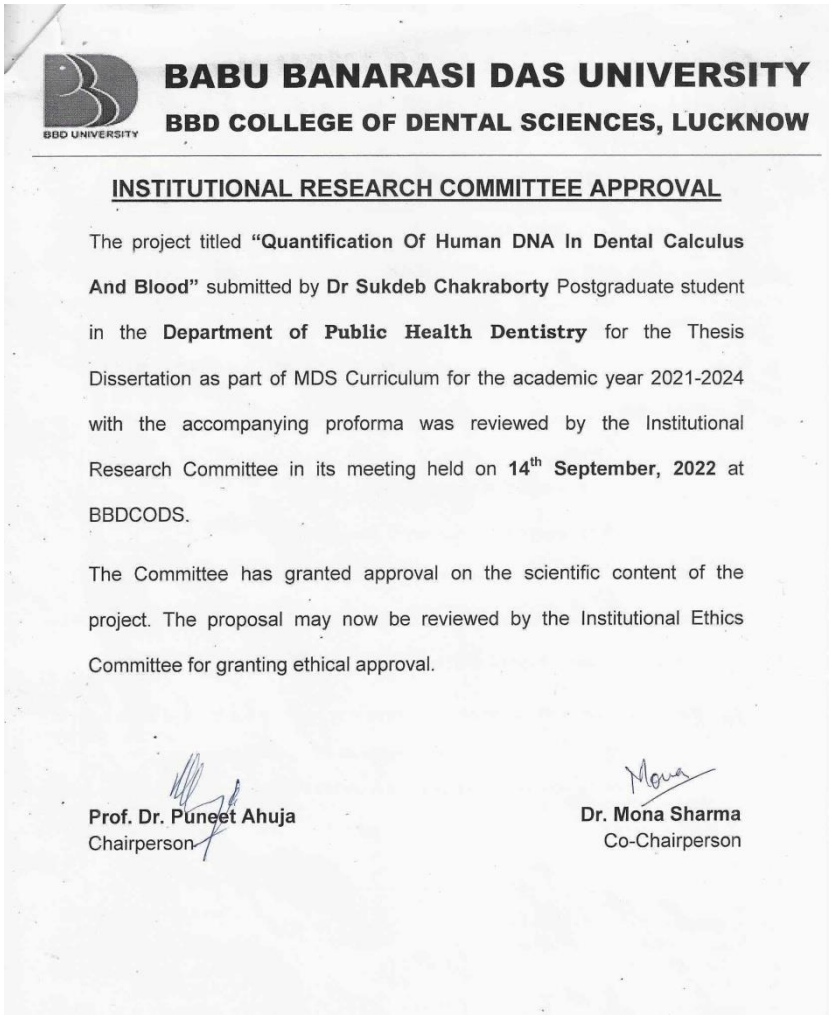
44. Gong R, Li S. Extraction of human genomic DNA from whole blood using a magnetic microsphere method. Int J Nanomedicine.2014 Aug 8;9:37819.

45. Rosinger S, Nutland S, Mickelson E, Varney MD, Boehm BO, Olsem GJ, Hansen JA, Nicholson I, Hilner JE, Perdue LH, Pierce JJ, Akolkar B, Nierras C, Steffes MW; T1DGC. Collection and processing of whole blood for transformation of peripheral blood mononuclear cells and extraction of DNA: the type 1 Diabetes Genetics Consortium. Clinical Trials 2010;7(1Suppl):S65-74.

ANNEXURE

ANNEXURE I

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL



BABU BANARASI DAS UNIVERSITY

BBD COLLEGE OF DENTAL SCIENCES, LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Quantification Of Human DNA In Dental Calculus And Blood"** submitted by **Dr Sukdeb Chakraborty** Postgraduate student in the **Department of Public Health Dentistry** for the Thesis Dissertation as part of MDS Curriculum for the academic year 2021-2024 with the accompanying proforma was reviewed by the Institutional Research Committee in its meeting held on **14th September, 2022** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Dr. Puneet Ahuja
Chairperson

Dr. Mona Sharma
Co-Chairperson

ANNEXURE II

ETHICAL CLEARANCE LETTER

BABU BANARASI DAS UNIVERSITY
BBD COLLEGE OF DENTAL SCIENCES, LUCKNOW

BBDCODS/IEC/09/2022 **Dated: 16th September, 2022**

Communication of the Decision of the Xth Institutional Ethics Sub-Committee Meeting

IEC Code: 23

Title of the Project: Quantification Of Human DNA In Dental Calculus And Blood.

Principal Investigator: Dr Sukdeb Chakraborty **Department:** Public Health Dentistry

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr Sukdeb Chakraborty,

The Institutional Ethics Sub-Committee meeting comprising following members was held on 15th September, 2022.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry
2.	Dr. Praveen Singh Samant Member	Prof. & Head, Department of Conservative Dentistry & Endodontics
3.	Dr. Jiji George Member	Prof. & Head, Department of Oral Pathology & Microbilogy
4.	Dr. Amrit Tandan Member	Professor, Department of Prosthodontics and Crown & Bridge
5.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopaedics

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI, thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Prof. Dr. Puneet Ahuja
Principal
BBD College of Dental Sciences
BBD University, Lucknow

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

Lakshmi Bala 16/09/22

Dr. Lakshmi Bala
Member-Secretary
Institutional Ethics Sub-Committee (IEC)
BBD College of Dental Sciences
BBD University, Lucknow

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

ANNEXURE III

CASE PERFORMA

QUANTIFICATION OF HUMAN DNA IN DENTAL CALCULUS AND BLOOD.

OPD NO.

1. **NAME-**
2. **PARENT'S NAME-**
3. **AGE/SEX-**
4. **ADDRESS-**
5. **CONTACT NO-**
6. **DATE OF SAMPLE COLLECTION-**

Signature of the investigator-

ANNEXURE IV

Certificate Of Incorporation Of DNA Research Centre

प्रारुप 1
पंजीकरण प्रमाण–पत्र

कॉर्पोरेट पहचान संख्या : U93000AP2008PTC060478 2008 - 2009

मैं एतदद्वारा सत्यापित करता हूँ कि मैसर्स
BIOAXIS DNA RESEARCH CENTRE PRIVATE LIMITED

का पंजीकरण, कम्पनी अधिनियम 1956 (1956 का 1) के अंतर्गत आज किया जाता है और यह कम्पनी प्राइवेट लिमिटेड है।

यह निगमन–पत्र आज दिनांक पांच अगस्त दो हजार आठ को मेरे हस्ताक्षर से हैदराबाद में जारी किया जाता है।

Form 1
Certificate of Incorporation

Corporate Identity Number : U93000AP2008PTC060478 2008 - 2009
I hereby certify that BIOAXIS DNA RESEARCH CENTRE PRIVATE LIMITED is this day incorporated under the Companies Act, 1956 (No. 1 of 1956) and that the company is private limited.

Given under my hand at Hyderabad this Fifth day of August Two Thousand Eight.

(RAMAKRISHNAN D)
सहायक कम्पनी रजिस्ट्रार / Assistant Registrar of Companies
आंध्र प्रदेश
Andhra Pradesh

कम्पनी रजिस्ट्रार के कार्यालय अभिलेख में उपलब्ध पत्राचार का पता :
Mailing Address as per record available in Registrar of Companies office:
BIOAXIS DNA RESEARCH CENTRE PRIVATE LIMITED
#12-13-1249, 1ST FLOOR, BESIDE ANDHRA BANK, NEAR ST.ANN'S HIGH SCHOOL, TARNAKA
SECUNDERABAD - 500017,
Andhra Pradesh, INDIA

DrillBit

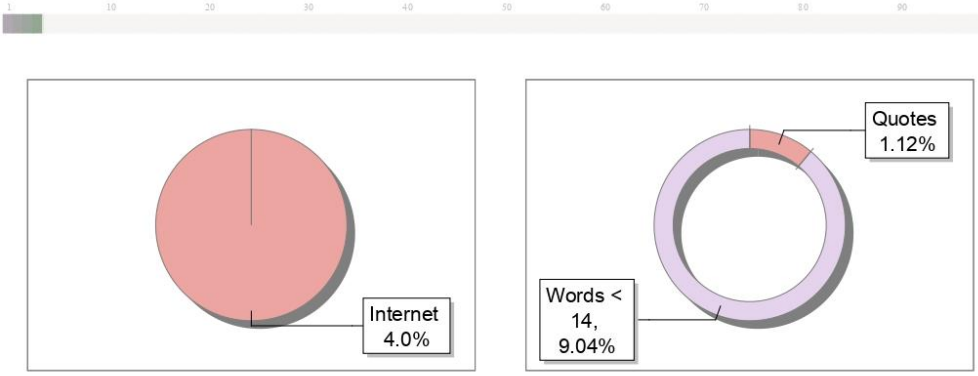
The Report is Generated by DrillBit Plagiarism Detection Software

Submission Information

Author Name	SUKDEB CHAKRABORTY
Title	QUANTIFICATION OF HUMAN DNA IN DENTAL CALCULUS A BLOOD : A CROSS-SECTIONAL, DESCRIPTIVE STUDY
Paper/Submission ID	1428416
Submitted by	amarpal.singh056@bbdu.ac.in
Submission Date	2024-02-13 12:59:19
Total Pages	3
Document type	Dissertation

Result Information

Similarity **4 %**



Exclude Information

Quotes	Excluded
References/Bibliography	Excluded
Sources: Less than 14 Words %	Excluded
Excluded Source	**0 %**
Excluded Phrases	Not Excluded

Database Selection

Language	English
Student Papers	Yes
Journals & publishers	Yes
Internet or Web	Yes
Institution Repository	Yes

A Unique QR Code use to View/Download/Share Pdf File