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Quantification of Human DNA in Dental Calculus and Blood: A Cross-Sectional, Descriptive Study

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KEYWORDS

Dental calculus, Blood, human DNA, DNA identification, mineralized plaque.

ABSTRACT:

Background: Whole blood is the most common sample type used for obtaining high purity DNA but previous studies have reported that DNA is also present in dental calculus.So 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 reporting to the OPD of Department of Public Health Dentistry, Babu Banarasi das college of Dental Sciences, Lucknow. The samples of dental calculus and blood were collected and underwent DNA analysis.

Results: The mean of concentration of DNA in blood (70.19±14.55µg/ml) was higher thatin dental calculus (54.92± 11.729µg/ml), but the difference in both the means were found to be non-significant ($p<0.218$).

Conclusion: 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.

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. Every human being is characterized based on the unique DNA sequence which are used in forensic identification.[1,2] It was first discovered by Friedrich Miescher in 1869, while he was investigating the composition of leukocytes, he isolated an unknown Substance that behaved differently to the proteins in solution what is now known as deoxyribonucleic acid (DNA).[3] 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]DNA profiling is a standard forensic DNA system used in human identification, criminal case work, as well as paternity testing, worldwide.[7] Calculus is derived from Greek word "Calcis," used for various kinds of stones. Dental calculus is a form of hardened dental plaque which is formed by precipitation of minerals from saliva and gingival crevicular fluid on the teeth.Calculus is composed of both inorganic (mineral) and organic (cellular and extracellular matrix) components.[8]Several studies have found that saliva, dental calculus, dentin and 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.[9] Once mineralized within dental calculus, human DNA, and proteins can preservefor 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

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require the destruction of bone or tooth tissue during analysis, and this has been a cause of concern for many native and indigenous communities.Using advanced sequencing technologies, anthropologists demonstrate that human DNA can be significantly enriched from dental calculus enabling the reconstruction of whole mitochondrial genomes for maternal ancestry analysis and also dental calculus is more resistant to environmental contamination than any other sources of DNA.[10]Since collection of blood samples may not be feasible in large epidemiologic studies as participants are dispersed and collection of blood samples is prohibitively expensive and painful.[11] Therefore, less invasive and more cost-efficient procedures for collecting DNA are needed. Thus, this study was conducted to compare the amount of human DNA in dental calculus and blood.

METHODOLOGY

This Cross-sectional, Descriptive 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. The s**tudy population comprised of** Males and females of age group 18-60 years from the OPD of Public Health Dentistry Department. A total of 35 patients, who fulfilled the eligibility criteria were recruited in the study as study samples using Convenient sampling method. The study was conducted over a period of three months from November 2022 to January 2023.

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.

Ethical clearance and Consent: This study was reviewed and approved by the ethical committee of BBD College Of Dental Sciences, Lucknow (BBDU). Written informed consent was obtained from all the participants.

Armamentarium Required: Plane Mouth Mirror, Dental Probe, Tweezers, Aluminium Foils, Sterile Plastic Pouches, Betadine, Sterile Cotton Rolls, Kidney Trays, Disposable Gloves, Disposable Mouth-mask, Disposable Salivary Ejector tips, 3ml EDTA sterile tubes, Dry-Ice, Portable Container

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. 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 Research Laboratoryfor human DNA analysis. For the collection of blood samples, the subjects were referred to the Department of Oral pathology. Three-millilitre whole blood samples were collected in 3ml EDTA collection sterile tubes and samples were stored at -20℃ until human DNA extraction.

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.

RESULTS

In the present study there were 35 subjects out of which 20 were female and 15 were male [Graph 1]. The amount of human DNA in dental calculus varied from 40 to 69 µg/ml with mean quantity of 54.92 µg/ml while in blood varied from 54 to 83 µg/ml with mean quantity of 70.19 µg/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 ± 17.172 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 ±

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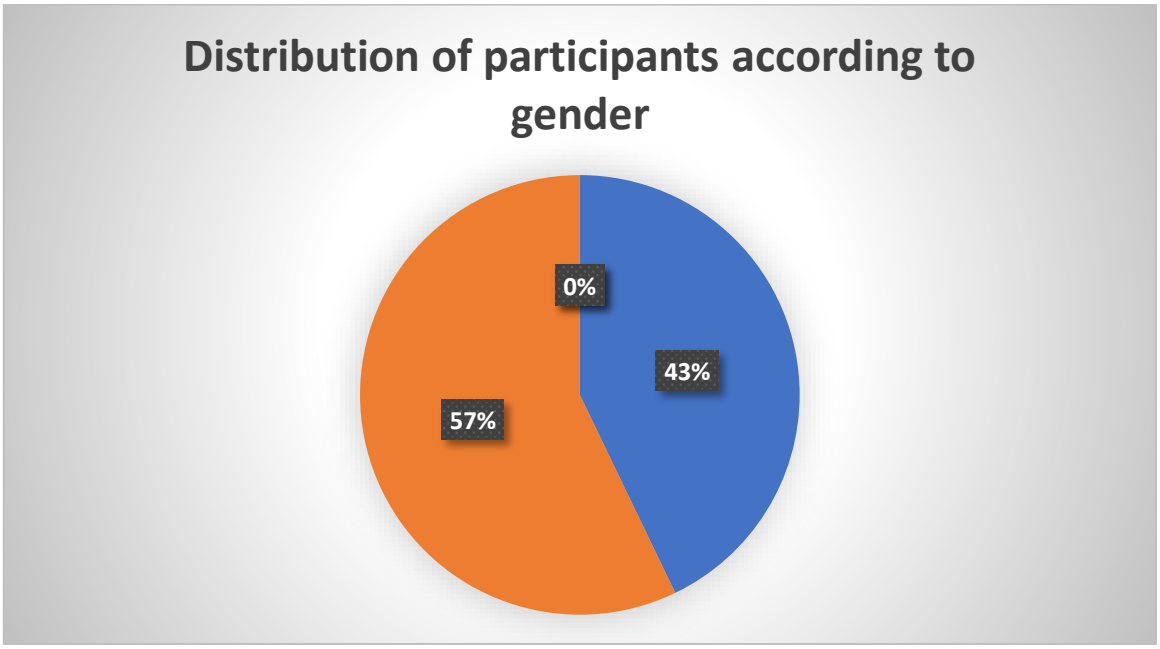
8.825 µg/ml) and in calculus was (57.14 ± 8.565 µg/ml) respectively and this difference was also found to be non-significant (p<0.23) [Table 1].

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 [Table 2].



Graph 1: Distribution of participants according to gender

Table 1: The distribution of mean of concentration in blood and calculus among male and female

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 2: The comparison of human DNA conc. in blood and dental calculus among male and female.

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. Forensic DNA analysis has played a crucial role in the investigation and resolution of thousands of crimes since the last 1980s.[12] Forensic scientists can use DNA in blood, skin, dental calculus, saliva or hair found at a crime scene to identify a matching DNA of an individual.[13] Characterization, or "typing" of blood and other body fluids has been used for forensic purpose for more than 50 years.[14] Blood is a fluid that moves through the vessels of a circulatory system. Whole

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blood is a common biological starting sample for DNA extraction as it contains red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma, with DNA found in the nuclei of WBCs.[15] DNA found in blood is of high quality and is used in forensics, cancer diagnoses, and various another biological test. 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 asource of DNA allows certain technical advantages over the use of blood.[16] 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.[17] 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.[18]Recent research on dental calculus has shown that it is the richest known source of DNA in the archaeological record, exceeding the DNA content found in bone and dentin by more than an order of magnitude.[19]

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)[20]** whereas in contrast other study done by Silke **Rosinger et al. (2010)**[21]which shows 20-25 mg/ml of DNA was 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 etal(2019)**[9]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)**[22] showed human DNA ranging from 21 to 37µg/ml in dental calculus which might be due to the collection and decontamination method used in the study.**Samantha H Blatt et al in 2022**[23] 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 found in DNA quantification values. DNA concentrations from blood were found to be in the optimal range while DNA obtained from dental calculus was near the optimal range. 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.

CONCLUSION

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.

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. 2005Feb 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. 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).

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www.jchr.org

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7. Buckleton J, Triggs CM, Wash SJ. Washington, D.C: CRC press.Forensic DNA evidence interpretation; 2005
8. Damle, Satyawan. (2016). Genetic determination through dental calculus: promise and hope! Contemporary Clinical Dentistry.
9. 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.
10. 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).
11. 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.
12. 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
13. 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.
14. Preus HR, Marvik OJ, Selvig KA, Bennike P. Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. Journal of Archaeological Science. 2011; 38(8): 1827 – 1831.
15. 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.
16. Weyrich, Laura & Dobney, Keith & cooper, Alan. Ancient DNA analysis of dental calculus. Journal of human evolution, 2014;79.
17. 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.
18. 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.
19. Lee K and Tripathi A (2020) parallel DNA Extraction From Whole Blood for Rapid Sample Generation in Genetic Epidemiological Studies. Front. Genet. 11:374.
20. 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.
21. 21. Rosinger S, Nutland S, Mickelson E, Varney MD, Boehm BO, Olsem GJ et al, 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.
22. 22.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.
23. 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.