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Original Research Article

Personnel identification using DNA extracted from saliva retrieved by Dental prosthesis

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Abstract

Background: Forensic odontology plays a pivotal role in human identification, particularly in scenarios where conventional methods are ineffective. Dental prosthesis, by virtue of their resilience and contact with oral fluids, can retain saliva containing epithelial cells, offering a viable source of DNA.
Aim: Personnel identification using DNA extracted from saliva retrieved by Dental prosthesis.
Materials and Methods: This observational cross-sectional study was conducted on 60 individuals wearing complete, removable partial, or fixed dental prosthesis. Saliva samples was collected using sterile swabs, and DNA was extracted using a silica column-based method. PCR amplification was performed for amelogenin gene-based sex determination and VNTR analysis for individual identification.
Results: The results indicated that DNA profiling was successful in 88.3% of samples. Fixed prosthesis were the most satisfactory in terms of DNA concentration and purity (mean concentration: 5.20 ng/µL; mean A260/280: 1.812), whereas complete dentures were less satisfactory across these parameters. Sex determination using the amelogenin gene was achievable in 97.3% of samples, and full STR profiles was obtained in 66.7% of cases. These findings highlighted the potential of fixed prosthesis as reliable sources for forensic DNA analysis.
Conclusion: Dental prosthesis can serve as reliable sources of forensic DNA evidence. Salivary DNA recovery from these prosthesis demonstrates strong potential in personal identification, particularly in mass disaster or unidentified body scenarios.

Keywords: Forensic odontology, DNA extraction, Dental prosthesis, Saliva, Personal identification, Amelogenin.

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1. Introduction

Forensic identification plays a vital role in legal and investigative processes, particularly in situations involving decomposed, burnt, or fragmented remains where conventional methods like fingerprinting or facial recognition are not viable. In such cases, forensic odontology becomes indispensable, leveraging the durability and uniqueness of dental structures for human identification.[1]

Dental prosthesis such as complete dentures, removable partial dentures, and fixed prosthesis are not only resilient to post-mortem degradation but also in continuous contact with saliva, a rich source of epithelial cells and nuclear DNA. Saliva offers a non-invasive, easily collectible, and infection-safe medium for DNA extraction, making it highly suitable for forensic purposes.[2]

With advancements in molecular techniques like polymerase chain reaction (PCR) and short tandem repeat (STR) analysis, even trace amounts of salivary DNA can now be amplified and profiled for personal identification and sex determination. Unlike denture labeling methods, which require preemptive action, DNA analysis from prosthesis provides a retrospective means of identification, even without prior patient information.[3]

Despite its potential, there remains limited research on the forensic viability of salivary DNA collected from dental

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prosthesis. Thus, this study aims to assess the personnel identification using DNA extracted from saliva retrieved by dental prosthesis. This study highlights the forensic potential of salivary DNA in associating individuals to dental prosthesis, emphasizing its importance in personal identification and legal investigations.

2. Objectives

The objectives of the study were to collect saliva from individuals wearing dental prosthesis, to evaluate the salivary DNA extract collected from these individuals, and to establish the association between the DNA extracted from the dental prosthesis and personnel identification.

3. Materials and Methods

This observational cross-sectional study is conducted in the Department of Prosthodontics and Crown & Bridge at Seema Dental College & Hospital, Rishikesh, Uttarakhand in collaboration with DNA labs, A Centre for Applied Sciences, Dehradun. The study assess the personnel identification using DNA extracted from saliva retrieved by dental prosthesis. Ethical clearance is obtained from the institutional review board, and informed consent is taken from all participants prior to sample collection.

3.1. Sample size and participant selection

A total of 60 participants are enrolled in the study based on a convenience sampling method. The sample size is calculated using a proportion-based statistical formula referencing a 92% DNA extraction success rate reported in earlier studies, with a 95% confidence level and a 5% margin of error. Individuals were eligible for inclusion if they were willing to participate and provided informed consent, and if they were wearing one of the following types of dental prostheses: complete denture, removable partial denture, or fixed denture prosthesis. Participants were excluded if they were undergoing chemotherapy or radiotherapy, had xerostomia, were uncooperative during clinical examination, or were unwilling to provide consent. The selected participants were then evenly divided into three groups based on the type of prosthesis worn, with 20 subjects in each group: complete denture, removable partial denture, and fixed prosthesis.

3.2. Study tools

The present study utilizes sterile sample collection kits (Pal Surgical and Medical: PSM) (**Figure 1**), a storage box with ice preservative solution (Aristo) (**Figure 2**), mouth mirror (**Figure 3**), autoclave (Max life: W.A-12365), disposable gloves (B'self health care), and masks (Asgard). Laboratory equipment includes a biosafety cabinet (UTECH: 84199090), deep refrigerator (Voltas, –20°C) (**Figure 4**A), Vortex (Tarson: 1801066) (**Figure 4**B), and RNA extraction machine (InstaNX Mag-32 by HiGenoMB, HIMEDIA). Reagents include Proteinase K (Bioron, Lot No. 172018741) (**Figure 5**A), Buffer AL (QIAGEN) (**Figure 5**B), and 0.9% sodium chloride solution (Hi Line-NS). PCR is performed using Rotor-Gene Q (QIAGEN: RD21911) (**Figure 6**A), and DNA visualization is done via UV Transilluminator (Bench Top Lab System: UV-1) (**Figure 6**B). Additional materials include rayon swabs, silica columns, primers, Taq polymerase, agarose, ethidium bromide, and dye. Documentation tools include consent forms, pens, and stamp pads.

Figure 1: Sterile sample collection kits for saliva retrieval

Figure 2: Storage box with preservative solution (Ice) to maintain sample integrity

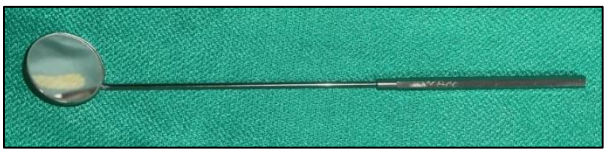

Figure 3: Mouth mirror

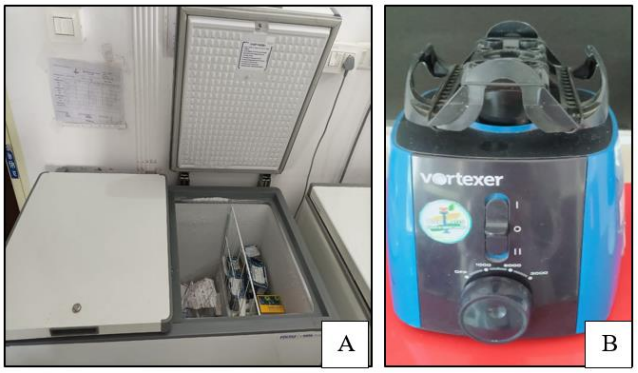

Figure 4: A): Deep fridge -20o C; **B):** Vortexer

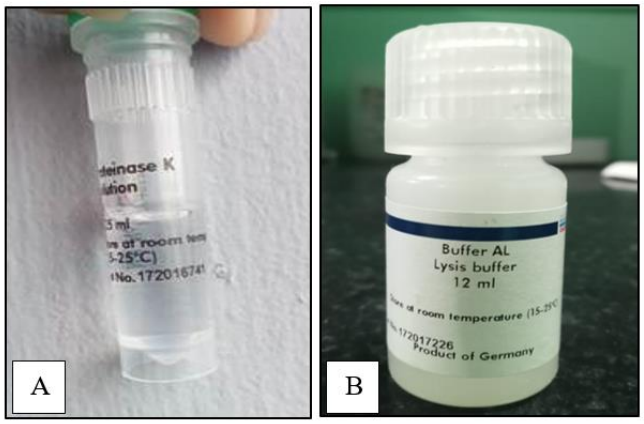

Figure 5: A): Proteinase K Solution 1.25 ml; **B):** Buffer AL Lysis buffer 12 ml

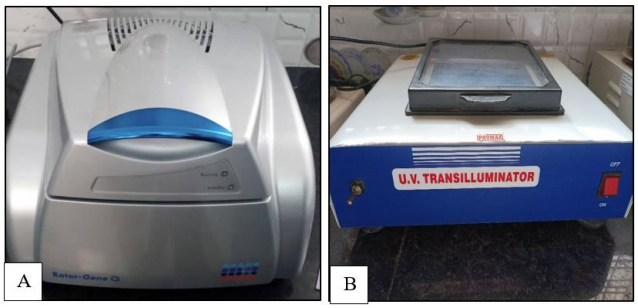

Figure 6: A): Real time PCR**; B):** UV Transilluminator for visualizations of DNA bands

3.3. Sample collection

Sterile rayon swabs were used to collect saliva from the intaglio surface of prostheses (**Figure 7**). Swabs were immediately transferred to vials containing 0.9% NaCl buffer and stored at –20°C until DNA extraction.

Figure 7: The prosthesis were gently wiped with a sterile cotton swab

3.4. DNA extraction and quantification

DNA was extracted using the silica column-based method (HiMedia InstaNX Mag-32). The protocol involved enzymatic lysis with Proteinase K and buffer AL, ethanol precipitation, washing with buffers AW1 and AW2, and elution in buffer AE. DNA concentration and purity were evaluated spectrophotometrically (A260/A280 ratio).

3.5. PCR amplification

PCR amplification was carried out using:

1. Amelogenin gene primers (AMEL-A/B) for sex determination (106 bp for X, 112 bp for Y)
2. GH436/437 primers targeting D4S43 VNTR locus for STR profiling

Thermal cycling conditions included initial denaturation (94°C, 5 min), 35 cycles of denaturation (94°C, 1 min), annealing (60°C, 1 min), extension (72°C, 1 min), and final elongation (72°C, 7 min). Products were visualized using 1.6% agarose gel electrophoresis with ethidium bromide staining under UV illumination.

3.6. Statistical analysis

Data were analyzed using SPSS v23.0. ANOVA and chi-square tests were employed for intergroup comparisons, with $p < 0.05$ considered statistically significant.

4. Results

Out of the 60 salivary samples collected from dental prosthesis, 53 samples (88.3%) yielded viable genomic DNA suitable for downstream analysis. Successful extraction was confirmed through agarose gel electrophoresis, which revealed well-defined bands indicating high-quality DNA integrity. Polymerase chain reaction (PCR) amplification was carried out using 35 thermal cycles. Amplification success was visually confirmed under UV illumination, with distinct electrophoretic bands indicating robust DNA replication.

4.1. Sex determination using amelogenin gene

Sex determination was successfully achieved in 58 of the 60 samples (97.3%) through PCR amplification of the amelogenin gene. The presence of two bands—106 bp (X allele) and 112 bp (Y allele)—was indicative of male individuals, while female individuals exhibited a single band at 106 bp corresponding to the X chromosome.

Two samples (2.7%) failed to produce discernible bands, likely due to DNA degradation or suboptimal concentration during extraction. Notably, samples retrieved from fixed prostheses exhibited more prominent and distinct bands, suggesting superior DNA preservation and integrity relative to other prosthesis types.

4.2. VNTR (D4S43 Locus) analysis

Variable number tandem repeat (VNTR) analysis targeting the D4S43 locus yielded partial short tandem repeat (STR) profiles in 50 out of 60 samples (83.3%). Full STR profiles were successfully obtained in 40 samples (66.7%).

Among the three denture groups, fixed prostheses demonstrated the highest profiling success:

1. Fixed dentures: Full STR profiles in 85% of cases

2. Removable dentures: 70% success
3. Complete dentures: 45% success

These findings underscore the impact of prosthesis type on DNA retention and subsequent profiling capability.

4.3. Electrophoretic band visualization

Figure 8 illustrate representative electrophoretic gel images depicting PCR bands corresponding to amelogenin gene-based sex identification and STR profiling from select samples. Distinct bands at 106 bp and 112 bp validate successful amplification and are consistent with gender-specific markers.

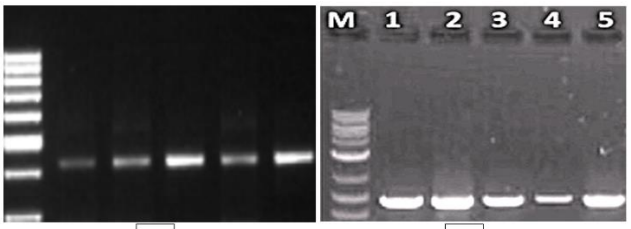

Figure 8: A,B): Agarose Gel Electrophoretic image depicting as below depicting 106 bp and 112 bp segments of the amelogenin gene

Further analysis of quantitative parameters revealed statistically significant differences among the three denture groups:

DNA yield (ng/µL): Table 1 and Graph 1 indicate that the fixed denture group exhibited the highest mean DNA concentration (5.20 ng/µL), followed by the removable denture (4.38 ng/µL) and complete denture (3.17 ng/µL) groups. ANOVA analysis showed a highly significant difference ($F = 650.379$, $p < 0.001$), confirming that prosthesis type significantly affects DNA yield.

DNA purity (A260/A280): As shown in Table 2 and Graph 2, the fixed denture group demonstrated the highest DNA purity (1.812), compared to removable (1.781) and complete denture groups (1.718). The ANOVA results indicated a significant difference among groups ($F = 165.331$, $p < 0.001$), with fixed dentures yielding DNA closest to the ideal purity range.

DNA integrity (%): **Table 3** and show that fixed dentures yielded the highest DNA integrity (92.00%), followed by removable (84.70%) and complete dentures (74.25%). ANOVA revealed a significant effect of prosthesis type on DNA integrity ($F = 360.133$, $p < 0.001$).

DNA profiling success: According to Table 4 and Graph 4, successful DNA profiling was achieved in 80% of complete denture, 90% of removable, and 95% of fixed denture samples. Although fixed dentures had the highest success rate, the association was not statistically significant ($\chi^2 = 2.264$, $p = 0.322$).

DNA extraction success: As shown in Table 5 and Graph 5, extraction success rates were 90% (complete), 95% (removable), and 100% (fixed) denture groups. Despite the apparent trend, chi-square analysis showed no significant association ($\chi^2 = 2.105$, $p = 0.349$).

Partial STR profiles: Table 6 and Graph 6 reveal that partial STR profiles were obtained in 70% of complete denture, 85% of removable, and 95% of fixed denture cases. This association was statistically significant ($\chi^2 = 4.560$, $p = 0.012$), indicating that denture type influences partial STR profiling success.

Full STR profiles: According to Table 7 and Graph 7, full STR profiles were obtained in 45% (complete), 70% (removable), and 85% (fixed) denture samples. Chi-square analysis showed a significant association between denture type and full STR profile success ($\chi^2 = 7.350$, $p = 0.025$), with fixed prostheses showing the highest success rates.

This observational cross-sectional study evaluated DNA extraction and profiling from saliva samples collected via complete, removable, and fixed dentures. There were no significant differences in age ($p = 0.056$) or gender distribution ($p = 0.934$) among groups. Fixed Dentures showed the highest DNA yield (5.20 ng/µL), purity (A260/A280 = 1.812), and integrity (92.00%), with statistically significant differences compared to other denture types ($p < 0.001$ for all). Although overall DNA profiling success was not significantly associated with denture type ($p = 0.322$), Fixed Dentures achieved higher rates of partial (95%, $p = 0.012$) and full STR profiles (85%, $p = 0.025$). These findings indicate that denture type particularly Fixed Dentures significantly affects the quality and success of DNA profiling from salivary samples.

Table 1: DNA yield (ng/µL)

Denture Type	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Complete Denture	20	3.1650	.20072	3.0711	3.2589
Removable Denture	20	4.3800	.18806	4.2920	4.4680
Fixed Denture	20	5.2000	.14510	5.1321	5.2679
'F' statistic	650.379				
df	2				
p-value	.000*				

Table 2: DNA purity (A260/A280 Ratio)

Denture Type	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Complete Denture	20	1.7180	.02016	1.7086	1.7274
Removable Denture	20	1.7810	.01619	1.7734	1.7886
Fixed Denture	20	1.8120	.01281	1.8060	1.8180
'F' statistic	165.331				
df	2				
p-value	.000*				

Table 3: DNA integrity (%)

Denture Type	N	Mean	Std. Deviation	95% Confidence Interval	
				Lower bound	Upper bound
Complete Denture	20	74.2500	2.33678	73.1564	75.3436
Removable Denture	20	84.7000	2.15455	83.6916	85.7084
Fixed Denture	20	92.0000	1.77705	91.1683	92.8317
'F' statistic	360.133				
df	2				
p-value	.000*				

Table 4: Overall DNA profiling success

Profiling	Complete Denture	Removable Denture	Fixed Denture	Total
No	4	2	1	7
	20.0%	10.0%	5.0%	11.7%
Yes	16	18	19	53
	80.0%	90.0%	95.0%	88.3%
Total	20	20	20	60
	33.3%	33.3%	33.3%	100.0%
Chi square statistic	2.264[a]			
df	2			
p-value	.322 (NS)			

Table 5: DNA extraction success

Profiling	Complete Denture	Removable Denture	Fixed Denture	Total
No	2	1	0	3
	10.0%	5.0%	0.0%	5.0%
Yes	18	19	20	57
	90.0%	95.0%	100.0%	95.0%
Total	20	20	20	60
	33.3%	33.3%	33.3%	100.0%
Chi square statistic	2.105[a]			
df	2			
p-value	.349 (NS)			

Table 6: Partial STR profiles

Profiling	Complete Denture	Removable Denture	Fixed Denture	Total
No	6	3	1	10
	30.0%	15.0%	5.0%	16.7%
Yes	14	17	19	50
	70.0%	85.0%	95.0%	83.3%
Total	20	20	20	60
	33.3%	33.3%	33.3%	100.0%
Chi square statistic	4.560[a]			
df	2			
p-value	.012*			

Table 7: Full STR profiles

Profiling	Complete Denture	Removable Denture	Fixed Denture	Total
No	11	6	3	20
	55.0%	30.0%	15.0%	33.3%
Yes	9	14	17	40
	45.0%	70.0%	85.0%	66.7%
Total	20	20	20	60
	33.3%	33.3%	33.3%	100.0%
Chi square statistic	7.350[a]			
df	2			
p-value	.025*			

5. Discussion

Forensic odontology is a significant outgrowth of forensic medicinal sciences, focusing on the examination, handling, and demonstration of dental evidence in legal settings.[4] Over the past decade, it has become increasingly important as it offers specialized methods for identifying individuals when conventional methods like fingerprinting are unavailable. Its primary application is in identifying deceased individuals, particularly in mass mortality events like air crashes and natural disasters, where dental information is often the most accurate identifier due to the resilience of dental tissues.[5] Forensic odontologists analyze remains at crime or disaster scenes, taking detailed photographs, cranial measurements, dental impressions, and X-rays.[6] They collect and document dental evidence, inspect the oral cavity for distinctive characteristics, and use specialized imaging methods to analyze dental patterns and bite marks,[7] which are instrumental in abuse or assault cases. However, forensic odontology relies on existing dental records for comparison, and bite mark analysis faces controversy due to variability in human dentition and potential misinterpretation. Recent developments in molecular biology, such as DNA extraction from dental tissue, have added to its usefulness, providing a source of genetic material even in harsh conditions.[8] Forensic odontologists also participate in national and international disaster victim identification (DVI) activities, with agencies like INTERPOL developing guidelines that include forensic odontology in disaster response planning.[9] Prosthodontics plays a crucial role in forensic identification through dental prosthesis like dentures, crowns, and implants, which are durable and tailor-made.[10] Labeling dentures with personal information allows prompt identification, and historical instances highlight the significance of prosthodontics in identification.[11] For unmarked dentures, forensic specialists compare recovered dentures with ante-mortem casts, and DNA analysis offers an alternative when physical comparison is impossible.[12] Saliva on dentures is an important source of DNA, and advances in molecular biology have improved DNA recovery from saliva in terms of reliability and speed.[13]

Studies by Inoue et al.[14] and Chauhan et al.[15] demonstrated the utility of dental prostheses as alternative DNA sources, especially when soft tissues are degraded or inaccessible. Inoue et al. successfully extracted DNA from acrylic resin prostheses, confirming their value for sex determination and personal identification via PCR amplification of epithelial cells, with the amelogenin gene effectively distinguishing males (two bands) from females (one band).[14] Chauhan et al. further validated that DNA from prostheses remains viable for forensic analysis and STR profiling, with fixed prostheses yielding better quality DNA due to closer mucosal contact.[15] However, both studies highlighted limitations such as variable DNA yield, storage effects, and contamination risks, suggesting refinement of retrieval protocols and the use of alternative approaches like ultrasonic agitation. Complementing this, Lee et al.[16] and Sherier AJ et al.[17] noted that alternative DNA extraction methods and next-generation sequencing (NGS) could improve recovery from degraded samples, while advanced amplification techniques enhance forensic applicability. Forouzesh further demonstrated that hard, non-porous surfaces like fixed dentures retain more epithelial cells, enabling superior DNA recovery and higher STR profiling success.[18] The present study corroborates these conclusions, as fixed prosthesis produced the highest DNA purity and integrity, ultimately resulting in the most successful STR analysis.

This study aimed to assess personnel identification using DNA extracted from saliva retrieved by dental prosthesis, investigating saliva as a viable DNA source when conventional samples are unavailable. The study collected saliva samples from 60 individuals wearing different types of dentures, stored them at -20°C, and extracted DNA using the Silica Column Method. The extracted DNA was amplified using PCR, with sex determination via the amelogenin gene and personal identification through VNTR analysis. The results demonstrated a high success rate in DNA recovery, with fixed dentures producing the highest quality DNA. Sex determination using the amelogenin gene was successful in most cases, confirming its reliability as a forensic tool. These findings are consistent with prior studies, confirming that fixed dental prosthesis provide superior DNA retention due to their greater contact with the oral mucosa, which facilitates better epithelial cell adherence.

6. Conclusion

Dental prosthesis, commonly used in prosthodontic treatments, show strong potential as forensic tools. This study confirms that salivary DNA retrieved from prosthesis is of adequate quality and integrity for personal identification. Fixed prosthesis yielded the highest DNA concentration, purity, and profiling success. Sex determination was successful in 97.3% of cases, with full STR profiles in 66.7% of samples. This non-invasive, cost-effective method may serve as a valuable aid in forensic investigations, especially in DVI and cases involving unidentified remains.

7. Source of Funding

None.

8. Conflict of Interest

None.

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