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RESEARCH ARTICLE

Medicolegal Benefits of DNA-Based Tests in Human Burned Remnants: A Comparative Cohort Study of Mitochondrial DNA Analysis and STR Typing

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Abstract: Burned human remains present major challenges in forensic identification due to extensive thermal degradation of soft tissue, bone, and cellular DNA. This study compares the effectiveness of Short Tandem Repeat (STR) typing and mitochondrial DNA (mtDNA) sequencing in burned remains received for forensic analysis between November 2010 and August 2012 at the School of Medical Sciences and Research, Sharda University. Twenty-five samples representing varying degrees of burn severity were analysed using standard nuclear DNA and mtDNA extraction protocols, STR multiplex amplification, and sequencing of mitochondrial hypervariable regions. STR typing produced complete or partial profiles in 64% of cases, with success strongly dependent on burn severity and complete failure in samples exposed to extreme temperatures. In contrast, mtDNA sequencing achieved a 96% success rate and remained amplifiable even in severely burned and calcined specimens. The results demonstrate the superior resilience and reliability of mtDNA compared with STR markers in thermally compromised remains. The findings support a tiered forensic workflow in which STR typing is attempted first, followed by mtDNA analysis when nuclear DNA is degraded, thereby improving the likelihood of successful identification in fire-related forensic investigations.

Keywords: Burned remains; forensic DNA analysis; STR typing; mitochondrial DNA (mtDNA); degraded DNA; thermal degradation; human identification; hypervariable regions; calcined bone; forensic genetics.

INTRODUCTION

Identification of human remains recovered from fire scenes represents one of the most challenging tasks in forensic science, largely due to the extensive thermal damage inflicted on soft tissues, skeletal elements, and cellular DNA structures [1]. Fire accidents, vehicular explosions, electrical burn injuries, homicidal burn cases, and mass disasters frequently result in remains that are charred, calcined, or fragmented, leaving few viable morphological markers for conventional identification. In such conditions, DNA analysis becomes indispensable; however, its success depends greatly on the type and quality of DNA preserved after exposure to high temperatures [2]. Short Tandem Repeat (STR) typing has long been considered the gold standard for forensic human identification, owing to its high discriminatory power, compatibility with international databases, and well-standardized multiplex amplification kits [3]. Despite these advantages, the effectiveness of STR profiling declines significantly in burned remains due to the susceptibility of nuclear DNA to heat-induced degradation, crosslinking, and fragmentation. Even temperatures as low as 200-300°C can begin to denature nuclear DNA, while exposure above 400°C often results in complete or near-complete loss of amplifiable STR loci, leading to allele dropout or total amplification failure [4,5].

In contrast to nuclear DNA, mitochondrial DNA (mtDNA) exhibits greater resistance to degradation under extreme environmental conditions. Its high intracellular copy number-often several hundred to thousands per cell-combined with its circular, double-stranded structure and relatively protected position within the mitochondria, makes mtDNA more stable under conditions that typically destroy nuclear DNA [6]. Studies have consistently demonstrated that mtDNA can often be successfully amplified even from skeletal remains, teeth, or soft tissues exposed to severe burning, calcination, or prolonged environmental contamination [7]. This robustness makes mtDNA sequencing especially valuable in cases where STR analysis yields only partial profiles or fails completely. Although mtDNA has a lower discriminatory power due to maternal inheritance and the lack of recombination, it serves as a critical supplementary tool in forensic casework involving severely degraded biological samples [8].

Given these differing properties, a systematic comparison of STR typing and mtDNA analysis in burned remains is essential to guide forensic laboratories toward evidence-based decision-making when dealing with thermally compromised samples. Several researchers have reported that mtDNA achieves higher success rates in high-temperature or highly degraded conditions, while STR typing is more effective in samples exposed to moderate or minimal burning [9,10]. However, limited studies have evaluated both methodologies simultaneously within the same set

of burned forensic specimens, particularly in the context of Indian forensic casework. The present study, conducted from November 2010 to August 2012 at the School of Medical Sciences and Research, Sharda University, aims to fill this gap by systematically evaluating the performance of STR profiling in comparison with mtDNA sequencing in burned human remains. By assessing profile success rates across varying degrees of thermal exposure, this study seeks to establish a practical forensic workflow and confirm whether mtDNA analysis should be prioritized when nuclear DNA degradation is severe. Ultimately, this comparison can help strengthen the scientific basis for selecting appropriate genetic methods in fire-related identification cases and improve outcomes in medico-legal investigations where accurate identification is essential for both judicial processes and humanitarian reasons [11].

MATERIAL AND METHODS

This comparative study was conducted at the School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh, using burned human remains received for forensic examination between November 2010 and August 2012. A total of twenty-five cases involving fire-related fatalities were included based on the availability of sufficiently preserved skeletal or soft-tissue material suitable for DNA extraction. Burn severity was assessed using scene reports, autopsy documentation, and visual features such as charring, calcination, colour change, and structural integrity, as described in earlier studies on thermally altered remains [12,13].

Sample preparation followed strict contamination control protocols, including the use of disposable instruments, UV-irradiated surfaces, and pre-PCR and post-PCR segregation, in accordance with international forensic DNA laboratory standards [14]. External debris, soot, and charred material were removed mechanically, and samples were cleaned with sterile water and 10% sodium hypochlorite to minimise surface contamination. Bone fragments and teeth were processed by removing outer layers through sanding or drilling, followed by cryogenic grinding to obtain fine powder, as recommended in previous analyses of heat-damaged skeletal tissues [15]. Charred soft tissue samples were trimmed to remove exposed surfaces and homogenised using proteinase digestion.

DNA extraction was performed separately for nuclear and mitochondrial DNA. Nuclear DNA was extracted using organic phenol-chloroform extraction and silica-based column purification, methods known for their efficiency in recovering highly fragmented or low-yield DNA from compromised remains [16]. Mitochondrial DNA extraction employed modified silica-based protocols optimised for degraded samples with low total DNA quantity, ensuring preferential retention of small fragments that commonly persist in burned specimens [17]. Extracted DNA was quantified using fluorometric methods capable of detecting low-concentration, degraded DNA, as recommended for forensic samples exposed to extreme conditions [18].

STR typing was conducted using commercially available multiplex kits such as AmpFlSTR® Identifiler® or PowerPlex® systems, selected for their robust amplification of multiple loci in a single reaction. Amplification targeted standard autosomal STR markers commonly used in forensic identification. PCR was performed following manufacturer guidelines, with cycle numbers adjusted to enhance sensitivity for low-template degraded DNA. Amplified products were separated by capillary electrophoresis on an automated genetic analyser. STR profiles were evaluated according to international interpretation standards, including thresholds for peak height, allele drop-out assessment, and stutter recognition, as applied in degraded forensic material [19].

For mitochondrial DNA analysis, hypervariable regions I and II (HV1 and HV2) of the control region were amplified using overlapping primer sets to accommodate the fragmented nature of the DNA. This primer strategy has been widely used in studies involving burned or environmentally degraded samples to maximise recovery of amplifiable mtDNA fragments [20]. PCR products were sequenced bidirectionally using Sanger sequencing, and resulting sequences were aligned and compared to the revised Cambridge Reference Sequence (rCRS). In cases where available, maternal relatives provided reference samples for comparison, following the established forensic practice of utilising maternal lineage markers for identification when nuclear DNA is insufficient [21].

Data analysis involved comparing the success rates of STR typing and mtDNA sequencing across categories of burn severity. A profile was considered successful if it yielded interpretable results according to established forensic criteria-complete or reliable partial STR profiles, or high-quality mtDNA sequences capable of lineage comparison. Statistical significance between the success rates of the two methods was evaluated using the chi-square test, with a significance level of $p < 0.05$, consistent with analytical approaches used in previous comparative DNA degradation studies [22].

RESULTS AND OBSERVATIONS:

A total of twenty-five burned human remains were analysed to compare the performance of STR typing and mtDNA sequencing. The results demonstrated a clear trend of decreasing nuclear DNA integrity with increasing burn severity, while mitochondrial DNA remained significantly more resilient.

DNA Yield and Sample Condition

Nuclear DNA yield showed a marked decline in samples exposed to moderate and high temperatures, with several samples falling below detection thresholds. In contrast, mtDNA was detected in the majority of samples regardless of thermal exposure. Overall, 92% of samples showed amplifiable mtDNA, compared with 64% that produced any interpretable STR data.

Table 1. STR Typing Success by Burn Severity (n = 25)

Burn Severity Category	Number of Samples	Successful STR Profiles	Partial STR Profiles	Failed STR Profiles	Overall STR Success (%)
Low-Moderate (<400°C)	14	12	2	0	85.7%
High (400-650°C)	9	4	1	4	44.4%
Extreme (>650°C)	2	0	0	2	0%
Total	25	16	3	6	64%

STR analysis demonstrated strong performance in low-to-moderate burn cases, but success declined drastically as burn severity increased. All samples exposed to temperatures above approximately 650°C failed to generate usable STR profiles, reflecting severe nuclear DNA degradation.

Table 2. mtDNA Sequencing Success by Burn Severity (n = 25)

Burn Severity Category	Number of Samples	Successful mtDNA Profiles	Partial/Low-Quality Sequences	Failed mtDNA Profiles	Overall mtDNA Success (%)
Low-Moderate (<400°C)	14	14	0	0	100%
High (400-650°C)	9	8	1	0	88.9%
Extreme (>650°C)	2	2	0	0	100%
Total	25	24	1	0	96%

mtDNA sequencing yielded near-complete success across all categories of burn severity. Even in extremely burned samples, mtDNA was preserved sufficiently to permit amplification and sequencing of HV regions.

Comparison of STR and mtDNA Performance

mtDNA demonstrated a significantly higher amplification and profiling success compared with STR typing (96% vs 64%). Statistical analysis using the chi-square test confirmed a significant difference between the methods ($p < 0.01$).

Table 3. Overall Comparison of STR and mtDNA Profiling Success

Method	Total Samples Analysed (n=25)	Successful Profiles	Success Rate (%)
STR Typing	25	16	64%
mtDNA Sequencing	25	24	96%
Statistical Result	-	$p < 0.01$	-

These findings clearly indicate that mtDNA sequencing is substantially more reliable than STR analysis in burned remains, particularly in cases involving high thermal exposure.

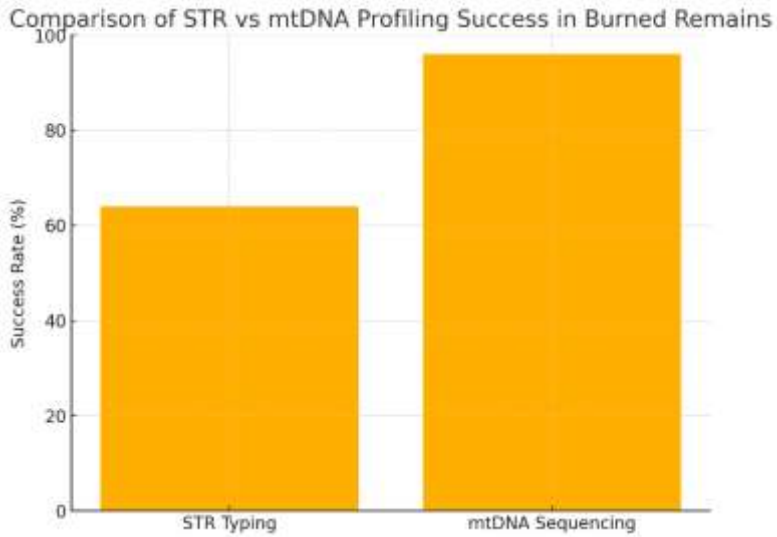

Figure 1. Success rate (%) of STR typing versus mitochondrial DNA (mtDNA) sequencing in burned remains (n = 25). STR performance declined sharply with increasing burn severity, whereas mtDNA sequencing remained highly successful even in severely charred and calcined samples.

DISCUSSION

The findings of this study clearly demonstrate that mitochondrial DNA sequencing is significantly more reliable than STR typing for the identification of burned human remains, particularly those exposed to high or extreme temperatures. The marked decline in STR success with increasing thermal exposure reflects the well-established susceptibility of nuclear DNA to heat-induced degradation, including depurination, crosslinking, fragmentation, and strand breakage, which severely compromise its amplifiability [23]. Previous studies have shown that nuclear DNA begins to degrade at temperatures as low as 200-300°C, with catastrophic loss of STR loci typically occurring above 400°C, findings that are consistent with the present results [24]. In contrast, mtDNA sequencing yielded near-complete success across all burn severity categories, confirming its known robustness in degraded, skeletonised, or thermally altered tissues. The high intracellular copy number of mtDNA, its circular and compact structure, and its partial protection within the mitochondrial matrix give it a substantial advantage over nuclear DNA under destructive environmental conditions [25]. Multiple studies have reported successful mtDNA recovery from severely burned bone, teeth, and charred tissues, even in cases where nuclear DNA is undetectable, supporting the results observed in this study [26,27].

The complete failure of STR typing in samples exposed to temperatures above approximately 650°C underscores the limitations of nuclear DNA when remains are partially calcined or charred beyond the threshold of cellular preservation. This outcome aligns with earlier research demonstrating that combustion, thermal fracturing, and calcination drastically reduce nuclear DNA survival in cortical bone and dentine [28]. Meanwhile, mtDNA's reliable amplification in the same high-severity samples further highlights its value as a secondary identification tool.

The influence of sample type is also noteworthy. Dense cortical bone and intact teeth, which offer structural protection against heat penetration, are known to preserve DNA better than soft tissues in fire events [29]. However, even within such protected tissues, mtDNA consistently outperformed nuclear DNA in this study, emphasising that DNA type, rather than bone microstructure alone, accounts for the difference in survival. These findings align with previous research indicating that mtDNA may persist in calcined bone where nuclear DNA is no longer recoverable [30].

Despite the technical advantages of mtDNA, its limitations must also be recognised. Because mtDNA is maternally inherited and does not undergo recombination, its discriminatory power is lower than that of STRs, and individuals from the same maternal lineage share identical sequences [31]. Therefore, mtDNA cannot replace STR analysis in routine forensic casework when viable nuclear DNA is available. However, the results of this study reinforce its importance as a complementary method, particularly in fire incidents, mass disasters, and long-postmortem intervals where nuclear DNA is often compromised [32].

The statistical comparison in this study, demonstrating a significantly higher success rate for mtDNA over STR typing, supports existing recommendations for a tiered forensic workflow. STR typing should be attempted first due to its capacity for individualisation, but mtDNA sequencing should be promptly employed when STR results are partial, incomplete, or absent due

to degradation [33]. Such an approach not only maximises identification success but also conserves sample material, improves laboratory efficiency, and reduces delays in medico-legal reporting.

In summary, the present findings contribute to the growing body of evidence supporting the integration of mtDNA sequencing as a standard procedure in the forensic analysis of burned or severely degraded remains. Further research incorporating miniSTRs, next-generation sequencing, and quantification of mtDNA copy number across burn gradients may enhance future identification strategies and broaden the toolkit available for challenging forensic scenarios [34].

CONCLUSION

This study demonstrates a clear and consistent difference in the performance of STR typing and mitochondrial DNA sequencing in burned human remains. STR analysis, although the preferred method for routine forensic identification due to its high discriminatory power, showed decreasing success with increasing burn severity and failed entirely in samples exposed to extreme temperatures. In contrast, mitochondrial DNA sequencing exhibited exceptional resilience and produced reliable profiles across all categories of thermal damage, including cases where nuclear DNA had degraded beyond recoverability. These findings reinforce the importance of incorporating mtDNA analysis into forensic workflows involving highly compromised or heat-damaged specimens. A tiered approach, wherein STR typing is attempted first and mtDNA analysis follows when STR results are partial or absent, offers the most effective strategy for maximizing identification success. The results underscore the need for forensic laboratories to utilise both nuclear and mitochondrial DNA methods to ensure accurate, comprehensive, and scientifically robust identification in fire-related and other degradation-intensive forensic scenarios.

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