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Impact of Chemical Exposure on the Quality of DNA Extracted from Teeth: A Potential Application for Forensic Study in Burkina Faso

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Abstract

Introduction: Forensic investigations involving the discovery of a corpse or human remains aim to identify the individual involved. Sometimes, the body is subjected to chemical solutions with the intention of disintegrating or completely altering it. This further complicates identification. Nevertheless, it is possible to recover sufficient DNA from dental tissues. The present study aims to determine the effects of chemical solutions on the physical structure of teeth and also on their DNA. **Methods:** Teeth from Sus scrofa pigs were subjected to acidic (NHO_3, H_2SO_4, and HCl) and basic (NaOH) solutions for specific durations (up to 144 h). Observations were recorded at regular intervals to document the effects on the tissues. After sampling, DNA was extracted using the PrepFiler® BTA Forensic DNA extraction kit. The extracted DNA was quantified using a BioDrop spectrometer and then amplified by conventional PCR using ACTB and mtDNA primers. The resulting amplicons were subjected to 2% agarose gel electrophoresis. After migration, the fragments were visualized under UV light in a trans-illuminator. **Results:** The teeth were completely dissolved in HCl and HNO_3 solutions after 8 hours of immersion. The other solutions had no significant impact on the physical integrity of the teeth. A total of 32 teeth samples were obtained after exposure to the chemical solutions. The DNA obtained was of sufficient quantity and acceptable purity for the majority of samples. **Conclusion**: This study has shown that chemical so-

lutions affect biological tissues and their DNA. Amplification of nuclear and mitochondrial DNA sequences by conventional PCR confirms that teeth remain the best source of DNA due to their resistance to degradation factors.

Keywords

Tooth, DNA, ACTB, mtDNA, PCR

1. Introduction

Perpetrators of murderous crimes display perfidy to prevent any identification of their victims. This perfidy involves abominable methods to hide or destroy the corpses of their victims. The ways in which perpetrators attempt to dispose of their victims range from carelessly leaving the corpse in a shallow grave to the total annihilation of the body through fire or even chemical means, such as Mexican-American drug cartels and other cases around the world [1]. Criminal minds use commercially available acids to destroy the body as a whole or its parts to avoid personal identification of the victim [2].

In this situation, genetic identification is the last resort to identify these victims, with other means of identification proving ineffective [3].

Teeth, being the most durable structures of the human body, persist even after the other skeletal structures have decayed [4], and withstand extreme physico-chemical conditions, representing a unique potential source of genetic material from which identification must be made [5]-[8].

In Burkina Faso, a West African country, victims are sometimes discovered as human remains, unidentifiable by conventional methods. Forensic teams thus face major difficulties in identifying these human remains. Several cases of human remain identification have been documented in the scientific literature. However, in Burkina Faso, there are no such data. We are trying to understand to what extent we can still hope to exploit the capabilities of molecular biology through the extraction of deoxyribonucleic acid (DNA) to identify individuals from teeth subjected to chemical solutions. To this end, Sus scrofa pig teeth were immersed in acidic and basic solutions to study their effects on both. We performed conventional PCR amplification of porcine ACTB and mtDNA sequences.

The aim of this study was therefore to provide data on the morphological effects on the teeth and the genetic analysis of degraded biological tissues.

2. Material and Methods

2.1. Teeth Samples

In the context of Burkina Faso, it is not easy to find sufficient quantities of human bones and teeth to conduct this study. Indeed, ethical and societal constraints complicate the acquisition of human tissues [9]. For this reason, we opted to use an animal model. The pig is the best animal model and has long been used as a

substitute for humans in several areas of scientific research [10] [11]. Furthermore, it exhibits physiological and genomic similarity to humans and is readily available and affordable [12]. In addition, the fact that the pig genome shares a high degree of sequence and chromosomal structure homology with humans is a significant advantage [13].

2.2. Samples Treatment

Each tooth sample was soaked in 25 ml of various chemical solutions prepared as follows:

- 37% hydrochloric acid solution: 14.6 ml of 37% hydrochloric acid + 10.4 ml of water;
- 95% sulfuric acid solution: 24 ml of 95% sulfuric acid + 1 ml of water;
- 65% nitric acid solution: 16.25 ml of nitric acid + 8.75 ml of water;
- 2 N sodium hydroxide solution: 2 g of NaOH crystals + 24 ml of water.

For each solution, an observation and sampling program was established at 1/2, 1, 2, 4, 8, 15, 24, 48, 72, 96, 120, and 144 hours.

2.3. DNA Extraction

After immersion, teeth samples were rinsed in deionised distilled water for 5 min, then air-dried for 24 hours. For each sample, 50 mg of powder obtained by cryogrinding with liquid nitrogen using a porcelain mortar were used for DNA extraction with PrepFiler® BTA Forensic DNA, using the manufacturer's protocol.

2.4. DNA Quantification

After extraction, DNA concentrations were quantified using the BioDrop µLite V7141 V1.0.2 spectrophotometer, and DNA purity was assessed by calculating the ratio of optical densities A260 and A280.

2.5. PCR Performing

PCR was performed using primers to amplify the Sus scrofa ACTB gene and mitochondrial DNA [12] (**Table 1**). The ACTB gene, which codes for β-actin, is the most stable of the reference genes and is widely used in porcine molecular biology studies [14] [15]. mtDNA, on the other hand, is frequently used in parentage studies, is abundant in cells, and is more resistant to degradation due to the protection offered by mitochondria. Searching for these genes in DNA obtained from samples thus allows us to determine the possibilities of resolving identification problems using human dental remains.

The amplification program was: 95˚C for 15 minutes for polymerase activation, followed by a phase of 40 cycles (95˚C for 1 minute for the DNA denaturation step, 68˚C for 1 minute corresponding to primer hybridization, and 72˚C for 1 minute corresponding to elongation), and finally a final extension at 72˚C for 7 minutes as described by Samsuwan *et al.*, 2018 [12]. PCR products were separated by gel electrophoresis using a 2% agarose gel.

Table 1. Primers used for PCR.

Gene	Primers	Sequences (5' 3')	ID Sequence NCBI	Size (pb)
ACTB	ACTB-F ACTB-R	AGATCGTGCGGGACATCAAG GAGAGAAGCCCGACTGAGC	DQ452569.1	273
mtDNA	MT DNA-F MT DNA-R	GGAGCAGTGTTCGCCATTAT TTCTCGTTTTGATGCGAATG	KT372134.1	294

3. Results

3.1. Effects of Chemical Solutions on Teeth

Samples resisted acids and bases in different ways. The effects of each chemical agent on biological tissues are described below.

3.1.1. Effects of 37% Hydrochloric Acid Solution

Teeth were collected after 1/2 hour, 1 hour, 2 hours, and 4 hours of immersion. Teeth sizes gradually decreased over time until complete dissolution after 8 hours of immersion. The colour of the solution changed, varying from yellowish to black (Figure 1).

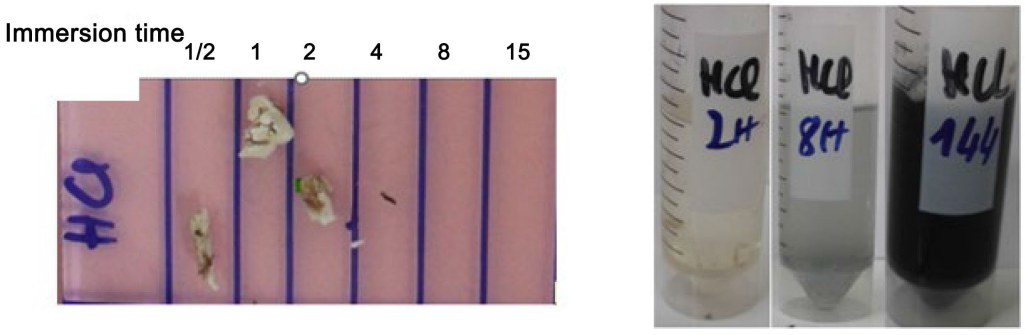

Figure 1. Effect of HCl on teeth and color change of acid solutions.

3.1.2. Effects of 95% Sulfuric Acid Solution

Teeth were still present after 144 hours of immersion in the sulfuric acid solution. Corrosion of the teeth and whitish precipitate formation were also observed (Figure 2, Figure 3).

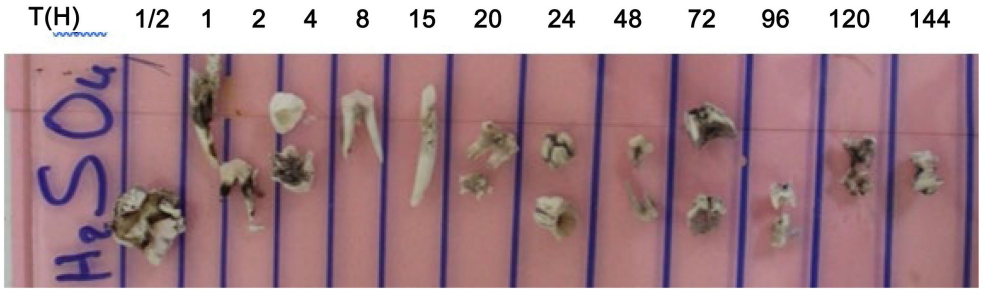

Figure 2. Effect of H_2SO_4 on teeth.

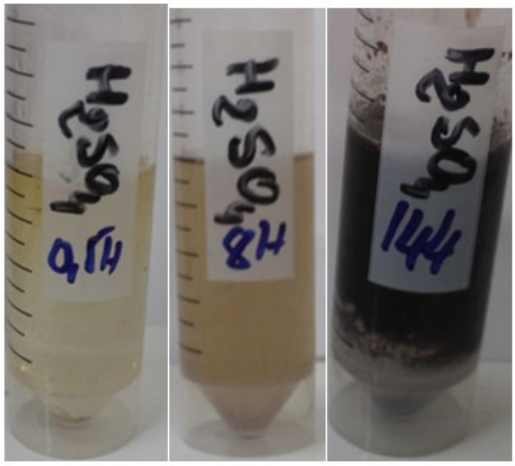

Figure 3. Colour change of H_2SO_4 solutions.

3.1.3. Effects of a 65% Nitric Acid Solution

Immersion in nitric acid also showed a decrease in tooth size over time (**Figure 4**). Complete dissolution was observed after 8 hours of immersion. Samples were collected only at 0.5, 1, 2, and 4 hours of immersion time. There was a discoloration of the solution to yellow (**Figure 5**).

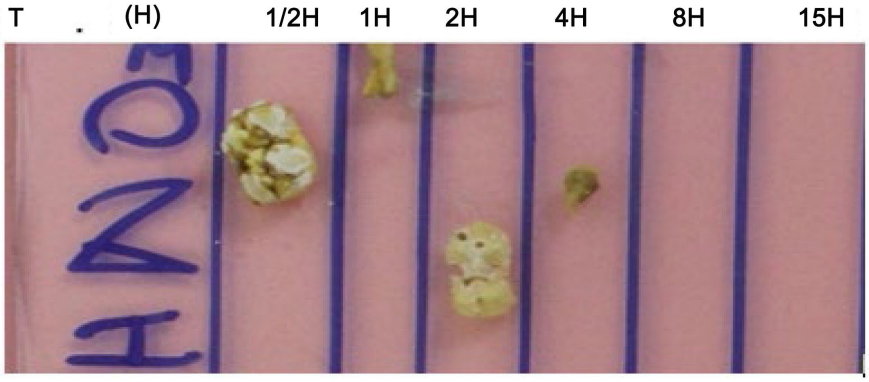

Figure 4. Effect of HNO_3 solution on teeth.

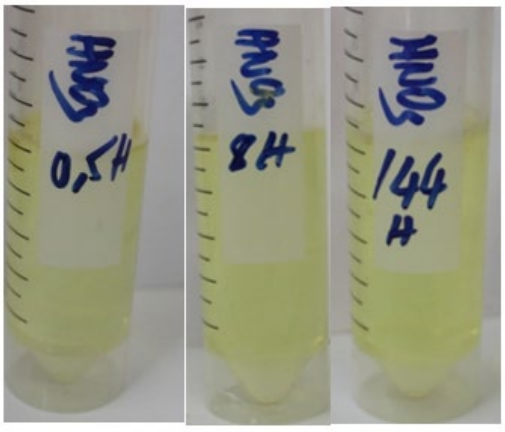

Figure 5. Color change of NHO_3 solutions.

3.1.4. Effects of Sodium Hydroxide

No significant change was observed over time. After immersion, teeth had a whitish colour and their enamel surfaces appeared polished (Figure 6).

Figure 6. Effect of NaOH solution on teeth.

3.2. Result of DNA Quantification

3.2.1. Positive Control

DNA extracted from a non-immersed tooth was taken as a positive control. DNA quantification showed 573.1 ng/µl as the DNA concentration and 2.003 as the ratio A260/A280.

3.2.2. Samples Soaked in Acid and Basic Solutions

After extraction, the DNA concentrations in the teeth from the different chemical solutions were measured and recorded in Table 2.

Table 2. DNA concentration after immersion in chemical solutions.

		DNA concentrations (ng/µl)			
		HCl	$HN0_3$	H_2SO_4	NaOH
Immersion time (H)	0.5	57.39	28.43	24.69	18.24
	1	20.67	14.43	17.99	0.00
	2	23.09	0.00	5.91	0.00
	4		1.20	0.68	0.35
	8		0.06		5.35
	15				4.11
	20				2.94
	24				19.94
	48	0.00	0.00	0.00	5.47
	72	0.00	0.00	0.00	5.62

The following figure compares the DNA concentration curves at various exposure times in different chemical solutions (Figure 7).

3.2.3. Agarose Gel Electrophoresis of PCR Products

PCR products were designated as positive or negative if visualized or not on a 2% agarose gel (Figures 8-10). ACTB amplification was positive for the majority of teeth. Positive results were found for mtDNA only with teeth immersed for 0.5 H

in hydrochloric acid, 1 H in sulfuric acid, 1 H in nitric acid, and 1 H in sodium hydroxide. Results are recorded in Table 3.

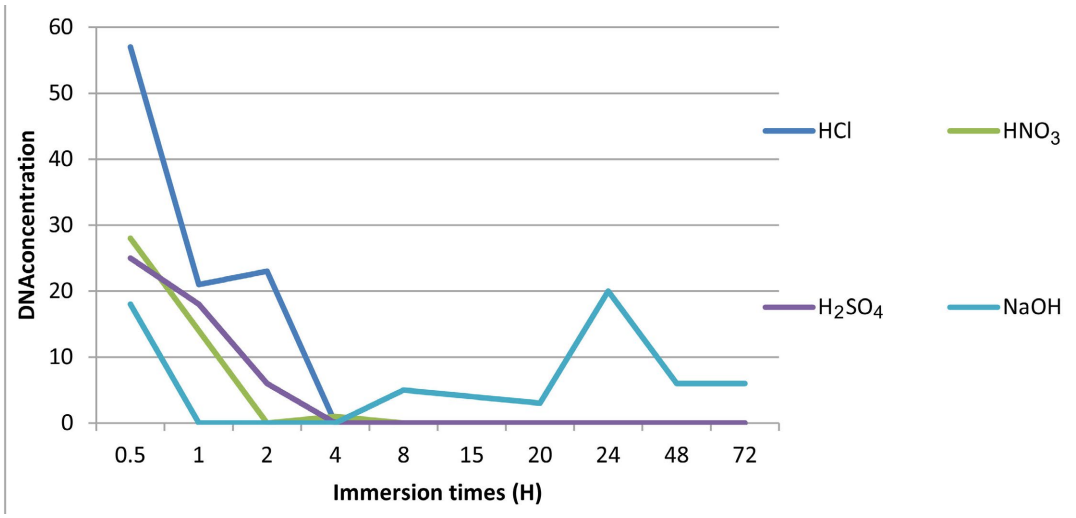

Figure 7. DNA concentration according to immersion times in different solutions.

Table 3. Detection of PCR products on 2% agarose gel.

			Chemical solutions																					
	C+	C−	HCl			HNO_3				H_2SO_4					NaOH									
Immersion time (h)			1/2	1	2	1/2	1	2	4	1/2	1	2	4	8	1/2	1	2	4	8	15	20	24	48	72
ACTB	+	−	+	+	+	+	−	+	+	+	+	0	0	0	+	0	0	0	+	+	+	+	+	+
mtDNA	+	−	+	−	−	−	+	−	−	−	+	0	0	0	−	0	0	0	−	−	−	−	−	−

Legend: + = positive; − = negative; C+ = Positive Control; C−: Negative Control; 0: No DNA after extraction.

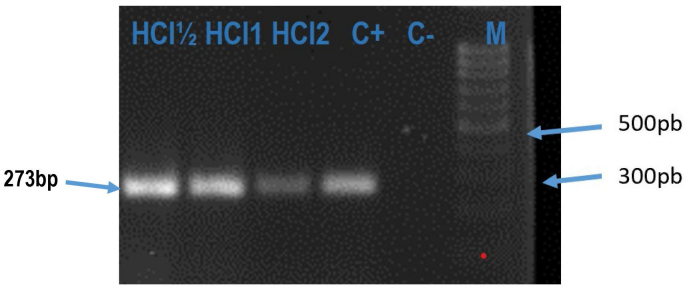

Figure 8. Electrophoresis of ACTB PCR products from teeth in HCl.

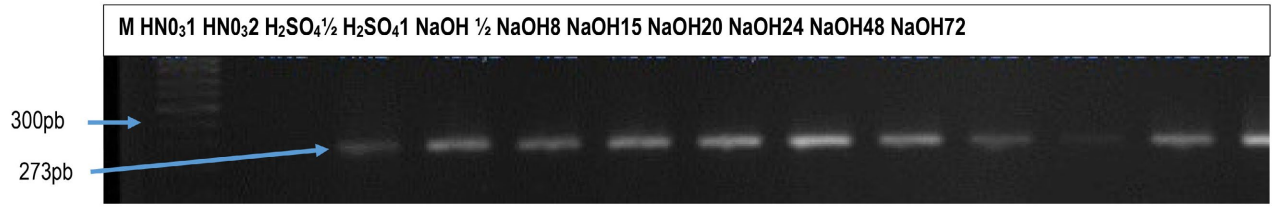

Figure 9. Electrophoresis of ACTB PCR products from teeth in other solutions.

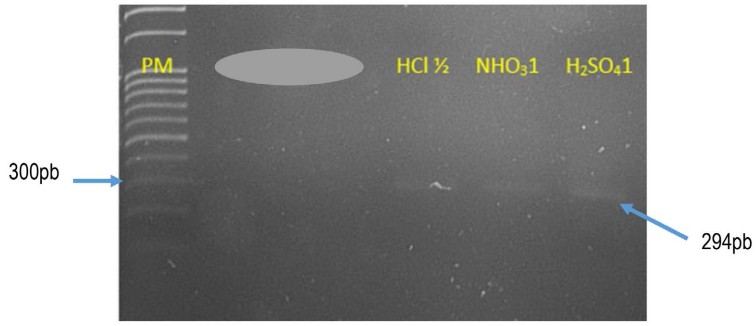

Figure 10. Electrophoresis of mtDNA PCR products from teeth in chemical solutions.

4. Discussion

4.1. Effect of Acidic Solutions on Teeth

Human remains identification is a major problem in some legal cases in Burkina Faso because of the deterioration of biological tissues. During crime scene investigations, human remains, mainly teeth, that have suffered physical and chemical attacks are found. Indeed, homicide perpetrators sometimes use strong acids and bases to dispose of their victims' bodies. These conditions of DNA degradation were reproduced in the present study.

We found that chemical degradation physically affected hard biological tissues. In acidic solutions, we noticed morphological changes depending on the type of acid. Hydrochloric acid and nitric acid completely dissolved teeth after 8 hours of immersion, whereas in sulfuric acid, teeth were removed after up to 144 hours with a whitish precipitate. The study conducted by Mazza *et al.*, 2005 [16], showed that a minimum of 10 days is required for complete dissolution of a tooth in sulfuric acid solution. However, it has been shown that 51% sulfuric acid, like battery acid, is more corrosive than 95% sulfuric acid [17]. In addition, our observations showed that hydrochloric acid and nitric acid are more corrosive than sulfuric acid, and Raj *et al.*, 2013 corroborate this as they also reported complete dissolution with HCl and NHO_3 after 8 hours of immersion [2].

In contrast, other studies have found different outcomes with immersion times of 13 hours for complete tooth dissolution in HCl and 18 hours for HNO_3 [18], and 15 and 20 hours, respectively, for the same acids [19]. Many reasons may explain these differences, including variations in the concentrations of the solutions used in the experiments and the size of the teeth.

Chemical reactions occurring in acidic solutions, upon contact with the teeth, explain some observations. In hydrochloric acid and nitric acid, the teeth form calcium chloride and calcium nitrate salts respectively, which are completely soluble. Consequently, no precipitate forms in these solutions. In contrast, teeth placed in sulfuric acid form an insoluble calcium sulfate salt, which fails to dissolve completely, forming an insoluble precipitate [16] [19].

4.2. Effect of Sodium Hydroxide Solution on Teeth

Sodium hydroxide did not significantly affect teeth. It proved to be the least effec-

tive at degrading teeth. Nevertheless, some surface changes were observed, such as tooth whitening, enamel polishing, and cracking. Our results are consistent with the findings of other authors [20] [21].

4.3. DNA Quantification

In general, immersion experiments in chemical solutions quantitatively affected DNA concentration compared to that found in unimmersed tooth. This reduction varied depending on the type of chemical solution and the immersion time. Depending on the nature of the solution, we were able to quantify DNA in the studied matrices for immersion times of up to 2 hours in hydrochloric acid solution, 4 hours in nitric acid solution, 8 hours in sulfuric acid solution, and 72 hours in sodium hydroxide solution.

We also calculated the A260/A280 absorbance ratios to assess the purity of the extracted DNA, and our analyses showed that the extracted DNA was of acceptable quality for further genetic analysis, with DNA considered pure if the ratio was between 1.8 and 2 [22]. According to the literature, the quality of DNA extracted from teeth depends on several external factors, such as temperature, and physiological factors such as tooth type and pulp weight [23].

It is known that mtDNA has a higher copy number than nuclear ACTB DNA. However, we found in our study that, unlike mtDNA, ACTB DNA was amplified in the majority of teeth samples. Therefore, is mtDNA more susceptible to degradation by chemical solutions than ACTB DNA? Several hypotheses can explain this. First, nuclear DNA is tightly bound to histones, forming chromatin. This structure provides significant physical and chemical protection against damage, including that caused by acids. In contrast, mtDNA is a naked circular molecule and is not enveloped by these protective proteins. Another explanation is the possible presence of PCR inhibitors [24]. These inhibitors may lead to false-negative detection.

5. Conclusion

The perfidy of criminals leads them to use stratagems to conceal their crimes. In Burkina Faso, some discovered human remains had been subjected to chemical attacks, thus complicating the identification process. The present study confirmed that, despite these chemical attacks, these tissues still remain DNA sources for genetic identification.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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