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Bone and Dental DNA Damage Due to Extreme High-Temperature Exposure Through STR-CODIS, Y-STR and MtDNA Examinations

Daño al ADN óseo y dental debido a la exposición a temperaturas extremadamente altas mediante exámenes STR-CODIS, Y-STR y MtDNA

Ahmad Yudianto[1,a,b,c], Wimbuh Tri Widodo[2,b,c], Sonny Kristianto[3,b,c], Fery Setiawan[4,b,d,e], Indah Nuraini Masjkur[5,c], Qurrota A'yunil Huda[6,c], Arif Rahman Nurdianto[7,f]

SUMMARY

Background: *Forensic experts play an important role in forensic science. Identification through DNA analysis is an accurate and stable diagnostic tool. However, along with the development of DNA material examination, problems arise since DNA undergoes degradation, commonly known as degraded DNA. High-temperature exposure is one factor in DNA degradation. Bone and dental tissues are among the materials most resistant to this degradation. Bone and teeth are the most solid parts of the human body because they contain hydroxyapatite and extracellular matrices that protect DNA (nuclear DNA and mtDNA). DNA degradation due to high-temperature exposure on bone and dental DNA samples in forensic identification has been extensively unknown.* **Objective***: This study aimed to analyze DNA damage derived from ribs and second molar teeth caused by extremely high temperatures using a short tandem repeat (STR) CODIS marker.* **Methods:** *This research analyzes DNA degradation of bone and dental materials due to effect of high-temperature exposures (500^{0}C, 750^{0}C, 1 000^{0}C, and 1 250^{0}C for 20, 30, and 40 minutes) based on the loci STR and mini-STR CODIS (CSF1PO, D18S51, D21S11, FGA, D8S1179, D5S820, D7S820, D13S317, D16S539), Y-STRs (DYS19, DYS389, DYS390) and 143-bp and 126-bp mtDNA. Samples consisted of 24 ribs and 24 molars from 7 cadavers.* **Results:** *The analysis showed that teeth were more resistant than bones in protecting DNA from high-temperature exposure. This could be seen in the number of presentations positively detected from loci STR, Y-STRs,*

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ORCID ID: 0000-0003-4754-768X[1]
ORCID ID: 0000-0003-4076-3339[2]
ORCID ID: 0000-0002-3790-2599[3]
ORCID ID: 0000-0002-1426-2074[4]
ORCID ID: 0000-0002-8364-7787[5]
ORCID ID: 0009-0004-1244-4693[6]
ORCID ID: 0000-0002-3178-7112[7]

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[a]Department of Forensic Medicine and Medicolegal, Faculty of Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia. [b]Forensic Science Program, Post Graduate School, Universitas Airlangga, Surabaya, 60115, Indonesia. [c] Human Genetic Study Group Institute of Tropical Disease, Universitas Airlangga, Surabaya, 60115, Indonesia. [d]Oral and Maxillofacial Pathology Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia. [e]Doctoral Program of Medical Science Faculty of Medicine. Universitas Airlangga, Surabaya, 60115, Indonesia. [f] Universitas Anwar Medika, Sidoarjo, Indonesia

*Corresponding author: Ahmad Yudianto
E-mail: yudi4n6sby@yahoo.co.id

and mtDNA. Loci of STR CODIS of bone materials detected by standard primer were D3S1358, D16S539 (1 250^{0}C-20') and CSF1PO (500^{0}C-40'); those of dental materials were D7S820, D8S1179 (1 250^{0}C-40'), D3S1358 (1 250^{0}C-20'), D13S317 (1 000^{0}C-40'), D16S539 (750^{0}C-40'), CSF1PO (750^{0}C-20'). Loci of STR CODIS of bone materials detected by mini primer were D16S539 (750^{0}C-40'), CSF1PO, D12S137 (500^{0}C-40'), and D3S358 (500^{0}C-30'); those of dental materials were CSF1PO (1 250^{0}C-40'), D16S539 (1 000^{0}C-20'), D13S317 (750^{0}C-40'), D3S1358 (750^{0}C-20'), D5S818, D7S820, D8S1179, D18S51 (500^{0}C-40'). The detected locus of Y-STRs of bone materials was DYS389I (1 250^{0}C-20'); that of dental materials was DYS389I (1 250^{0}C-40'). mtDNA was detected at 143 bp (750^{0}C-40' for bone materials and 1 250^{0}C-30' for dental materials) and 126 bp (750^{0}C-40'for bone materials and 1 000^{0}C-30' for dental materials). **Conclusion:** *Undetected mini primer on DNA amplification of high-temperature exposed bones and teeth might be due to complete degradation, resulting in DNA fragments losing their primer annealing sites. Differences in amplicon products and GC content of DNA templates content supported the successful detection of those loci at the maximum exposure of the research. The ratio of GC content for CSF1PO was 42.6 %, D8S1179 was 30.9 %, and D7S820 was 28.6 %, and had power discriminant of different. In conclusion, dental materials that remained capable of detection were loci D7S820 and D8S1179 with standard primer, CSF1PO with mini primer, and DYS389I at the maximum temperature exposure (1 250^{0}C for 40 minutes).*

Keywords: *High-temperature exposure, STR-mini STR CODIS, Y-STRs, mtDNA, bone and dental DNA, mortality.*

RESUMEN

Antecedentes: *Los expertos forenses desempeñan un papel importante en la ciencia forense. La identificación mediante análisis de ADN es una herramienta de diagnóstico precisa y estable. Sin embargo, junto con el desarrollo del examen de material de ADN, surgen problemas ya que el ADN sufre degradación, lo que comúnmente se conoce como ADN degradado. La exposición a altas temperaturas es un factor en la degradación del ADN. Los tejidos óseos y dentales se encuentran entre los materiales más resistentes a esta degradación del ADN. Los huesos y los dientes son los más sólidos del cuerpo humano debido a que contienen hidroxiapatita y matrices extracelulares que protegen el ADN (ADN nuclear y ADNmt). Hasta la fecha, la degradación del ADN debido a la exposición a altas temperaturas en muestras de ADN óseo y dental en la identificación forense ha sido ampliamente desconocida.* **Objetivo**: *Este estudio tuvo como objetivo analizar el daño en el ADN derivado de costillas y segundos molares causado por temperaturas extremadamente altas utilizando un marcador CODIS de repetición corta en tándem (STR).* **Métodos:** *En esta investigación se analiza la degradación del ADN de materiales óseos y dentales debido al efecto de exposiciones a altas temperaturas (500^{0}C, 750^{0}C, 1 000^{0}C y 1 250^{0}C durante 20, 30 y 40 minutos) basado en los loci STR y mini. -STR CODIS (CSF1PO, D18S51, D21S11, FGA, D8S1179, D5S820, D7S820, D13S317, D16S539), Y-STR (DYS19, DYS389, DYS390) y ADNmt de 143 pb y 126 pb. Las muestras consistieron en 24 costillas y 24 molares de 7 cadáveres.* **Resultados:** *Los resultados del análisis mostraron que los dientes eran más resistentes que los huesos a la hora de proteger el ADN de la exposición a altas temperaturas. Esto podría verse en la cantidad de presentaciones detectadas positivamente en los loci STR, Y-STR y mtDNA. Los loci de STR CODIS de materiales óseos detectados mediante cebador estándar fueron D3S1358, D16S539 (1 250^{0}C-20') y CSF1PO (500^{0}C-40'); los de materiales dentales fueron D7S820, D8S1179 (1 250^{0}C-40'), D3S1358 (12 500^{0}C-20'), D13S317 (1 000^{0}C-40'), D16S539 (750^{0}C-40'), CSF1PO (750^{0}C-20'). Los loci de STR CODIS de materiales óseos detectados por el mini cebador fueron D16S539 (750^{0}C-40'), CSF1PO, D12S137 (500^{0}C-40') y D3S358 (500^{0}C-30'); los de materiales dentales fueron CSF1PO (1 250^{0}C-40'), D16S539 (1 000^{0}C-20'), D13S317 (750^{0}C-40'), D3S1358 (750^{0}C-20'), D5S818, D7S820, D8S1179, D18S51 (500^{0}C-40'). El locus detectado de Y-STR de materiales óseos fue DYS389I (1 250^{0}C-20'); el de materiales dentales fue DYS389I (1 250^{0}C-40'). Se detectó ADNmt a 143 pb (750^{0}C-40' para materiales óseos y 1 250^{0}C-30' para materiales dentales) y 126 pb (750^{0}C-40' para materiales óseos y 1 000^{0}C-30' para materiales dentales).* **Conclusión:** *El minicebador no detectado en la amplificación de ADN de huesos y dientes expuestos a altas temperaturas podría deberse a una degradación completa que provoca que los fragmentos de ADN pierdan sus sitios de reconocimiento del cebador. La detección exitosa de esos loci en la exposición máxima estuvo respaldada por diferencias en los productos de amplicones y el contenido de GC de la plantilla del ADN. La proporción del contenido de GC para CSF1PO fue del 42,6 %, D8S1179 fue del 30,9 % y D7S820 fue del 28,6 %, y tenían un poder discriminante de diferentes. En conclusión, los materiales dentales que siguieron siendo capaces de detectarse fueron los loci D7S820 y D8S1179 con imprimador estándar, CSF1PO con mini imprimador y DYS389I a la temperatura máxima de exposición (1 250°C durante 40 minutos).*

Palabras clave: *Exposición a altas temperaturas, STR-mini STR CODIS, Y-STR, ADNmt, ADN óseo y dental, mortalidad.*

INTRODUCTION

During the DNA analysis, DNA material or specimens are often not fresh enough for DNA typing; this is known as degraded DNA (DNA degradation). DNA degradation is mainly found in cases with severely burned bodies. Specimen conditions that experience DNA degradation due to high-temperature exposure also hinder DNA analysis (1,2).

An attempt to overcome the identification with damaged DNA is by designing amplicon products shorter than those commonly used previously, implementing a mini primer set through a method to reduce the size of Short Tandem Repeat (STR) assays on DNA core locus examination. This mini primer STR on degraded DNA samples can still be amplified by Polymerase Chain Reaction (PCR). Therefore, forensic identification can still be conducted (3). Mini primers for mitochondrial DNA (mtDNA) nucleotide sequence are aimed in the hypervariable regions 1 and 2 (HV1 and HV2) displacement-loop (d-loop) regions. Therefore, a shorter amplicon size is obtained (4). Molecular forensics identification on DNA damage due to high-temperature exposure in bone and tooth DNA samples has yet to be fully understood. The result of this study is expected to help various forensic cases involving forensic DNA examinations with degraded nuclear and mitochondrial DNA specimens due to high-temperature exposure. It can provide information on bone and teeth resistance in protecting their DNA against high-temperature exposure (5-9).

Thus, this study aims to analyze bone and tooth DNA loci that can still be detected after exposure to temperatures of 500^{0}C, 750^{0}C, 1 000^{0}C, and 1 250^{0}C for 20, 30, and 40 minutes in DNA samples on molecular forensic identification, based on STR CODIS loci and STR CODIS mini primer (D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11), Y-STRs (DYS19, DYS389 and DYS390) and mtDNA 143 bp and 126 bp.

METHODS

This was a laboratory experimental study to analyze DNA damage from ribs and second molar teeth DNA samples due to extremely high-temperature exposure through STR CODIS loci [CSF1PO, TPOX, THO1, miniCSF1PO, miniTHO1, miniTPOX]. This study used a randomized, post-test-only control group design. The samples were taken from abandoned bodies in the Forensic Medicine and Medicolegal Department/Installation of Medical Faculty of Airlangga University/ Dr. Soetomo General Hospital, Surabaya. They had received approval for an ethical fit test. The calculation showed that the number of samples needed was 24 ribs and 24-second molar teeth. The samples were taken from 7 bodies. All the samples were heated using muffle furnace Naberthemtool at 500^{0}C, 750^{0}C, 1 000^{0}C, and 1 250^{0}C for 20, 30, and 40 minutes. DNA samples were exposed to extremely high temperatures and extracted and isolated. DNA extraction and isolation materials were DNAzol Reagent, Ethanol 100 %, and 70 % solution. Afterward, DNA samples were amplified using the Polymerase Chain Reaction [PCR]. Materials for PCR were PCR Mix [Dntp (ATP, CTP, TTP GTP), $MgCl_2$, Taq Polimerase], nuclease-free water, primer-primer and mini primer Short Tandem Repeats [STR] Combined DNA Index System [CODIS] locus-locus, consisted on STR CODIS loci and STR CODIS mini primer (D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11), Y-STRs (DYS19, DYS389 and DYS390) and mtDNA 143 bp and 126 bp. The amplification process began with DNA template preparation through extraction and isolation process using an extraction kit (DNAzol). This study used the DNAzol extraction method, followed by ethanol precipitation. The advantage of ethanol precipitation is that it can separate DNA through precipitation, while its disadvantage is that it can decrease the DNA level obtained through extraction. Before DNA extraction from the bones and teeth, decalcification was conducted to remove calcium content.

PCR amplification cycles for CSF1PO, THOI, TPOX STR loci were as follows: initial denaturation 96°C-2 minutes, for ten cycles

[subsequent denaturation 94^{0}C-1-minute, annealing 64^{0}C-1 minute, extension 70^{0}C–1 minute 30 seconds], for 30 cycles [denaturation 90^{0}C-1-minute, annealing 64^{0}C- 1 minute, extension 70^{0}C – 1 minute 30 seconds]. Whereas PCR amplification cycles for miniCSF1PO, miniTHOI, and miniTPOX locus were as follows: Initial denaturation 96^{0}C- 10 minutes, for 30 cycles [denaturation 94^{0}C -1-minute, annealing 55^{0}C-1 minute, extension 72^{0}C–1 minute], final extension 65°C-45 minutes. The PCR amplification product was visualized using Polyacrylamide agarose gel electrophorese [PAGE] and silver nitrate straining.

The statistical analysis was conducted using a sample t-test, while the non-parametric statistics comprised the Chi-Square Test, Pearson Chi-Square, and Fisher's Exact Test. Both parametric and non-parametric statistics depend on the data's normality.

RESULTS

The results of this study showed that the bone sample's weight was reduced by 65.1 %-91.8 % after various temperatures (500^{0}C, 750^{0}C, 1 000^{0}C, and 1 250^{0}C) and time (20', 30', and 40'), while the teeth sample's weight was reduced by 28.6 % -66.7 %. The results of DNA level measurement using a UV-spectrophotometer showed that temperature and time exposure significantly decreased bone (p=0.0001) and teeth (p=0.0001) DNA levels.

DNA STR CODIS loci detection results after high-temperature exposure (temperatures of 500^{0}C, 750^{0}C, 1 000^{0}C, and 1 250^{0}C for 20', 30' and 40 ') on bone material with standard primers, loci that were still detected were D3S1358 (41.67 %), CSF1PO (16.67 %) and D16S539 (58.53 %). Of the three loci, only the CSF1PO locus was shown to have a significant effect due to the treatment effect (temperature and time exposure) (p=0.018). On dental materials, DNA detection results after exposure (temperature 500^{0}C, 750^{0}C, 1 000^{0}C and 1 250^{0}C for 20', 30' and 40') were STR loci D3S1358 (41.67 %), CSF1PO (29.17 %), D7S820 (62.50.%), D8S1179 (66.67 %), D13S317 (37.50 %), D16S539 (41.67 %). Of the six loci, only the CSF1PO locus (p=0.037) showed a significant effect due to treatment (temperature and time exposure).

Table 1 shows the complete DNA STR CODIS loci detection results with standard primers on bone material exposed to high temperatures. Table 2 presents the complete DNA STR CODIS loci detection results with standard primers on teeth material exposed to high temperatures.

During mini primer use, DNA STR CODIS loci detection due to treatment loci were still detected in bone material were D3S1358 (8.33 %), CSF1PO (12.50 %), D13S317 (12.50 %) and D16S539 (37.50 %). In comparison, on dental material the loci were: D3S158 (16.67 %), FGA (7.12 %), CSF1PO (41.67 %), D5S818 (7.12 %), D7S820 (7.12 %), D8S1179 (7.12 %), D13S317 (45.63 %), D16S539 (29.17 %), D18S51 (7.12 %). Of the 9 STR CODIS loci on dental materials, only the D13S317 locus (p=0.033) showed a significant effect due to treatments (Table 3).

DNA Y-STRs CODIS loci detection results due to high-temperature exposure on bone material revealed only the DYS389I locus (58.30 %), while on dental materials, loci that were still detected are DYS19 (8.33 %), DYS389I (58.33 %), and DYS390 (4.16 %). Of the 3 Y-STR loci on dental materials, only DYS19 showed a significant effect due to treatment (p=0.018) (Tables 4 and 5).

mtDNA 143 bp due to high-temperature exposure was still detected on 25 % of bone material. In contrast, in dental material was 54.50 %, while for mtDNA, 126 bp in bone material was 25 %, and in dental material was 41.70 % (Table 6 and 7).

The results of the Chi-Square test on DNA detection strength of bone and tooth materials for STR CODIS and mtDNA loci with and without treatment showed that there were significant differences in loci detection of D7S820 (p= 0.0001), D8S1179 (p=0.0001) and D13S317 (p= 0.013) and 143 bp mtDNA detection (p=0.006). The Chi-Square test results on DNA detection strength of bone material for STR CODIS loci with standard primer and mini primer use with and without treatment showed a significant difference in bone material was only D3S1358 locus (p=0.020). In contrast, only D7S820 (p=0.001) and D8S1179 (p=0.0001) loci showed significant differences in bone material.

Table 1. DNA STR CODIS loci detection results on bone material due to temperature and time exposure with standard primer.

Exposure		D3S1358 Detection result		FGA Detection result		CSF1PO Detection result		D5S818 Detection result		D7S820 Detection result		D8S1179 Detection result		D13S317 Detection result		D16S539 Detection result		D18S51 Detection result		D21S11 Detection result		Total Det.+ (%)
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	
Exposure (-)		2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	100
500^{0}C	20'	1	1	0	2	2	0	0	2	0	2	0	2	0	2	2	0	0	2	0	2	25
	30'	1	1	0	2	2	0	0	2	0	2	0	2	0	2	2	0	0	2	0	2	25
	40'	1	1	0	2	0	0	0	2	0	2	0	2	0	2	2	0	0	2	0	2	15
750^{0}C	20'	1	1	0	2	0	0	0	2	0	2	0	2	0	2	2	0	0	2	0	2	15
	30'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	2	0	0	2	0	2	15
	40'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	1	1	0	2	0	2	10
1 000^{0}C	20'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	1	1	0	2	0	2	10
	30'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	1	1	0	2	0	2	10
	40'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	5
1 250^{0}C	20'	1	1	0	2	0	2	0	2	0	2	0	2	0	2	1	1	0	2	0	2	10
	30'	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
	40'	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
DNA Total + after exposure (%)		10/24 (41,67 %)		0/24 (0 %)		4/24 (16,67 %)		0/24 (0 %)		0/24 (0 %)		0/24 (0 %)		0/24 (0 %)		14/24 (58,53 %)		0/24 (0 %)		0/24 (0 %)		

Table 2. DNA STR CODIS loci detection results on teeth material due to temperature and time exposure with standard primer.

Exposure		D3S1358 Detection result		FGA Detection result		CSF1PO Detection result		D5S818 Detection result		D7S820 Detection result		D8S1179 Detection result		D13S317 Detection result		D16S539 Detection result		D18S51 Detection result		D21S11 Detection result		Total Det.+ (%)
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	
Exposure (-)		2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	100
500^{0}C	20’	1	1	0	2	2	0	0	2	2	0	2	0	1	1	2	0	0	2	0	2	50
	30’	1	1	0	2	2	0	0	2	2	0	2	0	1	1	2	0	0	2	0	2	50
	40’	1	1	0	2	2	0	0	2	2	0	2	0	1	1	2	0	0	2	0	2	50
750^{0}C	20’	1	1	0	2	1	1	0	2	1	1	2	0	1	1	2	0	0	2	0	2	40
	30’	1	1	0	2	0	2	0	2	1	1	1	1	1	1	1	1	0	2	0	2	25
	40’	1	1	0	2	0	2	0	2	1	1	1	1	1	1	1	1	0	2	0	2	25
1 000^{0}C	20’	1	1	0	2	0	2	0	2	1	1	1	1	1	1	0	2	0	2	0	2	20
	30’	1	1	0	2	0	2	0	2	1	1	1	1	1	2	0	2	0	2	0	2	20
	40’	1	1	0	2	0	2	0	2	1	1	1	1	1	1	0	2	0	2	0	2	20
1 250^{0}C	20’	1	1	0	2	0	2	0	2	1	1	1	1	0	2	0	2	0	2	0	2	15
	30’	0	2	0	2	0	2	0	2	1	1	1	1	0	2	0	2	0	2	0	2	10
	40’	0	2	0	2	0	2	0	2	1	1	1	1	0	2	0	2	0	2	0	2	10
DNA Total + after exposure (%)		10/24 41.67 %)		0/24 (0 %)		7/24 (29.17 %)		0/24 (0 %)		15/24 (62.50 %)		16/24 (66.67 %)		9/24 (37.50 %)		10/24 (41.67 %)		0/24 (0 %)		0/24 (0 %)		

Table 3. DNA STR CODIS loci detection results on bone material after high-temperature exposure at various temperatures and times with a mini primer.

Exposure		D3S1358 Detection result		FGA Detection result		CSF1PO Detection result		D5S818 Detection result		D7S820 Detection result		D8S1179 Detection result		D13S317 Detection result		D16S539 Detection result		D18S51 Detection result		D21S11 Detection result		Total Det.+ (%)
		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	
Exposure (-)		2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	100
500^{0}C	20’	1	1	0	2	1	1	0	2	0	2	0	2	1	1	1	1	0	2	0	2	25
	30’	1	1	0	2	1	1	0	2	0	2	0	2	1	1	1	1	0	2	0	2	25
	40’	0	2	0	2	1	1	0	2	0	2	0	2	1	1	1	1	0	2	0	2	20
750^{0}C	20’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	0	0	2	0	2	10
	30’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	0	0	2	0	2	10
	40’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	2	0	0	2	0	2	10
1 000^{0}C	20’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
	30’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
	40’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
1 250^{0}C	20’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
	30’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
	40’	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2	0
DNA Total + after exposure(%)		2/24 (8.33 %)		0/24 (0 %)		3/24 (12.50 %)		0/24 (0 %)		0/24 (0 %)		0/24 (0 %		3/24 (12.50 %)		9/24 (37.50 %)		0/24 (0 %)		0/24 (0 %)		

Table 4. DYS19, DYS389I, and DYS390 loci detection results on bone material after high-temperature exposure at various temperatures and times.

Exposure		DYS19 Detection result		DYS389I Detection result		DYS390 Detection result		Total Det. + (%)
		+	-	+	-	+	-	
Exposure(-)		2	0	2	0	2	0	100
500^{0}C	20’	0	2	2	0	0	2	33.3
	30’	0	2	2	0	0	2	33.3
	40’	0	2	2	0	0	2	33.3
750^{0}C	20’	0	2	2	0	0	2	33.3
	30’	0	2	1	1	0	2	16.7
	40’	0	2	1	1	0	2	16.7
1 000^{0}C	20’	0	2	1	1	0	2	16.7
	30’	0	2	1	1	0	2	16.7
	40’	0	2	1	1	0	2	16.7
1 250^{0}C	20’	0	2	1	1	0	2	16.7
	30’	0	2	0	2	0	2	0
	40’	0	2	0	2	0	2	0
DNA Total + after exposure (%)		0/24 (0 %)		14/24 (58.30 %)		0/24 (0 %)		

Table 5. DYS19, DYS389I, and DYS390 loci detection results on dental material after high-temperature exposure at various temperatures and times.

Exposure		DYS19 Detection result		DYS389I Detection result		DYS390 Detection result		Total Det + (%)
		+	-	+	-	+	-	
Exposure (-)		2	0	2	0	2	0	100
500ºC	20’	2	0	2	0	1	1	50
	30’	0	2	2	0	0	2	33.33
	40’	0	2	2	0	0	2	33.33
750ºC	20’	0	2	1	1	0	2	16.67
	30’	0	2	1	1	0	2	16.67
	40’	0	2	1	1	0	2	16.67
1 000ºC	20’	0	2	0	2	0	2	0
	30’	0	2	1	1	0	2	16.67
	40’	0	2	1	1	0	2	16.67
1 250ºC	20’	0	2	1	1	0	2	16.67
	30’	0	2	1	1	0	2	16.67
	40’	0	2	1	1	0	2	16.67
DNA Total + after exposure(%)		2/24 (8.33 %)		14/24 (58.33 %)		1/24 (4.16 %)		

Table 6. The detection results of mtDNA 143 bp and 126 bp amplicon products due to high-temperature exposure on bone material under various temperatures and time exposure.

Exposure		mtDNA 143 bp Detection results		mtDNA 126 bp Detection results		Total Det. + (%)
		+	-	+	-	
Exposure (-)		2	0	2	0	100
500^{0}C	20’	1	2	1	1	50
	30’	1	2	1	1	50
	40’	1	1	1	1	50
750^{0}C	20’	1	2	1	1	50
	30’	1	1	1	1	50
	40’	1	1	1	1	50
1 000^{0}C	20’	0	2	0	2	0
	30’	0	2	0	2	0
	40’	0	2	0	2	0
1 250^{0}C	20’	0	2	0	2	0
	30’	0	2	0	2	0
	40’	0	2	0	2	0
DNA Total + after exposure(%)		6/24 (25 %)		6/24 (25 %)		

Table 7. The detection results of mtDNA 143 bp and 126 bp amplicon products due to high-temperature exposure on dental material under various temperatures and time exposure.

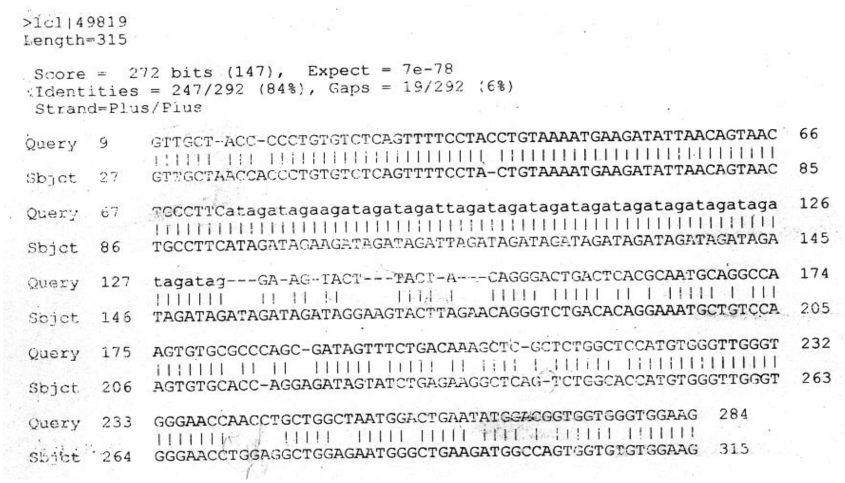
Exposure		mtDNA 143 bp Detection results		mtDNA 126 bp Detection results		Total Det. + (%)
		+	-	+	-	
Exposure (-)		2	0	2	0	100
500^{0}C	20’	2	0	2	0	100
	30’	2	0	2	0	100
	40’	1	1	1	1	50
750^{0}C	20’	1	1	1	1	50
	30’	1	1	1	1	50
	40’	1	1	1	1	50
1 000^{0}C	20’	1	1	1	1	50
	30’	1	1	1	1	50
	40’	1	1	0	2	25
1 250^{0}C	20’	1	1	0	2	25
	30’	1	1	0	2	25
	40’	0	2	0	2	0
DNA Total + after exposure(%)		13/24 (54.50 %)		10/24 (41.70 %)		

Several CODIS and Y-STRs STR loci were followed by sequencing analysis. CSF1PO locus from dental material was exposed to 500^{0}C for 20 minutes. The sequencing results can be seen below:

>1-primer1 CSF1PO

GGGTTCCAGTTGCTACCCCCTGTGTCTCAGTT
TTCCTACCTGTAAAATGAAGATATTAACAGT
AACTGCCTTCATAGATAGAAGATAGATAGAT
TAGATAGATAGATAGATAGATAGATAGATA
GATAGATAGGAAGTACTTACTACAGGGACT
GACTCACGCAATGCAGGCCAAGTGTGCGCCC
AGCGATAGTTTCTGACAAAGCTCGCTCTGGC
TCCATGTGGGTTGGGTGGGAACCAACCTGCT
GGCTAATGGACTGAATATGGACGGTGGTGG
GTGGAAGGAA.

Furthermore, GenBank data alignment was carried out for the CSF1PO locus as shown below:



From the sequencing results above, DNA from CSF1PO dental material showed that exposure to a temperature of 500^{0}C for 20 minutes could still be detected through PCR results visualization, where sequencing results showed 84 % identical if aligned/homologated with Genbank.

DISCUSSION

The breakdown of irreversible DNA hydrogen bonds causes DNA damage due to abnormal exposures, such as high temperatures. This

exposure results in damage to the DNA purine-pyrimidine pair. Purine-pyrimidine pairs are the main components of DNA structure, where adenine is always paired with thymine and guanine is always paired with cytosine. Environmental effects, such as temperature and time exposure, proved to affect DNA level as measured by spectrophotometry, which showed a significant decrease in bone and teeth samples. This decrease in DNA level is not an obstacle to further DNA examination because the remaining DNA levels were still adequate for DNA profiling. It was found that DNA profiling required a minimum DNA level of 50 ng. Another study found that the minimum amount of DNA required for a polymerase chain reaction (PCR) based STR examination was approximately 0.25-2 ng. In addition to DNA sample level in PCR-based DNA testing, it is also necessary to consider sufficient DNA quality. This means that the DNA used in the analysis must not be degraded. If DNA is severely degraded, the primer will be unable to attach (annealing) to the DNA target to be duplicated (8-10).

Short Tandem Repeats (STR) are multiple copies of a short identical DNA sequence arranged in direct succession in particular regions of chromosomes. In this study, STR analysis was carried out because, in general, 40 % of forensic samples that will be conducted with DNA testing had already experienced degradation or contamination. STR analysis on DNA with a core sequence of less than 1 kb (kilobase) is very effective and highly successful, especially on degraded/fragmented DNA into short fragments. Positive PCR proved this on unexposed DNA controls (9,10).

DNA fragment detection failure with STR CODIS primers due to high-temperature exposure above 1 000^{0}C was followed by examinations using STR CODIS mini primers, which amplify shorter DNA fragments. Mini primers had a high potential to detect these DNA fragments. This suggests that exposure to high temperatures that damage DNA can lead to failure in the overall identification process. MtDNA amplification with mini primers will produce fragments with shorter HV1 or HV2 regions (1,2,4,11).

Failure to detect in DNA examination using the PCR method might be caused by several factors, including a small amount of target DNA, target DNA that has experienced degradation or damage, insufficient DNA polymerase enzymes, a lack of PCR cycles, or the presence of PCR inhibitors (12-15).

Our results indicate an effect of high-temperature exposure on bone and tooth DNA levels, but several STR CODIS loci, Y-STRs, and mtDNA were still detected. DNA loci that were still detected at maximum exposure in this study (1 250^{0}C-40') using standard primers were only found in dental material: D7S820, D8S1179, DYS389I, and CSF1PO that used mini primer; therefore, the four loci were new findings in this study. The location of these loci is on the autosomal chromosome (STR: D7S820, D8S1179, CSF1PO) and Y-STRs (DYS389I); the examination method through this locus is the potential to be used for the identification process, especially in conditions where the sample is degraded due to high-temperature exposure (9,16).

The present results show that teeth are stronger due to their hydroxyapatite content and higher "hard tissue mineral" levels than bones; therefore, teeth can protect DNA. Besides, teeth also have important secondary minerals higher than bone, such as calcite, limonite, pyrite, and vivianite, making teeth stronger defenses against external exposure (17).

Teeth and bones are frequently the only DNA sources available for identifying degraded or fragmented human remains. The unique composition of teeth and their location in the jawbone provide additional protection to DNA compared to bones, making them a preferred source of DNA in many cases. Teeth are used for DNA analysis due to their mineral composition, low porosity, and resistance to taphonomy and contamination. In addition, STR loci differences that can be detected due to high-temperature exposure on bone and teeth DNA samples are due to differences in the GC content of each locus. GC content or guanine-cytosine bonds have a high level of stability to denaturation factors compared to adenine and thymine bonds (18).

The present results align with a similar study from Yudianto et al. (19), where they analyzed DNA damage patterns caused by an extremely high temperature using a short tandem repeat

(STR) CODIS marker. They demonstrated that PCR visualization using STR CODIS for costae showed the THO1 detection (+) at 1 250^{0}C- 40': 25 % and the TPOX detection (+) at 1 000^{0}C - 30': 50 %, whereas the results from molar teeth showed the THOI locus detection (+) in 1 250^{0}C-30': 25 % and TPOX in 1 000^{0}C-40': 50 %. Meanwhile, PCR visualization using mini-STR CODIS for the costae presented that the mini-THOI in 1 250^{0}C-20': 50 % (+) while for the molar tooth, the mini-THOI in 1 250^{0}C-30': 25 % (+) and mini-TPOX in 1 000^{0}C-40': 50 % (+). All loci were detected on the control group's costae and second molar teeth samples. Thus, supporting that extremely high-temperature exposure significantly decreased the DNA level of the second costae and second molar tooth.

According to Butler (2003), failure to detect DNA samples in forensic examination can be caused by DNA degradation, where DNA integrity is reduced, making DNA more difficult to amplify. DNA amplification failure with mini primers of bone and teeth DNA that are exposed to high temperatures is caused by severe degradation of DNA sample, making it no longer possible for mini primer to attach to DNA fragments. Mini primer is an alternative for standard primers in conditions where DNA is degraded, where standard primers give a low success rate under these conditions (1,2,7).

CONCLUSION

The effect of various temperature exposures are STR CODIS loci amplification results on bone material that were detected with standard primers are D3S1358 and D16S539 (temperature exposure 1 250^{0}C-20'); CSF1PO (temperature exposure 500^{0}C-40') and on dental materials: D7S820 and D8S1179 (temperature exposure 1 250^{0}C-40'); D3S1358 (temperature exposure 1 250^{0}C-20'); D13S317 (temperature exposure 1 000^{0}C-40'); D16S539 (temperature exposure 750^{0}C-40'); CSF1PO (temperature exposure 750^{0}C-20').

Detection of STR CODIS loci on bone material with mini primer are D16S539 (temperature exposure 750^{0}C-40'); CSF1PO and D13S317 (temperature exposure 500^{0}C-40'); D3S1358 (temperature exposure 500^{0}C-30'), while on dental materials: CSF1PO (temperature exposure 1 250^{0}C-40'); D16S539 (temperature exposure 1 000^{0}C-20'); D13S317 (temperature exposure 750^{0}C-40'); D3S1358 (temperature exposure 750^{0}C-20'); D5S818, D7S820, D8S1179 and D18S51 (temperature exposure 500^{0}C-40').

The Y-STR loci on bone material detected are DYS389I at temperature exposure 1 250^{0}C-20' and DYS389I on tooth material at temperature 1 250^{0}C-40'. For mtDNA, 143 bp from bone material is still detected at 750^{0}C-40' exposure and from dental material at 1 250^{0}C-30' exposure. In comparison, 126 bp mtDNA from bone material is still detected after 750^{0}C-40' exposure and dental materials after 1 000^{0}C-30' exposure.

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