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The Effect of Room Temperature Exposure on Sweat Spot-Derived DNA Samples Through Analysis of 143-bp mtDNA D-Loop HVS-1 (nt 16268-16410) And 126-bp HVS-2 (nt 34-159)

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Abstract

Context: Identification is an unavoidable process in forensic setting. DNA analysis is one of several scientifically recognized methods to identify somebody. As an alternative to the largely used DNA specimen, the last object frequently used by the perpetrator or victim can be used as well. Clothes or apparels stick to the outer skin; thus the epithelial cells of the skin can be expected to attach to them. One factor that may affect the quality of DNA is the duration of exposure. From the two DNA analysis methods, mitochondrial DNA (mtDNA) has better durability than nuclear DNA due to its relatively larger amount. Hence, mtDNA has some greater chance of success in the amplification process. *Aims:* From this study, we would like to understand of the effect of the duration of exposure to room temperature on the quality of DNA derived from sweat spots in Indonesia remaining that the data remains unavailable to date. *Setting and Design:* This is an analytical experimental research with time-series design. *Material and Method:* Sweat-spotted clothes that had been worn were subsequently exposed to room temperature for 0, 1, 7, 14 and 20 days. *Statistical analysis used:* nil. *Results:* Results showed that longer exposure markedly decreased the concentration of DNA from day 1 to 20 at $p < 0.005$. *Conclusions:* Longer duration of exposure to room temperature significantly decreased the quality of DNA derived from sweat spots on clothes. Visualization of PCR detection results show positive (+) detection only on day 0 of exposure on both 143-bp mtDNA HVS 1 and 126-bp mtDNA HVS 2.

Keywords: Sweat Spot; DNA Quality; Mitochondrial DNA; Identification.

Introduction

Personal identity is of paramount importance in order to obtain the fairest justice. According to Interpol Disaster Victim Identification (DVI) guide, there are two main groups of methods of identification; primary and secondary. Primary means of identification include friction ridge analysis, comparative dental analysis and DNA (Deoxyribonucleic Acid) analysis and secondary means of identification include personal description, medical findings, tattoos, as well as property and clothing found on the body [3].

Identification is frequently impeded by materials poor condition; thus, an alternative is pursued through the use of mini-primer set, in which the region of mitochondrial DNA amplification for use as the amplification product is reduced. The fact that there is a relationship between the size of a locus and the success of the amplification of degraded DNA obtained from the crime scene and in a mass disaster corroborates this situation. Mitochondrial DNA is used since it can determine density, thus it hastens the process of identification, especially in mass disaster cases with a large number of victims [1].

To date, however, there is not any specific research yet on successful use of the mini-primer sets as an

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alternative to forensic DNA identification using degraded DNA, especially mitochondrial DNA. It is necessary to determine the loci potentially useful for examination of degraded DNA. This mapping allows determination of the DNA loci that can be used for forensic DNA examination, either for the purpose of individual identification of a mass disaster's victims or revealing some criminal cases with attempts of evidences removal on victims and suspects.

In mitochondrial DNA, there are protein non-coding regions, called D-Loop, at nucleotide (nt) 16 024-576. This region is also called the control region since it is a segment that contains the elements of replication origin, transcription initiation and regulators. In D-Loop, there are segments called hypervariable segment I (HVS I) located at nt 16024-16383 (360 bp) and HVS 2 located at nt 57-372 (316 bp) [8,9].

A mechanical abuse will always leave a biological trace evidence or an object originating from the body of living things/humans. Biological evidence includes blood or blood spot on clothes or fabrics as well as sweat spot on worn clothes. So far, the effect of exposure to room temperature on the quality of DNA samples derived from sweat spot on clothes identified through mitochondrial DNA analysis has not been known.

A previous study by Yudianto (2006) showed that sweat spots could be an alternative to identification assays since perpetrators often ignore these spot. The length of exposure to the spots was in accordance with the statutory number of days for investigation process in the Criminal Code, which is 20 days. Thus, this study used the intervals of exposure length according to the repetition of day in the week (day 0, day 1, day 7, day 14 and day 20). The room temperature used was in accordance with the standard urban area, 29°C-32°C.

Material and Method

This study was of analytical experimental to analyze the feasibility of degraded mtDNA to be used in DNA assay). This study used a time-series design and samples were derived from clothes with sweat spots taken from respondents by means of the ethical feasibility test. Calculation of sample size indicated that 15 respondents were required in this study.

Sample Handling

The initial stage of the study was the preparation of the DNA templates for the Polymerase Chain Reaction (PCR) process. DNA templates for amplification were derived from the lytic human epithelial cells from clothes with sweat spots. They were cut, put into a conical tube, mixed with water free, and centrifuged at 10,000 G for 10 minutes. The pellet was taken and mixed with 1 ml of DNAZol and centrifuged at 10,000 G for 10 minutes at 4°C. Subsequently, 0.5 ml absolute ethanol (100%) was added to the viscous supernatant and centrifuged at 4,000 G for 1-2 min at 4°C. The pellet was washed with 8-10 ml of 75% ethanol. The DNA-containing pellet was dissolved with 0.2-0.3 ml of 8 mM NaOH solution and subjected to vortex sufficiently and stored at -20°C.

PCR Amplification

Materials for PCR amplification consisted of dNTP dNTP (ATP,CTP,TTP GTP), MgCl2 dan Taq Polimerase, Nuclease free water, and mtDNA primers HVS1-143 bp (nt 16268-16410) (*AFDI, primer)*: 5'CACTAGGATACCAACAAACC 3' and 5' GAGGATGGTCAA GGGAC 3' , HVS 2- 126 bp (nt 34-159) (*AFDIL. primer*) : 5' GGG AGC TCT CCA TGC ATT TGG TA 3' and 5' AAA TAA TAG GAT GAG GCA GGA ATC 3'.

- PCR amplification of mtDNA for HVS-1 143bp (Optimation PCR): Initial denaturation 96^0C - 4 minute, [35X : Subsequent denaturation 94^0C -1 minute, Annealing 61^0C - 1 minute, Extension 71^0C - 1 minute], elongation 72^0C - 5 minute.
- PCR amplification of mtDNA HVS 2- 126 bp (nt 34-159): Initial denaturation 95^0C - 3 minute, [30X : Denaturation 94^0C - 1 minute, Annealing 56^0C - 1 minute, Extension 72^0C - 1 minute], final Extension 72^0C - 3 minute.

Positive PCR results: purification, DNA quantity, preparing/labeling, purifying extension product/ precipitation and subsequently sequenced in the stage of purification and visualization (DNA Quantity), preparing/labeling, purifying extension product.

Sequence Analysis

Each nucleotide produced a peak with some different color on the electrophoregram, in which nucleotide G was black, nucleotide C was green and nucleotide T was red. Analysis of nucleotide sequencing results for determination of degradation to the nucleotide was carried out by labeling it with the letter 'N' on the sequencing results.

Result

There are two things to be analysed in this study. Firstly, we analysed the effect of the duration of exposure to room temperature on DNA concentration derived from sweat spots. The study began with exposing the sweat spot samples to room temperature (29.5°C-30°C). The length of exposure used in this study is 0, 1, 7, 14 and 20 days consecutively. DNA samples were extracted using DNAzol method. The DNA concentrations obtained from the extraction were measured using an UV-visible spectrophotometer and the results are shown on Table 1. From Table 1 we can clearly see that the longer the exposure to room temperature, the lower the concentrations of DNA were.

Secondly, we analysed the effect of exposure duration to room temperature on DNA derived from sweat spots through detection of 143-bp mtDNA HVS 1- (nt 16268-16410) and 126-bp HVS 2 (nt 34-159). To determine the effect of exposure to temperature and the extended exposure duration on bone DNA, the loci of 143-bp (nt 16268-16410) and 126-bp (nt 34-159) mtDNA amplicon products were examined. Results of PCR amplification by means of 2% agarose gel electrophoresis can be seen on figure 1 and 2. Both figures of PCR results show that DNA was detected only on day 0. Results of sequencing (Figure 3) indicated that there were DNA fragments detected. The reading of DNA sequencing after exposure to high temperature found a large number of the symbol 'N'. According to International Union of Pure and Applied Chemistry (IUPAC), the symbol 'N' is a method to mention the presence of ambiguities [5].

Table 1. Mean of DNA concentration derived from sweat spots Extended room-temperature exposure significantly reduced the DNA concentration from sweat spots (sig. (p) = 0.000; sig. limit p <0.05)

Sample	Quantity (x ± SD) (µg/ml)
Day 0	150.16 ± 5.71
Day 1	10.12 ± 5.46
Day 7	1.05 ± 0.72
Day 14	0.94 ± 0.61
Day 20	0.16 ± 0.31

x: mean of DNA concentration; SD: Standard deviation

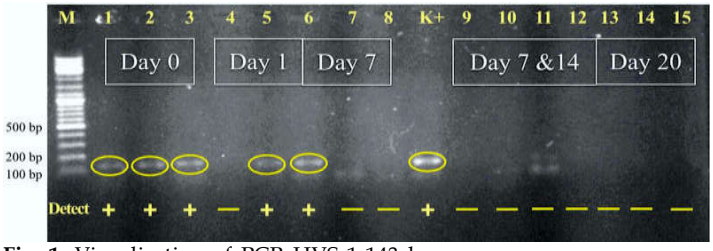

Fig. 1: Visualisation of PCR HVS 1-143 bp.
M is marker ladder of 100 bp; 1 to 15 represents the research samples; K+ is positive control; Detection + at mtDNA HVS 1-143 bp.

Fig. 2: Visualisation of PCR HVS 2-126 bp.
K+ is positive control.

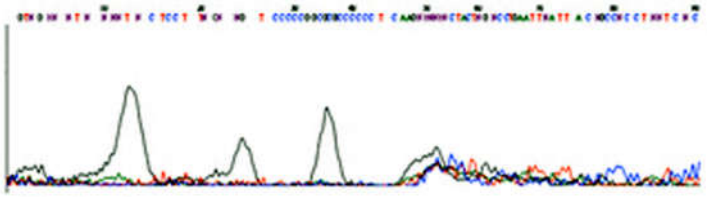

Fig. 3: Sequencing of HVS 2-126 bp

Discussion

Analysis of the Effect of the Duration of Exposure to Room Temperature on DNA Concentration Derived From Sweat Spots

The concentration of DNA constitutes an important factor in DNA analysis, particularly in regards of the success of DNA sample amplification. One nanogram reduction of DNA concentration potentially decreases STR detection capability up to 95% [1]. DNA integrity is important for forensic DNA assay. This implies that, despite the relatively high DNA concentrations in the assays, the high level of fragmented or degraded DNA will be less meaningful.

DNA degradation can be produced by both endogenous and exogenous factors. Endogenous factors originating in the cells are known as spontaneous degradation. Exogenous factors derive from the environment. Exogenous factors, such as humidity and room temperature, greatly affect DNA conditions. Another exogenous

factor is the presence of contaminants, such as bacteria. Tissues degraded by bacteria may subject to autolysis and spontaneous depurination [1,10,12]. Abnormal exposure to chemical agents, pH, temperature, or other exposures may lead to DNA degradation. Extended exposure to room temperature resulting in damaged conjugation bonds and detached nitrogen bases will lead to DNA fragmentation, even degradation.

Analysis Of The Effect Of Exposure Duration To Room Temperature On DNA Derived From Sweat Spots Through Detection Of 143-bp mtDNA HVS 1- [nt 16268-16410] And 126-bp HVS 2 [nt 34-159]

Undetected DNA in the visualization of PCR on these samples was due to the quality of DNA. The quality of the DNA includes DNA concentration, purity and condition. DNA quality for analysis should be good and, if degraded, the degradation should be as minimal as possible. Severely degraded DNA prevents primer from annealing the target DNA to be replicated. Thus, good quality of DNA is a fundamental prerequisite to the success of PCR reaction as a whole. According to Butler [1], sensitivity of PCR is a function of the number of cycles and concentrations and the integrity of DNA.

Severely degraded DNA prevents primer from annealing the target DNA to be replicated [5,6]. According to Muladno, obtaining adequate visualization requires adequate DNA purity and adequate DNA concentration, so that DNA can be used as material for DNA assays for identification and paternity tests [6].

DNA degradation is among the causes of failed DNA detection in DNA analysis using PCR method in this study. This is consistent with the notion of Yudianto (2010) concerning several possible causes for the failure of DNA detection, such as the minimum amount of target DNA, DNA degradation preventing primers from annealing, lacking DNA polymerase and PCR cycles and the presence of PCR inhibitors.

PCR cycles and insufficient DNA polymerase can be controlled by previous optimization. Failed amplification process is characterized by the absence of bands on the electrophoresis results. It can be overcome by the use of PCR master mix, which contains Mg^{2+}, taq DNA polymerase, dNTPs in quantities tailored to the requirement for optimal PCR reaction and has a proven reliability in PCR reaction, while inadequate cycles in PCR reactions have been overcome by PCR optimization of the primers used [1,12].

Exogenous DNA degradation caused by such exposures as X-rays, chemical agents, spontaneous instability, or extremely high temperature will result in various types of degradation, such as degraded double strands or single strands, base damage, sugar damage, and even DNA-DNA crosslink and DNA-protein crosslink. Those environmental factors lead to DNA degradation. Degradation can be fast or slow and it depends on the affecting factors and duration of exposure.

Conclusion

Duration of exposure to room temperature had an effect on the quality of DNA derived from sweat spots on clothes. It significantly decreases the DNA concentration at p <0.005. Visualization of PCR detection results show positive (+) detection only on day 0 of exposure on 143-bp mtDNA HVS 1 and 126-bp mtDNA HVS 2.

Key Messages

Sweat spot on clothes can be useful for DNA specimen in mtDNA analysis for identification purpose at an acceptable level. However, room temperature remarkably alters its availability to be a DNA specimen as well as several other factors.

Acknowledgement

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Conflict of Interest

None disclosed.

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