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THE EFFECT OF SOAKING PROCESS ON DNA LEVELS AND PURITY IN HUMAN NAIL SAMPLES

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ABSTRACT

Background: Generally, the primary sample used for DNA testing comes from blood. Improvements in existing analytical methodologies can reduce the need for DNA levels used in examinations so that samples used can come from other parts of the body with retrieval methods that are non-invasive, more straightforward, easier to store, and more resistant to decay, for example, nails. **Materials and methods:** Nail clipping samples were taken from the hands and feet of an unidentified corpse, which were then divided into 5 groups: the control group without treatment, the soaking group with fresh water and salt water on days 3 and 7. DNA extraction using chloroform-phenol, measurement of DNA levels, and purity using NanoDrop spectrophotometer. Each sample representative with the lowest and highest levels was further analyzed at D18S51 loci. **Results:** Average levels (µg/dl ± SD) of DNA nail clippings in the control group 299.68 ± 44.58, fresh water immersion group day 3 of 555.14 ± 192.12 and 7 of 429.82 ± 66.65, salt water immersion group day 3 of 228.04 ± 81.89 and day 7 of 400.76 ± 53.30. Polymerase Chain Reaction (PCR) visualization results from D18S51 loci show DNA bands or bands appear all 100%, with PCR product sizes of 290-366 bp. **Conclusion:** Quantitatively, there was no effect of decreasing DNA levels and purity on fresh and saltwater immersion, but qualitatively, DNA bands appeared thinner in the immersion group in fresh water.

Keywords: DNA, DNA Levels, DNA Purity, Nails, Soaking

INTRODUCTION

Forensic identification is an essential part of an investigation process for legal purposes, such as in cases of murder or disaster. A forensic pathologist will generally use the primary identification method, which uses fingerprint, tooth, and Deoxyribonucleic Acid (DNA) samples (1,2). In corpses that have decayed or submerged in water for a long time, identification through fingerprints will be difficult due to the skin's and underlying tissue's peeling, but nails can last longer (3). The development of existing DNA examination methodologies has led to a decrease in the amount of DNA needed in an examination, so the use of less invasive samples with lower DNA count results has attracted the interest of researchers, one of which is nails. Using nail samples has many benefits, including non-invasive, simple, and not requiring special storage tools. In addition, nail samples can be stored for quite a long time without special treatment before processing, even when nail clippings are stored at room temperature for over 20 years (4). A study found that complete DNA profiles can be obtained even from the nails of corpses that have been dead for more than six months (5). The effect of soaking on the DNA content and purity of human nails is not widely known.

MATERIALS AND METHODS

The samples in this study were pieces of fingernails and toenails without abnormalities derived from a corpse without identity and family. The nail clippings were then divided into 5 groups: the control group (without treatment), the soaking groups with fresh water for 3 days and 7 days, and the soaking groups with salt water for 3 days and 7 days. Each group contained 5 mg of nail clippings, and DNA extraction was carried out using the chloroform-phenol method. Measurement of DNA content and purity use the NanoDrop spectrophotometer. Each sample representative with

the lowest and highest levels was further analysed through a PCR amplification process at D18S51 loci. Furthermore, an electrophoresis process is carried out and painted with silver staining to display DNA bands so they can be read. Indonesia does not yet have a DNA population database of 13 STR CODIS, but several Indonesian population-specific alleles differ from other populations, including allele 7, one of which is D18S51 loci (6).

All procedures performed in studies involving human corpes were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amandements or comparable ethical standards and by the Health Research Ethical Clearance Commission of the Dr. Soetomo General Academic Hospital under the number 0825/KEPK/XI/2023.

RESULTS

The results of measuring DNA levels in human nail samples using the NanoDrop spectrophotometer from control and each treatment group can be seen in Table I.

Table I. Table of Average DNA Levels in Each Group

Treatment	Average DNA Level (ng/ µl) ± SD
Control	299.68 ± 44.58
Freshwater day-3	555.14 ± 192.12
Freshwater day-7	429.82 ± 66.65
Saltwater day-3	228.04 ± 81.89
Saltwater day-7	400.76 ± 53.30

Based on the table above shows that the average DNA content of human nail samples highest in the 3rd day freshwater immersion group was 555.14 ng/µl and the lowest average DNA level of human nail samples in the third-day salt water immersion group was 228.04 ng/µl. Based on the graph above, from the control group to the freshwater day-3 group, there was an increase in levels,

then a decrease in the salt water day-3 group, and there was an increase again in the freshwater day-7 group.

The results of measuring DNA purity in human nail samples using the NanoDrop spectrophotometer from control and each treatment group can be seen in Table II.

Table II. Table of Average DNA Purity in Each Group

Treatment	Average DNA Purity ± SD
Control (C)	1.48 ± 0.232
Freshwater day-3	1.76 ± 0.084
Freshwater day-7	1.81 ± 0.027
Saltwater day-3	1.57 ± 0.177
Saltwater day-7	1.79 ± 0.023

Based on the output of SPSS One Way ANOVA, the P-value was obtained from DNA levels of 0.01; because the P-value was $0.01 < 0.05$, it can be concluded that the variants of all groups, both the control group and the treatment group, there are differences. In contrast, a P-value of 0.03 was obtained in DNA purity analysis. Because of the P-value $0.03 < 0.05$, it can be concluded that the variance of all groups, both the control and the treatment groups, is different.

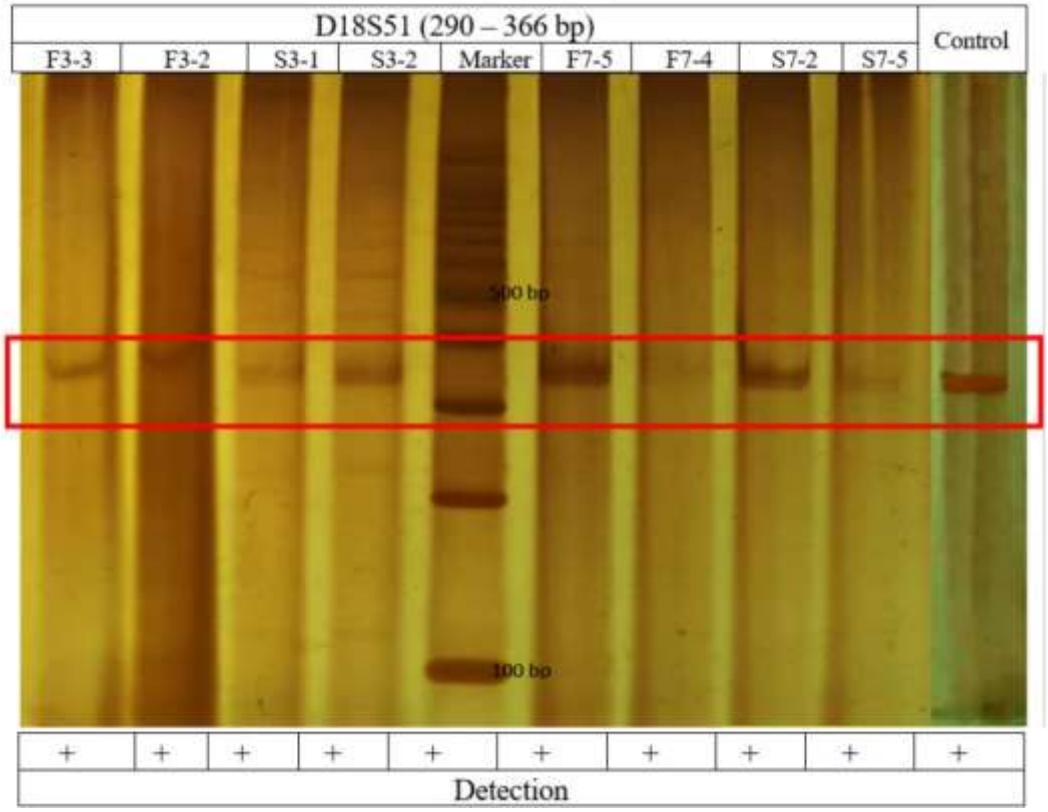

Fig 1. D18S51 loci PCR Visualization Result

The DNA size of the PCR product (bp) for the D18S51 loci is 290 – 366 bp. Figure 1 shows the results of PCR amplification of D18S51 loci on human nail samples and STR loci examination with a control group without treatment and a treatment group of freshwater immersion day 3, saltwater day 3, freshwater day 7, and saltwater day 7 showed positive results. From Figure 1, it can be seen that the size of the DNA band of each sample is different.

DISCUSSION

In the measurement of DNA levels of the control group, an average of 299.68 ± 44.58 (ng / µl) in the freshwater immersion group for 3 days did not decrease level instead the levels rose by 555.14 ± 192.12 (ng / µl). DNA levels in freshwater immersion for 7 days amounted to 429.82 ± 66.65 (ng / µl), while in the group of saltwater treatment for 3 days amounted to 228.04 ± 81.89 (ng /

µl), and saltwater immersion for 7 days amounted to 400.76 ± 53.30 (ng / µl). In the control group's DNA purity measurement, an average of 1.48 was found; in the 3-day fresh water immersion group was 1.76, and DNA levels in 7-day fresh water immersion was 1.81, while in the 3-day salt water treatment group were 1.57 and 7-day salt water immersion was 1.79. The above results show that the hypothesis related to a decrease in DNA levels of nail clippings after immersion treatment of fresh water and salt water does not occur; this is to the theory that nail keratin cells are very insoluble in water and organic solvents, thus protecting DNA from further damage (7).

The results of measuring the level and purity of human nail cut DNA both in the control and treatment groups with fresh and saltwater immersion by statistical data analysis using the One-way ANOVA test showed an average difference between groups. The level and purity of human nail cut DNA in the fresh and saltwater immersion treatment group did not decrease from the control group and tended to increase in levels. This can be caused when making nail clipping samples for each group not from the exact part/location of nail clippings, in this study, one group contains fingernails and toenails where the results of the levels of hand nail clippings and toenail clippings can vary; one of which is extraction protocol and measurement technical factors, in DNA samples nail clippings may be overestimated to varying degrees when assessed with a NanoDrop spectrophotometer (8).

The composition of samples in each group is also taken from different parts/locations of the nails so that it can cause variations in data from the level and purity of DNA, parts/locations of nail cutting from the outermost part of the distal nail (free edge) and mixed with the proximal part of the nail where the part is anatomically closer to the nail root and nail matrix. The nail matrix is responsible for the formation of the hard nail plate. It is the only part of the nail unit which contains melanocytes. Nail cells, called onychocytes, are driven superficially and distally to form the nail

plate (9) Suppose the outline is drawn in this study. In that case, there are 25 human nail DNA samples from both the control group and the treatment group, where each sample weighing an average of 5 mg obtained average DNA levels measured by NanoDrop showed a value of 382,668 ng / µl. In DNA purity measurements, showing the absorption ratio λ 260nm / λ 280nm ranging from 1.13 to 1.85 with an average of 1.68, indicating the presence of contaminants such as proteins, UV, phenols, and other substances in several products, DNA purity values usually range from 1.8-2.0. If the purity of DNA is less than 1.8, then the indication of contaminants from protein and UV, while if the purity of DNA is more than 2.0, then the indication of the presence of RNA, chloroform, and phenol contaminants (10).

The results of this study are broadly the same when compared to research conducted by Truong L et al. In 2015, using the same sample of live human nail clippings with an average sample weight of 4-10 mg from 14 volunteers, the nail clippings were washed with acetone before extraction. DNA was extracted using QIAamp MiniElute ccfDNA Kits (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. While in this study, the extraction used the chlorophyll-phenol technique. The research of Truong L et al. 2015 from a total of 14 samples of human nail clippings, where the average sample weight was 6.3 mg, resulted in DNA levels of 544.0 ng / µl or 91.6 ng/mg nail clippings and produced an average DNA purity of 1.43. In this study, broadly from a total of 25 human nail cut samples, where the average sample weight was 5.1 mg, it produced DNA levels of 382.68 ng / µl or 75.6 ng/mg nail clippings, and it produced an average DNA purity of 1.68.

From the description of the results and discussion above, the study results are still included in the category of having good purity to be used for the PCR amplification process. The DNA levels needed for each DNA analysis method are different. For the amplification process using Short

tandem repeat (STR) using PCR, 1-25 ng / µl is needed (11). So that when the PCR amplification process is continued and analysing STR D18S51 loci.

The results of PCR visualization are analysed descriptively by looking at the presence or absence of bands or band DNA corresponding to the size of the STR PCR product for each loci. The STR loci used in this study was D18S51. The choice of loci is because the loci Indonesia still needs to have a DNA population database of 13 STR CODIS. Several Indonesian population-specific alleles differ from others, including the 7 loci alleles D7S820 and D18S51 (12). The size of the D18S51 loci PCR product in each sample with the highest and lowest DNA levels in each group in this study can be seen in Table III.

Table III. The size of the PCR product of the D18S51 loci in each sample with the highest and lowest DNA levels

D18S51 loci	PCR result product size (bp)	The frequency of occurrence of DNA bands (%)	Information
Control		100%	Control
Sample F3-3		100%	Lowest rate (271.7 ng/µl)
Sample F3-2		100%	Highest rate (739.9 ng/µl)
Sample S3-1	290-366 bp	100%	Lowest rate (143.3 ng/µl)
Sample S3-2		100%	Highest rate (342.1 ng/µl)
Sample F7-5		100%	Lowest rate (329.4 ng/µl)
Sample F7-4		100%	Highest rate (491.9 ng/µl)
Sample S7-2		100%	Lowest rate (322.4 ng/µl)
Sample S7-5		100%	Highest rate (441.9 ng/µl)

PCR visualization results from the loci above in the highest and lowest samples show a band or band DNA appears 100% and there are some unclear or thin PCR visualization results in Figure 1; this can be due to degradation. If DNA degradation occurs, DNA cannot be amplified (13).

DNA degradation is mainly caused by environmental exposure, including high temperatures and humidity. In this study, although the results of measuring DNA levels were relatively high in the treatment group with fresh water immersion, the PCR visualization could have been more precise. The freshwater measurement showed a value of 6, which means acidic; water is one of the environmental factors; freshwater and saltwater conditions have a pH value (power hydron) variously, and acidic pH can increase degradation (14). The pH of freshwater ranges from 6.8 to 8.5, while the pH of salt water ranges from 7.8 to 8.2 (15).

Another cause that can cause thin visualization results is the short loci of chromosomes and the presence of telomerase processes. Chromosomes are easily degraded due to environmental influences, especially temperature and humidity. In comparison, the telomerase process is a natural process that occurs on chromosomes, in other words the shortening of chromosomes at the ends of chromosomes. The ends of these chromosomes are uncoded nucleoprotein structures (non-coding) called telomeres (16,17). Telomeres comprise guanine-rich hexanucleotide replicates (TTAGGG in vertebrate cells) and are enveloped by protein binders, forming a spin structure (18).

The function of telomeres is as a cover, essential for maintaining chromosomal stability by protecting the ends of chromosomes from recombination, fusion, and degradation. Therefore, loss of telomere function may majorly affect chromosome maintenance and integrity. In some studies, it is stated that telomere length is associated with the replication capacity of eukaryotic cells. It has been proven that there is a cause-and-effect relationship between telomere shortening and human cell replication due to the aging process wich is the life span of fibroblasts and epithelial cells can be extended by lengthening telomeres. Contrary to normal somatic cells, cancer cells are described as having undergone a delay in cell aging by lengthening telomeres, resulting in unlimited replication capacity. The natural process of telomerase on autosomes causes the size of

chromosomes to get shorter, so the area coding is also shortened. This can be caused when PCR is amplified, the primer cannot stick because the size of the template DNA does not correspond to the amplicon size (18)

CONCLUSIONS

This study showed that human nail clipping samples did not show a decrease in DNA levels and purity even though they were given immersion treatment of fresh and saltwater for 3 days and 7 days, respectively. In the visualization of electrophoresis results at the D18S51 loci, all representatives of all groups with the highest and lowest levels showed DNA bands, in representatives of the sample group immersed in fresh water thinner than other groups. In water pH measurement, PCR product size 290-366 bp shows freshwater pH of 6, and saltwater pH as significant.

Statistical analysis showed differences in average DNA levels and purity between groups, but none were smaller than the control group without immersion treatment. From a total of 25 human nail clipping samples, where the average sample weight was 5.1 mg, produced DNA levels of 382.68 ng/µl or 75.6 ng/mg nail cuts, resulting in an average DNA purity of 1.68.

Statement of Ethics: The Authors have no ethical conflicts to disclose

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