Analysis of Human Decomposition Effect on DNA Quality with Short Tandem Repeats [STRS] Combined Index DNA System [CODIS]

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Abstract

Introduction: Personal identification is a problem in criminal or civil cases. Exact determination of personal identity is very important in investigation as any error can be fatal in the judicial process. The process of identification that is often used is through DNA analysis. The problem that often becomes a serious problem for both forensic DNA expert and other DNA expert is the condition of degraded DNA.

Method and Materials: This study was conducted by analysing DNA damage patterns using CODIS STR (Short Tandem Repeat) markers to effect the decay process. The type of research used by experimental laboratories, with research design used is time series.

Results and Discussion: The result of DNA sample extraction from Psoas and Masseter muscle samples shows the average minimum range of DNA levels for DNA typing is 0.25 ng with purity of 1.8 – 2(1). The result of this study prove the effect of decomposition on DNA concentration on each muscle. This study is also showed a decrease in levels and purity in the samples of Psoas and Masseter muscle tissue buried in soil, sea/salt water and river/fresh water from day 1, 7, 14, 20 and 40.

Conclusion: Psoas and Masseter muscle can be alternative material for forensic identification. The success of sequence mapping pattern from STR loci are TPOX, THO1, and CS1PO according to each GC content. GC content of THO1 and TPOX has the same relative value of 0,48, compared to CSF1PO value of 0,33.

***Keywords*:** *Decomposed, Identification, STR CODIS*

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Introduction

Identification is to determine the truth or error of an accusation, including in criminal cases: identification of criminals, murderers, perpetrators in torture, rape and others. Civil matters involve insurance, inheritance rights and the alleged father of a child. Identification is generally divided into namely identification for victims of life and identification for people who have died either known or unknown.[1,2]

Identification through DNA analysis methods is an accurate and stable diagnostic tool. Individual genetic information has similarities to the whole cell and will remain unchanged even after death.[3]

In its development examination using DNA analysis method is not without obstacles. One obstacle that is often a serious problem for both forensic DNA expert and other DNA expert is the condition degraded DNA.[3,4]

Muscle tissue is an organ that has enough cell content so that there is DNA in the cell nucleus. One type of the muscle that is often used/recommendations in DNA analysis is the Psoas muscle and the Masseter muscle. Psoas muscle is a muscle located in the formation of the lower abdominal wall or posterior abdominal wall behind the diaphragm, associated with Quadratus lumborum in which there are arteries, veins and lymph nodes. While the Masseter muscle is a chewing muscle with vertical fibre model so that it will strengthen the direction of the bite/chew movement.[1,5]

Decomposition on the body is one of the obstacle in the DNA analysis method. Although DNA degraded is considered as a frightening thing for forensic DNA expert, forensic DNA experts overcome this situation by conducting research o research to create a way of examining DNA that under certain conditions. So far the effect of the decaying process of the body on the quality of DNA undergoing fragmentation in the examination of forensic identification are well known.[6,7]

In DNA analysis with a repetition unit length of less than 1 kbp is called a Short Tandem Repeat (STR). This STR is popular because it has a small allele size (less than 1 kbp) so it can be easily amplified by PCR and even degraded samples can be analysed. The FBI recommendations for identification are 13 STR-CODIS loci. The study used 3 STR loci [CSF1PO, THO1, TPOX] which have high discriminant power for Asian continental populations.[4]

Materials and Methods

This was an experimental laboratory with a time series research design. The sample came from psoas and masseter muscle. The used body was not identified in 2 x 24 hours [according to article 133 Law of Criminal Procedure]. Psoas and masseter muscles were stored in soil, sea/saltwater, river/fresh water for 1,7,14,20, and 40 day. The sample size is 24 psoas and 24 masseter muscle samples. This research have been approved by the ethical commite 33/hrecc.fodm/IV/2019.

Research material: psoas and masseter muscles, material for DNA extraction: DNAzol Reagent, 100% solution & 70% ethanol. Material for PCR: PCR mis (12.5 ml) consisting of dNTP (ATP< CTP< TTP< GTP), $MgCl_2$ and Taq Polymerase, DW sigma (Nuclease Free water), CSF1PO promer [Gen Bank Accession X14720]: 5'-AACCTGAGTCTGCCAAGGACTAGC-3' &5'-TTCCACACACCACTGGCCATCTTC-3', THO1 [Gen Bank Accession D00269]: 5'-CTGGGCACGTGAGGGCAGCGTCT-3' & 5'-TGCCGGAAGTCCATCCGCCAGC-CCC [CCG-CCG] ACCG-3 [CC-CCG]]' &5'-GGAGGACTGGGAACCACACAGGT-3'

Result and Discussion

Analysis of decomposition effect on DNA concentration

This study begins with the exposing samples with soil, sea water/saltwater, river water/fresh water within 1,7,14,20, and 40-day period. The exposed sample extracted with DNAzol method and were measured of DNA levels and DNA purity of the sample using UV-Visible Spectrophotometer.

The result of DNA extraction/isolation obtained DNA levels of Psoas muscle samples with average DNA levels of 572.89 ± 5.71 mg/ml with average DNA purity of 1.09 ± 0.21. Whereas DNA samples from the Masseter muscle produced an average DNA level of 589.19 ± 5.58 mg/ml with mean DNA purity of 1.29 ± 0.26. All of the average DNA samples were still in the minimum range of DNA levels for DNA typing, ie 0.25 hg with a purity of 1.8-2.[1]

From the results of this study prove the effect of the decomposition process on the DNA levels. It can be seen from the measurements of DNA levels through a UV spectrophotometer showing decreased of DNA levels of psoas muscle tissue samples that are buried in the soil and submerged in seawater where from days 1, 7, 14, 20 and 40.[1]

However, with the decreasing of DNA level, is not a

major problem since the remaining DNA levels are still possible to do a DNA profiling (minimum of 50 ng).

Water quality is tested by several parameters, such as physical parameters which are temperature, turbidity, dissolved solids, etc. Chemical parameters are pH, dissolved oxygen, BOD, metal content, and biological parameters namely the presence of plankton and bacteria. The pH and NaCl tests resultfrom two media (sea water and fresh water) at the Surabaya Health Laboratory Center stated that sea water media has high levels of NaCl which is 5.50 and 1,652.93 mg / L, compared to regosol soil media has a pH content at on 7.00 is 8.75 and NaCl 314.20 mg / L.[8,9,10]

DNA degradation of the human body can be caused by 2 factors, specifically endogenous and exogenous. Endogenous factors originate in the cell itself, which are also known as spontaneous degradation. Exogenous factors come from the environment. Postmortem decomposition of the human body is a very complex process, beginning with autolysis and decay and followed by aerobic and bacterial decomposition of organic matter. Environmental factors such as humidity and environmental temperature are very significant on the condition of DNA used as DNA identification material in the forensic field, as in DNA analysis in other fields. In general, forensic samples carried out by DNA testing, 40% have experienced degradation or contamination, so the STR analysis which has a core sequences of less than 1 kb (kilobases) is very effective and the success rate is high, especially on degraded DNA by producing short fragments.[4,11,12]

DNA level is an important factor in the DNA analysis, especially on the success of amplification of DNA samples. A decrease in DNA levels up to 1 ng has the potential to reduce the STR's detection ability to 95%. DNA integrity is a major concern for DNA forensic examination. This implies that even though the examination of DNA levels obtained is relatively high, if DNA has undergone fragmentation or degradation, then high DNA levels, become less meaningful.[2,9,13,14]

In addition to DNA levels, it is also necessary to have sufficient DNA quality, in other word, the degradation of DNA must be minimum. If the DNA degradation is severe, the primer cannot stick to the selected target DNA to be duplicated.[1,15]

Analysis of Visualized Amplification at loci: CSF1PO, THO1 and TPOX

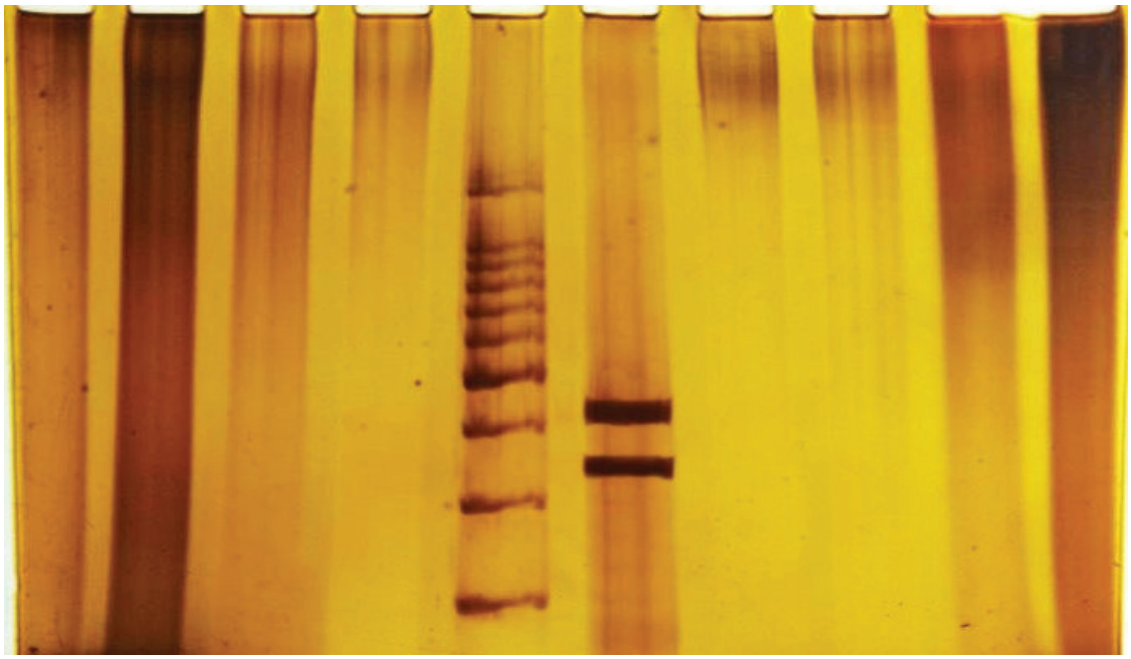

Figure 1. Results of PCR STR CODIS visualization locus CSF1PO [321bp - 357bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle, on day 7,14,20 on sea water media.

The amplification process begins with the preparation of the DNA template through the extraction and isolation process using an extraction kit (DNAzol). The PCR process uses primers at the STR CODIS locus [CSF1PO, THO1, TPOX] as shown by Figure 1, Figure 2, and Figure 3:

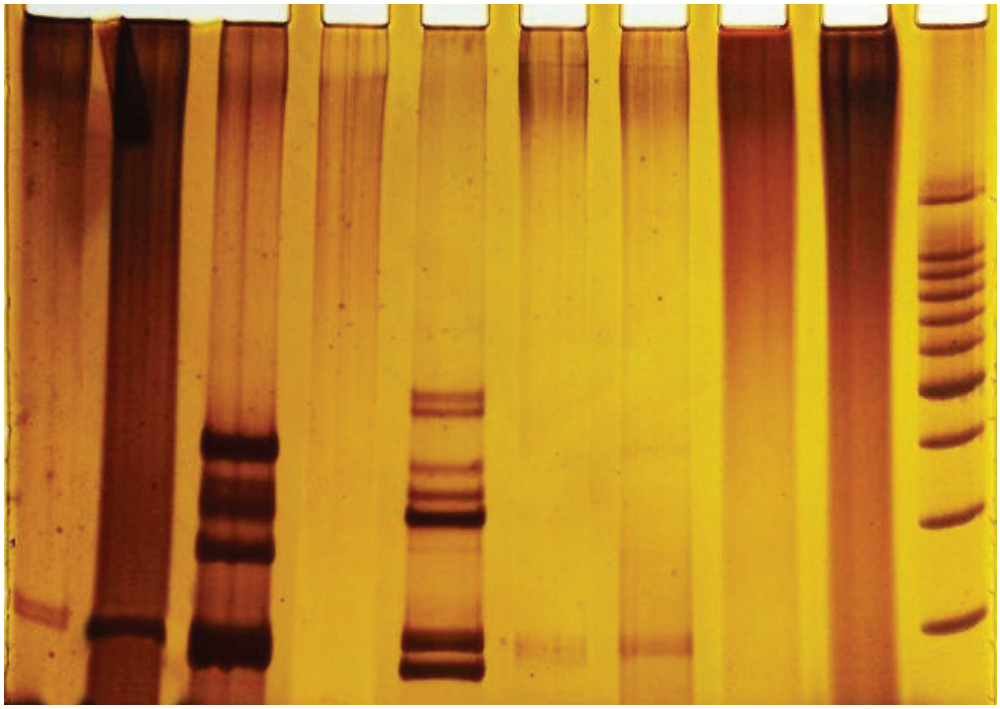

Figure 2. Results of PCR STR CODIS visualization locus CSF1PO [321bp - 357bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle, on day 1in the media Land, Sea Water and River Water.

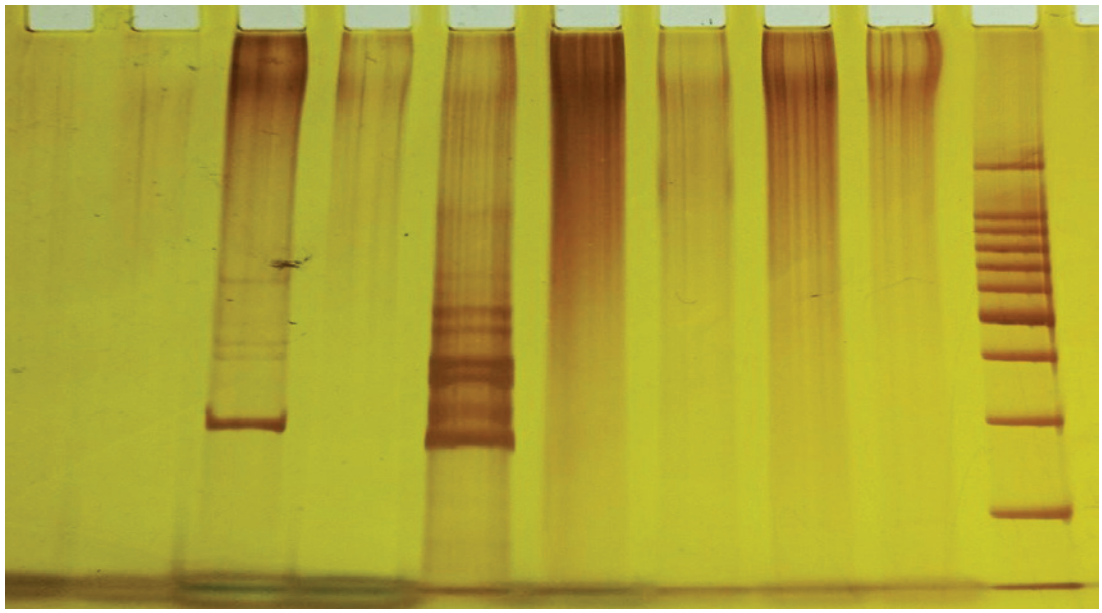

Figure 3. Results of PCR STR CODIS visualization of the TPOX locus [262bp - 290bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle,on day 7,14,20,40 River Water media

Table 1. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on Media Land

SAMPEL	Loci	CSF1PO					THOI					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%
Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%

Table 2. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on Sea Water Media

SAMPEL	Loci	CSF1PO					THOI					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	4/ 100%	4/ 100%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%

Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%	0%

Table 3. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on River Water Media

SAMPEL	Loci	CSF1PO					THO1					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%
Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	0%	4/ 100%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%

The table 1,2, and 3 above show negative detection results [-] on average from day 20 almost all STR CODIS loci: THOI, TPOX detected negative, except STR CODIS locus: CSF1PO detected negative [-] starting on day 7 on soil media, sea water and River water.

Study on STR CODIS as a general has not been reported, only a few primers. The results of related study of STR CODIS is solitary the pattern of mapping sequence of the STR locus are TPOX, THO1 and CSF1PO, based on GC content ratio. The calculation of the GC ratio of THO1 and TPOX content having the same relative value of 0.48, compared to CSF1PO of 0.33. Several studies have been conducted from several STR CODIS focus areas. The accuracy of research at the CSF1PO, THO1, TPOX locus has been reported in several studies including: chromosome populations and allele sequences at the THO1 locus, populations in Thailand with 8 STR loci including THO1, TPOX, CSF1PO and vWA and study of Chinese populations in Taiwan with STR. Genetic variation in Caucasia, examined genetic variation in population Filipinos and Thais living in Taiwan, use 9 STR loci. Whereas in Indonesia, THO1 allele pattern in the Batak population in Surabaya and D1S80 and D17S5 locus populations in Surabaya. The method of typing STR loci especially THO1 is a method that seem sensible, is convincing and efficient so that it is a useful method in forensic cases. 5 to 6 STR loci have a ratio of 1: 100 billion. In principle regarding the number of loci examined is that the more loci used for examination the better the accuracy.[16,17,18] Associated with the the success of the detected loci, it is because by differences in amplification products and the presence of GC content or guanine and cytosine bonds at each locus. GC content or guanine cytosine bonds have a high level of stability against denaturation factors compared to the bonds between Adenine and Thymine.[19,20,21,22]

CONCLUSION

Psoas and Masseter muscles can be an alternative component in forensic identification. The success pattern of mapping the sequence of the STR locus is TPOX, THO1 and CSF1PO. According to its GC content ratio. This is consistent with the calculation of GC THO1 and TPOX content ratios that have the same relative value of 0.48, compared to CSF1PO of 0.33.

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Ethics Approval and Consent to Participate

This study has been agreed by ethical commite which number 33/hrecc.fodm/IV/2019

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Conflict of Interests: The author and co-authors declare that they have no conflict of interest in publishing this article.

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