Identification of DNA Corpse in Ordo Diptera Familia Calliphoridae Larvae in CSF1PO, TH01, and TPOX Locus Using Polymerase Chain Reaction (PCR) Methods

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Abstract

Introduction: Biomolecular techniques using DNA are known to have high accuracy in the identification of decaying bodies. In the process of identification, larvae found in corpses that have undergone decay can be used as alternative biological evidence to estimate the time of death.

Aim: To identify the transfer of body DNA to the larvae of the fly ordo Diptera family Calliphoridae and the process of DNA transfer of decomposed bodies.

Method: blood from the postmortem corpse was taken as a control, then soft tissue was taken as a decomposition product for the breeding of Dipdo family Calliphoridae larvae. The protocol used is DNA profiling using the Short Tandem Repeat (STR) locus such as CSF1PO, TH01, and TPOX. The results of DNA extraction from the postmortem body and DNA extraction results obtained from the skin of the larvae and the digestive system of the larvae are then matched.

Result: The results of this study found that there was DNA transfer in larvae proved by the matching of DNA at the STR loci on the skin and the digestive system of larvae with the STR locus in blood taken from the postmortem body

Conclusion: the presence of decomposed corpse DNA decomposition to the larvae of members of the order called Calliphorideae family which can be detected through the results of nuclear DNA visualization at the CSF1PO, TPOX, and TH01 loci using the PCR method

Keywords: *corpse DNA, Diptera, Calliphoridae, Short Tandem Repeat (STR), polymerase chain reaction (PCR)*

Introduction

Death is the final stage of the life of every living thing including humans. In the world of forensic medicine, it is known that death can take place naturally or unnaturally (natural and unnatural death). Both natural death and unnatural death will undergo a process of decomposition (decomposition)[1]. The decay process is caused by the effect of proteolytic enzymes and microorganisms. Generally, the decay process starts 18 to 24 hours after someone dies[2].

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In the process of identifying corpses that have undergone decay, conventional methods such as visual methods and fingerprints cannot be used, so using DNA biomolecular analysis is a fast and appropriate method to use. The biomolecular method itself was only demonstrated by scientists in the early 1980s, by Jeffreys with DNA technology successfully demonstrated that parts of DNA can be used as a means of specific (personal) identification of a person so that since then various kinds of methods have begun to be applied to the investigation of cases forensics[3]. Compared to conventional methods that rely on serology and electrophoresis technology, DNA technology has advantages, especially in its potential for criminalization and sensitivity[4].

Identification towards the use of molecular forensics is increasingly needed. This method was first introduced by Sir Alex Jeffreys in 1985, who utilized medical and biological knowledge at the molecular or DNA level (deoxyribonucleic acid)[3]. Especially in cases of mutilation murders, victims of airplane accidents, suicide bombings that cause the victim's body to break into pieces, scorched and often found decomposed. These parts are difficult to recognize visually, so DNA analysis is an alternative method that can be used in the situation above because of its high level of accuracy[5].

The decayed products eaten by flies contain the nucleus of human cells[6]. The larvae found at the crime scene are one alternative biological evidence that needs to be analyzed. Considering the information obtained on the body of a flight will help police and investigators in determining the estimated time of death (estimation time of death) and help provide information on the possibility of fabricating a crime scene. Meanwhile, Welles and his colleagues in their research found that mitochondrial DNA (mtDNA) sequence data can be obtained from the intestine dissected from maggots that have been given to human tissue. This data can be used to identify both the human corpse upon which the maggot has eaten and the species of the maggot itself[7,8].

This study will use larva specimens (maggots) from the fly family of the Diptera Ordo results of postmortem soft tissue cultivation, with a view to knowing the process of transferring human DNA to fly larvae. In addition to knowing that the process of transferring human DNA into the larvae of the fly passes through the digestive system (migration occurs because the larvae feed on the corpse's soft tissue), or through the skin of the larvae in contact with the decomposed corpse's soft tissue. To be able to know the existence of decomposed human DNA and decomposed processes, it is necessary to extract the adult larvae in full and extract the inside (intrainstestinal digestion) rather than flies that are cultured from the soft tissue of decomposed human bodies. Considering that corpse cases that have undergone decay are very rarely found in the Department of Forensic Medicine/Soetomo Teaching Hospital, Surabaya. So that the cultivation of larvae is carried out by taking soft tissue samples of fresh human remains and allowed to rot to invite flies to lay their eggs. The larvae of the fly will live and eat the soft tissue of deceased human bodies.

The locus that will be taken for analysis is the Short Tandem Repeat (STR) locus: CSF1PO, TH01, TPOX which are some of the 13 loci determined by the Federal Bureau of Investigation (FBI). Examination using the PCR method because this method can multiply millions of DNA to billions of Kalli so that it is possible to conduct an analysis with a very minimal number of samples[9,10]. In addition to the PCR method is also able to analyze materials that have been partially degraded. This is important because DNA samples in the skin or in the digestive system of larvae will experience degradation due to temperature, PH, larval metabolic system, and contamination with other objects around the body[8]. The principle of PCR is selective exponential amplification of certain DNA fragments. The purpose of this study was to identify the transfer of body DNA to the larvae of the fly ordo Diptera family Calliphoridae and the process of DNA transfer of decomposed bodies.

Method

This type of research used in this study is an observational laboratory with a longitudinal study approach to analyzing the STR locus: CSF1PO, TPOX, and TH01. Some of these loci include loci recommended by the FBI (Federal Bureau of Investigation) with DNAzol extraction techniques. The results of this study are presented descriptively. The population in this study is the remains of humans who have undergone a process of decay. To obtain larvae, researchers still need to breed fresh bodies by autopsy. Whereas for the breeding of the order larvae, the Calliphoroda family is labeled to take part in soft tissue that is still fresh in the same body.

DNA samples were obtained from blood spots that would be used as a comparison material, and the larvae of the order were listed as the Calliphoroda family that grew in the soft tissue of the decomposed corpse. A sampling of research from the corpse has received permission from Forensic Medicine and Medicolegal Department/ Soetomo Teaching Hospital and thi ethical clearance permit from the ethics committee at Soetomo Teaching Hospital Surabaya, Indonesia. The variables of this study consisted of the independent variable (bodies that have undergone decay, fly larvae, larval exposure system) and the dependent variable (DNA degradation (core DNA) and DNA transfer to larvae).

Samples taken were larvae obtained from corpse soft tissue (postmortem) that had not been decomposed and decomposed, adult larvae of the order flies were put on the Calliphoridae family that were exposed to the corpse or soft tissue used as media (breeding) larvae. Sampling was conducted in the mortuary of the Soetomo Teaching Hospital Forensic and Medicolegal Department and sample examinations were carried out at the Human Genetic Study Group Laboratory, Institute of Tropical Disease (ITD) Universitas Airlangga.

Result

DNA Levels and Purity Measurement

DNA levels obtained in the skin and digestive tract of larvae were respectively: 0.2181 µg / ml and 0.0870 µg / ml, and the purity of DNA was 1.34 µg / ml and 1.02 µg / ml.

Table 1: DNA Content and Purity of Larval Isolation

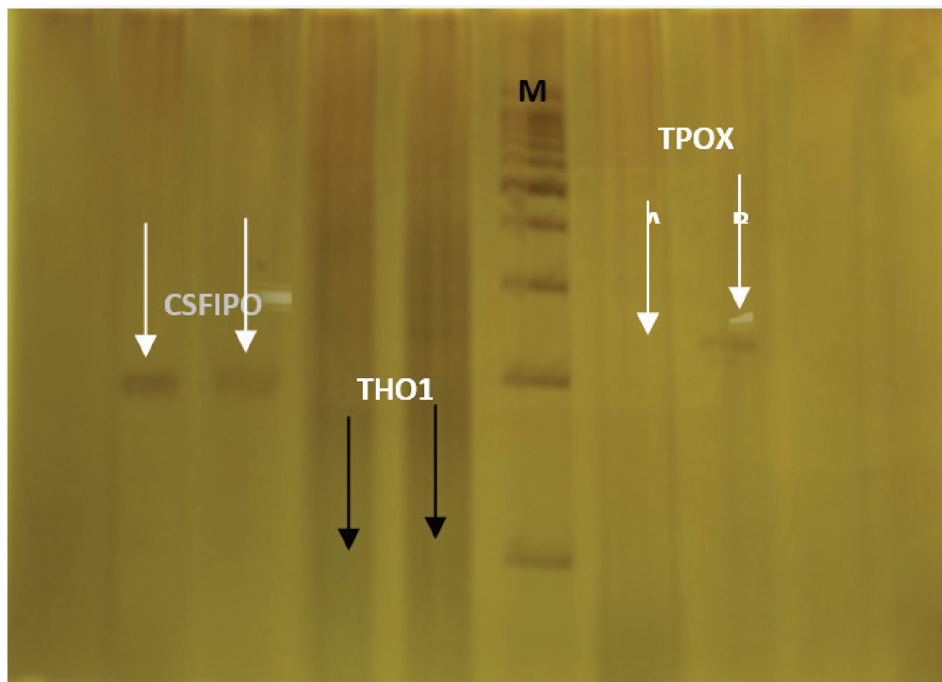
Sample	Absorbance		DNA levels (µg/ml) Absλ260x dilution x 50)	Purity of DNA (Abs λ 260 / Abs λ 280)
	λ 260	λ 280		
Fresh tissues	0.451	0.449	0.3268	1.46
Larvae skin	0.301	0,225	0.2181	1.34
Gastrointestinal tract	0.120	0.082	0.0870	1.02

DNA Visualization

After being colored, a picture of bands or ribbons from each locus will be examined with a marker tape.

Picture 1: Visualization of CSFIPO, THO1 & TPOX loci

A: Larvae skin, B: Gastrointestinal tract M: Marker ladder 100 bp



In electrophoresis visualization using polyacrylamide agarose gel composites in the form of a ribbon, it is determined whether the TH01 locus appears, by pulling the tape line from the sample towards the 100 bp marker, where the TH01 locus is between 179 - 203 bp. Then the tape is pulled towards the control (K562 marker as a positive amplication control) to determine the location of the allele whether under or over the control. The K562 marker is the allele marker of the CTT multiplex from Promega Corporation 2001. The TH01 locus of the multiplex CTT marker with alleles between 5-11 (K562 is located at alleles 9.3, 9.3). Then the larval skin bands and larvae are compared whether they are parallel (identical / consistent / matching) at the TH01 locus. Whereas the TPOX locus is between 224 to 252 basepair with allele variations ranging from 6 to 13 and CSF1PO locus is between 295 - 327 basepair, and contains T-A-G-A core sequence repeat with an allele range of 7 to 15 bp.

Table 2: Results of reading of electrophoresis visualization between larval skin and larvae with blood samples at the CSF1PO, THO1, and TPOX

No	Sample		THO1 Locus	TPOX Locus	CSF1PO Locus
1	1	Skin larvae	Identical	Identical	Identical
		Blood			
2	2	Gastrointestinal larvae	Identical	Identical	Identical
		Blood			

Discussion

DNA levels and purity are important stages in the PCR process as we know that the purity of DNA samples will affect amplification. The purity of DNA depends very much on the quality of the DNA samples taken, DNA purity 1-2 (ideally 1.8-2)[11]. DNA levels are an important factor in forensic DNA testing, which influences the success of STR and PCR on DNA samples. Decreasing levels up to 1 µg has the potential to decrease the STR's detection ability by 95%[12]. For obtaining adequate visualization results it requires adequate DNA purity and adequate DNA levels so that it can be used as a DNA examination material[13]. The amount of DNA needed in forensic DNA analysis varies depending on the needs and type of examination. The STR only requires a minimum DNA concentration of 1-25 µg. In addition to depending on DNA levels, the examination material also requires sufficient DNA quality that is DNA that is used in degraded conditions to a minimum[14]. If DNA is severely degraded, it will cause the primer to not be able to stick to the target DNA that will be duplicated[15].

To eliminate contamination from the outside attached to the skin of larvae, then to find out the transfer of DNA in the digestion of larvae, the skin of the larvae is rinsed with distilled water twice and sonified[16]. In this study using larvae as personal identification material, the results of this study found that larvae can be used as an alternative material in personal identification. The existence of the method of amplification of Polymerase Chain Reaction (PCR) with minimal DNA levels can be used typing checks. From various studies on the use of PCR in forensic medicine in amplifying target DNA, it is reported that the PCR technique has a very high success rate, because it requires relatively little DNA and relatively low 'freshness' levels compared to Restriction Fragment Length Polymorphisms (RFLP) techniques. The use of the TH01, TPOX and CSF1PO loci given that they were one of the first loci developed by the Forensic Science Service, and they have a probability of matching with a ratio of 1 in 50 million.

The results of the lottery or visualization of DNA eaten by larvae of members of the order listed Calliphoridae family were assessed (suitable) both on the skin and on the digestive system at the loci TH01, CSF1PO, and TPOX. The ribbon images in agarose determined at each locus differ in the thickness of the ribbon, this is related to several factors, according to the quality of the sample, the method of selecting larvae,

the technique of making extraction[20]. The results of the visualization or summary of DNA bands in agarose also show a difference, in which there is more band larvae than in the digestive tract. This is because in the stomach (plant) larvae of the fly, temporary liquid food and primary digestion do not occur in the stomach because proteolytic enzymes are not excreted in this area.

The results of this study can identify the three loci selected because they are part of the 13 STR loci that are used as a CODIS (Combined DNA Index System) data base identification system, and are the first loci available. These three loci rarely experience mutations because of the possibility of mutations of 1: 500, in the caucasian population these three loci have the least discrimination[1,12]. Thus it can be seen that the isolation of DNA from the larvae of members of the order family called the Calliphoridae family can still be an alternative material in forensic identification. Generally, DNA isolation from the larval digestive tract gets DNA levels or quantities low or even less, but DNA bands are still visible on the results of electrophoresis visualization. From these low levels, the identification test through the digestive tract of larvae has a low effectiveness value when compared with larval skin samples. However, DNA in the digestive tract can still be an alternative material for examination.

Conclusion

After analysis, the transfer of corpse's DNA to the larvae of members of the order is called the Calliphorideae family where it can be directly contacted by decaying results into the skin of the larvae or through the digestive tract.

Conflict of Interest: None

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References

1. Li R. Forensic biology. CRC Press; 2015.
2. Byers SN. Introduction to forensic anthropology. Taylor & Francis; 2016.
3. Wells JD, Stevens JR. Application of DNA-based methods in forensic entomology. Annu Rev Entomol. 2008;53:103–20.
4. Mukherjee S. DNA characterisation from gut content of larvae of Megaselia scalaris (Diptera, Phoridae) for forensic investigations. University of Huddersfield; 2019.
5. Jackson JK, Battle JM, White BP, Pilgrim EM, Stein ED, Miller PE, et al. Cryptic biodiversity in streams: a comparison of macroinvertebrate communities based on morphological and DNA barcode identifications. Freshw Sci. 2014;33(1):312–24.
6. Trebitz AS, Hoffman JC, Grant GW, Billehus TM, Pilgrim EM. Potential for DNA-based identification of Great Lakes fauna: match and mismatch between taxa inventories and DNA barcode libraries. Sci Rep. 2015;5:12162.
7. de Pancorbo MM, Castro A, Fernández-Fernández I, García-Orad A. Population genetics and forensic applications using multiplex PCR (CSF1PO, TPOX, and TH01) loci in the Basque Country. J Forensic Sci. 1998;43(6):1181-7.
8. Linville JG, Hayes J, Wells JD. Mitochondrial DNA and STR analyses of maggot crop contents: effect of specimen preservation technique. J Forensic Sci. 2004;49(2):1–4.
9. Choi E-J, Park K-W, Lee Y-H, Nam Y-H, Suren G, Ganbold U, et al. Forensic and population genetic analyses of the GlobalFiler STR loci in the Mongolian population. Genes Genomics. 2017;39(4):423–31.
10. Tahaa T, Elzalabanyb S, Shaker O, Fawz S. Genetic Variation for Nine Autosomal Short Tandem Repeat (STRs) in Egyptian Population. J Forensic Med. 2018;3(123):1026–2472.
11. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques. 1991;10(4):506–13.
12. Watson JD. Molecular biology of the gene. Vol. 1. Pearson Education India; 2004.
13. Chua TH, Chong Y V. Role of Polymerase Chain Reaction in Forensic Entomology. Polym Chain React Hernandez-Rodriguez P (red) InTech, Rijeka. 2012;51–64.
14. Nutton L. Detection of Male Human DNA from Whole Lucilia sericata (Meigen)(Diptera: Calliphoridae) Larvae using the Quantifiler® Trio DNA Quantification Kit. Murdoch University; 2017.
15. Haas NA. The Effects Hot Water Kill Time has on DNA Degradation and STR Profiling from Lucilia

sericata (Diptera: Calliphoridae) Crop Contents. 2019;

16. Chamoun CA, Couri MS, Garrido RG, Moura-Neto RS, Oliveira-Costa J. Recovery & identification of human Y-STR DNA from immatures of Chrysomya albiceps (Diptera: Calliphoridae). Simulation of Sexual Crime Investigation Involving Victim Corpse in State of Decay. Forensic Sci Int. 2020;110239.

17. Ago K, Orihara Y, Ago M, Nakagawa S, Ogata M. Evaluation of the species specificity for six human short tandem repeat loci CSF1PO, TPOX, TH01, F13A01, FESFPS and vWA, in the Japanese macaque. Leg Med. 2004;6(2):102–8.

18. Skowronek R, Tomsia M, Droździok K, Kabiesz J. Insects feeding on cadavers as an alternative source of human genetic material. Arch Med Sądowej i Kryminol Forensic Med Criminol. 2014;64(4):254–67.