DNA ISOLATION FROM SWEAT STAIN IN CLOTHES AS FORENSIC IDENTIFICATION MATERIAL

Ahmad Yudianto, Soekry Erfan Kusuma
Human Genetic Study Group
Airlangga University Tropical Disease Center (TDC)

ABSTRACT

DNA profiling is an instrument to assist the investigation of either criminal or civil cases. It is also highly useful in forensic identification. Until recently, personal identification using sweat stain in clothes with DNA analysis method (DNA profiling) has not been commonly used in Indonesia. A study has been conducted on the sweat stain in clothes using DNA analysis for forensic identification material. Loci in this study were CSF1PO, THO1, TPOX, vWA and D17S5. From 10 samples used, only 6 met the typing requirement (level >20ng/ml and purity 1- 2). As a comparison, we used blood from volunteers. Except the locus D17S5 which was not examined due to the small amount of DNA remains produced from sweat stain, in electrophoresis visualization that presenting as bands, the four loci in sweat and blood stain were identical/consistent. In conclusion, sweat stain in clothes can be used as alternative material for forensic identification using molecular forensic examination.

Keywords: *sweat stain in clothes, STR, forensic identification*

Correspondence: Ahmad Yudianto, Human Genetic Study Group, Airlangga University Tropical Disease Center (TDC), Campus C, Airlangga University, Mulyorejo, Surabaya, phone 62-31-5992446

INTRODUCTION

In forensic medicine, identification can be115 obtained from fingerprints (dactiloscopy), property, medical, dental, serologic, and photographic examinations. Today, identification method has been developed towards molecular forensic medicine. Molecular forensic was introduced firstly by Sir Alex Jefreys in 1985, using medical and biological science at molecular or DNA (deoxyribonucleotic acid) level. DNA is the smallest inherited unit, present in all living beings, from microorganisms to higher organisms, such as human, animals, and plants. Any part of human body can be taken as specimen since any cell nucleus in human body has identical DNA chain, in which a child essentially receives the same amount of genetic materials from his biological mother and father (Mendelian laws of heredity). Recently, specimen/samples commonly used in DNA examination for identification are blood stain/sperm stain, vaginal swab, buccal swab, and bone (Kusuma 2004).

In forensic medicine, one examination highly useful in investigation is the examination of evidence present in the body of the victim or the criminal and in the crime scene. Sometimes, the criminal often tries to eradicate or obfuscate the evidence from the victim or the criminal himself by, for example, washing the blood stain. In such case, the criminal only has his attention only to blood stain, while in clothes, in addition to blood stain, there is also sweat stain attached in particular areas, such as collar, sleeve, and armpit. Sweat stain in clothes is the result of secretion of 2 types of gland, aqueous (sweat glands) and oily substances (sebum from sebaceous gland). Besides, there is also dead and detached epidermis.

Sweat (sudoriferous) gland present in almost all parts of the body, numbering around 3 to 4 millions, with total height almost similar to that of a kidney. Fatty gland (sebaceous) gland present in almost all of skin surface, except palm, sole and side of the feet where there is no hair. The epithelial cells of this gland secrete their content averagely 7.4 days, so that the cell form is changed (Champion 1992). Surface epithelial cells, called epidermis, are always renewed through cellular mitosis in basal layer. The newly formed cells through cell proliferation in basal layer gradually shift to epidermal surface (for 20-30 days), designated as cytomorphosis (Fawcett 2002). Due to such composition, sweat stain in clothes contains nucleated somatic cells originated from the degradation of gland cells and dead skin epithelial cells, allowing the extraction of their DNA. Until recently, in Indonesia personal identification by using sweat stain in clothes with DNA analysis (DNA profiling) is still rarely performed, so that this study could provide answers in problems related with effectiveness of the use of sweat stain as forensic identification material.

MATERIALS AND METHODS

This was an observational study, undertaken in Human Genetic Study Group, Tropical Disease Center (TDC), Airlangga University, from November 2005 to February 2006. There were 10 samples in this study, the volunteers' sweat stain taken from their collar, armpit, and side of sleeve of the shirts they wore continuously for one week. The shirts were made from cotton and were not washed. The volunteers comprised male *becak* drivers, aged 30-65 years old. Blood from these persons were also taken for comparison. DNA level resulted from isolation for typing was minimally 20 ng/ml (Gatut et al. 2004; Notosoehardjo 1999), and the DNA purity was 1 - 2 (ideally 1.8-2) to enable the PCR (Muladno 2002). Materials needed were DNAzol Reagent, 100% ethanol , 70% ethanol, Bis acrylamid , Agarose, Temed, marker ladder 100 bp, K562, PCR Mix, and the primers were THO1, TPOX, CSF1PO, vWA and D17S5.

RESULTS

Table 1. DNA level and purity from sweat stain

Samples no.	λ 260	λ 280	Quality (λ 260/λ280)	Level (μg/ml)
1	0.301	0.264	1.139	20.77
2	0.569	0.295	1.928	39.26
3	0.245	0.114	2.149	16.91
4	0.586	0.354	1.655	40.43
5	0.222	0.128	1.734	15.32
6	0.404	0.253	1.501	27.88
7	0.293	0.254	1.153	20.22
8	0.271	0.135	2.007	18.70
9	0.401	0.218	1.839	27.67
10	0.304	0.149	2.040	20.98
			1.714 ± 0.36	24.814 ± 8.44

Table 1 shows that DNA results from isolation of sweat stain from the samples' clothes are varied, ranging between 15.32 and 40.43 mg/ml. Theoretically, such DNA level would be able to use in DNA profiling process, which requires about 20 ng/ml for typing. The DNA purity from isolation of sweat stain in samples' clothes was 1 - 2 (ideally 1.8), also enabled the PCR amplification. Therefore, according to these requirements, there were only 6 samples who were practically eligible for typing. They were samples no. 1, 2, 4, 6, 7 and 9.

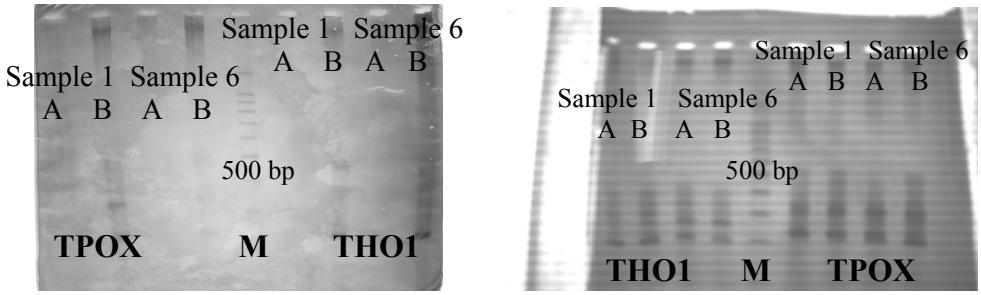

Figure 1. Visualization of loci TPOX and THO1 of sample no. 1 and 6 after first amplification PCR (left) and second PCR (right)
A : Sweat stain
B : blood
M : Marker Ladder, 100 bp

Table 2. Results of electrophoresis visualization reading between sweat and blood stain of the samples in loci CSF1PO, THO1, TPOX and vWA.

No	Sample	Loci CSF1PO	Loci THOI	Loci TPOX	Loci vWA
1	Sweat stain	Identical	Identical	Identical	Identical
1	Blood				
2	Sweat stain	Identical	Identical	Identical	Identical
2	Blood				
3	Sweat stain	Identical	Identical	Identical	Identical
4	Blood				
4	Sweat stain	Identical	Identical	Identical	Identical
6	Blood				
5	Sweat stain	Identical	Identical	Identical	Identical
7	Blood				
6	Sweat stain	Identical	Identical	Identical	Identical
9	Blood				

DISCUSSION

Studies on sweat stain have not been commonly performed. Many facts have not been disclosed, particularly those for forensic identification. Yamamoto K (1996) investigated cigarette butt as material for forensic identification. Sadad AR et al. (2004) carried out a study on mtDNA and cDNA content in urine. Kesay W et al. (2000) and Peter (2004) reported identification using sweat that attached to evidence with fingerprint examination. Seo Y et al. (2002) performed forensic identification using serumen found in earphone used by a burglar in Japan. Sosiawan A et al. (2004) studied the effectiveness of serumen as material for paternity examination.

In essence, sweat stain in clothes can be alternative material in forensic identification, as that used in forensic cases with samples from blood, vaginal secretions, skin scraping, or others. Sweat stain is suggested to have a high contribution in providing DNA from epidermal epithelial cells and glandular cells from sebaceous gland. The amount of sweat stain itself depends largely on the amount of secretion of sweat gland and the duration of the clothes being worn. In persons who are conducting activities (for example, sport) or exposed to heat 1-6 weeks, the amount of sweat is higher, increasing to 1.5-2 liter/hour (Guyton et al. 1994). In addition, activities induce adrenergic stimulation that results in the increasing sweat secretion. The level of humidity in air and ambient environment also affects the production of sweat.

Above factors may affect the amount of sweat stain attached in clothes, which directly affects the level of DNA produced. Lower DNA level from sweat stain in clothes affects the result of electrophoresis visualization, where the DNA bands of the sweat stain looks obscure. This is related with the lower number of certain DNA fragments (loci) amplified by PCR. After second amplification was done, DNA from sweat stain appeared clear and identical/consistent with DNA from blood. It can be therefore concluded that in certain trace evidence, sweat stain can be noticed as it may be very valuable as an alternative for identification. This study used STR analysis since generally 40% of forensic samples subjected to DNA examination have been degraded or contaminated (Notosoehardjo 1999). Therefore, Short Tandem Repeat (STR) analysis with core sequence of less than 1 kb (kilobasepair) is highly effective with a high success rate. Degraded and fragmented DNA may result in short fragments.

CONCLUSION

It is apparent that DNA from sweat stain can be used as alternative for forensic identification. Generally, DNA isolation from sweat stain had lower DNA level or quantity, so that repeated PCR amplification, averagely more than once, is needed. The loci used in this study were CSF1PO, THO1, TPOX, and vWA for amplification, all of which showed identical and consistence with those taken from blood as comparison.

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