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# DNA Methylation on Bloodstain as a Forensic Age Estimation Method

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***ABSTRACT--Forensic identification is an effort to help law enforcement in determining a person's identity. Personal identity is often a problem in criminal cases, civil cases, death without identity, and mass disasters. Age estimation is very important in forensic identification. DNA methylation is a potential epigenetic modification for age estimation because DNA in aging process resembles developments that are regulated in processes that are tightly controlled by specific epigenetic modifications. In most cases of violent crime, bloodstains can be found at the crime scene. Bloodstain may come from victims, perpetrators of crime, or even from both. Bloodstain can be used to help reveal the fact scientifically Correlation between DNA methylation from bloodstain and a person's age is unknown. The study aims to determine the correlation of DNA methylation from bloodstain and a person's age. The study was conducted at the Institute of Tropical Disease of Universitas Airlangga from July to October 2019. The research method used was analytic observational which was conducted on 10 samples with details of 5 male and 5 female samples. The results of the study, correlation of DNA methylation with age in male subjects correlation coefficient was 0.888 with a significance value of 0.04 and in female subjects correlation coefficient was 0.834 with a significance value of 0.079. The conclusion, there is a significant correlation between percent methylation with age in male subjects, whereas in female subjects there was no significant correlation.***

***Keywords: age, bloodstain, DNA methylation, estimation, forensic***

## I. INTRODUCTION

Identification in the field of forensic medicine is an effort to support law enforcement in determining a person's identity. Personal identity is a frequent case problem in criminal cases, civil cases, death without identity, and mass disasters[1]. Primary data used in identification are fingerprints, dental records, and deoxyribonucleic acid or deoxyribonucleic acid (DNA)[2]. Age estimation is very important in forensic analysis. Age estimation of the individual is often using bones and teeth. This can only be in cases of human skeletons identification[2]. Dental records are still rarely performed. The primary data that is very possible to be examined is DNA[3].

The accuracy of the identification method in terms of the estimated age of the individual obtained from the examination of biological evidence can provide important guidance for law enforcement when tracking down unidentified persons[4]. Developing tests related to age estimation is a challenge for scientists and practitioners of forensic medicine because they must be able to apply and validate using small or degraded samples consisting of various tissues and body fluids. As a first step, the generation of a reliable age prediction model is a must[5]. Recent studies have shown that human aging is related to changes in DNA methylation in specific location genomes, and this epigenetic modification can be used to estimate an individual's age[6]

DNA methylation is the best way of epigenetic modification in estimating the age of biological samples in humans. This is because, DNA in aging individuals resembles developments that are regulated in processes that are tightly controlled by specific epigenetic modifications. This epigenetic modification process is only found in position 5 of the pyrimidine ring of cytosine in the order of 5'-Cytosin-phosphate-Guanin-3 '(CpG) dinucleotide. 5-Methylcytosine from several CpG peaks in genomic DNA can be replicated during cell division by maintaining certain DNA methyltransferases (DNMT) as mediation in that DNA[3].

Bloodstain is one of the most frequently biological sample in forensic laboratories because blood is easily scattered in almost all forms of violence, the investigation of bloodstains is very useful to uncover a crime. In most of criminal cases, bloodstains can be found at the crime scene. Bloodstains may come from victims, perpetrators of crime, or even from both. Blood is important biological evidence because it is a biological sample with potential properties more specific to certain human groups It can be used to help reveal the event scientifically[7]. Based on these problems, this study aims to determine the estimated age using DNA methylation on bloodstain.

## II. RESEARCH METHOD

This study was conducted at the Human Genetic Laboratory, Institute of Tropical Disease, Universitas Airlangga during the period of July to October 2019. The ethical clearance is certified by Health Research Ethical Clearance Commission Faculty of Dental Medicine

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This is an observational laboratory research with a total of 10 samples with different age variations in each sex. Bloodstains were dissolved in 0.9% NaCl in a 15 mL Falcon tube then 6000rpm centrifuged for 20 minutes. DNA Extraction and Isolation using Invitrogen ™ DNAzol ™ Reagent. The results of DNA extraction continued with the bisulfite conversion process using the EZ DNA Methylation-GoldTM kit from Zymo Research, The result of bisulfite conversion is continued by electrophoresis using 1% agarose gel and then amplified by PCR using GoTaq® Green Master Mix from Promega. PCR products were sequenced using Applied Biosystems 3130 XL Genetic Analyzers to identify CpG sites, The sequencing results were analyzed with Bioedit®. CpG Island can be calculated using the Emboss CpGplot online application on the website http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/. Percent methylation is determined by dividing the number of CpG islands by the number of sequences. The correlation between percent methylation and age was analyzed using the correlation test through the IBM SPSS Statistics 24 program.

## III. FINDINGS AND DISCUSSION

DNA from bloodstain as illustrated in Table 1 below. The data are in accordance with UV light absorption spectrophotometer measurements at 260 nm and 280 nm optical densities.

Table 1. UV Spectrophotometer Results

| Sampel | OD 260 | 0D 280 | Concentration (µg/µl) | Purity |
|---|---|---|---|---|
| A | 0.238 | 0.206 | 833 | 1.16 |
| B | 0.213 | 0.188 | 745.5 | 1.13 |
| C | 0.3 | 0.256 | 1050 | 1.17 |
| D | 0.454 | 0.372 | 1589 | 1.22 |
| E | 0.436 | 0.363 | 1526 | 1.20 |
| F | 0.312 | 0.267 | 1092 | 1.17 |
| G | 0.233 | 0.196 | 815.5 | 1.19 |
| H | 0.295 | 0.249 | 1032.5 | 1.18 |
| I | 0.165 | 0.145 | 577.5 | 1.14 |
| J | 0.481 | 0.393 | 1683.5 | 1.22 |

Based on the table above, the lowest DNA level is 577.5 µg / µl and the highest DNA level is 1683.5 µg / µl. The average level of DNA in blood spots is 1094.45 µg / µl. The lowest DNA purity value is 1.13 and the largest purity value is 1.22. The average value of DNA purity in blood spots is 1.18.

After bisulfite conversion, to see the presence or absence of DNA in the results of methylation and PCR visualization using agarose 1% gel. From the results of visualization using agarose gel electrophoresis obtained bands of 10 samples which generally range between 150 bp and 300 bp.

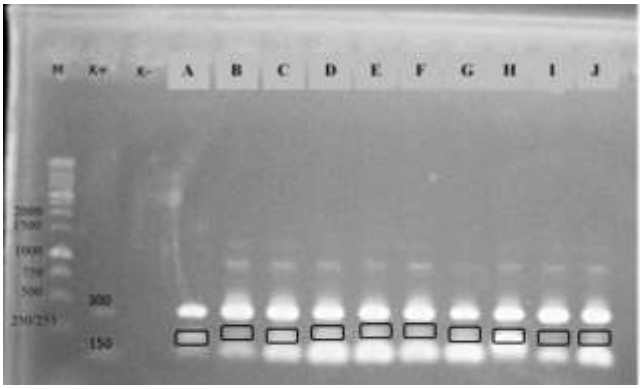


Figure 1. Electrophoresis results in agarose gel 1%

Information:
M = DNA marker 1 Kb
K+ = Positive control K562
K- = Negative control aquadest
A = 2 years old male.
B = 6 years old male.
C = 20 years old male.
D = 42 years old male.
E = 47 years old male.
F = 4 years old female.
G = 11 years old female.
H = 17 years old female.
I = 34 years old female.
J = 53 years old female

The results of DNA methylation were then sequenced using Applied Biosystems 3130 XL Genetic Analyzers in the form of FASTA to calculate percent methylation using Emboss CpGplot (http://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/). The results of the percent methylation calculation are presented in table 2.

Table 2 percent methylation at 300 bp

| Sample code | CpG site | Sequence | Percent Methylation (%) |
|---|---|---|---|
| A | 0 | 309 | 0 |
| B | 202 | 691 | 29,23 |
| C | 257 | 694 | 37,03 |
| D | 434 | 829 | 52,35 |
| E | 359 | 695 | 51,65 |
| F | 22 | 199 | 11,05 |
| G | 221 | 694 | 31,84 |
| H | 80 | 199 | 40,20 |
| I | 93 | 199 | 46,73 |
| J | 358 | 728 | 49,18 |

Data analysis using IBM SPSS Statistics 24, in male subjects, the r count value (Pearson Correlation) was 0.888 with a 2-tailed significance value of 0.04. Based on the R table, the R value for N = 5 with a significance of 5% is 0.878, because r counts are more than r tables and the significance value is less than 0.05 then there is a significant correlation between percent methylation and age in male subjects. In female subjects, the r count (Pearson Correlation) was 0.834 with a 2-tailed significance value of 0.079, because the r count was more than r tables but the significance value was more than 0.05, so there was no significant correlation between percent methylation and age in female subjects.

In this study, the lowest DNA level was 577.5 µg / µl and the highest DNA level was 1683.5 µg / µl. The average level of DNA is 1094.45 µg / µl. Optimal DNA levels needed for optimal results in bisulfite conversion are 200-500 ng[8]. DNA isolated from bloodstains in this study are in optimal levels for bisulfite conversion.

Percent methylation on male subjects tended to increase in line with age categorization but percent methylation actually decreased in elderly subjects. This is very likely influenced by lifestyle factors, the environment, and the illness. Whereas in female subjects the percent methylation consistently increased according to age categorization, although the rate of increase was not constantly measurable.

Between male and female, CpG sites have significantly different levels of DNA methylation. This CpG site is placed on an autosomal chromosome with absolute differences in methylation because the CpG site is located on the X chromosome so that differences in sex-specific methylation on the X chromosome tend to be more unstable. Women's susceptibility to stress and certain diseases also greatly affect the rate of DNA methylation, so that the factors that affect DNA methylation vary greatly between individuals[9]. Degenerative diseases and metabolic syndromes of each individual also greatly affect the results of percent methylated DNA. The process of the course of degenerative diseases targets elements of the cellular epigenetic machine, changing the expression and activity of the epigenetic machine so that it will affect changes in the epigenetic state of each individual[10].

The epigenome regulator which is often neglected is neuroendocrine, whereas DNA methylation can be a dynamic process, where the individual hormonal state greatly influences the role of hydroxymethylation of cytosine. The use of certain drugs which are oxidative and antioxidant will also have an effect on the rate of histone modification. This can occur, for example, through cytosine mutations in the case they are methylated in normal cells. The absence of methylated cytosine can cause modification of permissive histone and allow genes to be expressed. This type of mechanism can change the phenotype and behavior of cells[10]. Neoplastic, degenerative, metabolic, and even inflammatory diseases will cause oxidative stress which will affect the activation and inactivation of certain genes, as well as genome instability, which occurs with epigenetic mechanisms as well. Unlike genetic mutations, epimutation does not change the basic sequence of DNA and is potentially reversible[10].

## IV. CONCLUSION

From this study it can be concluded that DNA isolation in blood spots results in the lowest DNA level being 577.5 µg / µl and the highest DNA level is 1683.5 µg / µl. The average level of DNA in blood spots is 1094.45 µg / µl. The lowest DNA purity value is 1.13 and the largest purity value is 1.2. The average value of DNA purity in blood spots is 1.18. DNA methylation in blood spots was visualized using agarose gel 1% electrophoresis between 150 bp - 300 bp. There is a significant correlation between percent methylation with age in male subjects, whereas in female subjects there is no significant correlation.

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