MATERNALLY INHERITED NUCLEOTIDE VARIATION OF MITOCHONDRIAL DNA (mtDNA) D-LOOP REGION 126 pb (nt: 34-159 HVS II) IN MADURESE INDIVIDUALS

Ahmad Yudianto[1], Indah Nuraini M[2]
[1]Department of Forensic Medicine & Medicolegal
[2]Magister Program on Forensic Science
Faculty of Medicine, Airlangga University

ABSTRAK

mtDNA manusia memiliki tingkat polimorfism lebih tinggi daripada core-DNA, terutama di daerah D-loop, yang merupakan daerah non-coding dan yang paling polimorfik pada genom mitokondria. Analisis variasi urutan regio D-loop dapat digunakan menentukan individu atau etnis, juga hubungan keluarga dari garis ibu. Tujuan penelitian ini adalah menentukan variasi nukleotida dari garis ibu menggunakan swab bukal. Penelitian ini menggunakan metode observasional analitik. Hasil penelitian, 126 bp (HVSII, nt 59-134) fragmen daerah D-loop yang berhasil diamplifikasi dan ditemukan dari haplotipe: G101A, G107A, T118A, C129T, T160A. ***(FMI 2013;49:88-90)***

Kata kunci: *varian, DNA mitokondria, D-Loop*

ABSTRACT

Human mtDNA has higher polimorfism level than core-DNA, especially in the D-loop region, which is non coding region and the most polymorphic in the mitochondrial genom. The sequence variation analysis of D-loop region can be used determine the individual or ethnic , also maternal familiar relationship. the aims of the research, to determinant the nucleotide variation of maternal inherited using buccal swab. The method of research: analytic observasional. The result of research, 126 bp (HVSII, nt 59-134) fragment of the D-Loop region were successfully amplified and the found of haplotipe: G101A, G107A, T118A, C129T, T160A. ***(FMI 2013;49:88-90)***

Keywords: *Variants, mitochondrial DNA, D-Loop*

Correspondence: Ahmad Yudianto, Department of Forensic Medicine & Medicolegal, Faculty of Medicine, Airlangga University

INTRODUCTION

On the identification through DNA analysis is an attempt to compare the DNA profiles with comparative evidence, so it can be concluded whether the DNA profile matched evidence (match) with the comparison or not. Inheritance patterns that are specific to nuclear DNA and mitochondrial DNA determines technical DNA identification. On mitochondrial DNA (mtDNA) has a number of human genetic traits that distinguish it from the typical core genome. Mitochondrial DNA is passed down only through maternal lines without recombination (maternal inherited). Mitochondrial DNA in the daughter cells entirely contributed by the mother and the sperm do not contribute at all. The uniqueness of this inheritance system has been used in various fields, namely the determination of kinship, the study of evolution and global migration of modern humans, the field of forensics and identification of genetic diseases (Wallace 1997).

In the non-coding regions of mtDNA are called Displacement Loop (D-Loop), the region has the highest level of polymorphism in the mtDNA. As well as mtDNA is only passed down through the maternal lineage. Therefore, mtDNA variation in human populations have evolved. The process of evolution occurs through the accumulation of changes in DNA sequence, ie through the process of expanding the number of nucleotide substitutions as the development direction of the line of descent. So far the determination of mtDNA nucleotide variants in individual maternal lineage in a few generations Madura ethnic groups has not been known.

Madura island groups of the population are ethnic group that largely is still retain their ancestral mores coexist in harmony with their religion. Marriage in the society on the island of Madura in rural areas especially the smallest island of Madura is still going on among their own ranks (endogamy). Actually the reason they practically do the endogamy marriage the property that they have not lost/moved to another family, as well as

family ties are still strong. Endogamy marriage from the perspective of genetic genotype will increase the frequency of homozygote. Increased genetic homogeneity of this will appear if the marriage endogamy occurs continuously between generaasi up to the point where there is all alell homozygote in one locus or even on all loci (Syukriani 2012).

Based on this, it is interesting to do research in order to determine the presence of a new variant morph or on individual maternal lineage in the level of several generations of Madura ethnic groups, which can later be used as the basis in determining the genetic pattern of mtDNA in a given population. In this study the Madura swab lineage of women in the population with the pattern of marital endogamy Madura (Madura tribe), because the variants are seen in the pattern of decline in maternal lineage/female (maternal inherited). Based on this, it is interesting to do research in order to determine the presence of new or morph variants in individuals from the maternal lineage Madurese, which can later be used as the basis in determining the pattern of mtDNA genetic Madurese in a larger scale. Genetic variation differs between each population and a history of the occurrence of such variations, so research tentan genetic variation in the population is still relevant. According to Bodmer & Cavalli-Sforza (1976) concept of marriage between populations is the basis of population genetics analysis of the views of the gene frequency and explain the process peruba gene frequency in the population.

MATERIALS AND METHODS

Type of observational analytic study is to determine the nucleotide variation in the D-loop region of mtDNA on individual maternal lineage, with a cross-sectional survey research design. Materials on the study of DNA from buccal swabs from parents, grandmother/ grandfather, grandchildren, great-grandchildren whose mothers lineage specific population groups (parts of Madura Island).

The initial phase of the study was the preparation of template DNA for PCR process. Template DNA derived from the results of amplified lysis of human oral epithelial cells. Human oral epithelial cells obtained by oral swab (buccal swab). Buccal swab tip of the conical tube inserted added Water Free, sonication 2-3 hours. 10.000g-liquid centrifuges in 10 minutes. Pellet was mixed with 1 ml taken DNAZol. Centrifuged 10 min 10.000g-4ºC. Viscous supernatant to a new tube inserted. Add 0.5 ml of absolute ethanol (100%) 4.000g-centrifuge 1-2 minutes, the temperature of 4ºC. Wash pellet with 75% ethanol 0.8-1 ml 2 times. Dissolve the DNA pellet containing the NaOH solution as much as 0.2-0.3 ml-8mm stored at -20ºC.

In PCR amplification, using primer amplification reaction mtDNA 126 bp (nt 34-159. HVS II) (AFDIL, Gabriel et al 2001) M1: 5'-GGG AGC TCT CCA TGC ATT TGG TA-3 'and M2: 5 '- AAA TAA TAG GAT GCA GGA GAG ATC-3'. PCR process using a DNA Thermal Cycler Perkin Elmer artificial, performed with cycle stages, namely: Initial denaturation 95^{o}C-3 minutes, 30 cycles, each cycle consisting of: denaturation 94^{o}C-1 min, annealing 560C-1 minutes, Extension -1 min 72^{o}C and 72^{o}C Final Extension-3 minutes. PCR results were analyzed by agarose gel electrophoresis 1% (w/v)

Sequencing by the Sanger method

PCR results were positf purified (purification), DNA Quantity, Preparing/labeling, purifying extension products/precipitation. Extension purifying the sample results are added to the dried product made 25 μL Hi-Di Formamide, heated for 3 minutes in a 95^{o}C heating block, then the sample is introduced in the ice box for 5 minutes, then transfer to 0.5 ml tube and prepare the incoming pro-sequencing machine sequencing. Then the machine sequencing (ABI PRISM 310) run for 50 minutes persampel. Sequencing results seen on the monitor screen and obtained electrophenogram and nucleotide sequence of the samples examined.

Nucleotide Sequence Analysis Results

The results of a sequencing electropherogram readout scanner results against existing fragments in a polyacrylamide gel. Each nucleotide produces peaks with different colors depending on the electropherogram that adenine nucleotides are green, black guanine nucleotides, nucleotide Cytosin green and red Thymine nucleotides. The results of nucleotide sequences to determine the presence of a new variant compared with the Cambridge sequence.

RESULTS AND DISCUSSION

The process of PCR using the area Hipervariable region (HV) II D-Loop (nt 59-134), has been given a bright ribbon size 126 bp as shown in Figure 1.

To determine the nucleotide sequence of the PCR product size of 126 bp in the sequencing reaction used primer M1, this study has successfully determined the nucleotide sequence of the D-loop region of mtDNA from the sample as shown in Figure 2.

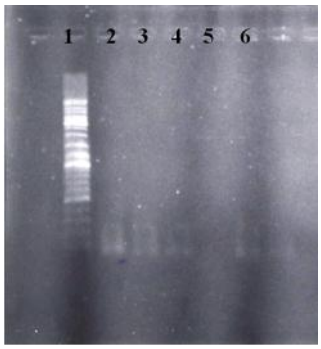

Figure 1. Results of the visualization of the results of 126 bp PCR (1) marker, (5) negative control, (6) positive control

Table 1. Variant data ormal D-loop region of mtDNA to rCRS found in samples

Position	101	107	118	129	160
rCRS	G	G	T	C	T
Grandmother	-	-	-	-	-
Mother	-	-	-	-	-
Grandchild	-	-	-	-	-
Great-Grandchild	A	A	A	T	A

The results of the analysis of nucleotide sequence homology to the sequence of samples rCRS Cambridge Reference Sequence reanalysis as the reference sequence (Table 1), indicating that found 5 or morph all kinds of variants at different levels with rCRS great-grandchildren. Five different variants or morph the 101G -> A, 107g -> A, 118T -> A, 129C -> T and 160T -> A.

CONCLUSION

MtDNA fragments sized 126 bp in the D-Loop region which lies in the region of nucleotides 59-134 of the individual parts of Madura have been successfully amplified by PCR with primers M1 and M2. The process of sequencing by the Sanger method using the results of PCR primer M1 managed to read the nucleotide sequence of 130 bp. The results of nucleotide sequence analysis hmologi rCRS obtained samples with 5 types of variants that occur at the level of great-grandchildren all the positions: 101G -> A, 107g -> A, 118T -> A, 129C -> T and 160T -> A.

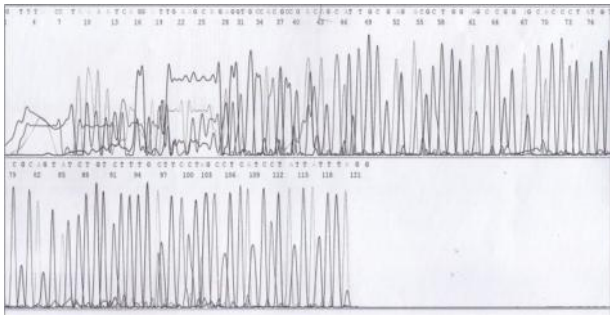

Figure 2. Electropherogram sequencing results

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