Forensic Research 2017, September 18-19, 2017, USA

Study of Molecular Genetic Changes of mitochondrial D-loop region in Iraqi ageing persons

Almuthana Kh. Hameed, Postgraduate student, Dep. of Biology, College of Education for Pure Science, University of Anbar, Iraq.
Athraa H. Hassoon, Assist. Prof. College of Education for Pure Science – Ibn Al Haitham, University of Baghdad, Iraq.
Harith K. Buniya, Assist. Prof. College of Education for Pure Science, University of Anbar, Iraq.

Introduction:

The mitochondrial DNA (mtDNA) is a small circular genome placed within the mitochondria in the cytoplasm of the cell, had a smaller 1.1 kbp fragment and called the displacement loop (D-loop). The D-loop is greatly polymorphic, and some polymorphisms are associated with aging (Muller et al., 2011).

Objectives:

This paper aims to study the D-loop region by using the sequencing technique and found the degree of variation characteristics of this fragment in blood and muscle samples of Iraqi ageing persons. In this work we tried to find the relationship between aging and the different types of mutations that may be occurred in D-loop region of mtDNA by using genetic analyses like polymerase chain reactions (PCR) and DNA sequences to determine the genetic polymorphism and variations in D-loop region of mtDNA in comparison with the data which published in National Center for Biotechnology Information (NCBI).

Methodology:

DNA from product according to (Zhang et al., 2013) by using forward (F) primer 5'-CCCCATGCTTACAAGCAAGT-3' and reverse (R) primer 5'-GCTTTGAGGAGGTAAGCTAC-3'. AccuPower® PCR PreMix (Bioneer, Korea) was prepared. The primers and DNA template adwad to PCR PreMix tubes and the final volume for PCR reaction was made up to 25 μL. The reaction mixers placed in thermal cycler with annealing temperature 60º C .The PCR products were sequenced in Macrogen Company (Korea). The DNA sequence data were analyzed using Mega 7 software and aligned with the Refseq, which published in NCBI databases.

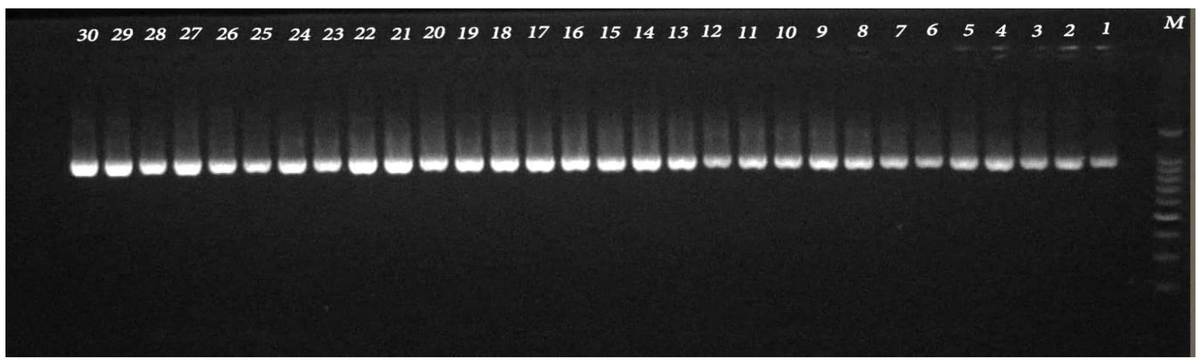

Figure (1): Amplification of mitochondrial DNA D-loop region of each sample:PCR products ≈ 982 bp of samples migrated on agarose 1.5% in 100 volt. For 1.30 min
M: 100 bp DNA Ladder. Lanes 1-5 for ages less than 20 years old, lanes 6-10 for 20-29 years old, lanes 11-15 for 30-39 years old, lanes 16-20 for 40-49 years old, lanes 21-23 for 50-59, lanes 24-27 for 60-69 years old and lanes 28-30 for 70-75 years old.

Table (2): The consolidated data for mutations observed in the D-Loop segment of the mitochondrial DNA for study samples with Muthana-1 (LC 229079.1)

Aged		8-10 years				35-75 years			
Type of sample		Blood	%	Muscle	%	Blood	%	Muscle	%
Mutation type	Nucleotide								
Deletion	------	3	100	10	100	156	100	20	100
	Total	3	8.1	10	17.2	156	23.3	20	21.5
Transition	T→C	3	30	8	44.4	105	42.3	16	48.4
	C→T	2	20	4	22.2	74	29.8	9	27.2
	G→A	2	20	4	22.2	35	14.1	5	15.1
	A→G	3	30	2	11.1	34	13.7	3	9
	Total	10	27	18	31	248	37.1	33	35.4
Chi-Square	----	---	14.75 **	---	11.64 **	---	10.23 **	---	10.82 **
Transvertion	G→C	0	0	4	20	34	14	6	15
	A→C	1	20	3	15	101	41.7	9	22.5
	A→T	1	20	4	20	56	23.1	7	17.5
	G→T	0	0	0	0	1	0.4	1	2.5
	C→A	1	20	5	25	30	12.3	8	20
	T→A	1	20	0	0	2	0.8	6	15
	T→G	0	0	1	5	14	5.7	2	5
	C→G	1	20	3	15	4	1.6	1	2.5
	Total	5	13.5	20	34.4	242	36.2	40	43
Chi-Square	----	---	8.24 **	---	8.63 **	---	10.35 **	---	8.07 **
Insertion	A	5	26.3	0	0	10	47.6	0	0
	T	4	21	0	0	2	9.5	0	0
	G	2	10.5	0	0	2	9.5	0	0
	C	8	42.1	0	0	7	33.3	0	0
	Total	19	51.3	0	0	21	3.1	0	0
Chi-Square	----	---	12.68 **	---	0.00 NS	---	11.53 **	---	0.00 NS
Total number of mutations		37	4.3	48	5.6	667	78	93	10.8
Number of samples		1		2		18		4	

****: High Significant (P< 0.01) NS: Non-Significant**

Conclusion: Different polymorphisms discovered in this region for both blood and muscle samples from Iraqi population. The accumulation of single nucleotide polymorphisms (SNPs) in the displacement loop of mtDNA may be associated with ageing. In this study, the SNPs in the mitochondrial D loop of blood and muscle samples were identified, and their association with ageing was estimated. The majority of the Polymorphism nucleotide were locations in the D-loop region. The nucleotide transition, transversion, insertion and deletion were causes the important variations in nucleotide sequencing. The total number of mutations in blood samples of young individuals was 37 mutation (4.3%) and 48 mutation (5.6%) in muscle samples for same individuals while the total number of mutations in blood samples of older individuals was 667 mutation (78%) and 93 mutation (10.8%) in muscle samples for same individuals. There are significant differences in the number of mutations in older people, specifically for blood samples incidence and frequency of mutations were greater than those of younger age groups. The analysis of genetic polymorphisms in the mitochondrial D -loop may help identify the most important variation in both young and adult Iraqi individuals (Al-Rashedi et al., 2016). The control sequences recorded in NCBI as an Iraqi genome, the accession number of the sequence was LC229079.1 and its name (Muthana-1).
venous blood and muscles were extracted by using DNA extraction Kit (Geneaid, Taiwan). The extracted DNA was resolved on 1% agarose gel. PCR technique was used for amplification of 982 bp .

References:

1. Zhang J, Guo Z, Bai Y, Cui L, Zhang H, Zhang S, Xu J. (2013). Identification of sequence polymorphisms in the displacement loop region of l mitochondrial DNA as a risk factor for renal cell carcinoma. Biomed Rep 1:563–6.
2. Al-Rashedi, N A.M; Jebor MA, Mousa TA. (2016). Mitochondrial DNA markers in Arabic Iraqi population. Eur J Forensic Sci.
3. Mueller EE, Eder W, Ebner S, Schwaiger E, Santic D, et al. (2011) The mitochondrial T16189C polymorphism is associated with coronary artery disease in Middle European populations. PLoS One 6: e16455.