DAHİLİ BİLİMLER / *MEDICAL SCIENCES*
Araştırma Makalesi / Research Article

Detection of Mitochondrial DNA Deletions in Heart Tissue with Acute Myocardial Infarction*

Akut Miyokardiyal İnfarktüslü Kalp Dokularında Mitokondriyal DNA Delesyonlarının Belirlenmesi

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Aim: The aim of this study was to determine 4977 bp and 7436 bp mitochondrial DNA (mtDNA) deletions and to investigate whether there is an association between the mtDNA deletions and myocardial infarction (MI) in cardiac muscle samples from autopsies.

Material and Method: In this study, a total number of 66 heart tissues from autopsies were studied, 33 of them were obtained from individuals with MI and the rest without MI. In order to determine 4977 and 7436 bp mtDNA deletions, mtDNAs in heart muscles from controls and heart tissues from MI region were isolated and amplified with polymerase chain reaction (PCR) technique. The amplified products separated on 2% agarose gel electrophoresis.

Results: 4977 bp mtDNA deletion was observed in 97% of the heart tissues of the individuals with MI. On the other hand, this ratio was only 39.4% for the control group ($p<0.001$). Similarly, the resultant deletion ratios for 7436 bp mtDNA were 97% for the heart tissues of subjects associated with MI, and 12.1% for the control group ($p<0.001$). Furthermore, there was a statistically significant association between the average age of all individuals and both deletions mentioned ($p<0.01$). Even though a strong effect of aging on deletions was identified; stronger effect of MI was undeniable.

Conclusion: In this study, it is showed that mtDNA deletions may contribute to diagnosis of MI in autopsies.

Key Words : ***Miyokardiyal infarktüs, mtDNA delesyonları, Otopsi***

Amaç: Bu çalışmanın amacı, 4977 bp ve 7436 bp mitokondriyal DNA (mtDNA) delesyonlarını belirlemek ve otopsi kalp kası örneklerindeki miyokardiyal infarktüs (MI) ve mtDNA delesyonları arasında ilişki olup olmadığını araştırmaktır.

Materyal ve Metod: Çalışmada 33 MI'lı ve 33 MI'sız kişiden alınan toplam 66 otopsi kalp dokusu kullanıldı. 4977 ve 7436 bp mtDNA delesyonlarını belirlemek için, kontrollerin kalp kaslarından ve MI'lı kalp dokularından mtDNA izole edildi ve polimeraz zincir reaksiyonu (PCR) tekniği ile çoğaltıldı. Çoğaltılan ürünler %2'lik agaroz jel elektroforezinde ayrıştırıldı.

Bulgular: MI'lı kişilerin kalp doklarının %97'sinde 4977 bp mtDNA delesyonu gözlemlendi. Diğer taraftan kontrol grubunda bu oran sadece %39.4'tü ($p<0.001$). 7436 bp mtDNA delesyon oranı da benzer şekildeydi; MI'lı kalp dokularında bu delesyonun oranı %97 iken, kontrol grubunda %12.1 bulundu ($p<0.001$). MI arasında istatistiksel olarak önemli ilişki saptandı ($p<0.001$). Ayrıca Bahsedilen her iki delesyonla kişilerin ortalama yaşı arasında da istatistiksel olarak önemli ilişki belirlendi ($p<0.01$). Delesyonlarda yaşlanmanın güçlü bir etkisi olduğu görülse de, MI'nın delesyonlardaki etkisinin de kuvvetli olduğu yadsınamaz.

Sonuç: Bu çalışmada, mtDNA delesyonlarının otopsi örneklerinde MI'ın tanısına katkıda bulunabileceği gösterilmiştir.

Anahtar Sözcükler: ***Myocardial infarction, mtDNA deletions, Autopsy***

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Human mitochondria contain their own genetic material in the form of mitochondrial DNA (mtDNA), which is the only extrachromosomal DNA in human cells. mtDNA is a super coiled, double-stranded circular molecule of 16,569-bp in size that encodes 13 polypeptides (7 NADH, 1 Cyt-b, 3 COX and 2 ATPase) which constitute the respiratory enzyme complexes (1).

The mitochondrial genome is more susceptible to increased oxidative damage than nuclear DNA due to its lack of histone protection, limited repair capacity, and close proximity to the

electron transport chain (2). Mitochondria are not the only major site of energy production in the cell, but also the major intracellular source of reactive oxygen species (ROS) and free radicals due to electron leakage from the respiratory chain. The free radicals cause peroxidation of membrane lipids, accumulation of oxidized dysfunctional proteins, and increased mtDNA damage. Especially impaired functions of energy dependent tissues such as heart are affected by mitochondrial dysfunction (3). The increased generation of reactive oxygen species is associated with mitochondrial damage and dysfunction in the failing heart, mtDNA defects may thus play an important role in the development and progression of myocardial failure (4). The accumulation of mtDNA mutations and oxidative damage is potential contributor to human diseases, such as age-related pathologies and cardiovascular disease (5). The most studied common mitochondrial DNA deletions are 4977 bp and 7436 bp mtDNA deletions (Figure 1). 4977 bp mtDNA deletion is detected at high frequencies in various tissues from aging humans as well as in ischemic myocardium (6). Also a significant accumulation of 4977 bp mtDNA has been demonstrated in the atrial tissue of patients with clinical atrial fibrillation (7). Some studies have documented the presence of mtDNA 4977 deletions in cardiac muscle from patients suffering from coronary atherosclerotic heart disease (8,9).

Myocardial infarction is defined as myocardial cell death due to prolonged ischemia. Cell death is categorized pathologically as either coagulation or contraction band necrosis, or both, which usually evolves through oncosis, but can result to a lesser degree from apoptosis. After the onset of myocardial ischemia, cell death is not immediate but takes a short period of time to develop. It takes 6 h before myocardial necrosis can be identified by standard macroscopic or microscopic postmortem examination (10). This may sometimes have considerable medicolegal importance, especially when cardiac diseases have been considered to cause a traffic or other accident and

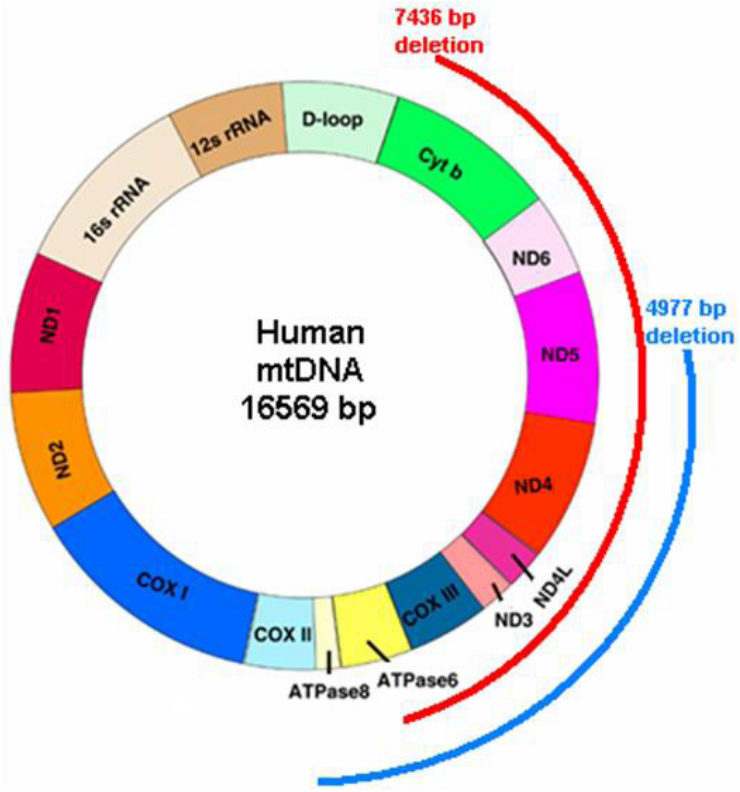

Figure 1. Schematic presentation of mitochondrial wild type genome and common deletions (4977 bp and 7436 bp mtDNA deletions).

in some criminal circumstances (11). It is very important to reach the exact diagnosis and many authors work on this issue especially for early stages of myocardial infarction (12,13).

The aim of the present study was to clarify the relationship between mtDNA deletion and acute myocardial infarction (MI) in cardiac muscle samples from autopsies.

Material and Method

In this study, we examined 66 heart tissues from autopsies, 33 of them obtained from individuals who had died because of MI and the rest from individuals died due to noncardiac cases (control group), all in the range of 25-89 years of age. All samples were stored at -20 °C until the analysis. The samples were acquired from The Council of Forensic Medicine, Ministry of Justice, Ankara. All of the autopsy cases were from the same ethnic and geographical origin, living in the Central Anatolia region of Turkey. A small questionnaire for gathering the demographic information was given to the family or relative of each autopsy cases. Only Turkish subjects were included in the study. The study design was approved by Ankara University Faculty of Medicine Ethics Committee (approval No: 121-3212 in 2007). Autopsy heart tissues were stored at -20 ºC before the analyses and autopsies were performed in accordance with the principles of The Declaration of Helsinki.

DNA Extraction

Total DNAs were extracted from 25 mg heart muscles from controls and heart tissues from MI region by "Qiagen DNA Mini Kit" according to the method recommended by the manufacturer.

Determination of 4977 bp and 7436 bp mtDNA deletions

In order to determine 4977 and 7436 bp mtDNA deletions, the regions were amplified with polymerase chain reaction (PCR) technique using the F1-R1, F2-R2 and F3-R3 primer pairs as performed in the previous study (14).

675 bp, 423 bp and 508 bp oligonucleotides were produced in the presence of total mtDNA, the 4977 bp dmtDNA and 7436 dmtDNA, respectively (Table 1). The polymerase chain reaction products were separated on a 2% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned and photographed using Syngene Monitoring System (Figure 2).

Amplification was carried out on a Techne Tc 512 PCR System in a 50 µl reaction mixture containing 200 µM of dNTPs, 10 pmol each of forward (F) and reverse (R) primers, 1 U Hot Star Taq DNA polymerase (Qiagen), 1 X PCR buffer (Qiagen) and 100 ng DNA. The PCR cycling conditions consisted of an initial denaturation step at 94 ºC for 5 min, followed by 33 cycles of 94 ºC for 30 s, 55 ºC for 30 s, 72 ºC for 30 s, and final extension step at 72 ºC for 10 min.

Statistical Analysis

Fisher's exact test was used for categorical variables to compare the differences of mtDNA deletions in with and without MI groups. Differences of mtDNA deletions among the age groups were assessed with one way anova technique. All statistical procedures were carried out by the Scientific Package for Social Sciences (SPSS), version 16.0, Statistical analysis software. Values of p less than 0.05 were considered as statistically significant.

Results

4977 bp mtDNA deletion was observed in 97% of the heart tissues of the individuals who had died because of MI whereas this ratio was only 39.4% for the control group. Similarly the resultant deletion ratios for 7436 bp mtDNA were 97% for the heart tissues of the individuals who had died because of MI, and 12.1% for the control group. There was a statistically significant difference between myocardial infarction and both deletions mentioned ($p<0.001$) (Table 2). Furthermore, a significant association was also found between the average age of control and patient group with 4977 bp and 7436 bp mtDNA deletions. It was determined that the mean ages of the control group with 4977 bp and 7436 bp mtDNA deletions were 70.15±8.88 and 77.50±9.04 respectively. On the other hand, the mean ages of the individuals without these deletions were 54.30±10.34 and 58.21±11.03 ($p<0.001$ and $p<0.01$, respectively). Also same statistical significant relation was found in the acute myocardial infarction patients. (Table 3)

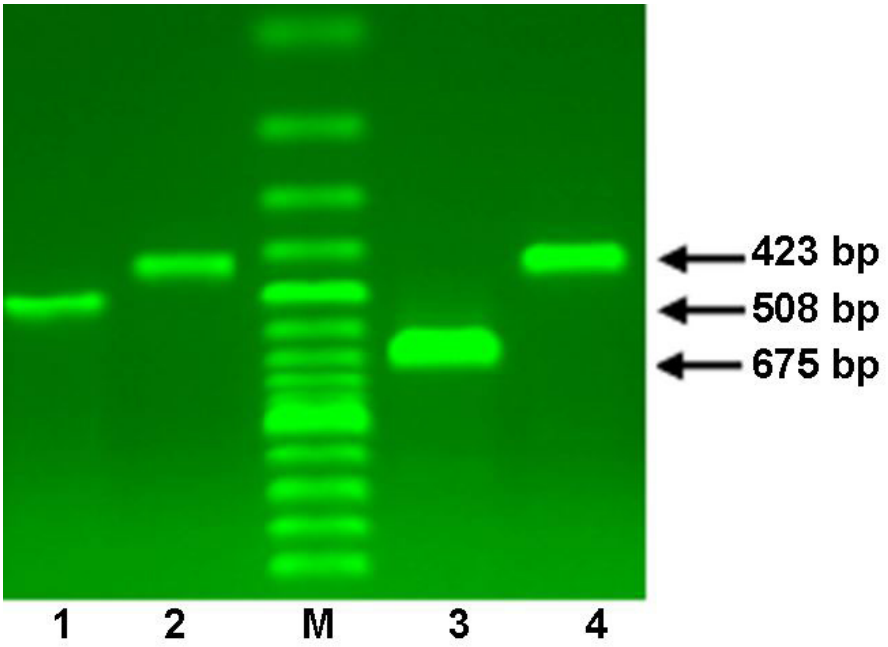

Figure 2. Representative gel image of 4977 bp and 7436 bp mtDNA deletions.
M=100 bp ladder;
Lane1= PCR products for 7436 bp mtDNA deletion (508 bp).
Lane2 and Lane4= PCR products for 4977 bp mtDNA deletion (423 bp).
Lane3= PCR products for total mtDNA (675 bp).

Conclusion

It is well known that up to 200 different deletions in the mitochondrial genomes are detectable in postmitotic tissues. However, among these 200, the

Table 1. Oligonucleotid primers used for PCR amplification of the mtDNA with 4977 bp and 7436 bp mtDNA deletions.

PRIMER PAIRS	PRIMER SEQUENCES	Size of the PCR product amplified from mtDNA with the length mutation (bp)	Aim of this PCR
F1-R1	F1: 5'-GGAGTAATCCAGGTCGGT-3' R1: 5'-AATGATGGCTAGGGTGACTT-3'	675	Determination of the total mtDNA
F2-R2	F2: 5'-GCCCGTATTTACCCTATAGC-3' R2: 5'-GGGGAAGCGAGGTTGACCTG-3'	423	Determination of the 4,977 bp dmtDNA
F3-R3	F3: 5'-CTCTAGAGCCCACTGTAAAG-3' R3: 5'-GTTGAGGGTTGATTGCTGTAC-3'	508	Determination of the 7436 bp dmtDNA

Table 2. The frequencies of occurence of 4977 bp and 7436 bp mtDNA deletions in acute myocardial infarction and control groups.

SAMPLES	N	Mean Ages±SE	DELETIONS					
			4977 bp mtDNA deletion			7436 bp mtDNA deletion		
			+	-	+%	+	-	+%
Acute myocardial infarction	33	61.58±18.21	32	1	97	32	1	97
Control group	33	60.55±12.45	13	20	39.4	4	29	12.1
P			**P<0.001**			**P<0.001**		

Table 3. The association between the mtDNA deletions and mean age of individuals in control and acute myocardial infarction groups.

SAMPLES	DELETIONS		N	MEAN AGES	Minimum	Maximum	p
Control group	4977 bp mtDNA	+	13	54.30±10.34	26	72	P<0.001
		-	20	70.15±8.88	52	89	
	7436 bp mtDNA	+	4	77.50±9.04	67	89	P<0.01
		-	29	58.21±11.03	26	77	
Acute myocardial infarction	4977 bp mtDNA and 7436 bp mtDNA	+	32	62.72±17.25	27	89	P<0.05
		-	1	25	25	25	
TOTAL	4977 bp mtDNA	+	45	64.87±15.58	27	89	P<0.01
		-	21	52.90±11.94	25	72	
	7436 bp mtDNA	+	36	64.36±17.11	27	89	
		-	30	57.10±12.42	25	77	

most studied common mitochondrial DNA deletions are 4977 bp and 7436 bp mtDNA deletions. Most studies showed these mtDNA deletions were important contributors to ageing and degenerative diseases. The age-related increase of the common mtDNA deletion is not universal, but clearly tissue-specific and different organs or even different areas of the same organ accumulate these deletions at different rates (15). Pathological mtDNA deletions are associated with ultrastructurally abnormal mitochondria; mitochondrial DNA defects reduce the specific mitochondrial enzyme activity levels and change the mtDNA integrity (16). Consequently, the accumulation of abnormal mtDNA contributes to the progression of mitochondrial diseases and heart failure (17).

The threshold for mutation and deletions in mtDNA causing symptoms varies between tissues, being lower in those tissues that are post-mitotic, highly metabolically active and dependent on oxidative phosphorylation for energy production, such as the heart muscle, brain, skeletal muscle etc. (18). The threshold level varies not only between tissues but also between different types of mutation. It has been demonstrated threshold level for mtDNA deletions is 60%. Most pathogenic mutations are heteroplasmic; if they were homoplasmic, they would often be lethal (19).

In forensic medicine, sensitive biochemical markers for the post-mortem diagnosis of acute myocardial infarction are needed. When the deleted mtDNA reaches the threshold level of 60-80%, the cell functions will be affected. However, when this ratio is homoplasmic, it will be lethal for the cell. In order to evaluate the mtDNA deletions as the biomarker for the MI diagnosis, it might also be better to determine heteroplasmic ratio quantitatively (20).

In our study, mtDNA deletions investigated and it has been showed that the result may contribute diagnosis of MI in autopsy specimens.

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