INT. J. BIOL. BIOTECH., 17 (2): 301-305, 2020.

IDENTIFICATION OF COMMON MITOCHONDRIAL MUTATIONS IN CERVICAL CANCER

Ayat Mohebifar[1], Kyumars Safinejad[*2] and Reihaneh Hoveida[3]

[1,3]*Department of Biology, Doroud branch, Islamic Azad University, Doroud, Iran*
[2]*Department of Biology, Borujerd branch, Islamic Azad University, Borujerd, Iran*
Corresponding author's Email: q_safinejad@yahoo.com

ABSTRACT

In order to investigate the frequency of common mitochondrial mutations in cervical cancer patients, the frequency of A1555G, A3243G and A7445G mutations in patients with cervical cancer in Lorestan province were studied. In this study, 50 women suffering from cervical cancer and 50 healthy women as control group were studied. Samples were screened by PCR-RFLP for common mtDNA mutations. The observed mutations were confirmed by sequencing. Frequency of alleles was analyzed using POPGENE Version 1.32 software and the results were analyzed using Prism 6 Graph Pad software. After analyzing the results, it was shown that there were no significant differences between the two groups for the A1555G, A7445G, and A3243G mutations. Regarding the relationship between the three mitochondrial genes with cervical cancer, it was found that there was no significant difference between the studied groups in the rate of studied mutations. The results of this study showed that there was no significant difference in the rate of mutations in the studied groups. In fact, according to the results of this study, mutation in A1555G, A7445G and A3243G cannot be considered as a diagnostic biomarker in cervical cancer.

Keywords: Cervical cancer, Mitochondrial DNA, Mutation, PCR-RFLP

INTRODUCTION

In developed countries such as the United States, Cervical cancer is the second leading cause of death after cardiovascular diseases (Vafaeinezhad *et al*., 2018). Among cancers, cervical cancer is the third most common tumor in women in the United States of America (Stepanenko *et al*., 2015). The disease is the seventh most common cancer and the sixth leading cause of malignant deaths in women and is the first or second most common female genital cancer in areas such as Africa, India, South and Central America and Southeast Asia. Throughout the world, approximately 500,000 people get the malignancy every year, and unfortunately 200,000 die (Lotfinejad *et al*., 2006). According to the World Health Organization (WHO) report, 85 percent of cervical cancer cases are in developing countries (Jafari *et al*., 2015).The median age of patients with this cancer is 52.2, while the median age for this cancer in Iran is 50-55 (Leece *et al*., 2010 and Horn *et al*., 2002).

With the emergence of the 21st century, two important misconceptions about cancer were eradicated: 1) the notion that cancer is a purely cellular disorder that results from epigenetic or genetic alterations (Porporato *et al*., 2018 and Lorenzo *et al*., 2015); and 2) the view that malignant cells often meet their bio energetic and anabolic needs that are mostly through aerobic glycolysis (Erez *et al*., 2015 and Danhier *et al*., 2017).

Therefore, nowadays, it is widely accepted that tumors are formed, developed, and respond in a complex, two-way interaction with the host immune system (Chen *et al*., 2017 and Galluzzi *et al*., 2015). For instance, the fundamental impact of mitochondrial metabolism at all stages of cancer such as malignant transformation, tumor progression, and response to treatment have been recognized formally (Vyas *et al*., 2016 and Wallace *et al*., 2012).

Human mtDNA is a 16569 bp double-stranded molecule containing 37 genes that encode 2 r RNAs, 22 t RNAs, and 13 polypeptides, which are present in high copy numbers in almost all cells (103 to 104 copies per cell) and majority of them are identical at birth. In addition, mtDNA is known to have a 10-fold higher mutation rate than nuclear genomic DNA due to the lack of protective histones, inefficient DNA modification systems, and exposure to mutagenic oxygen radicals produced by oxidative phosphorylation. The relationship between mtDNA mutations and metabolic neurological disorders has previously been reported in human cancers (Chang *et al*., 2018 and Wallace *et al*., 2012).These mutations include adenocarcinomas of the kidney, astrocytic cancer, thyroid tumors, breast tumors, ovarian tumors, prostate and bladder cancer, neuroblastomas , and oncocytomas.

The A3243G mutation, which is found in the mitochondrial tRNALeu gene (UUR) and is also known as MTTL, is one of the most common mitochondrial mutations (Lin *et al*., 2016 and Maa *et al*., 2009). The A7445G mutation, known in the previous articles as the T7445C, was first described in the Scottish family and was confirmed and established in two new generations of New Zealander and Japanese. The influence of this mutation is very low in the

Scottish race, while it is high in New Zealander and Japanese races (Finsterer *et al*., 2007 and Schapira *et al*., 2006). The A1555G mutation in the mitochondrial 12S rRNA gene is one of the most common causes of sensory lack of hearing and aminoglycoside-induced hearing loss. The mutation was first discovered in a large Israeli-Arab family and was then found with varying prevalence among different European, Asian and African ethnicities (Hutchin *et al*., 2000).

Because of the fact that no similar studies have been done in our country and due to the diversity of the population in Iran and the existence of different ethnicities, we decided to run a research with the aim of investigating the frequency of A7445G, A3243G and A1555G mutations in patients with cervical cancer in Lorestan province. The results of such studies will undoubtedly play a significant role in screening of cervical cancer and reducing its new incidence in diverse Iranian population.

MATERIALS AND METHODS

In this descriptive-laboratory study, 50 blood samples were collected from the Gynecology and Midwifery Hospital, with easy sampling method to identify common mitochondrial mutations in A7445G, A3243G and A1555G genes. 5 ml of blood was taken from all subjects in EDTA (0.5 mM) tubes. DNA was then extracted using the usual phenol-chloroform method and the concentration of extracted DNA was measured by Spectrophotometry (Unico 2100 USA) (Kleihues *et al*., 1997 and Prezant *et al*., 1993).

Genotype analysis of A7445G, A3243G and A1555G genes was performed by PCR-RFLP method (Kleihues *et al*., 1997). Using the mitochondrial genome sequence with access code of NC-012920 and Primer software 3, sequences of R and F primers were designed and purchased to detect 3 mtDNA molecule mutations. PCR was performed to detect the above-mentioned mutations on DNA samples using a thermo cycler (TECHNE TC-512 UK) according to the following reaction and thermal program: Primary denaturation at 94 ° C for 5 minutes, then 30 cycles, including: denaturation at 94 ° C for 30 seconds, 63 ° C for binding of primers to the target DNA for 30 seconds, 72 ° C to extend the supplementary strands for 50 seconds and finally the last expansion at 72 ° C for 6 minutes.

The PCR reaction conditions for the three mutations were: 1µL of R primer (10PM), 1µL of F primer (10PM), 1µL of Taq DNA polymerase (5U / µL), 0.5µL of mixed NTPs (10Mm), 2.5 µL of PCR buffer (10x), 1.5 µL of MgCL2 (50 mM) and 1 µL (100 ng) of DNA, which were reached to the volume of 25 µL with dH2O.

All PCR products were electrophoresed on 2% agarose gel with a voltage of 120 V for 1 hour and the obtained gel was stained with fluorescent dyes and PCR-RFLP method was used to investigate mutations. In each micro titer, 10µL of the PCR product was mixed with 1µl of the target restriction enzyme (10U / µL) and 2 µL of buffer and 7µL of distilled water and incubated at 27 ° C for 16 h. The products were then electrophoresed on 2% agar gel for 2 hours at 90 V.

RESULTS

Allelic frequencies in two groups were calculated using POPGENE Version 1.32 software for A1555G, A7445G, A3243G mutations. After analyzing the results using Prism 6 GraphPad software, it was shown that there was no significant difference between the two groups for 50 samples (Table 1).

Comparison of control and case groups in frequency of A1555G, A7445G, A3243G mutations was performed using Fisher's exact test. In this test there is no limitation for the sample size due to the exact probability of the relationship between the two variables or the difference between the ratios (Table 1).

Table 1. Frequency of A1555G and A3243G (by specific restriction enzyme; *HaeIII*) A7445G, (by specific restriction enzyme; Xba1) in two groups.

Allele	Control	Case	Control	Case	Control	Case
	(HaeIII) A1555G	(HaeIII) A1555G	(Xba1) A7445G	(Xba1) A7445G	(HaeIII) A3243G	(HaeIII) A3243G
A (wild Type)	1.0	0.943	0.98	0.906	1.00	0.981
G (Mutant)	0.0	0.057	0.02	0.094	0.00	0.019

The rate of mutations between two groups in three mitochondrial genes was investigated. The results of the above table show that since the P- values in all three gene loci were more than 0.05, there was no significant difference between the studied groups in the rate of mutations under study (Table 2). In fact, according to the results of this study, the mutations in this gene cannot be considered as a diagnostic biomarker in cervical cancer.

Table 2. Comparison of control and case groups in the frequency of common mitochondrial mutations.

Mutation	Allele Type	Control N = 51 (%)	Case N = 53 (%)	OR (% 95 CI)	P value
A1555G (HaeIII)	Wild	51 (100)	50 (94.3)	Vafaeinezhad *et al.*, 2018 7.1 (0.36 – 141.8)	**0.243**
	Mutant	0(0)	3 (5.7)		
A7445G (XbaI)	Wild	50 (98)	48 (90.6)	Vafaeinezhad *et al.*, 2018 5.2 (0.58 – 46.2)	**0.205**
	Mutant	1 (2)	5 (9.4)		
A3243G (HaeIII)	Wild	51 (100)	52 (98.1)	Vafaeinezhad *et al.*, 2018 2.9 (0.11 – 74.0)	**1.0**
	Mutant	0 (0)	1 (1.9)		

DISCUSSION

Somatic mutations in mtDNA have been increasingly observed in human cancers in recent years (Parrella *et al*., 2003 and Himani *et al*., 2005). The aim of this study was to identify and recognize common mitochondrial mutations including A7445G, A3243G, and A1555G and their relationship with cervical cancer. 50 patients with cervical cancer were studied in this research. These mutations are at a highly conserved 12S rRNA decoding site and are the most common type of mitochondrial mutation that can be related to lack of hearing. They are also involved in hearing loss caused by amino glycosides.

Progressive fracture of mitochondrial function with its failure may cause such problems (Stewart *et al*., 2008). While mitochondrial DNA mutations have been extensively studied in the field of rare genetic diseases, their role in carcinogenesis has been relatively less studied (Shu *et al*., 2019). There are strong empirical evidences for the role and driving factor of mitochondrial mutation in cancer, but experimental approaches to determine the role of mtDNA mutation in cancer-related mitochondrial dysfunction have not yet been concluded, due to the mitochondrial genome and therefore limited experimental tools in this field (Wang *et al*., 2016).

Despite these limitations, there is convincing data pointing to the nature of mtDNA-related mitochondrial dysfunction in cancer. Because mitochondria are the starting place of apoptosis, so its genomic mutation may play an important role in cancer. Reports on this subject seem contradictory in some cases. Some reports state that 70% of the studied colorectal cancers show mitochondrial DNA mutations (Lièvre *et al*., 2005).

Few studies have investigated the role of mitochondrial mutations in cervical cancer. Except for one study (Lim *et al*., 2012), the involvement of the mtDNA mutation in cervical cancer appears to have not been investigated and the searches for the relationship of cervical cancer with the common mitochondrial mutation (A7445G, A3243G, and A1555G) have reached no conclusion. Therefore, this study is a step in this direction. Mitochondrial mutations have been reported in other malignancies in women. In ovarian cancer, 60% of somatic mutations have been reported in D-strand, 12s rRNA, 16s rRNA and cyt. b (Penta *et al*., 2001). Most mutations were the transfer of T or C or G →A. For mtDNA mutations of cervical cancer, only in 35% of cases, there has reported a high frequency of D310 mutations (Lim *et al*., 2012).

According to the results of our studies, there was no significant difference between the two groups. Comparison of the control and case groups in the frequency of mutations also showed that since the P-Value in all three gene loci was more than 0.05, there was no significant difference in the rate of studied mutations. In fact, according to the results of this study, the mutation in these genes cannot be considered as a diagnostic biomarker in cervical cancer.

In this study, there was no significant difference in the frequency of common mitochondrial mutations between patients and case groups. We were not able to establish any relationship between the mutations and clinical features. In this regard, clinical studies focused on mtDNA mutation in patient groups have been classified. One such reports on patients with prostate cancer indicated mtDNA mutations (Patti *et al*., 2019). In addition, there is mtDNA mutation in primary, recurrent and metastatic tumors which play a real driving role for mtDNA mutations in thyroid cancer. However, the nature of such clinical data, although showing an important role in mtDNA mutations in cancer, cannot be invoked to infer its constitutive role. This study suggests that mitochondrial mutations in cervical cancer cells are indeed frequent and more work is needed in this regard. In addition, given the important role of

mitochondria in apoptosis, mutations in mtDNA in cancer cells may significantly affect the cellular apoptotic response to chemotherapeutic agents. However, no relationship was found between mtDNA mutations and apoptosis index or proteins and anti-apoptotic proteins related to data.

REFERENCES

Chang, Y., W. Xuefeng, H. Lifeng, T. Ying, C.H. Lili, W. Hailong, X. Qiang and K. Xiaoni (2018). Deciphering the Spectrum of Mitochondrial DNA Mutations in Hepatocellular Carcinoma Using High-Throughput Sequencing. *Gene Expression*, 18: 125–134.

Chen, D.S. and I. Mellman (2017). Elements of cancer immunity and the cancer-immune set point. *Nature*, 541: 321-330.

Danhier, P., P. Banski and V.L. Payen (2017). Cancer metabolism in space and time: beyond the Warburg effect. *Biochim Biophys Acta*, 1858: 556-572.

Erez, A. and R.J. De Berardinis (2015). Metabolic dysregulation in monogenic disorders and cancer - finding method in madness. *Nat Rev Cancer*, 15: 440-448.

Finsterer, J. (2007). Genetic, pathogenesis, and phenotypic implications of the mitochondrial A3243G tRNALeu (UUR) mutation. *Acta Neurol Scand*., 116: 1-14.

Galluzzi, L., A. Buque, O. Kepp, L. Zitvogel and G. Kroemer (2015). Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell*, 28: 690-714.

Himani, Sh., S. Archna, Sh. Chandresh, K. S. Kumar and S. Neeta (2005). Mutations in the mitochondrial DNA D-loop region are frequent in cervical cancer. *Cancer Cell International*, 5: 34.

Horn, L.C., G. Raptis and U. Fischer (2002). Familial cancer history in patients with carcinoma of the cervix uteri. *Europ J ObstetGynecol Reproduct Biol.*, 101(1): 54-57.

Hutchin, T. and G. Cortopassi (2000). Mitochondrial defects and hearing loss. *Cell Mol Life Sci*., 57: 1927-37.

Jafari, M., Y. Moradi, M. Khodadost, E. Sekhavati, H.A. Anabad and K. Mansori (2015). Trend of the esophageal cancer incidence in Iran. *Int J Travel Med Glob Health.*, 3(2): 127- 131.

Kleihues, P., B. Schauble, A. Zur Hausen, J. Estève and H. Ohgaki (1997). Tumors associated with p53 germline mutations: a synopsis of 91 families. *Am J Pathol*., 150(1): 1-13.

Leece, P., C. Kendall, C. Touchie, K. Pottie, J.B. Angel and J. Jaffey (2010). Cervical cancer screening among HIV-positive women. Retrospective cohort study from a tertiary care HIV clinic. *Can Fam Physician*, 56(12): e425-431.

Lièvre, A., C. Chapusot, A.M. Bouvier, F. Zinzindohoué, F. Piard, P. Roignot, L. Arnould, P. Beaune, J. Faivre and P. Laurent-Puig (2005). Clinical value of mitochondrial mutations in colorectal cancer. *J Clin Oncol*., 23: 3517–3525.

Lim, S.W., H.R. Kim, H.Y. Kim, J.W. Huh, Y.J. Kim, J.H. Shin, S.P. Suh, D.W. Ryang, H.R. Kim and M.G. Shin (2012). High-frequency minisatellite instability of the mitochondrial genome in colorectal cancer tissue associated with clinicopathological values. *Int J Cancer*, 131: 1332–1341.

Lin, C.S., H.T. Lee, M.H. Lee, S.C. Pan, C.Y. Ke, A.W. Chiu and Y.H. Wei (2016). Role of mitochondrial DNA copy number alteration in human renal cell carcinoma. *Int J Mol Sci*., 17(6): 814.

Lorenzo, G., G. Kroemer, L. Senovilla, L. Galluzzi, F. Andre and L. Zitvogel (2015). Natural and therapy-induced immune surveillance in breast cancer. *Nat Med*., 21: 1128-1138.

Lotfinejad, Sh. [Pap Smear] (2006). *1st. Urmia.* Urmia Medical Univercity Publishing. pp: 1-3 (Persian).

Maa, Y., F. Fang and Y. Yang (2009). The study of mitochondrial A3243G mutation in different samples. *Mitochondrion*, 9: 139-43.

Parrella, P., D. Seripa, M.G. Matera, C. Rabitti, M. Rinaldi, P. Mazzarelli, C. Gravina, M. Gallucci, V. Altomare, G. Flammia, B. Casalino, P.L. Benedetti-Panici and V.M. Fazio (2003). Mutations of the D310 mitochondrial mononucleotide repeat in primary tumors and cytological specimens. *Cancer Lett*., 190: 73-77.

Patti, O., N.B. Hirendra, V.P. Julie and D. Santanu (2019). Human Papilloma Virus-Associated Cervical Cancer and Health Disparities.*Cells*, 8(622): 1-12.

Penta, J.S., F.M. Johnson, J.T. Wachsman and W.C. Copeland (2001). Mitochondrial DNA in human malignancy. *Mutat Res.*, 488:119–133.

Porporato, P.E., N. Filigheddu, J.M. Bravo-San Pedro and G. Kroemer (2018). Mitochondrial metabolism and cancer. *Cell Research*, 28: 265-280.

Prezant, T.R., J.V. Agapian, M.C. Bohlman, X. Bu, S. Oztas and W.Q. Qiu (1993). Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet*., 4(3): 289-94.

Schapira, A. (2006). Mitochondrial disease. *Lancet*, 368: 70-82.

Shu, H.Y., H.C. Li, W.Q. Xie, B. Ni and H.Y.Z. Zhou (2019). Mitochondrial DNA variations in tongue squamous cell carcinoma. *Bimed Rep*., 10(1): 23-28.

Stepanenko, A.A. and V.V. Dmitrenko (2015). HEK293 in cell biology and cancer research: Phenotype, karyotype, tumorigenicity, and stress-induced genome-phenotype evolution. *Gene.*, 569(2): 182-90.

Stewart, J.B., C. Freyer, J.L. Elson and N.G. Larsson (2008). Purifying selection of mtDNA and its implications for understanding evolution and mitochondrial disease. *Nat Rev Genet*., 9: 657–662.

Vafaeinezhad, Z., Z. Kazemi, S.M. Mirmoeini, H. Piroti, E. Sadeghian, M. Mohammad Ali-Vajari, N. Fattah, M. Roza and M. Jafari (2018). Trends in Cervical Cancer Incidence in Iran According to National Cancer Registry. *J. Mazandaran Univ Med Sci*., 28 (161): 108-114.

Vyas, S., E. Zaganjor and M.C. Haigis (2016). Mitochondria and Cancer. *Cell*, 166: 555-566.

Wallace, D.C. (2012). Mitochondria and cancer. *Nat Rev Cancer*, 12(10): 685–98.

Wang, H.L., X. Lu, X. Yang and N. Xu (2016). Association of MBL2 exon1 polymorphisms with high-risk human papillomavirus infection and cervical cancers: A meta-analysis. *Arch. Gynecol. Obstet*., 294: 1109–1116.

(*Accepted for publication March 2020*)