DOI: https://doi.org/10.54393/pbmj.v6i08.928

PAKISTAN BIOMEDICAL JOURNAL

https://www.pakistanbmj.com/journal/index.php/pbmj/index

Volume 6, Issue 8 (August 2023)

Original Article

Comparative Analysis of Tyrosine tRNA (MT-TY) Gene Mutation Related to Lung Cancer Patients from District Peshawar, Khyber-Pakhtunkhwa, Pakistan During 2020-2022

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ARTICLE INFO

Key Words:
MT-TY, Gene Mutation, Lung Cancer

How to Cite:
Abbas, Z. ., Aslam, S., Nabeel, M. ., & Habib, F. . (2023). Comparative Analysis of Tyrosine tRNA (MT-TY) Gene Mutation Related to Lung Cancer Patients from District Peshawar, Khyber-Pakhtunkhwa, Pakistan During 2020-2022: Analysis of Tyrosine tRNA (MT-TY) Gene Mutation. Pakistan BioMedical Journal, 6(08). https://doi.org/10.54393/pbmj.v6i08.928

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Received Date: 9th August, 2023
Acceptance Date: 29th August, 2023
Published Date: 31st August, 2023

ABSTRACT

The uncontrolled growth of cells in the lungs is called lung cancer (LC). Majority of recorded LC cases is as a result of cigarette smoking. It is one of the most occurring types of cancer around the globe. **Objective:** To emphasizes on amplification of specific mitochondrial DNA (mtDNA) genes coding for tyrosine tRNA and to find out its association with LC patients of Peshawar. **Methods:** In 18 LC patients, the mitochondrially encoded tRNA tyrosine gene was studied. Samples were collected from the Institute of Radiotherapy & Nuclear Medicine (IRNUM), Peshawar, Khyber Pakhtunkhwa. The participants varied in age from 12 to 86 years. Patients had diabetes, hypertension, hepatitis C, bronchial asthma, dyspnea, hemoptysis, cardio myopathy, and stomach ulcers. DNA extraction and PCR of the tyr tRNA gene were performed. Along with gel electrophoresis. 8 samples were cleaned and sequenced. **Results**: The results were used for comparison with the reference DNA sequence of mitochondria i.e., revised Cambridge Reference Sequence (rCRS) Accession No 0129201. According to NCBI BLAST data, there were no variations in the nucleotide position 5826-5891 of the mitochondrially encoded tyrosine tRNA gene in LC subject. Chromatogram of tyrosine gene which was taken from UGENE software showed that there is no variation in Tyrosine gene subject sequence. **Conclusions:** We found no mutation in the mtDNA encoded tyr tRNA gene related to LC patient from District Peshawar Khyber-Pakhtunkhwa, Pakistan during 2020-2022.

INTRODUCTION

The uncontrolled growth of cells in the lungs is called lung cancer [1]. Majority of recorded LC cases is as a result of cigarette smoking [2]. Further, LC is divided into two types. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Based on the presence or absence of metastases, SCLC is divided into two clinically pathological stages: limited stage (LC) and extensive stage (ES) [3]. Small cell lung cancer is attributed to about 15% of lung cancer and non-small cell lung cancer is attributed to about 85% of LC cases [4]. It is one of the most occurring types of cancer around the globe (about 12.9%) [5]. In 2020, newly reported cases were around 2.2 million [6]. Tobacco smoking is one of the important causes which cause around 80-90%-increased risk in smokers of cigarette [7]. Nicotine addiction is the main source that helps to become addictive smokers. In the particles of tobacco smoke, there are so many lung-cancer-related cancer-causing particles and they are catalyzed before secretion but still can bind with DNA to form the cluster. The cluster of DNA leads to apoptosis or repair. If it is prolonged, mutations related to miscoding occur in RAS or P53 like key genes which results in genetic instability, leading eventually to cancer &

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mutational damage. Generally, a person who smokes for a lifetime has a 20-30% high chance to get cancer of the lung in comparison with a person who never smokes [8]. On the other hand, in Eastern Europe, the USA, and China, there are still smoke contagions that can become the main source newly causing about 10 to 1 million or more cases in the present century. In 2016 there were 0.828 million cases reported in china [9]. In 2012, LC was placed at 3rd most common cancer in Pakistan by Global Cancer Observatory, while in 2016 it was placed as 10th most common cancer in country according to Pakistan Health and Research Council [10]. In 2020 there were 2,210,000 cases reported from Pakistan [11]. Cancer of the lungs is still highly non-curable to all the cancerous types, and smoking prevention outcomes of fewer chances following about the 7-year duration [12]. About 17.8% of deaths of LC patients occurred in 2000, and around 1.1 million deaths occur around the world. However, there are about 11% of smokers heavily smoke and attain cancer of the lungs, disposing of the genetic factor that causes cancer of the lungs [13]. The frequently inherited LC risk generally comes from the small but major effects that arose from genetic polymorphisms that are familiar in that population [14]. The efficiency of the glutathione e-S transferase family was decreased by Polymorphism. Polymorphism in genetics is mainly concerned with the activation or detoxification of cancer-causing particles, it also monitors the host cell efficiency, and repairs the tobacco carcinogen-DNA damage [4].

METHODS

The blood samples were collected from IRNUM Hospital university campus Peshawar. Blood samples were collected in EDTA tubes from patients of LC. DNA extraction was done from blood according to the protocol modified from [15]. In EDTA tube 3cc of blood was taken from LC patients. First of all, we have taken 200 micro liter of blood sample in Eppendorf tubes. Then we added 200 micro liter dilution buffer in tubes and vertex it. Then added lysis buffer of 300 micro liter, protease k and 2 micro liters of BME. Then all samples were incubated for 3 hours at 65 degrees centigrade and then vertexes for 25-30 seconds. In the next step we added PCI 300 micro liter with ratio of 25:24:1 in each sample, vertex (25-30 sec) well. Centrifuged these specimens for twenty minutes at 8000rpm. In order to transferring the DNA into fresh tubes, we transport carefully the supernatants and discarded the pallets. After that we added ice cold isopropanol of same amount 300 micro liter and kept all samples in freezer overnight. Then again centrifuged the samples at 8000rpm for 20 minutes. The upper layer was then removed completely, and the pellets were rinsed with 70 percent ethanol in a volume of 500 micro liters. Again, centrifuged it for 5 minutes and supernatant discard once again. Then kept all samples for drying for 12 hours. After that 50 micro liters of double distilled H_2O was added. Extracted DNA was amplified by polymerase chain reaction (PCR) which was performed under these conditions with 35-40 cycles [16]. First step denaturation, at 95°C for 40 seconds the next was annealing, at 50-65°C for 1minute and the final was extension, at 72°C for 45seconds was performed. The amplification of mitochondrial encoded tyrosine tRNA gene from the NCBI database yielded forward and reverse primers. Double distilled water and primer were used to make the primer stock till the final volume reached 10 Pico mole/µl.

FORWARD: CCCTCACTAAAGGGAACAAAA
BACKWARD: CACTATAGGGCGAATTGGGTA

The amplified mitochondrial encoded tyrosine tRNA gene was sequenced and purified using 8 PCR product. The length of mitochondrially encoded tyrosine gene is 66-nucleotide shown below, with the beginning region at 5826 and the ending region at 5891. The following is the NCBI FASTA sequence for the tRNA gene for tyrosine.

GGTAAAATGGCTGAGTGAAGCATTGGACTGTAAATCTAAAG ACAGGGGTTAGGCCTCTTTTTACCA.

For analysis, we used the NCBI BLAST Nucleotide online database and UGENE. To match with the Revised Cambridge Reference sequence, the NCBI BLAST database was used. The sequence of FASTA files was completely aligned using the NCBI BLAST database. The ABI files were opened using the U-GENE program. The chromatographic tools in the UGENE program emphasized the accuracy of the sequence data in the form of distinct color lines that showed the nucleotide peaks that were taken into account for the final findings.

RESULTS

For examination of mutations in the mitochondrial encoded tRNA tyrosine gene. 8 LC individuals' blood samples were taken from the IRNUM Hospital in Peshawar and they had previously been diagnosed and were getting treatment. The ages of the participants ranged from 12 to 86 years. Diabetes, Hypertension, Hepatitis C, Bronchial Asthma, Dyspnea, Hemoptysis, Cardio Myopathy, and stomach ulcer were seen in patients. The clear findings of DNA extraction confirmation are shown in the following Figure 1. DNA was isolated from LC patients' blood samples at the molecular lab of Biotechnology and Genetic Engineering at Hazara University Mansehra, Pakistan.

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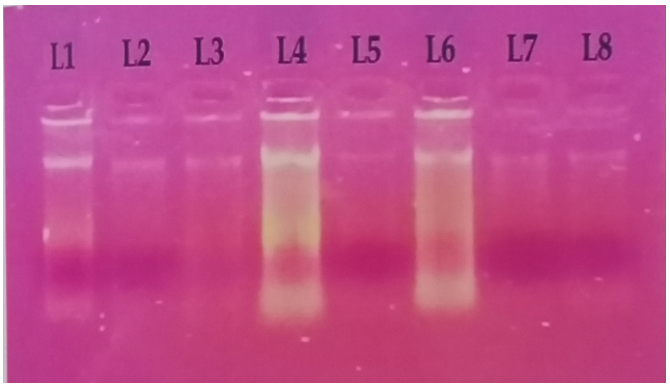

Figure 1: The appearance of genomic DNA taken from samples of LC patients was labeled with the letters L1, L2, L3, L4, L5, L6, L7, and L8

Amplification was carried out effectively using optimal PCR conditions, and the PCR product was run on a 1% agarose gel. Distinct bands of approximately 660bp fragments were amplified and compared with the marker indicated in Figure 2 on gel images produced from the gel documentation method.

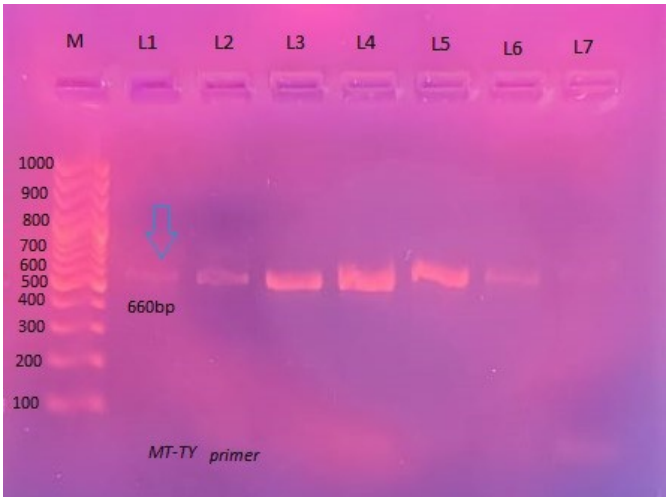

Figure 2: L1, L2, L3, L4, L5, L6, and L7 are alphabetically exhibited pictorial results of amplified PCR product and DNA markers

Samples from LC individuals are used in this method. At the age of 52, with a BMI of 63 kg and an onset age of 5 months, the person had stage-II type LC squamous cell carcinoma as well as dyspnea. The male subject's other family member is from Malakand and has a no family history of cancer. According to NCBI BLAST data, there are no variations in the nucleotide position 5826-5891 of the mitochondrially encoded tyrosine tRNA gene in LC subject. Figured 3 which was taken from NCBI Alignment results and Figure 2. Chromatogram of tyrosine gene which was taken from UGENE software showed that there is no variation in Tyrosine gene subject sequence.

Figure 3: Alignment and chromatograph results of samples L1-L8 of LC patients with different age, metabolic rate, weight and stage of LC with rCRS sequence of MT-TY gene Accession number NC-012920.1

DISCUSSION

This research study was done to find out the linkage of mitochondrially encoded tyrosine tRNA genes with LC from Peshawar. LC is one of the mostly occurring cancer types around the world (about 12.3%), in 2000 about new reported cases were around 1.2 million. Tobacco smoking is the one of the important factors which causes around 80 to 90% increase risk in smokers of cigarette [7]. LC has been recorded in 2.09 million cases, BC cancer has been reported in 2.093 million cases, prostate cancer has been reported in 1.28 million cases, skin cancer has been reported in 1.04 million cases, and stomach cancer has been reported in 1.03 million cases [17]. Squamous cell carcinoma (SqCC), adenocarcinoma, and large cell carcinoma are the most common subtypes of NSCLC. LC is thought to be a group of histologically and molecularly varied diseases that are currently divided into three histological subtypes: adenocarcinoma (ADC), squamous cell carcinoma (SqCC), and large cell carcinoma (LCC) [18]. In the particles of tobacco smoke there so many lungs cancer related cancer-causing particles like nicotine and they are catalyzed before secretion but still have ability to bind with DNA to form cluster. The cluster of DNA lead to

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apoptosis or repaired. If it prolonged, mutations related to miscoding occur in RAS or P53 like key genes which results to unstable the genes, which leads eventually to cancer & mutational damage [8]. Some chief tribal, geographical & sex dissimilarities in occurrence and there are several publications which shows the possibility that females also have high chances to attain cancer of lung by exposing to tobacco smoke. Generally, person who smoke for lifetime have 20-30% high chances to get cancer of lung comparison with the person who never smoke [8]. The smoke of tobacco contains about 20 types of known lung carcinogens, the most common are the tobacco-specific aromatic polycyclic hydrocarbons and nitrosamine. The efficiency of glutathione e-S transferase family was decreased by Polymorphism, which can inactivate cancer particles, and enhance family of P450 performance, that activates the previous, which outcome to high cancer rate receptiveness. Those cancer particles which active have chance to join the DNA, principal for alteration of DNA, especially the transfusion of Guanosine to thymine, that can repair, results to cell deaths/survive. Polymorphism in genetics is mainly concerned with the activation or detoxification of cancer-causing particles, it also regulates the host cell efficiency, and repair the tobacco carcinogen-DNA damage [4]. Cancer of lungs has now become one of the killers among cancers around the world. Although, cancer of lung is a major type which cause high mortality rate in US, LC frequency falling. About 2,105,020 newly reported person with LC in 2008, the mortality rate is about 161,840 by LC in the U. S [17]. The human mitochondrial DNA (mt-DNA) is a small, circular, double-stranded molecule with a size range of 16.5kb or 16569bp. Human mitochondrial DNA damage and somatic mutations can have a specific influence on the OXPHOS system's pairment activity, with the most important effect being that the increased production of ROS enhances the mt-DNA mutation. Mitochondria are the cell's powerhouse, performing synthesis and repair mechanisms with the help of enzymes. There are 37 genes in the human mitochondrial DNA genome, 13 of which are involved in oxidative phosphorylation complexes, 2 of which are involved in ribosomal RNAs, and 22 of which are involved in the production of tRNAs genes, that are all essential for energy formation [19].The nuclear genes which are associated with LC are KRAS, EGFR, ALK, MET, RET, BRAF, PIK3CA, LKB1, and ROS1 [20]. Mitochondrially encoded tyrosine tRNA gene is also known as MT-TY this official symbolic name was given by Human Genome organization gene Nomenclature Committee (HGNC). MT-TY gene having 66 nucleotides and transfer the amino acid tyrosine to growing chain at the ribosome site of protein synthesis during translation [21]. Mitochondrial DNA (mtDNA)-related disorders can be difficult to diagnose and need comprehensive clinical and laboratory investigations. Mitochondrial disease is caused by pathogenic variations in the mitochondrial tRNA gene MT-TY, which encodes the tRNATyr. A unique m.5860delTA anticodon mutation in the MT-TY gene has been identified in a patient who originally presented with symptoms similar to childhood onset myasthenic syndrome [22]. Genetic defects in mitochondrial DNA encoded tRNA genes impair mitochondrial translation with resulting defects in the oxidative phosphorylation system and respiratory chain of mitochondrial. The phenotypic spectrum of disease seen in mitochondrial tRNA defects is variable and proving pathogenicity of new variants is challenging. Only three pathogenic variants have been described previously in the mitochondrial $tRNA^{Tyr}$ gene MT-TY, with the reported phenotypes consisting largely of adult-onset myopathy and ptosis. We report a patient with a novel MT-TY acceptor stem variant m.5889A>G at low in blood, high heteroplasmy in muscle, and absent in the blood of mother. The phenotype consisted of a childhood-onset severe multi-system disorder characterized by a neurodegenerative course including combined myopathy and neuropathy, ataxia and seizures, hearing and vision loss and failure-to-thrive. Brain imaging showed basal ganglia calcifications and progressive atrophy. Mitochondrial biomarkers GDF15 and lactate were increased. Functional studies showed a deficient activity of the respiratory chain enzyme complexes containing mtDNA-encoded subunits I, III and IV. There were decreased steady state levels of these mitochondrial complex proteins, and presence of incompletely assembled complex V forms in muscle. These changes are typical of a mitochondrial translational defect [23]. The aim of this study was to determine the linkage of the mt-DNA encoded tyrosine tRNA gene in LC patients from Peshawar. LC patients' blood samples were collected from the IRNUM (Institute of Radiotherapy and Nuclear Medicine) hospital in Peshawar, their diagnosis and treatment are undergoing belonging to different geographic regions and families. DNA was extracted using the Phenol Chloroform protocol, and PCR was used to amplify the MT-TY gene almost 660bp was obtained. Clean and purified PCR products were sequenced. Out of 8 samples were sequenced and their sequence results were compared with rCRS of mitochondrial encoded tyrosine tRNA gene Accession No. NC 012920.1. Sequences BLAST in NCBI, and alignments were performed that showed 100 percent identity, concluded that no MT-TY gene variation was found in LC patients. On the basis of the current study, it is suggested that further research for finding the linkage of mitochondrially encoded tyrosine tRNA gene in subjects with LC may be possible in a large population size, and other

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mt-DNA genes also be studied for the association of mt-DNA variation and LC in Pakistan.

CONCLUSIONS

In 18 LC patients, the mitochondrially encoded tRNA tyrosine gene was studied. The specimens were taken from IRNUM, Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan. We found no variations linked to the MT-TY gene.

Authors Contribution

Conceptualization: ZA
Methodology: ZA
Formal Analysis: MN, FH
Writing-review and editing: SA, ZA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

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