ARTICLE / INVESTIGACIÓN

Analysis of Common Mutation of P53 Gene in Male with Lung Cancer in Mosul City

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DOI. 10.21931/RB/2022.07.03.52

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Abstract. TP53 gene plays a critical role in the follow-up of different cancer cases, including diagnosis and follow-up treatment and the mutation in the P53 gene. It harms the encoded P53 protein and the less function of P53 protein in different types of cancer due to the mutation in the TP53 gene. P53 protein has many mechanisms to eliminate cancer cells, like apoptosis, cell cycle arrest, and DNA repair. This study aims to detect the abnormality change in the sequence of P53 mutation and the correlation with cancer in Mosul city
DNA extraction depends on the manual description from blood, the Pro72Arg in exon 4 G\C allele mutation measurement by ARMS-PCR, and the analysis of common mutation in Exon 3,4,5 by DNA sequencing technique. The result of this study shows the observation of dif-ferent genotypes and allelic frequencies of Pro72Arg polymorphism in exon 4. It was the presence of wild-type genotype CC (pro\pro) 12%, hetero genotype CG (pro\arg) 72% and mutant genotype GG (arg\arg) 16% in patients with Lung cancer. While in healthy people, the wild genotype CC was 26 hetero genotype CG 66 and mutant genotype GG 8%. As for the result of DNA sequencing, this research doesn't find any change in the nucleotide of Exon 5 for the P53 gene of the case study. At the same time, the DNA sequence result of Exon 6 for the P53 gene in patients finds some changes in the nucleotide sequence with sequence. According to this study, the observation of different genotypes and allelic frequency of Pro72Arg pol-ymorphism in exon 4 for the P53 gene present significant variation between patients with lung cancer and healthy group male with Lung cancer.

Keywords: P53 gene, ARMS-PCR, Mutation, SNP, Lung cancer, Exon.

Introduction

Lung cancer is the second most prevalent cancer type after lung cancer (10.4 percent of all cancer incidence in both sexes) and the fifth most common cause of cancer death; It's an illness brought on by a mix of hereditary and environmental reasons [1].

The tumor marker is considered a vital marker found in the blood, tissues, and the body's urine may increase the levels of these markers with one type or more of cancer [1]. The tumor marker is produced from cancer or the host cell as a response to cancer. As well as the tumor marker plays an essential role in detecting the type and stage of cancer, which help in the early diagnosis of the tumor and the follow-up of chemotherapy and radiotherapy [2]. Few types of tumor markers are specific to one type of cancer. In contrast, most types of tumor markers are produced by many types of cancer in the same tissues, and these types of tumor markers present in very high levels and quantities in cancer patients compared with healthy people. Usually, these markers are considered valuable to detect the stage of the progressing tumor after initiation of treatment with chemotherapy and radiotherapy [3,4]. The first tumor marker was discovered in 1846 by Henry-jones [5].

Lung cancer was classified as an inherited disease, but many types of genes had a correlation with initiated cancer, and these genes are divided into two main types. The first type is called oncogenes, and the second type is suppressor genes. The P53 gene is considered a more common type of the second type [6].

Lung cancer is considered the most type of tumor widespread in the world. It is responsible for the death of more than 50% of all tumor patients worldwide. It's a disease caused by genetics and environmental factors [7,8].

A recent study detects 13 types of tumor markers. This study has a correlated role with the initiation and detection of human tumors. The most important type of this marker is P53, also called tumor protein P53, a nuclear protein that plays a vital role in cell cycle regulation. This function prevents tumors in the human body [9,10].

The P53 protein encoded by the gene is TP53, located on the short arms of chromosome 17 (17p13.1) and contains 11 exons. This protein consists of 393 amino acids and is present in all cell types in the human body but at a deficient level [11]. The study of P53 protein initiated science in 1974 and found a relationship with different types of cancers [12].

The TP53 gene mutation is considered the most genetic variation associated with human cancer. Many types of genetic mutation have been detected for tumor suppressor gene P53 for many cancers [13].

One of the most common SNPs in the TP53 gene is Pro72Arg (rs1042522), which is positioned in the proline region of p53 and is required for normal p53 function [12]. According to studies, the arginine (Arg) variation induces apoptosis faster and more efficiently than the proline (Pro) form, whereas the Pro variant initiates cycle arrest more effectively [13]. The TP53 gene's Pro72Arg SNP has been related to increased cancer risk.

Citation: Hamed OM. Analysis of Common Mutation of P53 Gene in Male with Lung Cancer in Mosul City. Revis Bionatura 2022;7(3) 52. http://dx.doi.org/10.21931/RB/2022.07.03.5212 April 2022 / Accepted: 13 June 2022 / Published:15 August 2022

In this type of tumor, TP53 gene mutation is associated with cancer development, and the frequency of these mutations increases with cancer progression, especially in solid cancer [14]. The primary type mutation of the TP53 gene is Missense mutation, located between 5-8 exons. The method that is most dependent on the detection of the mutation is the DNA sequins technique [15]. As well as, the type and frequent mutation of P53 differs from one type of cancer to another, varying from 10% to 57% depending on the causes, stage, and type of cancer [16].

P53 protein has many tumor suppressor mechanisms like increasing the activity of DNA repair proteins, stopping the cell's growth by cell cycle arrest and promoting the apoptosis of tumor cells [17].

The loss of function of the tumor suppressor gene is caused either by a mutation in the sequence of gene nucleotides or by any change in the structure of these genes. The normal P53 protein has a short half-life compared with the mutant P53 protein [18].

Also, the measurement of the P53 protein level is considered a marker for the progression stage of chemotherapy and radiotherapy because this treatment causes tumor cell death by apoptosis [19,20].

Many studies indicate the loss of function of P53 protein as a result of a mutation in the TP53 gene. It's considered the leading cause to occur and raise of cancer [21].

This study aims to detect the abnormality change in sequence of P53 mutation and the correlation with cancer in Mosul city.

Materials and Methods

Case study

The previous study contained (110) samples taken from women, which we separated into (80) samples from women with breast cancer and (30) samples from males as a control group in the same age group who did not have a problem with Lung cancer.

The blood venous was collected in two tubes, first, with an EDTA tube for DNA extraction and Genotyping test, and the second with Gel-tube to obtain the serum for biomarker test.

T-ARMS-PCR is considered an easy and rapid technique to detect genetic variation and allelic frequency. This technique uses four primers, two of which are called an outer primer, which is used for amplification of target gene and another primer, inner primer, is used to detect the wild and mutant type alleles. Four primers were put in the single PCR tube with master mix and template DNA, and the PCR product consisted of three different bands.

The genotyping test of this study includes detection of the Pro72Arg Polymorphism of the P53 gene in exon 4, which is done by adding 10 µic master mix, 1 µic, 4 µic DNA and complete the volume to 20 µic with DW. The PCR condition is 95°C for 6 minutes, then 35 cycles of 95°C for 45 seconds, 56°C for 1 minute, 72°C for 1 minute and a final extension of 72°C for 7 minutes [22].

Also, this study involves detection of the genetic variation of exon5 and exon 6 by sequencing technique with PCR conditions: 95°C for 5 minutes, then 35 cycles of 95°C for 1 minute, 59°C for 1.5 minutes, and 72°C for 1.5 minutes and final extension of 72°C for 7 minutes [23,24], table 1 explain the primers used in this study.

The biomarker test includes the measurement of some types of tumor markers that correlate with Lung cancer in this study determined the levels of some tumor markers such as AFP and CEA as an indicator of the tumor in patients.

Results and Discussion

The result of this study shows in table 2; the observation of different genotypes and allelic frequency of Pro72Arg polymorphism in exon 4 for the P53 gene present significant variation between the patient and healthy group. It is the presence of wild-type genotype CC (pro\pro) 12%, hetero genotype CG (pro\arg) 72% and mutant genotype GG (arg\arg) 16% in patients with Lung cancer. While in healthy people, table 3 showed the wild genotype CC was 26, hetero genotype CG 66 and mutant genotype GG 8% in the significant level P = 0.0440. These results also show the OR values, which is (6.000) for genotypes, which is more than (1.0) and is considered a risk factor for Lung cancer

polymorphism	Primer type	Sequence	Band size	Tm
Pro72Arg in exon 4 G\C allele	F-outer	5´TGCAGGGGGATACGGCCAGGCATTGAAGTC 3´	493 bp	56 °C
	R-outer	5´ TGGGGGGCTGAGGACCTGGTCCTCT3´		
	F-inner	5´ GCTGCTGGTGCAGGGGCCAGGG 3´	200 bp	
	R-inner	5´CCAGAATGCCAGAGGCTGCTCCGCG 3´	274 bp	
Exon 5	Forward	5´ TTC CTC TTC CTG CAG TAC TC 3´	210 bp	52 °C
	Reverse	5´ CAG CTG CTCACC ATC GCT AT 3´		
Exon 6	Forward	5´ ATTCCTCACTGATTGCTCC 3´	190 bp	59 °C
	Reverse	5´ TCCTCCCAGAGACCCCAGTT 3´		

Table 1. Primers were used in this study.

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO	%	P = 0.0440	6.000	1.0490 to 34.3184
CC	10	12	8	26			
CG	65	72	20	66			
GG	15	16	2	8			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
C	85	47	36	60	P = 0.0880	1.6765	0.9259 to 3.0354
G	95	53	24	40			

Table 2. Observation of different genotypes and allelic frequency of Pro72Arg polymorphism in exon 4

in patients. The mutant G allele of Pro72Arg polymorphism in exon 4 for the P53 gene is present in 53% of patients and 40% of control, so this allele has a significantly different distribution between the patients and control groups (O.R=1.67, P=0.0895% and CI: 0.9259 to 3.0354). Also, this study's result shows a significantly different distribution of wild C allele between patients with Lung cancer (12%) and healthy people (26%).

This research aims to detect the correlation between Pro\Arg polymorphism of the P53 gene with Lung cancer. These figures 1 and 2 can validate the association between genetic variation of P53 in patients and are considered risk

Groups	Tumor markers	
	AFP ng \ ml	CEA ng \ ml
Patients	67.1 ± 5.3	121 ± 7.5
Control	9.5 ± 1.7	5.8 ± 0.3

Table 1. Showed the wild genotype CC was 26, hetero genotype CG 66 and mutant genotype GG 8% in the significant level P = 0.0440

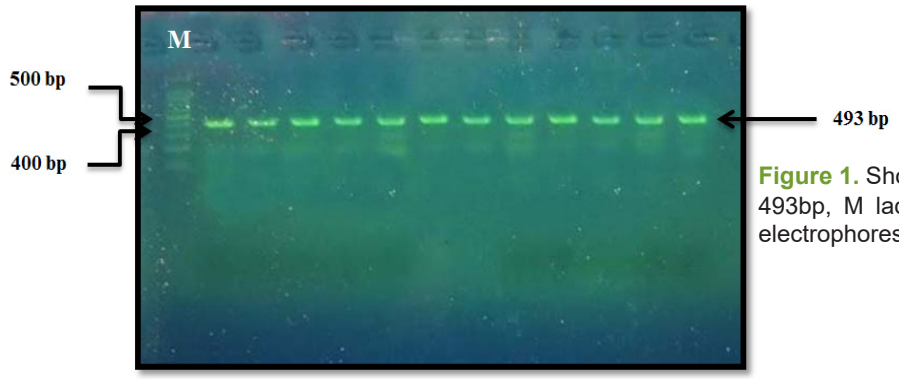

Figure 1. Shows the PCR product for Exon 4 with 493bp, M ladder separated by 2% agarose gel electrophoresis.

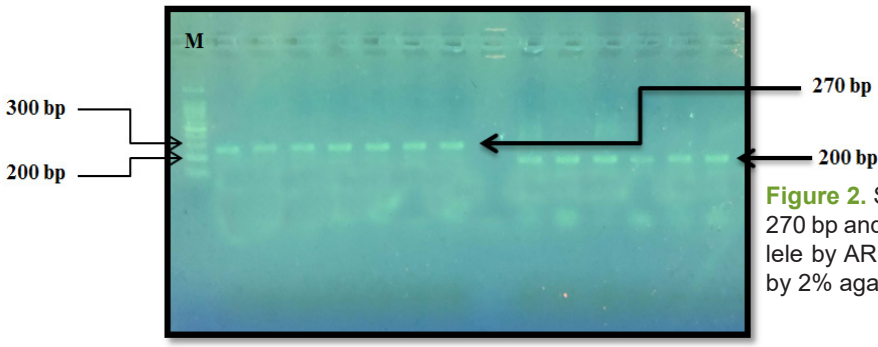

Figure 2. Show the PCR product for Mutant allele 270 bp and Wild type allele 200 bp for Wild type allele by ARMS-PCR technique, M ladder separate by 2% agarose gel electrophoresis.

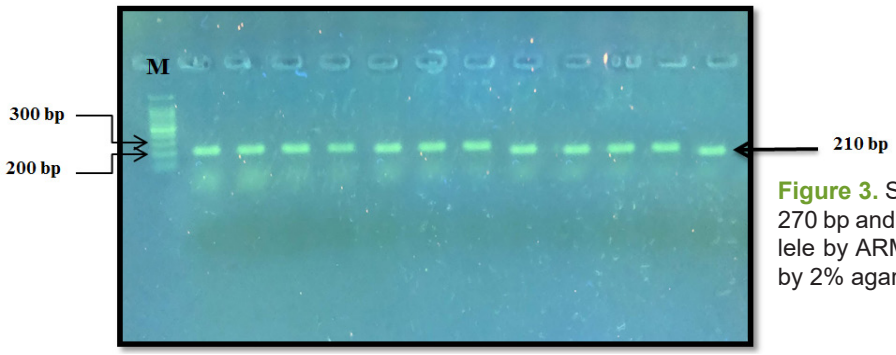

Figure 3. Show the PCR product for Mutant allele 270 bp and Wild type allele 200 bp for Wild type allele by ARMS-PCR technique, M ladder separate by 2% agarose gel electrophoresis.

factors. This result makes sure the Pro\Arg SNP of the P53 gene has a wide spread in Mosul city, as is the case with Caucasians, Chinese and African-Americans societies [25].

Previous studies have confirmed the presence of a multi-functional difference between P53 with Pro and P53 with Arg, such as the P53-Arg variant, which is more active in apoptosis. At the same time, the P53-Pro has an essential role in cell cycle arrest and DNA repair [14]. Other studies show the association between TP53 mutations with different types of cancer. One of these mutations is Pro\Arg in Exon 4, which includes replacement protein instead of Arginin in codon 72 of P53 [26, 27]. In this study, we evaluate the effect of Pro\Arg SNP on Lung cancer in Mosul city. The three different genotypes were identified, which is considered one of the Cancer causes.

Figure 3. The PCR shows the product for Exon 5 with 210bp for DNA sequencing, M ladder separated by 2% agarose gel electrophoresis.

As for the result of DNA sequencing in figure 3, also the result of this research in figure 4 doesn't find any change in the nucleotide of Exon 5 for the P53 gene of case study ID: EF554499.1 compared with the sequence of Exon 5 in National Centre of Biotechnology Information (NCBI) as shown in figure 4.

Download GenBank Graphics

Homo sapiens isolate 561 tumor protein p53 (TP53) gene, exons 5, 6 and partial cds

Sequence ID: EF445599.1 Length: 408 Number of Matches: 1

See 19 more title(s) See all Identical Proteins(IPG)

Range 1: 60 to 207 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
274 bits(148)	2e-69	148/148(100%)	0/148(0%)	Plus/Plus

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Query  1    CTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60   CTGCCCTGTGCAGCTGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCAT  119

Query  61   GGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  120  GGCCATCTACAAGCAGTCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGA  179

Query  121  GCGCTGCTCAGATAGCGATGGTGAGCAG  148
            ||||||||||||||||||||||||||||
Sbjct  180  GCGCTGCTCAGATAGCGATGGTGAGCAG  207
```

Figure 4. The alignment of Exon 5 for P53 gene in males with Lung cancer.

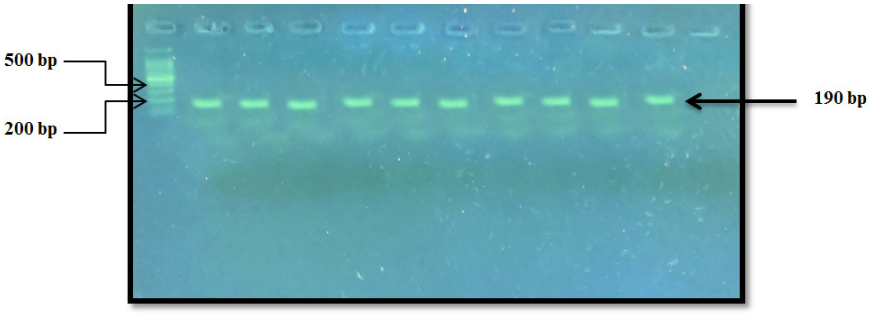

Figure 5. The PCR shows the product for Exon 4 with 190bp, M ladder separated by 2% agarose gel electrophoresis.

Download GenBank Graphics

Human p53 (TP53) gene, complete cds

Sequence ID: U94788.1 Length: 20303 Number of Matches: 1

Range 1: 13366 to 13463 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
167 bits(90)	2e-37	97/100(97%)	2/100(2%)	Plus/Plus

```
Query  15     TGTGGAGTATTTGGATGACAGAAACACTTTCTCCCACATAGTGTGGTGGTGCCCTATGAG  74
              ||||||||||||||||||||||||||||||| | | ||||||||||||||||||||||||
Sbjct  13366  TGTGGAGTATTTGGATGACAGAAACACTTT-T-CGACATAGTGTGGTGGTGCCCTATGAG  13423

Query  75     CCGCCTGAGGTCTGGTTTGCAACTGGGGTCTCTGGGAGGA  114
              ||||||||||||||||||||||||||||||||||||||||
Sbjct  13424  CCGCCTGAGGTCTGGTTTGCAACTGGGGTCTCTGGGAGGA  13463
```

Figure 6. The alignment of Exon 6 for P53 gene in males with Lung cancer.

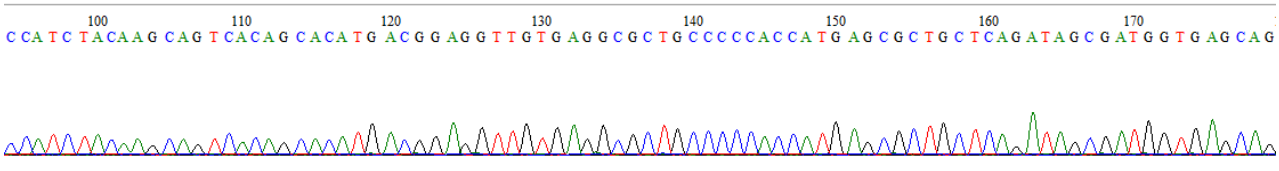

Figure 7. The electropherogram of the TP53 gene sequences

These changes in the nucleotide sequence of P53, shown in figures 5 and 6 considered the main reason for the lack of activity of P53 protein in tumor suppressive function and therefore increase the possibility of cancer injury.

The result of this study shows an increase in the levels of tumor marker AFP (67.1 ± 5.3) and CEA (121 ± 7.5) in patients with lung cancer compared with healthy males (9.5 ± 1.7) and (5.8 ± 0.3), respectively.

Tumor markers have changed the way oncologists practice. They can evaluate screening, diagnosis, prognosis, and therapy efficacy. According to a study, the Arg72 variant is more efficient in causing apoptosis. According to reports on tumor marker use, many clinicians believe that a biomarker for one cancer can be used effectively for all of these indications. This is a false assumption [24, 28]. Several recommendations have been produced to help doctors understand how to use these tests effectively [28].

Overall, this was the first investigation to look at the influence of the TP53 gene Pro72Arg SNP on lung cancer in a Mosul city. the present study discovered a link between TP53 gene variant and lung cancer, and this SNP is regarded to be a risk factor for lung cancer in men. However, further study is needed among Mosul's diverse ethnic communities to confirm these findings.

Conclusions

This study demonstrates that the polymorphisms rs1042522 and Exon 7 codon 249) Polymorphism of the P53 gene were associated with an increased risk of Breast Cancer among apparently healthy women in Mosul city.

ACKNOWLEDGEMENTS

I am grateful to the Science College at the University of Mosul for providing facilities that helped to increase the quality of this work.

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