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Research Article

Molecular study of *TP53* Gene in Iraqi Women suffered with Breast Cancer

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Abstract-Nearly 75% of malignancies have been associated with P53 protein failure, which typically results from *TP53* gene mutation. *TP53* is activated in response to DNA damage and hypoxia and repairs damaged DNA, which has an impact on cell aging and apoptosis. These actions are essential for tumor suppression in addition to modifying cellular responses associated with cell cycle regulation, which is a critical component of tumor suppression. Between September and November 2021, (96) referrals for female patients between the ages of 35 and 45 were sent to the Alternative Nuclear Medicine and Oncology Hospital in Mosul. The samples were split into two groups, one containing 71 women with breast cancer and the other 25 healthy women. This study identified the polymorphisms of *TP53* in codon 249 for exon 7 and (rs1042522) in exon 4, as well as the nucleotide sequences of the amplified parts using DNA sequencing technology, coupled with a variety of physiological variables and blood components. For instance, the levels of hemoglobin, urea, creatinine, red blood cells, white blood cells, and platelets. Exons 3, 4, and 6 had varied numbers of nucleotides, according to the findings of a sequencing test on the gene's amplified exons, however exon 5 had no change in nucleotides. Additionally, an unique genotype of the *TP53* gene with the GeneBank identifying number of LC682536.1 was discovered in the city of Mosul at the NCBI global gene site. A novel phenotype of the P53 tumor suppressor protein was also found in Mosul, and it was given the identification number GenBank: BDF83325.1. According to the study, the ratio between the levels of CA15-3 in patients and healthy controls was 23 (U/ml), while the patients' levels of urea were 38.2 (mg/dl). According to these findings, both the levels of urea and creatinine in the patients' blood plasma and the levels of CA15-3 in the blood plasma of breast cancer patients were significantly lower than those of healthy controls. The current investigation found that the total number of WBC, RBC, PL, and HB levels in the blood of breast cancer patients had dropped dramatically.

Keywords: TP53 Gene, ARMS-PCR, RFLP-PCR, Polymorphism and Breast Carcinoma.

I. INTRODUCTION

Cancer is a condition that is distinguished by the proliferation of cells with the ability to invade and destroy nearby tissues, or metastasize to distant tissues in a process called metastasis. These characteristics are characteristic of malignant tumors, in contrast to benign tumors, which are characterized by specific growths and cannot spread (Mattiuzzi and Lippi, 2019). However, benign

tumors can sometimes develop into malignant tumors. It is estimated that about 5-10% of cancers are caused by genetic mutations (Ferlay et al., 2021). Among the most prevalent cancers in women worldwide is breast cancer, and it is the cause of death for about 20% of women who die of cancer in the world (Ahmad, 2019). A cancerous growth that develops from breast cells is referred to as breast cancer. Breast cancer frequently begins in the cells of the lobules, which were formerly the milk-producing glands, or the ducts, which are the channels that convey milk from the lobules to the nipple. Breast stromal tissues, which include its fatty and fibrous connective tissues, are where breast cancer might arise in a different, less prevalent variety (Momenimovahed and Salehiniya, 2019). Cell cycle control systems closely regulate cell division in order to produce two daughter cells cells. DNA regulatory systems known as cell cycle checkpoints stop the aggregation and distribution of genetic mistakes during cell division. In reaction to irreversible DNA damage, checkpoints can slow down the course of the cell cycle or cause cell death (Takahashi and Kato, 2022), but in cancer cells, due to the presence of genetic mutations associated with the cell cycle, the cell cycle continues to divide continuously and abnormally (Liu et al., 2021). Apoptosis is an organized and coordinated cellular process that occurs under physiological and pathological conditions and it is important to understand the mechanism underlying apoptosis because it is quite important. in the pathogenesis of many diseases (Koff et al., 2015). There are two cases of programmed cell death, the first in which there is an increase in cell death as in degenerative diseases, while in the second case there is a decrease in cell death as in cancer (Mohammad et al., 2015). Any substance generated by cancer cells, other body cells in reaction to cancer, or some benign conditions is referred to as a tumor marker that provides information about the malignancy, including its aggressiveness and potential responses to various treatments as it can be measured in the blood, urine, tumors, or other tissues (Sokoll and Chan, 2020). Many different tumor markers have been described and some are associated with only one type of cancer while others are associated with different types of cancer and are proteins or other substances made by cancer cells in higher amounts than normal cells (Nagpal et al., 2016). Two important types of cancer-related genes have been discovered (Levine and Puzio-Kuter, 2010) : Oncogenes are genes that have the ability to make proteins believed to contribute to the formation of cancers. These genes are derived from genes called primary oncogenes that play a role in regulating normal cell division. Cancer can arise when a primary oncogene mutates due to genetic mutations into an oncogene that causes uncontrolled cell division and proliferation (Shortt and Johnstone, 2012). Tumor suppressor genes are important genes that control several cellular activities. These genes are grouped widely defining the roles in cell cycle development and progression, cell growth, DNA repair processes, and other cell - signaling tasks including apoptosis induction. And without those genes, there is a greater chance of uncontrolled cell proliferation, which is a recognized factor in the emergence of malignancies (Zhao et al., 2013). The *TP53* gene (Tumor Protein P53) is a tumor suppressor gene located on chromosome 17 (17p13.1) that encodes the p53 protein. This protein controls cell division by stopping cells from expanding and multiplying too fast or in an unpredictable way, which is how it functions as a tumor suppressor (Aubrey et al., 2016). The p53 protein acts in the nucleus of cells throughout the body where it binds directly to DNA and when DNA is damaged in a cell this protein plays an important role in determining whether to repair the DNA or destroy the damaged cell by itself, by preventing cells with damaged DNA from cleavage, p53 helps prevent the development of tumors (Olivier et al., 2010).

II. MATERRIAL AND METHOD

Case Study:

The Oncology and Alternative Nuclear Medicine Hospital in the city of Mosul received (96) referrals for the current research from women between the ages of (35-45) between September and November of 2021., the clinical cases of the disease were relied upon in selecting samples, the samples were divided into two groups based on the biochemical results:

The first group: This group included 25 ladies who weren't troubled in any way and were considered as a benchmark group.

The next category: Among this cohort were 71 women with breast cancer, based on biochemical results.

Collection of Blood sample:

Women were given 5.0 ml of venous blood, separated into two sections, the first of which was put in tubes containing the anticoagulant EDTA, and used it to isolate genomic DNA, and the other of which was put in tubes free of any anticoagulant and left for one hour until clotting then was centrifuged for (10) minutes at a speed of (3000) revolutions/minute to obtain the blood serum on which the various biochemical tests were conducted.

DNA extraction:

The DNA was recovered out from bloodstream of all (96) samples used in the research using the modified technique of (Iranpur and Esmailizadeh, 2010).

Genotyping:

PCR-RFLP Reactions:

The DNA concentration in each of the samples under examination was adjusted to the needed concentration for PCR reactions, which was (25) ng/microliter for each sample, by diluting with TE Buffer solution.

The DNA sample and the specific primer for each mutation were combined with the components of the master reaction mixture in a 0.2 ml Eppendorf tube provided by the American company Promega, and the reaction volume was set to 20 µl with distilled water. The mixture was then spun in a microfuge device for (3-5) seconds to completely mix the reaction components, next, using a unique protocol for each reaction, the thermocycler was filled with the reaction tubes to perform the replication procedure, then the PCR reaction product was incubated with the special cutting enzyme for each mutation for 3hr, next, using the volumetric guide Ladder DNA provided by Biolaps Company, the material was poured into the agarose gel pits at a concentration of 2%., then the samples are migrated by running the Electrophoresis device for (90-75) minutes and finally photographing the gel using the gel documentation.

Detection of the mutation (codon 249) of the *TP53* gene in exon No. 7:

The presence of the G ⟶ C mutation at the Exon 7 site (codon 249) was detected by adding 100 ng of template DNA and 10 Pico moles of the mutation primer prepared by Macrogen to the contents of the master mix (Vijayaraman et al., 2012).

Table 1: Shows the primers used to determine genetic variation in locus (codon 249) using PCR technique

Primer	Sequence	Band size	Annealing
Forward	5’ GGC-GAC-AGA-GCG-AGA-TTC-CA 3’	286 bp	55.0
Reverse	5’ GGG-TCA-GCG-GCA-AGC-AGA-GG 3’		

The reaction tubes are then placed in the thermocycler and the multiplication reaction is carried out using the particular program for the reactions, as shown:

One cycle of (5) minutes at a temperature of (95) °C for the initial denaturation of the DNA strand

Each doubling cycle includes (33) cycles, as shown:

(45) seconds at (95) °C for double strand denaturation, (1) minute at (55) °C for initiator binding to template DNA, and (1) minute at (72) °C for initiator elongation.

Then, a final cycle that includes (45) seconds at a temperature of (95) C, (1) minute at a temperature of (65) ˚C, and (7) minutes at (72) C to complete the elongation phase.

After the end of the reaction, the tubes were removed from the thermocycler device with a PCR yield of 286 bp and (5) microliters were withdrawn and incubated with the trimmer enzyme *(Hae-III)* at a concentration of 5 units supplied by Biolaps Company for a period of (3) hours at a temperature of (37) C.

Tetra-ARMS PCR reactions:

By diluting all research samples with TE Buffer solution, the DNA concentration was kept under control. and it was (25) ng/microliter for each sample. This concentration was necessary for PCR procedures. In order to produce the reaction of three bundles, four primers are added. The first bundle produces the whole gene segment, the second the natural allele, and the third the mutant allele. The DNA sample, the primer for each mutation, and the components of the master-mix were all combined in a 0.2 ml Eppendorf tube manufactured by the English company Biolaps to create the master reaction mixture for each PCR reaction. The reaction tubes were put into the thermocycler once the multiplication reaction was started using the unique algorithm for each reaction. To ensure the components of the reaction were mixed, the mixture was then thrown away in the Microfuge apparatus for (3-5) seconds. The sample was then loaded into the pits of the previously prepared agarose gel with a concentration of 2%, with the addition of the volumetric guide DNA Ladder prepared by Biolaps company in one of the pits, after which the samples were migrated by running the electrophoresis device for a period ranging from (70-60) minutes, and imaging was performed using the Gel Documentation device.

Determination of genetic variation of the *TP53* gene in situ (rs1042522) in exon 4 using Tetra–ARMS-PCR technique:

The presence of the C ⟶ G mutation at the rs1042522 site (pro72arg) was detected by adding 4 µl (100 nanogram) of template DNA and 1 µl (10 picomol) of each specific primer of rs1042522 mutation supplied by the Korean company Macrogen to the contents of the master mix (Asadi et al. , 2017).

Table 2: Shows the primers used to determine genetic variation in locus (rs1042522) using PCR technique

primer	sequence	Band size	annealing
F-outer	5′TGC-AGG-GGG-ATA-CGG-CCA-GGC-ATT-GAA-GTC3′	493 bp	59
R-outer	5′TGG-GGG-GCT-GAG-GAC-CTG-GTC-CTCT3′		
F-inner	5′ GCT-GCT-GGT-GCA-GGG-GCC-AGGG 3′	200bp	
R-inner	5′CCA-GAA-TGC-CAG-AGG-CTG-CTC-CGCG 3′	247 bp	

Then the reaction tubes were inserted into the thermocycler to conduct the multiplication reaction using the special program for the reaction as shown in Table 3:

Table 3: shows the program adopted in the PCR technique to identify the mutation (rs1042522)

No.	Stage	Temperature	Time	Cycle number
1.	Initial denaturation	95.0	5.0 min.	1
2.	denaturation	95.0	0.45 sec.	35
3.	Annealing	59.0	1.0 min.	
4.	Extension	72.0	1.0 min.	
5.	Final extension	72.0	7.0 min.	1
6.	Stop reaction	4.0	5.0 min	1

The optimal temperature for the primer bonding in this reaction was determined using the Gradient program on the thermocycler device; the gradient was (5) and the mean temperature was (62) C. The temperature of 61°C was used since it gave the best results. After that, a 2 percent concentration of agarose gel was run on the PCR result.

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology:

The sequence of the nitrogenous bases in the exons (3,4,5,6) of the *TP53* gene under investigation was established as the PCR products of the aforementioned genes were supplied together with the primers of the resultant package. The 3130 Genetic Analyzer instrument, made by the Japanese corporation Hitachi, was used to read the sequence for the genes.

The outcomes of the comparison between gene-specific sequences and those found in the NCBI database were looked at using BLAST software.

Table 4: shows the primers for the exons of the *TP53* gene on which the DNA sequencing test was conducted

Exon	Primer	Sequence
Exon 3 and 4	Forward	5′TGC-AGG-GGG-ATA-CGG-CCA-GGC-ATT-GAA-GTC3′
	Reverse	5′TGG-GGG-GCT-GAG-GAC-CTG-GTC-CTCT3′
Exon 5	Forward	5′ TTC-CTC-TTC-CTG-CAG-TAC-TC3′
	Reverse	5′ CAG-CTG-CTC-ACC-ATC-GCT-AT3′
Exon 6	Forward	5′ ATT-CCT-CAC-TGA-TTG-CTCC3′
	Reverse	5′ TCC-TCC-CAG-AGA-CCC-CAG-TT3′

III. RESULT AND DISCUSSION

Detection of the mutation (codon 249) of the *TP53* gene in exon No. 7:

The results showed, as shown in Figures (1) and (2), there is a relationship between the incidence of breast cancer in women and the genetic mutation of the *TP53* gene at the site (codon 249) in exon 7, When observed in Figure (2) after *Hae-III* cutting enzyme cleavage, the result of the PCR reaction in three packages is 309 and 158 for the normal GG genotype, 309 and 249 for the mutant CC genotype, and 309, 249 and 158 for the heterozygous genotype, and this indicates the emergence of genetic variation for the *TP53* gene. And with all genotypes and in different percentages, as shown in Table (5).

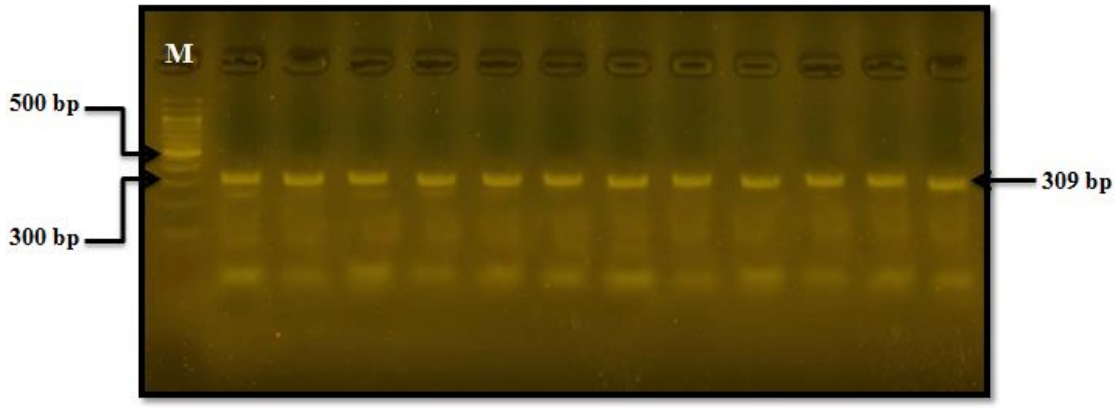

Figure 1: The PCR product for the genetic variation (codon 249) of exon 7 of the *TP53* gene and the reaction product 309 bp, M is the volume guide of 100 bp, supplied by Biolabs and separated by 2% agarose gel.

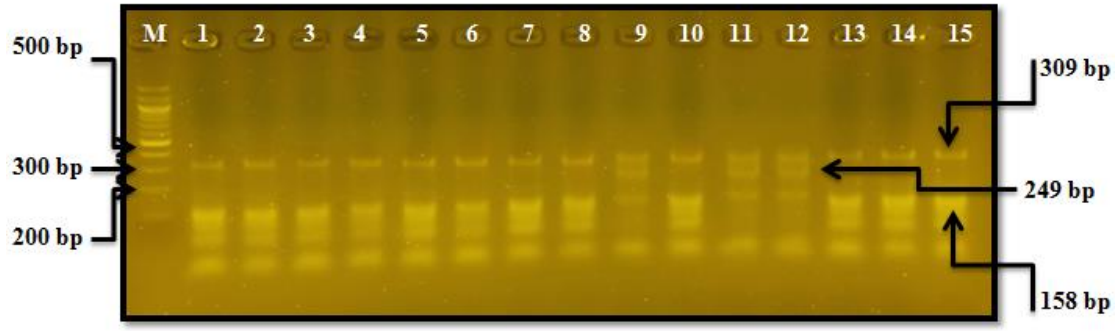

Figure 2: shows the result of the RFLP-PCR reaction for the genetic variation (codon 249) of exon No. (7) of the *TP53* gene. The result was a reaction containing 3 bundles, the first with a size of 309 bp for the main gene, the second with a size of 158 bp for the natural allele, and the third bundle with a size of 249 bp For the mutant allele, M is the 100 BP volume guide supplied by Biolabs and separated by 2% agarose gel.

Table 5: shows the allelic ratio and the different genotypes of the *TP53* gene at the site (codon 249) in exon 7

Genotypes	**Patients**		**Control**		**P Value**	**OR**	**(95%Cl)**
	NO.	**%**	**NO.**	**%**			
GG	8	11	13	61	P = 0.0001	84.500	9.6875 to 737.057
GC	14	20	7	34			
CC	52	69	1	5			
Alleles	**NO.**	**%**	**NO.**	**%**	**P Value**	**OR**	**(95%Cl)**
G	30	39	33	78	P < 0.0001	14.422	6.2327 to 33.3727
C	118	61	9	22			

According to the study's results, the proportion of patients who observed the mutant genotype CC was largest (69%) and the proportion who observed the normal genotype GG was lowest (11%), and the proportion of observing for the heterogeneous CG was 20 percent comparison to healthy group, where the mutant genotype CC was the lowest 5 percent and the normal GG genotype was the highest percentage of observations. The percentage of observations for the heterogeneous genotype CG were 34 percent, The allelic observation rate in breast cancer patients was 61 percent for the mutant C allele and 39 percent for the normal G allele. The percentage of the mutant allele seen relative to the control group was 22%, as opposed to 78% for the normal allele.

As already known, the occurrence of a mutation in this *TP53* gene leads to a defect in the mechanism of action of the main protein encoded by the gene (Vijayaraman et al., 2012), as the p53 protein works to repair damaged DNA as a result of external environmental influences or due to oxidative stress, and increases the effectiveness of The process of apoptosis, thus suppressing tumor formation by eliminating all abnormal cells, also has an important role in the cellular response as it is considered one of the regulators of the cell cycle (Wang et al., 2022). P53-dependent apoptosis contributes to chemotherapy-induced cell death. It has been found that programmed cell death contributes to the action of chemotherapy, which increases the sensitivity of cancer cells and significantly reduces the doses of chemotherapy and possibly its harmful side effects (Levine, 2019). Where breast cancer is associated with genetic mutations within exon 7 of the *TP53* gene, these mutations are primarily mutations in the CG dinucleotide, so mutations in the *TP53* gene lead to a defect in the work of the protein and then it will lose its function, which is one of the causes of cancer (Bouaoun et al., 2016).

The study's findings also revealed that the OR value of the mutant genotype was very high (O.R = 84.5 at the level of probability P = 0.0001), and the OR value of the mutant allele was O.R = 14.2 at the level of probability P = 0.0001, which is regarded a health hazard and one of the causes of breast cancer caused by p53 protein imbalance.

Determination of genetic variation of the *TP53* gene in situ (rs1042522) in exon 4 using Tetra–ARMS–PCR technique:

The results showed, as in Figure (3), a relationship between breast cancer incidence and genetic variation in the *TP53* gene at the site (rs1042522) in exon No 4, It is evident from the results of the PCR reaction that the genetic variation of the gene appears in the three different genotypes, CC, CG, and GG, in different percentages, as shown in Table (5).

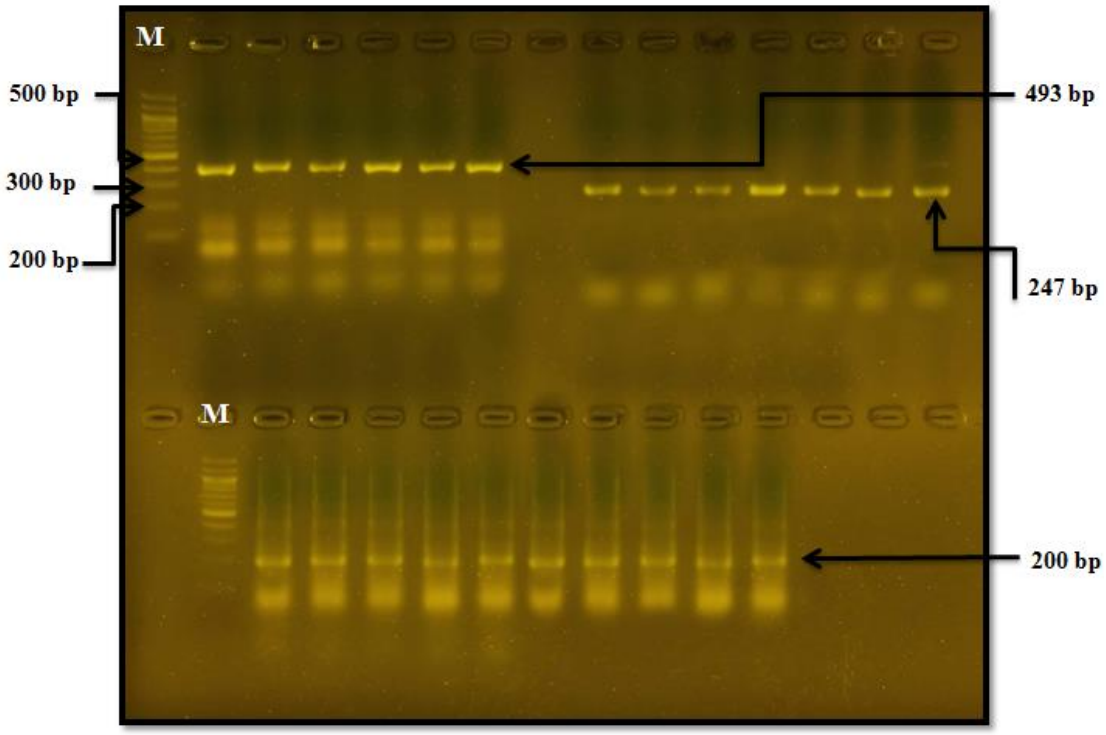

Figure 3: shows the result of the PCR reaction for the genetic variation (rs1042522) of exon number 4 of the *TP53* gene. The result was a reaction containing 3 bundles, the first with a size of 493 bp for the main gene, the second with a size of 247 bp for the normal allele, and the third bundle with a size of 200 bp for the mutant allele. M is the 100 BP volume guide supplied by Biolabs and separated by 2% agarose gel

Table 6: Distribution of allelic viewing and genotype of the *TP53* gene at the locus (rs1042522) in exon 4 among the group of healthy women and women with breast cancer, noting that the C allele is the normal allele and the G allele is the mutant allele

Genotypes	Patients		Control		P Value	OR	(95%Cl)
	NO.	%	NO.	%			
CC	26	35	15	70	P = 0.0897	4.0385	0.8055 to 20.2474
CG	34	56	4	20			
GG	14	19	2	10			
Alleles	NO.	%	NO.	%	P Value	OR	(95%Cl)
C	86	58	34	80	P = 0.0087	3.0640	1.3273 to 7.0728
G	62	42	8	20			

Table (6) shows the frequency of the various *TP53* genotypes and the proportion of allelic observations at the location (rs1042522) in exon No. 4 of the *TP53* gene. The results for breast cancer in women showed that the mutant GG genotype had the highest risk of recurrence among them, at 19 percent, compared to the 10 percent mutant genotype in the reference group. In the control group, where the percentage of people with the normal genotype was large (70%) the results for breast cancer in women also showed that the mutation was associated with a higher risk of recurrence. women with breast cancer had the lowest proportion of the normal genotype CC, which was down by 35%, In the sick group, 56 percent of the heterogeneous CG genotype was seen, as opposed to 20 percent in the control group. Regarding allelic recurrence, the findings revealed that a higher number of patients (42%) than in the control group (20%) had the mutant G allele. In terms of the normal allele, patients had a 58 percent prevalence compared to the control group's 80 percent.

According to earlier research, p53 is an intracellular protein that regulates gene expression for a certain cell cycle stage. The cell cycle is prevented if these genes are not expressed, which essentially prevents cell division. It is also a protein responsible for DNA repair processes, which prevents cells from replicating with their mutations. P53 sends a signal that causes apoptosis when DNA is altered or disrupted and cannot be fixed, stopping cells from proliferating and transforming into tumors (Mishra et al., 2013). It is worth noting that the occurrence of mutations in the *TP53* gene at the site (rs1042522) in exon No. 4 leads to a defect in the work of the p53 protein, and then the biological function of this protein will be lost, this leads to the production of the p53 protein, which has a defect in its main function, which is one of the causes of cancer (Dahabreh et al., 2013).

The study's findings also indicated that the odds level for the mutant GG genotype was O.R=4.038 at a probability level of P=0.089 and that it was O.R=3.06 at a probability level of P=0.008 for the mutant allele, both of which are considered risk factors for breast cancer because they are higher than (1).

Haplotype analysis:

Haplotype analysis plays an important role in identifying new genetic associations between the alleles of different genetic variations for a particular gene. Through this study, 4 possible genotypes were identified resulting from the allelic associations of the genetic mutations (rs1042522), (codon 249), as shown in Table No (6).

Table 7: shows the genotypes and their frequencies resulting from the Haplotype test

Genotype	Frequency (patient)	Frequency (control)	Odd. Ratio	CI (95%)
CC	62.73(0.424)	20.38(0.162)	(3.814)	2.145~6.781
CG	23.27(0.157)	81.62(0.648)	(0.101)	0.057~0.180
GC	55.27(0.373)	6.62(0.053)	(10.740)	4.589~25.136
GG	6.73(0.045)	17.38(0.138)	(0.298)	0.118~0.751

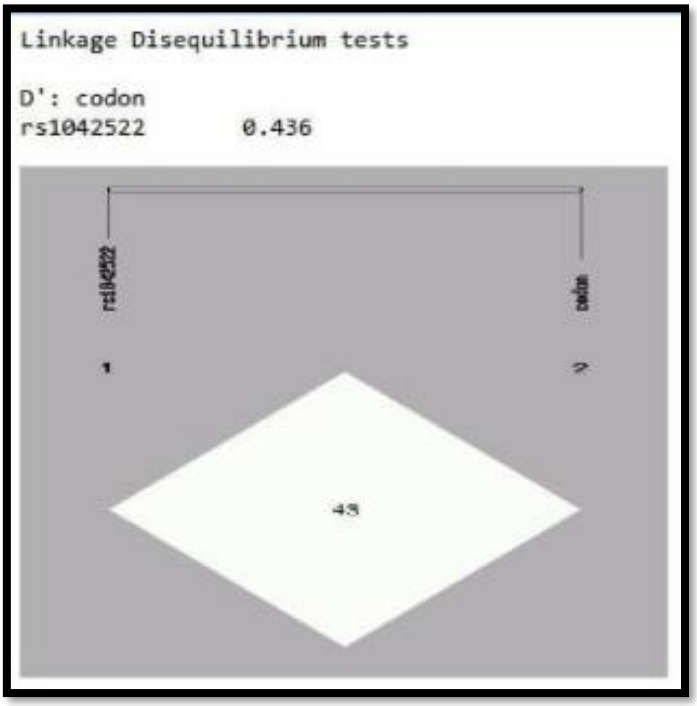

Figure 4: Overlap of *TP53* gene variants by haplotype analysis

It is clear from the above table that the GC genotype was of greater importance than the rest of the genotypes, as its frequency in patients was 55.27 (0.373) compared to its frequency in the control group 6.62 (0.053) when the Hardy-Weinberg equiliprium test for genetic variance at the site (rs1042522) was conducted. It was found that the P value = 0.234, which is less than 0.5, and this indicates that this group is not balanced and is not subject to the HWE law because of this mutation that affected the *TP53* gene. Whereas, when conducting the Hardy-Weinberg equiliprium test for genetic variance at the site (Codon 249), it was found that the value of P = 12.7, which is higher than 0.5, and this indicates that this group is balanced and subject to the law of HWE.

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technology:

Figures (5), (6) and (7) show the output of the PCR reaction 493 bp, 230 bp, 180 bp for exons 3,4,5,6 respectively, on which the nucleotide sequence identification test was performed.

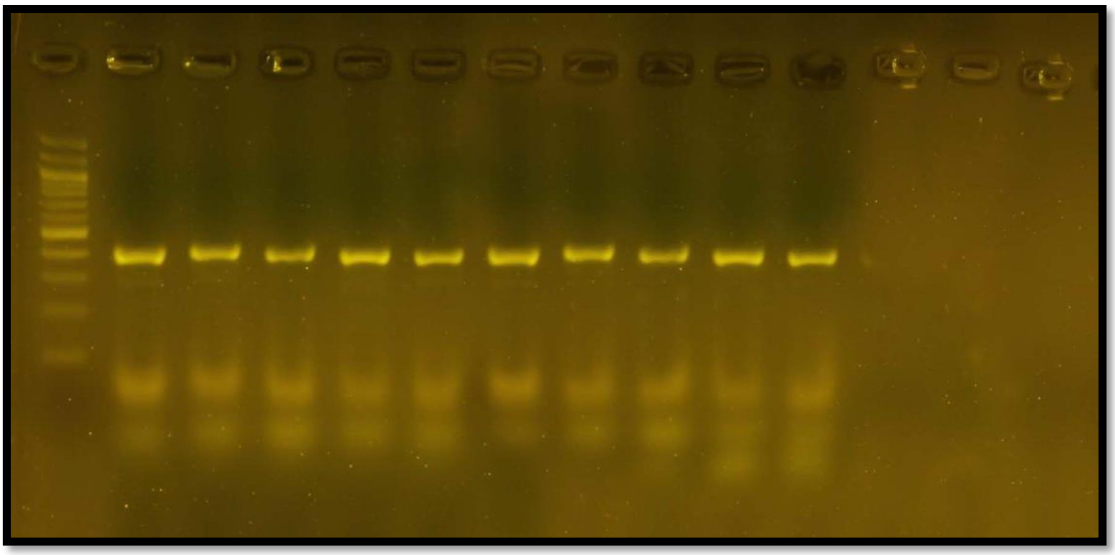

Figure 5: shows the PCR reaction product of exons No. (3,4) of the *TP53* gene and the reaction product 493 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

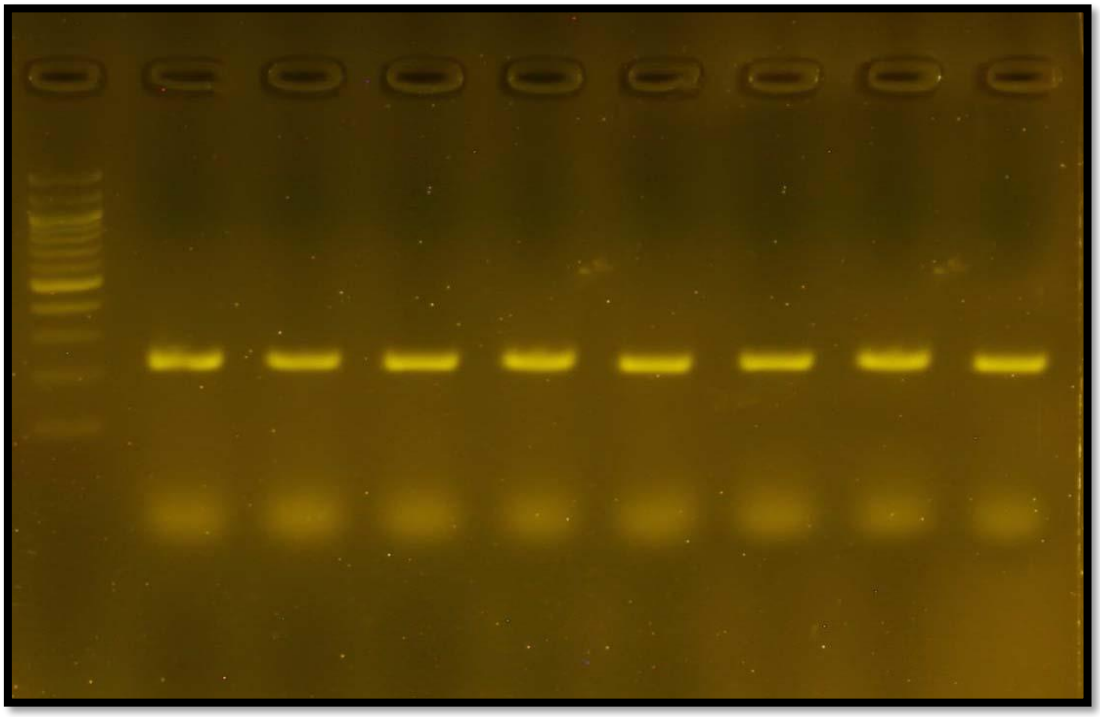

Figure 6: The PCR product of exon No. (5) of the *TP53* gene and the reaction product is 230 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

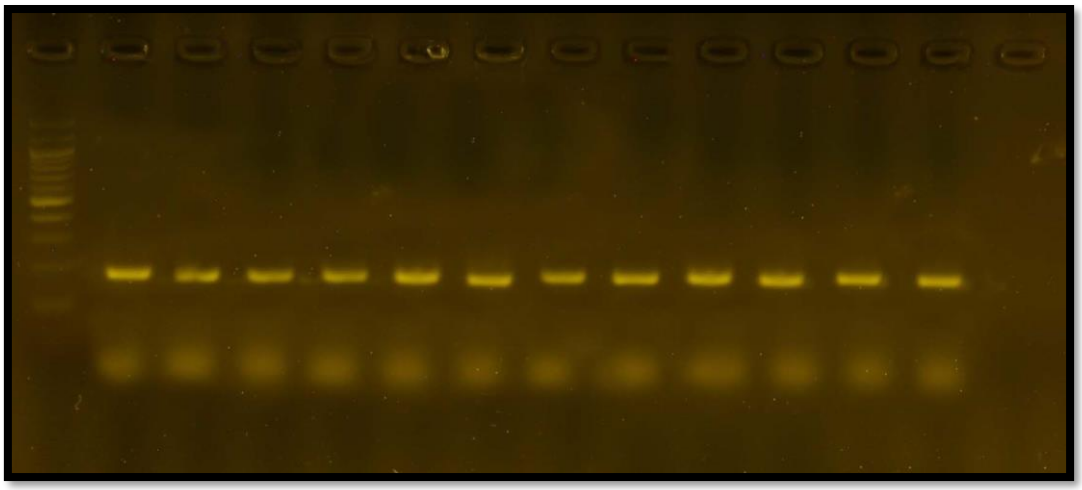

Figure 7: The PCR product of exon number 6 of the *TP53* gene shows the result of the reaction 180 bp, M is the volume guide with a size of 100 bp, which was prepared by Biolabs and separated by 2% agarose gel

The results of a sequencing test for the amplified gene's exons revealed that exons 3, 4, and 6 had differences in a number of nucleotides, however exon 5 had no differences. These findings are depicted in the following figures:

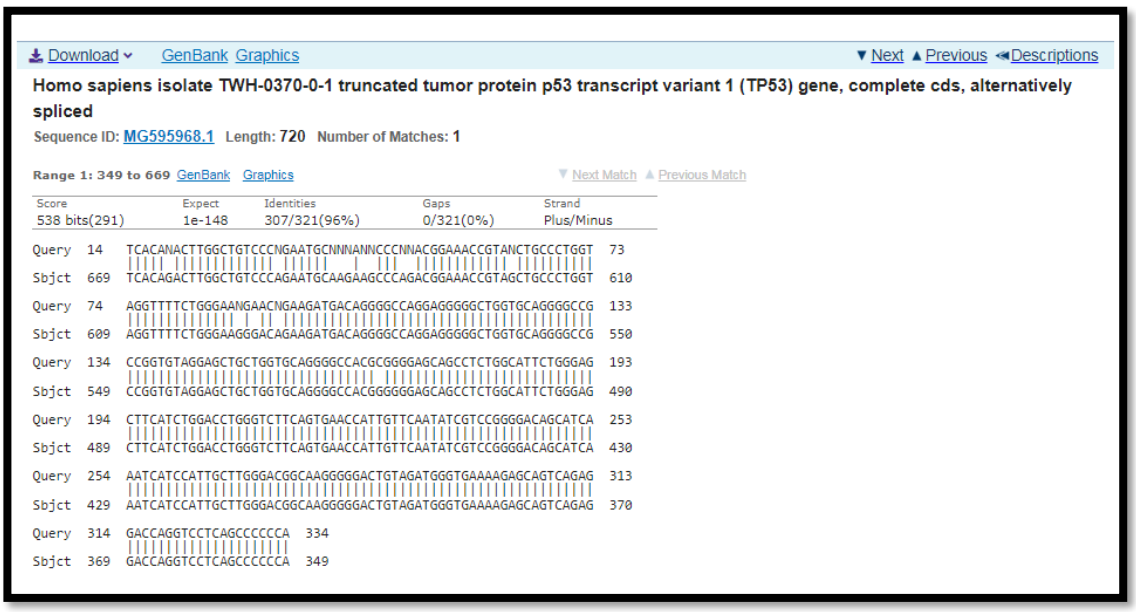

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Figure 8: The result of matching with the nucleotide sequence of exon 3 of the *TP53* gene was compared with the sequences of the original gene at the NCBI site

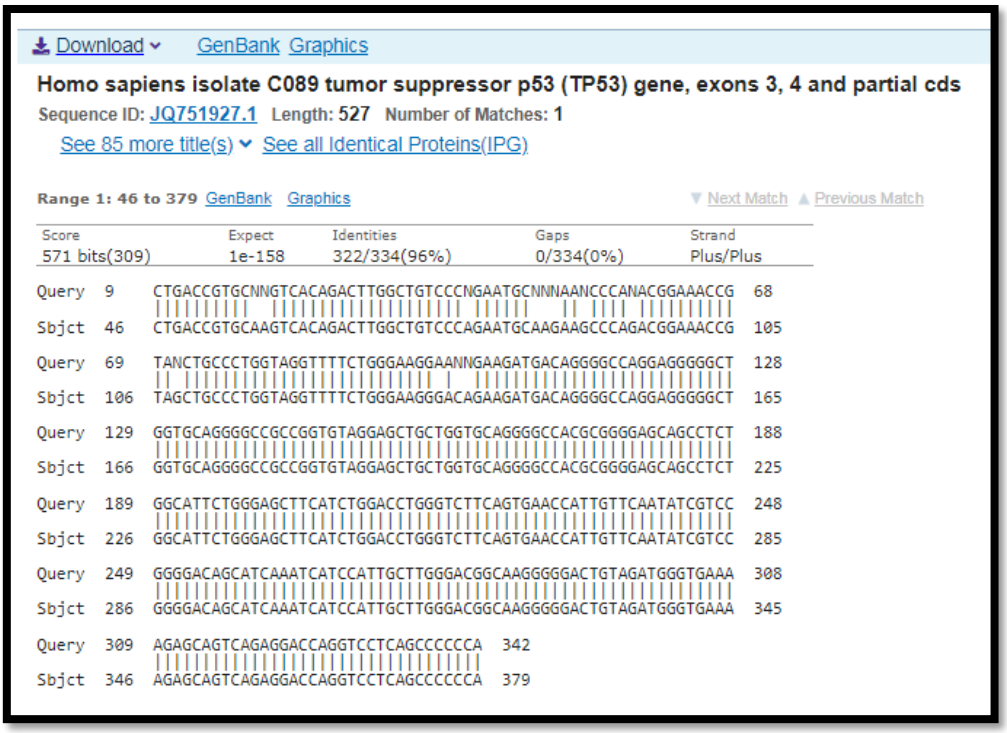

Figure 9: The result of matching with the nucleotide sequence of exon 4 of the *TP53* gene was compared with the sequences of the original gene at the NCBI site

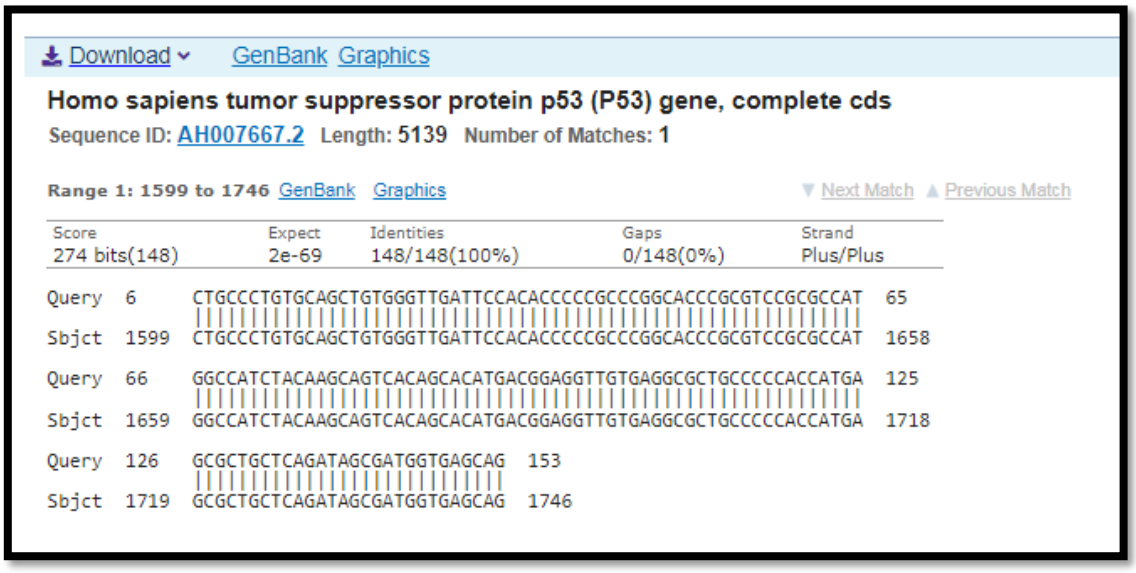

Figure 10: shows the result of matching with the nucleotide sequence of exon 5 of the *TP53* gene that was compared with the sequences of the original gene at the NCBI site

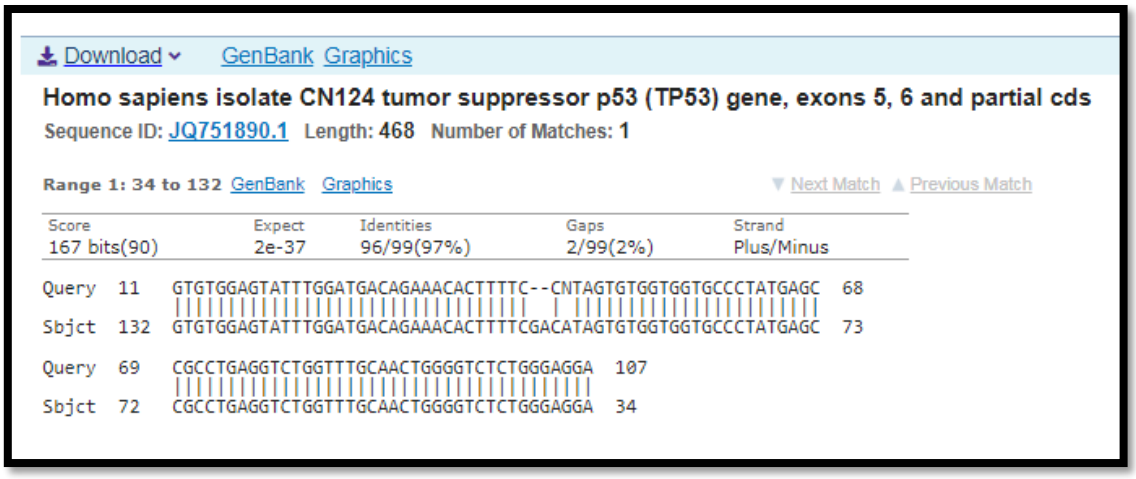

Figure 11: shows the result of matching with the nucleotide sequence of exon 6 of the *TP53* gene that was compared with the sequences of the original gene at the NCBI site

Table 8: illustrates the locations and kinds of alterations in the *TP53* gene in breast cancer patients

Exon	ID sequince	Nucliotide	Location	Mutation type	Idendity	Gaps
(3)	(JQ751927.1)	A → G	(135)	(Transition)	(96%)	(0)
(3)	(JQ752213.1)	A → G	(138)	(Transition)	(97%)	(0)
(4)	(JQ752208.1)	G - → -	(90)	(Deletion)	(98%)	(1)
(4)	(JQ752208.1)	G → C	(216)	(Transversion)	(98%)	(1)
(4)	(MG595968.1)	G → A	(623)	(Transition)	(96%)	(0)
(4)	(MG595968.1)	G → C	(580)	(Transversion)	(96%)	(0)
(5)	(AH007665.2)	C - → -	(1615)	(Deletion)	(99%)	(1)
(5)	(JQ751838.1)	T - → -	(407)	(Deletion)	(99%)	(1)
(6)	(HM236157.1)	.-- → N	(354)	(Adition)	(99%)	(1)
(6)	(JQ751846.1)	→ N	(170)	(Adition)	(96%)	(1)
(6)	(JQ751890.1)	G → --	(171)	(Deletion)	(97%)	(2)
(6)	(JQ751890.1)	A → --	(172)	(Deletion)	(97%)	(2)

When the table 8 is observed, it shows the different types of genetic variations and their locations on the exons of the *TP53* gene after conducting a sequencing test and comparing them with the gene sequences at the NCBI site.

According to research, the *TP53* gene is also polymorphic, with single nucleotide polymorphisms (SNPs) occurring when one single nucleotide is changed by another and being the most prevalent sort of mutation in DNA (Doffe et al., 2021). Loss of p53 function predisposes cells to malignant mutations and may result in genomic instability. P53 inactivation arises mostly through point mutations, however gene

deletions or nucleotide insertions have also been observed (Kim and Lozano, 2018). These alterations may impair p53 function, increasing the risk of cancer or impairing therapy response.

IV. CONCLUSION

According to this study, the observation of different genotypes and allelic frequency of G $\longrightarrow$ C and C $\longrightarrow$ G polymorphism in exon 7 and exon 4 sequentially for *TP53* gene present significant variation between patient with Breast cancer and healthy group.

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