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Mutation of P53 Gene in Breast Cancer Patients in Nineveh Province

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Abstract:
p53, a gene that encodes a nuclear phosphoprotein with anticancer properties, is one of the most researched genes. Mutations in this gene typically occur in its most conserved regions, and these mutations can impair the protein's ability to regulate cell growth. Although p53 mutations have been identified in a small percentage of primary breast carcinomas, their overall prevalence remains unknown. According to existing evidence, the risk of developing breast cancer has been linked to genetic and socioeconomic factors. Patterns of p53 mutations in breast cancer have been found to vary by geographic region, possibly influenced by factors such as race and environment. Tissue samples were collected from 63 female breast cancer patients at the Oncology and Nuclear Medicine Hospital in Mosul City, Nineveh Governorate. A questionnaire was also conducted with patients who tested positive a few months after their diagnosis. Data on potential and established risk factors for breast cancer were analyzed, including lifetime alcohol consumption, menstruation and childbirth history, hormonal use, adult body size, past medical history, and family history of breast cancer.DNA was extracted from the tissue samples, and the PCR technique was used to determine whether a mutation in the p53 gene was present, using specific primers. Sequencing and histological examination techniques were then performed on the samples. Fifteen percent of these mutations showed more than one alteration, and p53 overexpression was observed.Of the tumor samples we sequenced, 30 (48%) also exhibited mutations in the p53 gene. We identified two significant changes in exon 4 of p53, and our population-centered sample of breast cancer patients revealed an association between the p53 gene and the prevalence of p53 mutations in tumor tissues. Our findings led us to conclude that the ratio of breast cancer to population size may decrease after accounting for factors such as endocrine status, alcohol use, drug use, and lifestyle changes.
Keywords: *p53 Gene Mutation, Breast Cancer Patients*

الطفرة الحاصلة في جين 53P لمرضى سرطان الثدي في محافظة نينوى

الخلاصة
يعد الجين 53p، وهو الجين الذي يشفر البروتين الفوسفوري النووي المضاد للسرطان، أحد الجينات الأكثر بحثًا. تميل الطفرات إلى التجمع في الأجزاء الأكثر حفظًا من الجين، ويمكن أن تؤثر على قدرة البروتين في تنظيم تطور الخلايا. على الرغم من العثور على طفرات 53p بنسبة صغيرة في سرطانات الثدي الأولية، إلا أن انتشارها لا يزال غير معروف. وفقا للأدلة المتاحة، تم ربط خطر الإصابة بسرطان الثدي بالوراثة والحالة الاجتماعية والاقتصادية. وجد أن أنماط طفرات سرطان الثدي 53p تختلف حسب المنطقة، والتي قد تتأثر بعوامل مثل العرق والبيئة. تم جمع عينات النسيج من 63 شخصا مصابين بسرطان الثدي في مستشفى الأورام والطب النووي في مدينة الموصل، محافظة نينوى. كما تم اجراء استبيان للمرضى الذين اثبتت اصابتهم بعد بضعة أشهر من التشخيص. تم تحليل البيانات المتعلقة بعوامل الخطر المحتملة والمثبتة لسرطان الثدي. وشملت هذه العوامل استهلاك الكحول مدى الحياة، , الحيض , الإنجاب , الاستخدام الهرموني، كتلة الجسم حسب عمر البالغين، الأحداث التاريخية الطبية السابقة، والتاريخ العائلي لسرطان الثدي. تم استخلاص عينات الدي ان اي من النسيج واجريت تقنية البي سي ار لاثبات وجود الطفرة في جين 53p من عدمها وذلك باستخدام بادئات خاصة من ثم اجريت تقنية تحديد التتابع و تقنية الفحص النسيجي للعينات، خمسة عشر بالمائة من هذه الطفرات كان لها أكثر من تغيير واحد كما تم العثور على زيادة في التعبير عن 53p. وأظهرت ثلاثون (48٪) من عينات الورم التي قمنا بتسلسلها طفرات في الجين 53p. لقد وجدنا تعديلين مهمين في الاكسون 4 لـ 53p وكشفت العينة التي تركز على السكان من مرضى سرطان الثدي عن وجود علاقة بين الجين 53p وانتشار طفرات 53p في أنسجة الورم. قادنا هذا إلى استنتاج أن نسبة سرطان الثدي إلى حجم السكان ستنخفض بعد التحكم في وضع الغدد الصماء، وتعاطي الكحول، وتعاطي المخدرات الأخرى فضلا عن تغيير نمط الحياة.

INTRODUCTION

One of the worldwide dangerous and global health concern is breast cancer where incidence rates is high in the area like Nineveh Province, Iraq [1]. Molecular genetic pathways involved in breast carcinoma have been greatly studied in an attempt to link the disease to its progression over the last decade. The p53 gene, a tumor suppressing gene, and a regulator of growth of cell, is one of the most studied genes in this context [2]. A number of mutations in p53 gene, especially in the highly

conserved regions of p53 gene, have been detected in a number of cancers such as breast cancer [3]. These mutations can also make it difficult for the protein to regulate cell growth and therefore hinder uncontrolled tumor development [4].Around the world, breast cancer is the most common cancer in women and about 10% of American women by age 75 are diagnosed [5]. It has been shown by research that there are both genetic and environmental factors involved in breast cancer risk. This can, in fact, be exemplified by an increased risk of genetic mutations of certain types of breast cancer with cigarette smoking [6]. Moreover, the p53 mutations pattern is different among different ethnic groups: it is more frequent in some mutations in African American women, than in Caucasian women [7]. A crude understanding of these differences is crucial for the development of specific treatments and better patient outcomes.Breast cancer is highly prevalent in Iraq and especially in Nineveh Province, so it urgently has to be analyzed genetically in detail, to improve early detection, and as treatment strategies [8]. The p53 gene is very important in maintaining genomic stability in a way that prevents cellular transformation and carcinogenesis through DNA repair processes and apoptosis [9]. Common mutations of the p53 genes have been found in many human malignancies, such as breast cancer, rendering the gene inactive and thus unable to regulate the growth of tumor [10]. According to studies, the incidence of p53 mutations in breast cancer ranged from 17% – 45%, [11] reflecting the variations based on location (geographical) and ethnicity. In addition, these mutations have been associated with aggressive tumor behavior, poor prognosis and lower patient survival [12].The need to know the frequency and types of p53 mutations in the breast cancer patients in Nineveh Province is very crucial. Secondly, this information reveals key information about the genetic make up of breast cancer in this part of the world, a tool that can be used in the development of even better targeted, more specific treatments [13]. Second, some p53 mutations can become prognostic markers by assisting the prediction of progression and outcomes of the disease in patients [14]. Finally, [15] this information can be useful to public health programmes on genetic counseling, risk assessment and early diagnosis for people at risk of breast cancer.The association between p53 mutations and a number of factors have been considered in previous research, including genetic syndromes such as Li-Fraumeni syndrome, which was inherited and resulted in p53 mutations [16]. [P] The role of environmental factors, e.g. UV radiation and contaminants of cigarette smoke and alcoholic beverages in P53 mutation triggering [17] [18] has also been studied. The common method to identify mutant p53 proteins data about protein expressions [19][20] is immunohistochemical staining. In addition, in spite of conflictionary reports, the association between p53 mutations and survival has not been correlated in several malignancies [21][22]. Additional research is evidently needed to determine the significance of p53 mutations in terms of predictive value in prognosis of breast cancer [23].

OBJECTIVE

The aim of the study is to study the range and frequency of p53 mutations in breast cancer in patients treated in Nineveh Province. Molecular techniques will be used to goal for p53 gene alterations in tumor samples from proven cases of breast cancer. The findings from this study will help us better understand how p53 functions in the development of breast in this population and pave the path for implementing more potent therapeutic and diagnostic strategies for the benefit of patients with breast cancer in Nineveh Province.

METHODOLOGY

Subject Selection

The study selected patients with a mean age of 53°t9 (between 30 and 70 years old) with an invasive ductal carcinoma diagnosis. The study consisted of 57 females, 6 males, and 63 total patients. For the female participants, the mean age was 52±9 years and 56±8 years for the male participants. The study included both male and female cases, and the results are analyzed separately in the Results section for the purpose of taking account of physiological differences between gender. All of our patients were selected from Oncology and Nuclear Medicine Hospital and Mosul City, with the collection of full medical history from

each of the participants. The study did not include patients who were receiving cancer treatments such as chemotherapy or radiation. The Institutional Review Board (IRB) approval was received for the study.

Control Group

All patients had control biopsies taken from tissue at the side of the tumor so that a baseline could be established for analysis. 30 control samples were collected in total, guaranteeing the representative samples of a general population.

Data Collection

The mastectomy surgeries provided tumor biopsies. Further analysis of the samples was preserved in phosphate buffer saline. With a sterile disposable scalpel each tumor was bisected. A portion of the viable tissue from 1 half was stored for DNA extraction and sequencing, the rest of the half was sliced evely and fixed in 10% formalin for histology. Viability of DNA was preserved through storage of the tumor tissue sample at -80°C.

DNA Isolation

A well-established method [31] was used to extract genomic DNA from the breast tumor tissue by proteinase K digestion, phenol chloroform extraction, ethanol precipitation. The DNA concentration and purity was also determined using the standard spectrophotometric techniques and a 1% agarose gel, stained with ethidium bromide.

Polymerase Chain Reaction (PCR)

PCR amplification of DNA from the tumor samples was done using traditional protocol." The ingredients of the mix used were, 5.0 µl of 10× PCR buffer, 3.0 il of 25 mM MgCl2, 0.5 il of 10 mM dNTPs, 0.5 µl of 10 pmol forward primer, 0.5 µl of 10 pmol reverse primer, 0.5 il Taq DNA polymerase (1 U µL-1) added to the mixture containing 100–200 ng DNA and autoclaved distilled water to a final of 50 µl. The primers (Table 1) used for this amplification 'were for exonic regions 4–8 of the p53 gene.

Amplification Primers for p53 Exons 4-8

p53 Exon	Primer Sequence F= forward sequence R= reverse sequence
4	F: TGACTGCTCTTTTCACCCAT R: GGAAGCCAGCCCCTCAGGGC
5	F: TGTTCACTTGTGCCCTGACT R: CAGCCCTGTCGTCTCTCCAG
6	F: GCCTCTGATTCCTCACTGAT R: TTAACCCCTCCTCCCAGAGA
7	F: ACTGGCCTCATCTTGGGCCT R: TGTGCAGGGTGGCAAGTGGC
8	F: TAAATGGGACAGGTAGGACC R: TCCACCGCTTCTTGTCCTGC

Table 1: Amplification primers for exons 4–8 of the p53 gene.

Single-Strand Conformation Polymorphism (SSCP) Analysis

DNA from PCR products was analyzed for single-strand conformation polymorphism (SSCP). Denaturing was performed by mixing 4±1.2 mL of PCR product with 1 mL of a denaturation solution composed of 800 µL formamide, 100 µL of 1% Bromophenol Blue, 100 µL of 1% xylene cyanol, 2 µL of 0.5 M EDTA, and 1 µL of 10 M NaOH. The mixture was denatured at 93°C for 5 minutes and immediately cooled in ice water. A 10% polyacrylamide gel with a 0.5 mm thickness was prepared, and the samples were electrophoresed in 1X TBE buffer at 4°C using 19 milliamperes. Multiple electrophoresis runs were performed to confirm reproducibility and avoid false positives. Bands were visualized via silver staining, following the method described by Bourn et al. [32].

Sequencing

PCR products showing mobility shifts on SSCP, along with randomly selected samples, were purified and submitted for sequencing. Sequencing was performed using ABI's AmpliTaq FS dye terminator cycle Sanger sequencing chemistry on an ABI 3100 Genetic Analyzer (Bangalore Genei).

Histological Sample Processing

Tumor slices preserved in paraffin were mounted on poly-L-lysine-coated slides, dewaxed in xylene, and rehydrated in ethanol and distilled water. Antigen retrieval was enhanced via microwave treatment at 750W for two 10-minute sessions. Immunohistochemistry (IHC) was used to assess the presence of p53, Estrogen Receptor (ER), Progesterone Receptor (PR), and HER2 (Human Epidermal Growth Factor Receptor-2) in the samples. Staining intensity and extent were graded on a scale from 1 to 3, and Allred scoring was used for p53, ER, and PR, while the Hercep Test was applied for HER2 expression. Primary antibodies and the HRP detection system were purchased from Biogenex (USA). A pathologist evaluated and graded all slides under a microscope.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, Version 16. Chi-square tests were used to compare categorical data between groups, with statistical significance set at a p-value ≤ 0.05. Detailed statistical results, including p-values and confidence intervals, are presented in the Results section.

RESULTS

The clinical and pathological characteristics of the 63 breast cancer patients in the study are presented in Table 2. Patients were aged 53±9 years. Among the majority of patients (76%) the tumor stage was T2 and 48% of tumours consisted of p53 mutations. Statistical analysis in detail revealed significant associations of p53 mutations, and certain clinical features â" age, and menopausal status.

Table 2: Clinical and Pathological Characteristics of Breast Cancer Sufferers

Parameters	N (Value %)	Females (N=57)	Males (N=6)
Breast Side			
Left	30/63 (47.6%)	27/57 (47.4%)	3/6 (50.0%)
Right	33/63 (52.4%)	30/57 (52.6%)	3/6 (50.0%)
Menstrual Status			
Premenopausal	20/57 (35.1%)	20/57 (35.1%)	n/a
Postmenopausal	37/57 (64.9%)	37/57 (64.9%)	n/a
Age at Menarche			
≤14	23/57 (40.4%)	23/57 (40.4%)	n/a
>14	34/57 (59.6%)	34/57 (59.6%)	n/a
Parity			
Nulliparous	10/57 (17.5%)	10/57 (17.5%)	n/a
1-3 Children	30/57 (52.6%)	30/57 (52.6%)	n/a
>3 Children	17/57 (29.8%)	17/57 (29.8%)	n/a
Lifetime Duration of Breastfeeding			
<35 months	25/57 (43.9%)	25/57 (43.9%)	n/a
35-55 months	11/57 (19.3%)	11/57 (19.3%)	n/a
56-79 months	9/57 (15.8%)	9/57 (15.8%)	n/a
>79 months	12/57 (21.1%)	12/57 (21.1%)	n/a
Paget's Disease			
Yes	6/63 (9.5%)	5/57 (8.8%)	1/6 (16.7%)
No	57/63 (90.5%)	52/57 (91.2%)	5/6 (83.3%)
Tumor Stage			
T2	48/63 (76.2%)	43/57 (75.4%)	5/6 (83.3%)
T3	15/63 (23.8%)	14/57 (24.6%)	1/6 (16.7%)
T4	0/63 (0%)	0/57 (0%)	0/6 (0%)
Histological Grade			
G I	0/63 (0%)	0/57 (0%)	0/6 (0%)
G II	45/63 (71.4%)	43/57 (75.4%)	2/6 (33.3%)
G III	18/63 (28.6%)	14/57 (24.6%)	4/6 (66.7%)

No. of Axillary Lymph Nodes			
0	19/63 (30.2%)	18/57 (31.6%)	1/6 (16.7%)
1-4	27/63 (42.9%)	25/57 (43.9%)	2/6 (33.3%)
>4	17/63 (27.0%)	14/57 (24.6%)	3/6 (50.0%)

Distribution of p53 Mutations by Age and Gender

Table 3 presents a detailed overview of the distribution of patients with and without p53 mutations, stratified by age groups and sex. The analysis reveals significant insights into the presence of p53 mutations in relation to the patients' age.

p53 IHC Status by Age Group and Gender

Age Group	p53 Status	Total Count	Females Count	Females %	Males Count	Males %	Total %
≤51	Positive	18	16	88.89%	2	11.11%	100%
	Negative	17	15	88.24%	2	11.76%	100%
	Total	**35**	**31**	**88.57%**	**4**	**11.43%**	**100%**
>51	Positive	12	11	91.67%	1	8.33%	100%
	Negative	16	15	93.75%	1	6.25%	100%
	Total	**28**	**26**	**92.86%**	**2**	**7.14%**	**100%**
Overall	**Total**	**63**	**57**	**90.48%**	**6**	**9.52%**	**100%**

Table 3: Distribution of Patients with and without p53 Mutations by Age

Age Group ≤51 Years:

In the age group of 51 years or younger, a total of 35 patients were assessed. Among them, 18 were identified as p53 positive, comprising 16 females and 2 males. This reflects an 88.89% proportion of females among the positive cases and an 11.11% proportion of males. Conversely, 17 patients were p53 negative, consisting of 15 females and 2 males, resulting in an 88.24% proportion of females and 11.76% of males within the negative cohort. Overall, the total distribution in this age group shows 88.57% females and 11.43% males among all p53 cases, indicating a higher prevalence of p53 mutations in females.

Age Group >51 Years:

For patients over the age of 51, the total number of assessed individuals was 28. Among them, 12 were p53 positive, comprising 11 females and 1 male. This translates to a remarkable 91.67% of females among the positive cases, with males constituting only 8.33%. In the negative category, 16 patients were identified, with 15 females and 1 male, leading to an 93.75% proportion of females and 6.25% of males. The total percentages indicate that in this age group, females account for 92.86% while males comprise 7.14% of the overall p53 status.

Combining both age groups, the overall total includes 63 patients, of which 57 were females and 6 were males. This results in a 90.48% representation of females and 9.52% representation of males within the total patient population. These results underscore the gender disparities in p53 mutations, with females showing a consistently higher prevalence across all age groups.

Figure 1 illustrates the immunohistochemical labeling of key receptors in breast cancer tissue samples. The staining for estrogen receptor (ER, 1a), progesterone receptor (PR, 1b), and human epidermal growth factor receptor 2 (HER2, c2) was performed at a magnification of 400X. The patterns observed in these receptors provide further context for understanding the biological implications of p53 mutations in the patients studied.

Immunohistochemical Labelling (Figure 1)

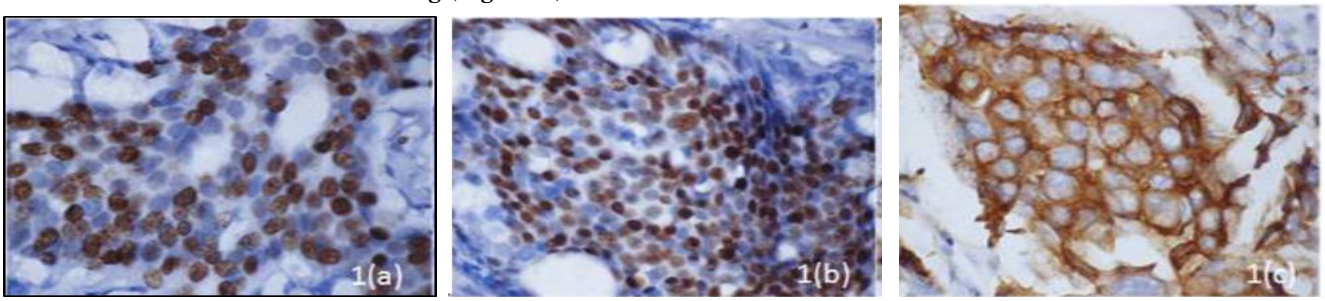


Figure: 1: immunohistochemical labeling of estrogen receptor (ER, 1a), progesterone receptor (PR, 1b), and human epidermal growth factor receptor 2 (HER2, c2) was performed at 400X.

As shown in Table 3, gender and prevalence of p53 mutations also show a marked correlation, female prevalence of p53 mutations was significantly higher than male prevalence in both age groups. The need for further examination of these mutations in relation to breast cancer pathology and treatment strategies is demonstrated by this distribution. Figure 1 supports these findings by showing the immunohistochemical analysis of the patients' tumors' receptor profiles, which provide an insight into breast cancer biology.

Analysis of p53 Mutations in Breast Cancer Patients

Table 3 summarizes the detail analyses of p53 mutations in exon 4 which contains vital mutations accountable for occurrence of breast cancer. This table packs important parameters including, the number of codons, the nucleotide position, change of base, change of amino acid and the types of mutations found. All together, 33 patients were studied, and they were almost all (30) females and few (3) males. The analysis also demonstrated two important mutations among these patients; a nonsense mutation at codon 107, which causes a stop codon and a missense mutation at codon 72, which brings about an amino acid change from proline (Pro) to arginine (Arg). Particularly, the nonsense mutation was detected in 2 females and 1 male, and the missense mutation in 28 females and 2 males.

In spite of this, this gender-based breakdown points out on the prevalence of p53 mutations in the studied population and puts the importance of taking gender into consideration during mutation analysis. The study emphasizes the need for sequencing and complementary single-strand conformation polymorphism (SSCP) analysis in order to guarantee that the data obtained are accurate. Importantly, there was no cross between any of the patient samples to the equivalent control biopsies in exons 5, 6, 7 or 8. Further finding the sequencing results are corroborated by the sequence SSCP analysis (Fig. 2), thereby validating the identified mutations.

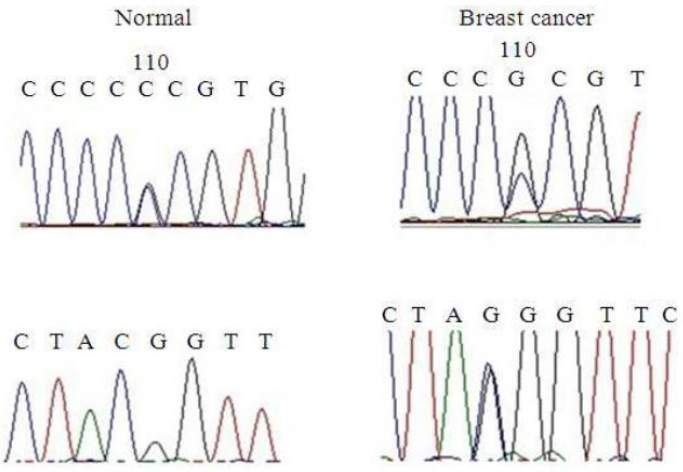


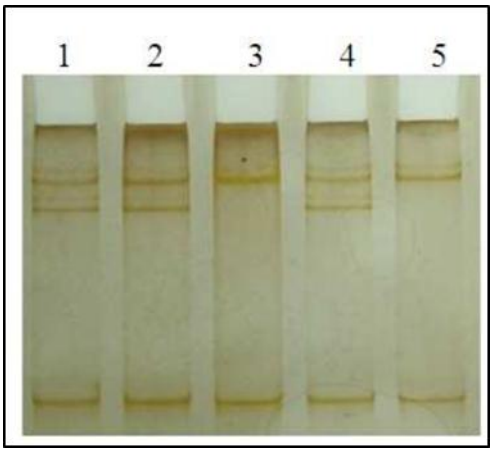
Figure 2: SSCP Analysis Results Corroborating p53 Sequencing Data

No. of Patients	Exon Containing Mutations	Codon Number	Nucleotide Number	Base Change	Amino Acid Change	Mutation Types	Females Count	Males Count
3	4	107	1149	TAC → TAG	Tyr → Stop	Nonsense	2	1
30	4	72	1138	CCC → CGC	Pro → Arg	Missense	28	2
Total							30	3

Table 4: The types of p53 mutations seen in individuals with sporadic breast cancer

It was discovered that a nonsense mutation at codon 107 accompanied the missense mutation at codon 72. PCR-SSCP analysis was utilized as a screening method to look for useful mutations of the p53 gene. The following accession numbers were acquired after submitting both the altered and wild-type sequences to NCBI:

This table presents the p53 gene mutations identified in breast cancer patients, along with the corresponding accession numbers submitted to NCBI for both the wild-type and mutated sequences. The table details the exon affected by the mutation, the specific BankIt ID associated with each submission, the sequence ID, and the unique accession number assigned by NCBI for reference. Notably, exon 4 exhibits both wild-type and multiple mutations (mutation A and mutation B), while exons 5 through 8 only reflect the wild-type sequences, as no mutations were detected in these regions.



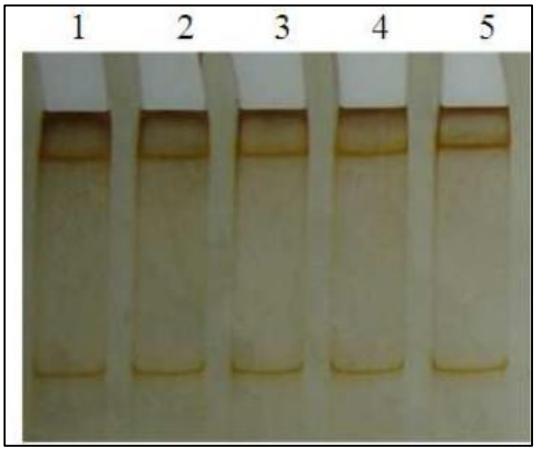


Table 5: p53 Gene Mutations and Corresponding Accession Numbers in Breast Cancer Patients

Electrophoretic Mobility Shift of p53 Exon 4 in Tumor Samples

Mutations in the p53 gene's exon 4 were analyzed using electrophoretic mobility shift assays. Figure 3 illustrates the comparison between tumor samples and their corresponding normal biopsies on a 10% polyacrylamide gel. Genetic abnormalities are visibly apparent in tumor samples 1, 2, and 4, showing a clear shift in mobility, which indicates the presence of mutations in exon 4 of the p53 gene. These shifts provide further validation of the mutations identified through sequencing analysis

Tumor biopsy Control biopsy

Figure 3: Electrophoretic Mobility Shift of p53 Exon 4 in Tumor Samples

The data presented in the tables illustrate a compelling relationship between clinical features and the prevalence of p53 mutations, with notable distinctions based on gender and age. In the analysis of age, individuals aged ≤51 years represent 36.7% of the p53 mutant population, with a slight majority being females (37.0%) compared to males (33.3%). This indicates that younger women may have a higher susceptibility to p53 mutations in comparison to their male counterparts. On the other hand, those over 51 years constitute a significant 63.3% of the cohort, suggesting that p53 mutations may become more prevalent with advancing age. Interestingly, the gender distribution remains relatively balanced in this older age group, with females at 63.0% and males at 66.7%, indicating that age is a critical factor influencing mutation occurrence in both sexes.

Table 6: Female-Male Distribution of Clinical Features in p53 Mutants

Clinical Features	Total (N=30)	Females (N=27)	Males (N=3)
Age (years)			
≤51	11/30 (36.7%)	10/27 (37.0%)	1/3 (33.3%)
>49	19/30 (63.3%)	17/27 (63.0%)	2/3 (66.7%)
Residence			
Rural	4/30 (13.3%)	3/27 (11.1%)	1/3 (33.3%)
Urban	26/30 (86.7%)	24/27 (88.9%)	2/3 (66.7%)
Menopausal Status			
Pre	3/26 (11.5%)	3/27 (11.1%)	n/a

Post	23/26 (88.5%)	24/27 (88.9%)	n/a
Breast Involved			
Left	11/30 (36.7%)	10/27 (37.0%)	1/3 (33.3%)
Right	19/30 (63.3%)	17/27 (63.0%)	2/3 (66.7%)
Provisional Diagnosis			
IDC	30/30 (100%)	27/27 (100%)	3/3 (100%)
Paget's Diseases	3/30 (10%)	2/27 (7.4%)	1/3 (33.3%)
Lymph Nodes			
Yes	20/30 (66.7%)	18/27 (66.7%)	2/3 (66.7%)
No	10/30 (33.3%)	9/27 (33.3%)	1/3 (33.3%)
Histological Grades			
G I	0/30 (0%)	0/27 (0%)	0/3 (0%)
G II	19/30 (63.3%)	18/27 (66.7%)	1/3 (33.3%)
G III	11/30 (36.7%)	9/27 (33.3%)	2/3 (66.7%)
Tumor Stage			
T2	0/30 (0%)	0/27 (0%)	0/3 (0%)
T3	18/30 (60%)	16/27 (59.3%)	2/3 (66.7%)
T4	12/30 (40%)	11/27 (40.7%)	1/3 (33.3%)

The patterns of residence disclose that 86.7% of patients live in urban, with 88.9% of these being urban females. The presence of p53 mutations in these urban specimens may imply that the environmental and lifestyle characteristic of an urban life style can enhance the development of such mutations.

Further depth is added to the analysis by menopausal status, 11.5% of p53 mutants are pre-menopausal where all of them are female. Conversely, 88.5% are post menopausal suggesting the hormonal changes may have a significant role in p53 mutation expression. This relationship also serves as an indication of the relation between p53 mutations and an increased incidence in older women.

The situation with breast involvement is instructive as in the 36.7% cases it involved the left breast while in 63.3% the right breast, showing a minor bias for right-sided tumor development in the case of p53 mutants. The pattern may simply represent underlying biological factors or anatomical predispositions for tumor development.The provisional diagnoses are that all patients have Invasive Ductal Carcinoma (IDC), and this confirms the idea that p53 mutations are linked with this particularly aggressive subtype of breast cancer. The presence of Paget's disease in 10% of cases, particularly among males, underscores the complexity of breast cancer manifestations in relation to p53 mutations.Positive lymph node involvement is another main issue as 66.7% of patients have positive status for regional lymph nodes (a clear sign of aggressive p53 mutant tumors with high potential to metastasize). This finding is not gender specific, indicating that p53 mutations increase the risk of lymphatic spread universally. Histologically, the distribution of grades reveals that Grade II tumors are the most common at 63.3%, while Grade III tumors account for 36.7%. The absence of Grade I tumors among p53 mutants may indicate that these mutations are linked to more aggressive tumor characteristics. Regarding tumor staging, the data show no cases classified as T2, while T3 and T4 stages account for 60% and 40%, respectively. This suggests that p53 mutations are associated with more advanced tumor stages at diagnosis, which may impact prognosis and treatment options.

p53 IHC Status by Age Group and Gender

The analysis presented in Table 7 reveals a significant relationship between p53 expression and patient age groups, with noteworthy distinctions between genders. The total count of patients with p53 expression was 63, with a breakdown of 35 females and 28 males.

In the younger age group (≤51 years), 88.57% of females exhibited Negative p53 status, while only 5.71% of males fell into the same category. This indicates a markedly higher prevalence of p53 expression among females, particularly as 28.57% showed a +3 expression level, while no males exhibited this level of expression. The prevalence of p53 status among females in this age group was 55.56% overall. In contrast, the older age group (>51 years) had a higher percentage of females (53.57%) showing Negative p53 status compared to males (3.57%). However, 21.43% of females displayed a +1 expression, emphasizing the increasing complexity of p53 expression patterns with age. Overall, 92.86% of females had some level of

p53 expression compared to 7.14% of males, with a total prevalence of p53 expression among females being 57 out of 63 (90.48%) and males at 6 out of 63 (9.52%).

The immunohistochemical (IHC) analysis of p53 expression across age and gender groups further emphasizes the relationship between age and gender and mutation prevalence.

Moreover, a substantial relationship was identified between menstrual status and the expression of p53, with a p-value of 0.031, as well as with HER2 expression, which had a p-value of 0.001. These findings underscore the significance of both age and gender in the prevalence of p53 expression among patients, providing critical insights into the factors influencing breast cancer pathology.

Table 7: A Statistical analysis of the prevalence of p53 expression across patient age groups and gender

Age Group	p53 Status	Total Count (n)	Females Count (n)	Females %	Males Count (n)	Males %	Total %
≤51	Negative	17/63	15/35	42.86%	2/35	5.71%	26.98%
	+1	3/63	2/35	5.71%	1/35	2.86%	4.76%
	+2	5/63	4/35	11.43%	1/35	2.86%	7.94%
	+3	10/63	10/35	28.57%	0/35	0.00%	15.87%
	Total	**35**	**31**	**88.57%**	**4**	**11.43%**	**55.56%**
>51	Negative	16/63	15/28	53.57%	1/28	3.57%	25.40%
	+1	7/63	6/28	21.43%	1/28	3.57%	11.11%
	+2	2/63	2/28	7.14%	0/28	0.00%	3.17%
	+3	3/63	3/28	10.71%	0/28	0.00%	4.76%
	Total	**28**	**26**	**92.86%**	**2**	**7.14%**	**44.44%**
Overall	**Total**	**63**	**57**	**57/63 (90.48%)**	**6/63 (9.52%)**	**100%**	**100%**

The clinical characteristics of p53 mutants are outlined in Table 6, which also examines variables like age, gender, place of residence, menopausal status, breast involvement, tentative diagnosis, Paget's diseases, lymph nodes, histological grades, and tumor stage. The detailed examination of this data adds to a more sophisticated comprehension of the many clinical presentations linked to p53 mutations.

Problems Addressed

- Finding and characterizing p53 mutations in patients with breast cancer.
- Correlation between clinicopathological markers and positive for p53 mutations.
- Investigation of p53 protein expression using immunohistochemistry.
- Identification and examination of certain mutations at codon locations 107 and 72.
- Investigation of the connections between HER2 status, age, gender, and p53 expression.

Researchers in the area of cancer biology have focused on p53, a well-established tumor suppressor gene, because of its capacity to control multiple key physiological processes, including cell cycle arrest, senescence, apoptosis, DNA repair, and angiogenesis [33]. Researchers have looked at the role of p53 in human breast cancer extensively [34]. Mutations, with or without Loss of Heterozygosity (LOH), were discovered early in cell lines and primary breast tumors, validating p53's role as a tumor suppressor gene in breast cancer. There is a wealth of data about breast cancer in the West thanks to epidemiological research [35], but less is known about the disease in Nineveh. Nineveh is a large subcontinent home to many people of different races, cultures, and ethnicities. Breast cancer epidemiology, risk factors, and susceptibility are topics addressed in various ways across groups and locations. Different regions of Nineveh have studied the correlation between p53 expression and breast cancer risk [36]. This study expands previous research conducted in India on sequence aberration in the p53 gene and its protein expression and linkage with demographic and clinical results in a population from the central and northeastern parts of the country. There were 63 people in our research diagnosed with invasive ductal carcinoma of the breast. Consistent with previous results in the literature ("Pathohistological and Immunohistochemical Analysis of Primary Invasive Ductal Breast Carcinoma With Signet-Ring Cell Differentiation- Differential Diagnosis, Prognosis, and Complex Treatment," 2021), we detected a substantial association between the p53 mutation and gender, postmenopausal status, histological grade, and ALN involvement. Thirty (48%) of the tumor specimens we sequenced showed mutations in the p53 gene. We found two important alterations in p53's exonic region 4 (Figs. 2 and 3). It has been found that

the missense mutation at codon 72 (pro to Arg) is not equally distributed across all populations [37]. Around 50% of our breast cancer patients have this mutation. Cancer risk may be increased in individuals with the p53 Arg homozygous genotype, as has been shown in previous studies [38]. Furthermore, increased expression of the p53 protein was detected by immunohistochemistry examination, which coincided with our mutation results (Fig. 4). The p53 protein is constantly being made and broken down in human cells. MDM2 binding is linked to p53 protein degradation. In a self-reinforcing cycle, the p53 protein induces MDM2 [39]. The mutant p53 protein, on the other hand, is frequently able to accumulate at very high quantities and hence identifiable by IHC since it does not activate MDM2. We discovered an additional unique nonsense mutation leading to a stop codon at position 107. Despite the low frequency with which this mutation occurs, we were unable to detect p53 protein expression by histological examination, which is consistent with the translation of shortened p53 protein and, in turn, protein breakdown [40].

Limitations of Research

Selection bias and sample size: With only 63 participants, the study's sample size may be too small to make firm conclusions from, and the results could not be entirely typical of the general public. Furthermore, the sample was taken from a particular Mosul City hospital, which could introduce selection bias and limit the applicability of the findings to the population of Nineveh Province as a whole.Regional and Ethnic Distinctiveness: Patients with breast cancer from Iraq's Nineveh Province are the subject of the study. Different geographical areas and ethnic groups may have different genetic and environmental risk factors for breast cancer. As such, the results of the study could not be immediately applicable to people that originate from different ethnic backgrounds or different geographic areas.

Cross Sectional Design – Cross sectional study design takes data at one point in time. It becomes more challenging to determine what mutation caused a p53 mutation or how p53 mutations dynamically change over time. In longitudinal studies, one might obtain a more thorough knowledge of the temporal characteristics of p53 mutations in breast cancer progression.

Exclusion of Treated Patients

Those who were receiving radiation or chemotherapy because of cancer were not included in the analysis. It is unfortunate that this exclusion restricts the p53 alterations that can be studied in patients following or who have received cancer therapy; however, it may concentrate the analysis on untreated cases.The molecular subtypes of breast cancer, such as HER2/neu expression and hormone receptor status (ER, PR), are not explored in this study. Gaining knowledge of the relationship between particular subtypes and p53 mutations may offer more complex insights into the variability of breast cancer.

Limited Scope of p53 Analysis: The majority of the study is concerned with p53 mutations in exons 4 through 8. Thorough investigation of the whole p53 gene may identify more mutation hotspots, which could lead to a deeper comprehension of the genetic changes in breast cancer.

Immunohistochemical Ambiguity: The investigation uses immunohistochemical labeling to find the expression of the p53 protein. But uncertainty is introduced by the possible variation in staining techniques and interpretation. The consistency of immunohistochemical techniques may improve the accuracy of analyses of protein expression.

Aspects of the Environment and Lifestyle: Although nutrition and alcohol consumption are lifestyle factors that are linked to an increased risk of breast cancer, the study does not go into detail about these relationships. A more thorough examination of lifestyle decisions and environmental exposures could improve our knowledge of the aetiology of breast cancer in Nineveh Province.

Focus on a Single Gene: Much of the research is focused on p53 mutations. Breast cancer is a complex illness that is affected by different hereditary and environmental variables. A more complete comprehension of the genetic landscape may be gained by examining a wider range of genes associated to breast cancer.

Generalizability: the result of the study may not be externally valid outside of research setting. It is necessary to exercise cautions when interpreting these findings to different population and regional context.

Implications and Future Research

Despite having limitations, the findings of the study have important implication or future research and clinical practice. Knowing the incidence and types of p53 mutation in Nineveh Province breast cancer patient help in paving way for individualized and successful treatment approaches in addition to offering important insight into the genetic makeup of disease in a particular community. The discovery of specific mutation including codon position 72 and 107 encourages additional studies into the functional importance and possible effect on the course of the disease and outcome of the patients.

The relationship found between clinical pathological indicators, protein expression and p53 mutation highlight the need for further research to understand the complicated molecular pathways behind the breast cancer in different culture. Given the intricate relationship between genetic and environment factors, future research is necessary to broaden the scope of genetic studies and to include wider range of gene associated with breast cancer. Furthermore, examining the distinct pattern of mutation of molecular subtypes of breast cancer can improve our understanding of the heterogeneity of disease.

Clinically, the observed p53 mutation may be used as a prognostic indicator to help with risk evaluation and selection of treatment. To determine the clinical usefulness of these mutations as indicators of treatment response and disease progression, more validation research is necessary. The incorporation of genetic data into standard clinical practice may open the door to more individualized methods of managing breast cancer. The influence of lifestyle and environmental factors on the development of breast cancer in Nineveh Province should be considered in future research projects. More levels of complexity might be revealed by examining gene-environment interactions and how they affect the likelihood of developing a disease. Studies that monitor the changes in p53 mutations over time and in different stages of breast cancer may provide a dynamic viewpoint that improves our capacity to discover possible places of intervention and determine causal links.

CONCLUSION

Therefore, our findings suggest that the p53 gene may be used as a predictive marker in the early detection of breast cancer; however, further clinical and epidemiological data from the Nineveh and Asian countries is needed to support this assertion.

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