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The Impact of Herceptin on p53 Gene Expression and Biochemical Markers in Breast Cancer Patients and Healthy Individuals

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Abstract

Background: Breast cancer is a complex disease characterized by the abnormal growth of cells in the breast tissue. Gene p53 gene expression is a tumor suppressor gene that, when mutated, is associated with the development of hereditary breast cancers. In sporadic tumors, although inherent gene mutations are rare, the study aimed to assess the sensitivity and specificity of the Herceptin effect on serum renal function, liver function, and P53 gene expression in detecting disease recurrence in postoperative breast cancer patients.

Methods: A total of 120 female participants were enrolled in this study. The participants were divided into two groups: the experimental group, 60 breast cancer patients with HER2-positive tumors receiving Herceptin treatment, and 60 healthy females. Serum samples were collected from all participants. Biomarker levels and the expression levels of the p53 gene were assessed using quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Result: According to the demographic characteristics of patients with breast cancer and the control group, hematological characteristics, renal function, and liver function tests, there was no significant difference in mean serum creatinine between patients with breast carcinoma and the control group, In addition, the mean hemoglobin level was significantly lower in the patients' group compared to the control group. While level p53 is increasing in serum patients, the average fold change in the compared groups expressed the p53 promoter gene mean fold change between patients with breast cancer and the control group.

Conclusion: Normal ALT and AST levels suggested that patients with breast cancer had no hazards of heart or liver function. Normal ALP level may be associated with osteomalacia and liver impairment in breast cancer patients. The effect of Herceptin on p53 gene expression was investigated by extracting p53 protein and analyzing its effects using quantitative polymerase chain reaction (qPCR). qPCR analysis revealed alterations in the levels of p53 mRNA in response to Herceptin treatment.

Keywords: Biochemical Markers, Renal and Liver function test, PCR techniques, and hematological characteristics

Introduction

Breast cancer (BC) is one of the most common human neoplasms. There are many different tumor subtypes, risk factors, and treatment options for BC (Anstey, EH. et al., 2017). When cancerous cells multiply in the breast, BC develops. This illness is classified as cancer when malignant cells form in the lining of the breast's milk-producing glands or ducts. The ability of cancer cells to invade nearby healthy tissue or spread throughout the body, a process known as metastasis, and their unregulated division, which leads to abnormal development, distinguish them from other types of cells (Takahashi H, et al., 2020). Breast cancer is the most prevalent cancer that kills women and affects them (Ali, JK. et al., 2018). Males can also develop breast cancer, although women are more than 100 times more likely to do so than men. In America, there will be 42,260 breast cancer deaths in 2019, according to estimates (Flaig, TW. et al., 2019). Currently, after cardiovascular diseases, breast cancer is the second largest cause of mortality for women in Iraq (Al-Abassi HM. et al., 2018). Whereas the Iraqi Cancer Council reported that the proportion of females with breast cancer is 34.06% higher than that of other types, i.e., the illness killed 23.02% of Iraqi women and had a 32.31 / 100,000 infection rate or 6,018 / 100,000 in 2018, and the prevalence of it is rising in women between the ages of 35 and 75 (Pandey RJ. et al., 2021). The precise causes of breast cancer are not well understood, and a single cause does not bring them on, but rather the interaction of a number of factors—age, menstruation, alcohol use, hormonal factors, inadequate nutrition, environmental factors, hereditary factors, obesity, and others—all raise the risk of acquiring cancer (Kamińska M. et al., 2015).

p53 is the most extensively studied tumor suppressor gene, encoding a sequence-specific transcriptional regulator that controls a plethora of biological functions, including cell-cycle progression, senescence, differentiation, DNA repair, and apoptosis (Aylon, Y., & Oren, M. 2016). Under typical circumstances, human double minute 2 protein-mediated targeted degradation maintains low levels of p53. Oncogene activation, DNA damage, and hypoxia are examples of cellular stressors that cause p53 activity to rise in order to perform

its role as a transcription factor, setting off a series of events that eventually stop tumor development. (Willis, A. et al., 204Given the importance of p53 in tumor suppression, it is no surprise that the p53 gene is the most often altered in human cancers. (Hollstein et al., 2021). Independent of the wild-type p53 protein, several biochemical and biological functions of mutant p53 proteins have also been identified. For instance, it has been demonstrated that the mutant p53 protein activates a large number of genes. (Webster, M. R. et al., 2020). that inhibiting the p53 pathway may increase the chemotherapy response in advanced breast tumors. (Bertheau, P. et al.,202). p53-induced apoptosis and delineating functional interplay between p53 family members.

Materials and Methods

Considerations for Ethical Behavior

The study comprised the collection of blood samples as well as experimental methodologies approved by the Al-Diwaniyah Teaching Hospital and Cancer Centre's ethics committee. Before the samples were collected, all of the research participants gave their permission to the University of Al-Qadisiyah. Furthermore, all techniques and protocols were carried out in accordance with the guidelines and regulations of the Ethical Committee of the College of Medicine, University of Al-Qadisiyah**.**

The Subjects

The samples were taken from people at Al-Diwaniyah Teaching Hospital and the Al-Diwaniyah Cancer Centre between 1/2/2023 and 20/7/2023. The blood samples were 120 blood samples acquired from Iraqi subjects. The participants included 60 healthy people as a control group (G1) and 60 breast cancer patients as a treatment group (G2). The biochemical investigations were done by a fully automated machine (the biochemistry analyzer Cobas). The hematological investigations were done by a fully automated machine (hematology analyzer CBC), which, based on the principle of electrical impedance and utilizing a one-step RT-qPCR technique, allowed for the detection of RNA expression levels. Molecules of the Analysis Gene Expression of P53 Promote.

The laboratory tests of blood samples

Measurement Techniques A. Alanine Aminotransferase (ALT) 1. Principle of the UV enzymatic technique 2. Kinetic colorimetric technique used for ALT measurement B. Aspartate Aminotransferase (AST) 1. Principle of the kinetic colorimetric technique used for AST measurement C. Alkaline Phosphatase (ALP) 1. Principle of the kinetic colorimetric technique used for ALP measurement D. Total Bilirubin (T Bill) 1. Principle of the measurement technique used for T Bill E. Creatinine Test 1. Principle of the measurement

technique used for creatinine F. Urea 1. Principle of the measurement technique used for urea G. Complete Blood Picture (CBP) 1. Measurement techniques for white blood cell count (WBC) 2. Measurement techniques for red blood cell count (RBC) 3. Measurement techniques for hemoglobin levels.

Real – time quantitative PCR techniques

Serum samples: after being removed from liquid nitrogen and melting at ambient temperature, the samples are well mixed by a vortex. Next, the RNA is extracted. It is also possible to prevent errors brought on by standard dilutions when constructing a standard curve. Additionally, relative gene amounts between two treatment groups are occasionally of greater relevance than precise DNA/RNA molecular counts. As a result, relative quantification is frequently used. The Pfaffl equation was used to compute gene expression, gene fold, or RQ (Relative Quantification) values (Pfaffl, 2001): RQ = 2-(ΔΔCT). The gene fold was first estimated by gathering the average CT value (CT - cycle threshold) from a real-time PC device for each sample that was performed in triplicate. The ΔCT value was then calculated for each sample as follows:

Δ CT = CT (gene of interest) – CT (reference gene)

ΔCT is the difference in CT values between the gene of interest and the reference gene in a particular sample. This is required to normalize the gene of interest to a gene that is unaffected by the experiment. The following formula is used to calculate the ΔΔCT value:

ΔΔ CT = Δ CT (treated sample) – Δ CT (untreated sample (control)

Following the calculation of ΔΔ CT for all samples, the following equation is used to quantify gene expression (fold change): **Fold gene expression RQ = 2-(ΔΔCT)**

Results

Demographic characteristics in breast cancer patients and in the control group

Demographic characteristics of patients with breast cancer and control group are shown in (Table 1) there was no significant difference in mean age between patients group and control group, 46.9 ±10.11 years versus 43.9 ±9.91 years, respectively (p = 0.109).

Hematological characteristics in patient with breasts cancers and controls groups

A comparison of some hematological characteristics between breast cancer patients and the control group is shown in (Table 1) (Figure 1). There was no significant difference in mean WBC count between the patients' and the control groups, 6.8 ± 2 $X10^9$/L versus 6.7 ± 1.3 $X10^9$/L, respectively (p = 0.56). However, the mean RBC count was significantly lower in the patients' group compared to the control group, 4.3 ± 0.61$x10^{12}$/L versus 4.7 ± 0.64 $x10^{12}$/L, respectively (p = 0.0057). In addition, the mean hemoglobin level was significantly lower in the

patients' group compared to the control group, 13 ±1.5 g/dl versus 14 ± 0.84 g/dl, respectively ($p < 0.001$). There was no significant difference in mean platelet count between the patients' and the control groups, 290.11 ±158.65 $x10^9$/L versus 293.00 ± 67.89 $x10^9$/L, respectively ($p = 0.66$).

Renal function tests in breast cancer patients and the control group

A comparison of blood urea and serum Creatinine between patients with invasive ductal carcinoma and the control group is shown in (Table 1) (Figure 1). There was significant difference in mean blood urea between patients with breast carcinoma and the control group 32 ± 6.7 mg/dl versus 26 ± 9.1 mg/dl, respectively ($p = 0.005$). Moreover, there was significant difference in mean serum Creatinine between patients with breast carcinoma and the control group, 0.77 ± 0.31 mg/dl versus 0.63 ±0.23 mg/dl, respectively ($p = 0.0054$).

Liver function test in patients with breasts cancers and controls groups.

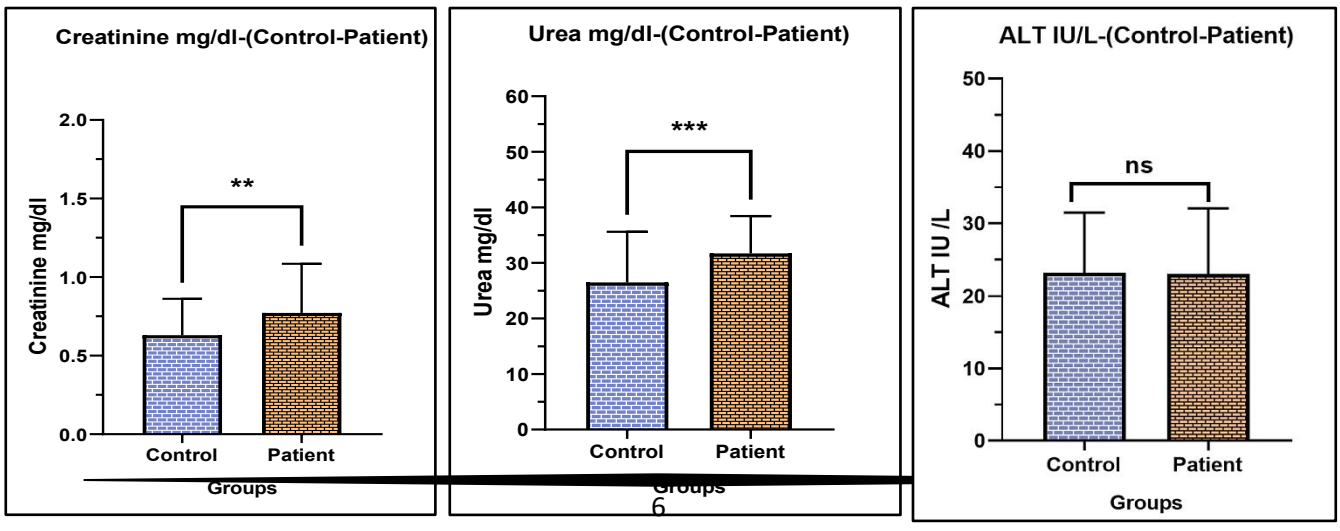
A comparison of liver function tests between patients with invasive ductal carcinoma and the control group is shown in (Table 1)(Figure 1) was no significant difference in mean ALT level in patients with breast cancer compared to the control group, 23 ± 9.0 IU/L versus 23 ± 8.3 IU/L, respectively ($p = 0.94$). However, there was no significant difference in mean AST between patients with breast cancer and the control group, 26 ± 12 IU/L versus 24 ± 8.3 IU/L, respectively ($p = 0.23$). In addition, there was no significant difference in mean ALP between patients with breast cancer and the control group, 206 ± 27 IU/L versus 201 ± 24 IU/L, respectively ($p = 0.37$). Moreover, there was no significant difference in mean TSB between patients with breast cancer and the control group, 0.78 ±0.22 mg/dl versus 0.76 ± 0.22 mg/dl, respectively ($p = 0.62$).

Table 1: Comparison of demographic hematological characteristics, blood urea, serum creatinine, TSB, and liver function, between breast cancer and control group.

Characteristic	Control group *n* = 60	Breast Cancer *n* = 60	*P*
Age (years)			
Mean ±SD	43.9 ± 9.91	46.9 ± 10.11	0.109 I NS
Range	25 – 66	25 – 67	
WBC X10^9/L			
Mean ±SD	6.7 ± 1.3	6.8 ± 2	0.56 I NS
Range	4.7 - 9.4	3.5 –-9.8	
RBC X10^{12}/L			
Mean ±SD	4.7 ± 0.64	4.3 ± 0.61	**0.0057 I ****
Range	3.5 - 5.6	3.4 - 5.8	
Hemoglobin (g/dl)			
Mean ±SD	14 ± 0.84	13 ± 1.5	**<0.001 I ****

Range	12 – 16	10 – 15	
Platelet count X10^9/L			
Mean ±SD	293.00 ± 67.89	290.11 ± 158.65	0.66 I NS
Range	194 – 405	68 – 910	
Blood urea (mg/dl)			
Mean ±SD	26 ± 9.1	32 ± 6.7	**0.005 I ****
Range	10 – 40	20 – 43	
Serum Creatinine (mg/dl)			
Mean ±SD	0.63 ± 0.23	0.77 ± 0.31	**0.0054 I ****
Range	0.4 - 1.4	0.5 -1.6	
ALT (IU/L)			
Mean ±SD	23 ± 8.3	23 ± 9.0	0.94 I NS
Range	11 – 40	11 – 40	
KS	> 0.1	0.08	
AST (IU/L)			
Mean ±SD	24 ± 8.3	26 ± 12	0.23 I NS
Range	11 – 40	12 – 89	
KS	> 0.1	0.07	
ALP (IU/L)			
Mean ±SD	201 ± 24	206 ± 27	0.37 I NS
Range	170 – 245	170 – 255	
KS	> 0.1	> 0.1	
TSB (mg/dl)			
Mean ±SD	0.76 ± 0.22	0.78 ± 0.22	0.62 I NS
Range	0.4 – 1.3	0.4 – 1.4	
KS	> 0.1	> 0.1	

n: number of cases; SD: standard deviation; urea, Creatinine. WBC: white blood cells; RBC: red blood corpuscles significant at $p \leq 0.001$, age, AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TSB: total serum bilirubin; I: independent samples t-test; NS: not significant.



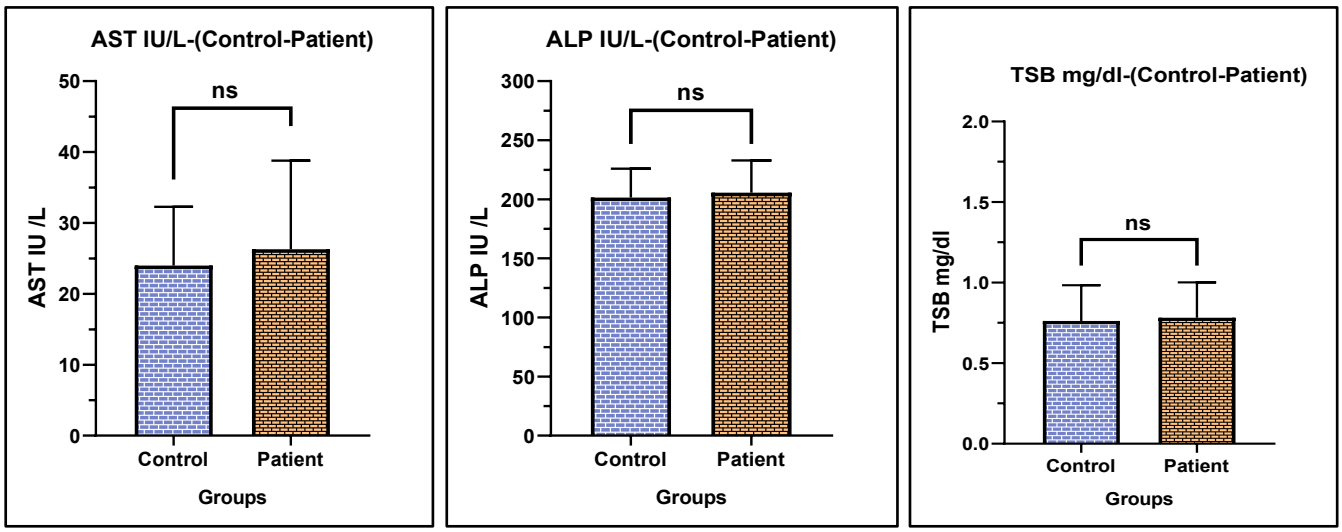


Figure 1: The bar chart for creatinine, urea, ALT, AST, ALP, and TSB) between control and patient

Molecular Analysis

Efficiency of the assay's amplification P53 promoter gene:

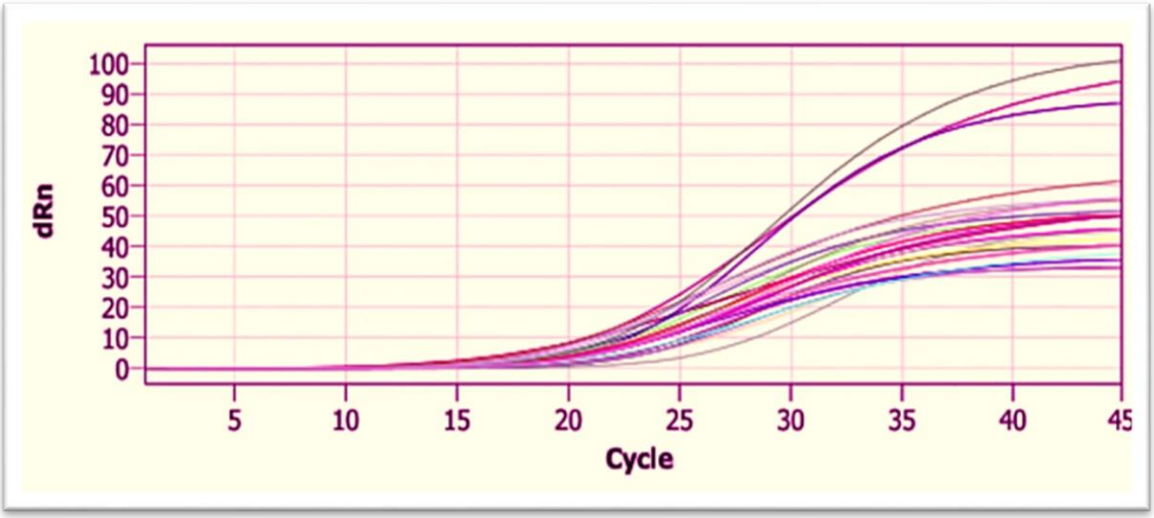


Figure 1: Amplification curve of the tested samples represents the P53 gene. This indicates a successful RNA extraction and cDNA synthesis.

The amplified Ct values for P53 promoter gene and Internal reference gene (housekeeping gene U6) for patient group: The results of a gene sample taken from breast cancer patients, 60 samples compared to statistical women, showed that the expression of the healthy gene was 2.25 and that of the p53 patients was 9.578, meaning that the expression of the gene increased by 9.578 compared to the control 2.25 (Figure 2).

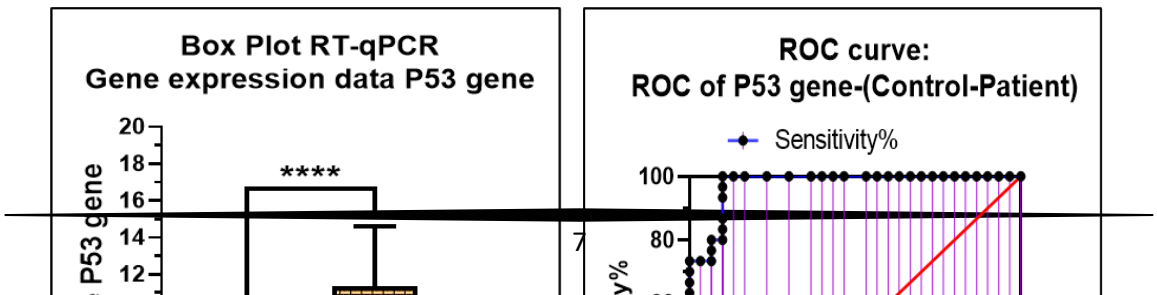


Figure 2: Fold change comparison between the control and patients expressing P53 promoter gene. This shows a significant down regulation in the expression of P53 gene in control group in comparison with patient group. The Receiver Operating Characteristic (ROC) curve, which show cases the relation and the efficacy of the P53 gene in detecting and diagnosing breast cancer.

Discussion

The mean age of patients with invasive ductal carcinoma in the present study is comparable to that obtained by (Wang, L., et al 209), who collected registered data for breast cancer cases form the Iraqi Cancer Registry/Ministry of Health, enrolling 23,792 patients. They found that the mean age was 52 years. In addition, the mean age in this study was approximately similar to that obtained by (Stiff, A., et al 2018), who carried out a retrospective descriptive study in which medical notes and histopathological reports of patients with a confirmed diagnosis of breast cancer between January 2011 and December 2015 were reviewed in Basra and they found a mean age of 50 years.

In line with the present study results, (Akinbami, A. et al., 2013) WBC levels were higher in breast cancer patients than in controls, according to one study. Nonetheless, according to (Park, T., et al., 2019), they compared WBC counts between patients with breast cancer (n = 4,402) and controls (n = 4,402) chosen from the Korean National Health and Nutrition Examination Survey using a sizable case-control study; they found that WBC count mean in patient's group was significantly lower than that of the control group in clear controversy to current study finding. Evidence is mounting that chronic low-grade inflammation may contribute to the etiology of several malignancies (LM, C., 2002). White blood cell (WBC) count, an inflammatory biomarker, has emerged as a helpful indicator of infection and a predictor of other diseases (Park, T., et al., 2019). Even within the normal range, a high WBC count has been linked to atherosclerotic cardiovascular disorders, cancer incidence, and mortality (Lyman GH, et al., 2015).

In the present study results, it has been show that mean RBC count and mean hemoglobin are lower in patients with invasive ductal carcinoma compared to the control group by several previous reports (Rana NP, 2015). Anaemia is frequently seen in cancer patients, with proportions ranging from 22.7% (122) to 63% (123) and up to 89% after chemotherapy (Xu H, Song J, et al., 2016) increased incidence of anemia has been associated with reduced quality of life and treatment response in patients with breast cancer (Al-Ansi AM, et al., 2021). Regarding platelet count, and in agreement with the current study finding, (Shahryari, S., et al., 2021) found no significant difference in mean platelet count between patients with breast cancer and control subjects; however, (Rathore, S. S., et al., 2022) reported significantly less mean platelet count in women with breast carcinoma compared to the control group and this is inconsistent with current study observation. Indeed, most previous authors linked the platelet count to disease behavior such as grade of disease, stage of sease and survival rates (Garmi, N., Nasrallah, S. et al.2020).

There is much evidence to suggest that cancer patients frequently have renal impairment. Reports show this renal insufficiency is linked to worse overall survival and higher cancer-related mortality. onsequently, it is crucial to check for renal insufficiency in cancer patients using a suitable and accurate method of estimating renal function (Wanchoo, R., et al., 2016) . In the study of (Hasbahceci, M. et al., 2018), blood urea and serum Creatinine levels were measured in a group of women with breast carcinoma and compared to a control group; the results revealed statistically significant difference in mean blood urea or serum Creatinine between the two groups. Thus, the present study agrees with that of (Hasbahceci, et al., 2018). In addition, the current study results are consistent with that of (El-attar AZ, et al., 2022), who found no significant difference in blood urea and serum Creatinine between women with breast carcinoma and the control group.

Indeed, current results agree with (Xu, H., et al., 2016), who found no significant difference in mean TSB but a significantly higher level of AST in breast cancer patients compared to the control group. Still, it disagrees with them in the finding of significantly higher ALT levels in the cancer group compared to the control group. Previous reports evaluated the liver function test in breast cancer patients in association with liver metastasis (Ermshaus, A., Schäfer, P., et al. (2023).

Molecular Analysis

In this study, the results of a gene sample taken from breast cancer patients, 60 samples compared to statistical women, showed that the expression of the healthy gene was 1.00 and that of the p53 patients was 9.578, meaning that the expression of the gene increased by 9.578 compared to the control 1.000. Use the U6 (housekeeping gene) whenever expressing a gene. The housekeeping gene is an essential gene in a living cell that the cell constantly needs

and does not need to be activated. We use housekeeping in every sample to correct errors because it can correct errors in gene expression that can happen. The human P53 gene is abundantly expressed specifically in breast cancer is characterized by increase production of patients with breast cancer and comparison healthy, this agrees with (Jautz, J. (2019).

As it is shown in (Figure 2), the results of the present study showed a significant higher regulation in the expression of P53 in patients with breast cancer group in comparison to the control group. This higher regulation can be interpreted as that there were two key sequences in the initiation of transcription in p53 gene promoter: G:C→T: A boxes, alteration in these sequences caused a increase in the affinity of the transcription factors such as EK-LF or Sp1, limiting the transcription of the mRNA. This was either because the C: G→T: A A box that determined the starting point was altered or because the CAAT box and the duplicates CACCCC (distal and proximal) that would determine the starting frequency that were affected. this agrees with (Ali Syeda, Z., Langden, S. S. S., et al., 2020).

The consequences of the current study were consistent with the results of other trials. this agrees with (Brown, D. et al., 2022),which showed that high regulation of the P53 gene can result from a wide range of genetic lesions, including modest HBB-specific deletions, point mutations, and large deletions of the entire P53 cluster. this agrees with r.(Cao A et al., 2010).As it is shown in table 3.5 and table 3.6, a comparative CT method (ΔΔCt) used reference gene and gene of interest to determine the relative quantity of target nucleic acid sequence in samples where ΔΔCt = Δ Ct (sample) – Δ Ct (reference gene) (Morzaev D. et al., 2015) .In the current study, this method demonstrated that the patients with breast cancer exhibited higher expression of the gene of interest as it was compared with internal reference gene.

Conclusion

In conclusion, this study compared various demographic and laboratory characteristics between patients with breast cancer and a control group. The findings revealed that there were no significant differences in mean age and white blood cell count between the two groups. However, the patients' group had significantly lower mean red blood cell count and hemoglobin levels compared to the control group. Furthermore, there were significant differences in blood urea and serum creatinine levels, indicating potential renal dysfunction in the patients' group. On the other hand, there were no significant differences in liver function tests between the two groups. These results provide valuable insights into the demographic and physiological characteristics of patients with breast cancer, highlighting the importance of monitoring hematological and renal parameters in this patient population. The

effect of Herceptin on p53 gene expression was investigated by extracting p53 protein and analyzing its effects using quantitative polymerase chain reaction (qPCR). The findings of this study suggest that Herceptin treatment has an impact on p53 gene expression. qPCR analysis revealed alterations in the levels of p53 mRNA in response to Herceptin treatment. These changes may indicate a potential regulatory effect of Herceptin on the p53 gene.

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