Comparison Gene Expression of BARCA1 and BARCA2 and complete blood counts in the Blood Serum of Women with Breast Cancer in Ramadi City

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Abstract
Breast Cancer is one of cancer types which appear in the breast tissues. The current study aimed to study and evaluate the level of gene expression of the BARCA1 and BARCA2 genes and the concentration levels of these genes in the blood serum of women with breast cancer in Ramadi city, the center of Anbar Province. Then, to compare these variables with companions and healthy people as a control group. In addition, to showing changes in physiological blood characteristics of breast cancer patients and the companion group, and comparing them with healthy as a control group. The results of gene expression between the BARCA1 and BARCA2 genes showed that there were significant differences at the level of probability (P0.05), with an increase in the level of gene expression of the BARCA2 gene by (2.002) in the diseased group compared to the healthy ones, with the presence of significant differences between the two genes compared to the control group, and the high level of expression coincided The gene expression of the BARCA2 gene was associated with a decrease in blood tests in the blood plasma of breast cancer patients. Therefore, the obtained results of the study showed that there is a close relationship between the level of gene expression and the blood biochemical characteristics of breast cancer patients.
Keywords: Breast Cancer, Gene expression, BARCA1, and BARCA2

1. Introduction

Cancer represents a major human health problem all over the world. The term cancer is applied to a group of diseases that are characterized by abnormal growth and reproduction of cells, which lead to the destruction of other healthy cells in the body. When cancer occurs in the tissues and cells of the breast, it is known as breast cancer. Breast cancer, which stands for BC, is a heterogeneous hormone-dependent cancer. It represents about 22.9% of all female cancers (Thompson, et al., 2005). It arises from breast tissue, particularly the milk ducts (80% of cases are ductal carcinoma) and the lobules. Cancer originating from a ductal area is known as ductal carcinoma while cancer originating from the lobules of the breast is known as lobular carcinoma (Sharma, 2021).

The general risk factors for female breast cancer are age, infertility, age of first full-term pregnancy, menopause, hereditary factor, genetic mutation in the BRCA1/ BRCA2 breast cancer gene, and hormones (estrogens and/or progestin) in post menopause. The incidence of cancer increases approximately in the fifth decade of life (Yang et al., 2020).

The BRCA1 gene located on chromosome q12-2117 is identified and cloned by Miki et al. (1994) after an extensive international effort. Hereditary breast cancers represent 5-10% of all breast cancers, and inherited mutations of the BRCA1 gene represent about 40-45% of these hereditary cancers, however BRCA1 mutations represent about 80% of families whose members suffer from a high incidence of breast and ovarian cancer (Chen et al., 2018). BRCA1-associated breast cancers differ from tumors not associated with BRCA mutations with regard to pathological features, for example: the estrogen receptor are negative, high-grade, and have a higher frequency of somatic abnormalities in important genes such as p53. (Honrado et al., 2022). The biological theory of BRCA1 and BRCA2 and the different pathological aspects of BRCA1-related tumors support the hypothesis that patients who carry a BRCA1 and/or BRCA2 mutation may have a worse prognosis for breast cancer than non-carriers (Narod et al., 2002).

The primary function of BRCA2 is to facilitate homologous recombination in cells deficient in BRCA1 or BRCA2 and incapable of repairing DNA double-strand breaks through error-free homologous recombination leading to error repair through the non-homologous end-joining pathway (NHEJ) that is prone to error and thus chromosomal instability (Roostaeian et al., 2011). Thus, the current paper aimed to study the relationship of gene expression of the BRCA1 and BRCA2 genes with the levels of physiological characteristics of the blood of women with breast cancer.

2. Materials and Methods

Collecting of sample

This study consists of (60) female as samples. The

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samples were divided into three categories: The first category included (20) samples of women with these samples were collected from Al-Anbar specialized center for the treatment of tumors for a period of 3 months as (patients groups). The age of the infected women ranged from (30-55) years. The ages of the women accompanying the sick women (Escorts groups) ranged from (20-60) years and the healthy women, their ages ranged from (25-45) years as control groups. The blood was drawn by a sterile syringe with a capacity of 5 ml, then the blood was divided into two parts: the first part 3 ml, was placed in an EDTA tube for later molecular examinations, while the second part was placed in a gel tube with light shaking, then it was placed in a centrifuge to separate the plasma from the blood and take 500 µl of plasma for the purposes of blood analysis

Extraction RNA and real time-PCR (qRT-PCR)

500 µL of blood plasma was used and 100 µL of Trizol reagent was added to extract the RNA. The RNA was converted into cDNA using a special kit supplied by the Korean company BIONEER according to the company's instructions. Then real-time quantitative reverse transcription (Real-Time q RT-PCR) technology was used to quantify the gene expression of the study gene BARCA1 and BARCA2 and the titration gene β-actin as shown in(Table 1) . The data were partially calculated using the Livak equation and according to the method (Livak Schmittgen, 2001).

(Table 1) show the primers used in the molecular study .

Human Gene	Sequence 5' – 3'	NCBI Reference Squence	BP
ß– actin	5 '-GCGAGAAGATGACCCAGA-3' 5 '-CAGAGGCGTACAGGGATA-3'	NM_ 001101.5	90
BRCA 1	5 '-AATGGAAGGAGAGTGCTTGGG- 3' 5 '-GCCTCAGAATTTCCTCCCCAA - 3'	672	105
BRCA 2	5 '-AATGGCAGACTGACAGTTGGT – 3' 5 '-ATTCTGGGGCTTCAAGAGGTG – 3'	675	94

Sample analysis of complete picture of the blood

3 ml of blood is taken and placed in a special tube containing EDTA anticoagulant, then left on the shaker device for 5 minutes to ensure that there is no blood clotting, then the tube is placed in the SWELAB device with the chemical (Lyse, Deunal) supplied by the company (SWELAB) Then the result is given after one minute, as a complete picture of the blood.

Statistical analysis

The results were statistically analyzed using the SPSS program 23 and using the Analysis of Variance (ANOVA TABLE) to calculate the variance, test the averages using the least significant difference test L.S.D at the probability level of 0.05. The averages were also compared using the standard error (Steel& Torri, 1980).

3. Results and Discussion

Gene expression of the BRCA 1 and BRCA 2 genes

The gene expression results shown in Figure (1) showed that there was a significant difference at the level of probability ($P \leq 0.05$) in relation to the level of gene expression of BARCA 1 and BRCA 2 genes in breast cancer patients compared to the healthy (control group). The level of BRCA2 gene expression increased by (0.02) in the blood serum of breast cancer patients compared to the healthy as a control group, with a significant difference between the two genes compared to the control group (the healthy). There were also significant differences for both genes in the Escorts group compared to the healthy group

	C		BRCA-1 P		BRCA-1 PE		BRCA-2 P		BRCA-2 PE	
	2-ΔΔct	Folding	2-ΔΔct	Folding	2-ΔΔct	Folding	2-ΔΔct	Folding	2-ΔΔct	Folding
AV	1.00	1.00	1.53	0.53	1.72	0.72	2.84	2.43	1.87	2.78
SD	0.00	0.00	0.44	0.44	0.73	0.73	2.02	1.47	1.82	2.76
P≤ 0.05	c	bc	ab	c	ab	c	a	ab	ab	a
P≤ 0.01	N.S	ab	N.S	b	N.S	ab	N.S	ab	N.S	a
n=8										

Figure (1) Expression of BARCA 1 and BRCA 2 genes in breast cancer patients compared to the healthy group and Escorts group. Different lowercase letters indicate significant differences. C=Control (healthy groups), BARCA 1P= patients and BARCA 1PE= Escorts. lowercase letters indicate significant differences at the level (P ≤ 0.05, P ≤ 0.01).

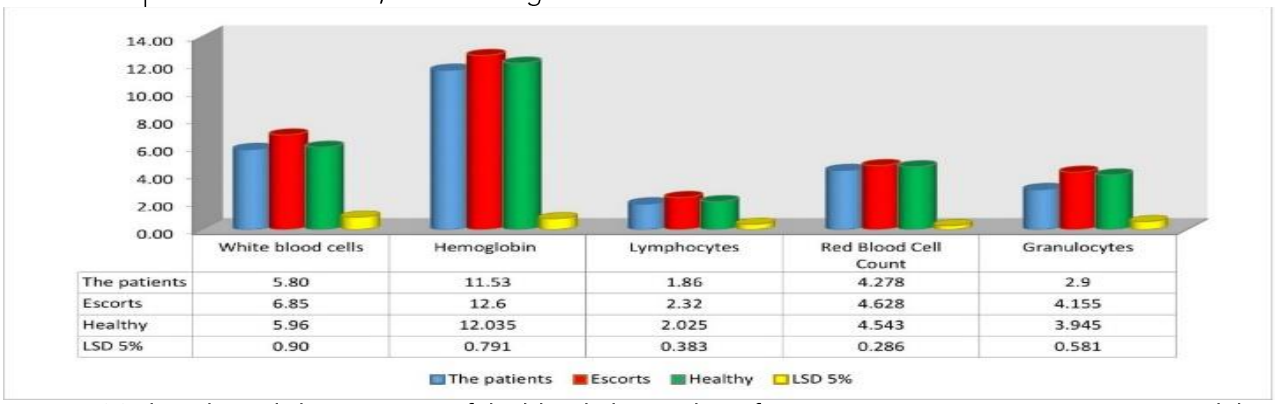
The result of this study was consistent with the findings of (Massimo et al., 2019), as he indicated in his study on the differential expression of a group of DNA repair mechanisms in tumors associated with BRCA genes, that the level of gene expression of the BRCA 2 gene was high, while a significant statistical decrease was found for the BRCA1 gene. Tumor samples compared to their non-tumor counterparts. While (Taylor et al., 1998) indicated a strong relationship between the degree of breast cancer development and low expression of the BRCA1 gene in his study that included the use of (142) samples of breast cancer tumors, and this is similar to what (Russell et al., 2000) indicated. They mentioned that the low expression of the BRCA1 gene led to the progression and development of breast and ovarian cancer, given that this gene is considered a tumor suppressor in the breast and ovarian tissue. These results indicate that the loss of the BRCA1 gene through mutations or decrease its gene expression leads to tumor formation and development in the target tissues, and its high gene expression leads to tumor growth cessation and programmed cell death (Thompson et al., 1995).

The dysregulation of oncogenes and tumor suppressor genes is considered the main cause of cancer, as understanding the regulatory mechanism of these genes will provide clues about their interaction with cancer, as BRCA1 and BRCA2 genes are responsible for most cases of familial (hereditary) breast cancer (Paull et al., 2001). The BRCA1 and BRCA2 genes are associate with many cellular processes in the cell nucleus such as DNA repair, recombination, as well as the regulation of DNA replication, in addition to their role in widely stimulating of chromatin proliferation, which plays a vital role in the process of transcription and repair (Ye et al., 2001).

Breast tumors related to the BRCA1 and BRCA2 genes suffer from a deficiency in the repair factors of DNA defects. It has been proven that both hereditary and sporadic breast cancers suffer from a decrease in the factors regulating repair, which provided an opportunity to exploit this deficiency, whether acquired or inherited, and cell growth Abnormal (cancerous). The cells of mammals are subject to high amounts of genotoxicity, whether from inside or outside of the cell, as errors in the transcription process, chemical damage to bases, and oxygenation generated from metabolic processes contribute to DNA damage from within the cell itself, while ultraviolet radiation and exposure Chemicals damage of DNA from outside the cell (Lilia et al., 2008). The repairing mechanisms contribute in limiting DNA damage, as disorders in the repair pathways (mechanisms) lead to the accumulation of DNA damage and the occurrence of cancer, and mutations play a role in genes. Carcinogens, tumor suppressor genes, and other genes that contribute to cell survival and growth which lead to the continuation and progression of cancer.

Blood test results

The result of Figure (2) comparing CBC between groups showed that there were significant differences in the average values of some variables of the CBC image at the level of probability (P0.05). The white blood cells showed significant differences at the level of probability (P0.05) with an average value of (5.79%) among the infected compared to the companions (6.84%) and the healthy (5.95%) as a control group, while the red blood cells did not show statistically significant differences at the level of probability (P0.05) between the infected (4.27%) and the companions (4.62%) compared to the healthy (4.54%) as a control group. The result of lymphocytes indicated the emergence of significant differences, as the average value was (1.86%) for the infected and (2.32%) for the companions, compared to the healthy (2.02%) as a control group, and the value of platelets did not show any significant differences between The infected (26.30%) and the companions (27.25%) compared to the healthy (25.75%) as a control group, in contrast to the HGB, as it showed significant differences among the infected (11.5%) and the companions (12.6%) compared to the healthy (12.0%) as a control group.



	White blood cells	Hemoglobin	Lymphocytes	Red Blood Cell Count	Granulocytes
The patients	5.80	11.53	1.86	4.278	2.9
Escorts	6.85	12.6	2.32	4.628	4.155
Healthy	5.96	12.035	2.025	4.543	3.945
LSD 5%	0.90	0.791	0.383	0.286	0.581

Figure (2) Physiological characteristics of the blood: the number of WBC, RBC, PLT, HGB concentration, and the volume of HCT

The result of this study was identical to what was dealt in a study conducted between 2015 and 2020 that included 735 patients who had a CBC image before and after chemotherapy, as its results indicated a decrease in the vital values of the CBC image as an indicator of the risk factors associated

with chemotherapy (Chen et al., 2021). It is consistent with the findings of (Standish et al., 2008) in a small study conducted on 14 patients, which indicated to a decrease in the CBC vital values index affects the psychological state and the immune system and thus survival as stated in (Sun et al., 2020).

4. Conclusion

We conclude from our study that the BRCA1 and BRCA2 genes are elevated in breast cancer patients and their comorbidities. This means family members are more likely to have a BRCA1 or BRCA2 mutation if family has a strong history of breast cancer. Family members who inherit BRCA1 and BRCA2 mutations usually share the same mutation. If one of family members has a known BRCA1 or BRCA2 mutation, other family members who get genetic testing should be checked for that mutation using a molecular marker.

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