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Detection the Expression of IGF1 Gene and Evolution The Genotoxicity Using Some Genetic Test and Some Vital Variables in Breast Cancer Women

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Abstract: One key participant in carcinogenesis is the insulin-like growth factor 1 (IGF1) pathway. Previous research used prolonged administration of several IGF1 receptor agonists to animals. The second leading cause of cancer-related mortality among females is breast cancer, which also happens to be the most prevalent malignancy among women. Nearly 40% of all female cancers have this description. In order to evaluate genetic damage in a group of breast cancer patients, this research set out to investigate these assays and their applicability. Methods: All recorded cancer cases from March to October 2024. A total of 90 samples were obtained from females (ranging in age from 50 to 70 years) at the Oncology Center of the Saladin Health Department in Tikrit. Of these, 30 served as controls and 60 had breast cancer, a diagnosis that was confirmed by histopathological reports. The genotoxicity doses assessed in oral epithelial cells using the Micronucleus assay and Comet assay for DNA damage in peripheral lymphocytes demonstrated a significant rise in white blood cell concentration (8.30*±1.50) compared to the healthy control group (7.06±1.88). On the other hand, a decrease in PCV concentration (33.50*±1.13) compared to the healthy control group (36.40±3.16) and Hbg/DL(10.53±1.20) (9.86*±1.80) was observed ($P \leq 0.05$). Breast cancer patients had an average of 155.8 ± 4.75 mean micronuclei, which is greater than the healthy control group's 29.25 ± 4.37 average abnormalities in nuclei. The findings highlight an average rise in the number of impacted cells (*6.64± 1.2) and an average total number of cells (51.66 ± 18.19). There was a significant difference between the control group and the patients in terms of the average amount of damage (* 31.33 ± 12.15) and the IGF1 gene. These findings point to the possibility that micronucleus and comet estimates may be useful in estimating cellular genetic damage in breast cancer patients.

Keywords: Breast cancer, micronucleus, micronucleus assay, Comet assay

Introduction:

An essential function of the insulin-like growth factor 1 (IGF1) pathway is to prevent cell death, promote cell proliferation, and maintain cell viability. The fact that epigenetic and transcriptional alterations in IGF1 signaling might promote cancer initiation and progression is, therefore, not unexpected .

One typical use of exogenous insulin is in the treatment of type I and type II diabetes utilizing novel insulin-like molecules. New insulin molecules have had their chemical structures tweaked slightly to boost stability and temporal bioavailability while simultaneously improving the original molecule's pharmacokinetic characteristics. Receptor binding affinities, such as those for insulin (IR) or insulin-like growth factor 1 (IGF1R), may be enhanced or maintained by these molecular alterations. In light of this binding affinity, insulin analogues may enhance mitogenesis ability or not. Insulin X10 is a well-known insulin analogue that binds more strongly to IGF1R. Therefore, this molecule (insulin X10) does not find its way into the pharmaceutical industry because of its potentially heightened cancer capability. Insulin glargine is now the most widely used insulin treatment on a global scale since it is a recognized insulin analogue with a greater binding affinity at IGF1R. Whether or not glargine poses a carcinogenic danger is an ongoing concern for some. As the second leading cause of cancer-related mortality in women, about 40% of all female cancer cases are breast cancer. It is also the most common malignancy in women. Worldwide, 2.261 million new instances of breast cancer were identified in women in 2020, accounting for 11.7% of all cancer sites. 0.685 million women lost their battle with breast cancer that year, making it 6.9% of all cancer sites (1). Cancer of the breast is known to manifest in a variety of ways. With a high risk of recurrence and metastasis, it is among the most dangerous malignancies. The idea that a single actor is responsible is not clearly implied. There are a number of known risk factors for breast cancer, including both internal and external factors. Age, however, is the single most important risk factor for this illness. Approximately two-thirds of newly diagnosed cases occur in those over the age of 55. Additionally, compared to women under 65, those over 65 had a somewhat higher risk than 4.0. The number of known breast cancer risk factors has grown substantially. Certain risk factors, such age, BRCA1 and BRCA2 gene mutations, family history, and reproductive history, cannot be changed. Some, including excessive endogenous estrogens, hormone treatment, obesity, and alcohol intake, may be amenable to change. Some of these variables are inherent to the body and increase with age, such as the time between menstruation and menopause (the younger the age of first menarche, the greater the risk). Additionally, there are environmental and exogenous risk variables to consider. Lifestyle and behavioral issues, for example, include things like drinking too much, eating poorly, using the pill for birth control, and hormone replacement treatment. being overweight or obese lack of sleep and lack of physical exercise. Up to half of all cases of breast cancer may have their origins in a person's food and the environment (6). A vegetarian diet and one low in fat (7). reduce sex-steroid hormone levels. These findings suggested that lowering blood sex-steroid hormone levels might lower the breast cancer risk. Breast development, including the typical physiological processes of growth, proliferation, and differentiation, and cancer of the breast (8). ERα and ERβ, which are members of the nuclear receptor superfamily, mediate these processes when they bind to 17β-estradiol (E2) and similar substances. When ligands attach to ERs, they modify their conformation, allowing for interaction with chromatin and the control of transcription of target genes (10). In breast cancer, there is a shift in the range of changes that may inhibit DNA synthesis and RNA transcription. This can happen by cross-linking DNA or by binding to DNA bases and forcing repair enzymes to break them. cause nucleotide mispairing as well. As the mode of action depends on the genetic structure, a means of detecting changes in DNA structure is needed(11). We performed the following assays to estimate genomic alterations. The micronuclei test for epithelial cells is a biological approach for assessing DNA damage and evidence of cell death in the oral epithelium (12). The single-cell gel electrophoresis (SCGE) or comet test detects DNA damage in individual eukaryotic cells. The theory behind the comet test is as follows: Undamaged DNA remains relatively compact and structured in the nucleus, but becomes slightly loose when the cell is damaged. Suitable repairing both single-strand and double-strand breakage, it features a simple and economical setup (13).)

Materials and methods

The example handle Between March and October 2024, 90 samples were collected from women. Thirty of these women acted as controls, while sixty were diagnosed with breast cancer based on histological results. Their ages range from 50 to 70.Blood collection and human blood samples

human blood samples were collected by venipuncture using a 21-gauge needle and syringe, from the antecubital vein (~ 5 ml), and immediately transferred into tubes containing sterile EDTA solution (to a final concentration of 1.6 mg EDTA/ml of blood). Urine was processed promptly for complete blood count and comet assay.(14)

Complete blood count (CBC)

The complete count of blood elements was measured using the CBC device, which is an auto analyzer device from the Canadian company GenoTek to measure all types of blood variables. This device is characterized by speed and accuracy in the results and gives approximately 19 blood variables are measured using the following steps. 1 ml of counted venous blood was taken and placed in a test tube containing an anticoagulan

EDTA, The tube was placed on the shaker for 5 minutes to mix the blood with the components of the tube. Then place the tube in the complete blood test device, as the device withdraws an amount of blood using a probe. The device also contains three solutions: lyse, diluent, rinse, and then. The results appear on the device screen and are printed using the device's printer.

Micronucleus assay in Oral epithelial cells:

The test was performed according to the method described by Gopal and Padma, 2018 (15) as follows: People were asked to wash their mouth with water and using a sterile dry polypropylene cotton swab to scrape cells from the lining of each cheeks and pressed on a clean microscope slide. It was dried with air and fixed with methyl alcohol. It was stained by May-Grunwald and then dyed with giemsa. The frequency of micronuclei was recorded as 2000 cells per capita were recorded in each case to determine the percentage of MN.

Evaluation of DNA damage in peripheral blood lymphocytes

Sample preparation

Peripheral blood lymphocytes extracted from freshly obtained blood were centrifuged and subsequently rinsed three times in phosphate-buffered saline .

Viability assay

A viability experiment ought to be conducted in order to determine the amount of the test substance that produces at least 90% viability. This was achieved by mixing 5 µl of trypan blue with 10 l of 106 cells/ml in a microcentrifuge tube. Place on a slide and cover with a cover slip once it has stood for at least two minutes. Calculate the number of living (shiny) and dead (blue) cells in 100 cells

comet assay:

When planning and evaluating the comet test, we stuck to the standard procedure. Since an alkaline environment may identify alkalilabile sites, single-strand breaks, and double-strand breaks, it was used to conduct the comet test. A 20 volume percent (wt/vol) solution of STM buffer comprising 250 mmol/L sucrose, 50 mmol/L Tris-HCl, pH 7.4, 5 mmol/L MgSO4, and 0.5 mmol/L phenylmethylsulfonyl fluoride was used to homogenize the cells. The cells were mixed with 95 µL of low melting point agarose (0.75%), which was developed for blood samples, to make the slides. After that, 500 µL of 1% ordinary melting agarose was spread out on a microscope slide and completely covered with the cells and agarose. When the slides were dry, we took off the cover slip and soaked it in lysis solution for a day. With the addition of fresh 1% Triton X100 and 10% DMSO, the pH of this mixture was brought to 10.0-10.5; it already included 2.5 M NaCl, 100 mM EDTA, and 10 mM Tris. A freshly made alkaline buffer containing 300 mM NaOH and 1 mM EDTA, with a pH of 12.6, is then used to soak the slides for a duration of 10 minutes. Twenty minutes of electrophoresis at 25 V (0.90 V/cm) and 300 mA is applied to DNA in a pH-12.6 alkaline environment. Upon completion, neutralize it to a pH of 7.5 by adding 0.4 M Tris. Lastly, use Cybergreen dye to colorize the DNA. To guarantee the process's dependability, each electrophoresis experiment included a positive and a negative control.

Measuring gene expression

mRNA was isolated from the samples as a basic step of the real-time polymerase chain reaction real-time PCR). In order to estimate the level of gene expression, an RNA extraction kit was used Transzol Up plus (RNA Kit) Supplied by the company TRANS

IGF1	F	TGATTTTTGTGCACTCTGCTC	21
IGF1	R	GAATACCCAAGGGGGATTTG	20

Annealing 58 product 122

Housekeeping gene

GADPH	F	TGCCACCCAGAAGACTGTGG	20
GADPH	R	TTCAGCTCAGGGATGACCTT	20

Quantitative Real-Time PCR (qRT-PCR) Run

The Real-time PCR technique was used to do quantitative real-time PCR, also known as qRT-PCR. By measuring the threshold cycle (Ct) using the components of the TransStart® Top qPCR SuperMix Kits, the levels of gene expression and fold change were detected. Using real-time cycler software, the Ct threshold cycle was determined for every sample. We ran each sample twice and averaged the results. - Housekeeping genes were used to normalize the expression data of chosen genes. The following is an expression of the findings as a folding change in gene expression, obtained by following the suggested procedure for data analysis: The ΔCt value, which measures the difference between the target gene and housekeeping gene Ct values, was calculated for every sample.

Statistical Analysis

A statistical application called SPSS V20 was used to investigate the data. The mean ± standard error (SE) was used to display the data. Separate samples To examine differences in means, T tests were employed. [10] Significant differences were defined as those with p-values of 0.05 or below. We used these formulas to find the fold change.

1. ΔCt (control) = Ct (gene) – Ct (HKG)

2. ΔCt (patient) = Ct (gene) – Ct (HKG)

4 ΔΔCT=ΔCT of each sample – average control ΔCT.

Then the fold change was calculated using the Livak method [11]

5. Fold change = 2-ΔΔCt

Results and discussion

Level of WBC in blood serum. The results of the statistical analysis in Table 1 showed a significant increase at the level in the concentration of white blood cells WBC for patients with breast cancer(8.30*±1.50) compared with Healthy control (7.06±1.88) (P≤0.05).

Table 1. Measuring some blood variables such as (WBCs) The percentage of red blood cells (PCV) and hemoglobin (Hbg) in breast cancer women with menopause and control group.

Group	**WBCs (×10*9/L)**	**PCV %**	**Hbg/DL**
Healthy control	7.06±1.88	36.40±3.16	10.53±1.20
breast cancer patients	8.30*±1.50	33.50*±1.13	9.86*±1.80

standard deviation ± Mean, * significant at the (P≤0.05)

The results of the current study are consistent with Mimica et al.,2016 The reason for the high number of white blood cells WBC is related to low-grade chronic infections, which may It causes an increase in WBC, and this increase may be a result of insulin resistance, obesity, or menstrual disorders What is meant by chronic low-grade inflammation is a mild inflammatory condition that is sustained over a long period of time Unlike acute inflammation, which is an immediate and strong response to injury or infection, chronic low-level inflammation It is less obvious, but it is persistent, and this condition can affect the body’s tissues and organs over time (18). Table 1 displays the statistical analysis findings, which demonstrate a substantial drop in PCV concentration for breast cancer patients (33.50*±1.13) as compared to the healthy control group (36.40±3.16) and Hbg/DL 10.53±1.20 (9.86*±1.80) (P≤0.05).There is a decrease in the level of red blood cells (PCV) in patients for patients with breast cancer) can be caused by a deficiency of iron, vitamin B12, or folic acid, some women may suffer from heavy bleeding as a result of the disorder Menstrual cycle which leads to loss of large amounts of blood and a decrease in. (19)

The results of MN test in Oral epithelial cells.

Comparisons between patient groups and healthy controls were provided in Table (2) and Figure (1) (2) for mn in oral epithelial cells. According to the findings, the frequency of mn in the epithelial cells of the breast cancer patient group was higher than the control group's basic level, as shown by the repetition of the mn. In comparison to the healthy control group, patients with breast cancer had the highest mean numbers (155.8 ± 4.75), according to the data. Patients with breast cancer have significantly greater rates of all nuclear alterations compared to a healthy control group.

Table 2. The mean differences of the frequency of the micronuclei in the in exfoliated Oral epithelial cells which evaluated for the control and breast cancer patients

Anomalies	**Study groups M ± SD**	
	Healthy control	**breast cancer patients**
Binucleated cell	5.05 ± 3.50	30.05 ± 5.62
Condensed chromatin	9.40 ± 3.02	11.50 ± 3.89
Broken eggs	1.90 ± 1.17	4.06 ± 1.77
Basal cell	2.00 ± 1.16	5.85 ± 1.98
Karyorrhetic cell	0.09 ±0.04	10.04 ± 1.78
Karyolytic cell	10.60± 2.06	90.10 ± 2.69
Mono nucleated cell with Micronucleus	2.50 ±1.05	13.50 ± 1.43
Percentage of nuclear anomalies in total	29.25 ± 4.37	155.8 ± 4.75

S.E. Stands for standard error, and the mean difference is significant at the 0.05 level (t-test). Total micronucleus.

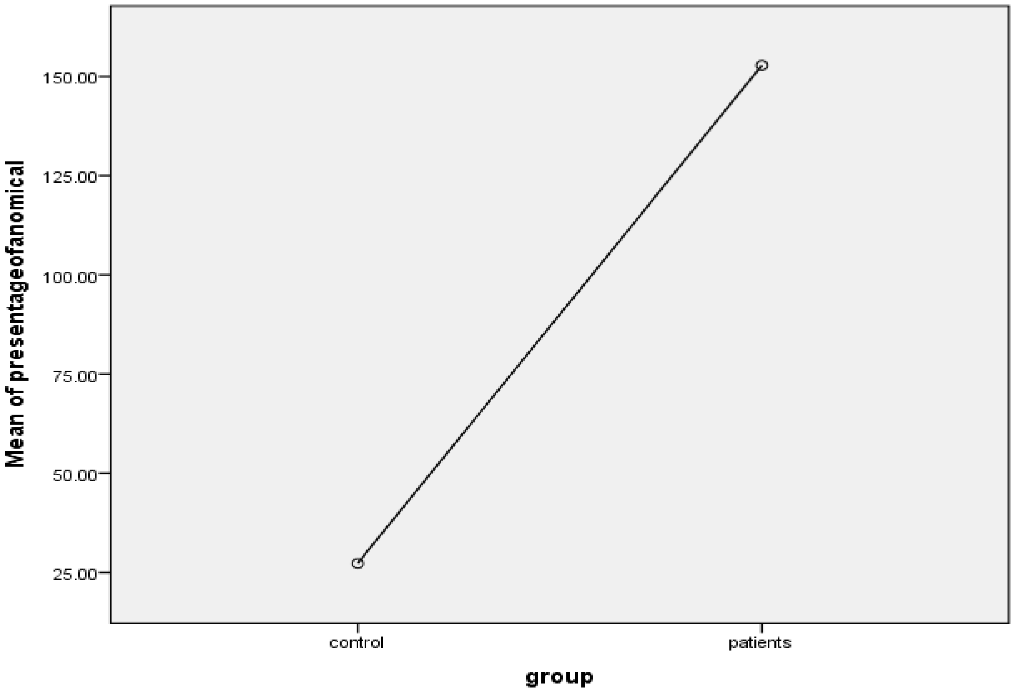

Figure 1. The mean difference is significant at the 0.05 level (t-test) between control and in breast cancer women.

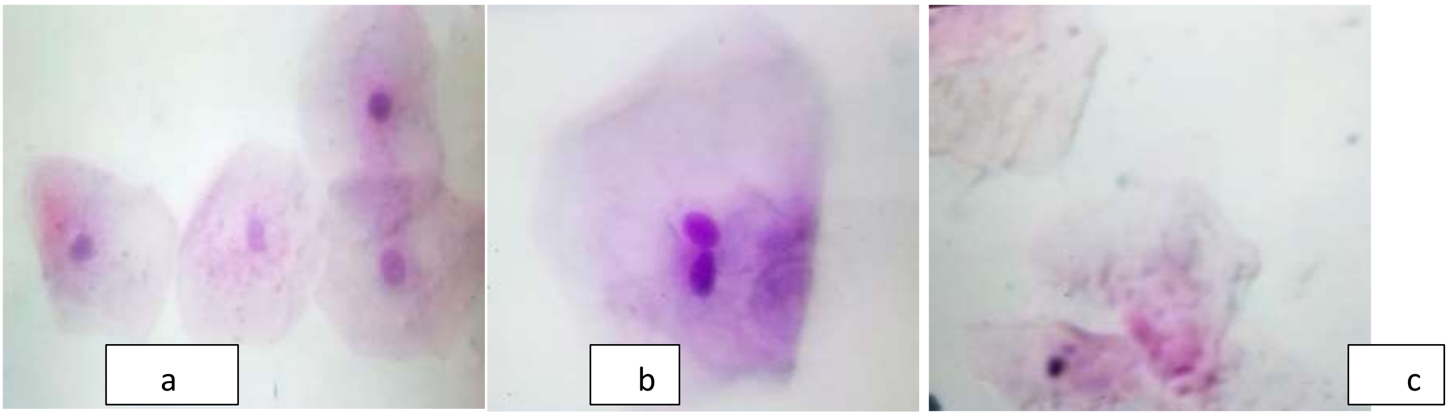

Figure 2. A: normal cell b: cell with condenced chromatin Binucleated cell, c: Karyolysis cell and karyorrhetic cell Maygrünwald + Gemsa stain 40X.

The examination of micronuclei performed on the epithelial cells of Oral is to detect biomarkers of early biological effects (20). Test of the micronuclei of epithelial cells is a cellular technique for measuring DNA damage and signs of cell death in the oral epithelium (21). Epithelial cells of the endothelium form the first barrier to inhalation or ingestion and are able to metabolize carcinogens near reactive products (22). The possibility of decomposition or degeneration in a cell in the form of condensed chromatin, pyknotic, loss of nuclear materials karyolitic, which is in the form of the socalled ghost.In rare cases, some cells can also appear with two nuclei in the same cytoplasm stage, or form a nuclear bud or broken egg or form small nuclei (MN) near nuclei in the same cytoplasm. These biomarkers for DNA damage (eg, MN, nuclear buds) and cell death (such as apoptosis, karyolysis) can be observed in epithelial cells, and through it can assess the genetic toxicity and effects of cell inhibition (23).

Determination of DNA damage in peripheral lymphocytes by comet assay technology:

Table 3 and figure 3 show Evaluate the mean differences of the affected DNA cells significant differences $p \leq 0.05$ were noticed in control and breast cancer patients.

Table 3. Percentage values of cells by DNA damage of in breast cancer women .

	Anomalies SD ± M	**Average of damage anomalies SE ± MD**	**Average of damage anomalies SE ± MD**	**Average of damage total cells SE ± MD**
control	48.33 ±6.05	-	72.45 ± 71.24	-
breast cancer women	39.48±5.03	*6.64± 1.2	21.02 ± 6.49	* 31.33 ± 12.15

* Significant at the P< 0. 05 (Independent Samples Test), M Average test, S.D standard deviation, MD average differences, S.E standard error

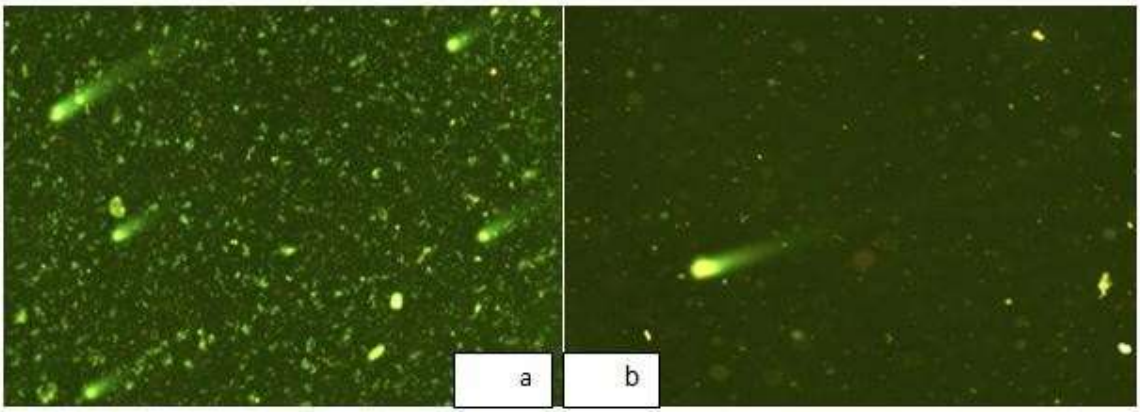

Figure 3. a and b Different levels of DNA damage in in peripheral lymphocytes in in breast cancer women: 100 X Cybergreen stain

This test helps find many main DNA lesions that other methods overlook as well as DNA damage. Applied to many different tissues or cell types sensitive to modest amounts of DNA damage, it requires relatively few cells per sample and finishes really quickly (24). This test is a priceless experimental tool for mapping DNA damage to human cells in vivo for therapeutic, environmental, and professional uses (e.g., pre-transplant storage, tissue engineering, and previous in vivo experimental testing). Furthermore, the comet test helps research many cell kinds for evaluation of DNA damage. Many organs include epithelial cells, one kind of cell type. These cells might be used for assessing genetic toxicity and for use as biological indicators for early impact (25). Given 80% of cancers start in epithelial cells, investigating DNA damage in these tissues is very essential. Studies like comet testing in epithelial cells help to investigate genetic toxicity both in the body and in laboratory environments and clearly have therapeutic value (26).

IGF1 gene expression

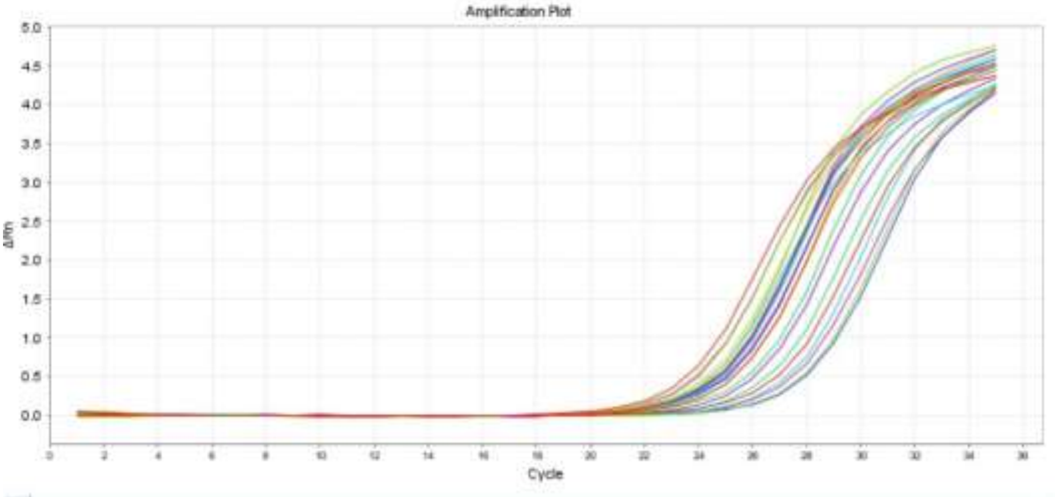

Figure 4. Rt-PCR results of related mRNA expression tested by threshold cycle (Ct)

The results revealed that IGF1 gene expression was significantly higher in breast cancer women with menopause compared to controls (p = 0.005), as shown in Table 4.

Table 4. IGF1 gene expression in breast cancer women and control.

mRNA	**Mean ΔCT± ES for control**	**Mean ΔCT± ES for Patients**	**p-value**	**ΔΔCt**	**Folding expression**
IGF1 gene mRNA	25.13±0.47	27.91±0.61*	0.05	-1.01	3.099

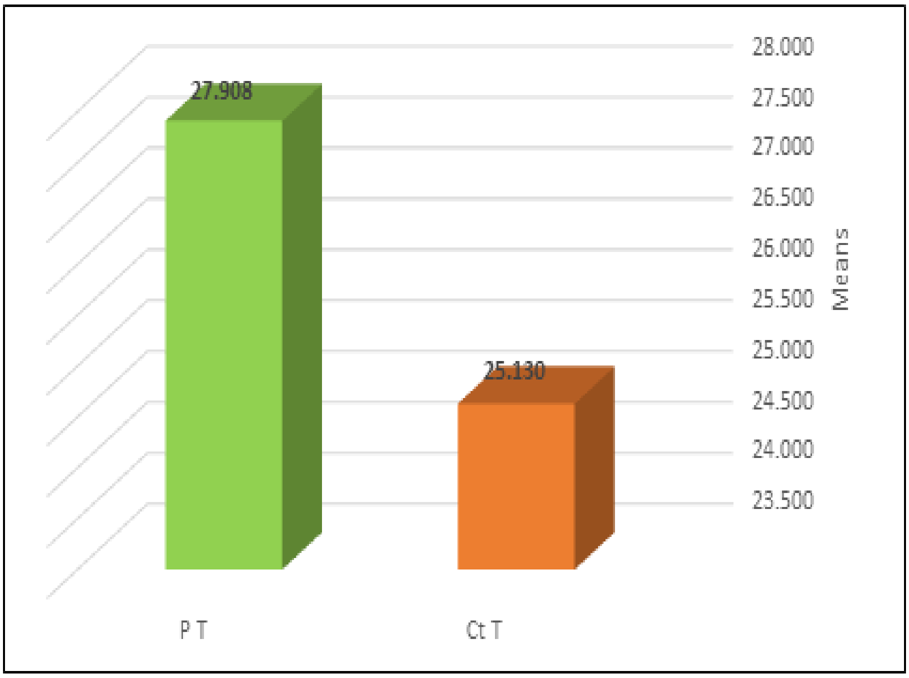

Figure 5. IGF1 gene expression in breast cancer women with menopause and control

Activation of the IGF1 receptor promotes the formation of tumors in the mammary glands by increasing glycolysis and, by extension, biomass production (27). We still don't know the fundamental mechanisms that link MetS and IGF-1. The Warburg effect, which involves hyperactivated glucose uptake and aerobic glycolysis, is believed to be one of six cancer hallmarks, along with colorectal cancer. The role of insulin/IGF-1 signaling in the development of CRC cells' Warburg metabolic traits is still not fully understood. It is highly probable that it is the outcome of various processes that are either directly or indirectly controlled by IGF-1. These processes include activation of various signaling pathways, such as PI3K/Akt/mTORC and Raf/MAPK, activation of glucose transporters (like GLUT1), activation of important glycolytic enzymes (like LDHA, LDH5, HK II, and PFKFB3), abnormal expression of oncogenes (like MYC and KRAS) and/or overexpression of signaling proteins (like HIF-1, TGF-β1, PI3K, ERK, Akt, and mTOR, among others (31,32,33,34).

Conclusions

The study revealed that IGF1 gene expression was significantly higher in breast cancer women compared to controls and confirmed the existence of significant genetic damage observed in breast cancer patients with as evidenced by elevated micronucleus and comet assay results compared to healthy controls. These findings underscore the need for enhanced public health strategies to address the implications of breast cancer patients-related cellular damage in the region.

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