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Molecular detection of GSTM and GSTT genes with biomarkers studies in samples of Iraqi breast cancer patients

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Abstract

To identify genes like GSTT and GSTM's impact on breast cancer development, as well as their relationship with age, blood group, white blood cell count, erythrocyte sedimentation rate, and lipoprotein levels. Genomic DNA was extracted from blood samples of 75 breast cancer cases and 40 control subjects, and detection was performed using multiplex PCR-based methods and biomarker examination. Gel electrophoresis showed significant differences in nucleic acid bands from GSTT and GSTM genes in patients with breast cancer. The Type O blood group was more prevalent, with a significantly higher age (51.74 ±1.56) compared to the control groups (34.50 ±3.03). White blood cell count was also significantly higher in the breast cancer group. The study revealed significant differences in erythrocyte sedimentation rate, high-density lipoprotein, and low-density lipoprotein levels in breast cancer patients compared to control groups, with both groups showing higher levels of these markers. The study indicates that GSTT and GSTM may increase breast cancer susceptibility, with the Type O blood group being more prevalent in patients. Other factors, such as white blood cell count, erythrocyte sedimentation rate, high-density lipoprotein, low-density lipoprotein, immunoglobulin G, and blood sugar, were also significantly higher in patients with breast cancer.

Keywords: Breast cancer risk, GSTM, GSTT, Iraq.

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1. Introduction

A group of more than a hundred disorders known as cancer occurs when some body cells develop uncontrollably and spread to other parts of the body. Cancer can start almost everywhere in the human body. Cancer can be caused by a variety of things, including physical agents, chemicals, hormones, oncoviruses, radiation, and hereditary factors [1]. Despite the fact that the frequency varies by nation and ethnic group, breast cancer (BC) is one of the most prevalent diseases and a significant public health problem among women worldwide [2]. Even though they only contribute to one-third of BC cases, age, family history, and a number of reproductive characteristics are all known risk factors [3].

Breast cancer is a multifactorial, polygenic disease that can be influenced by both environmental and genetic factors Ng et al. [4] and Gallegos-Arreola et al. [5] and the pathologic mechanism is yet unknown. As a result, studies on gene polymorphisms have gained importance in the overall growth of BC [6, 7]. Recently, certain genes have been identified as likely cancer susceptibility genes. A number of key physiological processes in the human body, including the detoxification of hazardous, potentially cancer-causing substances, depend on GSTs [8, 9]. Eight types of GST enzymes alpha (A), kappa (K), mu (M), omega (O), pi (P), sigma (S), theta (T), and zeta (Z) are found in humans [10, 11]. Depending on where within the cell they are found, GSTs are categorized as cytoplasmic (A, P, M, S, T, or Z), mitochondrial (K), or membrane-bound (M) (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism).

Due to the polymorphism of several GST genes, specific allelic variants are linked to altered risk (or outcome) of a number of disorders. Different populations have reported having these polymorphic GST gene variations. It has been established that the levels of the proteins GSTT and GST activity are much higher in tumor tissue than in healthy breast tissue. Additionally, GSTs function as cancer chemotherapy agents, which increases tumor resistance to these drugs [8].

The unique gene or collection of genes (such as GSTA, GSTM, GSTP, GSTS, GSTT, and GSTZ genes). GSTs may therefore be clinically useful in the case of some malignant tumors. Based on studies examining the distribution of GSTT and GSTM variants in patients with histologically diagnosed breast cancer in comparison to controls to explore the potential association of GST genotypes and risk of breast cancer, as well as the role of GSTs in inactivating endogenous metabolites during oxidative stress and its influence on the normal functions of mammalian tissues, some studies investigated the distribution of GSTT and GSTM variants in patients with histologically diagnosed breast cancer in comparison to controls [10-13]. The three gene changes may increase the risk of BC because of their biological effects [14, 15]. The combined effects of the three genes on breast cancer risk were not examined in their meta-analyses, nor were statistically significant associations evaluated. In addition, a large number of new studies have been published, necessitating a meta-analysis and re-analysis of earlier meta-analyses in order to better understand the individual and combined effects of these genes on breast cancer risk [16-18].

The aim of this study is to determine the degree to which various genes, including GSTT and GSTM, may be involved in the development of new breast cancer. Additionally, the country of Iraq has experienced numerous wars over the past forty years, which have caused widespread pollution.

2. Materials and Methods

2.1. Samples

Seventy-five blood samples were collected from infected subjects and fifty blood samples were collected from non-infected subjects (Oncology Center - Merjan Hospital) in Babylon Province, Iraq. Blood samples were collected from infected subjects of different ages. Clinical signs were also recorded, such as age and blood groups. In addition, some of the test analyses were recorded, such as lipid profile analysis (HDL and LDL), blood sugar (fasting blood glucose), IgG, ESR, and WBC count, to clarify how laboratory testing and breast cancer are related. The blood samples were preserved at -20 ℃ for molecular analysis.

2.2. Extraction of Genomic DNA for GSTT and GSTM Genes Using Multiplex PCR

Genomic DNA was extracted from whole blood using the "gSYNCTM DNA Extraction Kit" and stored at -80°C until use. Multiplex PCR was performed on the PCR system (Applied Biosystems) with a reaction volume of 25 µL, including 5 µL of Maxter Mix, 1 µL of each primer pair GSTM and GSTT (Forward 5′-GCTTCACGTGTTATGGAGGTTC-3′, Reverse 5′-GAGATGAAGTCCTCCAGATTT-3′, and Forward 5′-TTCCTTACTGGTCCTCACATCTC-3′, Reverse 5′-TCACCGGATCATGGCCAGCA-3′ respectively) and B-globin as control (Forward 5′-CAACTTCATCCACGTTCACC-3′, Reverse 5′-GAAGAGCCAAGGACAGGTAC-3′), 16.5 µL of nuclease-free water, and 1.5 µL of DNA. The amplification program was as follows: initial denaturation at 94°C for 15 min; denaturation-2 at 94°C for 120 sec., annealing at 60°C for 40 sec., extension 1 at 72°C for 11 min; and extra extension at 72°C for 10 min. The PCR products were subjected to 2% agarose gel electrophoresis at 70 volts for 30 min, using the UV transilluminator to visualize DNA bands.

2.3. Ethical Approval

All procedures followed the guidelines for the sake of scientific integrity; the ethical, human, and scientific aspects were taken into account in the process of collecting samples, especially since it was approved by the official authorities.

3. Results

3.1. Results of multiplex PCR for the detection the amplicon of DNA at the GSTT and GSTM genes

Gel electrophoresis revealed bands of extracted nucleic acid from GSTT and GSTM genes from patients with breast cancer (Figure 1) and the GSTT gene from patients with breast cancer with β-globin (Figure 2).

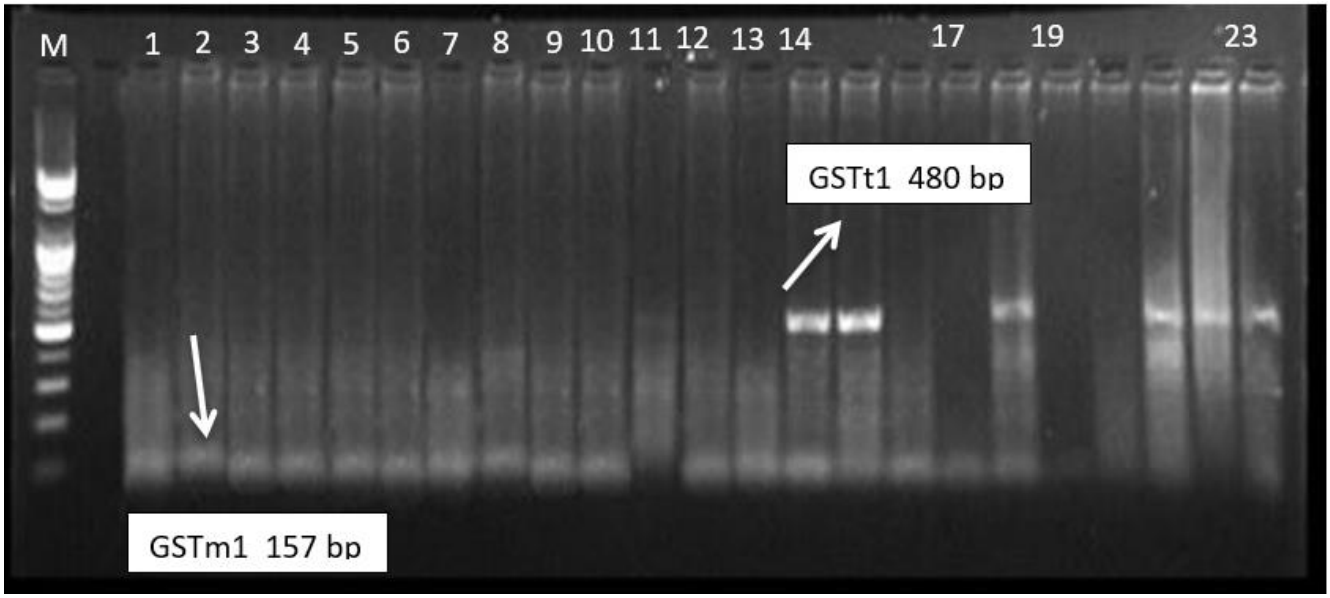

Figure 1.
PCR-multiplex electrophoresis gel. M = Molecular weight marker (100 bp); 1-10, 12, 13, 17, 16, 20= one present genotypes of GSTm; 14, 15, 18, 21, 22, 23= double GSTm, and GSTt; 19 = null genotypes.

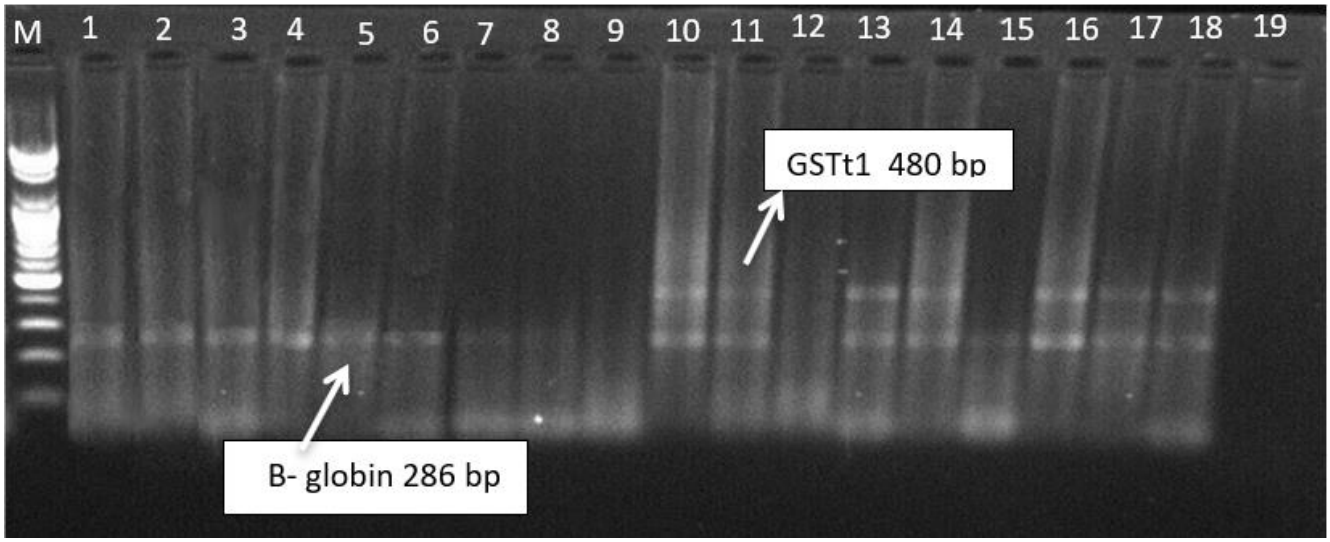

Figure 2.
PCR-multiplex electrophoresis gel. M = Molecular weight marker (100 bp); 1-9, 12, 15= genotypes of B-globin; 10, 11, 13, 14, 16-18= genotype GSTt and B-globin present; 19 = null genotypes.

3.2. Correlation Between Blood Group and Age at Healthy and Patients with Breast Cancer

Blood groups in patient group with breast cancer were statistically significant at (0.0382) in comparison with control groups (Table 1).

Type O of blood group in patient group were statistically significant at ($P \leq 0.05$) more than other phenotypes of blood group, while O type of blood group in patients with breast cancer at (27 out of 75 [34.00%]) in comparison with control groups at (16 out of 40 [40.00%]) (Table 1). Type A and B of blood group in patient group were statistically significant at ($P \leq 0.05$) more than AB type of blood group and less than O phenotype of blood group, while A and B types of blood group in patients with breast cancer at (18[24.00%]) in comparison with control groups at (8[20.00%]) (Table 1).

Type AB of blood group in patient group were decreased statistically significant at ($P \leq 0.05$) than other type of blood group, while AB type of blood group in patients with breast cancer at (12 out of 75 [18.00%]) in comparison with control groups at (8 out of 40 [20.00%]), (Table 1). The age in the patient group with breast cancer was highly statistically significant at (51.74 ±1.56) in comparison with the control groups at (34.50 ±3.03) (Table 1).

Table 1.
Distribution of sample study according to Blood group and age in patients and control.

Factors		Patients (No= 75)	Control (No= 40)	P-value
Blood group: No (%)	A	18 (24.00%)	8 (20.00%)	0.0382 *
	B	18 (24.00%)	8 (20.00%)	
	AB	12 (18.00%)	8 (20.00%)	
	O	27 (34.00%)	16 (40.00%)	
Age (year)	Mean ± SE	51.74 ±1.56	34.50 ±3.03	0.0001 **

Note: * (P≤0.05), ** (P≤0.01).

3.3. Correlation Between ESR And WBC In Healthy Individuals and Patients with Breast Cancer

White blood cell counts in the patient group with breast cancer were highly statistically significant at (P≤0.01) in comparison with control groups. White blood cells in the patient group at (21.55 ±0.37) were highly statistically significantly more than the control group at (5.32 ±0.25) (Table 2) (Figure 3). Similarly, the erythrocyte sedimentation rate (ESR) in the patient group with breast cancer was highly statistically significant at (P≤0.01) in comparison with the control groups. ESR in the patient group at (121.56 ±0.92) was highly statistically significant more than the control group at (13.30 ±1.33) (Table 2) (Figure 4).

Table 2.
Comparison between patients and control groups in ESR and WBC.

Group	Mean ± SE	
	ESR	WBC x 10^3
Patients	121.56 ±0.92	21.55 ±0.37
Control	13.30 ±1.33	5.32 ±0.25
T-test	4.311 **	1.717 **
P-value	0.0001	0.0001

Note: ** (P≤0.01).

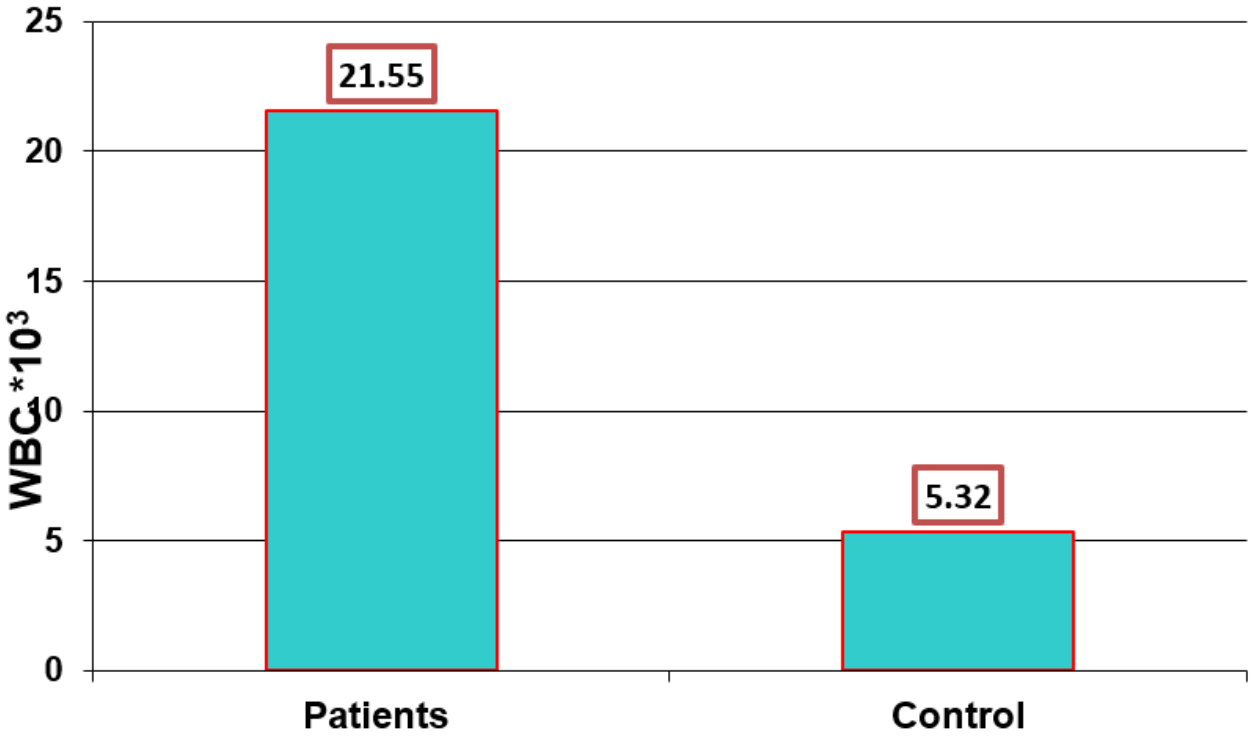

Figure 3.
Comparison between patients and control groups in WBC.

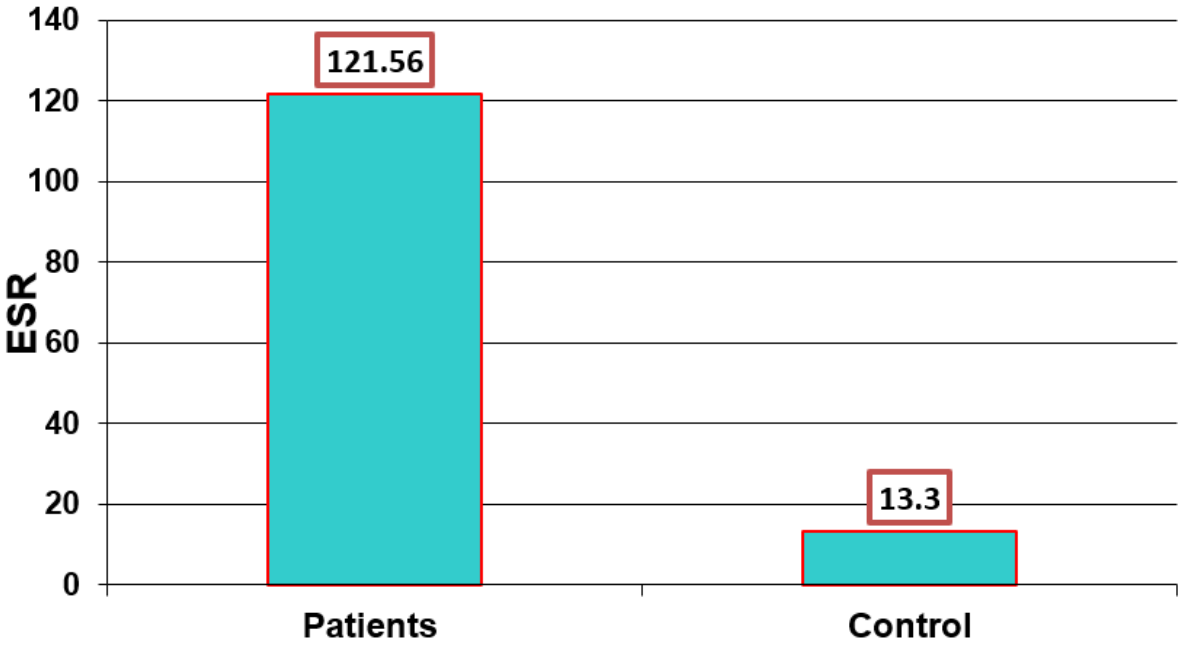

Figure 4.
Comparison between patients and control groups in ESR.

3.4. Correlation Between HDL and LDL at Healthy and Patients with Breast Cancer

The high-density lipoprotein (HDL) in the patient group with breast cancer was highly statistically significant at (P≤0.01) in comparison with the control groups. HDL in the patient group at (187.36 ±2.25) was highly statistically significant compared to the control group at (29.20 ±1.30) (Table 3) (Figure 5).

Likewise, the low-density lipoprotein (LDL) in patient group with breast cancer were highly statistically significant at (P≤0.01) in comparison with the control groups. LDL in patient group at (93.36 ±1.10) were highly statistically significant, more than control group at (25.00 ±2.87) (Table 3) (Figure 6).

Table 3.
Comparison between patients and control groups in HDL and LDL.

Group	**Mean ± SE**	
	HDL	**LDL**
Patients	187.36 ±2.25	93.36 ±1.10
Control	29.20 ±1.30	25.00 ±2.87
T-test	10.21 **	5.570 **
P-value	0.0001	0.0001

** (P≤0.01).

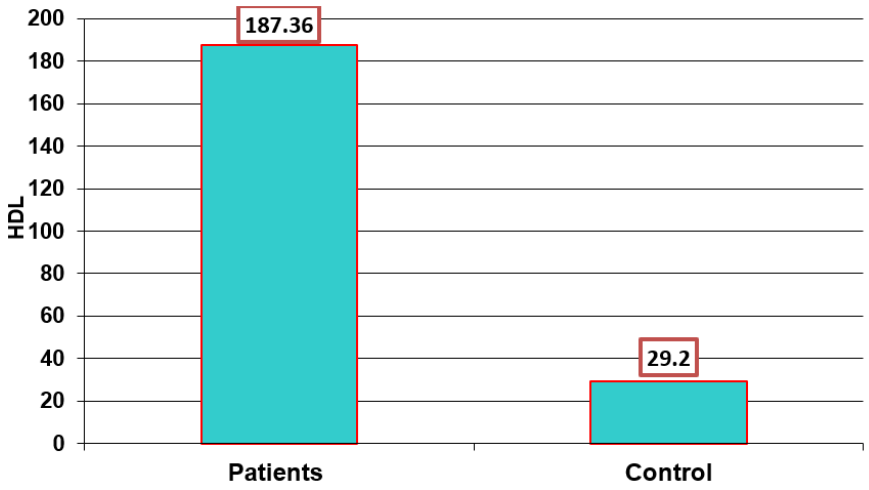

Figure 5.
Comparison between patients and control groups in HDL.

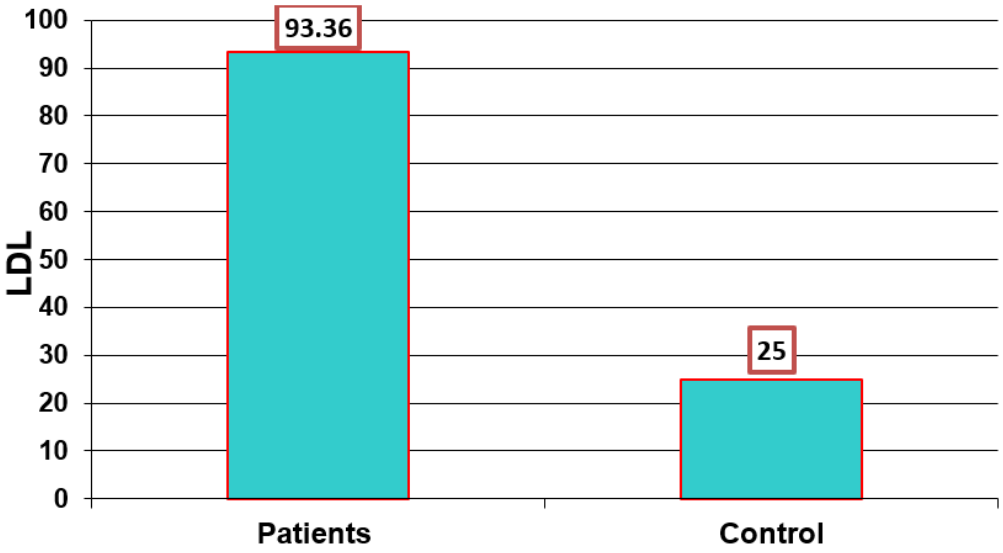

Figure 6.
Comparison between patients and control groups in LDL.

3.5. Correlation Between IgG and Blood Sugar in Healthy Individuals and Patients with Breast Cancer

The immunoglobulin G (IgG) in the patient group with breast cancer were highly statistically significant at (P≤0.01) in comparison with control groups. IgG in patient group at (5994.00 ±243.01) were highly statistically significant more than control group at (880.00 ±53.33) (Table 4) (Figure 7). As well, the Blood sugar in patient group with breast cancer were highly statistically significant at (P≤0.01) in comparison with control groups. Blood sugar in patient group at (152.66 ±5.73) were highly statistically significant more than control group at (117.10 ±4.76) (Table 4) (Figure 8).

Table 4.
Comparison between patients and control groups in IgG and Blood sugar.

	Mean ± SE	
Group	**IgG**	**Blood sugar**
Patients	5994.00 ±243.01	152.66 ±5.73
Control	880.00 ±53.33	117.10 ±4.76
T-test	1096.10 **	26.151 **
P-value	0.0001	0.0086

Note: ** (P≤0.01).

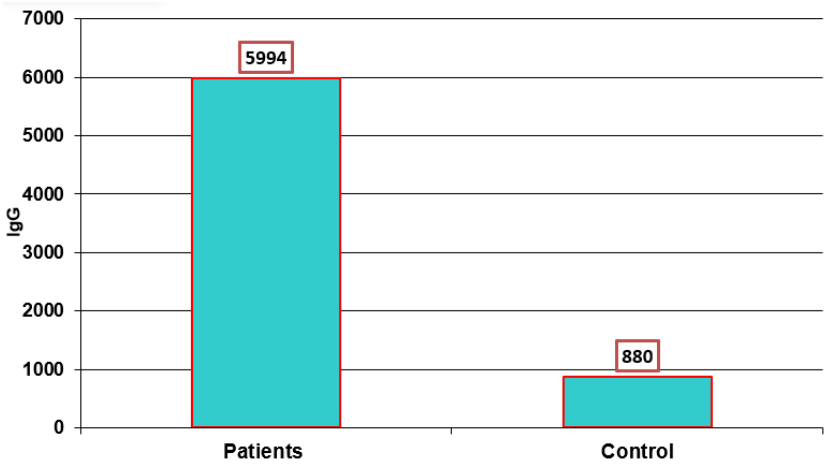

Figure 7.
Comparison between patients and control groups in IgG.

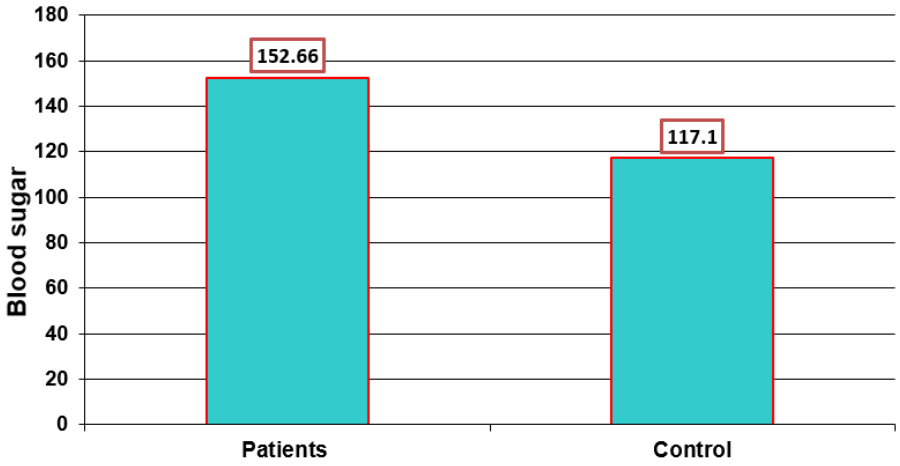

Figure 8.
Comparison between patients and control groups in Blood sugar.

4. Discussion

The results of this study of multiplex PCR electrophoresis gel revealed some bands as one present genotype of GSTm and others was double GSTm, and GSTt, and one of them was null genotype. A other study revealed no correlation between the GSTt-null genotype and overall breast cancer risk, while the GSTM1-null genotype has been linked to an elevated risk of breast cancer. These findings imply that variations in low-penetrance genes, including GSTm and GSTt, are linked to an increased risk of breast cancer [19]. However, a combined genetic variability in members of the GST gene family may be associated with an increased susceptibility to breast cancer [20]. In a Turkish study, neither the GSTT1-null genotype nor the GSTM1-null genotype was significantly associated with an increased risk of developing breast cancer. Strong evidence supporting earlier research comes from a Chinese meta-analysis that found no general correlation between the GSTM and GSTT deletion variants [21]. The GSTT/GSTM double null genotype, on the other hand, did not correlate with chemotherapy response in an Indian population but was found to be protective against the development of breast cancer [22].

Furthermore, our findings supported the hypothesis that the ABO blood group is related to breast cancer risk or survival. Additionally, we detected a strong correlation between the serologic ABO blood type and the overall incidence of breast cancer at ($P \leq 0.05$). Even though strong links between the ABO blood group and breast cancer risk have been found in several earlier research.

Some studies that have been published to date detected no connection with ABO blood group are explained by Dede et al. [23]. While Ronco et al. [24] reported substantial relationships in several studies. ABO blood type was disclosed in two research by Momenimovahed and Salehiniya [25]. One study indicated a positive link between type A and risk of ductal subtype breast cancer, while the other study reported a positive association between type O and breast cancer risk.

Additionally, the study of Bothou et al. [26] indicated that women with a family history of breast cancer showed positive connections with type A or B. The correlations between blood group and breast cancer survival have also been inconsistent, with two studies indicating worse outcomes for patients with blood type AB or B or any other non-O blood group and a third claiming no correlation. The tiny sample size used in the analysis constrained many earlier investigations. The use of retrospective cases, hospital-based controls, or other variations in demographic characteristics is further cause for the heterogeneity between studies [25].

The physiological mechanisms behind these relationships are still unknown, despite recent research by Abegaz [27] showing that the ABO blood group has been associated with risk for many tumor forms, including pancreatic and gastric cancer. Cid et al. [28] discovered the ABO gene encodes a glycosyltransferase with three primary variant alleles (A, B, and O) that each have a different substrate specificity. The H antigen is a protein with a backbone that the A, B, and O glycosyltransferases modify with N-acetylgalactosamine, D-galactose, or no sugar residue, respectively.

Red blood cells and many other tissues throughout the body, including breast ductal and lobular cells, display blood group antigens on their surfaces [29]. Concerning breast cancer and other tumor types, Croce [30] shed light on how malignant cells' surface ABO antigen expression differs from that of normal epithelium. The altered expression of blood group antigens on the surface of cancer cells was described by Le Pendu et al. [31] and has significant repercussions for the development of malignancy. ABO genotype has also been linked to circulating levels of tumor necrosis factor-alpha, E-selectin, and P-selectin, according to recent research by Groot et al. [29] raising the possibility that blood group antigens may affect the body's inflammatory response.

Dede et al. [23] revealed the chronic inflammation is another way that ABO antigens may affect cancer risk. Chronic inflammation has been strongly associated with the beginning and spread of cancer. While Gates et al. [32] explained no correlation was found between ABO blood group and incidence or survival of breast cancer. This is the first prospective study we are aware of looking at breast cancer risk in Iraq and the ABO blood group. In conclusion, our findings point to a link between the ABO blood group and the likelihood of developing or surviving breast cancer.

Asiri et al. [33] revealed that every year, about 80% of women with breast cancer are 45 years of age or older, and around 43% are 65 years of age or older. Think about this. Breast cancer has a one in 69 chance of occurring in women between the ages of 40 and 50. That risk rises to one in 43 between the ages of 50 and 60. While Duffy et al. [34] showed that age raises

the risk of breast cancer. After age 50, most breast cancer cases are discovered genetic changes. Breast and ovarian cancer risk is increased in women who have hereditary changes (mutations) to specific genes, such as BRCA1 and BRCA2.

In addition, our results showed that elevated WBC count in patients with breast cancer compared with non-patients. These results may depend on menopausal status, BMI, and ER/PR status, or may depend on metabolic syndrome and insulin resistance, such as inflammatory conditions. Park et al. [35] explained that breast cancer associated with non-obese patients and with ER+/PR+ breast cancer had raised WBC counts compared with postmenopausal obese women with ER−/PR− breast cancer. White et al. [36] suggest that chronic inflammation plays an important role in cancer development and obesity can play a crucial role in the development of breast cancer .

Lahmann et al. [37] demonstrated that the optimistic associations between breast cancer risk and obesity have been detected in postmenopausal women. WBCs, including neutrophils, monocytes, and eosinophils, produce reactive oxygen species (ROS) and nitric oxide species (NO), which are chemically reactive molecules. Okoh et al. [38] showed that the antioxidant defense system fails to neutralize ROS and NOS effectively; they can damage cellular proteins, lipids, and DNA, which can result in the accumulation of genetic instability, impact SNPs, or activate the PI3K-Akt pathway for carcinogenesis. Akinbami et al. [39] reported that WBC counts were higher in patients with breast cancer than in controls, but their study did not include information about menopausal status .

Okuturlar et al. [40] showed that neutrophil levels were associated with the risk of breast cancer, including Stage IV breast cancer. Several studies evaluated the correlation between breast cancer and WBC counts, El-Schich et al. [41]. Park et al. [35] illuminated that patients with breast cancer had a higher neutrophil/lymphocyte ratio. Hanker et al. [42] reported that because of the interaction between ER and PR status and inflammation, which can also affect endocrine resistance, ER and PR status can act as a catalyst for the development of breast cancer. Tumor necrosis factor alpha (TNF-α) and other pro-inflammatory cytokines raise the transcriptional activity of the NF-κB and JNK pathways, which may lead to the development of tumors or the development of resistance to hormone therapy.

According to the study's findings, ESR levels were statistically significantly higher in breast cancer patients than in control persons. It has been discovered that high ESR levels are associated with a generally bad prognosis for several cancers, including breast cancer. An increased ESR may still be a good indicator of an impending recurrence in some studies of patients with breast cancer, particularly if the value remained elevated after chemotherapy or failed to return to normal after six months of treatment Eboreime et al. [43].

Bochen et al. [44] reported the inflammatory cytokines, including those linked to C-reactive protein, are known to promote metastatic spread by stimulating angiogenesis, increasing vascular permeability, and functioning as an endothelial cell mitogen, a high ESR with C-reactive protein may indicate a high metastatic potential. Strojnik et al. [45] resulted of prospective epidemiologic research are inconsistent, with some studies demonstrating a link between high ESR levels and poor prognosis and other studies demonstrating no such link Eboreime et al. [43].

Stoerkel et al. [46] explained that women treated for early-stage breast cancer were found to have elevated levels of ESR measured two and a half years after the time of diagnosis and were associated with poor prognostic outcomes. This was linked to a lower overall survival rate. Velidedeoglu et al. [47] showed that another study in the same field found that invasive breast cancer patients with high ESR levels at diagnosis had a 1–7-fold higher probability of dying from the disease than those with low CRP levels. There have been encouraging reports about the prognostic importance of ESR in various cancers. Izuegbuna et al. [48] reported an increase in ESR levels in patients with chronic myeloid leukemia and came to the conclusion that ESR could be a helpful indicator of disease progression or a way to track how well a patient is responding to treatment. Patients with high ESR as seen in the index study, were reported by Al-Bairmany [49] in a comparable study from the year 2022.

Besides, our findings demonstrate that higher levels of HDL and LDL are linked to a higher risk of breast cancer (BC). Individual studies have reported either a positive relationship with HDL Martin et al. [50] or no relationship with HDL or LDL, contrary to previous meta-analyses of observational studies of BC risk and lipids that reported a negative association with HDL and no relationship with LDL Touvier et al. [51] and Borgquist et al. [52].

The study of Zhang et al. [53] showed these contradictory findings and inferred an impact direction, which is not achievable in observational studies due to the possibility of reverse causality. Additionally, they discover evidence of connection at the ABO locus and between several lipid characteristics and BC. While Berisa and Pickrell [54] revealed that the haplotype patterns indicate that ABO gene expression, not blood group, may be the causal mechanism, several research studies have established a relationship between blood group and BC risk.

Johnson et al. [55] revealed that genetically elevated plasma HDL and LDL levels appear to be associated with increased BC risk and the effects of HDL, BMI, and age at menarche. Fadiel and colleagues, Beeghly-Fadiel et al. [56] found a relationship between HDL-cholesterol and ER+ BC, whereas we found a relationship between HDL-cholesterol and risk for all BCs. Nelson [57] indicated do not provide proof for a particular carcinogenesis process, but they do draw fresh attention to putative mechanisms that need further functional investigation. Breast tumorigenesis may be directly influenced by cholesterol and its oxysterol metabolites, whether in the circulatory system or the local mammary microenvironment . These results suggest a causal link between elevated HDL cholesterol and a higher chance of developing BC, and this theory merits additional investigation. Statins are frequently used to lower LDL levels, however, they also raise HDL levels. If additional research confirms the link between greater HDL levels and a higher chance of developing BC, the conventional wisdom that HDL is "good cholesterol" or has beneficial effects may need to be reassessed.

In this discovery study, higher levels of IgG and linked to a higher risk of breast cancer. Breast cancer, like other tumor forms, includes a variety of cell types in the tumor microenvironment in addition to being heterogeneous in terms of patients. On their cell surfaces or after disintegrating, cancer cells, tumor-associated fibroblasts, endothelial cells, pericytes,

inflammatory cells, and extracellular matrix components can all exhibit a wide variety of antigens. Because cancer cells accumulate genetic and epigenetic alterations (such as growing aneuploidy) that result in a significant amount of aberrantly produced proteins, the issue only gets more complicated as the disease progresses. In the best-case scenario, cytotoxic T-lymphocytes and natural killer cells may eliminate cancer cells if the immune system can identify these tumor-associated antigens as "non-self." Regrettably, using the 'immune editing mechanism', Waks and Winer [58].

Gu et al. [59] improved the cancer cells can avoid identification and eradication. We could detect evidence of both escape and elimination events when comparing the IgG levels between the cancer and control groups. This is related to the negative control of T-cell function that CTLA-4-mediated signaling in CTLs, which is unfavorable in the case of cancer. Alraouji et al. [60] revealed the FDA has approved the use of monoclonal antibodies to disrupt CTLA-4's interaction with its ligand, which is known as the earliest discovered immune checkpoint molecule. LAG-3 is a member of the immunoglobulin superfamily and, like CTLA-4, negatively controls T cell function. Burugu et al. [61] showed that LAG-3 has emerged as an intriguing novel target for immunological therapy, as it was demonstrated that the administration of anti-LAG-3 antibodies could impair T-reg-mediated immune suppression.

Analysis of these proteins also revealed that they are involved in tumor angiogenesis, which is regarded as a key characteristic of cancer Ciccone et al. [62]. Kontomanolis et al. [63] revealed that both immune-inflammatory cells (such as macrophages) and tumor cells can release vascular endothelial growth factor, which can stimulate angiogenesis that promotes tumor growth. Furthermore, proteases that break down the extracellular matrix can release matrix-bound latent VEGF ligand. Additionally, the PTEN and mTOR pathways are recognized [64]. These are often overexpressed (mTOR) or inactivated (PTEN) in a variety of tumors, including breast cancer, and are closely related to the AKT/PI3P signal transduction pathway. Currently, the development of cancer is characterized by the presence of immunological inflammatory cells in tumor locations Ciccone et al. [62] and Wang et al. [64].

Garaud et al. [65] discovered that the increased macrophage activity could signify more tumor-associated macrophages or an increase in the clearance of apoptotic/necrotic cells or cell debris (TAM). Given that they have been linked to a worse outcome in several cancer forms, these macrophages ultimately enhance tumor development and spread by facilitating angiogenesis and matrix remodeling. Increased IgG levels (5994.00 ±243.01) from our dataset are also a sign of inflammatory conditions, albeit it is challenging to determine how tumor-promoting or tumor-antagonistic processes occur.

Charitha et al. [66] found that LKB1-mediated signaling is one of the mechanisms that are highly represented. LKB1 is important in the process of contact inhibition with regard to its role in epithelial polarity because suppression of its expression compromises epithelial integrity and makes cells vulnerable to Myc-induced transformation. Yuan et al. [67] showed that the molecular association composition analysis of the LKB1 pathway revealed Ezrin (EZR) to be a distinct protein. The actin microfilament-associated EZR plays a significant role in tumor-induced lymphangiogenesis and is a critical regulator of Src activity [66, 67]. Additionally, CYLD protein was found in breast cancer sample interactions mediated by Beta1-integrin and signaling mediated by LKB1. The primary way that CYLD controls inflammation is by inhibiting the activation of NF-kappa-B signaling by TNFR.

The study of Reddy et al. [68] revealed the influence of cell division and proliferation by activating signaling pathways including Akt, MAPK, or Wnt/Beta-Catenin. Andalib et al. [69] showed that the loss of CYLD increases NFKB signaling and promotes breast cancer metastasis. While the study of Janostiak et al. [70] presented that the c-Kit receptor is a member of the receptor tyrosine-kinase family and can regulate apoptosis, proliferation, differentiation, and cell motility in different types of blood cells when it binds to its ligand stem cell factor (SCF) (red blood cell, T-cells, mast-cells). Additionally, it plays a significant role in the development of gametes, melanin, and the gastrointestinal tract's Cajal cells. Although its precise function in the development of breast cancer is yet unknown, c-Kit overexpression was frequently detected in triple-negative breast cancer Beneventi et al. [71].

Biswas et al. [72] demonstrated that the Aurora-A signaling pathway is yet another signaling cascade. Aurora-A has been demonstrated to be required for centrosome separation and mitotic entry, and it is typically overexpressed in breast cancer and other cancer types but not in benign breast lesions. The fact that Aurora A exhibits both tumor suppressive and oncogenic properties has made it more difficult to create inhibitors for this pleiotropic protein recently [73]. In conclusion of this study, the AKT/PI3K/mTOR pathway, PTEN, c-Kit, and Aurora A-signaling may be the pathways that were most closely connected to breast cancer when the IgG increased in patients with breast cancer and control samples were compared. Therefore, some signaling is then being driven by these signaling, which may compromise cell adhesion, contact inhibition, and vascularization. The invading immune cells could be a sign of inflammation, with leukocyte and cancer cell interactions influencing the immune response.

5. Conclusion

In this study, that greater levels of blood sugar and linked to a higher risk of breast cancer. Increased blood glucose levels (152.66 ±5.73) compared with the control group (117.10 ±4.76) from our dataset. For more than 50 years, people have believed that since more glucose is available in the circulation, potentially cancerous cells may grow faster because they need glucose to advance [74]. But some studies Boyle et al. [75], As shown that in the treatment of breast cancer, glucose levels are crucial. Numerous studies have looked at the impact of hyperglycemia on tumor cell biology and therapy. Additionally, some investigations revealed a correlation between hyperglycemia and higher toxicity after chemotherapy for solid and hematologic malignancies [76]. Therefore, the study of Shu et al. [77] revealed that fasting hyperglycemia has been linked to an independent role in carcinogenic processes with a range of putative mechanisms, including the production of free radicals and the stimulation of DNA repair enzyme damage.

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