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Welcome to the Int J Cancer and Biomedical Research (IJCBR)!

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

A handwritten signature in blue ink that reads "Mohamed L. Salem".

**Mohamed L. Salem,**

Editor in Chief

# Glucose Transporter 4 and Interleukin 8 expression in hormone receptor-negative/HER2 overexpressing breast carcinoma subtype: Correlation with the biological behavior of the tumor cells and prognostic parameters

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## ABSTRACT

**Background:** Breast carcinoma (BC) is the most commonly occurring cancer. Nearly 15–20% of breast carcinomas are HER2 overexpressing subtype. Patients with this subtype have a poor prognosis with fewer therapeutic options. Increased glucose transporters expression is crucial for enhancing glucose uptake observed in several tumors. Overexpression of IL-8 has been found in neoplastic epithelial cells of various malignancies which are involved in tumor cell proliferation and invasion. **Aim:** Study the immunohistochemical (IHC) expression of GLUT4 and IL-8 in ER-, PR-/HER2+ BC subtype to correlate their expression with the biological behavior of tumor cells and prognosis. **Materials & Methods:** Fifty-eight ER-, PR-/HER2+ primary invasive ductal breast carcinoma NOS paraffin blocks were collected from archive of Pathology department, Faculty of medicine, Tanta University and private labs. Cut sections were stained with primary anti-GLUT4, anti-IL-8, and anti-Ki67 antibodies. **Results:** High GLUT4 expression was significantly associated with advanced TN staging and lympho-vascular invasion (LVI). A significant relationship was found between high IL-8 immuno-expression and TN staging and LVI. A significant positive correlation was observed between high GLUT4 and IL-8 expressions on one hand and positive Ki67 immunoreactivity in tumor cells on the other hand. No statistically significant association was detected between either GLUT4 or IL-8 expression and patient age, tumor grade, or M stage. **Conclusion:** GLUT4 and IL-8 expression in ER-, PR-/HER2+ BC were associated with poor prognostic parameters, thus downregulation of GLUT4 and IL-8 might provide alternative therapeutic choices for patients with this subtype.

**Keywords:** ER-/HER2 positive breast carcinoma; GLUT4; IL-8; immunohistochemistry cell biology

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## INTRODUCTION

Breast carcinoma is the most commonly occurring cancer representing about 11.7% of all cancer, and the second cause of cancer-related death in females worldwide (Sung et al., 2021). In Egypt, carcinoma of the breast is the most common malignant tumor among women, with the number of cases diagnosed in 2018 was 23,081 (35.1%) and deaths were 9,254 (10.8 %) (Bray et al., 2018).

According to molecular profiles, breast carcinomas have been divided into five subtypes: luminal A (ER+, PR+ /HER2-), luminal

B (ER+, PR-/HER2+), HER2 positive/enriched subgroup (ER-, PR- /HER2+), the basal-like (ER-, PR-/ HER2-) and normal-like (Wirapati et al., 2008; Parker et al., 2009).

Human Epidermal Growth Factor Receptor 2 (HER2), is a member of the EGF receptor (EGFR) family with tyrosine kinase activity which is encoded by ERBB2/HER2 gene (Ferrari et al., 2016). HER2 activates cell proliferation, angiogenesis, and invasiveness resulting in an aggressive neoplasm with poor prognosis and adverse outcome (Godoy-Ortiz et al., 2019). Nearly 15–20% of breast carcinomas are HER 2 overexpressing subtype, these tumors are

called HER2 positive breast carcinoma (Wolff et al., 2018). Several therapeutic approaches have been upgraded for the treatment of ER-/HER2+ carcinoma, to improve the prognosis. Though, there has been a discrepancy in the determination of the clinical status and outcome of ER-/HER2+ patients till now (Cejalvo et al., 2018).

Aberrant cellular metabolism has been identified as an important feature of cancer cells to facilitate tumor growth and progression. Normal cells depend on oxidative phosphorylation of glucose for ATP generation and energy production. In contrast, cancer cells divert their metabolism to anaerobic glycolysis for a generation of energy needed for cell division even in the presence of sufficient oxygen. This was known as the Warburg effect (Hanahan and Weinberg, 2011). As glycolysis is approximately 18 times less efficient in ATP production compared to oxidative phosphorylation, the cancer cells have to increase glucose uptake compared to normal cells to compensate for low ATP (Qian et al., 2014).

Moving glucose across the cell membrane to enter the cells is an initial crucial step in glycolysis, which may occur via passive and active transport. Passive transport is mediated by a family of glucose transporters (GLUTs) which consists of fourteen isoforms: GLUT-1 to GLUT-14 (Mueckler and Thorens, 2013).

As glycolysis has a significant role in tumor cell survival and proliferation, consequently cancer cells have to increase the expression of GLUTs to meet the demand of increased rates of glucose uptake (Herling et al., 2011). One of the important isoforms is GLUT4 (human SLC2A4: 17p13) because it carries an essential role in glucose homeostasis throughout the body (Herman and Kahn, 2006). GLUT4 is expressed in insulin-sensitive tissues as adipose tissues and skeletal muscles (Ancey et al., 2018). It has been also expressed in other tissues not sensitive to insulin as colon, breast, thyroid, pancreatic, and gastric carcinomas (Medina et al., 2003; Garrido et al., 2013; Moreira et al., 2013), meaning that non-sensitive insulin tissues can express GLUT4 when they turn malignant (Barrona et al., 2016).

Interleukin-8 (IL-8); also known as CXCL8 is a potent chemotactic cytokine for neutrophils. It is secreted by activated macrophages, lymphocytes, neutrophils, endothelial, and various normal epithelial cells. IL-8 activates multiple intracellular signaling processes via two cell-surface receptors, CXCR1 and CXCR2 (Liu et al., 2016). Overexpression of IL-8 has been found in neoplastic epithelial cells of various malignancies which are involved in tumor cell proliferation, survival, invasion, migration, and angiogenesis through several pathways (Waugh and Wilson, 2008). Increased IL-8 expression was detected in neoplastic breast tissues compared to normal tissue. Among the 5 subtypes of breast carcinoma, IL-8 is expressed highly in ER-negative, PR-negative, and HER2-positive breast cancers than in hormone receptor-positive subtypes, and this overexpression was associated with tumor metastasis, advanced stage, and poor prognosis (Chavey et al., 2007). This work aims to study the immunohistochemical expression of GLUT4 and IL-8 in ER-, PR-/HER2 positive breast carcinoma subtype to correlate the expression of these two markers with the biological behavior of tumor cells and prognostic parameters.

## MATERIALS AND METHODS

After the acceptance of the Research Ethics Committee-Faculty of Medicine-Tanta University, cases were collected from the archive of the Pathology Department, Faculty of Medicine, Tanta University, and private laboratories. The selected 58 cases were ER-,PR-/HER2 overexpressing primary invasive ductal breast carcinoma NOS subtype (IDC NOS).

None of the cases had received neoadjuvant therapies. All specimens were modified radical mastectomy ones. Classic pathological data as histological type, tumor size, degree of differentiation, and axillary lymph node status were obtained from the pathology reports, in addition to biomarker IHC profile (the hormone receptors status and HER2 expression). Only HER2-positive (defined by complete intense homogenous membrane staining in > 10% of tumor cells; score 3+ Figure 1) were included in the study (Wolff et al., 2018). Tumors have been graded and staged



according to the Nottingham system and the International Union Against Cancer TNM Classification respectively (Tan et al., 2020).

### Immunohistochemistry

The paraffin-embedded tissue blocks were cut into 5- $\mu$ m-thick sections, deparaffinized with xylene, and then rehydrated using descending grades of ethanol. The sections were incubated in citrate buffer (pH 6.0) for retrieving antigen. Incubation at 4°C with GLUT4 polyclonal antibody (1:100; ABclonal, USA), IL-8 polyclonal antibody (1:100; ABclonal, USA) and Ki67 polyclonal antibody (1:100; ABclonal, USA) was performed. The slides were then stained with hematoxylin.

### Evaluation of IHC staining and statistical analysis

#### 1. GLUT4 IHC evaluation

Five fields were randomly selected (x400) and visualized. The quantitative analysis of GLUT4 protein expression was performed. The cells with brown particles in the cell membrane or cytoplasm were considered as positive cells: 0% of cells positive (-, negative), < 25% of cells positive (+, weakly positive), 25%–50% of cells positive (++, moderately positive), > 50% of cells positive (+++, strongly positive). Negative and weakly positive were evaluated as low expression, and moderately and strongly positive were evaluated as a high expression (Zhai et al., 2012). Skeletal muscles were used as positive control.

#### 2. IL-8 IHC evaluation

Cytoplasmic and membranous staining for IL-8 was evaluated, the intensity of staining was scored as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The percentage of stained cells was grouped as no staining = 0, 1–10% of stained cells = 1, 11–50% = 2, 51–80% = 3, and 81–100% = 4. Value of <4 and  $\geq$  4 divide the tumors into low and high IL-8 expression groups respectively (Lee et al., 2012).

#### 3. Ki67 IHC evaluation

The number of cells showing nuclear staining was counted and the percentage was obtained as the number of Ki67 positive cells over the total number of counted tumor cells.

The cutoff value of Ki67 was defined as at least 10% as approved by several studies (Inwald et al., 2013; Kamranzadeh et al., 2019). Tumors with <10% of stained cells were considered negative, and those with 10% or more of stained cells were considered positive Ki67 expression.

### Statistical analysis

Statistical analysis of the present study was conducted, using SPSS (version 20) (Chicago, IL, USA). The association between clinicopathologic factors and expression of GLUT4, IL-8, and Ki67 was determined using the Chi-square test. The correlation between biomarkers expression was performed using Spearman correlation (*r*). The results were considered statistically significant if the *p*-value was < 0.05.

## RESULTS

### Clinicopathological characteristics

The study was carried on 58 cases of ER-, PR-/HER2+ IDC NOS subtype. The age of the patients was ranged from 43-72 years with a mean age (57.31 $\pm$ 8.87). Most of the cases were postmenopausal  $\geq$  50 (69%). Grade II tumors were seen in 32 cases (55.2%) and grade III in 26 cases (44.8%). According to TNM classification, most of the cases were of T<sub>2</sub> stage (46.5%), while T<sub>1</sub> and T<sub>3</sub> stages were found in 12 cases (20.7%) and 19 cases (32.8%) respectively. Axillary lymph node metastasis was found in 52 cases (89.7%), lympho-vascular invasion (LVI) was detected in 38 cases (65.5%), and distant metastasis in 15 cases (25.9%). Ki67 positive nuclear immunoreactivity was found in 41 tumors (70.7%) (Figure 2) (Table 1).

### GLUT4 expression and its relation to clinicopathological characteristics

The majority of studied tumors (42/58) showed high cytoplasmic and /or membranous GLUT4 expression in their cells (72.4%). High GLUT4 immunolabeling was found in 30 postmenopausal cases (71.4%) and 12 premenopausal cases (28.6%). Twenty-two grade II cases (52.4%) and 20 grade III cases (47.6%) showed high GLUT4 immunostaining.

**Table 1.** Clinicopathologic characteristics of studied cases

Clinicopathologic characteristics	Number (%)
Patient's age	Mean± SD
< 50	57.31±8.87
≥ 50	18 (31) 40 (69)
Degree of tumor differentiation	
Grade II	32 (55.2)
Grade III	26 (44.8)
Tumor size (T stage)	
T <sub>1</sub>	12 (20.7)
T <sub>2</sub>	27 (46.5)
T <sub>3</sub>	19 (32.8)
Axillary lymph node status (N stage)	
N <sub>0</sub>	6 (10.3)
N <sub>1</sub>	16 (27.6)
N <sub>2</sub>	25 (43.1)
N <sub>3</sub>	11 (19)
Lympho-vascular invasion (LVI)	
Present	38 (65.5)
Absent	20 (34.5)
Distant metastasis (M stage)	
M <sub>0</sub>	43 (74.1)
M <sub>1</sub>	15 (25.9)
Ki-67	
Negative	17 (29.3)
Positive	41 (70.7)

High GLUT4 expression was noticed in 22 (52.3%) T<sub>2</sub> cases and 15 (35.7%) T<sub>3</sub> cases respectively, whereas only 5 (12%) T<sub>1</sub> cases showed high GLUT4 immunostaining. High GLUT4 immuno-expression was detected more frequently in tumors with LVI (78.6%) than tumors without (21.4%). Most of the axillary lymph node positive cases showed high GLUT4 immunohistochemical expression in which 9 (21.4%) N<sub>1</sub>, 21(50%) N<sub>2</sub>, and 10 (23.8%) N<sub>3</sub> cases were of high immunoreactivity. While only two N<sub>0</sub> cases (4.8%) exhibited high GLUT4 expression. The majority of M<sub>1</sub> studied cases (12/15) showed high GLUT4 expression, and 30/43 M<sub>0</sub> cases showed high GLUT4 expression (Figure 3). There was a significant relationship between GLUT4 immunostaining and tumor size (p=0.027), LVI (p=0.001), and axillary lymph node status (p=0.017). On the other hand, no significant association was detected between GLUT4 expression and patient's age (p=0.361), tumor grade (p=0.348), or distant metastasis (p=0.445) (Table 2).

#### **Correlation between GLUT4 and Ki67 expression in breast carcinoma studied cases**

A strong positive significant correlation (r=0.704, p=0.0001) was found between immunohistochemical expression of GLUT4 and

positive Ki67 nuclear reactivity in ER-,PR-/HER2+ BC cases. Tumors with high GLUT4 immunolabeling expressed a higher rate of Ki67 positivity than tumors with low GLUT4 expression. In which 38 cases (90.5%) of high GLUT4 immunostaining tumors were Ki67 positive compared to 3 cases (18.7%) of low GLUT4 expression that were Ki67 positive (Table 3).

#### **IL-8 expression and its relation to clinicopathologic characteristics**

The relationship between IL-8 immunohistochemical staining and the clinicopathological characteristics of studied cases was summarized in Table 4. Forty cases (70%) showed positive cytoplasmic and membranous IL-8 staining. High IL-8 expression was found in 28 cases (70%) of the postmenopausal age group and 12 cases (30%) of premenopausal one with no statistically significant difference between the two age groups (p=0.515) and IL-8 expression. Grade II tumors (60%) showed high IL-8 immuno-expression more frequent than grade III tumors (40%), however, no significant statistical relationship was found between IL-8 immunostaining and tumor grade (p=0.207) (Figure 4). Significant statistical relationship was detected between TN staging and IL-8 expression, in which IL-8 positivity was increased with increased tumor size (7.5% T<sub>1</sub>, 52.5% T<sub>2</sub>& 40% T<sub>3</sub>) (p=0.001), and positive lymph node status (97.5%) (p=0.002). Also, tumors with LVI showed significantly high IL-8 expression (77.5%) compared to tumors without LVI (p=0.004). IL-8 immuno-expression did not significantly differ between M<sub>0</sub> cases (67.5%) and M<sub>1</sub> cases (32.5%) (p=0.085).

#### **Correlation between IL-8 and Ki67 immunohistochemical expression**

Thirty-three out of 40 tumors with high IL-8 immunostaining (82.5%) showed positive Ki-67 expression as well, compared to 7 (17.5%) cases with high IL-8 immuno-expressive tumors showed <10% of Ki67 immunoreactive cells. There was a significant positive correlation between high IL-8 expression and Ki67 positive immunoreactivity in ER-, PR-/HER2 overexpressing tumors (r=0.387, p=0.003) (Table 5).

**Table 2.** GLUT4 immunohistochemical expression and its relation to clinicopathologic characteristics

	GLUT4 expression n (%)		Chi-square	p-value
	Low n=16	High n=42		
Age				
< 50	6 (37.5)	12 (28.6)	0.432	0.361
≥ 50	10 (62.5)	30 (71.4)		
Degree of tumor differentiation				
Grade II	10 (62.5)	22 (52.4)	0.489	0.348
Grade III	6 (37.5)	20 (47.6)		
T stage				
T <sub>1</sub>	7 (43.8)	5 (12)	7.169	0.027*
T <sub>2</sub>	5 (31.2)	22 (52.3)		
T <sub>3</sub>	4 (25)	15 (35.7)		
LVI				
Present	5 (31.3)	33(78.6)	11.484	0.001*
Absent	11 (68.7)	9 (21.4)		
N stage				
N <sub>0</sub>	4 (25)	2 (4.8)	10.244	0.017*
N <sub>1</sub>	7 (43.7)	9 (21.4)		
N <sub>2</sub>	4 (25)	21(50)		
N <sub>3</sub>	1 (6.3)	10 (23.8)		
M stage				
M <sub>0</sub>	13 (81.3)	30(71.4)	0.583	0.445
M <sub>1</sub>	3 (18.8)	12(28.6)		

LVI: lympho-vascular invasion, \*: statistically significant

**Table 3.** Correlation between GLUT4 and Ki67 expression in breast carcinoma studied cases

	GLUT4 expression n (%)		r	p-value
	Low n=16	High n=42		
<b>Ki67 expression</b>				
<b>Negative</b>	13 (81.3)	4 (9.5)	0.704	0.0001*
<b>Positive</b>	3 (18.7)	38 (90.5)		

r: Spearman correlation, \*: statistically significant

**Table 4.** IL-8 expression and its relation to clinicopathologic characteristics

	IL-8 IHC expression n (%)		Chi-square	p value
	Negative n=18	Positive n=40		
Age				
< 50	6(33.3)	12(30)	0.064	0.515
≥ 50	12(66.7)	28(70)		
Degree of tumor differentiation				
Grade II	8 (44.4)	24 (60)	1.215	0.207
Grade III	10 (55.6)	16 (40)		
T stage				
T <sub>1</sub>	9(50)	3(7.5)	13.880	0.001*
T <sub>2</sub>	6(33.3)	21(52.5)		
T <sub>3</sub>	3(16.7)	16(40)		
LVI				
Present	7(38.9)	31(77.5)	8.192	0.004*
Absent	11(61.1)	9(22.5)		
N stage				
N <sub>0</sub>	5(27.8)	1(2.5)	12.365	0.002*
N <sub>1</sub>	7(38.9)	9(22.5)		
N <sub>2</sub>	4(22.2)	21(52.5)		
N <sub>3</sub>	2(11.1)	9(22.5)		
M stage				
M <sub>0</sub>	16(88.9)	27(67.5)	2.962	0.085
M <sub>1</sub>	2(11.1)	13(32.5)		

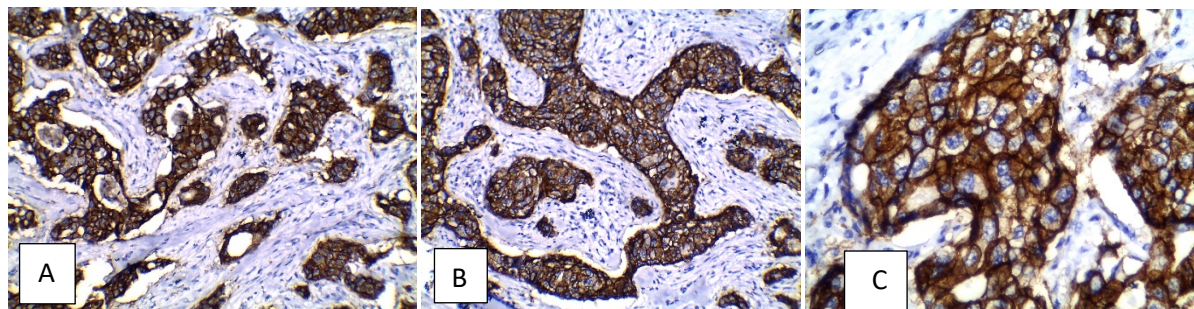
LVI: lympho-vascular invasion, \*: statistically significant



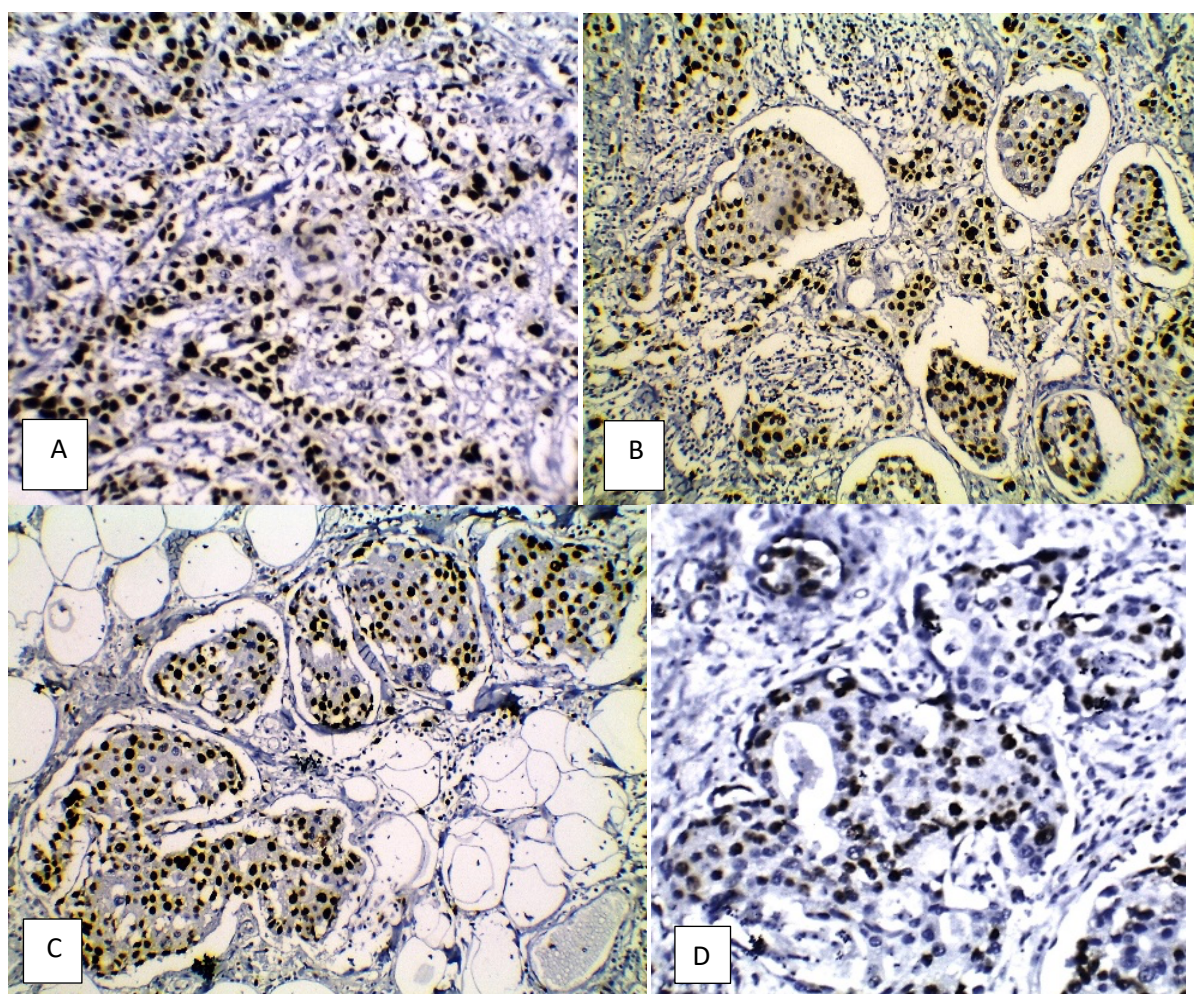
**Table 5.** Correlation between IL-8 and Ki67 expression in the studied cases

	IL-8 expression n(%)		r	p value
	Low n=18	High n=40		
<b>Ki-67 IHC expression</b>				
<b>Negative</b>	10(55.6)	7(17.5)	0.387	0.003*
<b>Positive</b>	8(44.4)	33(82.5)		

r: Spearman correlation, \*: statistically significant

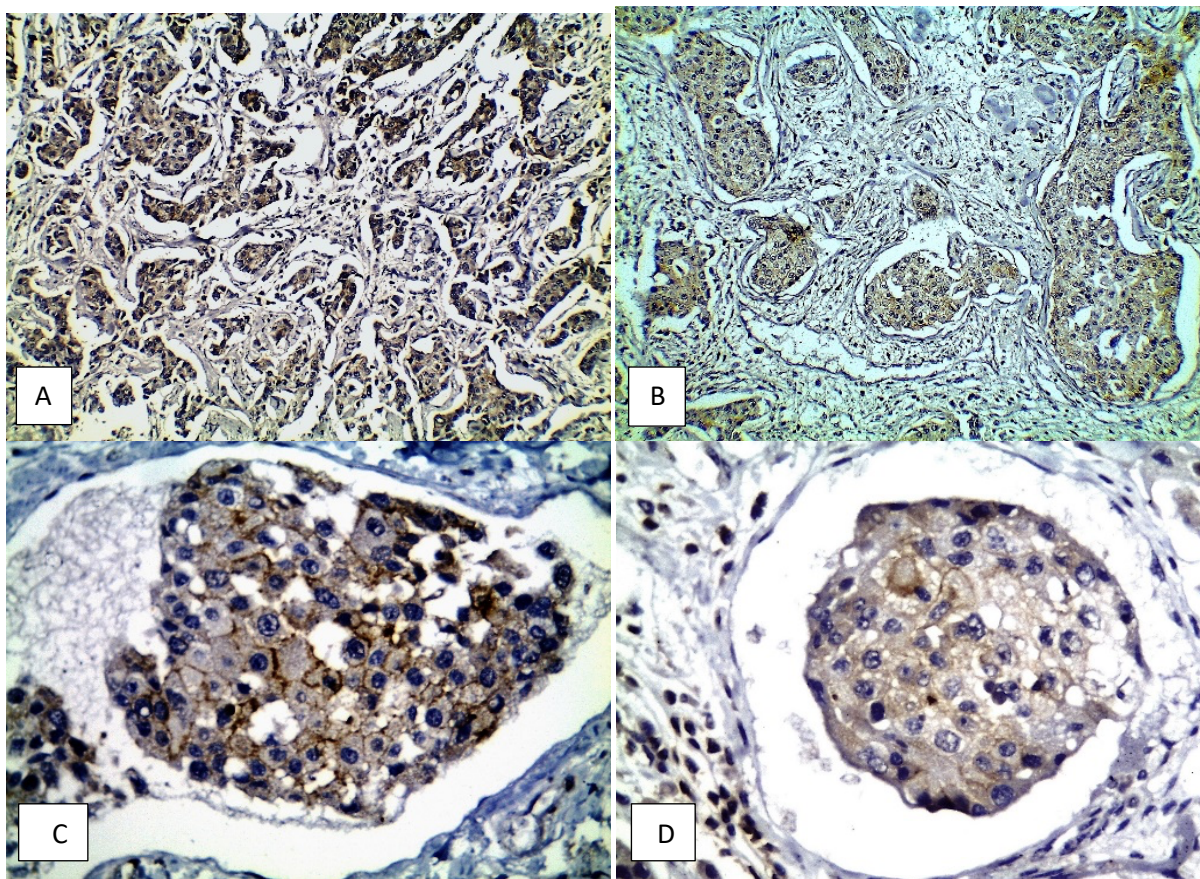


**Figure 1.** Immunohistochemical staining of HER2 in ER-, PR-/HER2+ breast carcinoma showing strong complete membranous staining in >10% of tumor cells, (A): Grade II breast carcinoma (×200), (B): Grade III tumor (×200), (C): Grade III tumor (×400).

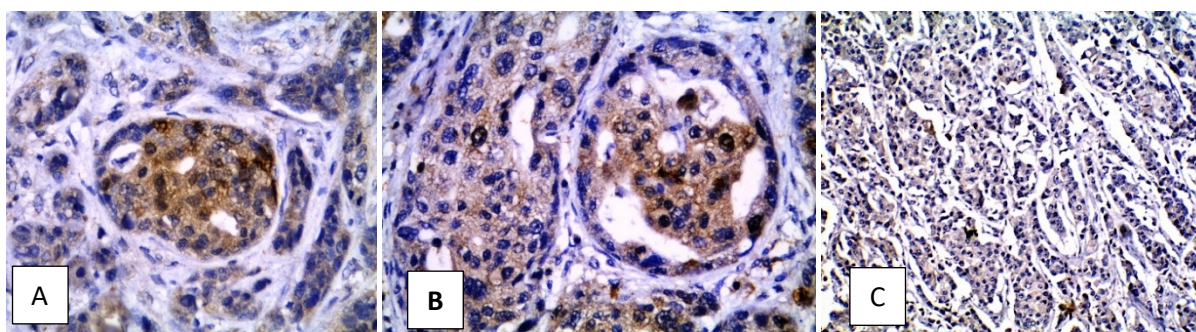


**Figure 2.** Immunohistochemical expression of Ki67 in ER-, PR-/HER2+ breast carcinoma, (A): Strong nuclear reactivity in grade II tumor (×200), (B): Strong expression in grade III tumor (×200), (C): Strong immunostaining in grade III tumor (×200), (D): Ki-67 staining in <10% of tumor cells in grade II breast carcinoma (×400).





**Figure 3.** GLUT4 immunohistochemical expression in breast carcinoma studied cases. (A): High cytoplasmic expression in grade II tumor ( $\times 100$ ), (B): High cytoplasmic expression in grade III tumor ( $\times 100$ ), (C): High membranous and cytoplasmic expression in grade III tumor ( $\times 400$ ), (D): Tumor emboli with high membranous and cytoplasmic expression ( $\times 400$ ).



**Figure 4.** IL-8 immunohistochemical expression in studied cases. (A): High cytoplasmic expression in grade II tumor ( $\times 400$ ), (B): High cytoplasmic expression in grade III tumor ( $\times 400$ ), (C): Low cytoplasmic expression in grade II tumors ( $\times 200$ ).

#### Correlation between IL-8 and GLUT4 immunohistochemical expression

A significant strong positive correlation was observed between IL-8 and GLUT4 immunohistochemical expression in tumor cells ( $r=0.670$ ,  $p=0.0001$ ), where 37 cases (92.5%) of high IL-8 expression showed high GLUT4 immunostaining in tumor cells. Furthermore 13 cases (72.2%) of low IL-8 expression showed low GLUT4 immunoreactivity in tumor cells (Table 6).

#### DISCUSSION

The classical definition of cancer is an abnormal proliferation of cells but according to emerging evidence that proved the metabolic disturbances occurred in cancer cells, cancer should be considered as a metabolic disorder (Pavlova and Thompson, 2016).

**Table 6.** Correlation between IL-8 and GLUT4 immunohistochemical expression

	IL-8 expression n (%)		r	p value
	Low n=18	High n=40		
<b>GLUT4 expression</b>				
<b>Low (n=16)</b>	13 (72.2)	3 (7.5)	0.670	0.0001*
<b>High (n=42)</b>	5 (27.8)	37 (92.5)		

r: Spearman correlation, \*: statistically significant

Breast cancer is the most common prevalent malignant tumor among females worldwide with an increasing number of cases yearly (Siegel et al., 2018). Despite the use of additional predictive measures as IHC expression of hormone receptors and HER2 as well as classic pathological parameters, such as histological type, tumor size, grade, and axillary lymph node status to assess the prognosis and plan for therapeutic targets, the number of deaths is still not decreasing as expected (DeSantis et al., 2019). Therefore understanding the altered intracellular metabolic processes of malignant cells is crucial for predicting prognosis and for designing an effective therapy especially for ER-,PR-/HER2 positive breast carcinoma subtype which is a well-known type with more aggressive behavior than ER+,PR+/HER2 – and few treatment options. One of the most important altered cellular metabolism occurring in malignant cells is the transformation of glucose to lactic acid (anaerobic glycolysis) to produce energy instead of ATP generation via oxidative phosphorylation even in the presence of oxygen (Dhup et al., 2012). To maintain energy production for glycolysis-dependent cancer cells, used for proliferation and synthesis of important anabolic protein molecules, cancer cells increase the expression of different glucose transporters (GLUTs) to enhance glucose uptake (Barbosa and Marte, 2020).

One of the important GLUTs is GLUT4 because it has a high affinity for glucose, granting transport of glucose at a high rate under physiological conditions (Macheda et al., 2005).

GLUT4 is primarily expressed in insulin-sensitive tissues, but it was found to be expressed in other tissues as well as in several malignant tumors. Several studies demonstrated that GLUT4 is expressed in breast cancer cells (Medina et al., 2003; Garrido et al., 2013; Moreira et al., 2013), however, limited

information is known about the involvement of this transporter in breast cancer biology. IL-8 is a chemotactic cytokine that becomes visible as a possible factor that could share in the progression of breast cancer through its mitogenic and angiogenic properties. Upregulation of IL-8 is considered an unfavorable prognostic factor (Liu et al., 2016).

In this study, GLUT4 and IL-8 immunohistochemical expression in ER-,PR-/HER2+ breast carcinoma subtype were investigated to assess the relation of their expression with biological tumor cell behavior and prognosis. GLUT4 immunohistochemical expression was evaluated in 58 cases of ER-,PR-/HER2+ breast carcinoma subtype, high expression was detected in 72.4% of tumors. The expression was mostly cytoplasmic and membranous as GLUT4 is present in intracellular cytoplasmic vesicles and upon stimulation, it is translocated to the plasma membrane of the cells. High GLUT4 expression was significantly associated with advanced tumor size, positive lymph node status, and LVI. Also, the postmenopausal age group was found to express GLUT4 at a higher rate than premenopausal one and in tumors with a poor degree of differentiation than grade II tumors, however, this difference did not reach statistical significance. Also, most tumors with distant metastasis expressed high GLUT4 immunostaining, but without statistically significant difference. In addition to previous findings in the current study, high GLUT4 expression was found to be significantly correlated with positive Ki67 immunostaining in breast cancer cells, where tumors with high GLUT4 expression showed positive Ki67 immunoreactivity more frequently than tumors with low GLUT4 expression.

The findings of the present study are in complete agreement with other studies which proved that increased glucose uptake in breast

cancer cells was significantly linked to high GLUT4 expression which was associated with poor prognostic parameters and poor survival outcome (DeBerardinis et al., 2008; Garrido et al., 2015; Zeng et al., 2020). Moreover, Garrido et al. (2015) proved that loss of GLUT4 impairs the viability of breast cancer cells via metabolic reprogramming of tumor cells. On the other hand, Zeng et al. (2020) showed that downregulation of GLUT4 in breast cancer cells was associated with poor overall survival.

Previous studies proved that breast carcinoma subtypes that express Epidermal Growth Factor (EGF) receptor are strongly associated with increased glucose uptake via increasing the expression of GLUTs (Su et al., 2006; Xu et al., 2011; Jung et al., 2019). One of these studies showed that EGF stimulation of in vitro experimental breast cancer cells (T47D) caused activation of multiple intracellular signaling pathways which in turn led to the activation of transcription factors (Jung et al., 2019). One of these factors is facilitative sugar transporter (GLUT) with subsequent enhancement of cellular proliferation and survival. In addition inhibition of EGF/EGFR resulted in decreased cellular proliferation by decreasing the expression of GLUTs with the resultant diminished glucose influx into tumor cells. As HER2 is a component of the EGF receptor family, thus overexpression of HER2 in breast cancer cells was significantly correlated with high GLUT4 expression which was associated with an increased rate of cellular proliferation detected by Ki67 as observed in this study.

High IL-8 immuno-expression was detected in the majority of studied tumors in the present study (70%). This high positivity was significantly related to large tumor size, LVI, and presence of axillary lymph node metastasis. While, IL-8 expression in tumor cells was not significantly related to patients' age, tumor grade, or presence of distant metastasis, although the majority of postmenopausal age group, grade III tumors, and tumors associated with distant metastasis exhibited high IL-8 expression. In agreement with these results, Milovanovic et al. (2013) observed that IL-8 was highly expressed in primary breast carcinoma HER2 positive subtype, and this expression was correlated to high tumor grade, negative ER

expression, and poor prognosis. Other researchers proved that IL-8 upregulation enhances angiogenesis and tumor invasion (Brat et al., 2005; Matsuo et al., 2009; Singh et al., 2013). Besides, Singh et al. (2006) found that IL-8 expression in ER-negative breast cancer cells aids in bone resorption through stimulation of osteoclasts. Also, it was found that patients with lymph node negative breast cancers and high IL-8 expression were associated with poor survival outcome (Rakovic and Milovanovic, 2013).

High IL-8 expression was found to be associated with ER-/HER2 positive breast cancers more frequently than with ER+/HER2 negative tumors by various studies and this expression was linked to histologic grading, LVI, lymph node status, and metastatic potential (Malonia et al., 2014; Lyon et al., 2008; Sheikhpour, 2017). Similarly, Bendrik and Dabrosin (2009) showed that ER-/HER2 positive breast cancer cells expressed high levels of IL-8 and were associated with higher LVI and metastatic potential than tumors with ER+/HER2-. Added to that, Singh et al. (2013) revealed that increased IL-8 expression was linked to an increase in the activity of breast cancer-like-stem cells by activation of HER2. These stem cells have various roles in breast cancer propagation. Furthermore, Aceto et al. (2012) tested the effect of HER2 overexpression on IL-8 upregulation in breast cancer, and they found that IL-8 was 11-fold overexpressed than in HER2- breast cancers. Contrarily, Yao et al. (2007) observed that IL-8 expression in ER-/HER2+ tumors enhanced a decrease in tumor growth.

The previous findings of the present study can be explained by that the overexpression of HER2 activates signal transduction pathways ending in IL-8 upregulation in breast cancer cells giving these cells an alternative way to maintain cellular proliferation in absence of estrogen.

A strong positive significant correlation was detected between IL-8 expression and positive Ki-67 immunoreactivity in the breast cancer cases in this study, in which 82.5% of high IL-8 tumors showed positive Ki67 nuclear immunoreactivity which supports the mitogenic role of IL-8 in breast cancer cells. The role of IL-



8 in enhancing cellular proliferation was disclosed by Liu et al. (2016) who found that IL-8 expression stimulates the expression of several initiators of the cell cycle as cyclin D1 and down-regulation of IL-8 caused arrest of the cell cycle at G<sub>1</sub>/S phase. On the other hand, Yao et al. (2007) found that the downregulation of IL-8 in ER-negative breast cancers did not affect the cell cycle and cellular proliferation.

Regarding the relationship of GLUT4 and IL-8 expressions in breast cancer studied cases, a strong significant positive correlation was observed between these two biomarkers expression. This finding was agreed with Xu et al. (2017) who proved that increased expression of IL-8 by colorectal cancer cells leading to increase glucose uptake and glycolysis, while the inhibition of IL-8 expression decreased the glucose consumption. Additionally, glucose deprivation in breast cancer cells led to the upregulation of IL-8 (Marjon et al., 2004).

## CONCLUSION

Considering the findings of the present study which demonstrate the crucial role of GLUT4 and IL-8 in ER-,PR-/HER2+ breast cancer cell biology, it was concluded that increased expression of GLUT4 and IL-8 in ER-,PR-/HER2 positive breast carcinoma subtype provides surrogate pathways for tumors cells to proliferate and infiltrate in absence of estrogen/ER growth signals by fact that their expression was associated with poor prognostic factors namely: high cellular proliferation rate, large tumor size, LVI, axillary lymph node positive status, and distant metastasis.

So, downregulation of GLUT4 and IL-8 via blocking of GLUT4 and immunotherapy that targeting IL-8 might offer an alternative therapeutic approach for ER-,PR-/HER2+ breast cancer patients through metabolic reprogramming of cancer cells which will affect tumor cell proliferation, invasion and survival.

## CONFLICTS OF INTEREST

All authors declared no conflicts of interest.

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