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Immunohistochemical Expression of Leptin in Mammary Carcinoma

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Abstract

Background: Leptin is a multifunctional adipose regulatory peptide that has various actions in carcinogenesis. Mammary carcinoma is one of the leading cancers in the world with tempted aspects to explore new prognostic factors.

Aim of Study: We attempted to leptin expression in mammary carcinoma and its clinicopathological correlation.

Material and Methods: Thirty-five cases of breast carcinomas were studied for correlating leptin immunohistochemical expression with other available parameters.

Results: The included cases' mean age is 57 years ±SD 11.7. The majority of cases were invasive duct carcinoma. As regards clinical pathological stages, stage IA (48.6%), stage IB (14.3%), stage IIA (14.3%), stage IIIA (2.9%), stage IIIB (14.3%), and finally stage IIIC (5.7%). Leptin immunostain was positive in 60% of studied mammary carcinoma cases and negative in 40%. Five cases of peritumoral fibrocystic disease were studied; all showed leptin positivity in ductal cells. Correlating leptin immunohistochemistry results with other findings, we found that significant relationship was found between leptin staining and mammary carcinoma histopathological type. Most invasive duct carcinoma (NST) showed positive leptin immunostaining (67.9%). On the other hand, most cases of the other types (lobular and mixed carcinomas) showed negative staining (71.4%) (*p*-value 0.027). Leptin immunostaing had insignificant correlation to T stage, grade, tumor lymphovascular emboli and perineural infiltration. As regarding tumor lymph nodal metastasis, most of cases without nodal metastasis (N0) were leptin-positive (76.5%) with significant *p*-value of 0.025. A significant relationship was detected between leptin immunostaining and clinicopathologic staging as well as molecular subtype. Estrogen-positive cases significantly correlate with leptin, with *p*-value of 0.0001. This also was observed with progesterone positivity, with *p*-value of 0.002. No significant relationship was found between leptin and Her-2 as well as Ki-67.

Conclusion: Leptin immunostain can be of an extreme help in mammary carcinoma prognosis.

Key Words: *Leptin – Breast carcinoma – Immunohistochemistry.*

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Introduction

BREAST cancer is a worldwide notorious health problem that ranks the first in females' cancer-caused mortality. According to GLOBOCAN 2018 world incidence, it accounts for 24.2% of cancer cases and 15% of cancer death **[1]**. In Egypt; according to GLOBOCAN regional cancer registry 2018; it accounted for 35.1% of all new cancer cases and the second in cancer-caused mortality in females **[1]**. Risk factors for breast cancers are many, some are constitutional and about 20% is modifiable, including overweight and obesity **[2,3]**.

Obesity; has a distressing incidence worldwide, according to WHO fact sheets; about 2 billion adults are considered as overweight and about 600 million people are categorized as obese worldwide **[4]**. Overweight and obesity are known to be risk factors for different malignancies including breast cancer in postmenopausal females **[5,6]** who will have a 12% higher risk for each 5kg/m^2 increase in Body Mass Index (BMI) **[7]**. They are also condemned for the more dismal prognosis, which is independent of the menopausal status **[8,9]**.

The role of obesity in breast carcinogenesis is complex, it involves dysregulated production of adipokines, exaggerated steroid hormones action, altered response to insulin, Insulin-like Growth Factor (IGF) I, and a sustained chronic local inflammatory response **[10]**. Adipokines are a family of energy regulating peptides that include leptin and adiponectin. It is mainly produced by adipose tissue. Adiposity is associated with increased secretion of leptin and decreased adiponectin levels. This disturbed ratio was proved to increase the risk of breast cancer **[11,12]**. Adiponectin is known to have an anti-neoplastic effect through inhibiting cell growth, invasion, and migration and it also promotes apoptosis **[13,14]**. Leptin is a multifunctional adipo-regulatory peptide. It also con-

tributes to fetal development, sex maturation, lactation, hematopoiesis, and immune responses **[15-19]**.

The biological actions of leptin are mediated through the leptin receptor that activates several intracellular signaling pathways **[20,21]**. Higher serum leptin and overexpression of its receptors in breast cancer are correlated with hormone receptor, tumor size, and higher tumor grade **[22,23]**. Leptin is considered as a pleuri-potent pro-carcinogenic factor that promotes all stages of breast cancer development. It contributes directly to expanding malignant cell mass by promoting epithelial cell proliferation, and transformation through amplification of ER signaling **[24,25]** and trans-activating HER2 **[26]** and it inhibits apoptosis through a Bcl-2-dependent mechanism **[27]**. The indirect effect is exerted on the tumor micro-environment through its angiogenic capability **[28]**, and pro-inflammatory effect **[29]**.

Leptin can induce several signaling pathways that are involved in breast carcinogenesis like JAK/STAT which induces cyclin D1 expression **[30]**, and up-regulating human Telomerase Reverse Transcriptase (hTERT) activity **[31]**, PI3K/AKT that controls specific signals on the insulin receptor substrate **[32]** enhancing the cellular proliferation, modifying the expression of the key regulators of Epithelial-Mesenchymal Transition (EMT) facilitating invasion and metastasis **[33]**. It is anticipated to up-regulate Acetyl-CoA Acetyltransferase 2 (ACAT2) concerned with the proliferation, migration, and invasion of cancer cells **[34]** and regulating epithelial-mesenchymal transition mediated by IL-8 **[35]**.

Notably; leptin can modify the main hormonal influencers of breast cancer; through ERK signaling; it induces stromal aromatase enzyme, thereby increasing estrogen level in breast tissue **[36]** and also stimulates the estrogen receptor alpha (ER(x) **[37,38]**. It also augments the Epidermal Growth Factor Receptor (EGFR) signaling pathways through IGF-1 signaling **[39]**. A dual beneficial relationship exists between HER2 and leptin as HER2 induces the expression of leptin in mammary cancer cells **[40]** and leptin pays back through trans-activating HER2 mediated by the activation of the EGFR and JAK2, resulting in overexpression of HER2 in mammary cancer cells **[41,42]**.

Solid malignant tumors are often hypoxic with the production of Hypoxia-Inducible Factor (HIF-1) that induces leptin expression in adipocytes and fibroblasts **[43,44]**. Leptin exerts a pro-angiogenic effect through MAPK/ERK 1/2, p38, and JNK signaling pathways. It facilitates angiogenesis either alone or combined with Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF-2) **[45]**. It also can increase the levels and promote the action of Matrix Metalloproteinases (MMPs) 2 and 9; involved in angiogenesis **[46,47]**.

Leptin can trigger a pro-inflammatory micro-environment at the tumor bed, known as Crown-like Structures (CLS). It is formed of activated macrophages and a plethora of inflammatory mediators (e.g. TNF-(x and IL-6) **[48]** that activate the aromatase enzyme **[49,50]**, stimulate angiogenesis, promote metastatic potential, facilitate the evasion of the immune system, and influence the response to therapy **[51,52]**.

Cancer Stem Cells (CSCs) have a fundamental role in breast carcinogenesis. They show selective expression of the leptin receptor and an increased response to its action **[53]**. Many studies have shed light on the role of the leptin signaling pathways (i.e., Notch, Wnt, mTOR, STAT3, HER2/Erb, and IGF pathways) and its transcription factors (i.e., NF_B and hypoxia-inducible factor) in influencing CSC activity **[54-56]**.

A growing list of evidence supports the antagonizing role of leptin during the treatment stage; It increases the resistance to chemotherapy by the expression of enzymes essential for acid (-oxidation pathways **[57]**, through JAK/STAT signaling pathway, it also increases the activity of HER2, making the malignant cells less sensitive to monoclonal trastuzumab treatments **[58]**. Moreover; it stabilizes the Estrogen Receptor alpha (ERa), sustaining ERa-dependent transcription in malignant cells despite the presence of anti-estrogens **[59]**.

Aim of work: To study leptin immunostain in breast carcinoma and to correlate it with the available clinicopathological factors.

Material and Methods

This retrospective institutional study was conducted by collecting data and tissue blocks from Kasr Al-Ainy Hospital, Cairo University. Pathologic records of patients who underwent mastectomy were assessed. The study was approved by the Ethics Committee of the Faculty of Medicine, Cairo University for use of patients' samples for research purposes.

We retrieved 35 cases of breast carcinomas during the interval between 2018 and 2019. Tu-

moral paraffin-embedded cell blocks were collected. Hematoxylin and Eosin (H & E) slides were reviewed. Selecting the tumor block with representable preserved invasive viable tissue was done.

Serial sections (4-gm) were prepared from the tumor blocks and mounted on adhesive-coated glass slides for Estrogen (ER), Progesterone (PR), HER2/neu, Ki67 and leptin staining. Commercially available ER (1: 50; Dako), PR (1: 10; Dako), HER2/neu (1: 10; Dako), and Ki67 (1: 300, Cat. #RB-9043-P; Lab Vision, Thermo Fisher Scientific, Fremont, USA) were used as primary antibodies. Leptin primary antibody used is a rabbit antihuman polyclonal anti-leptin antibody (Chongqing Biospes Co, Jiangbei District, Chongqing, China) at 1:100 dilution. Immunohistochemistry autostainer (BenchMark ULTRA, Ventana, Arizona, USA) was used for immunohistochemistry staining.

Briefly, 5-gm-thick tissue sections were deparaffinized in xylene and rehydrated in graded alcohol, and subsequently microwave-treated in sodium citrate buffer (pH 6.0) twice. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 15min, followed by washing with Tris-buffered saline. The sections were then incubated with diluted primary antibody (NBP2-22204; Novus Biologicals, Littleton, USA). Thereafter, the sections were refrigerated at 4ºC overnight in a humid closed chamber. The sections were again washed in Tris-buffered saline and incubated with avidin-biotin-peroxidase system (Dako, Dako Corporation, Carpinteria, CA, USA) for 30min. The diaminobenzidine was used as a chromogen and hematoxylin as a counterstain.

For leptin stain, internal positive control of mammary adipose tissue was included. As for estrogen and progesterone, surrounding normal breast tissue act as an internal positive control; as for HER2 and Ki67, a known overexpressing breast carcinoma was used. Negative control is done by omitting the primary antibodies. The immunostained entire slide had been evaluated and subsequently scored by light microscopy; provided that the staining did not represent background or artifact. Hematoxylin and eosin stained sections were evaluated by Leica light microscopy along with the prepared immunohistochemically stained slides.

Histologic nuclear grading of the surgical specimens was performed according to the Bloom-Richardson's criteria **[60]**. A standard histopathologic examination was done that included determining the type of cancer and the pathologic tumor stage, which were assessed according to the criteria established by the 8th edition AJCC staging manual **[61]**.

A cutoff value of 1% for the stained nuclei was used to define ER and PR positivity. Evaluation of the percentage of tumor nuclei stained and intensity of staining was done, classifying it to strong and weak expressions. Membranous staining for HER2 with strong complete staining in 10% of the tumor cells was regarded as HER2 overexpression. Samples with scores 3+ were regarded as positive for HER-2/neu. The Ki-67 labeling index was expressed as a percentage and was graded as "high" if the number of positive cells was >_20% **[61]**.

The available data were collected and tabulated; including patient's age, gender and history of neoadjuvant chemotherapy treatment, as well as pathological factors as tumor size, tumor grade, lymph node metastasis, tumor perineural/lymphovascular invasion, presence of in situ component and surrounding fibrocystic disease. We classified cases into its appropriate stage and molecular subtype.

A five high power field was used for leptin evaluation. The expression of leptin was localized to the cytoplasm. The evaluation was assessed by semi-quantitative method. Immunohistochemical leptin staining evaluation was done by scoring leptin stain intensity and percentage of stained tumor cells. Staining was subjectively scored to tiered system (0- Nil, 1- Weak focal <10%, 2- Moderate focal 11-50%, 3- Marked diffuse >50%). For the statistical purposes, the negative/weak focal staining was considered negative; while moderate/strong was considered positive **[62]**.

Data were analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24 (SPSS Inc., Chicago, IL, USA). Numerical data were described as mean and standard deviation or median and range. Categorical data were described as numbers and percentages. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons between two groups for numeric variables were done using the Student's *t*-test w. Comparisons between categorical variables were performed using the chi square test and fisher exact test as appropriate. A *p*-value less than or equal to 0.05 were considered statistically significant. All tests were two tailed.

Results

Leptin is an important cytokine for lipid metabolism and energy balance, with related postula-

tion to the development of breast cancer. However, the relationship between leptin and breast cancer prognosis is still controverted. In this study, we investigated leptin immunohistochemistry in 35 cases of mammary carcinomas, tried to find relations with hormonal and proliferation indices as well as tumor invasiveness and staging.

The included cases' mean age is 57 years ±SD 11.7, with range 30-78 years and median of 55. Only female gender was found in studied cases (100%). Regarding the size of breast tumors, the mean size is 3.6cm ±SD 2.1, with range 1.2 to 10.5cm and median of 3cm.

Only four of the studied cases have history of neoadjuvant chemotherapy (11.4%). Regarding the T stage; 7 cases were T1 (20.0%), 22 cases were T2 (62.9%) and 6 cases were T3 (17.1%). The majority of cases (28 cases, 80.0%) were invasive duct carcinoma (NST). The rest of cases exhibit lobular carcinoma type (4 cases, 11.4%), invasive mixed ductal and lobular carcinoma (2 cases, 5.7%) and only one case of invasive papillary carcinoma (2.9%). Seventeen cases showed in situ ductal carcinoma (48.6%). Nine cases showed evidence of tumor lympho-vascular invasion (25.7%); while five cases showed perineural tumor infiltration (14.3%). As regards lymph nodes metastasis, eighteen cases showed positive lymph nodal metastasis (51.4%). Nodal staging is as follows; N0: 17 cases (48.6%), N1: 10 cases (28.6%), N2: 3 cases (9.6%), and N3: 5 cases (14.3%). Concentrating on tumor grading, ten cases were grade I (28.6%); while most cases were grade II (25 cases, 71.4%). No case showed grade III.

By classifying cases into clinical pathological stages, we find 17 cases (48.6%) were stage IA, 5 cases were of stage IB (14.3%). Total cases of stage I is 22 cases (62.9%). Five cases were of stage IIA (14.3%). Only one case was of stage IIIA (2.9%), five cases were of stage IIIB (14.3%), and finally 2 cases were of stage IIIC (5.7%). Total cases of stage III was 8 cases (22.9%).

As regards immnuno staining, all cases were stained with estrogen, progesterone, Her-2, Ki67 and leptin. Estrogen-positive cases were 26 (74.3%), while 9 cases were negative (25.7%). Eighteen cases showed strong estrogen labeling intensity (51.4%), while eight showed a weak one (22.9%). Progesterone-positive cases were 20 cases (57.1 %), of which half of them showed strong labeling intensity and another half showed a weak one (10 cases each, 28.6%). Only one case showed evident Her-2 expression (2.9%). Most cases showed low mitotic activity as indicated by Ki-67 (24 cases, 68.6%); while the rest showed high Ki-67 expression (11 cases, 31.4%). The mean expression of Ki-67 is 20.8% ±SD 22.1, with median 12% and range 2% to 80%. By summation of all previous results, molecular subtype was assumed as: Luminal A 19 cases (54.3%), luminal B 7 cases (20.0%) and triple negative 9 cases (25.7%).

Leptin immunostain was positive in 21 cases out of the 35 studied mammary carcinoma cases (60%) and negative in 14 cases (40%). Moderate leptin staining intensity was noted in 9 cases (25.7%); while strong staining intensity was noted in 12 cases (34.3%). Five cases of peri-tumoral fibrocystic disease were studied; all showed leptin positivity in ductal cells.

Correlating leptin immunohistochemistry results with other findings, we found that significant relationship was found between leptin staining and mammary carcinoma histopathological type. Most invasive duct carcinoma (NST) showed positive leptin immunostaining (19/28 cases, 67.9%). On the other hand, most cases of the other types (lobular and mixed carcinomas) showed negative staining (5/7 cases, 71.4%), with only two cases of lobular and papillary carcinomas showing moderate and strong leptin immunostaining respectively (*p*-value 0.027).

Most cases with neoadjuvant chemotherapy were leptin-negative (3/4 cases, 75.0%) with insignificant *p*-value of 0.292. Leptin immunostaing had no correlation to T stage; most cases of T 1 were leptin-positive (4/7 cases, 57.1%); while most cases of T2 were also leptin-positive (14/22 cases, 63.6%). On contrary, most cases of T3 were leptin-negative (4/6 cases, 66.7%).

As regards cases with in situ component, leptin positivity was noted in 9/17 cases (52.9%) (*p*-value of 0.625). Concentrating on leptin positivity inside the in situ carcinoma regions, we found that 8/12 (66.7%) showed leptin-positive anaplastic cells. It is worthy to say that the four leptin-negative in situ cellular regions were in mixed and invasive lobular carcinoma cases (*p*-value 0.131).

The majority of studied carcinoma cases with tumor lymphovascular emboli were leptin-negative (6/9 cases, 66.7%); also those with tumor perineural infiltration (4/5, 80.0%), with *p*-values 0.094 and 0. 141 respectively.

As regarding tumor lymph nodal metastasis, most of cases without nodal metastasis (N0) were leptin-positive (13/17 cases, 76.5%) with significant

p-value of 0.025. N1 showed equal proportion between leptin negativity and positivity (5/10 cases, 50.0% each), while all cases of N2 were leptin-negative (3/3 cases, 100.0%) and most of N3 cases were also leptin-negative (3/5 cases, 60.0%).

The majority of grade-I was leptin-positive (8/10 cases, 80.0%), while leptin-positive cases in grade-II represent only 48.0% (12/25 cases) with more grade-II cases expressing leptin negativity (13/25 cases, 52.0%) (*p*-value of 0.084).

A significant relationship was detected between leptin immunostaining and tumor staging with most cases exhibiting lower stages I and II express leptin positivity (18/27 cases, 66.7%) and on contrary, the minority of cases exhibiting higher stage III express leptin positivity (2/8 cases only, 25.0%) with *p*-value of 0.051. Leptin positivity in stage IA was (13/17 cases, 76.5%), stage IB was (4/5 cases, 80.0%), stage II was (1/5 cases, 20.0%), stage IIIA was (1/1 case, 100.0%), stage IIIB was (1/5 cases, 20.0%) and none in stage IIIC (0/2 cases, 0%).

Focusing on other immune markers and leptin, we found that estrogen-positive cases significantly correlate with leptin. Most estrogen-positive cases were leptin-positive (19/26 cases, 73.1%) with *p*-value of 0.0001. Leptin positivity correlates also with estrogen intensity, being noted in 14/18 cases with strong estrogen expression (77.8%), with *p*-value of 0.004. This also was observed with progesterone positivity. Most progesterone-positive cases were leptin-positive (16/20 cases, 80.0%), with *p*-value of 0.002. Progesterone intensity was also correlated with leptin, 9/10 cases with strong progesterone expression were leptin-positive (90.0%) with *p*-value of 0.004. No significant relationship was found between Her-2 and leptin. It was noted that 19/34 cases of negative Her-2 cases were positive to leptin immune staining (55.9%), and meanwhile the only case of positive Her-2 was positive to leptin (1/1 case, 100.0%) (*p*-value 1).

As regards Ki-67, most cases with high mitotic index were leptin-negative (7/11 cases, 63.6%); on the other side, most cases with low mitotic index were leptin-positive (16/24 cases, 66.7%) but *p*-value was insignificant of 0.093.

A significant correlation between leptin and molecular subtype was encountered (*p*-value 0.016). Most cases of luminal A and luminal B types were leptin-positive (15/19 cases, 78.9% & 4/7 cases, 57.1% respectively). On the contrary, most triple negative cases were leptin-negative (7/9 cases, 77.8%).

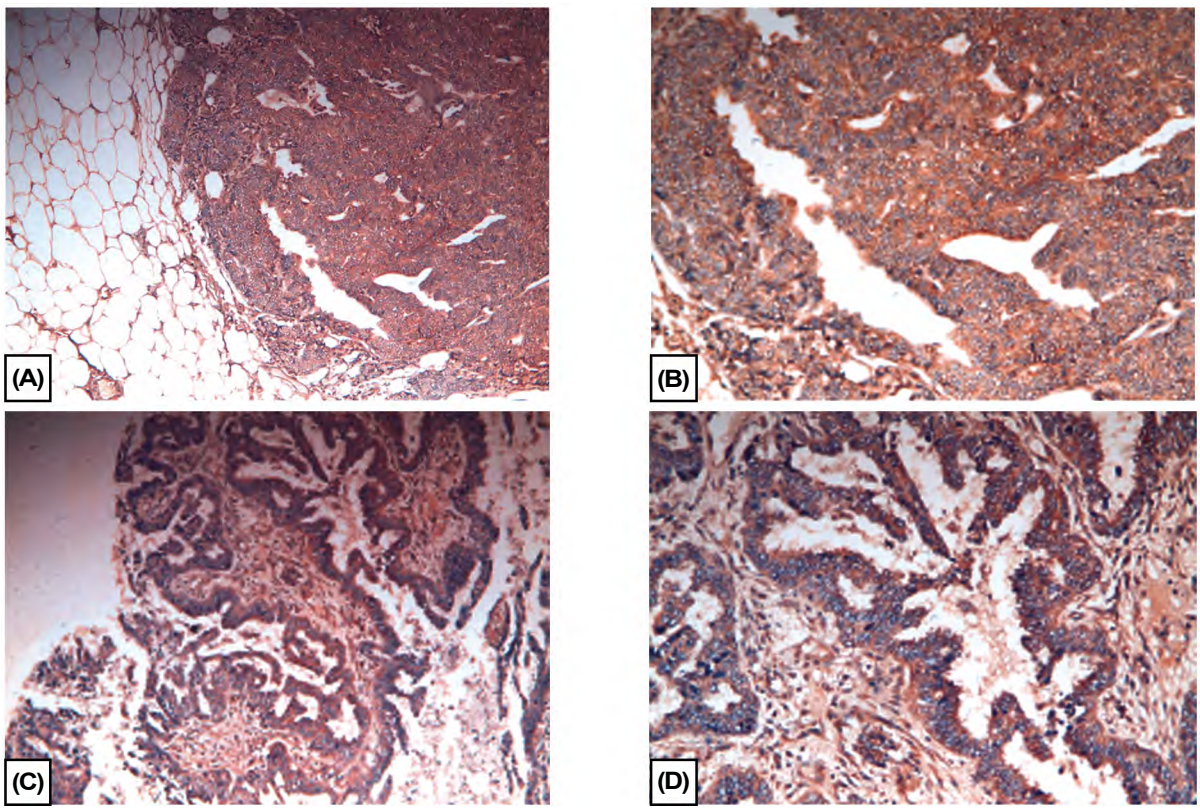

Fig. (1): Positive leptin strong and diffuse expression in mammary invasive carcinoma. (A & B): Invasive duct carcinoma,(C & D): Invasive papillary carcinoma (X100 & X200, Leptin immune stain).

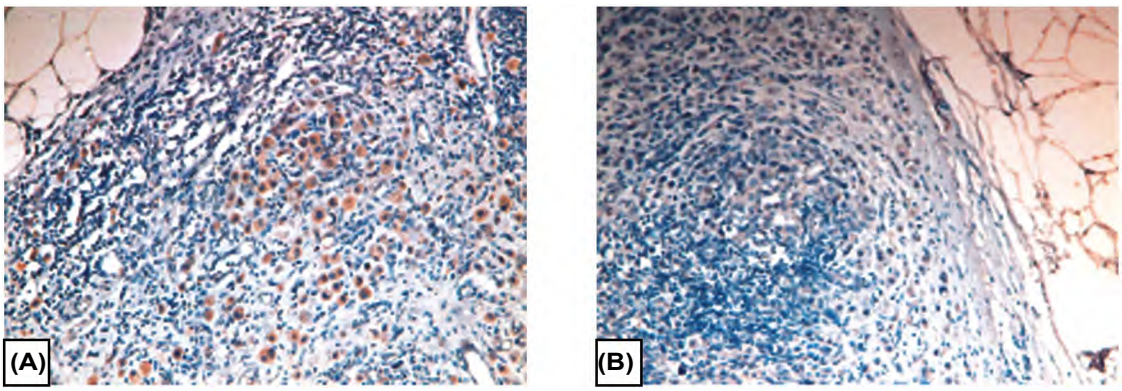

Fig. (2): (A) Positive leptin expression & (B) Negative leptin expression in invasive lobular carcinoma (X100, Leptin immune stain).

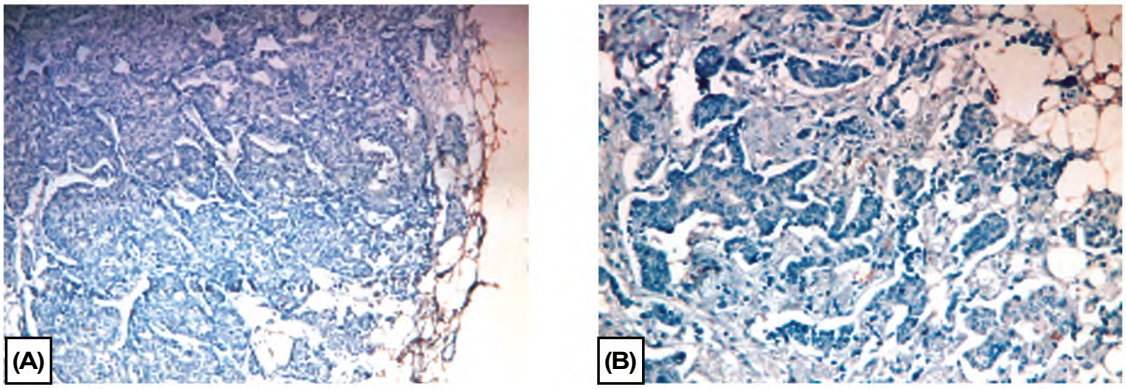

Fig. (3): Negative leptin expression in invasive duct carcinoma (X100 & X200, Leptin immune stain)

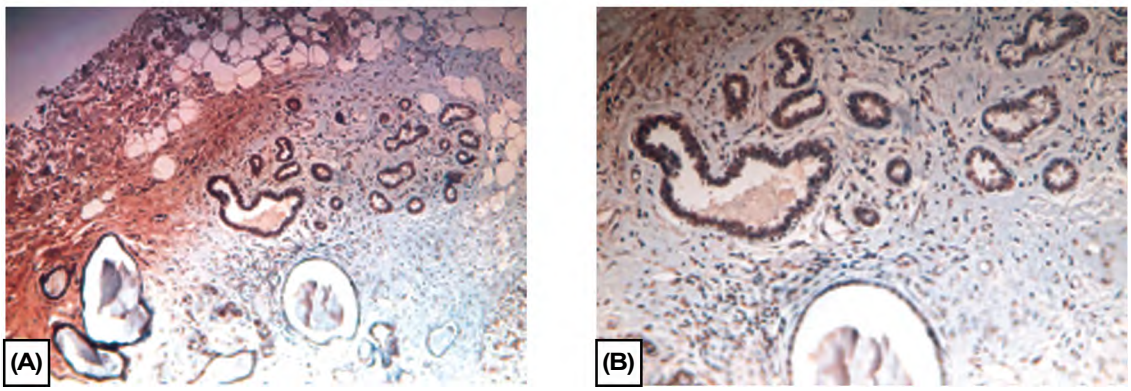

Fig. (4): Positive leptin expression in peri-tumoral fibrocystic disease (X100 & X200, Leptin immune stain).

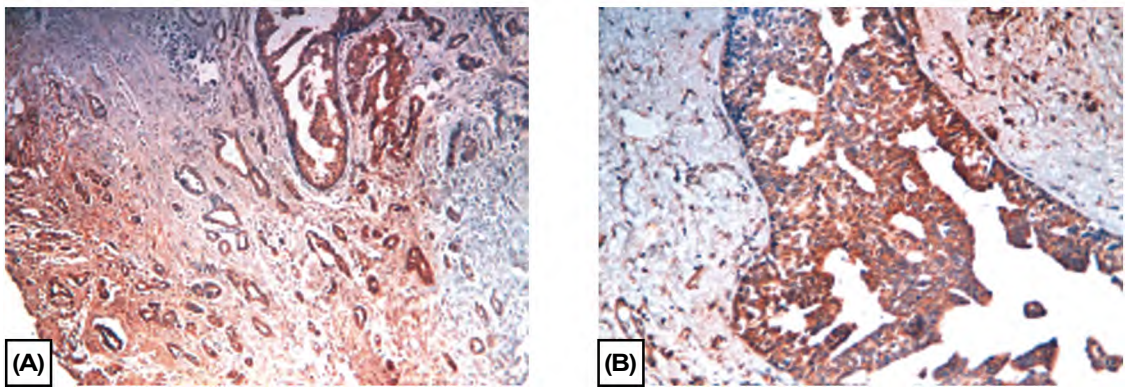

Fig. (5): Positive leptin expression as regards in situ duct carcinoma with surrounding invasive component (X100 & X200, Leptin immune stain).

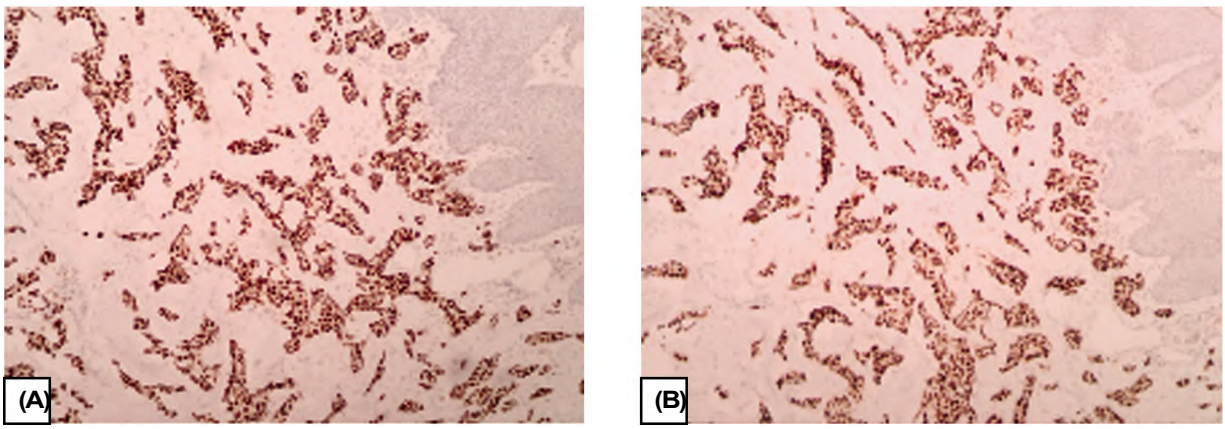

Fig. (6): Positive estrogen and progesterone expression in invasive duct carcinoma (X100, A- Estrogen, B- Progesterone immune stains).

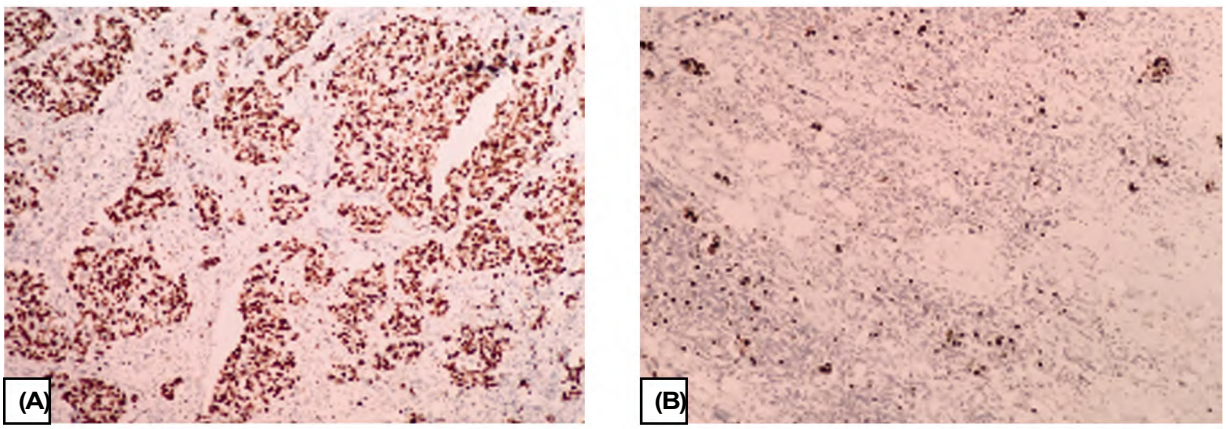

Fig. (7): Ki-67 high and low expressions in invasive duct carcinoma (X100, Ki-67 immune stain)

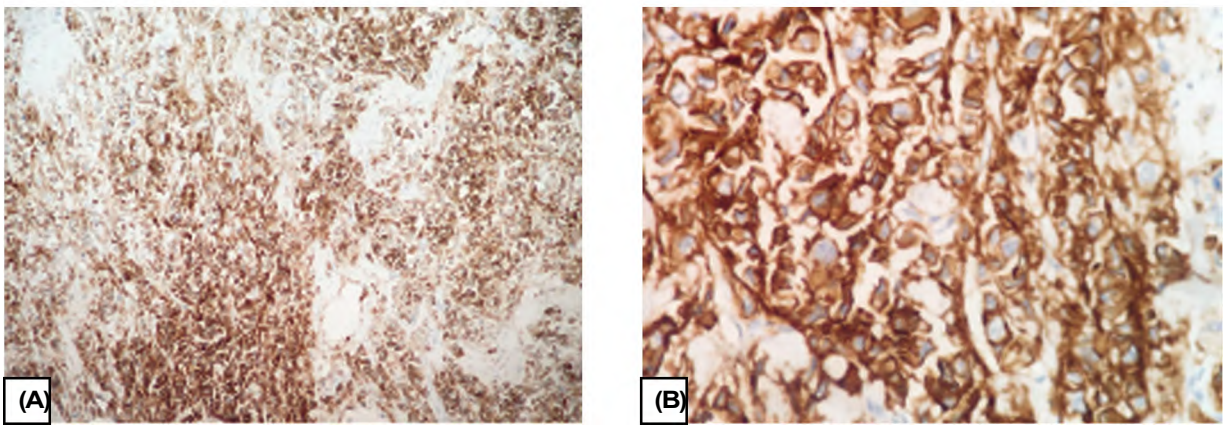

Fig. (8): Positive Her-2 expression in invasive duct carcinoma (X100 & X200 Her-2 immune stain).

Discussion

Although breast cancer treatment has wide steps in progress, the 5-year relative survival for tumor can be less than 17%. This may be due to early diagnosis retardation, large population with preliminary advanced-stage BC, and failed treatment regimens in some types **[63,64]**. Therefore, it is life-saving to identify new prognostic factors and therapeutic targets for breast carcinoma; in order to stratify patients, check tumor progression, and make early diagnosis feasible.

Leptin is a circulating satiety hormone which is produced primarily by white adipose tissue. It has a crucial role in regulating energy metabolism and cellular proliferation. Leptin can provoke leptin receptor, different signaling pathways, and enzyme aromatase. Aberrant expression of leptin is involved in pathogenesis of many carcinomas, even can be

used in their differential diagnosis and prognosis **[65]**. It exerts its proliferative effects on malignant epithelial cells, which may activate carcinogenesis of breast tissue and promote the proliferation as well as angiogenesis of cancer cells **[66-68]**.

In this study, we aimed to explore leptin relations in breast carcinoma with its different clinicopathologic parameters. Thirty-five female mammary carcinoma cases were studied. By keeping immnunohistochemical data together, molecular subtype was assumed as: Luminal A (54.3%), luminal B (20.0%) and triple negative (25.7%). Leptin immunostain was positive in 21 cases out of the 35 studied mammary carcinoma cases (60%) and negative in 14 cases (40%). Moderate leptin staining intensity was noted in 9 cases (25.7%); while strong staining intensity was noted in 12 cases (34.3%). Five cases of peri-tumoral fibrocystic disease were studied; all showed leptin positivity in ductal cells.

Our study showed lower leptin-positive percentage of breast cancer than that found by Ishikawa et al., who found that overexpression of leptin, as determined by staining intensity, was observed in 92% of studied breast cancer cases but in no normal epithelium and also that distant metastasis occurred more frequently with the increasing expression of leptin. They postulated that leptin may have a promoting effect on the carcinogenesis and metastasis of breast cancer, possibly in an autocrine manner. Functional inhibition of leptin may be effective for the prevention and treatment of breast cancer **[69]**. Their higher detected percentage of breast cancer can be attributed to more number of exclusively studied invasive duct carcinoma cases and the semi-quantitative reading of immunostaining. Also Yongnam Kim et al., found that 84% of their studied carcinoma cases showed a leptin expression **[70]**. This is due to lone postmenopausalpatients' study category.

Correlating leptin immunohistochemistry results with other findings, we found that significant relationship was found between leptin staining and mammary carcinoma histopathological type. Most invasive duct carcinoma (NST) showed positive leptin immunostaining (67.9%). On the other hand, most cases of the other types showed negative staining (5/7 cases, 71.4%), with only two cases of lobular and papillary carcinomas showing moderate and strong leptin immunostaining respectively (*p*-value 0.027).

Most cases with neoadjuvant chemotherapy were leptin-negative (75.0%) with insignificant *p*-value of 0.292. This is corresponding to Yongnam Kim et al., who found insignificant relation between leptin and chemotherapeutic treatment **[70]**. Kong et al., also stated that positive expression of leptin was superior in the Pathological Complete Response (PCR) group to that in the progressive disease group (p=0.049, p=0.025) **[71]**. On the other hand, Del Fabbro et al., reported overweight patients receiving neoadjuvant therapy had lower (PCR) rates. Karatas et al., suggested leptin was an independent prognostic factor for the reduction of PCR to neoadjuvant chemotherapy in breast cancer patients. This can be attributed to their larger sample of patients receiving neoadjuvant treatment **[72,73]**.

Leptin immunostaing had no correlation to T stage; most cases of T 1 were leptin-positive (57.1%); while most cases of T2 were also leptin-positive (14/22 cases, 63.6%). On contrary, most cases of T3 were leptin-negative (4/6 cases, 66.7%). This was similar to Khabaz et al., who also found no significant association of leptin immunostaining with tumor size **[74]**. As regards cases with in situ component, leptin positivity was noted in (52.9%) (*p*-value of 0.625). Concentrating on leptin positivity inside the in situ carcinoma regions, we found that (66.7%) showed leptin-positive dysplastic cells. It is worthy to say that the four leptin-negative in situ cellular regions were in mixed and invasive lobular carcinoma cases (*p*-value 0.131). All the cases with peri-tumoral fibrocystic disease showed leptin positivity in this region.

Leptin positivity was also evident in DCIS in previous studies. Caldefie-Chézet et al., declared that leptin was expressed in 50% of cases in DISC. Since leptin expression corresponded to 80% and 75% of the cases of IDC grades I and II, respectively, and was only revealed in 60% of the cases of IDC grade III studied. Leptin was expressed by all modified, benign or malignant breast cells and not only by breast cancer cells. It was expressed in the vicinity of a ductal breast lesion. This suggests that leptin may be a proliferative factor for the development of ductal breast cancer **[75-77]**.

The majority of studied carcinoma cases with tumor lymphovascular emboli were leptin-negative (66.7%); also those with tumor perineural infiltration (80.0%), with *p*-values 0.094 and 0.141 respectively. As regarding tumor lymph nodal metastasis, most of cases without nodal metastasis (N0) were leptin-positive (76.5%) with significant *p*-value of 0.025. The majority of grade-I was leptin-positive (80.0%), while leptin-positive cases in grade-II represent only 48.0% (*p*-value of 0.084). A significant relationship was detected between

leptin immunostaining and tumor staging with most cases exhibiting lower stages I and II express leptin positivity (66.7%) and on contrary, the minority of cases exhibiting higher stage III express leptin positivity (25.0%) with *p*-value of 0.051. This was similar to Khabaz et al., who reported a considerable fraction of stage IIb and stage III were found to be common with low leptin immunostaining. Significantly, more cases with metastases in lymph nodes were observed in low score staining (p=0.0300) **[74]**. This is contradicted with what Yongnam Kim et al., reported, as they found that disease stage was not different; according to the expression of leptin in the postmenopausal patients **[70]**. Again this can be attributed to their restricted postmenopausal patients study.

Estrogen-positive cases significantly correlate with leptin. Most estrogen-positive cases were leptin-positive (73.1%) with *p*-value of 0.0001. This also was observed with progesterone positivity. Most progesterone-positive cases were leptin-positive (80.0%), with *p*-value of 0.002. No significant relationship was found between Her-2 and leptin. It was noted that (55.9%) of negative Her-2 cases were positive to leptin immune staining, and meanwhile the only case of positive Her-2 was positive to leptin (100.0%) (*p*-value 1). As regards Ki-67, most cases with high mitotic index were leptin-negative (63.6%); on the other side, most cases with low mitotic index were leptin-positive (66.7%) but *p*-value was insignificant of 0.093. A significant correlation between leptin and molecular subtype was encountered (*p*-value 0.003). Most cases of luminal A and luminal B types were leptinpositive (78.9% & 57.1% respectively). On the contrary, most triple negative cases were leptinnegative (77. 8%).

This is in concordance to Yongnam Kim et al., as well as Khabaz et al., who stated that most HR-positive studied patients showed a leptin expression **[70,74]**. HER-2/neu expression, lymphatic invasion and histologic nuclear grade were not different according to the expression of leptin **[70]**. Our findings are different from previous studies that declared high leptin expression was significantly associated with high Ki-67 expression and triple negative breast carcinoma **[76-78]**. This can be related to more studied triple negative cases.

As deduced from all the above data, leptin is a conflicting topic in breast carcinoma. Still, studies with broader panel of cases and unified standardized way of immune markers assessment are certainly of great value for assessing the value of leptin immunostaining in mammary carcinomas.

Conclusions and Recommendations:

Leptin immune stain is a helpful method in outlining the prognosis of breast carcinoma. Our study proposes that leptin could have a role in identifying the mammary carcinoma type, stage, grade and treatment in BC, due to its association with many clinicopathological factors.

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التعبير الكيميائى الهستولوچى المناعى للبتين فى سرطان الثدى

اللبتين هو ببتيد شحمى متعدد الوظائف له إجراءات مختلفة فى التسرطن. إن سرطان الثدى هو آحد السرطانات المنتشرة فى العالم التى تدفع لإستكشفاف عوامل تنبؤية جديدة للعلاج. فى هذا البحث حاولنا دراسة التعبير عن اللبتين فى سرطان الثدى وإرتباطه الإكينيكى بالعوامل المختلفة. تم دراسة خمس وثلاثين حالة من سرطان الثدى، بمتوسط عمر ٥٧ سنة ±١١.٧. كانت غالبية الحالات عبارة عن سرطان القناة الغازية. كان التعبير المناعى لليبتين موجباً فى ٦٠٪ من حالات سرطان الثدى المدروسة وسلبياً فى ٤٠٪. من خلال ربط نتائج الكيمياء الهيستولوجية المناعية للبتين بالنتائج الآخرى، فقد وجدنا آن هناك علاقة إحصائية بين التلوين المناعى للبتين ونوع السرطان المرضى. آظهر معظم سرطان القناة الغازية تلويناً مناعياً إيجابياً (٦٧.٩٪). من ناحية آخرى، آظهرت معظم حالات الآنواع الآخرى تلويناً مناعياً سلبياً (٧١.٤٪). لم يكن للبتين المناعى إرتباط إحصائى بمرحلة آو درجة الورم، وكذلك آيضاً الإنتشار الورمى الوعائى اللمفاوى والتسلل حول العصب. فيما يتعلق بالإنتشار الورمى الخبيث العقدى الليمفاوى، فإن معظم الحالات التى لا تحتوى على ورم خبيث عقدى N0 كانت إيجابية للبتين (٧٦.٥٪). تم الكشف عن علاقة ذات دلالة إحصائية بين التلوين المناعى للبتين ودرجة الورم وكذلك النوع الجزيئى. ترتبط الحالات الإيجابية للإستروجين والبروجسترون بشكل كبير مع الليبتين. لم يتم العثور على علاقة ذات دلالة إحصائية بين اللبتين وHer-2 وكذلك Ki-67. لهذا فإن التعبير المناعى للبتين له آهمية فى دلالة العوامل المؤثرة فى سرطان الثدى.