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Histological changes in breast Cancer patients and the physiological role of Leptin and Interleukin-6 in blood serum

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ABSTRACT

The current study included two aspects,which were histological physiological. (75) blood samples from women with breast cancer, in addition to 15 blood samples for healthy women as control were grouped. In addition to collecting tissue biopsy from the mammary glands of some women whose blood samples were drawn Included were also. Women diagnosed with breast cancer, ages ranged between 38-68 years. The study was conducted from 1 May 2019 to 2 October 2019. The results of microscopic examination showed the presence of invasive breast cancer, there were types of breast cancer, including infiltrative carcinoma, and metaplastic carcinoma. Another types of gland cancer were medullary carcinoma, and invasive Ductal carcinoma, which is breast cancer the musinaise type. The results also revealed the presence of ductal carcinoma *in situ* DCIS (the sold type). The results also showed the spread of cancerous Paget disease to the dermal layer of the skin. The physiological results showed hematological changes characterized by a high level of Leptin hormone ($P \leq 0.01$) in the blood of women with breast cancer group 3, compared with the control group, while there was a significant increase ($P \leq 0.05$) in women with a concentration of hormone ($P \leq 0.05$) in injuries in groups 2 and 5, compared with the control group, and no significant changes occurred in groups 4 and 6 compared to the control group. Also significant increase ($P \leq 0.01$) in the concentration of interleukin-6 group of women with breast cancer group 6 compared to the control group, while the group 2 and 3 showed a significant increase ($P \leq 0.05$) compared to the control group, while group 4 recorded significant decrease ($P \leq 0.05$) in interleukin-6 concentration compared to control group. From the results of the current study, Conclusion can be made that, breast cancer has severe negative effects on the histological composition of breast tissue, which negatively affects the functional role of the Leptin hormone and interleukin-6 in the blood serum.

Keywords: Breast cancer, interleukin-6, leptin hormone.

Introduction

Cancer is an increased number and growth of cells, as the cells increase quickly and without stopping, and they are cussing pressure on the affected organ and the so-called tumor. This causes a disturbance in the mechanism of internal regulation, which results in cell growth outside the control of the body (1,2). Breast cancer is the most common in the first place in Iraq and the Arab world, but it is less frequent in Africa, and it is the second in terms of deaths after lung cancer in the United States of America, Australia, New Zealand and Western Europe (3). As breast cancer affects more than 5.3 million people annually in the world, as it affects women equally after the age of fifty years, and that the chances of developing it increase with age (4). As breast cancer is a common and serious disease in the world that affects the breast in women it usually appears in the milk glands and their ducts (5). This type of tumor represents 18% of all tumors that affect women and is the third disease in terms of spread globally (6). It is a cause of death between the ages of 55-35 years, for example in the United Kingdom there are 150,000 deaths annually due to breast cancer and about 25,000 injuries diagnosed annually, and more than 50% of injuries occur at the age of 50-64 years, and that the injury varies globally according to the site Geography, the western countries have the highest rates of infection, but they are lower in Asia and Africa and this may be due to the severity of environmental factors (7), the disease is the most common cause of death in women and is diagnosed Million new cases each year, as these figures have made frightening scientists and researchers are looking for proven methods to help in the diagnosis, prevention and treatment, as well as studying the factors and causes that lead to the metastasis (8).

In Iraq, this type of cancer is the most common of all types of malignant tumors for women, which represents almost a third of women's cancers, according to the latest Iraqi cancer registry (9). It is estimated that in 2018, 627,000 women died from that cancer, or about 15% of all cancer deaths among the female category (10).

This study aimed to detect the Histological changes and Physiological variations (Leptin hormone and interleukin-6) associated with breast cancer in the blood serum of infected women.

Patients and methods

75 blood samples of serum blood of women with breast cancer, in addition to 15 blood samples for healthy women, were used 5ml for each sample. The study was conducted at the Teaching Oncology Hospital- Medical City/Baghdad from the period 1 May 2019 to 2 October 2019. Blood samples were drawn using a 5ml medical syringe, they were placed in dry plastic tubes (Gel Tubes) and left for 15 minutes at room temperature, and then placed in a centrifuge for 10 minutes at a speed of 3000 r/min for the purpose of separation, and the serum was withdrawn using micro-pipette, the serum samples were distributed to small quantities in Eppendoff tubes and the serum was kept in these tubes at a temperature (-20°C) until physiological tests were conducted. Tissue biopsy was collected from the mammary glands of some women from whom blood samples were previously drawn, and whose ages ranged 38-68 years. Samples were taken in to a 10% dilute formalin solution for a period of 24 hours (11) for the purpose of preparing tissue sections (12). Moreover, the blood samples were distributed into groups as flow: The first group (includes 15 samples) as a control group from women with a healthy condition. The second group (includes 16 samples) as it represents the newly diagnosed women. The third group (includes 15 samples) as it represents women with breast cancer and they have an initial progression of the disease, where pain and swelling were observed under the axilla and before treatment. The fourth group (includes 15 samples), included women who are taking chemotherapy with limits less than five doses (without metastasis). The fifth group (includes 17 samples) and this group represents women taking chemotherapy more than seven doses with radiation therapy (without metastasis). The sixth group (includes 12 samples), as this group is considered the advanced group in terms of infection (Metastasis), the transmission of infection to other organs, including the bone, lung, liver and spine. Estimation of Leptin hormone in the women's blood serum following the steps attached with the ready-made test kit of the German company LDN and according to the instructions of the manufacturer of Elisa technology (13). The concentration of Interleukin-6 in the blood serum was also estimated following the steps provided with the ready-made test kit for the Chinese company Crystalday, according to the manufacturer's instructions for Elisa Technology (14).

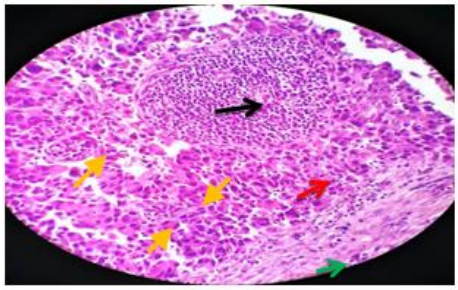
Statistical analysis

The results were statistically analyzed using the Minitap Ver-17 statistical program according to the ANOVA test, and the arithmetic mean was compared to the Duncan polynomial test at the probability level $P< 0.05$ (15).

Results and discussion

Microscopic and Histological changes

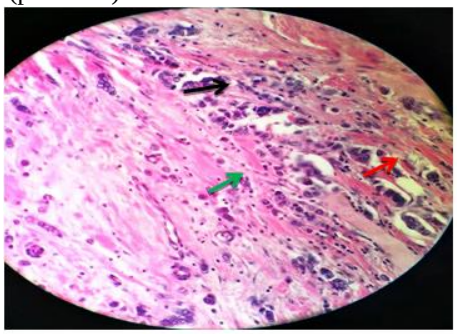
The results of microscopic examination of the women Biopsy showed with breast cancer the presence of invasive breast carcinoma, where cancerous cells were observed in huge numbers in the gland with lymphocyte infiltration in spherical nodules in the gland, picture 1.



Picture (1) Breast tissue showing invasive breast cancer (common type NOS), where invasive cancer cells ( ) with lymphocyte infiltration in a spherical nodule in the gland board ( ), and fibers ( ) surrounded by cancer cells ( ), (H&E color, zoom power 400).

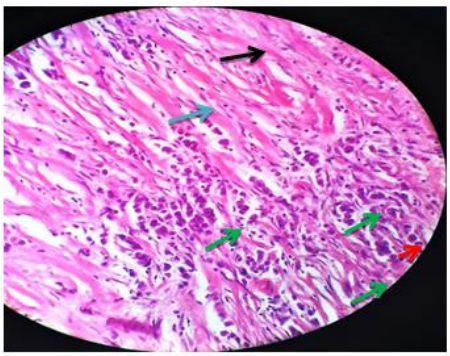
Among the observations that may support the role of the immune system in the surface of tumor cells and this malignant cancer is what is observed from the presence of cellular leaching in the broad nodular in the tissue of the gland, (picture 1).

Other type of cancer, which is infiltrative carcinoma, where the epithelial cell transformation of the gland and the ducts was observed, the presence of central necrosis, and the dissolution of most cells in the gland cavities and ducts surrounded by lymphocyte and epithelial cells. Scattered within the fibrous colloid tissue (picture 2).



Picture (2) infiltrated breast cancer, G2, showing the epithelial transformation of the gland and ducts into tumor cells ( ) with a central necrosis ( ), fibrosis ( ), (H&E color, zoom power 400).

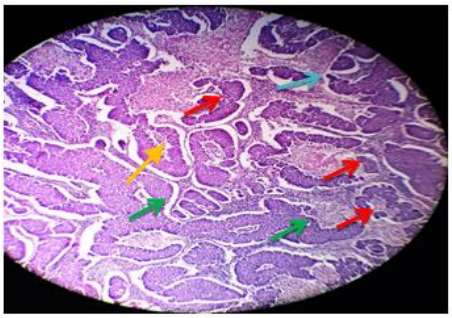
Infiltrative carcinoma is the transition of metastatic tumor cells and their passage in the lobules of the gland, fibrous interstitial tissue and ductal gland. This condition appeared with cellular necrosis, which means that tumor cells either have a role in necrosis of healthy epithelial cells and then isolation or even the tumor cells got necrosis by the immune response of the body and as in the previous picture 2, (16). The presence of metaplastic carcinoma, was observed in the epithelial cells of the glandular veins and ducts and tumor cells found in the cavities of the glands and ducts in the form of degenerated and lumpy cellular masses surrounded by the longitudinal colloidal fibers (picture 3).



Picture (3) metastatic breast cancer, distinguished by the presence of epithelial cells transforming into tumor cells ( ) in the cavities of glands and ducts ( ), fibrosis ( ) and peripheral fiber bundles ( ), (H&E color, the zoom power 400).

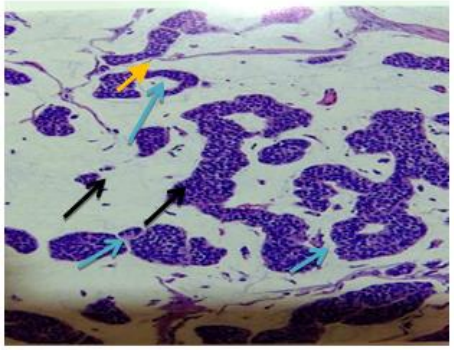
The results were consistent with the results of other advanced studies, in that, the tumor forms of mutant cancerous cells showed that they are of large nuclei, and that the cytoplasm is homogeneous, and this is an indication of the high effectiveness of cancer cells on the high programmatic division and proliferation and metastasis (17).

The presence of medullary carcinoma, another type of breast cancer. It contained degenerative and recurrent epithelial cells carcinoma was observed in picture 4.



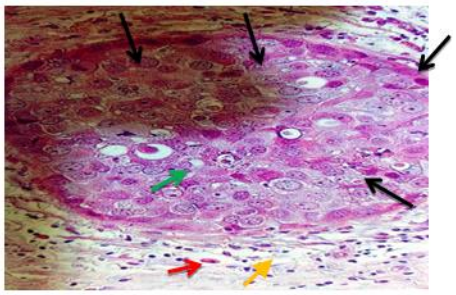
Picture (4) Invasive medullary B.C, G2, showing the entire gland contains the cancer cells ( ) with the presence of hyperplasia of epithelial cells ( ) and the presence of degenerative cancerous epithelial cells ( ) , Necrosis ( ), (H&E color, zoom power 100).

Invasive medullary carcinoma confirms the ability of a tumor to invade all of the gland tissue and convert it into an irreversible malignant tumor, (picture above 4), and this condition is considered a killer of cancer patients with this disease (18, 19). The musinaise type, is another type of invasive ductal carcinoma. What distinguishes this type is that the cancer cells secrete the mucin that occupies the space between cancer cells, (picture 5).



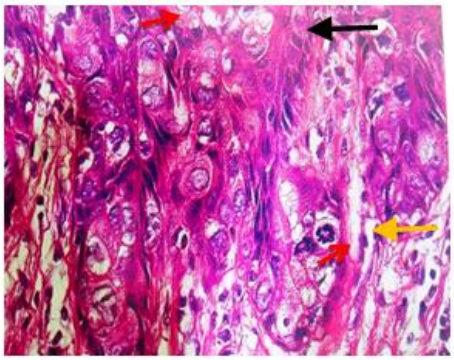
Picture (5) Musinaise cancer type, showing the presenceof tumor cells ( ), and note the presence of mucin ( ), which occupies the space between the cancer cells ( ), (H&E color, zoom power 200).

Ductal carcinoma *in situ* (DCIS), is the sold type which is non-invasive carcinoma, and notice that the entire canal cavity is filled with cancerous cells and the canal cavity was not obvious with a high division rate, (picture 6).



Picture (6) DCIS, sold type, where the cavity of the duct is full of cancer cells ( ) with penetration of the basal membrane ( ), and fibrosis ( ) with a high mitosis ( ), (H&E color, zoom power 400).

The metastasis of Paget's disease to the dermis of the skin in the form of separate cancer cells between the normal cells of the skin, and this is usually associated with the presence of ductal carcinoma and appears clinically in the form of ulceration in the nipple skin, (picture 7).



Picture (7) Metastasis of Paget's cells ( ) in the dermis layer ( ) of the skin in the form of cancer cells separated between the normal cells ( ), (H&E color, zoom power 400).

The Physiological variations

Figure 1, the high concentration of the Leptin hormone was highly significant (P≤0.01) in the blood of women with breast cancer group 3 (33.290±4.87)ng/ml, compared with the control group (23.213±1.696)ng/ml, while the concentration of leptin hormone increased significantly (P≤0.05) in group 2 (26.113±3.040)ng/ml, and the group 5 (24.847±2.476)ng/ml, compared to the control group, no significant changes occurred in groups 4 and 6 compared to the control group. Group 3 was distinguished by the fact that they did not take any chemotherapy treatment and they had an initial progression of the disease, and the results of the current study were consistent with a study conducted by (20), who found elevated levels of leptin in women with breast cancer and attributed the reason for leptin to being a factor that contributes to the formation and development of cancer and help growth of cancer cells, and in another study conducted by Jarde (21), they also found an increase in Leptin levels in women with breast cancer, while the researcher indicated Fusco (22) to the absence of any significant relationship between leptin levels and breast cancer, and this contradicts the results of the current study.

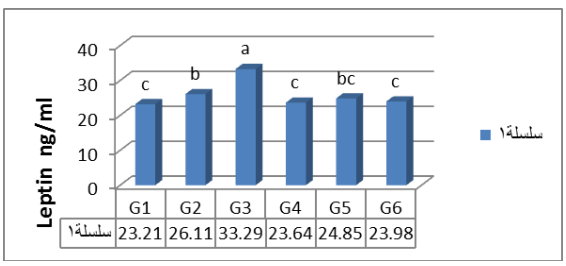


Figure (1): The concentrations of Leptin hormone among different groups of women with breast cancer, compared to the control group.

Many reports confirm that Leptin has an anti-death effect of programmed cells and an incremental effect (23). Obesity increases the risk of developing types of cancer in both males and females in general, however, the role of leptin is important in recent studies on mice have shown, that mice with a deficiency of this hormone have been observed to decrease tumor growth compared to mice with a high rate of this the hormone, as it has a significant impact on the formation, and development of tumors in obese patients (24). Studies in this field have shown that the breast is a tissue rich in fat, and adipocytes are an important source of leptin (25). The high level of leptin hormone in breast cancer may be caused by the pro-inflammatory environment (26). Recent studies have revealed a high concentration of leptin in the blood of patients with breast cancer compared to the control group (27, 28). By this, all women can change their lifestyles by following a low-fat diet and exercising to achieve a healthy weight and prevent the risk of obesity. The levels of the Leptin hormone in the serum of patients with breast cancer, which was observed to rise at the beginning of the early infection compared to the control group, and these came in line with the results of other studies that also showed a relationship between the Leptin hormone and an increased risk of cancer (29). Evidence suggests that leptin and estrogen may collaborate in maintaining the growth of estrogen-dependent breast cancer (30). Figure 2, shows a highly significant increase (P≤ 0.01) in the interleukin-6 concentration of women with breast cancer group 6 (303.20±13.3)ng/L, compared to the control group (173.05±6.93)ng/L, while the group 2 and 3 showed a significant increase (P≤ 0.05) in concentration (196.83±14.98)ng/L, (199.76±10.13)ng/L respectively compared to the control (173.05± 6.93)ng/L, while group 4 recorded a significant decrease (P≤ 0.05) in the interleukin-6 concentration (158.03±12.37)ng/L compared to the control. When compared the infection groups, it observed a high level of interleukin-6 with the onset of the injury and when the injury progresses compared to the control group, but in the advanced group of injury, the tumor and cancer cells transfer to other parts of the body, and these results are consistent with the results of other studies (31), where the levels of IL-6 are much higher in patients with breast cancer compared to healthy women. It was found that elevated levels of Interleukin-6 were associated with strong tumor invasion and poor prognosis (32). The results of the current study are consistent with the findings of Yunfeng (33), where between high levels of Interleukin-6 in the serum as well as Interleukin-8 was observed higher in ductal Breast carcinoma patients compared to the control. Cytokines may be able to serve as the biological marker of metastasis and invasive tumors. It is possible for tumor breast cells to produce Interleukin-6, and these may constitute a small part of the total IL-6 content in the serum, in addition to Tumor cells, T cells, Macrophages and B cells are able to secrete it (34). Thus, IL-6 production by immune cells is likely to increase serum levels in patients with breast cancer (35). Ravishankaran and Karunanithi mentioned that levels of expression of Interleukin-6 in the serum

correlate with the extent of the tumor invasion, and the lymph node metastasis (36), and this matches the results of the current study. It should be clear that, the higher risk of breast cancer is associated with overweight and obesity in women, especially in Postmenopausal patients (37). The presence of tumor cells spread to several organs, and to the liver may stimulate Kupffer cells to produce a variety of cytokines, including Interleukin-6, which may modify the synthesis of albumin by liver cells (38).

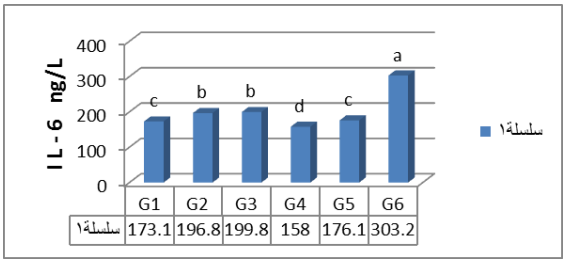


Figure (2): The concentrations of interleukin-6 among different injury groups of women with breast cancer, compared to the control group as a normal case.

As the role of interleukins in contributing to the formation of the angiogenesis for tumor, interleukin-6 plays an important role in cell differentiation and is believed to contribute to tumor development as a growth factor. It has been shown that high levels of interleukin-6 in the serum are related to its association with disease staging and negative clinical outcomes in women with metastatic breast cancer (38).

Conclusions

Through the results of the current study, the study conclude:

1. The occurrence of breast cancer in women has a direct negative impact, as we refer to the observation of some changes that appeared at the level of the breast skin such as redness, itching, peeling skin, and the appearance of the skin with a rough and wrinkled texture on the surface of the breast, and a change in the shape and appearance of the breast compared to the normal case of the breast.

2. The negative impact on breast tissue was also shown through the microscopic examination, where the presence of infiltrative carcinoma and metaplastic carcinoma, and medullary carcinoma were observed.

3. The results of the examination showed presence of invasive ductal carcinoma, which is breast cancer musinaise type, and also a presence of another type of ductal carcinoma *in situ* (DCIS), which is the sold type. The results of examination also showed the spread of cancerous Paget disease to the dermis layer of the skin. Finally, we confirm that not all boils and pain are symptoms of breast cancer. As women may complain of many changes in the level of the breasts during menstruation, pregnancy and lactation, but they are normal. However, the changes and symptoms that have been observed if they are accompanied by relapses of the nipple and a change in skin color and breast tissue.

4. Also, it was found that the occurrence of hematological changes represented by a high concentration of interleukin-6 in most groups of women with breast cancer, which shows that the stage of advanced infection (metastases) was the highest and influencing the immune side.

5. In addition, the level of the Leptin hormone is higher than normal in infected women with the initial progression of the infection.

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التغيرات النسجية في مريضات سرطان الثدي والدور الفسيولوجي لهرمون اللبتين والانترلوكين-6 في مصل الدم

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[4]كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

تضمنت الدراسة الحالية جانبين، أحدهما نسيجي والأخر فسلجي. تم جمع 75 عينة دم من نساء مصابات بسرطان الثدي بالإضافة إلى 15 عينة دم للنساء الأصحاء كمجموعة سيطرة. بالإضافة إلى جمع خزعات نسجية من الغدد اللبنية لبعض النساء اللواتي تم سحب عينات دم منهن مسبقا والمشخصات بسرطان الثدي وبأعمار تراوحت بين 38-68 سنة النساء. أجريت الدراسة من الفترة 1 أيـــار 2019 الـى 2 تشرين الاول 2019. أظهرت نتائج الفحص المجهري وجود سرطان الثدي الغازي، وهناك أنواع من سرطان الثدي منها السرطان الارتشاحي Infiltrative carcinoma والسرطان المتحول Metaplastic Carcinoma، وهناك نوع أخر من سرطان الغدد هو السرطان اللبي Medullary carcinoma، وسرطان الثدي القنوي الغازي، وهو النوع المخاطي musinaise type. أظهرت النتائج أيضًا وجود سرطان القنوي الموقعي DCIS (النوع sold type). كما أظهرت النتائج انتشار مرض باجيت السرطاني Paget's diseaseفي طبقة الادمة من الجلد. أظهرت النتائج الفسيولوجية تغيرات كيموحيوية اتصفت بارتفاع تركيز هرمون اللبتين بشكل عالي المعنوية ($P\leq 0.01$)في دم النساء المصابات المجموعة G3، مقارنةً بمجموعة السيطرة، بينما حصل ارتفع معنوي ($P\leq 0.05$) في تركيز هرمون اللبتين لدى النساء المصابات في المجموعتين G2 و G5 ، مقارنةً مع مجموعة السيطرة، ولم تحدث تغيرات معنوية في المجموعتين G4 و G6 مقارنةً مع مجموعة السيطرة. كما أظهرت حدوث زيادة معنوية ($P\leq 0.01$) في تركيز الانترلوكين-6 لنساء المصابات بسرطان الثدي مجموعة G6 مقارنة بمجموعة السيطرة، بينما اظهرت المجموعة G2 و G3 زيادة معنوية ($P\leq 0.05$) مقارنة مع مجموعة السيطرة، بينما سجلت المجموعة G4 انخفاضا معنويا ($P\leq 0.05$) في تركيز الانترلوكين-6 مقارنة بمجموعة السيطرة. من نتائج الدراسة الحالية يمكن استنتاج ان لسرطان الثدي تأثيرات سلبية شديدة على التركيب النسجي لأنسجة الثدي مما يؤثر سلبا على الدور الوظيفي لهرمون اللبتين والانترلوكين-6 في مصل ا