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Histological and Physiological assessment of Endothelin-1 and Cholesterol in breast cancer of women

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ABSTRACT

The current study included two aspects, one of which is histological and the other physiological. It included (75) blood samples from women with breast cancer, in addition to (15) blood samples for healthy women as control, in addition to collecting tissue biopsy from the mammary glands of some women whose blood samples were drawn Including them in advance. Those diagnosed with breast cancer and ages ranged between 38-68 years, where the study was conducted from 1 May 2019 to 2 October 2019. Gland tissue and the owner of those tumours swelling of the axillary lymph nodes. Another cancer that was found was papillary carcinoma, where epithelial hyperplasia appeared in the form of large papillae that filled the cavity of the gland. There is a large number of white blood cells and severe hematoma. Another type of gland cancer discovered is cribriform carcinoma, which is a rare case. Invasive cancer is the most dangerous, as it is observed in the glandular tissue with the presence of agglutination and crowding of tumour cells and tumour cysts. The study also showed haematological changes characterized by a highly significant concentration of Endothelin-1 ($P \leq 0.01$) in the blood of women with breast cancer group 5 and group 4, compared to the control group as a usual case. At the same time, there was a significant increase ($P \leq 0.05$) in the concentration of Endothelin-1 in infected women in groups 3, 2 and 6 compared to the control group. The study showed a very significant decrease ($P \leq 0.01$) in the cholesterol concentration of women with breast cancer of group 5, and group 6 compared to the control group, while its level decreased significantly ($P \leq 0.05$) in-group 4 compared to the control group.

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INTRODUCTION

Cancer is an abnormal growth of cells. Instead of replacing only damaged cells, the cells increase dramatically and without stopping, overwhelming the affected organ, forming what is called a tumour—causing an imbalance in the mechanism of internal regulation that results in cell growth outside the control of the body [1]. Breast cancer comes primarily in terms of prevalence in Iraq and the Arab world. Still, it is less frequent in Africa, and it is the second in terms of death after lung cancer in the

United States of America, Western Europe, Australia and New Zealand [2]. Breast cancer affects more than 5.3 million people annually in the world, and it affects women equally after the age of fifty years, and the chances of developing it increase with age [3, 4]. Breast cancer is one of the most dangerous diseases in the world affecting breast tissue, in women and usually appears in the ducts and milk glands [5]. It represents 18% of all tumours affecting women and is the third disease in terms of spreading 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. About 25,000 cases of diagnosis annually, more than 50% of injuries occur at the age of 64-50 years. The damage varies globally according to the geographical location of the western countries. It has the highest rates of infection. Still, it is lower in Asia and Africa and may be due to the severity of environmental factors [7].

In the United States, the proportion is terrifying, as a woman out of every eight women diagnoses breast cancer in a period of her life. Still, on a global scale, this disease is more Diseases cause the death of women and diagnose about one million new cases every year or, these frightening numbers made scientists look for certain methods to diagnose the disease and to prevent and treat it and study the factors and causes that lead to its spread [2].

In this type, where the cells forming the breast tissue can begin to grow unchecked and crowding out the natural milk-producing cells, these cells may storm the surrounding tissues, pushing around them and forming a tissue cluster known as a mass or tumour, and if cancer stays in place and does not invade the surrounding platelets or parts others of the body are classified as a benign tumour, but if it continues to invade the surrounding breast tissue and spread to the lymph nodes, then it is classified as a malignant or cancerous tumour.

In 2015, nearly 8.8 million women were diagnosed with cancer worldwide, compared to 571,000 breast cancer deaths. In Iraq, breast cancer is the most common form of malignancy for women, which represents almost a third of women's cancers, according to the latest Iraqi cancer registry, that is, it is the second leading cause of cancer deaths in Iraq [8]. It is estimated that 2018 saw 627,000 women die from that cancer - about 15% of all cancer deaths among women while the incidence of breast cancer is higher among women in more developed regions. However, some believe that this cancer is a disease of the developed world.

The study aimed to assess the role of endothelin-1 and cholesterol in women with breast cancer and to demonstrate the effect of breast cancer on the histological composition of breast tissue.

MATERIALS AND METHODS

In this study, 75 blood samples from women with breast cancer were used in this study, in addition to 15 blood samples for healthy women as a control with a capacity of 5 ml for each sample, where the research was conducted in the Teaching Oncology Hospital / Medical City from the period 1 May 2019 to 2 October 2019. Blood samples by a 5 ml medical syringe, these samples were placed in dry and clean 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. The serum was withdrawn by Accurate pipette and neglected precipitate. Then the serum samples were distributed to small quantities in Eppendorf tubes. The serum was kept in these tubes at a temperature of (-40°C) until physiological tests were performed, in addition to collecting tissue biopsy from the mammary glands of some women from whom blood samples were previously drawn, and ages ranged between 38- 68 years, by needle or by surgical removal of neoplastic masses to confirm the diagnosis, as these samples were taken from women patients in a 10% dilute formalin solution for a period of 24hours and installed in an ethyl alcohol concentration of 70% [9] to prepare tissue sections Depending on the method [10]. The blood samples were distributed into groups as follows. The first group (15 samples) includes the control group, that is, women with a healthy condition and those without cancer. The second group (16 samples), as this group includes women with newly diagnosed and reviews for the first time, as it was noticed in this group the apparent appearance symptoms, including the appearance of red spots on the skin of the breast and around the nipple, as well as dreamy secretions and others that indicate breast cancer. The third group (15 samples) this group includes women with breast cancer (the presence of a lump inside the breast), and they have an initial progression of the disease where swelling (swelling) of the glands under the armpit was observed with pain in the axillary region and before treatment. The fourth group (15 samples) this group includes infected women and those taking chemotherapy, up to 5 doses and less (without spread). The fifth group (17 samples), as this group includes infected women and those taking chemotherapy, seven doses and more, with radiation therapy (without spread). The sixth group (12 samples), as this last group is considered the advanced group in terms of infection,

metastasis, as it was transferred to other organs, including the liver, lung, bone and spine.

Where it was estimated the concentration of Human Endothelin-1 (ET-1) in the blood serum of women, by following the steps attached with the ready-made test kit from the Chinese company SunLong and according to the instructions of the manufacturer of the technology [11], and also the concentration of Total serum cholesterol using Human Test Kit (Human, INF-1001701-Germany), and using the method [12].

Statistical analysis

The results were statistically analyzed using the Minitap Ver-17 statistical program according to the ANOVA test, and the arithmetic means compared to the Duncan polynomial test at the probability level $P< 0.05$ [13].

RESULTS AND DISCUSSION

Symptoms of breast cancer patients

From the total observations and changes recorded for women with breast cancer and the reviews for the Teaching Oncology Hospital as follows:

1. Note the appearance of a solid mass or thickening in the breast and on the level of the armpit that differs from the surrounding tissues, as the cancerous lumps are distinguished from others by their solid strength and rough surface at most women.

2. The occurrence of general inactivity and weakness in the bodies of women with breast cancer, as well as some biochemical changes and anaemia, and the weakness was more severe in the advanced group of the infection, i.e. those who had a previous injury and then moved to other organs, through their observation, and when measuring the control group as a normal case.

3. A dreamy discharge was observed, i.e. the appearance of a transparent or blood-like (bloody) discharge from the nipple without pressure on the breast, due to the appearance of the tumour in the breast (breast cancer itself) or the presence of papilloma's, which were tumours that increase the risk of breast cancer.

4. There were cases of deformities in the nipple that were represented by relapses and changes at the nipple level, the nipple retracted into the breast, and its deep vacuoles (as if inverted nipple).

Microscopic and Histological changes

The results of the microscopic examination showed the presence of invasive breast carcinoma. The study revealed the presence of large numbers of tumour cells in different shapes and sizes in the gland tissue. Many of these tumour cells were large with huge numbers of inflammatory white blood cells on the opposite side of the gland, as well The tumour cells infiltrated the numbers of those white blood cells, as shown in Figure 1.

Where other advanced studies have shown that the incidence of these tumours in the breast in women may be attributed to many reasons, including environmental, nutritional factors, poor health awareness, exposure to radiation hazards (uranium in particular), and this was documented by Lam et al. [14]. As most of the cases that have been observed upon review to the Teaching Oncology Hospital were swelling in the armpits where a person has been diagnosed by specialists with the presence of swelling of the axillary lymph nodes, where their enlargement was an indication of the relationship with the mammary gland tumour through the flow of lymph from the gland to the axillary lymph nodes, as It was observed through the questionnaire form for patients that they did not want to eat food because of the psychological condition that accompanied the injury, as well as taking anti-cancer drugs among some of them after the diagnosis that weakens the appetite with anaemia and this corresponds to what he mentioned [15].

The results of the microscopic examination also showed another type of breast cancer, which was papillary carcinoma, as the gland tissue contains epithelial cell hyperplasia, as it appeared in the form of large papillae that filled the gland cavity, where each lobule formed from the infiltration of vast numbers of metastatic tumour cells. Which were formed from cells of different shapes, some small and large from the same papilla and surrounding atrophic lumbar ducts, and there was limited lymphocytic infiltration at the extremities of papillae as in Figure 2.

As papillary carcinoma was observed through the results of the study, it had a widespread in the gland tissue. Tumour cells were different forms of heterogeneity, which means an excessive activity of these cells and their invasion of the tissue of the gland was considered to be of the third grade (G3), and this corresponds to What he mentioned [16, 17].

The results of the microscopic examination also showed lobular and ductal epithelial hyperplasia, where massive leaching of metastatic cells in lobules of the lymph nodes and ducts was observed

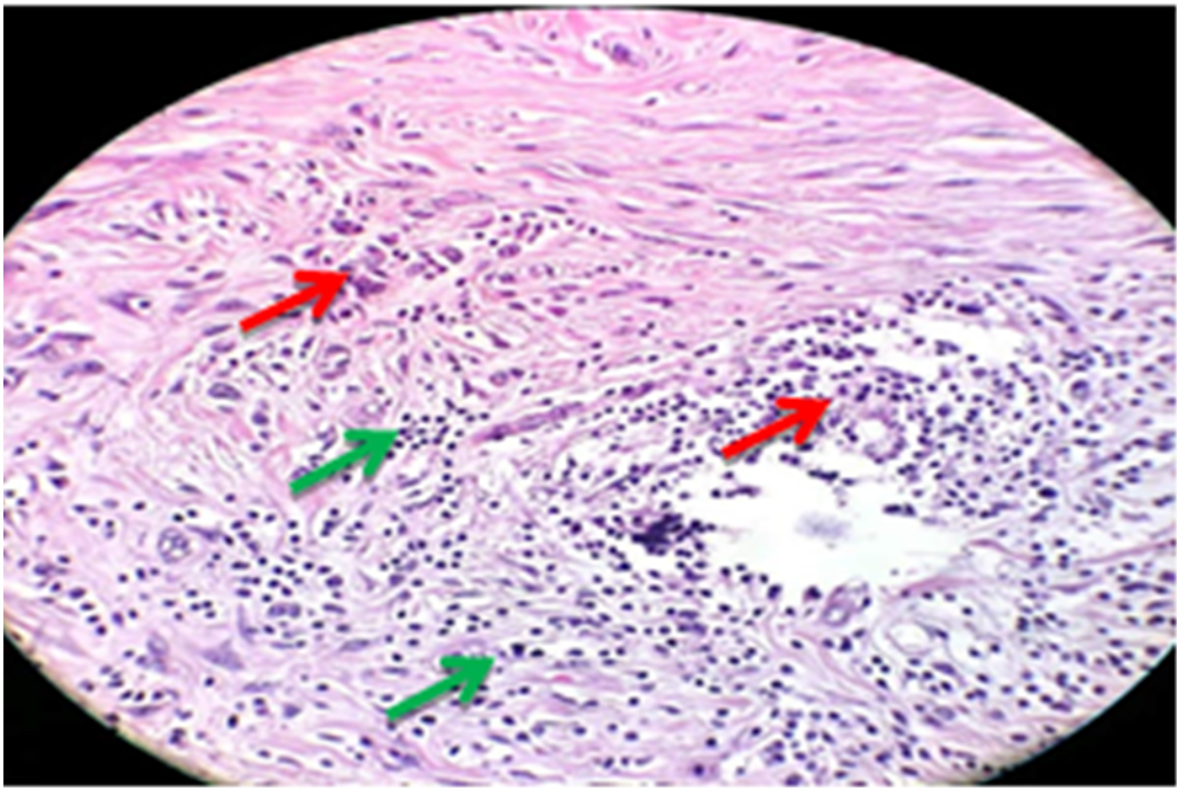

Figure 1: Cross section in the breast tissue showing breast cancer, invasive type G3,characterized by the presence of tumor cells different sizes (↗) in the gland tissue with the presence of numbers of inflammatory white blood cells (↗), the color of H&E, the zoom power(200)

with the presence of large numbers of white blood cells between them. Severe hyperemia was found in the blood vessels in the gland with increasing vessel size The hematoma and its expansion, and the presence in the lumen of some alveolar glands, a uniform, leaky red colour in the lumen of those alveoli, and as shown in Figure 3.

As the emergence of ductal lobular hyperplasia coincided with the presence of blood congestion and hyperemia in the blood vessels, which means that the blood plays an essential role in carrying white blood cells to help curb the tumour, as well as it may have a role in the transfer of tumour cells to other areas of the body and this corresponds to what was mentioned before [18].

The results of the histological examination also showed the presence of a type of breast cancer, which was the cribriform carcinoma, where the epithelium tumour cells were found in many of the acini, where it emerged as contiguous holes with each other and spaced out, which is surrounded by the areolar tissue. The dense and numbers of white blood cells, especially lymphocytes in a leaky form, spread around many acini, as adipose tissue surrounding the end of the gland and fatty cells appeared degenerated and some of them were removed from the walls where the cells communicated with each other to appear as alveoli as in Figure 4.

In the results of the previous image above, another tumour type of breast cancer appeared, which is Cribriform Carcinoma, and this is one of the rare cases of breast cancer. The reason may be attributed to the spread of tumour cells through the spreading duct system at different parts of the gland, and this is consistent with the study [19].

The results of the histological examination showed the presence of the most dangerous type of breast cancer, Invasive Carcinoma (which is characterized by its breakage of the basal membrane and the colloid of other tissues), where the emergence of vast numbers of epithelial cells with large nuclei of these cells together with each other, with the presence of reduction in some of the cytoplasm, with the edges of these tumour epithelial cells appearing in the form of tapered-shaped heads toward the depth of the areolar tissue of the gland, in addition to the accumulation of tumour cysts (adenoid cysts) in the gland board with small masses of degenerated cells surrounded by gaps or fossils with borders clear,

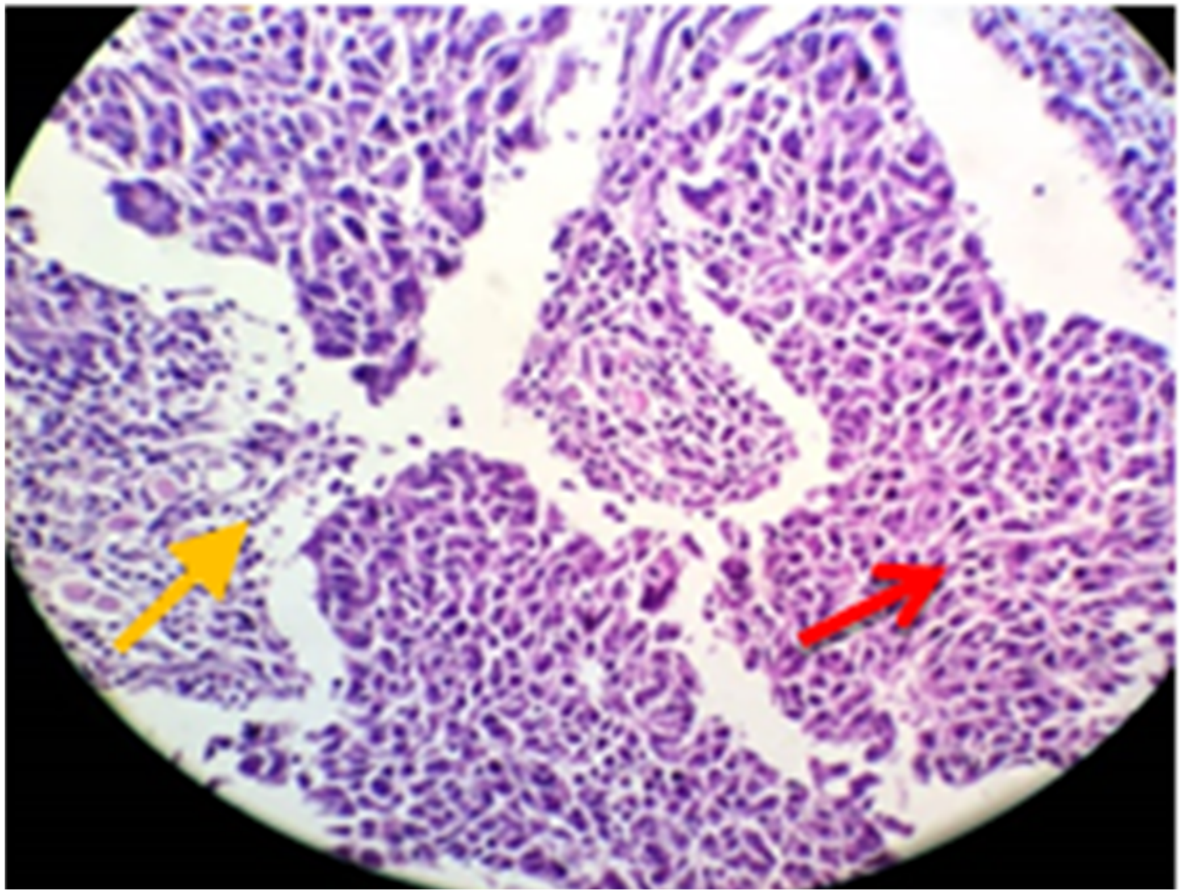

Figure 2: Section in the breast tissue showing papillary breast cancer (type NOS) G3, showing that gland tissue containing epithelial hyperplasia (↗) and the presence of limited lymphocytic infiltration (↗), color H&E, zoom power (400)

as noted the disintegration of the fibrous tissue due to the degeneration of some colloidal fibres and the infiltration of some white blood cells, especially the lymphocytes, into the spaces and vacuole in the tissue, as shown in Figure 5.

The results of histological examination also showed the presence of lobular adenocarcinoma, as this type is distinguished by the appearance of epithelial adenocarcinoma cells in different forms and with large nuclei and a large mass compact with each other adjacent to the fibrous tissue at the other end of the gland, in which many lymphocytes and cells appeared. A limited tumour around the glandular duct, as cellular necrosis appeared in small ducts, as in Figure 6.

As the results of the histological examination also indicated the presence of one or more lobes in which there was an unpublished cellular and tumour transformation into other lobules, and the reason may be attributed to the role of the immune system as well in limiting the spread of the tumour, as this may mean an early stage of the spread of this tumour to other lobules, and this was also noticeable before [19] and Figure 6 shows that.

Physiological variation in breast cancer patients

The current study showed, as shown in Figure 7, the significantly higher concentration of endothelin-1 ($P \leq 0.01$) in the blood of women with breast cancer group 5 at a rate of (101.59±4.058)pg/ml and group 4 at a rate of (97.63±4.77)pg/ml, compared to the control group as a usual case, as it was at a rate (72.29±6.08)pg/ml.

In contrast, a significant increase ($P \leq 0.05$) occurred in the concentration of endothelin-1 in affected women in group 3, 2 and 6 at a rate of (91.3±6.44)pg/ml, (79.56±9.76)pg/ml, (79.42±6.34)pg/ml, respectively, compared to the control group at the rate of (72.29±6.08)pg/ml.

This may be due to the spread of injury outside the breast tissue, i.e. to the axillary region. The current results were in agreement with the results of the Tamkus et al. (2013) [20], which showed an increase in the levels of endothelin-1 in breast can-

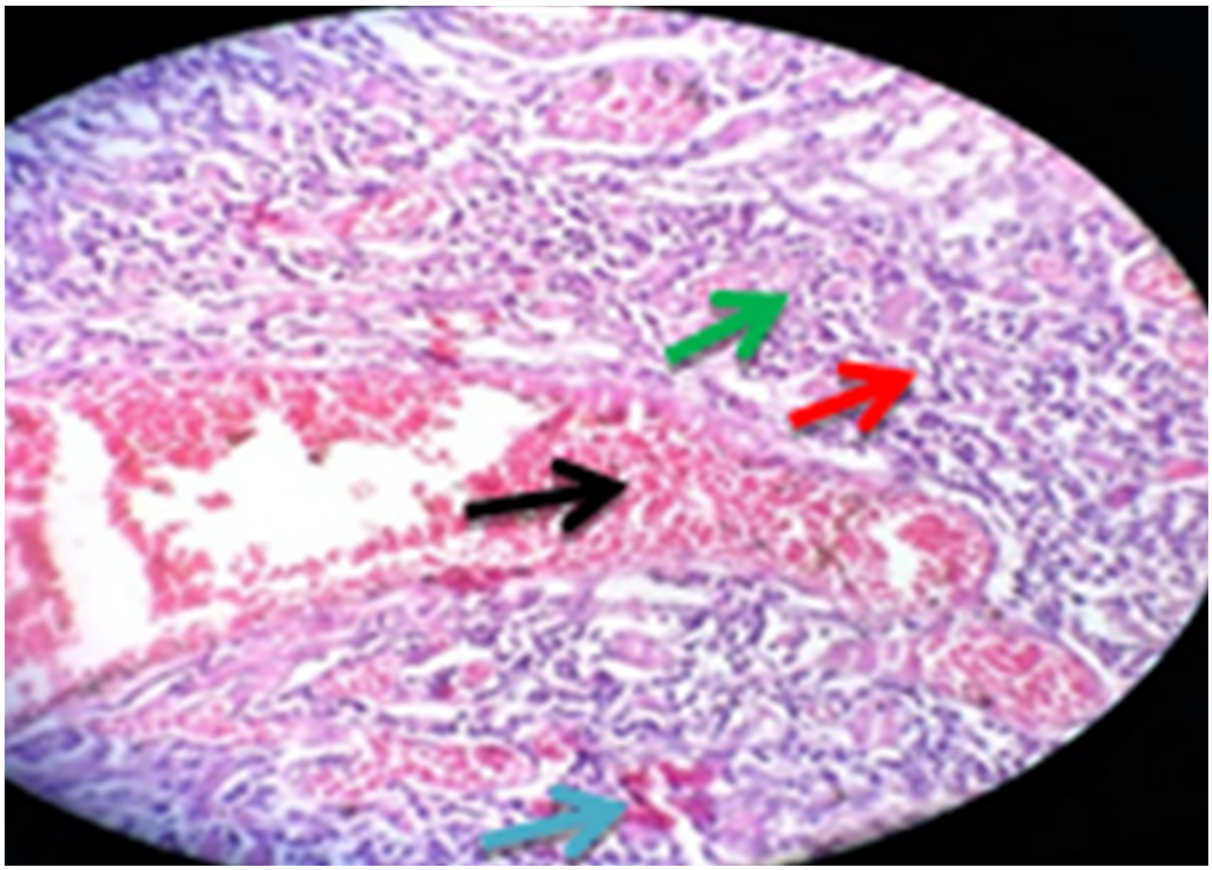

Figure 3: The section showing breast cancer, G3, notice the transfusion of tumor metastatic cells in a tumor (↗), with a large infiltration of white blood cells (↗) and the presence of hyperemia in the blood vessels. (↗), and notice the presence of a homogeneous red color leak in the alveoli cavities (↗), color with H&E, the zoom power(200)

cer patients, and this increase included an increase in the number of lymph nodules in conjunction with the progression of the infection. In blood vessels [21], it is secreted in response to shear stress cases [22]. Endothelin-1 was known as a mitogenic factor for smooth muscle cells [21] and cancer cells [23]. Endothelin-1 was expressed in excessive quantities in carcinoma of the breast, and this term is associated with invasion and spread of the lymph nodes [24, 25].

The mechanism by which endothelin increases the invasion process includes two Autocrine and Paracrine mechanisms that increase the Chemotaxis of tumour cells and the production of proteases [26].

In recent days, it was found that the pathway of endothelin is involved in the carcinogenic process by activating the Proliferation, migration of invasive cells (that is, it helps to invade) and the formation of blood vessels, where advanced studies indicated that endothelin (ET-1) enhances the progression of breast cancer, and quantities of increasing of this peptide in the process of invasion and metastasis [27]. It was found that women with breast cancer show a significantly higher statistical expression of endothelin-1 compared to women who do not have breast cancer, although the physiological role of ET-1 in human breast cancer has not been well known, its increased level has implications for Tumor growth and cell growth, works by ETA and ETB receptors [28]. The current study showed high levels of ET-1 in the blood serum of women with breast cancer, and this result indicates that the presence of the tumour causes an increase in ET-1 levels. As previous studies did not find any correlation between breast cancer warning factors such as tumour size, lymph nodes and ET-1 level, other studies conducted measuring ET-1 levels in patients with liver cancer, where there was a significant increase in levels and concentration of this peptide also, in the colon and rectal cancer, there was a rise in its levels. That increased production is more likely to epithelial cancer cells, as it was found in these patients with diffuse cancer that the percentage of this peptide was much higher than others with local infection [28].

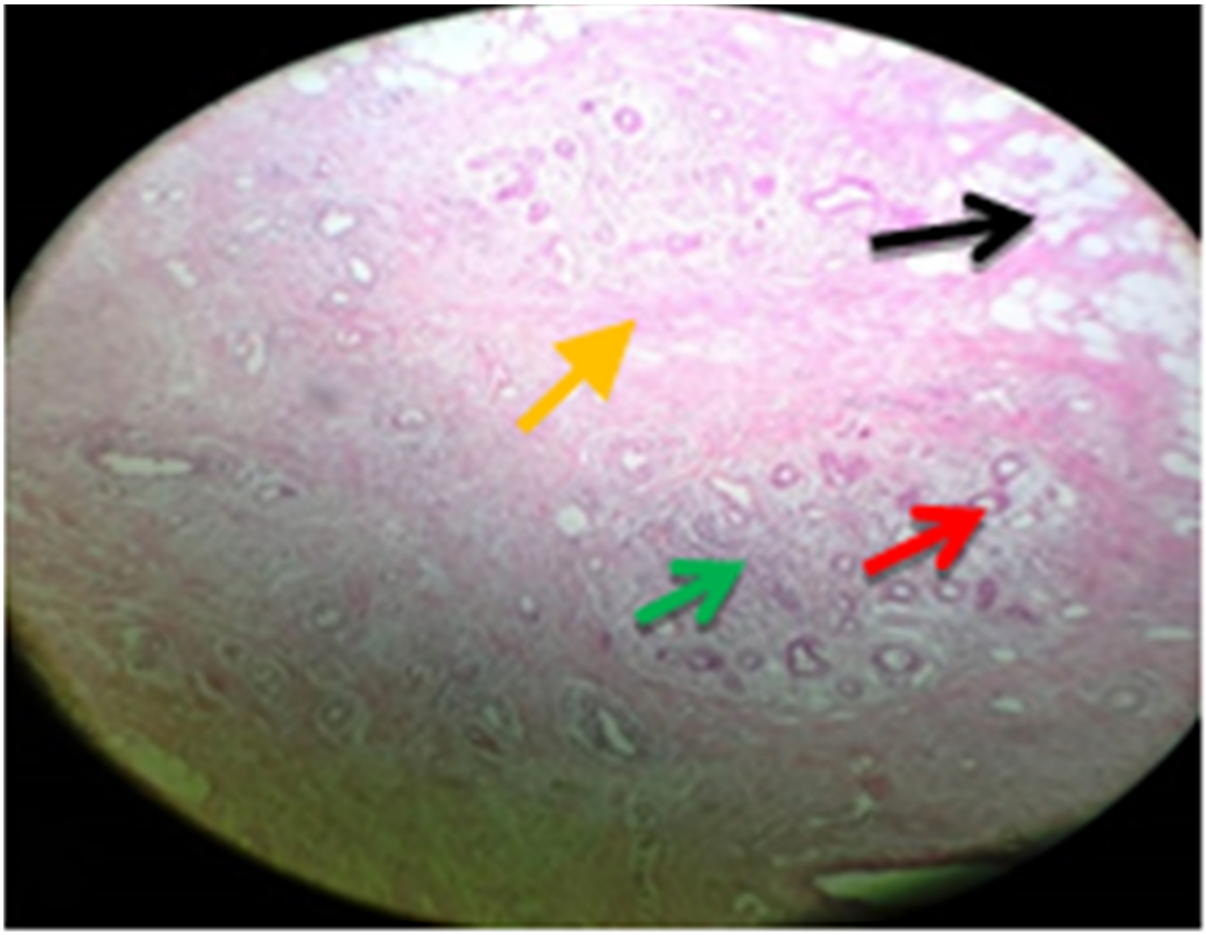

Figure 4: Section of the breast tissue showing the cribriform carcinoma of breast cancer, G2,which is characterized by the presence of epithelial and tumor cells (↗) and white lymphocytes (↗), the presence of degenerative fat cells (↗), fibrosis (↗), H&E color, zoom power (100)

By contrast, in the current study, its rate was higher in the early stages of infection. They underwent initial eradication operations and their treatment with specific therapeutic doses compared to patients who had advanced injury and metastases to other regions. This difference may be due to the effect of topical treatment and systemic anti-tumour given to treating cancer Breast at ET-1 levels in these patients, and this may indicate that levels of this peptide appear to change with treatment and progression of the infection. [25] signal that the expression of ET-1 receptors (ETA and ETB) is associated with expression of the vascular endothelial factor and the generation of new vessels, and thus ET-1 affects the possibility of metastasis to cancer cells by stimulating them to form new blood vessels.

Where clinical studies have demonstrated the role of endothelin in the growth of malignant cells as well as tumour invasion and vascular generation [29], about breast cancer, as the expression of ET-1 and its receptors was associated with the conversion of normal cells into malignant cells [30].

The results of the current study indicate a gradual increase in the levels of expression of ET-1 in patients with breast cancer depending on the development of the disease and this corresponds to Kalles et al.(2019) [29]. Alanen et al.(2000) [31] described elevated peptide ET-1 in samples from women with malignant breast cancer compared to the moderate level of healthy and benign women. It should be noted that the Angiogenesis process is a fundamental process related to the natural history of tumour growth.

Therefore, anti-vascular therapy has a benefit in curbing breast cancer. The vascular endothelial growth factor (VEGF) is one of the most critical factors involved in vascular activity and formation in breast cancer.

Therefore the effect of ET-1 and its receptors on the formation of blood vessels for rising cancer may be achieved in cooperation with the term VEGF [29].

The nether blood variables, as the current study showed, as in Figure 8, the presence of a highly sig-

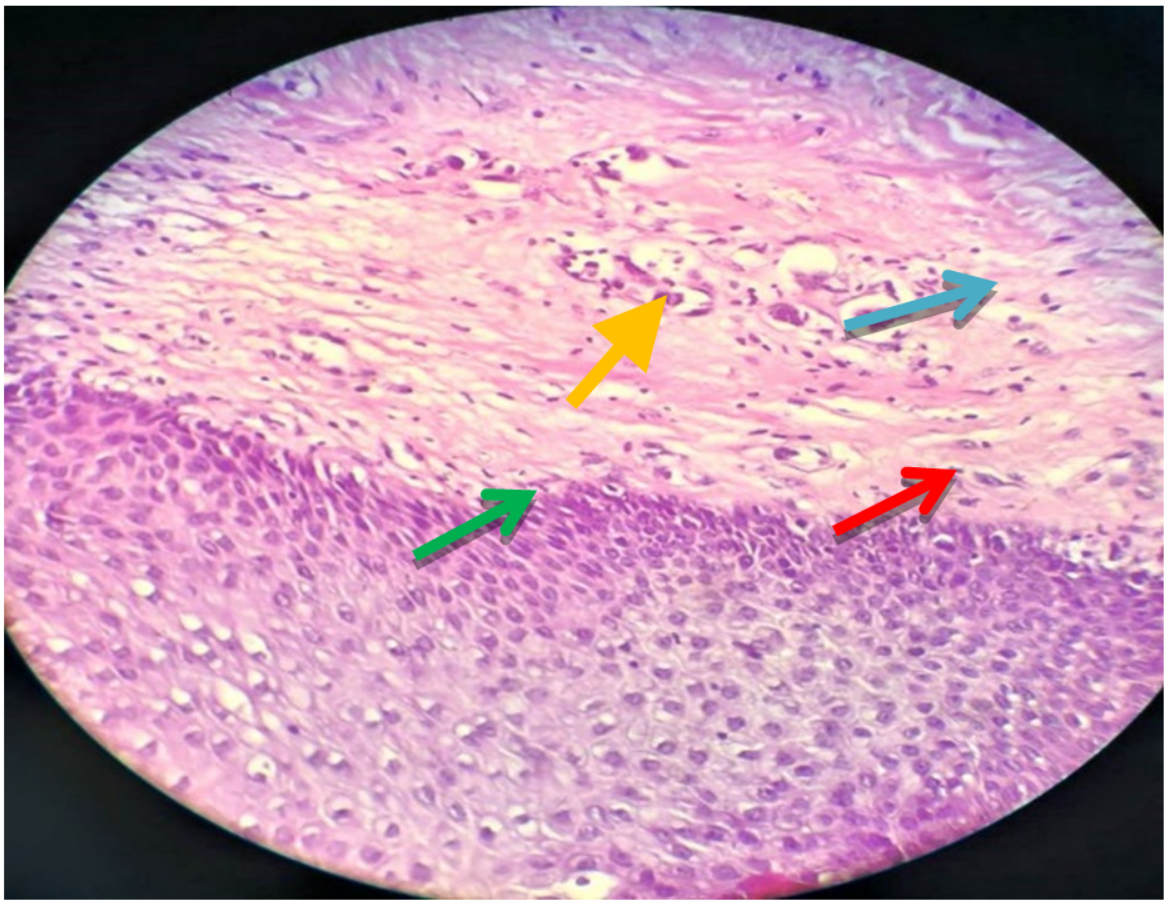

Figure 5: Section of the breast tissue showing breast cancer invasive carcinoma, G2, where the number of invasive tumor cells (↗) is shown in the tissue with the edges of these tumor cells appearing in the form of gas pointed heads (↗) and tumor adenoid cysts (↗), with infiltration of white lymphocytes (↗), H&E color, zoom power (200).

nificant decrease ($P \leq 0.01$) in the cholesterol concentration of women with breast cancer for group 5 at a rate of (160.61±6.34)mg/dl, and group 6 at a rate of (160.9±5.22)mg/dl compared to the control group, it was at the rate of (201.13±1.435)mg/dl.

In contrast, its concentration decreased significantly ($P \leq 0.05$) in the group 4 at the rate of (169.69±3.731)mg/dl compared to the control group at the rate of (201.13±1.435)mg/dl, where the results showed that there was no increase in the level of cholesterol for the groups of women with breast cancer compared to the control group and the low cholesterol level over the range. It is normal for the body.

These results correspond to the results reported by Riley et al. (2016) [32] as they indicated in their findings that the percentage of cholesterol does not affect breast cancer and its survival within the normal range.

Where Haritwal et al. (2016) [33] found an increase in the concentration of cholesterol and lipid in women with breast cancer compared to the control group, this difference can be attributed to the fat metabolism between breast cancer patients and the control group, and this is contrary to the results of the current study.

Sharma et al. (2016) [34] stated that chemotherapy significantly changes plasma fat in breast cancer patients. Sharma et al. (2016) [34] found that many chemotherapy drugs for cancer patients show some change in the features of the lipid substance. However, this mechanism is unknown, as they studied the levels of lipids in the blood serum of breast cancer patients, as their results showed a significant decrease in the level of cholesterol and fat after patients took chemotherapy, they attributed the reason to the fact that the chemical compounds used in the treatment affect the genes responsible for the metabolism of lipids in the liver cells, with a difference in the effect of a compound from another.

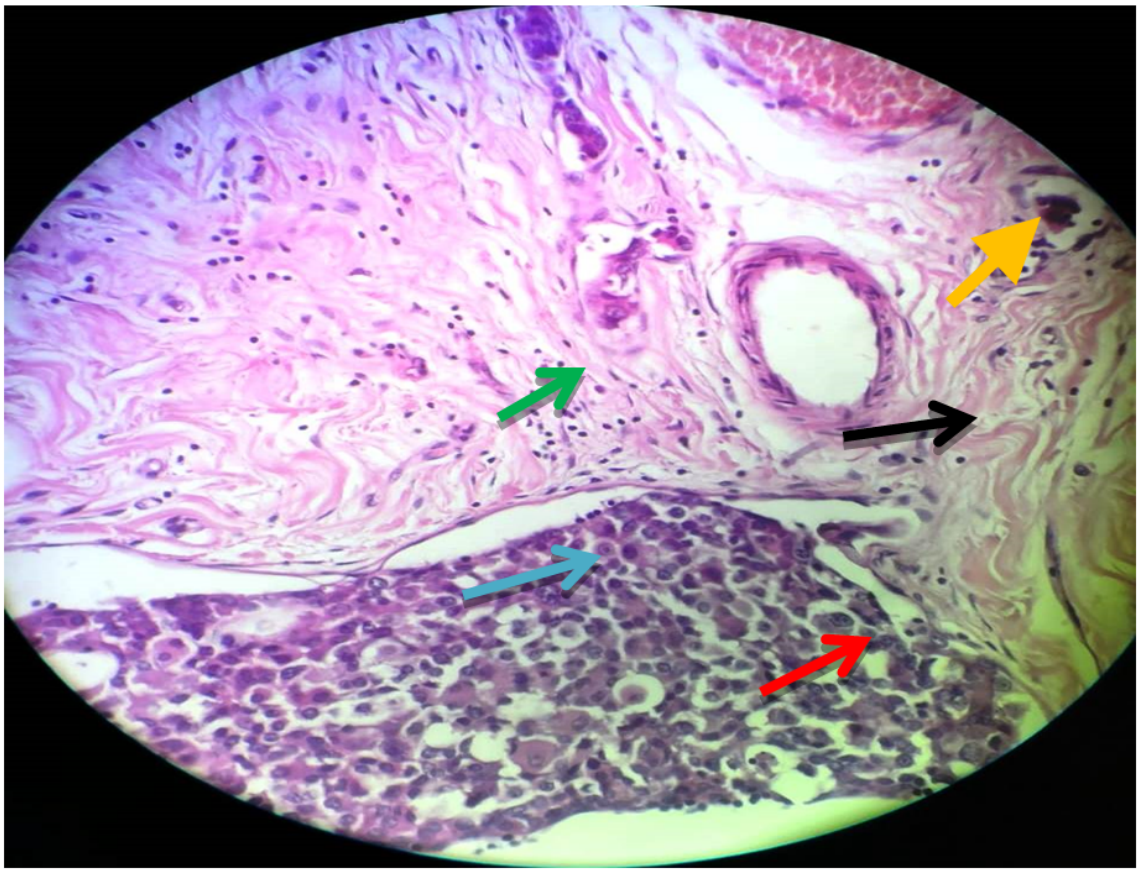

Figure 6: Section of the breast tissue showing lobular carcinoma (adenocarcinoma) G2, where it shows the emergence of cancerous epithelial cells (↗) with large thickening nuclei (↗) and the spread of lymphocytes (↗) and the presence of cellular necrosis (↗), fibrosis (↗), H&E color, zoom power (400)

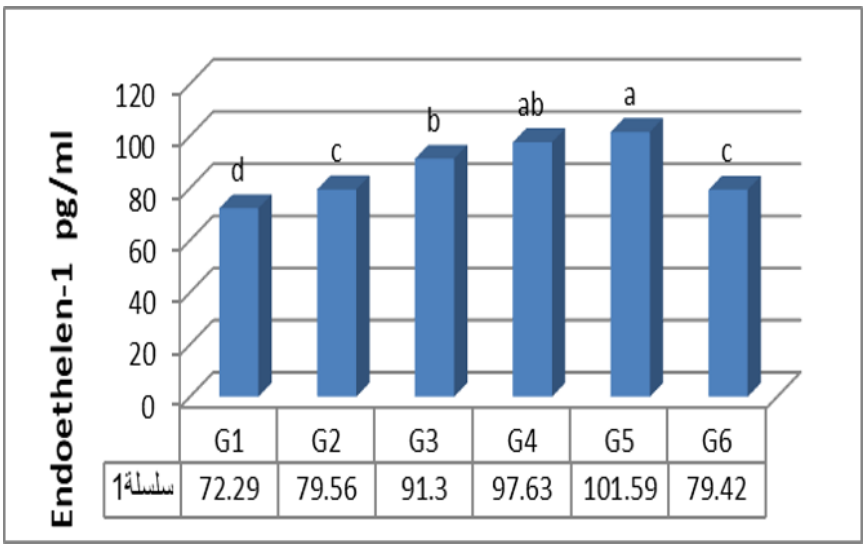

Figure 7: Shows the changes in the concentrations ofendothelin-1 among different injury groups of women with breast cancer, compared to the control group as a normal case

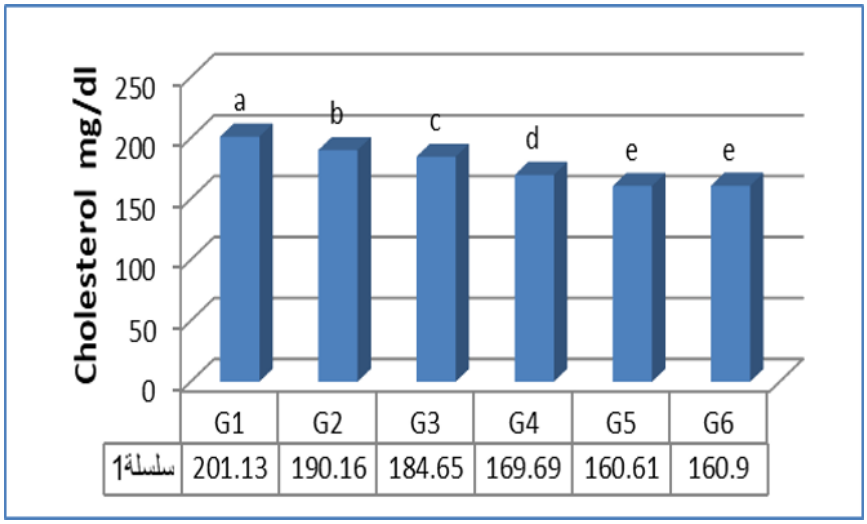

Figure 8: The changes in cholesterol concentrations indifferent groups of women with breast cancer, compared to the control group as a normal case

Some previous studies assume that changes in the serum lipid concentration in breast cancer patients can lead to increased production of tumour necrosis factor [35].

CONCLUSION

Through the results of the current study, we conclude that women's breast cancer has a direct negative impact as it leads to general inactivity and weakness in women and sometimes deaths. Breast cancer affects many biochemical variables, causing anaemia, and multiple histological effects (necrosis, congestion, and recurring inflammation). The histological examination also showed the presence of various forms of malignant tumours, among them lobular and ductal epithelial hyperplasia of breast cancer, papillary tumour, and cribriform carcinoma. Also, it was found that haematological changes were represented by a high concentration of Endothelin-1 at all stages of infection in women with breast cancer, which reflects the extent of the suffering of the blood vessel walls from disease or treatment, or both, as well as lower cholesterol than the normal range of the body.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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