

Physiological and Histological Study of Breast Cancer Patients in Al-Muthanna Governorate–Iraq

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ABSTRACT

Background: Cancer is a very deadly disease and the most prevalent cancer in women around the globe is breast cancer. Second to cardiovascular disease in the list of deaths in Iraq is breast cancer. This study was carried out at Al-Muthanna Governorate in Iraq. **The Aim:** This current study aimed to assess the variations in hematological and biochemical parameters in cases of breast cancer, and also to bring out the histological changes that are unique to breast cancer patients. **Methods:** This study incorporated a total of 60 clinical samples, involving women of various age groups (30-60 years) diagnosed with breast cancer and control, all of whom were patients at Al-Sadiq Hospital and daily clinics in Al-Muthanaa province. **Results:** Our study results indicate that. The results showed that there were no significant differences between the patient group and the control group in terms of the hematological parameters which were evaluated (RBCs, HB, PCV, WBCs, Platelet), and slight differences were observed regarding biochemical parameters (ALT, AST, Cholesterol) between the two groups, with $P < 0.05$ significance. However, the study did show effects of breast cancer on these parameters. However, the levels of Progesterone, Estrogen, and Prolactin showed significant differences ($P < 0.05$) between the patient group and control group. Also, we found important differences in the histology, morphology of the breast tissue when compared with healthy breast tissue. **Conclusions:** The changes consisted of abnormal cell growth, enlarged breast structures, abnormal clusters of cells, blockage of blood, elongated areas with occasional bleeding, necrotic lesions and other changes that were noticeable. All these changes are indicative of the existence of breast cancer.

Keywords: Breast cancer, Biochemical, Hormonal, Histopathological.

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INTRODUCTION

Cancer is characterized by the abnormal proliferation of cells, resulting from various alterations in gene expression, these changes disrupt the normal balance between cell growth and death, ultimately this process gives rise to cells that acquire invasive and metastatic properties, enabling them to infiltrate surrounding tissues and disseminate to distant sites. this invasive behavior leads to substantial morbidity if untreated (Gomes, 2021). Breast cancer is the most prevalent cancer in females and the second most fatal cancer, following lung cancer. Breast cancer has increased worldwide over the years and over one million new cases are reported each year. (Houssein et al, 2021).

The majority of breast cancers arise in the breast tissue due to the excessive proliferation of ducts connecting lobules to the nipple. Continuous stimulation by various carcinogenic risk factors leads to the transformation of these cells into localized tumors or, in more advanced cases, metastatic carcinomas (Hosseini H et al, 2019).

A risk factor refers to anything that increases the chances of developing a disease. Every woman is susceptible to developing breast cancer. Several significant risk factors for breast cancer that affect a large portion of the population have been recognized for a long time. However, the majority of breast cancer cases occur in women who do not have any

identifiable risk factors other than their gender (Feng et al, 2019). In Iraq, the first cancer in ranking diagnosed in women (Arkan, 2016).

Histopathologically, a hallmark is the presence of many giant cells in the stromal tissue as well as sometimes in the glandular lumina. It's crucial to emphasize that these giant cells are not considered neoplastic. They are distinctly large, have abundant cytoplasm and have several central nuclei, some of which may be visible with nucleoli. The carcinoma cells can assume different patterns, including tubules, glands, papillae and may also show mucinous or lobular patterns. There are several inflammatory cells in the stromal tissue, lymphocytes, monocytes and histiocytes, and numerous hemosiderin deposits and extra-vascular red blood cells. These hemosiderins make an important part of the characteristic brown colour of the tumour (d'Amati et al, 2022).

Biochemical and hematological parameters, involving diverse enzymes, biomolecules, and blood cell types (including WBC, RBC, and PLT), provide important information about the body's overall health. A comprehensive blood analysis enables healthcare professionals to evaluate symptoms such as pain, fatigue, weakness, or any other indications of illness (Khan et al, 2017).

METHODS

The total number of clinical samples included in this study was 60 consisting of elderly women and younger women diagnosed with breast cancer. These were patients of private Al-Sadiq Hospital and daily clinic of Al-Muthanaa province. A total of sixty samples were taken, and divided into groups of people with breast cancer and a control group. The age of the patients and healthy subjects ranged from (30 to 60 years).

After careful examination of the health record of each sample, and confirm the diagnosis of the disease by histology, the samples which do not meet the study criterion or are not relevant to the disease were excluded. This was done with hospital administration and attending physician approval and complete informed consent of the patients and with assurances that patient information would be kept confidential.

Study Samples

Blood sample

For each participant in both the patient and control groups, 5 mL of venous blood was withdrawn and subsequently divided into two tubes. Specifically, 2 mL of blood was collected in an EDTA tube for hematological parameter tests, and 3 mL of blood placed in a gel tube. After letting the blood samples stand for 15 minutes, they underwent centrifugation at speed of 3000 rpm for duration of 10 minutes. The resulting serum was then frozen at -20°C until it was prepared for use in the measurement of biochemical parameters.

Biopsy Sample

It was placed in a plastic container with 10% neutral buffered formalin (NBF) and processed through an automatic tissue processor (Histoline, Italy) under the supervision of Dr. Haider Khudair in his laboratory, Muthanna, Iraq. The purpose of this procedure was to produce formalin-fixed, paraffin-embedded (FFPE) blocks to be examined histologically. After removal of the organs from the patients, the tissue samples were immediately collected and fixed in 10% formalin. Following fixation, the samples were carefully sliced with a special scalpel and mounted into tissue cassettes with labels. Tissues were treated with a series of alcohol solutions of varying strengths (70%, 95%, and 100%) to remove the fixation agent and water. After this, paraffin block with the tissue was precisely cut to show the target area and then finely cut tissue slices, about 5 micrometers thick were cut using a microtome machine. The purpose of this was to enhance the visibility and clarity of tissue structures to be assessed. The sections of tissue were more often than not subjected to both (H&E) and PAS-stains.

RESULTS

Demographic characteristics

There were no statistically significant differences between the mean ages of patient group and the control group ($P < 0.05$) (Table 1). Likewise, no significant differences were seen within the different age groups between patients and control groups ($P = 0.739$). Additionally, no significant difference was found between patient and control groups in terms of residence (urban and rural) ($P = 0.654$). In this case-control study, it is important to recognize that the

distribution of the individuals in both groups in terms of age and residency are important factors to not only be considered but also confirm that there are not statistically significant differences between the groups in terms of age and

residency. The present findings show that 22.5% of breast cancer patients have a family history of breast cancer. On the other hand, (77.5%) does not have family history of breast cancer as shown in (Table 1).

Table (1): Demographic characteristics of patients with Breast Cancer and healthy control subjects

Subjects

Characteristics	Patients <i>n</i> = 40	Healthy control <i>n</i> = 20	<i>P</i>
Age (years)			
Mean ±SD	40.15 ± 13.87	40.50 ± 10.70	0.941 † NS
Range	30– 60	31– 58	
30-39, <i>n</i> (%)	12 (30.0%)	8 (40.0%)	0.739 ¥ NS
40-49, <i>n</i> (%)	19 (47.5%)	8 (40.0%)	
50-60, <i>n</i> (%)	9 (22.5%)	4 (20.0%)	
Residence			
Urban, <i>n</i> (%)	27 (67.5 %)	12 (60.0 %)	0.654 ¥ NS
Rural, <i>n</i> (%)	13 (32.5%)	8 (40.0%)	
Family History			
Positive	9 (22.5%)		
Negative	31 (77.5%)		

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P > 0.05$

Physiological Examination

Hematological Parameter

The current study showed in (Table 2) no significant difference at ($P < 0.05$) in the white blood cell levels, lymphocyte counts, MID levels and granulocyte counts in the patient groups but slightly lower than those in the control group ($P = 0.954$), ($P = 0.951$), ($P = 0.549$) and ($P = 0.328$) respectively.

Furthermore, the findings from table (2) reveal that there is no significant difference at ($P < 0.05$) in the levels of red blood cells, hemoglobin (Hb), and packed cell volume (PCV) in the patient groups, but

these values are slightly lower compared to those in the control group ($P = 0.557$, $P = 0.485$, and $P = 0.824$ respectively).

Finally, the study indicate no significant difference at ($P < 0.05$) in mean of platelet count in patient groups was somewhat higher compared with control group ($P = 0.093$).

Table (2): Mean levels of some hematological parameters in patients and control group.

Hematological parameters	Cases –control comparison		<i>P</i>
	Patients <i>n</i> = 40	Healthy control <i>n</i> = 20	
White Blood Count (WBC)			
Mean± SD	7.57 ± 1.69	7.61 ± 2.69	0.954 † NS
Range	4.91 – 14.17	5.30- 14.10	
Lymphocytes Count			
Mean± SD	0.282 ± 0.077	0.280 ± 0.083	0.951 † NS
Range	0.13 – 0.49	0.13- 0.42	
MID			
Mean± SD	0.182 ± 0.123	0.226 ± 0.211	0.549 NS
Range	0.01 -0.70	0.01-0.75	

Hematological parameters	Cases –control comparison		P
	Patients n = 40	Healthy control n = 20	
Granulocyte count			
Mean± SD	0.648 ± 0.076	0.619 ± 0.106	0.328 † NS
Range	0.5 -0.8	0.48-0.86	
Red Blood Cell (RBC) Count			
Mean± SD	4.46 ± 0.361	4.54 ± 0.51	0.557 † NS
Range	3.70 -5.51	3.60-5.21	
Hemoglobin levels			
Mean± SD	10.76 ± 1.56	11.36± 1.78	0.485 † NS
Range	8.30 -14.30	8.40-14.10	
Packed cell volume (PCV)			
Mean± SD	34. 84 ± 3.82	35. 53± 4.60	0.824 † NS
Range	27.70 -42.70	28.50-44.0	
Platelet count			
Mean± SD	284.82 ± 77.19	237.70± 80.08	0.093 † NS
Range	150.0 -455.0	126.0-380.0	

n: number of cases; SD: standard deviation; †: independent samples t-test; NS: Non-significant at $P < 0.05$.

Biochemical Parameters

The research indicates that there is no statistically significant disparity at ($P < 0.05$) in the average levels of alanine transaminase (ALT) between the patient groups and control group, although slightly elevated levels are observed in the patient group ($P = 0.255$). Similarly, the study demonstrates no statistically significant difference at ($P < 0.05$) in the mean levels of aspartate aminotransferase (AST) between the patient groups and the

control group, with slightly higher levels observed in the patient group ($P = 0.493$). Moreover, the study findings suggest that there is no statistically significant difference at ($P < 0.05$) in the mean cholesterol levels between the patients and control groups. However, the cholesterol levels in the patient groups were marginally lower ($P = 0.217$), as indicated in the results (Table 3).

Table (3): Mean levels of some biochemical markers in patients and healthy control.

Biochemical markers	Cases –control comparison		P value
	Patients n = 40	Healthy control n = 20	
Alanine transaminase (ALT) (U/L)			
Mean± SD	16.67 ± 8.68	14.20 ± 3.76	0.255 † NS
Range	7.0 – 39.0	8.0- 20.0	
Aspartate aminotransferase (AST) (U/L)			
Mean± SD	19.82 ± 15.43	16.40 ± 4.32	0.493 † NS
Range	8.0 – 66.0	9.0 – 24.0	
Cholesterol (mg/dl)			
Mean± SD	150.32 ± 36.16	164.80 ± 6.17	0.217 † NS
Range	58.0 -209.0	155.0 -174.0	

n: number of cases; SD: standard deviation; †: independent samples t-test; NS: Non-significant at $P > 0.05$.

Hormonal Parameters

Progesterone levels were assessed in all participants, and the outcomes are presented in (Table 4). The average progesterone levels were elevated in individuals with breast cancer compared

to control group ($P < 0.05$), and this discrepancy was statistically significant ($P = 0.007$).

Estrogen results are shown in the same table, The mean estrogen levels differed significantly between the patient group and control group ($P < 0.05$). Breast

cancer patients showed elevated average estrogen levels compared to the control group, and this disparity was statistically significant ($P=0.001$).

Prolactin levels were assessed in all participants, and those diagnosed with breast cancer displayed

higher average prolactin levels compared to control group. This difference was identified as statistically significant ($P = 0.001$), as illustrated in the results presented in (Table 4).

Table (4): Serum hormones level in in patients and controls.

Hormonal Parameters	Cases–control comparison		<i>P value</i>
	Patients <i>n</i> = 40	Healthy control <i>n</i> = 20	
Progesterone levels ng/MI			
Mean± SD	3.14 ± 0.53	2.51 ± 0.51	0.002 † S
Range	1.91 – 4.40	1.60- 3.10	
Estrogen level ng/mL			
Mean± SD	71.43 ± 11.44	57.16 ± 15.35	<0.001 † S
Range	40.20 – 85.40	34.20 – 74.90	
Prolactin level			
Mean± SD	31.33 ± 18.71	13.22 ± 3.95	< 0.001 † S
Range	5.17 – 100.55	8.20 – 20.10	

n: number of cases; SD: standard deviation; †: independent samples t-test; S: significant at $P > 0.05$.

Histological Examination

Histological examinations performed on tissue sections showed clear histological changes in the breast and significant external and internal changes. The lactiferous duct has acute tissue changes in the epithelial layer that lining the inner surface of the duct, all cells have irregular nuclei, and acidophilic cytoplasm, and most lumen was closed because of the abnormal cellular growth of the epithelial cell (Figure 2).

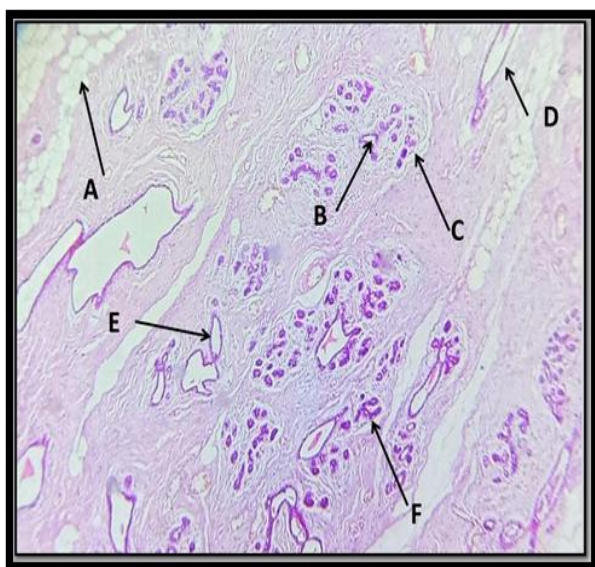


Figure (1) :Tissue section of normal breast shows : A- adipose tissue, B- interlobular ducts, C- lobule D- lactiferous ducts, E- terminal interlobular ducts F- acini H&E (4X).

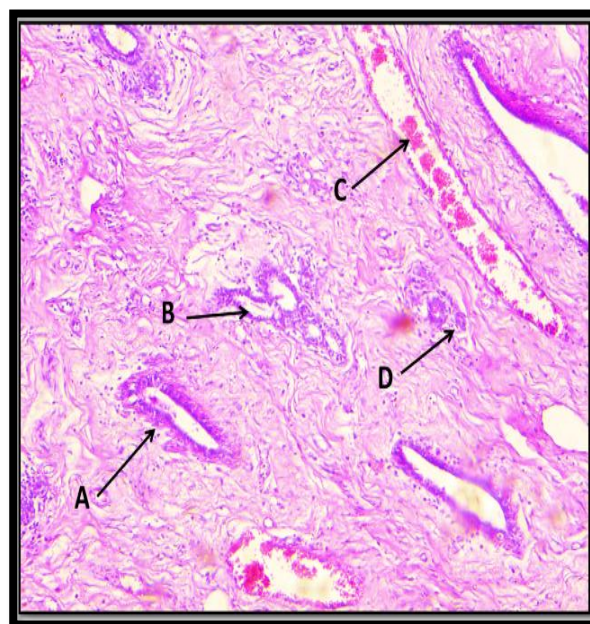


Figure (2): Tissue section of breast cancer shows A- acute tissue changes, B- irregular nuclei, C- acidophilic cytoplasm, D- abnormal cellular growth H&E (10X).

The tissue section of the infected breast showed many irregular dilations filled with thick fluid, some of these dilations have many R.B.CS, all these dilations surrounded by adipose tissue, so clear cellular proliferation between the dilations, these dilations have different shapes which randomly spread through breast stroma (Figure 3).

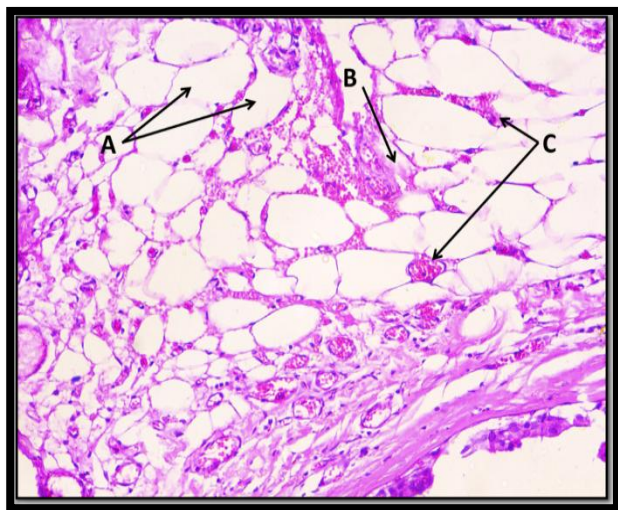


Figure (3): Tissue section of breast cancer shows A- adipose tissue, B- thick fluid, C- R.B.C.S, H&E (20X).

In the histological specimen, the presence of histopathologic lesion which revealed that there was necrotic lesion present in various parts of the breast, where cellular proliferation occurred (Figure 4).

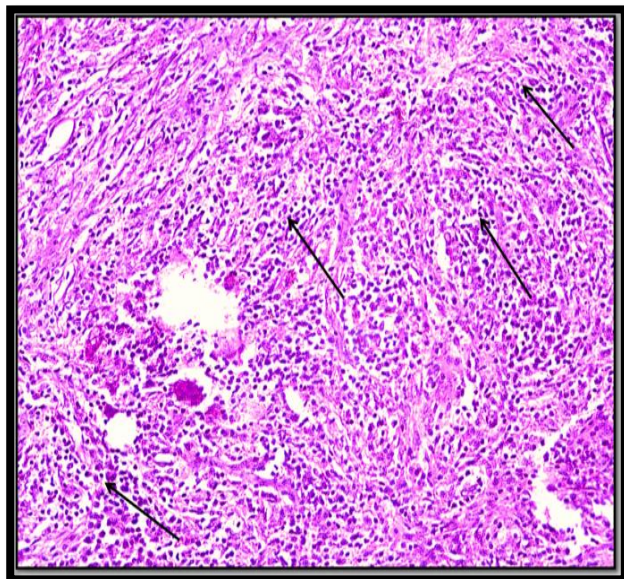


Figure (4): Tissue section of breast cancer shows necrosis lesions H&E (10X).

The histological result noted prominent fibrosis between lobules, with a high amount of fibers with infiltrated cells between elastic and collagen fibers in the breast parenchyma, the connective tissue fibers between lobules were arranged as long broad bands between lobules, and the tissue field appeared wide empty spaces beside the broad band of the connective tissue fibers, tissue necrosis in many locations of breast between lobules (Figure 5).

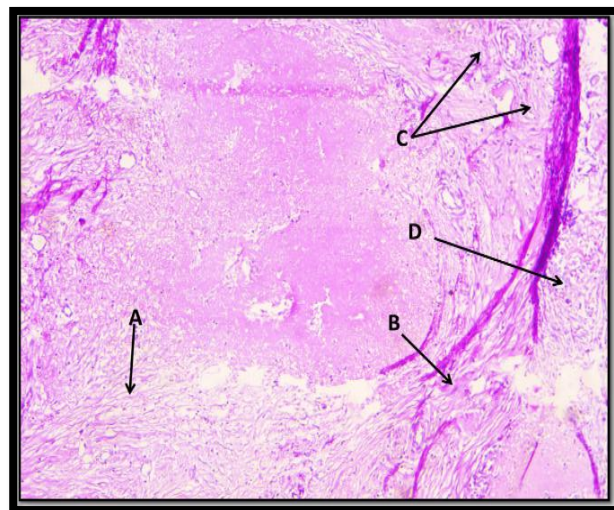


Figure (5): Tissue section of breast cancer shows A- elastic fibers, B- collagen fibers, C- fibrosis, D- tissue necrosis H&E (4X).

DISCUSSION

Breast cancer is the most common type of cancer in women, and is regarded as the second leading cause of deaths. In the past few years, the number of cases associated with breast cancer have increased globally (Houssein et al, 2021). A more aggressive manifestation of cancer is frequently associated with onset of breast cancer at a younger age. In our study, we noted that the highest incidence of cases occurred among individuals aged 40 to 49 years, constituting 47.5% of the total patients. This observation is consistent with the outcomes of earlier studies that reported Iraq having the highest risk of breast cancer among all nations, possibly linked to the sociopolitical conditions in the country, such as chemical warfare and bombings.

This might explain why southern governorates like Basra and Thi-Qar exhibit a heightened risk of breast cancer (Alwan, 2010). In addition to this, we found out that nearly 67.5% of the women affected by breast cancer dwelled in urban regions, while about 32.5% of the women lived in the rural regions. This is in agreement with the findings from previous studies (Aynalem et al, 2022). Several reasons might explain why these trends have been observed. Firstly, considering the greater population concentration in urban regions as well as constant emission of gases and smokes

from industries located there, the greater prevalence of automobiles, and the lack of agricultural practices, one might say that the urban regions present quite a polluted environment compared to the rural regions. In our study, we noted a lower percentage of patients (22.5%) reporting positive family history, in contrast to a higher proportion (77.30%) with a negative family history. These observations align with the results of a previous research study, the heightened risk linked to having positive family history may be attributed to various inherited factors. All of these factors may help to cause both estrogen receptor positive (ER-positive) and estrogen receptor negative (ER-negative) tumors (Khalaf et al, 2022). The low white blood cells can be attributed to a side effect of take certain medical drugs [11].

The average counts of lymphocytes and granulocyte were found to be higher in the cases compared to the controls, but the MID was lower compared to the control. The increased counts can be attributed to presence of cancer cells in neoplasms (Aynalem et al 2022). values were also smaller in those cases compared with the healthy individuals because hemoglobin and packed cell volume values are indicators of the existence of anemia, and the major problem experienced by most cancer patients due to the disease is anemia. This small value in the blood components could be a result of immunosuppression or bone marrow depression. The other reason may be that these indicators could be drastically lowered if the patients had pre- or postoperative problems. Additionally, patients' clinical circumstances and dietary state may also have a significant impact on the lowering of such levels (Aynalem et al 2022).

The increase platelete might be explained by the reactive thrombocytosis that occurs in the majority of breast cancer patients as result of anemia caused by the disease (Akinbami et al, 2013). Also, the increased activity of transaminase in breast cancer patients as compared to the healthy individuals signifies that there is an elevation in the levels of AST and ALT. which can indicate liver dysfunction, tissue damage, and necrosis. It is possible that the elevation in liver enzymes is attributed to the metastasis of breast cancer to the liver,

leading to cellular inflammation in the liver tissues (Hassen et al. 2021). And this decrease in cholesterol may be attributed to the dietary habits adopted by women undergoing breast cancer treatment. A significant decrease in the total cholesterol level was observed in breast cancer patients compared to the control group, which can be attributed to the increased utilization of cholesterol by tumor cells for the formation of a new biofilm (Alkindi et al, 2022). increased ovarian secretion of the progesterone hormone may be contributing factor to the development of the breast cancer in many women. The down-regulation of the progesterone receptors in the breast tissue might possibly account for their negative implications. It has been suggested that progesterone may be linked to the protection of the body from and increased threat of breast cancer. Progesterone levels have been shown to play a key role in the occurrence of breast cancer before and after menopause, being inversely proportional to the occurrence of breast tumors (Abbas & Alsamarai. 2022).

Serum estrogen levels were found to be significantly higher in females with breast cancer compared to control group. The presence of elevated estrogen levels can contribute to tumor development by promoting cell proliferation in the presence of existing mutations or potentially increasing the likelihood of mutation regulation of the proliferation and differentiation processes of the mammary gland. The described process could be an important one in the formation of breast cancer. As another potential pathway for action, it could be involved in the metabolism of estrogen, which can cause oxidative stress, thus affecting the proliferation of the mammary cancer cells.

Metabolites of estrogen can act in interaction with DNA mutations or reactive oxygen species (ROS), leading to accumulation of genomic alterations that are essential for mammary tumorigenesis (Abbas & Alsamarai. 2022). a correlation between prolactin levels and breast cancer. The observed difference highlights the potential specificity of prolactin as a diagnostic and prognostic marker for this disease. The evidence indicates that prolactin plays crucial role in the growth and regulation of both normal and malignant breast cells.

Prolactin is believed to contribute to the carcinogenic processes of the breast, potentially through its stimulatory effects on the immune system and its proliferative and anti-apoptotic properties (St-Hilaire et al , 2011).

As for the histological field, aberrant cells may be present within the lining of breast ducts, yet they haven't extended beyond the duct itself, indicating a condition known as ductal carcinoma in situ (DCIS), form of breast cancer, these observed changes, such as irregular nuclei and abnormal cell growth, are indicative of this disorder (Ibrahim , 2017). Also, abnormal expansions within the breast tissue accompanied by dense fluid and red blood cells indicate the presence of cystic formations or expanded ducts.

The proliferation of cells indicates that the tissue is reacting to either inflammatory or potentially cancerous situations. It's common to find adipose tissue surrounding these expansions. The dispersed pattern of occurrence implies widespread involvement rather than being restricted to a specific localized lesion (Turashvili & Li, 2023), necrosis and irregular cell growth in infected breast tissue suggest a multifaceted pathological reaction that involves tissue damage, an inflammatory response, and uncontrolled cell proliferation. Necrosis could potentially lead to additional tissue damage, while abnormal proliferation signifies unrestrained cell growth (D'arcy, 2019). certain forms of breast cancer, like invasive ductal carcinoma, have the potential to induce fibrotic alterations and the penetration of cancer cells amid collagen and elastic fibers, aggressive carcinomas may also exhibit tissue necrosis as a distinctive characteristic (Collins, 2016).

CONCLUSION

The results of our research are also consistent with some of the previous studies. We found that there were some changes in the physiological parameters that we examined for the CBC, as well as minor changes in the biochemical parameters, as well as noticeable changes in the hormonal tests. On the histological side, there were significant and severe changes in the affected breast, including noticeable phenotypic changes and significant changes in breast structure, including: such modifications involved unusual cell

proliferation, the development of the mammary tissues, the appearance of unusual cell clusters, congestion of blood, long zones having periodic bleeding, necrotic zones, and many other obvious changes. Overall, such modifications indicate the existence of breast cancer.

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