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A HISTOLOGICAL STUDY OF BREAST CANCER PATIENTS IN DIYALA SOCIETY, IRAQ

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Abstract

This study was conducted on a group of women patients diagnosed with breast cancer attending the Tumors Cancer Center at Baquba Teaching Hospital in Diyala Governorate. This study included 100 samples that were divided into 50 women diagnosed with breast cancer and 50 normal women without the disease as a control group. The obtained results of this study showed that the women diagnosed with breast cancer in the age group (40-49) is the highest class of disease infected, as it reached (42%) among the other age classes of affected women, with a significant difference at the probability level of P≤0.05. The results also showed that there is a significant difference at the probability level of P≤0.01 among the women infected by disease and smoking. The percentage of women who did not smoke and suffering from disease was 74%, while 26% of the affected women had shown a relationship between smoking and disease. Meanwhile, the invasive ductal carcinoma was the most common and prevalence type than the other types with a ratio of 80%, while the less common type was the tubular type, at a ratio of 2%. The results of the histological aspect of the study showed that invasive ductal carcinoma cells are characterized by a dark nucleus with shapes and sizes that differ from one another and are multinucleated. On the other hand, the cancer cells appear clustered together as groups in multiple places where they form nests separated from other groups by connective tissue. The results indicated the presence of the second type of invasive lobular breast cancer, where the cancer cells were characterized by being small in size, regular and inconsistent and similar to the Indian file in shape. The carcinoma tubular type of cancer was 2% of the total samples examined in the current study, and the distinguishing feature of this type is that the majority of the tubules is open and made up of a single layer of epithelial cells containing a clear cavity.

Keywords: Carcinoma Tubular, Invasive ductal carcinoma, Indian file

Introduction

The human body consists of a large number of diverse cells that grow and divide during their life duration and then differentiate according to their types until they reach the end of their life and then die sequentially. The growth process takes place when the body needs to increase the number of cells, as the growth process is controlled by a specific balance between the signals of growth stimulating and the stopping, and when needed to compensate for the decrease in the number of cells, the balance is shifted towards the increased growth. The surplus cells are collecting in the body's tissues together as lumps (mass) called a tumor (Cancer) (ACTS, 2013). These lumps are common in the breast and are of two types, either benign or malignant, or what is known as cancer and a small percentage of the lumps are cancerous (malignant) and the rest are not dangerous and not fatal. The tumor may occur in a specific tissue and stop at it and then it is called the benign type (benign tumor), or it is possible that spread (Metastases) as a result of the attack on other tissues of the body near or even distant and in this case is called malignant tumor (WHO, 2010). There are many types of cancer, and one of the most important of these types is breast cancer, as it is one of the most common types of cancer among women, as it represents a major health problem and is the second major cause of death in the world, as it killed 8.8 million women in 2015 (OMS, 2018). Cancer cells in breast tissue are characterized by having the ability to rapidly spread and invade the surrounding tissues and then spread to the rest of the body tissues, and this disease occurs especially in women and affects men, but in a smaller proportion, as it affects breast tissue, especially the milk ducts and lobules (Ho *et al.,* 2009). Hereditary breast cancer accounts for about 5-10% of all cases, as mutations in the BRCA1 and BRCA2 gene are the most relevant mutations in terms of changes in the gene that are

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responsible for the risk increasing of breast cancer (Veltman *et al.,* 2008). The factors that cause breast cancer are not known, but there are several factors called risk factors that may play an important role in increasing the incidence of infection, the most important of which is smoking, which is considered as one of the cancer causes in humans, due to the relationship between smoking and the expansion of the milk ducts among women diagnosed with breast cancer who are smokers (Tawfeeq, 2014). The age factor also played an essential role in increasing the risk of infecting by breast cancer, because the probability of infection by this disease increases with age progress and rarely occurs in the age of twenties and most often the infection occurs after menopause in women (Hayes, 2013). The aim of the study is to detect the prevalence of breast cancer and to identify the tissue changes of the breast gland caused by breast cancer.

Material and Methods

Collecting and dissecting of samples

The current study was conducted during the period from October 2019 to January 2020 and included 100 samples that were divided into 50 women diagnosed with breast cancer and 50 normal women without the disease as a control group. This study was designed to include two parts; the first part was collected data from 50 women diagnosed with breast cancer and included these data (age, smoking and family history of the injury). The second part of the study was collected the tissue samples from patients with breast cancer from tissue laboratory of Baquba Teaching Hospital/Diyala Governorate. The biopsy taken from the site of injury was fixed in formalin solution for 24 hours for the purpose of the study and making tissue sections for examination using the Hematoxylen-Eosin stain.

Results and Discussion

The Prevalence of Breast Cancer

Distribution of women diagnosed with breast cancer according to age groups

The ages of women who suffered from breast cancer that was included in the present study ranged between (30-70) years, and they were divided according to age groups and as shown in table 1.

The results of our study showed that the highest ratio of infection was in the age class (40-49) years, while the age class of (70-79) years was lowest infection ratio among the total number of women diagnosed with breast cancer, with a significant difference at a probability level $P \leq 0.05$. The results of the current study were in agreement with the study of (Alghaliby, 2015), where the results of his study showed that the age class (40-49) years was the highest ratio class of breast cancer, by 41.17%, while the class of lowest rate was 70-year-old group, by 5.88%. These results were not in agreement with the results of (Al-Shammari, 2017) study, which showed that the highest infected women of breast cancer was represented by the class (50-59) years by 48%, which is the class that increases the risk of infection as the rate of breast cancer increases with age. The reason for the difference between classes may be due to the ages of the patients that fall within the samples that have been studied, or it may be that the reason for the difference may be due to pathogenic radiation exposure as a result of the circumstances or wars that the country was exposed to them.

Table 1: The percentage of the study women according to the age groups.

Class	Age of woman	Percentage
30-39	6	12%
40-49	21	42%
50-59	12	24%
60-69	9	18%
70-79	2	4%
Total	50	100
Probability Level	P<0.05	

Distribution the women diagnosed with breast cancer according to family history of infection:

The results of this current study showed that there is no relationship between patients who have a family history of breast cancer, as 32% of the affected women have a family history of the infection and it is considered the lowest percentage of 68% of the patients who do not have a family history of breast cancer.

The study of (Alghaliby, 2015) was in agreement with the results of the current study, as it showed that 36.47% of the studied cases had a positive family history of breast

Table 2: The percentage of the study women according to family history of infection.

Family history ofinfection	Woman Patients	Percentage
Found	16	32%
Not found	34	68%
Probability Level	P<0.05	

Table 3: The percentage of the study women according to smoking.

Smoking	Woman Patients	Percentage
Smoking	37	74%
Not smoking	13	26%
Probability Level	P<0.001	

cancer, while the cases with a negative family history of breast cancer were 63.52%. There is a significant difference between the two groups at the probability level P <0.05, while the results of (Al-Khyaat *et al.,* 2008) study shows there was a relationship between the infection of breast cancer and a family history and at a very high probability level P = 0.000.

Distribution the women diagnosed with breast cancer according to smoking:

The statistical analysis results of the present study showed that there is a high significant difference at P<0.001. The percentage of women diagnosed with breast cancer was 74% of women who had never smoked, while 26% of women with breast cancer had a relationship between smoking and the disease. Therefore there was no high correlation between smoking and the breast cancer disease.

These results are in agreement with the results of (AL-Aragy, 2003) study, which showed that smoking was not related to the risk of breast cancer, as the highest percentage was 86.8% of patients who had no relevance with smoking, while the ratio of women who had a rapport to active smoking were 13.2%. The results of this study do not agree with the results of (DeVita *et al.,* 2011) study, which they indicated the existence of a relationship between smoking and the risk of breast cancer infection, as smoking is considered as one of the most important factors causing breast cancer. The reason of this explanation was carcinogens, such as nicotine in a cigarette, cause DNA damage through mutations in Tumor Protein (P53), which cause an imbalance in the cell growth process and lack of control, causing cancer. Or through binding to receptors and activating (Akt-Pka), which in turn leads to inhibition of apoptosis and cancer, or by inhibiting the functioning of tumor suppressor genes by enhancing the action of carcinogens.

Histopathological changes in breast cancer women patients

The results of the histological examination of the current study showed that the most common type of breast cancer was Ductal Carcinoma Invasive, where it was found by 80% of the study samples examined and 10% was the tissue tumor of the invasive lobular carcinoma, while it was found that 2% is the percentage of tubular cancer. This result is consistent with the findings of the researcher (Yalda, 2013), who observed in his study during the diagnosis of breast cancer that invasive ductal carcinoma is the most common type in affected women, with a ratio of 89.5%, while the rest of the other types include invasive lobular carcinoma, local ductal carcinoma, lobular carcinoma in situ. (Souhila, 2011) stated in his study on breast cancer that invasive ductal carcinoma is the most common type with 85% while invasive lobular carcinoma represents only about 6%.One of the most common and diagnosed types of cancer is invasive ductal carcinoma compared with the rest of the other types, and the reason for this result is due to the shape of the cancer cell which is considered as one of the most important factors that the diagnosis of breast cancer depends. Sometimes the shape of a cancer cell includes more than one type that cannot be classified, but due to the location of the cancer disease and the shape of the cancer cell that is similar to the shape of cancer cells in the breast ducts. This is why the term invasive ductal carcinoma is used to diagnose this type of cancer in breast tissue. The results of the current study showed the occurrence of histological changes in the shape of cells and the structure of the tissue affected by invasive ductal carcinoma, where the nucleus of the cancer cells was dark in color, in contrast to the normal cells that were of light stain and also large in size and about twice the normal size of the nucleus and dense chromatin. The nuclei of the cells were multiple irregular arranged and could be distinguished by the thumping cell behavior that constituted an invasion of the tissue, especially the connective tissue cells, which worked to prevent the spread of cancerous cells to the rest of the other parts of the body. Dense collagen fibers can also be observed inside the stroma, which adds stiffness to the affected breast tissue, and this is a characteristic of cancer-affected breast tissue as it can be distinguished from a normal breast when touched during the clinical examination, as shown in Fig. 1.

This conclusion is consistent with the study of (Koss

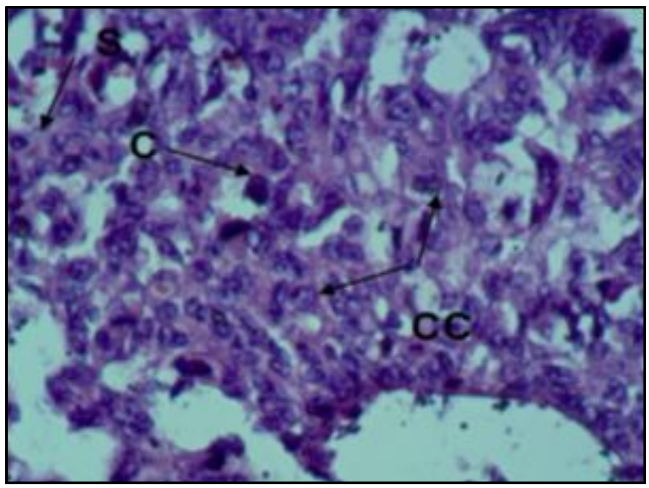

Fig. 1: Cross-section of female human breast tissue with invasive ductal carcinoma shows: S stroma, CC cancer cells, C chromatin stained (E & H ×40).

and Melamed, 2006) who indicated that the cancer cells in the mammary gland tissue affected by invasive ductal carcinoma are clear and easily distinguishable where the cells are large in size and the peripheral cytoplasmic blobs appear with a speckled shape. Also, they noted that the nucleus is large, as if it is swollen, and the nucleus is prominent inside the nucleus and can be seen easily during the histological examination. During the microscopic examination, pathological tissue changes were found, represented by the presence of many small-sized lymphocytes with cancer cells widespread in the breast tissue, and this is evidence of an immune response against cancer, as shown in Fig. 2. This study showed that cancer cells were found inside the blood vessels and lymph, and this indicates the spread of cancer cells to the rest of the other parts of the body, as this is a characteristic of breast cancer spread through the bloodstream as in Fig. 3.

These results are consistent with those of (Mahdi *et al.,* 2009) who demonstrated during microscopic examination that invading cancer cells are very clear and chromatin dense and those they have the ability to invade and spread to the rest of the epithelial tissues surrounding the tumor and to connective tissues. The results of the current study showed that cancer cells may appear clustered together in groups in multiple places, as is evident in Fig. 4, where they are in the form of nests separated from each other by connective tissue, and the fiber-generating cells are clear, compact, dark pink in color and arranged in rows with collagen fibers.

This is in agreement with the results of (Rabban, 2010), who found, during his study of sections of breast tissue affected by invasive ductal carcinoma, that mostly, cancer cells are clustered with each other, forming a shape

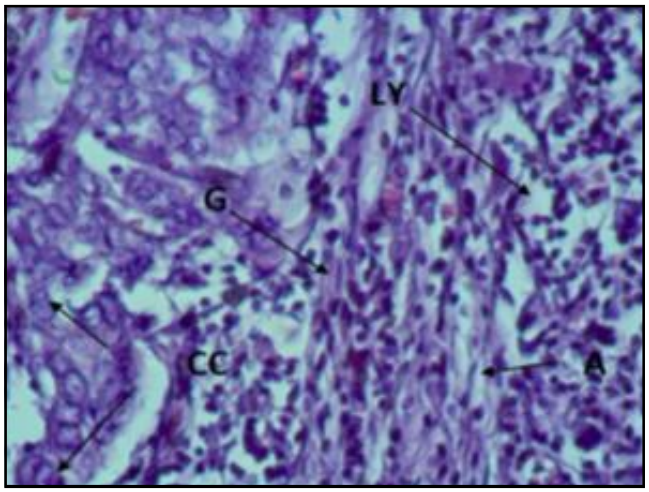

Fig. 2: Cross section of female human breast tissue with invasive ductal carcinoma shows: LY lymphocytes, CC cancer cells, G collagen fibers, A fibroblasts stained (E & H ×40).

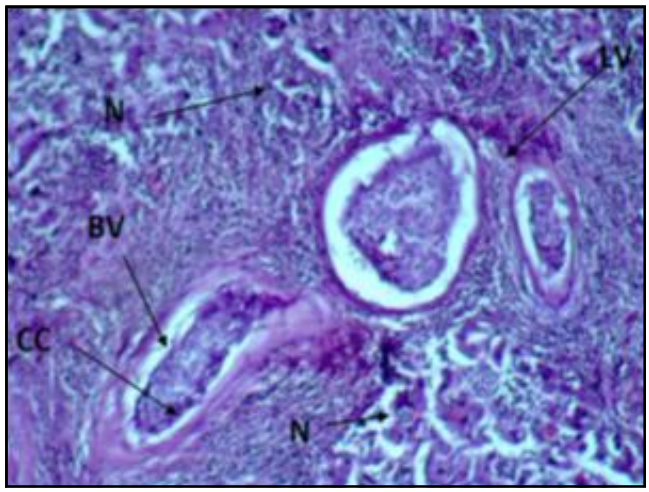

Fig. 3: Cross section of female human breast tissue with invasive ductal carcinoma shows: CC cancer cells, metastatic intravascular, BV, vascular, N necrosis and neoplasm, LV lymphatic vessel stained (E & H ×40).

similar to a very small ball and gives the appearance of multinucleate and the cells are sometimes single and scattered. Such changes are difficult to distinguish from normal cells. The results of the current study showed the second type of breast cancer, the invasive lobular type, in which the cancer cells were characterized by their small size, regularity, and loosely coherent. On the other hand, cancer cells appear individually and in a single-file pattern that leads to the formation of linear filaments similar to the Indian file between the normal breast tissues.

The reason that carcinomas are loosely coherent in invasive lobular carcinomas and lobular carcinomas in situ is because this pattern is partly related to the fact that the expression of the adhesion molecule E-cadherin

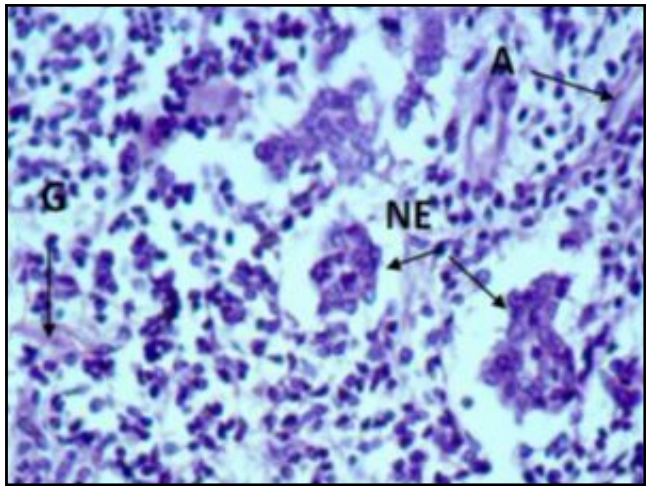

Fig. 4: Cross-section of a human breast tissue with invasive ductal carcinoma shows: NE tumor cells to form nests, G collagen fibers, A Fiber-generating cells stained (E & H ×40).

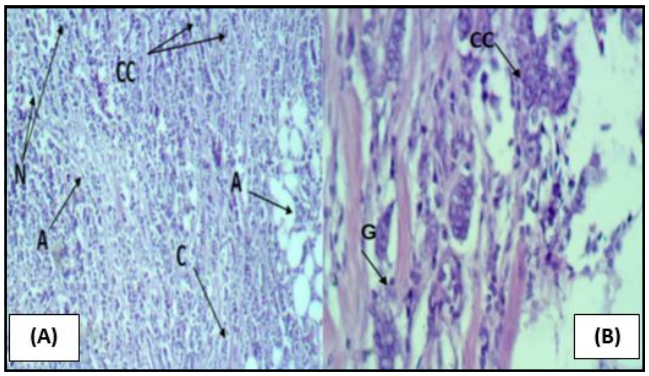

Fig. 5: Cross-section of human female breast tissue with invasive lobular carcinoma shows: CC the proliferation of cancer cell groups similar to the Indian file, A fat cells, C collagen fibers, A fibroblasts, N necrosis stained (E & H ×40).

is lost, and in many cases this is related to mutations that occur in the gene encoding this protein or perhaps due to a loss of heterozygosity on chromosome 16q22.1, E-cadherin gene region (Shah *et al.,* 2017). This result is consistent with the results of (Limaiem and Budh, 2020) in their study on invasive lobular breast cancer, as they indicated that cancer cells are characterized by their small size and regularity, and the cancer cells appear singly and in a single-file pattern that leads to the formation of linear strands between the normal breast tissue. The current study indicated the existence of another type of cancer, which is the tubular carcinoma. The distinctive feature of this type is that the majority of the tubules are open and consist of a single layer of epithelial cells containing a clear lumen and that the tubules are composed of oval or round cells that are randomly arranged and the cells are small or medium in size. The polymorphism of nuclei is small, beside the nuclei are not clear, and the muscle epithelial cells are absent. Also, in this type of cancer, fibrous tissue formation has been observed in the tissue stroma as shown in Fig. 6.

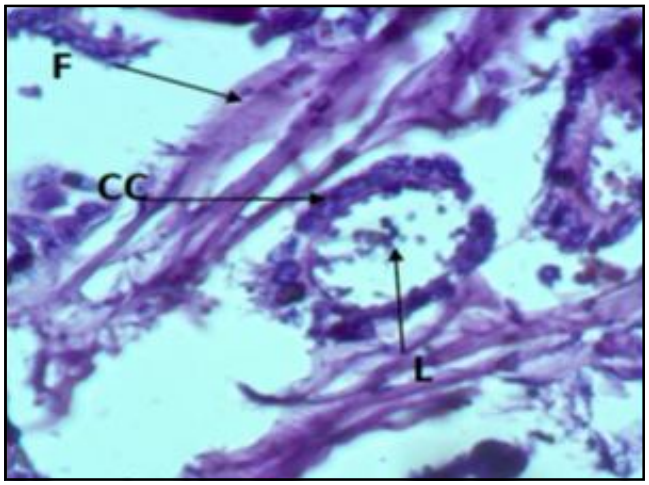

Fig. 6: Cross-section of the breast tissue of a female human with tubular carcinoma shows: F fibrous tissue, CC cancer cells, L lumen tubule.

This results of study is in agreement with the findings of (Fernández-Aguilar *et al.,* 2005; Limaiem and Budh, 2020) in their studies, in which they indicated that the characteristic feature of tubular carcinomas is the predominance of open tubes composed of a single layer of epithelial cells containing a clear lumen. The tubules are generally a combination of oval or round and compound shapes randomly arranged. The cells are graduated from small to medium in size, regular with little variation in the nucleus shapes, in addition to the nuclei being indistinct, and the mitotic shapes are very few.

Conclusions

The following conclusions can be withdraw from this study

1. The results of the current study showed that the age group 40-49 years is the group most affected by breast cancer, by 42%, and the age group 70-79 years is the lowest group in the incidence of the disease, by 4%.

2. The results of the study showed through a histological examination that invasive ductal carcinoma is the most common type, while the tubular carcinoma is the lowest prevalence.

3. There was no clear association between the family history of disease, smoking and breast cancer for the sample of women included in the study.

4. There are many histological changes of cancer cells of the invasive ductal type was observed through the shape and size of the large nucleus, polymorphism, dense chromatin and dense collagen fibers, while the invasive lobular type was characterized by the small size of the cancer cells and their regularity similar to the Indian coil and the presence of intercellular necrosis.

Also, it was observed the emergence of tissue changes represented by the proliferation of lymphocytes with cancer cells in addition to the presence of cancer cells clustered in the form of nests of Nests and the presence of infiltration between inflammatory cells between the lactic ducts.

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