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Women applications of a comparative bilateral female rats' breast carcinoma findings

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ABSTRACT

Background and objective: Breast carcinoma is the second most common type of cancer in women, and its incidences and consequences depend on internal congenital factors and various environmental chromosomal mutational factors. Multiple studies had evident heterogeneity in ipsilateral and contralateral breast cancers, of these the higher incidence of left breast cancer than right for indistinct reasons. This review article is concerned with the possibility of gathering the results of a recent research in comparison of bilateral rats' mammary cancer as a clue in evaluating the variations of the women's right and left breast tumors. Bilateral breast's gross, microscopical consistency and the pathological, immunohistochemical expressions of both sides of breast tumors were studied, hoping to explain the disparity in bilateral breast cancer incidence and may exploit it for prophylactic purposes.

Material and methods: The study used two hundred virgin female Sprague Dawley rats aged 7 weeks, weighing 100–120g. The rats were divided into two groups: 50 rats used as a control group, which received a placebo, and 150 rats carcinogenic exposed group, which received a single oral dose of (7.12-Dimsthylbenz [a]anthracene (DMBA). Animals were weighed and monitored weekly until the end of the study. The lumps bilaterally appeared after induction and were excised for gross structural, histological, and immunohistochemical examinations. The study was also supplemented by normal women's bilateral mammography computerized technique (MATLAB) software analysis for gross anatomical study, as this was impossible in rats. Statistics data groups were analyzed using SPSS version 22.

Results: Among (150) rats exposed to DMBA, only (87) developed tumors as first noticed after (13-16) weeks. Tumors were distributed as 43.68% (38/87) on the right breasts, and 56.32% (49/87) on the left, including (5/87) bilateral, moreover to (11) right breast's fibroadenoma. The weight and size of the tumors were greater on the right than left. Histopathological features of rat's mammary cancer induced by DMBA were similar to those of human breast cancers regarding location, and histopathologic modifications, such as ductal carcinoma in situ, Invasive ductal carcinoma, lobular carcinoma in situ, and invasive lobular carcinoma. On the right side, Lobular carcinoma was observed tightly packed in clusters forming thin strands of cells arranged in a linear design (Indian file) with abundant eosinophilic infiltration, while on the left side carcinoma were disseminated irregularly in dense fibrous tissue and fat with less or damaged eosinophilic infiltrations.

The immunohistochemistry scores matched the tumor growth, susceptibility, and type. Estrogen receptors, progesterone receptors, and Kiel clone 67 (Ki67) expressions were higher with invasive ductal carcinoma. The invasive lobular carcinoma was negative for Human Epidermal Growth Factor Receptor 2 (HER2) and KI67. No triple-negative results were observed. The results of the women's supplementary study by mammography analysis revealed that the majority of Iraqi females were heterogenous type III, and the left breast was denser than the right.

Conclusion: Among 150 rats only 58% developed various breast tumors after a single dose of carcinogens. The lower incidence of right breast carcinoma than the left is supposed to be due to the lower density, more fatty tissues, and lower immunity of the left breast. The left breast carcinomas were multifocal while the right breast carcinomas were localized in a single sector but characterized by a more aggressive cellular multiplication. Accordingly, if the revealed results are applied to women's breast carcinoma, it may lead to some important hints in understanding some secrets and mapping new management in breast carcinoma in the future.

Keywords: Breast carcinoma- eosinophils response- ductal carcinoma- lobular carcinoma- bilateral breast cancer.

Introduction

Breast carcinoma (BC) is the second main cause of morbidity and mortality in females[1]. Overall, it calculated that each women have a risk of about 13% in the USA developing breast cancer in her life[2]. Recently according to various global breast carcinoma studies a continuously growing rate of about (0.6%) per year with the progress of the complexity of life, The rise in incidence rates is a little steeper in women younger than 50 (1.0%)[3]. when the breast is embryologically traced, it is no more than a modified sweat gland, but by a wider focusing view the breast is a very complex machine having the stress of a dozen of Bosse's orders including hormones, chemicals, physical build, and stress, ethnics and races, aging, morbidities, harsh environmental factors, etc.[4]. Although all the breast carcinomas are due to genetic mutation and promotion the congenital factors have a very limited role in the scenario of breast carcinoma[1]. Due to various previous studies, only the first generation of breast carcinoma is well-differentiated and is called luminal-A type in the early stages at which rarely the victims are alert of it, subsequent descendants carcinomatous cells are gradually will be adultery and not assemble parent cells, here heterogenicity story starts (luminal-B, or C) and advancing staging even complex laboratory studies of immunohistochemistry show them a triple negative in their optimally advanced stages, the claimed cause for that may be due to easily-fragile, delicate and weak original mother cells[(5)].

Carcinoma of the breast is heterogenous in everything including carcinoma inside one breast and contralateral breasts, carcinogens response, type, ages, zones of breasts, species, geographical factors, body frame, environment, and prognosis moreover to metastasis, recurrences, and survival rate, accordingly, it is a nonpredictable malignancy[6,7].

In a review of many international studies, the higher incidence of left breast carcinoma by a very limited rate than right breast is documented[6]. The cause of this incidental variation is not explained yet. The research was conducted as a recommended proposal for PhD study candidate in the College of Medicine of Hawler Medical University- Basic Sciences Department which was published partially[8], as a continuation of a previous study titled (Pilot Study of Breast Cancer Patients with a Long Term Follow up in Erbil-Iraq[9].

Rats were picked to perform this study as the rats closely mimic human beings structurally among mammalians. Rats have six pairs of mammary glands and nipples, three pairs of cervical and pectoral regions, and remnant three pairs in the abdomen and inguinal region.

Ethical approval

This study was approved by the Research Ethics Committee and Institutional Animal Care of the Hawler Medical University, College of Medicine Erbil-Iraq, ID (HMU 4,2)

Material and methods

The study was conducted in Hawler Medical University College of Medicine -Basic Science Department, from March 2021 until June 2022.

This study's components were performed on rats, except for supplementary comparative women's breasts bilateral densities mammography as the gross rats' radiological anatomy studies were impossible in rats. The carcinogen DMBA was obtained in powder form (Sigma-Aldrich, USA). DMBA was diluted in sesamoid oil in a ratio of (1000 mg of DMBA and 50 ml of Sesamoid oil). Two hundred virgin female Sprague Dawley rats (7 weeks) aged and weighing 100-120 grams were used. they have been divided into two groups. (control group) were 50 rats received normal saline as a placebo), while the second group where 150 rats orally received a single dose of 7,12 dimethylbenzene(a)anthracene (DMBA) in a dose of 80mg/Kg body weight dissolved in sesamoid oil. Continuous daily and weekly follow up performed. On weeks 13-16 breast masses were detected in the thoracic-cervical and abdomen-inguinal regions, breast excised fixed in formalin then stained in

hematoxylin-eosin stain for histopathological and immunohistochemical studies.

Immunohistochemistry, was performed for Progesterone receptor (PR), Estrogen receptors (ER), Ki-67, and HER2 antigens.

Regarding the second part of the study 1056 female human mammography films were gathered for two different radiologists' viewing and report in one hand and special computerized programed (MATLAB-software) breast density studies in the other hand.

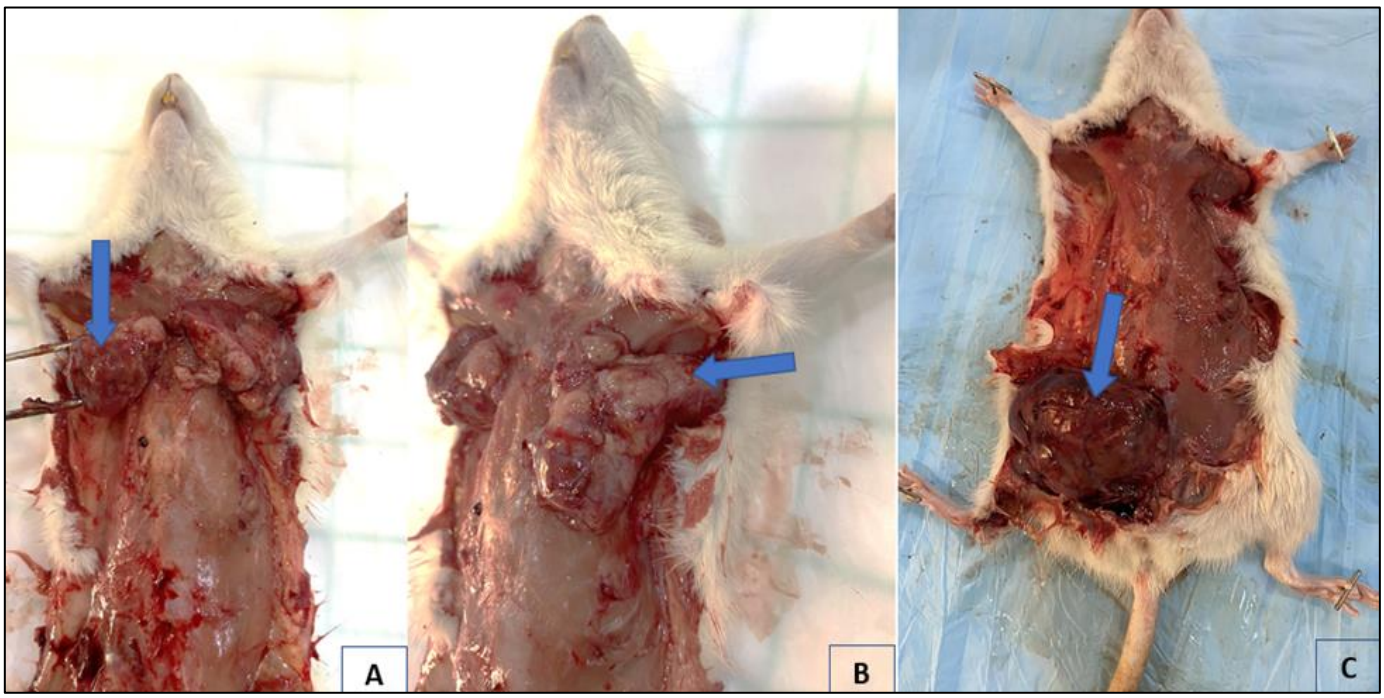
Statistical analysis: mainly unpaired Personal Chi-square used to investigate variation in the location of tumors after induction by DMBA (P-value ≤ 0.05). Cohen's Kappa is used for sleuthing the agreement of mammography observers.

Results

All the rats significantly gained weight from weeks 1-10, after that the treated rats with a single dose of (DMBA) followed by loss of weight indicating the initiation stage of the carcinoma, followed by the promotion stage of tumor appearance especially between weeks (13-16). Ultimate body weight ranged between (235 and 250) g with a 90% survival rate. Controlled animals gained weight ranging between (300-350) g and were tumor-free throughout the study. The tumors were lobulated or discoid, solid to firm consistency (firm lumps contained necrotic tissues) with a white-gray to pinkish color.

Out of 150 rats, 87 developed tumors induced by (DMBA), where 39(44.83%) on the right breast chain of the rats, and 43(49.43%) on the left mammary chain. Meanwhile, of these 5(5.87%) are bilaterally distributed. Positionally, the tumors distributed as 54.2 (47/87) in the cervical-pectoral region, and 45.98% (40/87) in the abdomen-inguinal areas respectively, The observed differences between these regions were statistically non-significant in Fig 1 and Table 1.

Fig. 1: Gross view of rat's mammary gland in right and left breasts.



The significantly bigger sized (25.51 ± 0.8616 and 22.49 ± 0.8355) and heaviest weighted (2.576 ± 0.1288 and 2.186 ± 0.1226) tumor was found in the right and the left sides respectively of the Inguino-abdominal region of rat's mammary gland with (P-Value =0.004) vs. the mammary tumor developed in the cervicothoracic area revealed the smaller sized (23.45±0.9542 and 20.48 ± 0.8623) and lesser weighted (2.272 ± 0.08481 and 1.969 ± 0.08346) in the right and left side respectively with (P-value =0.001). The mammary gland tumor in the right region was bigger and heavier in weight (non-significantly statistically) in comparison to the left side in both regions as shown in Table 1.

Table 1: Measurement of tumor size (cm) and weight (gm) in two regions on the Right and Left rat's mammary gland.

Tumor's location		Right mammary tumor	Left mammary tumor	P=Value
Cervicothoracic region	Size (mm)	23.45±0.9542	20.48 ± 0.8623	0.023
	Weight (gm)	2.272 ± 0.08481	1.969 ± 0.08346	0.013
Inguinoabdominal region	Size (mm)	25.51 ± 0.8616	22.49 ± 0.8355	0.021
	Weight (gm)	2.576 ± 0.1288	2.186 ± 0.1226	0.031
All	Size (mm)	25.66 ± 0.8421	22.22 ± 0.8206	0.004
	Weight (gm)	2.408 ± 0.07600	2.067 ± 0.07245	0.001

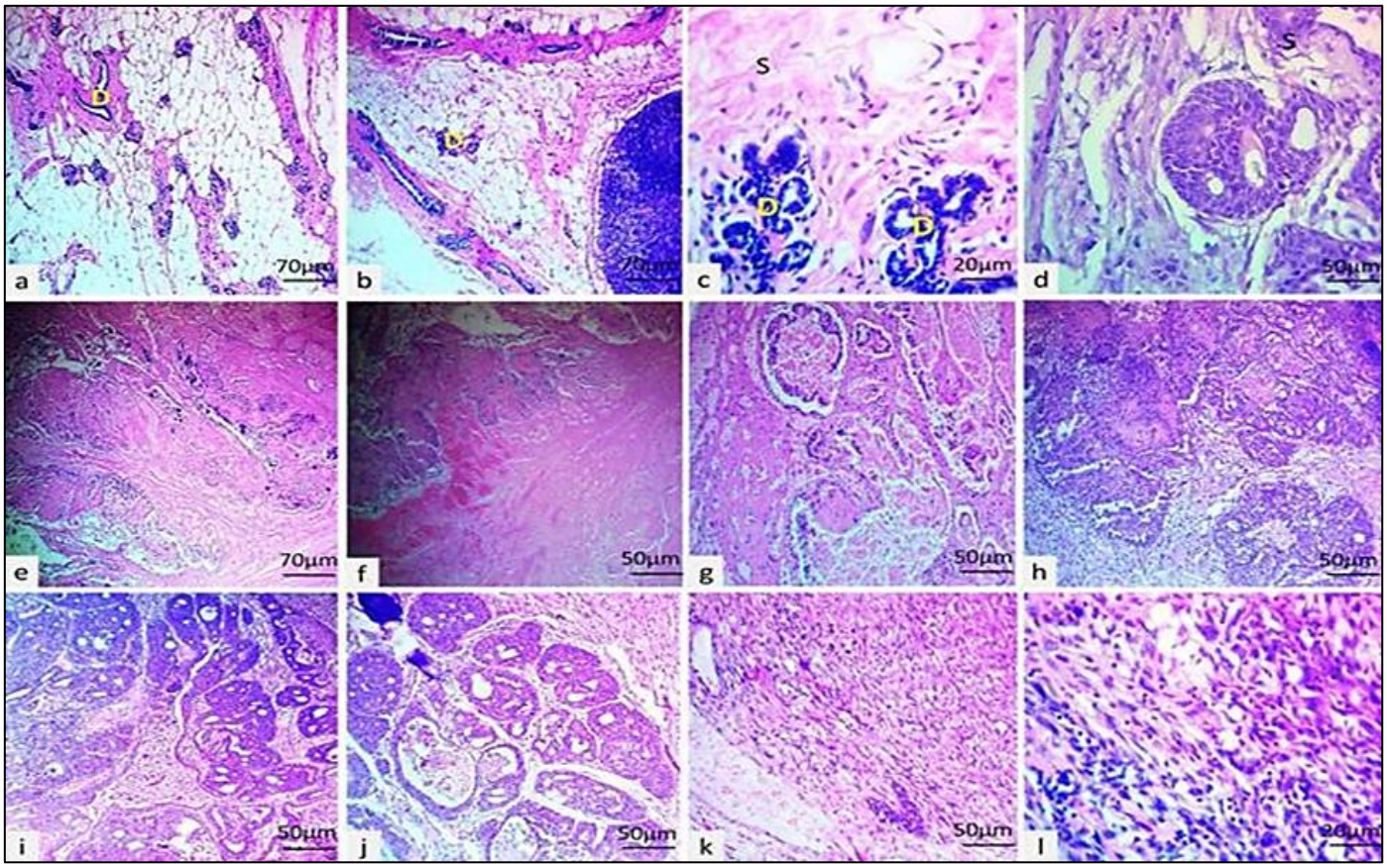
*Values are Mean ± SE. An unpaired T-test was used for the statistical analysis and **p<0.05.*

Histopathologic features

All the rat's mammary glands were excised, and a histological study was performed. Microscopic sections of the right and left mammary gland regions in the controlled group showed normal histologic features as in (Fig. 1a- c and 2a, b) respectively. The mammary gland is composed of lobules of secretory acini with the ductal system (terminal lobular duct and large duct) that is surrounded by stroma. The acini were lined by a single layer of dark cells with myoepithelial cells. Normal ducts had a three-layer structure that comprised ductal epithelial cells, myoepithelial cells, and a basement membrane. The interstitial tissues were mostly composed of fatty tissues. In the experimental group, the histopathological analyses revealed extremely heterogeneous mammary tumors. Among eighty-seven tumors induced by DMBA various changes with different percentages as shown in Fig. 2.

Ductal carcinoma in situ (DCIS) was observed with a higher incidence on the left side, with 9 cases (10.34%), compared to the right which had 6(6.9%) cases. Invasive ductal carcinoma (IDC) also showed a greater prevalence on the left side, with 27 (31.1%) cases compared to 13(14.94%) cases on the right. Lobular carcinoma in situ (LCIS) were 2(2.98%) cases on the left and 1(1.49%) case on the right. Similarly invasive lobular carcinoma (ILC) had a higher incidence on the left side, with 11(12.64%) cases versus 8 cases (9.18%) on the right. Interestingly, fibroadenoma was exclusively found on the right side of the mammary gland, with 10 (11.5%) cases (Table 2) and Fig 2.

Fig. 2: Microscopic section of the right mammary gland in rat showed; a-c: Normal histologic structure of mammary nodule, terminal ductules (D), and stroma (S) with fatty tissue. (H&E stain)



The histopathological findings viewed in in (Fig 2 d-e); Lobular carcinoma in situ (LCIS), proliferated cells form an adenoid shape containing microcystic eosinophilic secretion within the lumen. In ductal carcinoma in situ (DCIS) Neoplastic cells proliferate and form large tubular to cystic forms with abundant pale eosinophilic (hyaline) secretory material in their lumen with calcification and abundant stroma, fibroadenoma as seen in (Fig 2 f-g). Invasive ductal carcinoma (IDC) showed a tubular and cystic shape that is filled with necrotic debris or eosinophilic secretion within the lumen of the proliferated cells with secondary lumina as observed in (fig 2 h-j). Moreover, invasive lobular carcinoma showed tumor cells arranged in single files or cords that diffused throughout mammary tissue (fig.2 K-l).

The lobular carcinoma in situ (LCIS) in both locations was seen to have the monomorphic, dispersive proliferation of monotonous cells, round-oval nuclei in the cell's center, regular nuclear membranes, uniform chromatin, and sparse cytoplasm without mitotic division. Proliferating cells are organized in a glandular pattern with an eosinophilic material-containing cystic dilatation in the center of each cell (Fig. 2d and Fig. 3c, d).

The majority of fibroadenomas are papillary-cystic growth patterns The dilated ducts are filled with papillary ingrowths (dark purple), and only the right region of the mammary gland can identify eosinophilic material within these ingrowths (Fig. 3e-g).

Invasive ductal carcinoma (DCIS) microscopic study showed strong epithelial cell growth in a distended duct that was divided by secondary lumina with central necrosis. Highly pleomorphic cuboidal to oval cells with protruding nucleoli and spherical, vesicular nuclei can take on a variety of morphologies, such as varying cyst sizes, or be grouped to create regular cribriform gaps (Fig. 2 h- j and Fig. 3 e- j).

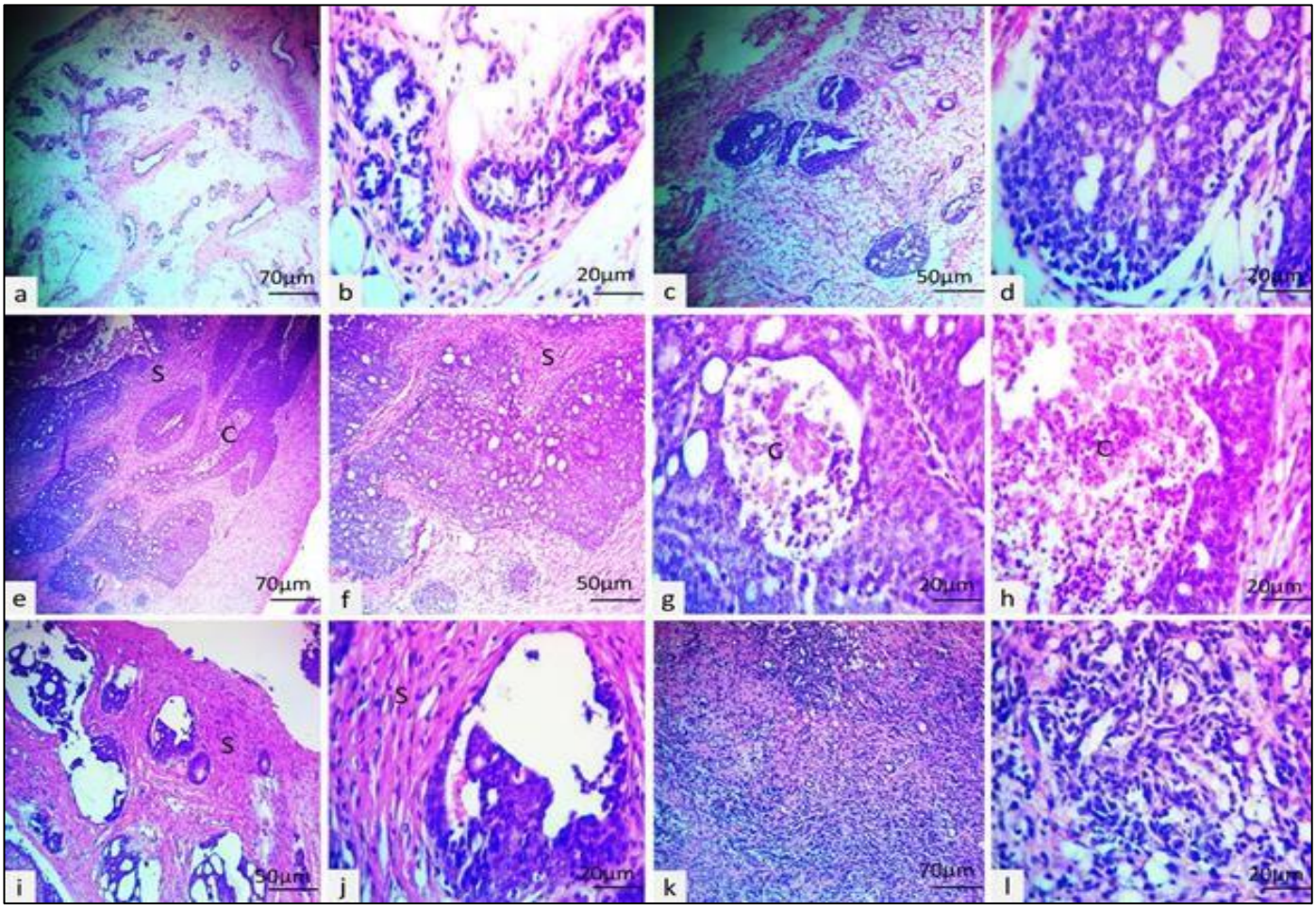
Microscopic features of Invasive lobular carcinoma in the right breasts, showed that the tumor cells are tightly packed in small clusters, forming a pattern of thin strands of cells arranged linearly (Indian file), while in the left the breasts tumor cells dispersed

irregularly in a densely fibrotic stroma, which is seen diffusely infiltrating breast tissue and fat (Fig. 2 k, l and Fig. 3k, i).

Table 2: Distribution of the tumors in the DMBA-treated rats

Neoplasms types	Right breast	%	Left breast	%	Bilateral tumors	P-value
DCis	6	6.9	9	10.34	5(5.74%)	0.0008
IDC	13	14.94	27	31.1		
LCis	1	1.49	2	2.98		
ILC	8	9.18	11	12.64		
Fibroadenoma	10	11.5	0	0		
Total tumor/ side	38	43.68	49	56.32		
All tumor	87	43.68	87	56.32	5	

Fig. 3: Microscopic section of the left mammary gland in rat revealed; a and b: Normal histologic morphology of mammary gland nodule, terminal Ductule (D), and stroma (S) with fatty tissue and musculature. (H&E stain)[8]



Histopathological findings of the left breast tumors as seen in (Fig 3 c-d); Lobular carcinoma in situ (LCIS), neoplastic cells form an adenoid structure containing microcystic eosinophilic secretion. Invasive ductal carcinoma (DCIS), the solid proliferation of pleomorphic cuboidal to oval cells with abundant eosinophilic granular cytoplasm, round vesicular nuclei, and prominent nucleoli in a distended duct with central comedo necrosis (Fig 3 e-h). Invasive ductal carcinoma (DCIS) with predominantly papillary and partly clinging type manner or cribriform structure with secondary lumina formation (Fig 3 I-j). Invasive lobular carcinoma showed tumor cells arranged in slender strands and cords are seen diffusely infiltrating mammary parenchyma (Fig 3K- l).

Immunohistochemistry analysis

For all markers, the mammary gland in the control sections showed no staining (score 0) as in (Fig. 4 a, e, h, k). The ER marker showed a variable level of nuclear expression and stain intensity according to different types of tumors, as shown in Table 3.

Table 3: The scoring system and percentages for all positive antibody markers staining among different types of tumors.

Neoplasms types	ER	PR	Ki-67	HER2
Ductal carcinoma in-situ	(score 1, 10%)	(score 8, 78%)	(score 8, 70%)	(score 4, 25%)
Invasive ductal carcinoma	(score 6, 23%)	(score 12, 85%)	(score 8, 75%)	(score 8, 70%)
Invasive lobular carcinoma	(score 8, 75%)	(score 8, 75%)	(score 0, %)	(score 0, %)

Weak expression was found in the LCIS vs. the DCIS that revealed focal strong positive staining (Fig. 5b, c), while the invasive lobular carcinoma showed diffuse-moderate expression for both ER and PR antibodies markers (score 8, 75%) (Fig. 5d). The brownish nuclear staining for the PR marker was expressed by moderate-diffuse staining in the LCIS vs. the DCIS that were highly expressed and showed diffuse strong PR staining (score12). Generally speaking, no significant differences were observed regarding IHC bilaterally.

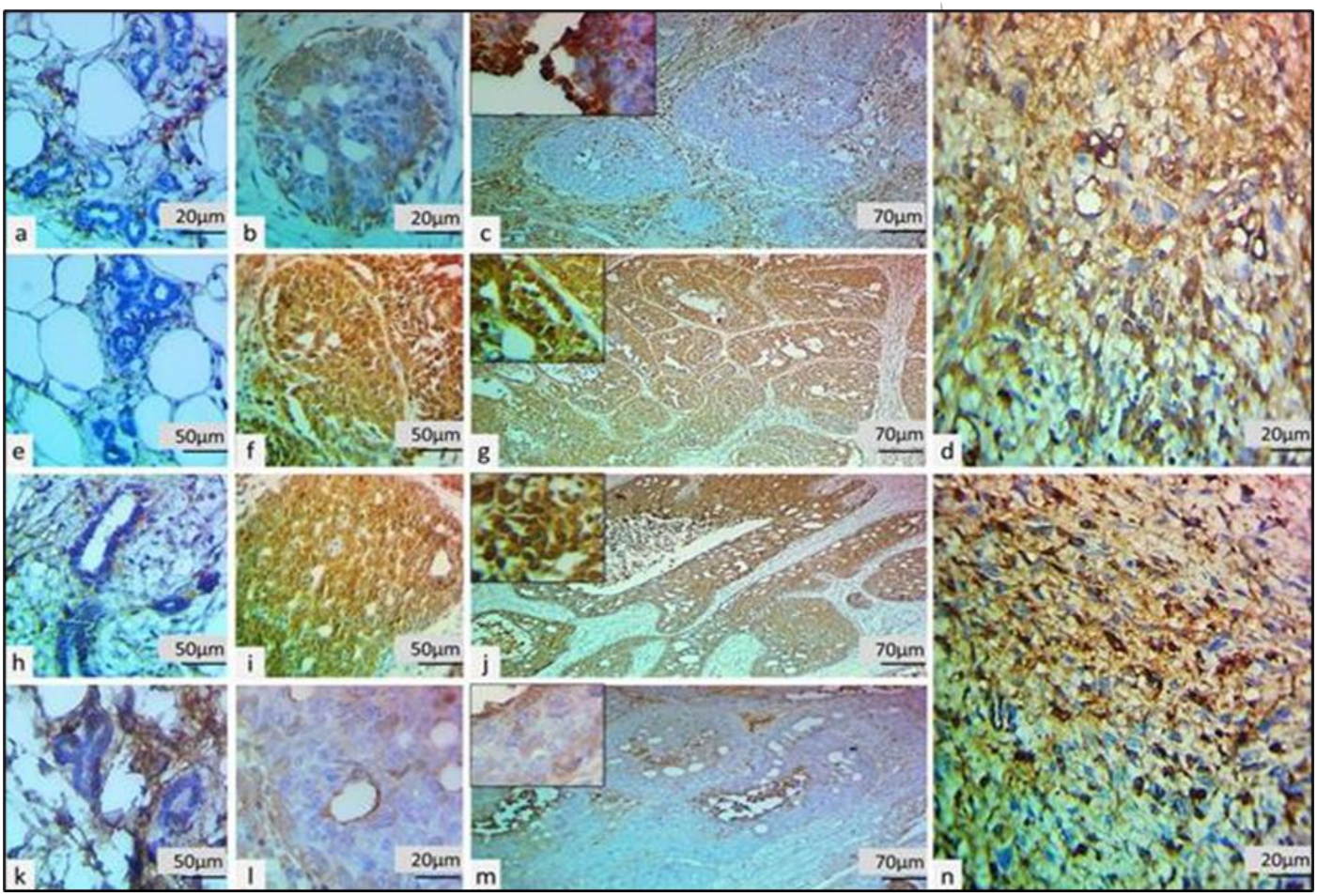
Immunohistochemical staining of ER, PR, Ki-67, and HER2 nuclear-membranous immunostaining as seen image (Fig. 4).

Both the right and left flank regions of the rat's mammary glands. **a:** No ER staining was present (score 0) in the control group. **b:** Weak ER staining (score 1) in the LCIS. **c:** Focal-strong ER staining (score 6) in the DCIS. **d:** Diffuse moderate ER and PR staining (score 8) in the invasive lobular carcinoma. **e:** No PR staining (score 0) in the control group. **f:** Moderate-diffuse PR staining (score 8) in the LCIS. **g:** Diffuse-strong PR staining (score 12) in the DCIS. **h:** No Ki-67 staining (score 0) in the control negative group. **i:** Moderate-diffuse Ki-67 staining (score 8) in the LCIS. **j:** Moderate-diffuse Ki-67 staining (score 8) in the DCIS. **k:** No 8. HER2 staining (score 0) in the control negative group. **l:** Moderate-focal HER2 staining (score 4) in the LCIS. **m:** Moderate-focal HER2 staining (score 4) in the DCIS **n:** No Ki-67 and HER2 staining (score 0) in the invasive lobular carcinoma.

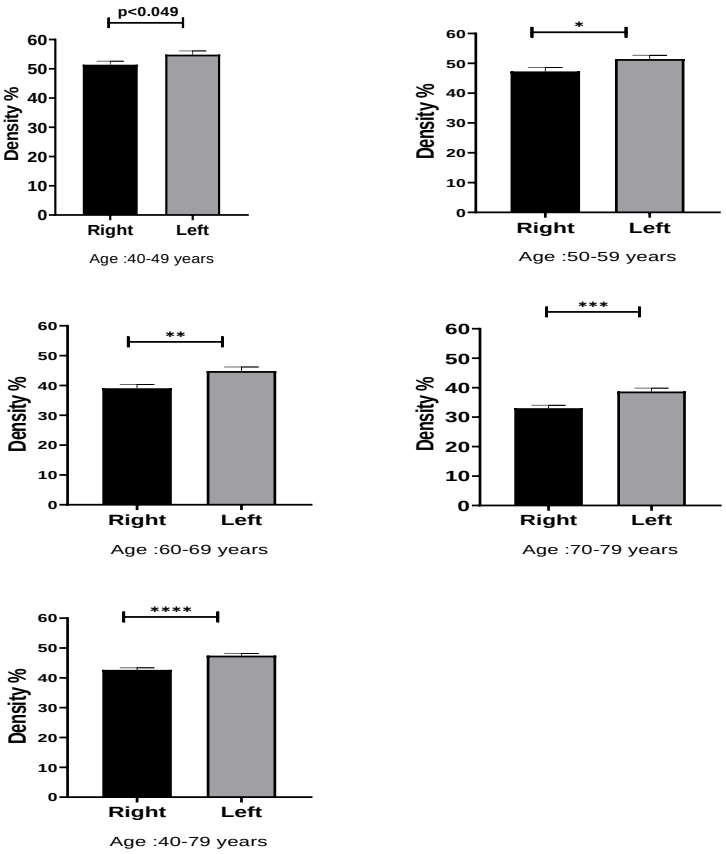
Breast density study results

Comparison of bilateral normal breasts mammographic density of 1056 women, ages were (40-90) years, within the scope of this study comparison studies by two approaches, first by visual mammographic density measurement by two different radiologists no significant variation found between both, and the comparative result statistically found almost perfect. Digital image process (MATLB) showed significantly denser left breast mammography than right (Graph 1), with a decrease the density with age advancing.

Fig. 4: Various Immunohistochemical staining of tumors



Graph 1- Demonstrating the differences in breast density between the right and left breasts according to the age [8]



The measurement of the breast density between women ages 40 -70 years old, showed the density of the left breast in comparison to the right was (47.46 ± 0.65 Vs 42.69± 0.64) Table 4, 5, and Graph 1 revealed the density levels on the right and left breasts according to the age groups.

The results of a study conducted on 1056 mammogram samples of women between the ages of 40 and 79 revealed that the most frequently observed class of density on the right and left breast was heterogeneous, accounting for 46%; the next most common class was scattered, with 27%; then almost entirely fatty, with 15%; and finally, extremely dense, with 12%. The details are shown in Table 5 and Graph 1.

Table 4: breast density in various age groups of bilateral breasts[8]

Breast density	Age groups					P- Value
	40-49	50-59	60-69	70-79	40-79	
Right breast	51.41± 1.210	47.27 ± 1.332	39.05± 1.276	33.03± 1.044	42.692± 0.64	0.001
Left breast	54.84± 1.252	51.43± 1.278	44.84± 1.352	38.71± 1.147	47.46± 0.65	

Table 5: Age distribution of breast Density[8]

Ages	Sides	Class 1[a]	Class 2[b]	Class 3[c]	Class 4[d]
40-49	Rt. breast density	32 (12%)	72(27%)	104 (40%)	56 (21%)
	Lt. breast density	23 (9%)	64 (24%)	128 (48%)	49 (19%)
50-59	Rt. breast density	47 (18%)	73 (28%)	120 (45%)	24 (9%)
	Lt. breast density	23(9%)	88(33%)	104 (39%)	49 (19%)
60-69	Rt. breast density	48 (18%)	72 (27%)	120 (46%)	24 (9%)
	Lt. breast density	27 (10%)	88 (33%)	105 (40%)	44 (17%)
70-79	Rt. breast density	64 (24%)	51 (19%)	142 (54%)	7 (3%)
	Lt. breast density	62 (23)	52 (20%)	144 (55%)	6 (2%)

Discussion

The variations in the characters and incidences among right and left-sided BC are not well understood. A few studies have been conducted on bilateral differences and the frequency of BC studies on the morphological differences between right and left-sided BC are also rare. Breast carcinoma in women is more likely to develop on the left side [1].

According to most of the research conducted to find the reasons for BC the acquired genetic cellular mutation was found as the main cause in nearly 94-96% while congenital causes for BC were found in only 4-6%, this fact giving lion-share to the environmental factors[8,9]. Out of 150 rats, 87 developed tumors induced by DMBA, this indicates the heterogenicity in the body response of the rats to

carcinogens, indicating that only 58% established genetic mutation and developed carcinoma, others are not attacked by carcinoma, due to either their higher immunity or resisted chromosomal mutation for unknown causes [4].

Due to the results of the current study tumors of the thoraco-cervical region were less aggressive, lighter in weight, and smaller in size Vs. abdomen-inguinal, the possible interpretation of this is that the thoracic-cervical breast in rats are normally smaller in size and rudimentary as the rats depend mainly on the abdomen-inguinal breast for lactating purposes[11]. Mammary carcinoma in the left breast is more frequent in the right, with ratios ranging from 1.05 to 1.26. The difference is not big, but it's also not considered small[12]. To find this fact and underlying factors this study conducted which is lasted for 24-26 months. In the comparison of the bilateral breasts' normal mammography of human female breasts found the larger surface area density of the left breast Vs. the right by using MATLAB's precise computerized technique. According to previous studies, the interpretation of mammograms using digital image processing is more accurate and efficient. MATLAB software is frequently used for this process[13]. Although the radiologists' readings in the current study found no significant differences. The heterogeneous breast was dominantly observed (46%), in the current study among 1056 mammogram samples of women of (40-79) years of age or BI-RAD III)[15], this aligns with other studies performed in Jourdan and China[14,15]. Various studies found the promising and show the proposed deep learning-based techniques can produce a clinically useful computer-aided tool for breast density analysis by digital mammography with precision, sensitivity, and specificity of 97.85%, 97.85%, and 99.28%, respectively. Still, there are trials of digital breast density classification with the aid of the conditional Generative Adversarial Networks (cGAN) network is applied to segment the dense tissues in mammograms[15].

The interval for the initiation stage of the tumors was observed that all the rats significantly gained weight from weeks (1-10), after that the single oral dose DMBA-treated rats started losing weight, especially between weeks (13-16). When this is applied to a human being estimating that each month of a rat's age = to (2.5-3) years of the human being's age[5]. Accordingly, a human being exposure to carcinogens triggering tumor initiation needs (7.5-10) years which is highly applicable to analyses of the causative agent (e.g. four friends of Mosul medical college) who shared the character of a feminine face and rented for six years an old house in Mosul (north ancient city in Iraq) were attacked by very aggressive BC (IDC) during the same period and died nearly equal period in different countries, were their study period was in (1980-1986) developed BC in (1996-1997) died after 2-3 years by brain and bone metastasis or recurrences approximately same time[9]. This story may be a live example of environmental factors enhancing BC. On the other hand, knowing tumor initiation time allows for the earlier search for malignancies in a toxic environment.

Among all the tumors induced in this study distributed into both breasts with higher attacks of the left one 38 (43.68%) and 49 (56.32%) right and left consequently, including 5 (5.78%) Bilateral tumors. These findings let the tumor curve shift to the left. This finding has been confirmed by previous studies[18]. In the current research, lobular carcinomas in the right breast were characterized by being more localized in a single sector of the breast and not associated with other companion tumors, but faster growing proved by significantly bigger sized (Rt.= 25.51 ± 0.8616 and Lt.= 22.49 ± 0.8355) mm and heavier weighted (Rt. = 2.576 ± 0.1288 and Lt. = 2.186 ± 0.1226) gm respectively. The isolated heterogeneous finding observed in the contralateral breast morphologically and microscopically cases of (ILC) in the right breasts; showed that the tumor cells are tightly packed in small clusters, forming a pattern of thin strands of cells arranged linearly (Indian-file), while in the left the breasts (ILC) the tumor cells dispersed irregularly in a densely fibrotic stroma, which is seen diffusely infiltrating breast tissue and fat putting in mind this finding gives left breast carcinoma poorer prognosis than right[19]. On

the other hand, this kind of histopathology indicates the higher sheltering immunological capability of the right breast, which localizes the tumor in one sector but makes the imprisoned tumor cells brutal (as observed the localized tumors of the right breast were bigger and heavier). The previously mentioned feature in the current study may change the plans for the management of breast carcinoma both on the right and left. In the right breast, it may need only the affected sector or quadrant excision ± axillary management no adjuvant therapy just a follow-up. while the left breast needs more aggressive excision ± axillary clearance and chemo or radiotherapy[20]. Although controversial results are documented by other articles regarding the left breast being more aggressive in comparison to the left[21,28]. Another point that emphasizes this higher immunity of the right breast is the incidence of 11 (9.57%) cases of fibroadenoma solely observed on the right breast with abundant eosinophilic infiltration, which may explain higher immunity of the right breast that prohibits malignant transformation of the induced cells. It has been documented that fibroadenoma may change to carcinoma with time and other associated comorbidities such as (DCIS). Complex fibroadenomas are more related to malignant change. old age and strong family history are risk factors for the malignant transformation of fibroadenomas[21]. This indicates the right breast fibroadenomas benign tumor liability to mutational changes to malignancy, and vice versa right breast has higher immunity that prohibits carcinogenic cellular mutational changes but needs continuous follow-up before its malignant transformation[22].

The same finding of abundant eosinophilic infiltration was also observed surrounding malignant tumors in the right breast, while on the left breast, scattered destructed eosinophilic cell secretions were observed. These microscopical observations may indicate the higher capability of the right breast to fight carcinogens. However, on the right breast attendance of damaged eosinophilic secretions indicates an earlier game-over signal of the breast tissues or cells to carcinogenic materials.

All types of malignant tumors were developed on both the right and left breast among the treated rats, and several benign fibroadenomas were also induced on the right side unexpectedly. Eosinophilic aggregation surrounding the tumors on the right breast was exclusively observed in the current study, which may suggest higher immunity of the right breast than the left for carcinogenic agents. The eosinophilic aggregation around tumors had been confirmed in certain studies observations, which revealed that eosinophils display regulatory functions towards other immune cell subsets in the tumor microenvironment or direct cytotoxic functions against tumor cells, leading to either antitumor or protumor effects. This dual role of eosinophils as an immunological response might depend on the different tumor microenvironmental factors[23,25]. In addition, the clinical relevance of these cells has been recently addressed. In most cases, the accumulation of eosinophils both in the tumor tissue called tumor-associated tissue eosinophilia, and in the peripheral blood were reported to be prognostic markers for a better outcome of cancer patients[19]. In immunotherapy of cancer, particularly in therapy with immune checkpoint inhibitors, eosinophils were even shown to be a potential predictive marker for a beneficial clinical response. A better understanding of eosinophils' role in cancer progression will help to establish them as prognostic and predictive markers and to design strategies for targeting eosinophils. In review right breast showed more attentively responding with aggressive resistance. Moreover, eosinophil infiltration in tumor tissue is considered an independent prognostic factor[25]. Eosinophils are often recruited to tumor sites, where eosinophil granule proteins and cytokines are released upon activation, damaging and killing tumor cells[24]. The absence or damaged eosinophilic response in the left breast may be attributed to the variation in lymphatic drainage and variability of blood supply of contralateral breasts. Through clinical practice, we observed increased eosinophils in the peripheral blood not specifically for breast tumors but also in (3) cases of gastrointestinal tumors which suggests further studies.

Regarding breast cancer types, subtypes of the proteins on the malignant cell membrane receptors are controlled by multiple interfering factors including local, anatomical, histopathological, and immunohistochemical. Our study results showed 78 tumor cases including invasive ductal carcinoma (DCIS); the left breast showed the greatest number in comparison to the right area, lobular carcinoma in situ (LCIS) was nearly equal, invasive ductal carcinoma (DCIS) in the left breast showed the greatest number versus the right breast (table 2). Fibroadenomas are only found in the right mammary gland 11 (9.57%). Invasive lobular carcinoma was a little higher on the left than on the right side.

Immunohistochemical staining of ER, PR, Ki-67, and HER2 showed no significant differences between contralateral breasts. As seen in (table 3) tumors of DCis tumors exhibited high scores of (PR), low scores of (ER), and (Ki-67) and medium scores of HER2, indicating luminal early ductal multifocal and multi-factorial changes[25]. While in the invasive ductal carcinoma, all the tumor markers scores were high including HER2 indicating more aggressive changes and starting the loss of normal ductal cellular identification. In cases of ILC ER and PR were high scored but negative HER2 and Ki-67 these findings as also mentioned by previous studies make ILC liable for hormone therapy although often obtain resistance[26,28].

The possible explanation for heterogeneous characters of the incidences and variability of immunohistochemical staining of tumors in carcinogen-attacked rat breasts is that the type of tumor depends on the subdivisional sequences of the mutated cell from the inner basal layer of polarized luminal epithelial cells as the intrinsic subtype should be similar to the subtype based on mRNA gene expression profiling alone[27,29].

Conclusions

About (58-60) % of breast cancers are mainly due to chemical or heavy metallic compound exposure, while (40-42) % of remnant females are higher auto-immunized for breast carcinoma.

Larger surface area density of the left breast Vs. the right, preferring interpretation of mammograms by digital image processing is more accurate and yields higher degrees of sensitivity, and specificity of 97.85%, and 99.28%, respectively.

In human beings, the initiation stage of the tumor interval after a single exposure to carcinogens triggering chromosomal mutation and cancer formation needs 7.5 -10 years.

Breast carcinoma attack probability after exposure to carcinogens is nearly 39 (44.83%) Vs. 43 (49.43%) right and left breasts consequently. With 5(5.75%) bilateral, this confirms that the tumor frequency curve is more shifted to the left.

Histopathological characteristics of BC of contralateral breasts are variable, the right breast is more resistant to carcinogenic fighting and shelters the carcinomatous cells in a limited sector by predominance of eosinophils, while the left breast missing an organized defense mechanism and the tumor cells sparsely diffuse all over the breast. This may change the methodology of contralateral BC management.

On the other hand, the right single malignant sector cells, showed more aggressive and wild character than in the left breast, due to game-over protumor characteristics of the eosinophils.

In general, the DC incidences were more than LC and the IMH staining showed and proved heterogenicity of contralateral breast tumors due to multifocal basic cellular chromosomal mutations. Higher numbers of rat candidates advised for further studies decreasing errors and more confident results.

Conflict of interest:

None

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