Research Square

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Quantitative Immunohistochemical Expression of GP88 in Invasive Ductal Carcinoma (IDC) of Human Breast Cancer

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Abstract

Background: Progranulin or acrogranin is an 88-kDa glycoprotein identified by a biological screen for protein targets associated with high tumorigenicity. This work was aimed to investigate the digital expression of GP88, and HER2/neu as a predictive biomarker in human invasive ductal carcinoma (IDC) versus benign tumors and normal breast tissues, as well as, its correlation with different pathological parameters.

Methods: The immunohistochemical avidin-biotin complex protocol of the paraffin section was used to detect the expression of GP88 and HER2/neu in IDC of 60 patients, 30 benign, and ten normal breast tissues.

Results: the study showed a high expression of GP88 in IDC comparing to normal and benign breast tissues. A higher significant statistical correlation between the expression of GP88 and large tumor size, tumor grade, and lymph node metastasis (LNM). While a negative statistical correlation was noticed between the expression of GP88 and ER, PR, and HER2/neu status.

Conclusion: It could be concluded that GP88 glycoprotein may be a valuable predictive and therapeutic marker in human IDC patients.

Introduction

Breast cancer represents a major scientific, clinical and societal problem. It is the most common malignancy and the second leading cause of cancer death in females following lung cancer [1, 2] with more than million new cases and 370,000 deaths yearly worldwide [3]. In many developing countries, the incidence of breast cancer is now rising sharply due to changes in reproductive factors, lifestyle, and increased life expectancy [4]. The invasive ductal carcinoma is the most common type of breast cancer, and comprises 70–80% of all cases. It starts in a milk duct of the breast, breaks through the wall of the duct, and grows into the fatty tissue of the breast, that it may be able to spread (metastasize) to other parts of the body through the lymphatic system and blood stream[5]. The cells invasive must occur from detached and penetrate the basement membrane, adherent cells lose their normal attachment to the substrate[6]. Proliferation is highly sensitive to the extracellular matrix maintain an actively proliferating phenotype in the newly encountered matrix [7]. Ductal carcinoma is an ill-defined mass, sometimes adherent to the skin or underlying muscle. The tumor is of varying size and may be associated with calcification. The histomorphology of the tumor is highly variable, ranging from a low-grade tumor showing mildly pleomorphic tumor cells arranged in tubules with little mitotic activity to a high-grade tumor showing highly pleomorphic tumor morphology, with the tumor cells arranged in solid sheets and groups, brisk mitotic activity and abundant tumor necrosis [8]. The Nuclear morphology is evaluated in variation of nuclear size, regularity of the nuclear border, hyperchromasia, and prominence of nucleolus. Also, it has been reported that histologic grade for breast cancer remains a prognostic factor despite changes in tumor size and number of positive lymph nodes [9]. Histological grade is commonly employed

and has been shown to be of independent prognostic significance. The studies showed very strong correlation with prognosis; patients with grade I tumors have a significantly better survival than those with grade II and III tumors ($p < .0001$) [10]

Progranulin is 88-kDa glycoprotein and known as GP88, PC-cell derived growth factor or acrogranin, GP88 gene is located on the 21q portion of chromosome 17, while the mouse gene was found on chromosome 11[11]. The autocrine growth factor GP88 is abundantly expressed in epithelial cells, immune cells, neurons, and chondrocytes[11]. Also, involved in a variety of biological processes, including embryogenesis, inflammation [12], wound healing[13], host defense [14]and cartilage development and degradation[15]. The importance of the Progranulin (GP88) is no unique receptor for GP88 has been identified[16]. GP88 has been shown to bind the membrane proteins sortilin and tumor necrosis factor receptors (TNFR) 1 & 2[17]. It is thought to exert its mitogenic effect through the stimulation of both the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) pathways[18]. GP88 stimulates the expression of the angiogenic growth factor (VEGF) in breast cancer cell lines, the breast cancers with elevated GP88 levels have higher VEGF levels and microvascular density [19]. GP88 is an important marker in breast cancer, it can be measured in healthy and breast cancer patients[20]. Increased secretion of GP88 is associated with more aggressive forms of breast, brain, renal, cervical and other tumors [12]

HER-2/neu (also called c-erbB-2) is a proto-oncogene located on chromosome 17, and encodes a 185-kd transmembrane glycoprotein receptor (p185 HER2) which has a tyrosine kinase activity and related to the epidermal growth factor receptor (EGFR). It is amplified or overexpressed in about 25–30% of human breast cancer, which is associated with tumor aggressiveness [11], and maybe related to variability in the interpretation of protein expression levels [21]. The predictive value of HER-2/neu status with respect to response to therapy as hormonal therapy, resistance to alkylating agent-based chemotherapy and taxanes [22, 23]. So, the aim of the present work was designed to study the Immunohistochemical expression of the GP88 as a novel prognostic biomarker in human invasive breast carcinoma.

Materials And Methods

Tissue samples were obtained from patients diagnosed with breast tumors in the Department of Pathology, Medical Research Institute (MRI), Alexandria University, Egypt, Formalin-fixed and paraffin embedded tissue specimens from 60 patients diagnosed with IDC, 30 patients diagnosed with benign breast tumor and 10 were taken from normal breast tissue adjacent to the benign tumors were included. All the cases were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study according to the guide ethics of institute MRI (IORG#: IORG0008812). Samples from all studied cases were subjected to the following: **1-Clinical parameters** included patients' age, tumor size, lymph node metastasis (LNM) status, **2-Histopathological examination** by hematoxlin and eosin (H&E) stained slides for each patient and reviewed by two pathologists (Diagnosis of the specimens was made according to the WHO classification of the Tumors) [24]. 3- Biological markers of (ER, PR). **3-Immunohistochemical technique:** the detection of both GP88 and HER2/neu protein used

avidin biotin complex protocol (ABC) and DAB stain [25] Immunohistochemical method was utilized to study the expression of GP88 and HER2/neu. in 100 paraffin-embedded breast tissues. In brief; paraffin-embedded specimens were cut into 5µm sections. The sections were deparaffinized using 2 changes of xylene and rehydrated. The sections were submerged in antigen retrieval (citrate buffer saline pH 6) in an oven at 95°C for 20 minutes and then left at room temperature for 20 minutes to cool. The sections were treated with 3% H_2O_2 in PBS to quench the endogenous peroxidase activity, followed by incubation with serum blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibody for GP88 (Biorbyt Company, London, UK) and HER2/neu (Novus Biologicals Company, USA) at 4ºC overnight. The sections were treated with conjugated 2^{ry} antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzedine (DAB) and counter stained with hematoxlyen. For negative controls, antibody was replaced with PBS. Each step was followed by PBS washing. Immunohistochemical evaluation of results was arbitrarily graded as negative (0), weak (+1), moderate (+2) and strong (+3). **4- Image analysis**: Integrated optical density (IOD) of GP88 and HER2/neu in normal, benign and IDC groups was analyzed using digital image analyzer. Images were viewed and recorded using Olympus microscope equipped with spot digital camera and image J software. Maximum, minimum and integrity of intensity color based on Gray-level acquisition, analysis of data were carried out by reading ten image for each case. The mean values of each reaction were based on the mean of pixel number [26]. IOD based on Gray-level transition probabilities in digitized images was graded from dark to light (0 up to 250). The average score across the whole image should be taken; IOD in digitized images was calibrated from strong to light (180 down to 70) by pixel. This calculation was proceed after subtract the pixel value from 250, the pick of lighter elimination

Results

1- Histopathological investigation:

The paraffin section photomicrograph illustrated the histopathological changes of the studies groups. The normal breast tissue showed acini lined by epithelial cells, an intact myoepithelial cell layer attached the basement membrane of acini (Figure 1A). A mild adenosis and cyst formation with mild epithelial hyperplasia in the adjacent duct and intact myoepithelial cell layer in fibrocystic disease of the benign breast case (Figure 1B). The tumor cells of invasive ductal carcinoma cases have abundant eosinophlic cytoplasm and large moderately pleomorphic round to ovoid vesicular nuclei arranged in cords and some contain small nucleoli. Infiltrated tumor cells in desmoplastic stroma represented GII (Figure1C). The neoplastic growth formed of nests and tubules and hyperchromatic nuclei and largely pleomorphic vesicular nuclei were exhibiting mitotic activity in ductal epithelial cells with many pyknotic cells, as well as, many residual nuclei was seen in GIII (Figure 1D)

The histopathological diagnosis of studing cases found that the benign cases revealed as the majority (83%, 25/30) of fibroadenoma, while 17% (5/30) cases were represented with fibrocystic disease. At malignant cases, there was a majority of invasive ductal carcinoma (IDC) (98%, 59/60), while 2% (1/50) of the malignant cases were represented with invasive lobular carcinoma (ILC). In addition, according to

Scarf'-Bloom-Richardson histological grading system of breast cancer, the majority of the malignant cases diagnosed as grade II (45/60, 75%), grade III cases were represented by 22% (13/60) and the minority cases were grade I (2/60, 3%) of the malignant cases as in the (Table 1).

2- Clinicopathological parameters:

The age ranges of benign and malignant groups were 15-64 and 15-74 years with a mean ± SD of 36 ± 11 and 52 ± 10 years respectively. The data reported of the benign group cases 57% (17/30) at age range > 35-45 years versus of the malignant group cases 31.5% (19/60) at age ranges > 35-45 and > 45-55 years respectively. Neither of the benign cases were above 65 years nor of malignant cases under 25 years as shown in (Table 2)

According to the tumor size assessed according to TNM (Tumor, Node, Metastasis) staging system of breast cancer, there was 80% (24/30) of the benign cases were T2 (> 2-5 cm) versus 66% (27/45 grade II and 11/13 grade III) of the malignant cases were T2 (> 2-5 cm) as illustrated in the (Table 3). Whereas the percentage of positive vascular invasion (VI) was 98% (59/60) were distributed as in the (Table 4), as well as, Lymph Node Metastasis (LNM) was illustrated in the (Table 5). Also, Noticed the majority positive cases were represented (44/60, 3%) and free of lymph node metastasis cases were 16/60 (27%).

3- Semi-qualitative immunohistochemical staining:

Hormonal status of both estrogen and progesterone receptor (ER&PR|) used as a biological marker in the malignant cases were differentiated to malignant grade II &III and illustrated in (Table: 6&7) respectively. According to epidermal growth factor receptor-2 (HER2\neu) expression, noticed that 53% (24/45) of IDC grade II were weak positive (-ve), while 38% (5/13) of IDC grade III were strong positive (3+), as shown in (Table: 8). As regard to GP88 immunostaining expression resulted a moderate positive expression (2+) in 71% (32/45) of grade II IDC and strong (3+) in 77% (10/13) of grade III IDC as illustrated in (Table:9)

4- Immunohistochemical staining results :

Immunostaining expression of HER2/nuw was detected as diffuse brown color detected in cell membrane of the ductal epithelial cells as shown in (Figure: 2 A,B.C.D,).

Immunostaining expression of GP88 was detected as diffuse, homogenous brown color detected in the cytoplasm and membrane of the ductal epithelial cells of the studied groups as shown in (Figure: 3 A,B.C.D,E,F) .

5- Immunostaining optical density (IOD)

A gray value detection of the binary evaluation at the 8-bit image of the stain density were quantitative digitally by pixel in the integrated region of optical density (ROD) as following:

a- HER2/neu

The mean and SD values of HER2/neu IOD for breast lesions illustrated the different ranges from 42. ±3.82, 63.±5.34, 143±5.49 and 169±4.46 as control, benign and IDC grade II and III were respectively. There was statistically significant difference between benign group and malignant one, as well as between the malignant grade II and III of IDC ($p < 0.001$), as shown (Figure:4)

b- GP88

The mean and SD values of GP88 IOD for control, benign and IDC grade II and III were 35 ± 3, 41 ± 4, 140 ± 8 and 162 ± 7 respectively. A significant difference was noticed between GP88 IOD of grade II and III IDC and GP88 IOD of both control and benign groups ($p < 0.001$), but there was no statistical significant difference between GP88 IOD of control and benign groups ($p = 0.1$) as shown (Figure : 5)

c- Correlation between GP88 IOD and histopathological parameters:

There was no statistical significant correlation between the GP88 IOD and patients' age (r= -.14, *P* = .28), ER status (r= -.09, P = .50), PR status (r= .06, *P*= .65) and HER2/neu status (r.22, *P*= .08) of the studied breast cancer cases, while highly statistical significant correlation was noticed between the GP88 IOD and tumor size (r= .354**, *P*= .006), tumor grade (r= .353**, *P*= .006) and LNM status (r.493**, *P* = .000) as shown in table (10).

Discussion

Progranulin or GP88 is an autocrine growth factor and pleiotropic regulatory protein that has been shown to play role in tumorigenesis, including proliferation, survival, migration, angiogenesis invasion and matrix metallo-protease activity [27], as well as, in wound healing and in inflammation in normal tissues [28]. The study was evaluated the role of GP88 through the digital immunostaining density under the correlation with the clinical pathological parameter in the breast cancer lesions.

The study evaluated a statistical significant increase in the immunohistochemical expression of GP88 in IDC, versus, normal tissues and benign tumors ($p < .001$). This result is in alignment with previous finding reported a high level of GP88 expression in breast cancer biopsies versus benign lesions and normal mammary epithelial tissues [29, 30]. The pathological studies with 203 formalin-fixed paraffin-embedded human breast cancer tissue biopsies indicated that GP88 was preferentially expressed in ductal carcinoma with little expression in lobular carcinoma while benign lesions and normal mammary epithelial tissues were negative [31]. In addition, circulating GP88 level in the serum of breast cancer patients was increased in compared to healthy volunteers[28]. Moreover, the parental SW13 cells (cell derive from an adrenal carcinoma) poorly tumorigenic in *vivo*; however, by over-producing GP88, they become highly tumorigenic in mice [32, 33]. This finding revealed that the SW13 cells are highly sensitive to the proliferative effects of GP88. So, the digital analysis (IOD) of the GP88 in the present work revealed the role of the GP88 expression associated with the increased tumor grade in the breast cancer and detected its role in the proliferation and invasive of the tumor cell in breast tissue. Also, the immunostaining positivity of GP88 in this work revealed the marked and intensity was present in

malignant lesion and differentiated in both grade II and grade III, this results were indeed the role of the GP88 expression induced and associated with division and invasive tumor cells. This finding was confirmed by He, et al,[33] who reported that the pgrn (GP88) stimulate cell division, invasion, and survival demonstrates, other revealed the role of GP88 expression for regulates multiple processes or stages in carcinoma progression and suggested the GP88 may be a possible future therapeutic target [34].

However, there was no statistically significant correlation between immunohistochemial expression of GP88 and patients' age (r = -0.1, P = 0.3). This result is consistent with the results of other previous studies [28, 35]. who reported the clinical and tumor characteristics (p-value risk ratio 95%) confidence interval lower upper age in years Age ≤ 50 compared to > 50. Therefore, it could be agreed that the tissue GP88 level is predictive of recurrence in patients with hormone-responsive BC [36], while the patients with early-stage BC would be suggested that serum GP88 determination has the potential to have additional clinical utility.

The large tumor size, high tumor grade, and positive lymph nodes are all signs of poor prognosis and elevated GP88 expression have shown to be associated with tumorigenesis and poor prognosis in several cancer types including ovarian [30], renal [37], prostate [38], liver [39], esophageal [40, 41] and breast cancers [42]. The current study showed a highly statistically significant correlation between the immunohistochemical expression of GP88 and tumor size (r = 0.354**, P = 0.006) of malignant breast cases. This result is going in accordance with Li et, al., [38][35], but contrasted with Serrero et, al. [29] and DeMorrow, [42]. Moreover, a highly statistically significant correlation was observed in the current study between GP88's immunohistochemical expression and tumor histological grade (r = .353**, P = .006). This result is contrasted with Serrero et, al.,[29], but consistent with the other who reported that overexpression of GP88 in 80% invasive ductal carcinoma, where it correlated with the clinical variables of poor prognosis such as tumor grade, p53 expression and Ki67index[31, 35]. Immunohistochemical expression of GP88 in the present study exhibited a highly statistically significant correlation with LNM of the studied breast cancer cases (r = .493**, P = .000). A previous study found that high levels of GP88 expression were closely correlated with clinicopathological parameters such as lymph node metastasis [35]. The histopathological features are independent predictors of clinical outcomes such as lymph node status, tumor size and tumor grade for invasive ductal carcinoma patients were based evidence that IHC analysis of GP88 expression maybe help a predict treatment outcomes in a prognostic role. At the correlation between the GP88 and hormonal status as a routine marker and necessary for the treatment and therapy of BC, our results in IDC patients found no statistical significant correlation between the digital quantitative immunohistochemical expression of GP88 and ER (r = .09, P = .50) and PR (r = .06, *P* = .65) status. These results are conflicted with Song et. al., [43] who reported that GP88 positive staining was more common in ER/PR negative samples than that in ER/PR positive tumors. The current results are going in accordance with Tkaczuk, *et al.*, [28] who observed no statistical correlation between GP88 expression and ER and PR levels. However, screening of GP88 expression in human breast cancer cell lines indicated that GP88 was highly expressed in both ER positive and ER negative human breast carcinomas[29, 44]. This lack of correlation between GP88's immunohistochemical expression and ER

status indicated that the growth factor receptors promotes proliferation without requiring ER-mediated growth signaling [45].

It has been reported that level of G P88 expression was a major determinant of the intrinsic activity of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3’-kinase (PI3K) in cell line studies suggesting that the mitogen-activated protein kinase and phosphatidylinositol 3’-kinase signaling pathways may be involved in the promotion of tumor invasion and migration by GP88 [16, 33]. However, the current study revealed no statistical significant correlation between the immunohistochemical expression of GP88 and HER2/neu status (r = 0.2, *P* = .08) of the studied breast cancer cases. This result is going in accordance with many studies reported that there was no correlation between expression of GP88 and HER2/neu, and indicated that GP88 and HER2/neu were independent biomarkers [28, 31]. Other investigator found that 25% of HER2-overexpressing biopsies (3 + by IHC) were strongly positive for GP88 [46] and the treatment of HER2-overexpressing cells with Trastuzumab decreased the levels of MAPK phosphorylation [47]. The results is going to that the activation of mitogen-activated protein kinase (MAPK) signaling pathways in breast cancer cells by the over-production of growth factors [43]. So, GP88 can activate MAPK signaling pathway and phosphorylated MAPK regardless of the HER2 expression levels, thereby GP88 is not necessarily dependent on HER2 overexpression, because it has its own ability to activate MAPK pathway [48]. This may be indicated that HER2/neu and GP88 are independent biomarkers.

Finally, the study found that high progranulin expression was associated with higher breast histopathological features, which are strong independent predictors of clinical outcomes such as lymph node status, tumor size and tumor grade for patients with invasive ductal carcinoma, as well as, the digital quantitative immunostaining of GP88(IOD) in IDC cases was evident a high statistically significant correlation compared to the benign and malignant cases as well as between the IDC grade II and grade III (p = 0.0001). Beside of no correlation between GP88 with ER, PR, and HER2/neu expression.

Conclusion

It may be suggested that the GP88 involved in processes of cells invasive, regulate tumor cell progression and a biomarker dependent as a therapeutic target.

Declarations

Ethics consents:

All the cases were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study according to the guide ethics of institute MRI (IORG#: IORG0008812).

Consent for publication: the co-author's consent to the publication .

Availability of supporting data: not applicable

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Help us for collecting sample, made the diagnosis of sample and reviewed the present results.

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Tables

Table (1): Distribution of breast cancer regarding tumor grade

Tumor grade	No of the cases	%
Grade I	2	3
Grade II	45	75
Grade III	13	22
Total	60	100

Table (2): Age distribution among the benign and malignant groups

Age categories	Benign group		Malignant group		Total	
	No	%	No	%	No	%
15-25	8	27	0	0	8	9
>25-35	3	10	2	3	5	6
>35-45	17	57	19	31.5	36	40
>45-55	1	3	19	31.5	20	22
>55-65	1	3	16	27	17	19
>65	0	0	4	7	4	4
Total no. of cases	30	100	60	100	90	100%
Range	16-64		35-74		16-74	
Mean ±SD	35.6 ± 11.23		51.53 ± 9.6		F: 49.1, $p < 0.001$**	
$X^2 = 35.7$ p < 0.001*						

X2: Chi square test, *P*: statistically significant ** Calculated by the F-test for two means

Table (3): Tumor size distribution between the benign and breast cancer cases

Tumor size (cm)	Benign group		Malignant group			
			Grade II		Grade III	
	No	%	No	%	No	%
TI ≤ 2	2	7	5	11	1	8
T2 > 2-5	24	80	27	60	11	84
T3 > 5	4	13	13	29	1	8
Total	30	100	45	100	13	100
$X^2 = 5.22$, $P = 0.3$ (test is not significant)						

Table (4): Distribution of vascular invasion (VI) among breast cancer cases

vascular invasion	Grade I		Grade II		Grade III		Total	%
	No.	%	No.	%	No.	%		
Positive	2	100	44	98	13	100	59	98
Negative	0	0	1	2	0	0	1	2
Total	2	100	45	100	13	100	60	100

Table (5): Lymph node metastasis (LNM) status of breast cancer cases

Lymph node metastasis	Grade I		Grade II		Grade III		Total	%
	No.	%	No.	%	No.	%		
Positive	0	0	35	78	9	69	44	73
Negative	2	100	10	22	4	31	16	27
Total	2	100	45	100	13	100	60	100

Table (6): Estrogen Receptor (ER) distribution among breast cancer grades

Estrogen Receptor (ER)	Grade II		Grade III	
	No	%	No	%
Negative (-ve)	5	11	3	23
Weak positive (1+)	10	22	6	46
Moderate positive (2+)	18	40	3	23
Strong positive (3+)	12	27	1	8
Total	45	100	13	100
$X^2 = 5.6$, p = 0.14 (statistically not significant)				

X^2 : Chi square test

Table (7): Progesterone Receptor (ER) distribution among breast cancer grades

Progesterone Receptor (PR)	Grade II		Grade III	
	No	%	No	%
Negateve (-ve)	7	16	3	23
Weak positive (1+)	8	18	7	54
Moderate positive (2+)	21	46	2	15
Strong positive (3+)	9	20	1	8
Total	45	100	13	100
$X^2 = 5.04$ p = 0.2 (statistically not significant)				

Table (8): HER2\neu distribution among breast cancer cases

HER2/neu	Control group		Benign group		Malignant group				Total	
					Grade II		Grade III			
	No	%	No	%	No	%	No	%	No	%
Negative (-ve)	8	80	12	40	8	18	2	16	30	31
Weak +ve(+1)	2	20	16	54	24	53	2	16	44	45
Moderate +ve(+2)	0	0	2	6	9	20	4	30	15	15
Strong +ve(+3)	0	0	0	0	4	9	5	38	9	9
Total	10	100	30	100	45	100	13	100	98	100

X^2 : Chi square test (X^2=9.5 p=0.2 (no statistical significant)

Table (9): GP88 immunostaining reactivity in the different studied groups

GP88	Control group		Benign group		Malignant group				Total	
					Grade II		Grade III			
	No	%	No	%	No	%	No	%	No	%
Negative (-ve)	8	80	17	57	3	7	1	8	29	30
Weak +ve(+1)	2	20	11	37	4	9	0	0	17	17
Moderate +ve(+2)	0	0	1	3	32	71	2	15	35	36
Strong +ve(+3)	0	0	1	3	6	13	10	77	17	17
Total	10	100	30	100	45	100	13	100	98	100

X^2 : Chi square test (X^2=98.5 p=0.0001 (statistically significant)

Table (10): Correlation between GP88 IOD and histopathological parameter of cases studies

Pathological parameters	Cath-D IOD	
Age	r	0.05
	p	0.68
Tumor size	r_s	0.04
	p	0.77
Grades	r_s	.257*
	p	0.05
LNM	r_s	0.351**
	p	0.006
ER status	r_s	-0.12
	p	0.35
PR status	r_s	-0.17
	p	0.2
Her2/status	r_s	0.301*
	p	0.02

Figures

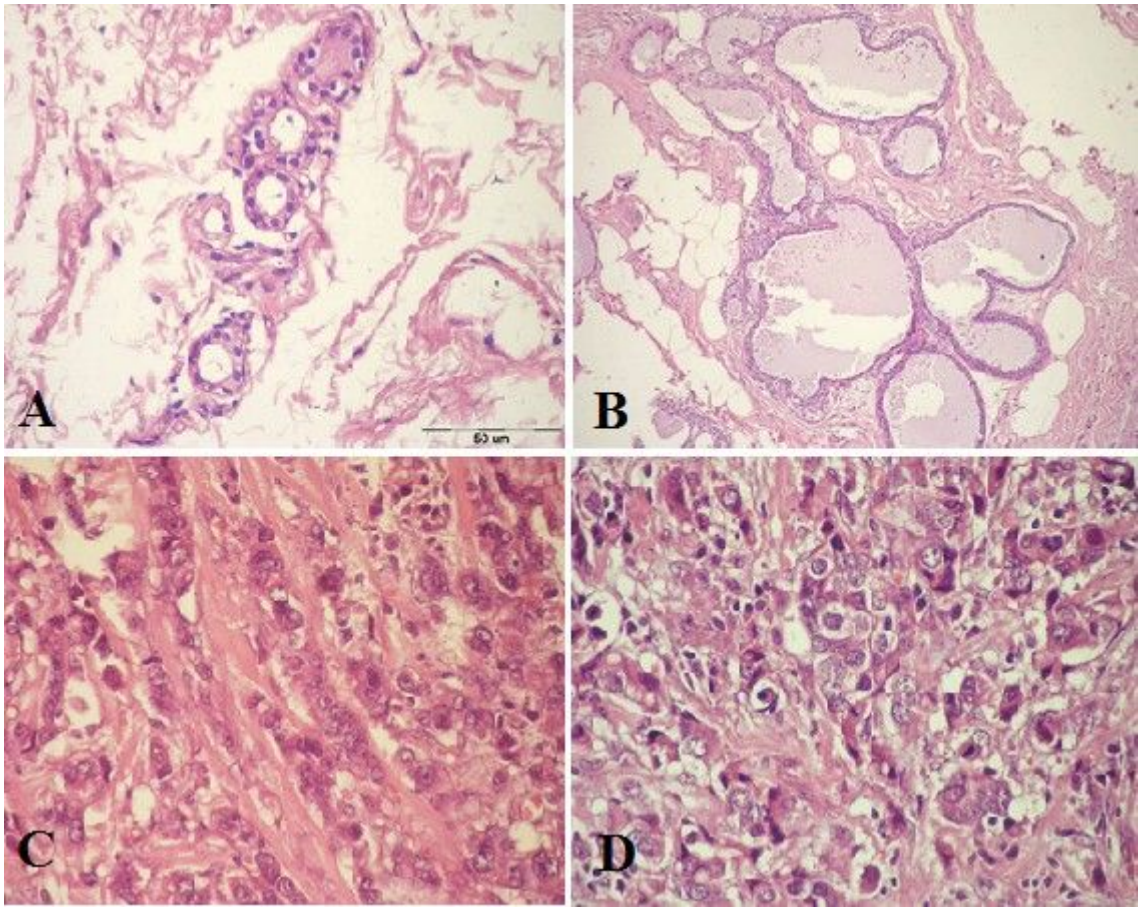

Figure 1

Paraffin section photomicrograph stained by (H&E stains, Bar=50 µm). A- Note the acini present in a normal lobule with dark-staining epithelium cells nuclei and homogenous pink cytoplasm and the underlying myoepithelial cells with clear cytoplasm, wild and edema connective tissue a]was seen. B- A fibrocystic case of benign lesssion with apocrine cyst formation and mild epithelial hyperplasia in the adjacent duct and intact myoepithelial cell layer. C- IDC grade II case, show tumor cells have abundant eosinophlic cytoplasm and large moderately pleomorphic round to ovoid vesicular nuclei arranged in cords and some contain small nucleoli. There are tumor cells infiltrated by desmoplastic stroma. D- IDC case grade III, show solid nests of tumor cells with large pleomorphic nuclei and some prominent nucleoli. There are numerous mitotic figures, large vesiculated nuclei and many residual nuclei and vacuolated cytoplasm.

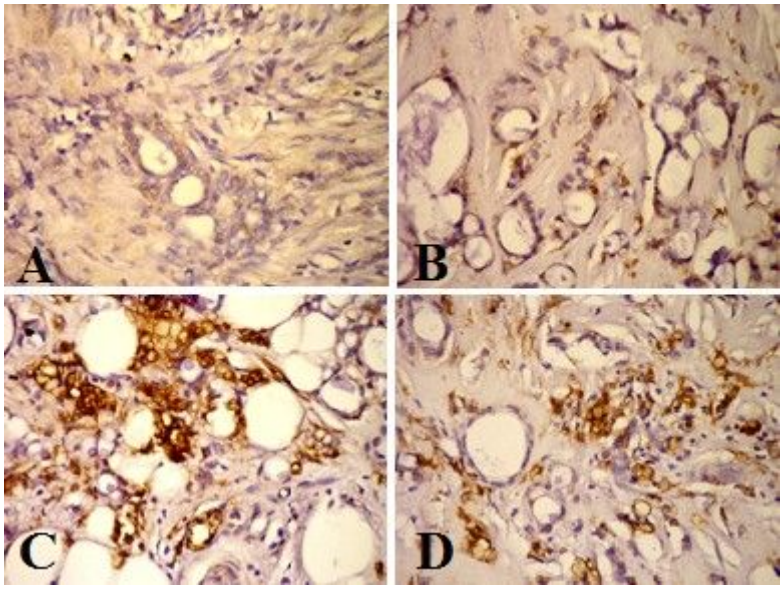

Figure 2

HER2/neu Immunohistochemical staining photomicrograph of breast paraffin section used ABC protocol (DAB stain, Bar =50 µm) show: A- control breast tissue very weak immunoactive at epithelial ductal cells. B- Benign case with mild expression of HER2/neu in the cell membrane of the ductal epithelial cell <10 cells. C- IDC grade II, strong positive immunostaining of HER2/neu (+3) in the epithelial cell membrane > 50. D- Moderate positive expression (+2) of HER2/neu in the ductal epithelia cells membrane >30.

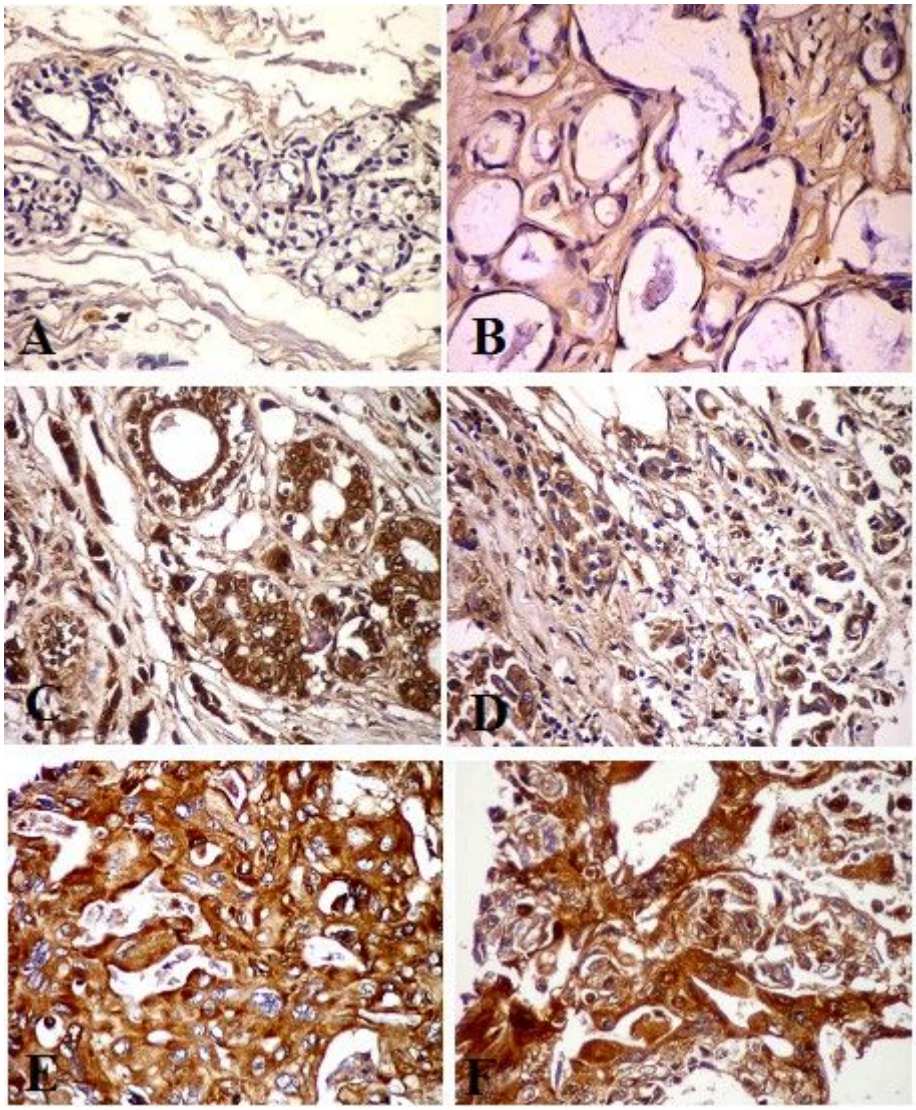

Figure 3

GP88 Immunohistochemical staining photomicrograph of breast paraffin section used ABC protocol (DAB stain, Bar =50 μm) show : A- control breast tissue and B- benign case with negative expression of GP88 in the cytoplasm of the ductal epithelial cell and weak expression in stromal tissue. C&D - IDC grade II, strong immunostaining of GP88 (+3) in the epithelial cell at the luminal surface. D- Marked positive expression (+2) of GP88 in the cytoplasm of the ductal epithelia cells. E&F- IDC grade III , note strong expression (+3) of GP88 as the homogenous expression of GP88 in the ductal epithelial cell and surrounding stromal tissue (E), a high expression of the GP88 with coarse and homogenate immunostaining at the upper surface of the epithelial ductal cell(F).

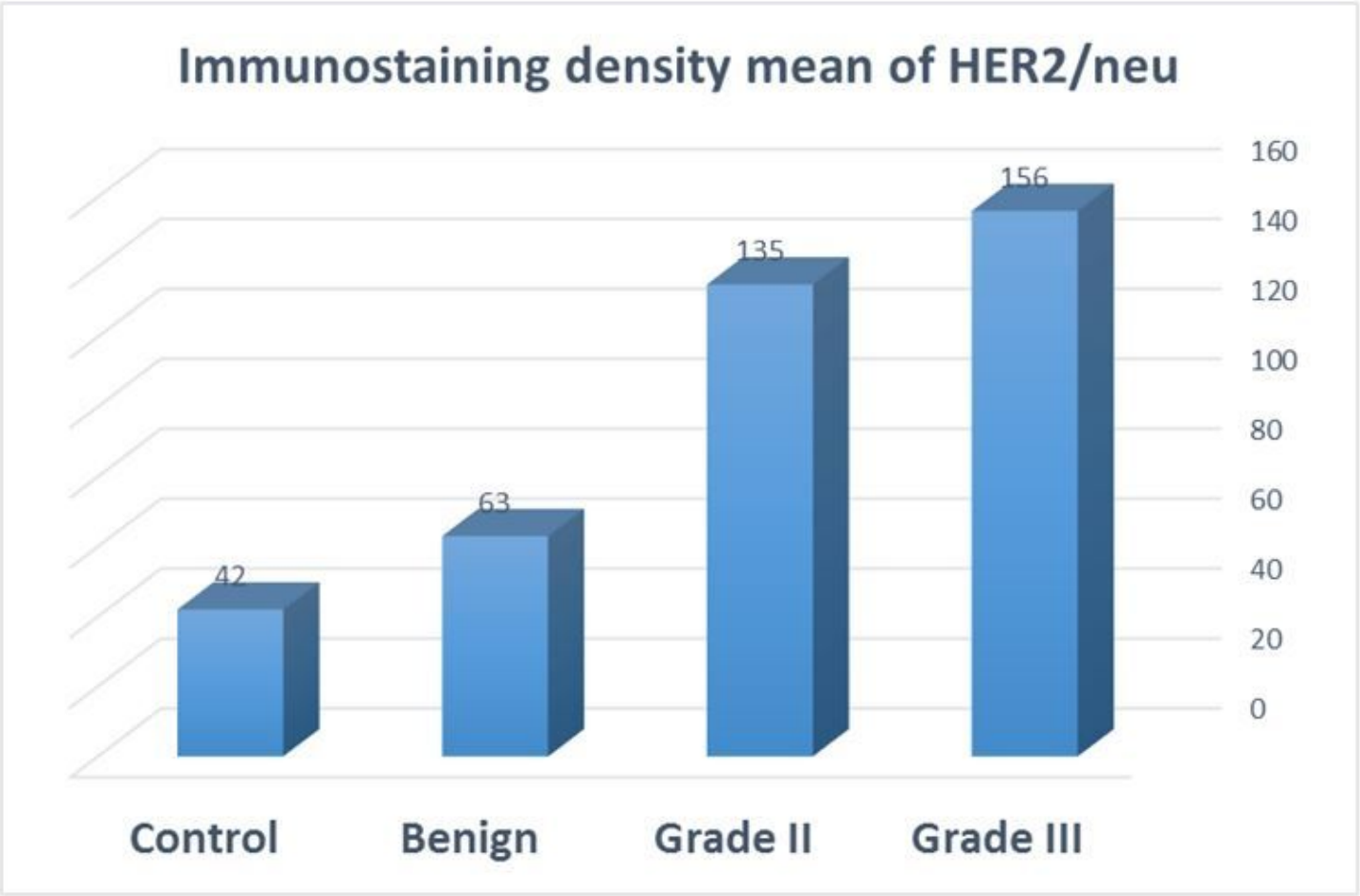

Figure 4

The mean value of HER/2neu IOD in the different studied groups.

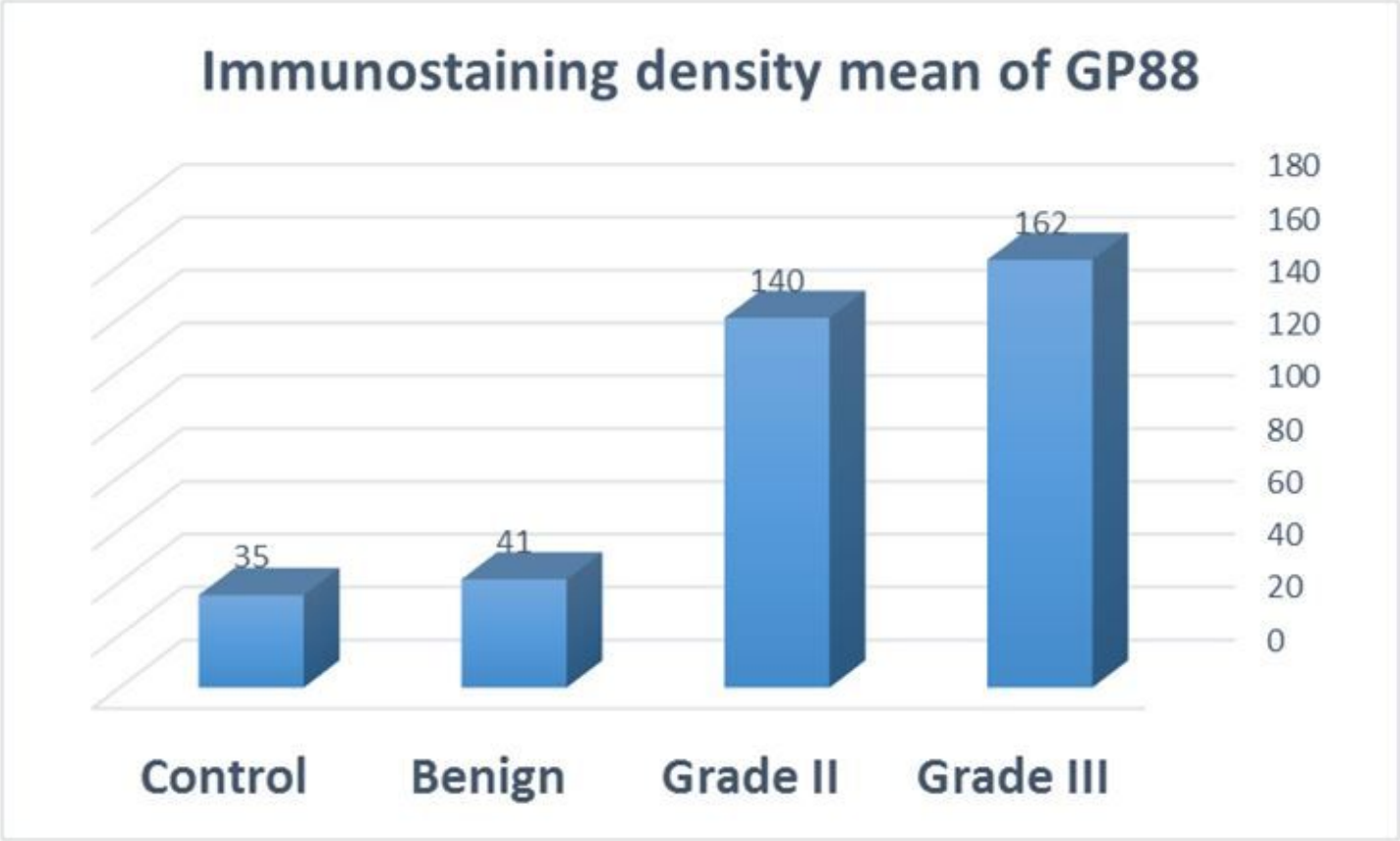

Figure 5

The mean value of GP88 IOD in the different studied groups.