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COMPARATIVE STUDY OF IMMUNOHISTOCHEMICAL EXPRESSION OF GP88 AND Cath-D AS PROGNOSTIC MARKERS AND CORRELATION WITH PATHOLOGICAL AND HISTOLOGICAL PARAMETERS IN HUMAN BREAST CANCER

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Abstract

Progranulin or acrogranin is an 88-kDa glycoprotein identified by a biological screen for protein targets associated with high tumorigenicity. Cathepsin D (Cath-D) is a soluble lysosomal aspartyl glycoprotease that can degrade the protein components of the matrix and free growth factors therein embedded, thus favoring tumor growth, invasion, and angiogenesis. The present work aimed to compare the expression of GP88 and Cath-D as novel prognostic biomarkers in human invasive ductal carcinoma (IDC) versus benign tumors and normal breast tissues as well as their correlation with different pathological and histological parameters. The immunohistochemical technique was used to examine the expression of GP88 and Cath-D in normal, benign, and IDC. Present results showed higher expression of GP88, and Cath-D in IDC compared to normal and benign breast tissues.

Keywords: GP88 and Cath-D prognostic marker.

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Introduction

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in women worldwide [(1)]. 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 [(2)] with more than 1,000,000 new cases and 370,000 deaths yearly worldwide [(3)].

Progranulin is an 88-kDa glycoprotein 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. The autocrine growth factor GP88 is abundantly expressed in epithelial cells, immune cells, neurons, and chondrocytes [(4)].

Cathepsin D (Cath-D) is a soluble lysosomal aspartyl glycoprotease that can degrade the protein components of the matrix and free growth factors therein embedded, thus favoring tumor growth, invasion, and angiogenesis. The aspartic protease Cath-D, a poor prognostic indicator of breast cancer, is abundantly secreted as pro-Cath-D by human breast cancer cells and self-activates at low pH in vitro, giving rise to catalytically active Cath-D [(5)].

In the present study, the expression of GP88 and Cath-D in an invasive ductal carcinoma (IDC) was investigated using an immunohistochemical technique, and the intensity of immunostaining was quantitatively estimated using an image optical density (IOD) analyzer.

Material and Methods

Tissue samples were obtained from patients diagnosed with breast tumors in the Department of Pathology, Medical Research Institute, Alexandria University, Egypt. Formalin-fixed and paraffin-embedded tissue specimens from 60 patients diagnosed with IDC, 30 patients diagnosed with benign breast tumors and 10 were taken from normal breast tissue adjacent to the tumors were included. All the cases were asked to freely volunteer for the study and informed written consent was gathered before their inclusion in the study. Hematoxylin and eosin (H&E) stained slides for each patient were reviewed by two pathologists. Diagnosis of the specimens was made according to the WHO classification of the Tumors. Clinical parameters included patients' age, tumor size, and lymph node metastasis (LNM).

Immunohistochemical investigation of GP88 and Cath-D: The immunohistochemical method was utilized to study the expression of GP88 and Cath-D in 60 paraffin-embedded breast tissues. In brief, paraffin-embedded specimens were cut into 5µm thick sections. The sections were deparaffinized using 2 changes of xylen and rehydrated. The sections were submerged in an 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 and then incubated with serum serum-blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibodies for GP88 and Cath-D (Biorbyt Company, London, UK) at 4ºC overnight. Sections were treated with conjugated 2nd antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzidine (DAB), and counter-stained with hematoxylin. For negative controls, the antibody was replaced with PBS. Each step was followed by PBS washing. Evaluation of GP88 and Cath-D immunohistochemical results was arbitrarily graded as negative (0), weak (+1), moderate (+2), and strong (+3).

Statistical Analysis: Data were normally distributed according to the Kolmogorov-Smirnov (K-S) normality test, and then analyzed using statistical software package SPSS 20. P values$\leq$ 0.05 were considered statistically significant.

Results:

A -Histopathological results:

a. Haematoxylin and Eosin (H&E) staining of control, benign and malignant breast tissues:

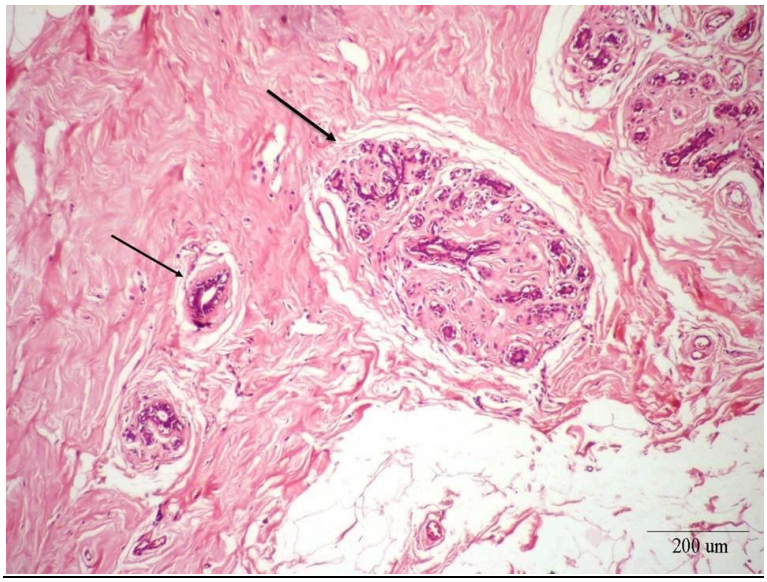


Figure (1): A section of control breast tissue showing normal ducts (thin arrow) and lobules (thick arrow) (H & E. Bar =200 µm).

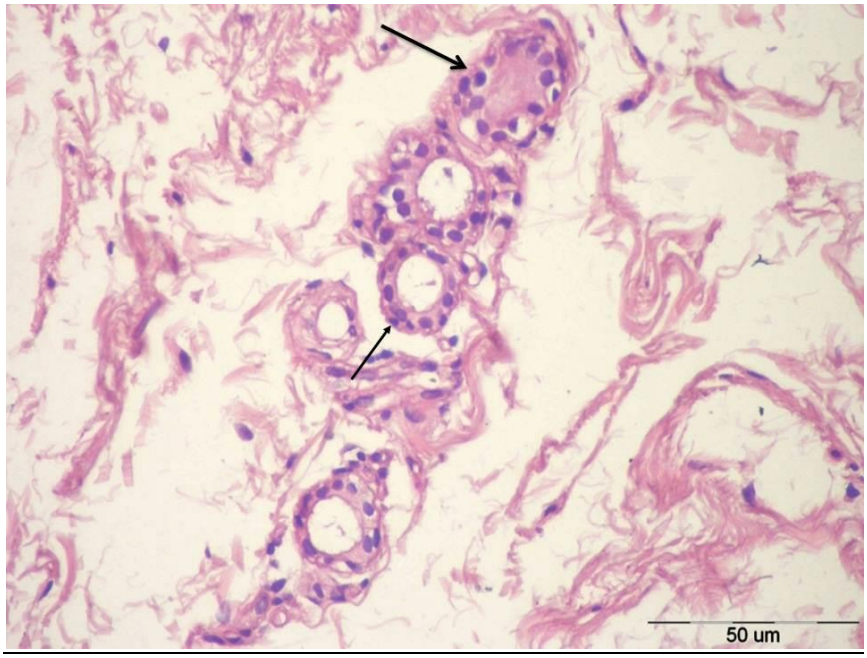


Figure (2): A view of the acini present in a normal lobule. The acini are lined by cuboidal epithelium (thick arrow) with underlying myoepithelial cells having clear cytoplasm (thin arrow) (H & E. Bar =50 µm).

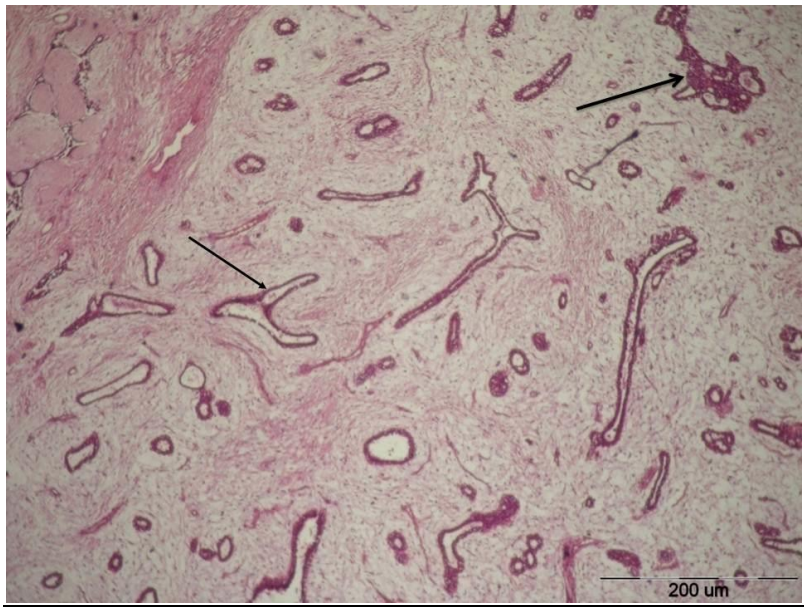


Figure (3): A section of fibroadenoma showing both periductal (thin arrow) and intraductal (thick arrow) patterns (H &E. Bar =200 µm).

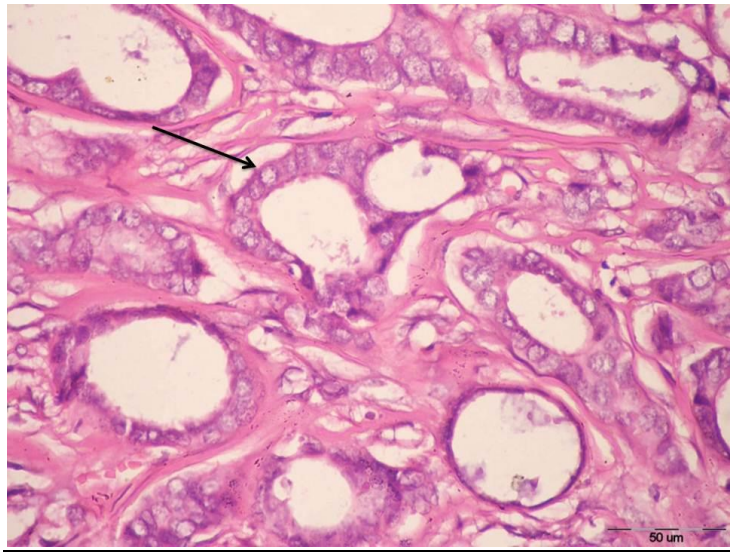


Figure (4): A section of IDC grade I showing well-defined ducts lined by cuboidal epithelial cells with vesicular nuclei (H&E. Bar =50 µm).

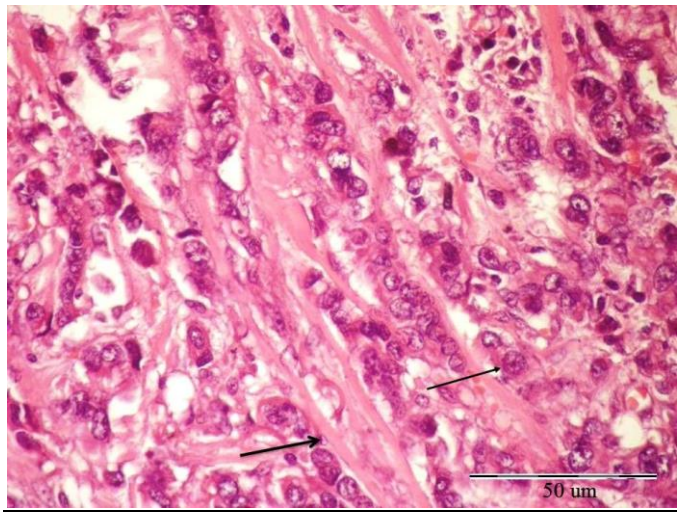


Figure (5): A section of IDC grade II showing tumor cells with abundant eosinophilic cytoplasm and pleomorphic round-to-ovoid vesicular nuclei (thin arrow). The cells that are arranged in cords infiltrate the desmoplastic stroma (thick arrow) (H&E. Bar =50 µm).

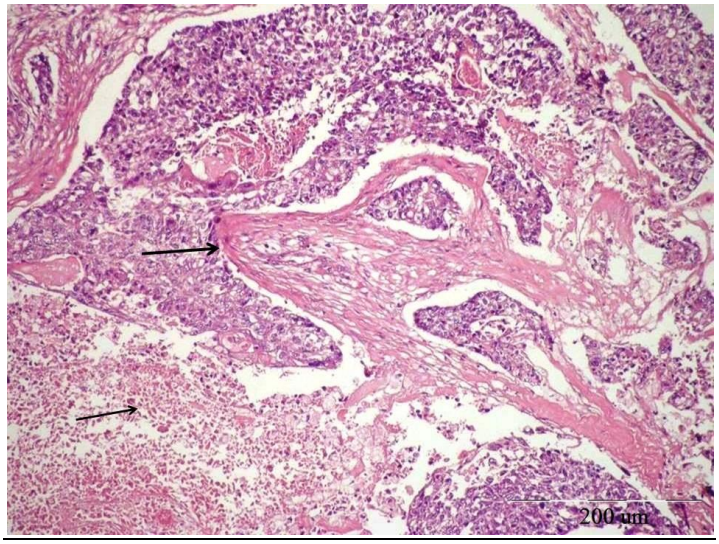


Figure (6): A section of IDC grade III (Atypical medullary variant) showing sheets of malignant ductal cells with wide areas of necrosis (thin arrow) and lymphocytes infiltration separated by fibrous tissue septa (thick arrow) (H&E. Bar=200 µm).

B-Immunohistochemical results:

I-GP88:

a. Immunohistochemical reactivity of GP88:

- Immunostaining reactivity of GP88 was detected as a diffuse, homogenous brown color in the cytoplasm and membrane of the ductal epithelial cells of the studied groups.
- GP88 immunostaining reactivity was negative (-ve) in 80% (8/10) of the control group and 57% (17/30) of the benign group, while it was moderate (2+) in 71% (32/45) of grade II IDC and strong (3+) in 77% (10/13) of grade III IDC as illustrated in figures (7).

- **Table (1): 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	32	33
Weak +ve (1+)	2	20	11	37	4	9	0	0	19	19
Moderate +ve (2+)	0	0	1	3	32	71	2	15	35	36
Strong +ve (3+)	0	0	1	3	6	13	10	77	12	12
Total	10	100	30	100	45	100	13	100	98	100
$X^2 = 98.5$, $p = 0.000$ (statistically significant)										

X^2: Chi-square test

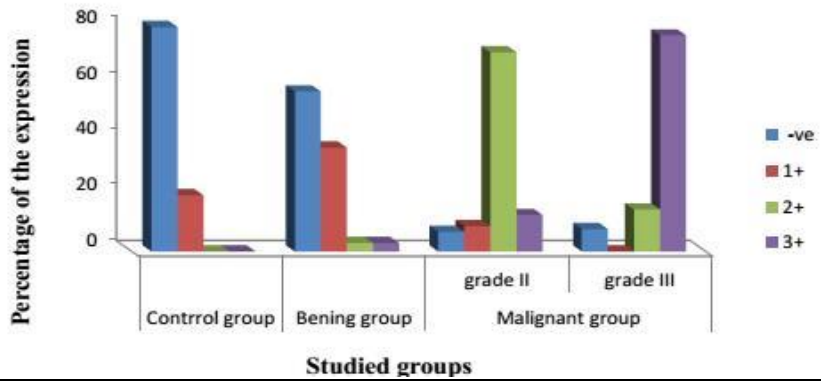


Figure (7): Immunostaining reactivity of GP88 in the different studied Groups. GP88 was overexpressed in the malignant group versus control and benign groups.

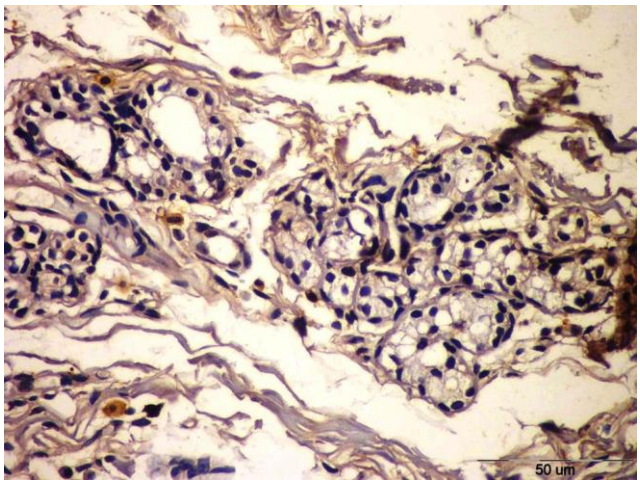


Figure (8): Immunohistochemical staining of control breast tissue showing negative expression of GP88 in the cytoplasm of the ductal epithelial cell (Bar =50 µm).

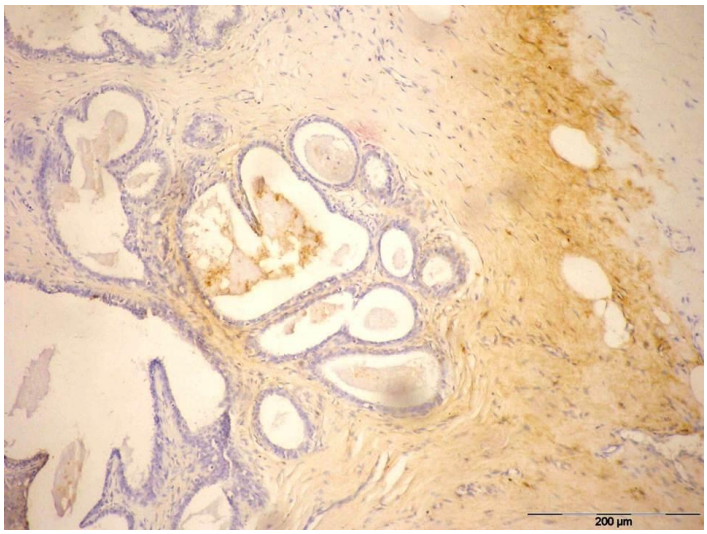


Figure (9): A benign breast tissue showing negative (-ve) expression of GP88 in the cytoplasm of the ductal epithelial cells (Bar =50 µm).

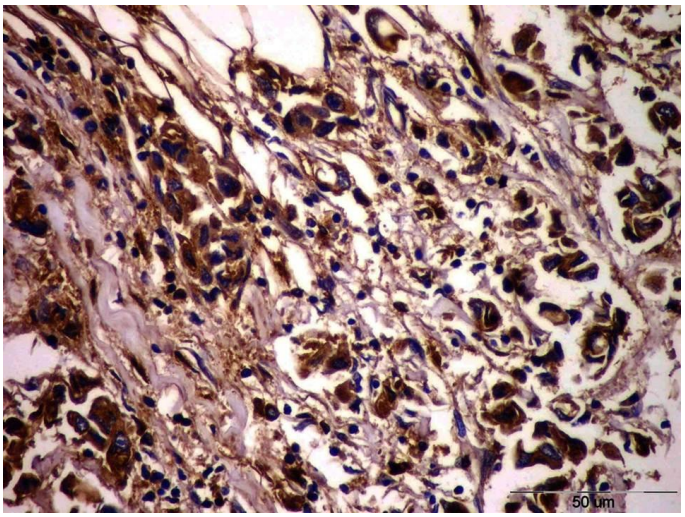


Figure (10): An IDC grade II breast tissue showing moderate expression (2+) of GP88 in the cytoplasm of the ductal epithelial cells (Bar=50 µm).

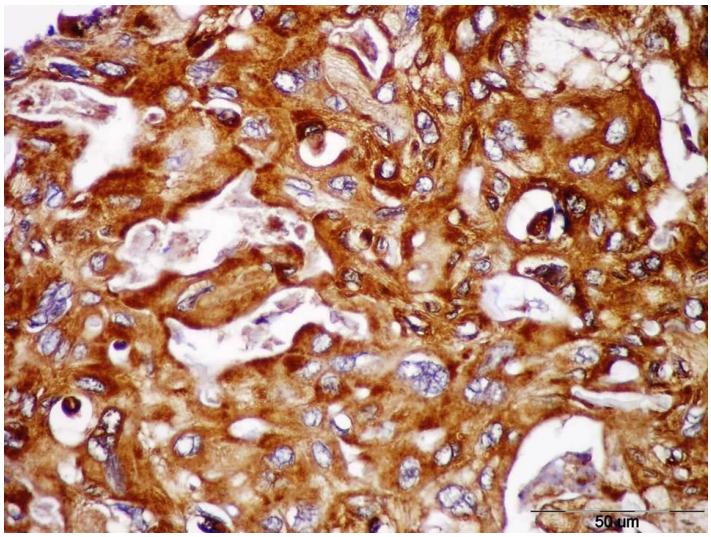


Figure (11): An IDC grade III breast tissue showing strong expression (3+) of GP88 in the cytoplasm of the ductal epithelial cell (Bar=50 µm).

b. Integrated optical density (IOD) of GP88 in the different studied groups:

The mean 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 ($p < 0.00$), but there was no statistically significant difference between GP88 IOD of control and benign groups ($p = 0.1$) as shown in table (2) and figure (12).

Table (2): The mean and SD of GP88 IOD in the different studied groups

GP88	Control (n = 10)	Benign (n = 28)	Grade II (n = 38)	Grade III (n = 12)	F	p
Min. – Max. Mean ± SD.	29 – 40 35 ± 3	35 – 48 41 ± 4	130 – 155 140 ± 8	146– 166 162 ± 7	1983	<0.001*
p_1		0.1	<0.001*	<0.001*		
p_2			<0.001*	<0.001*		
p_3			<0.001*			

F: F test (ANOVA)
p_1: p-value for Post Hoc test (Scheffe) for comparing between control and each other group.
p_2: p-value for Post Hoc test (Scheffe) comparing between benign and each other group.
p_3: p-value for Post Hoc test (Scheffe) for comparing grade II with grade III.
*: Statistically significant at $p < 0.05$

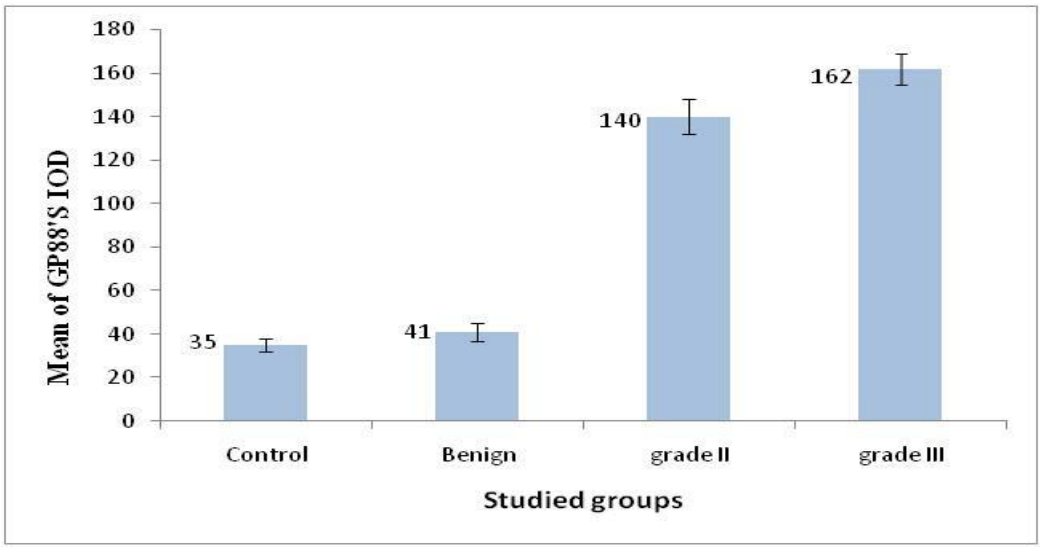


Figure (12): The mean and SD of GP88 IOD in the different studied groups

C. Correlation between GP88 IOD and histopathological parameters in breast cancer cases:

There was no statistically 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 the highly statistically 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 (3).

Table (3): Correlation between GP88 IOD and histopathological parameter in breast cancer cases

Pathological parameters	GP88 IOD	
Age	r	-0.14
	p	0.28
Tumor size	r_s	.354**
	p	0.006
Grades	r_s	.353**
	p	0.006
LNM	r_s	.493**
	p	.000
ER status	r_s	-0.09
	p	0.5
PR status	r_s	0.06
	p	.65
Her2/status	r_s	0.22
	p	.08

r: Pearson coefficient
r_s: Spearman coefficient
*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

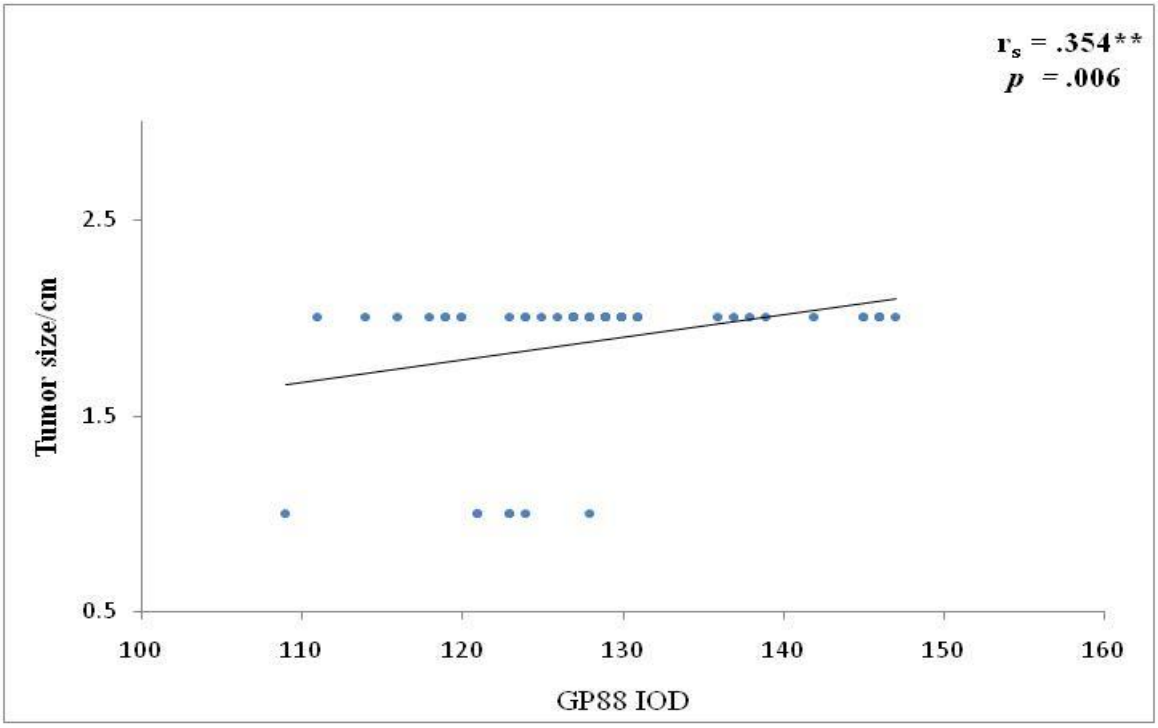


Figure (13): Correlation between GP88 IOD and tumor size/cm of the breast cancer cases.

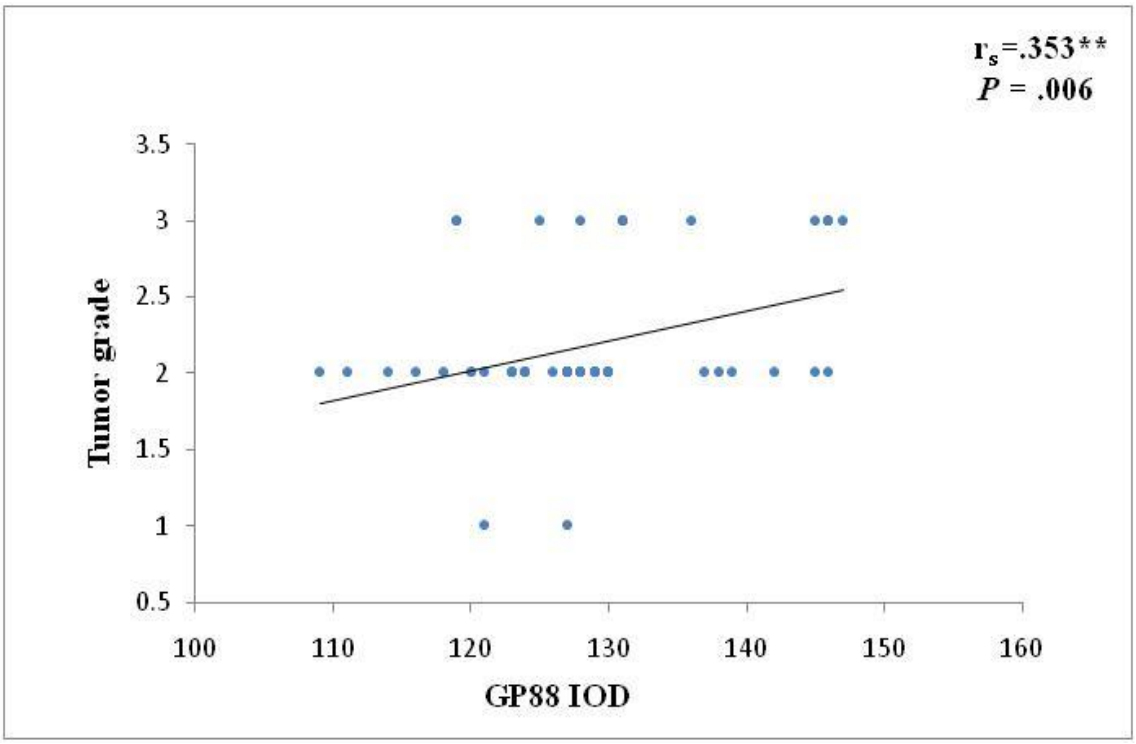


Figure (14): Correlation between GP88 IOD and tumor grade of breast cancer cases.

II- Cath-D:

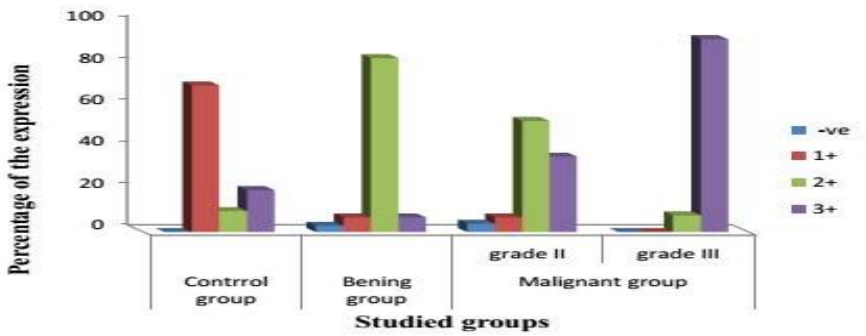
a. Immunohistochemical reactivity of Cath-D:

- Immunoreactivity of Cath-D was detected as a brown course, or tiny granules detected in the cytoplasm of the ductal epithelial cells of the studied groups.
- Cath-D immunostaining reactivity was weak +ve (1+) in 70% (7/10) of control group, moderate +ve (2+) in 83% (25/30) and 53% (24/45) of benign and grade II IDC groups respectively, while it was strong +ve (3+) in 36% (16/45) and 92% (12/13) of grade II and grade III IDC respectively as illustrated in table (4) and figures (15).

- **Table (4): Cath-D immunostaining reactivity in the different studied groups**

Cath-D	Control group		Benign group		Malignant group				Total	
					Grade II		Grade III			
	No	%	No	%	No	%	No	%	No	%
Negateve (-ve)	0	0	1	3	2	4	0	0	3	3
Weak +ve (1+)	7	70	2	7	3	7	0	0	7	7
Moderate +ve (2+)	1	10	25	83	24	53	1	8	57	58
Strong +ve (3+)	2	20	2	7	16	36	12	92	31	32
Total	10	100	30	100	45	100	13	100	98	100
$X^2 = 66.7$, $p = 0.000$ (statistically significant)										

- X^2: chi-square



- **Figure (15):** Cath-D immunostaining reactivity in the different studied groups

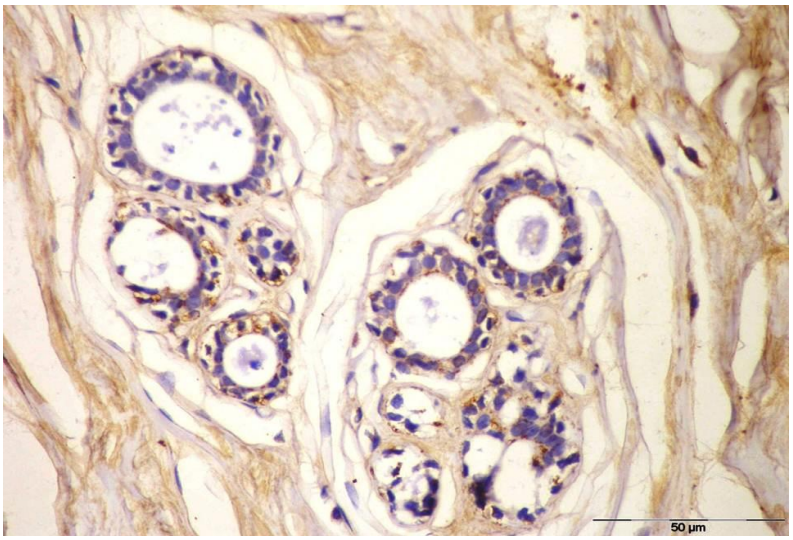


Figure (16): Immunohistochemical staining of a control breast tissue showing weak (1+) expression of Cath-D (Bar=50 µm).

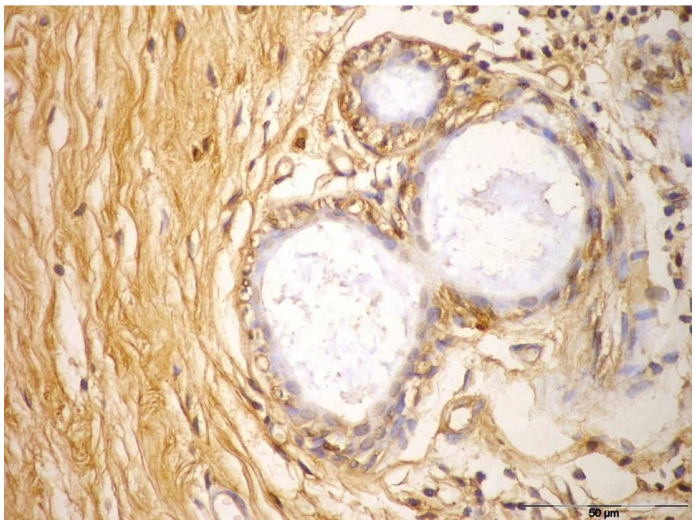


Figure (17): A benign breast tissue showing moderate (2+) expression of Cath-D enzyme in the cytoplasm of the ductal epithelial cells and surrounding extracellular matrix (Bar=50 µm).

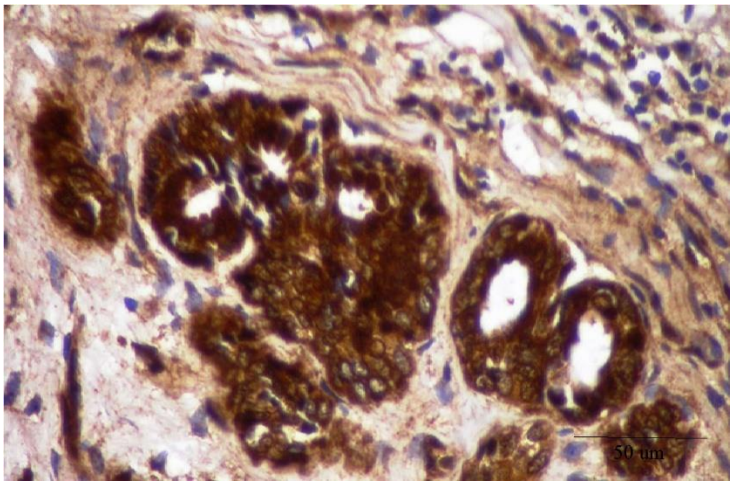


Figure (18): IDC grade I show a strong positive expression (3+) of Cath-D in the ductal epithelial cells (Bar=50 µm).

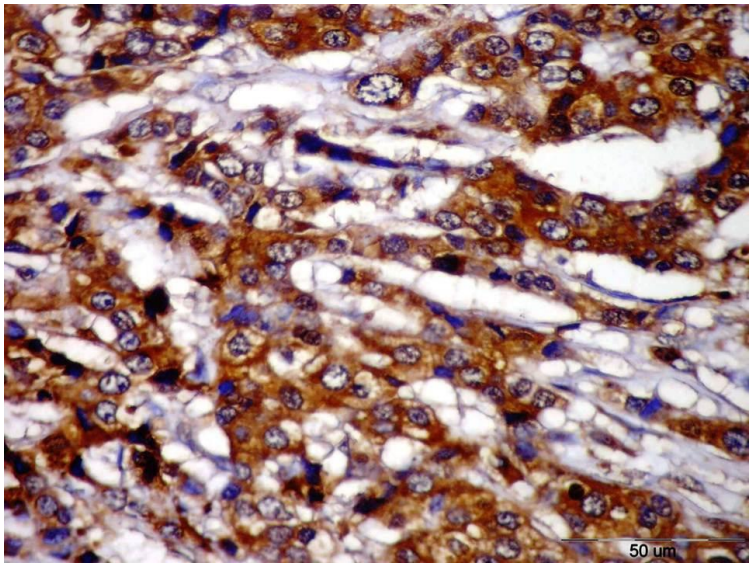


Figure (19): An IDC grade II breast tissue showing moderate (2+) expression of Cath-D in the cytoplasm of the ductal epithelial cells (Bar=50 µm).

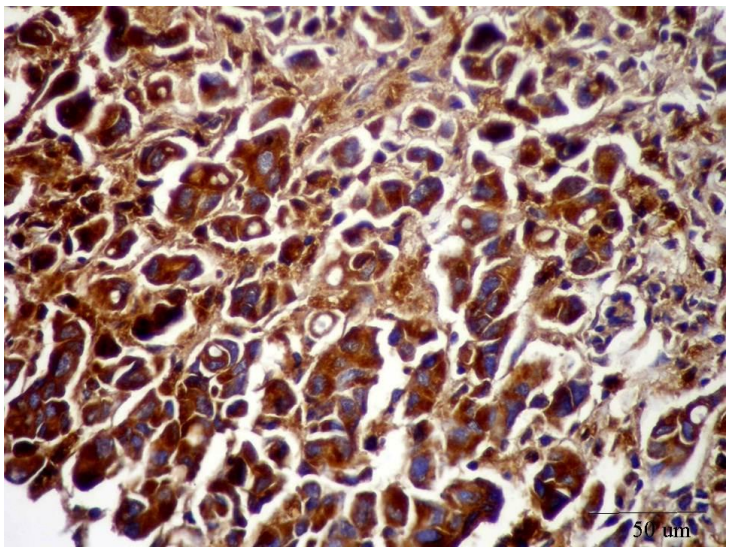


Figure (20): An IDC grade III breast tissue showing strong (3+) expression of Cath-D in the cytoplasm of the ductal epithelial cells (Bar=50 µm).

b. Integrated optical density (IOD) of Cath-D in the different studied groups:

The mean values of Cath-D IOD for control, benign, and IDC grade II and III were 30 ± 3, 124 ± 3, 159 ± 9, and 168 ± 3 respectively. A statistically significant difference ($p < 0.00$) was noticed between the studied groups as shown in table (5) and figure (21).

Table (5): Comparison between the studied groups according to Cath-D IOD

Cath-D	Control (n = 7)	Benign (n = 25)	Grade II (n = 40)	Grade III (n = 13)	F	P
Min. – Max. Mean ± SD.	26 – 33 30 ± 3	120 – 130 124 ± 3	150 – 173 159 ± 9	162 – 171 168 ± 3	882	<0.001*
p_1		<0.001*	<0.001*	<0.001*		
p_2			<0.001*	<0.001*		
P_3			0.03*			

F: F test (ANOVA)
p_1: p-value for Post Hoc test (Scheffe) for comparing between control and each other group.
p_2: p-value for Post Hoc test (Scheffe) comparing between benign and each other group.
p_3: p-value for Post Hoc test (Scheffe) for comparing grade II with grade III.
*: Statistically significant at $p \leq 0.05$

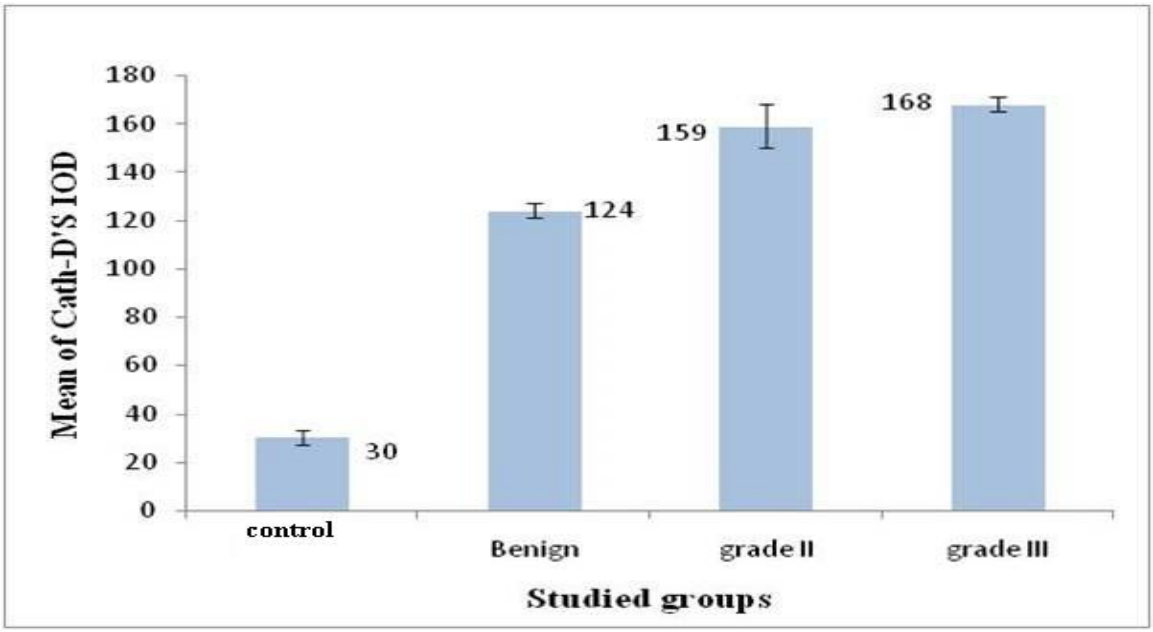


Figure (21): The mean and SD of Cath-D IOD in the different studied groups

C. Correlation between Cath-D IOD and histopathological parameters in the breast cancer cases:

There was no statistically significant correlation between Cath-D IOD and patients' age (r = 0.05, *P*= 0.68), tumor size (r = 0.04, *P* = 0.77), ER (r= -0.12, *P*= 0.35) and PR (r= -0.17, *P* = .20) status of the studied cancer cases, while a highly statistically significant correlation was recorded between Cath-D IOD and LNM (r= .351**, *P* = .006), and a statistically significant correlation was noticed between Cath-D IOD and tumor grade (r= .257*, *P* = .05), and HER2/neu status (r= .301*, *P* =.02), as shown in table table (6).

Table (6): Correlation between Cath-D IOD and histopathological parameters in the breast cancer cases.

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

r: Pearson coefficient
r_s: Spearman coefficient
*. Correlation is significant at the 0.05
**. Correlation is significant at the 0.01 level

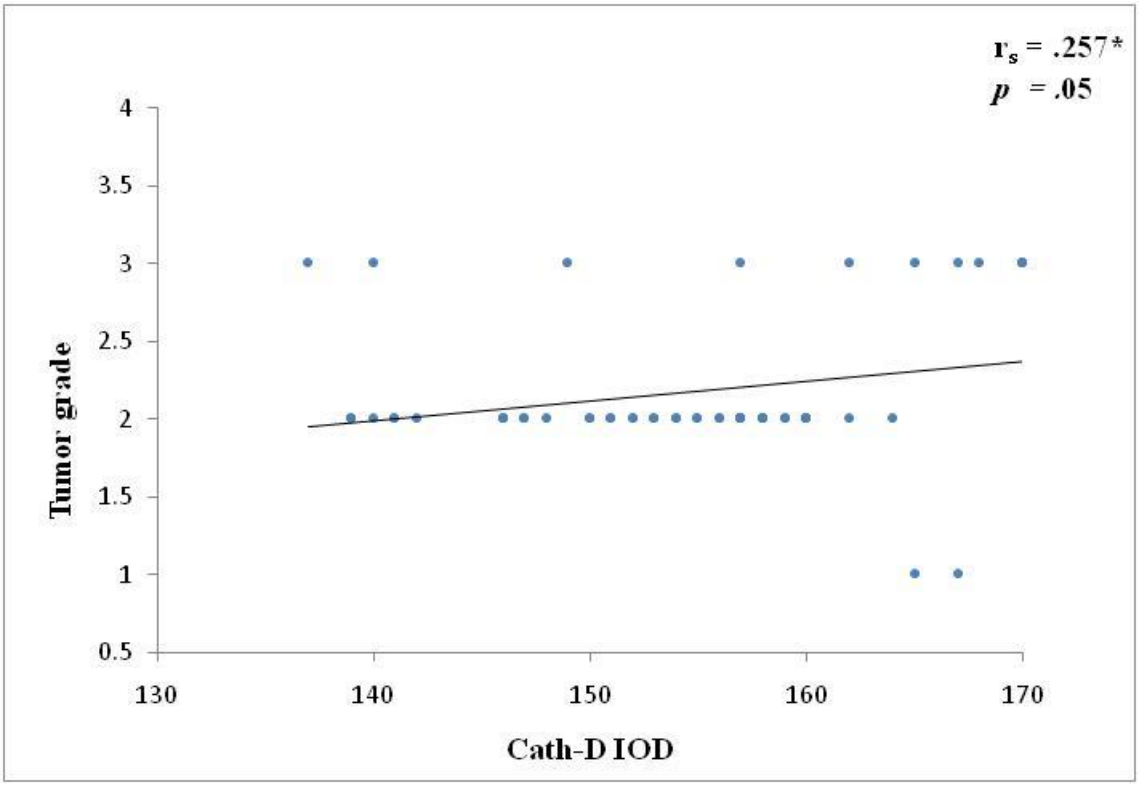


Figure (22): Correlation between Cath-D IOD and tumor grade of the breast cancer cases.

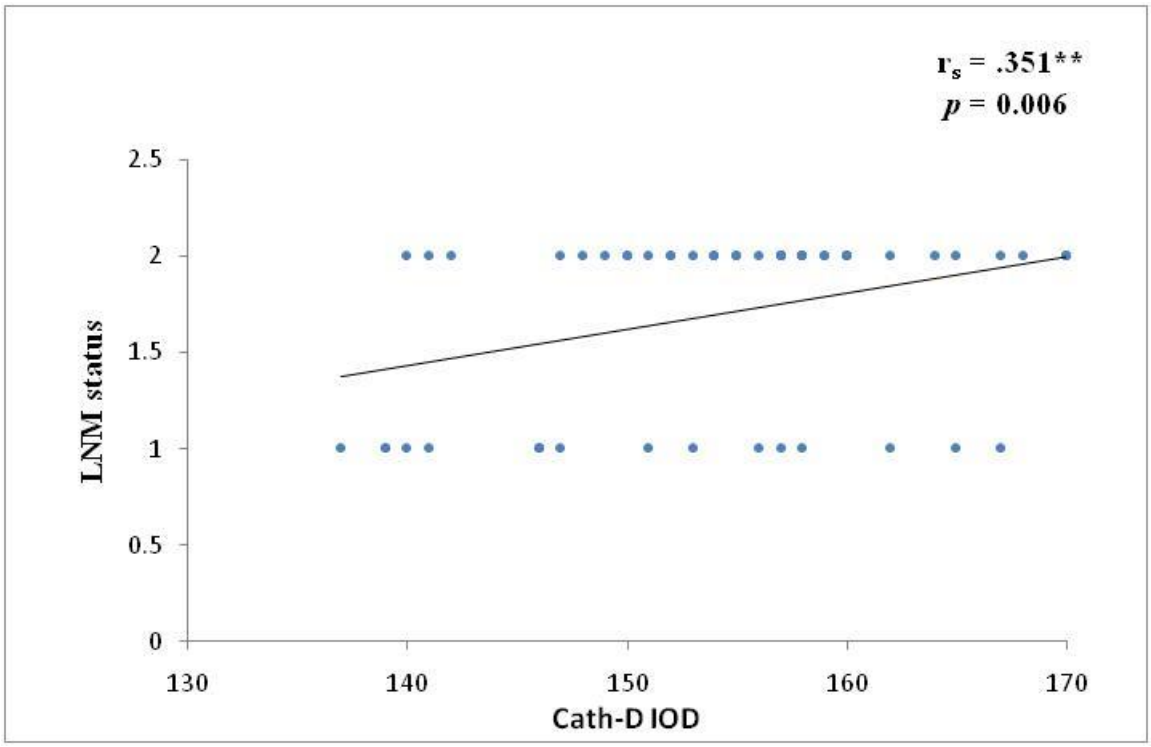


Figure (23): Correlation between Cath-D IOD and HER2/neu status of the breast cancer cases.

DISCUSSION

Breast cancer remains a major scientific, clinical, and societal challenge. It is the most common malignancy and the second leading cause of cancer death in females worldwide following lung cancer [6,7]. In many developing countries, the incidence of breast cancer is now rising sharply due to changes in reproductive factors, lifestyle, and increased life expectancy [8].

The results of the present study showed that 98% of the breast cancer cases were invasive ductal carcinoma (IDC), most of which were allocated to the age range of (>35-55) years. These results are consistent with several previous studies that reported that IDC is the most common histological type of invasive breast cancer, and in the developing world, it is characterized by an early peak age of onset [9, 10].

The present study was undertaken to assess the immunohistochemical expression of GP88 and Cath-D in human breast invasive carcinoma versus normal control and benign breast tumors, as well as to investigate the correlation of their immunohistochemical expression with clinicopathological parameters.

The ability of cancer cells to produce and respond to their own (autocrine) growth factors is important in the proliferation and progression of cancer cells [11].

Progranulin or GP88 is an autocrine growth factor and pleiotropic regulatory protein that has been shown to play a role in tumorigenesis, including proliferation, survival, migration, angiogenesis invasion, and matrix metalloprotease activity, in addition to its role in wound healing and inflammation in normal tissues [12].

The results of the present study showed a statistically 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 findings that reported a high level of GP88 expression in breast cancer biopsies versus benign lesions and normal mammary epithelial tissues [13]. In addition, 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 [14].

Cath-D is a lysosomal aspartic protease that is overexpressed by epithelial and stromal breast cancer cells. This protease is an independent marker of poor prognosis in breast cancer as it is correlated with the incidence of clinical metastasis [15].

The results of the current study showed that Cath-D expression was increased in breast cancer cases than in normal and benign cases. A Previous study reported that normal lobular or ductal epithelia both from non-tumoral and tumoral lesions showed no Cath-D specific Staining [16].

Interestingly, the present results showed a statistically significant difference between the expression of Cath-D in normal and benign cases. This finding agreed with Brujan, *et al*., (2009) [17], who noticed that the expression of Cath-D in benign breast tumors was higher than in normal breast tissues, but still lesser than in malignant breast tumors.

Several approaches, such as immunohistochemistry, in situ hybridization, cytosolic immunoassay, and Northern and Western blot analyses have indicated that in most breast cancer tumors Cath-D is overexpressed 2- to 50-fold compared to its concentration in other cells such as fibroblasts or normal mammary glands [18].

The current results showed no statistically significant correlation between the immunohistochemical expression of Cath-D and patients' age (r = .22, P = .09). This lack of correlation between the expression of Cath-D and patients' age was also reported by several previous studies [19].

The results of the current study showed no statistically significant correlation between the immunohistochemical expression of Cath-D and tumor size (r = .04, *P* = .77). This result is consistent with Gion, *et al*., (1995) [20] and Brujan, *et al*. (2009) [21], but contrasted with Ruibal, *et al*., (2012) [22] who found that cytosolic concentration of Cath-D was associated with large tumors.

The represented data showed a statistically significant correlation between the immunohistochemical expression of Cath-D and tumor histological grade (r = 0.3*, *P* = 0.05). This result follows Paksoy, *et al*., (2011) [23] and Ruibal, *et al*., (2012) [24], but contrasted with Carrascosa Lloret, *et al*., (2002) [25] and Huang, et al., (2013) [26].

Cath-D is involved in the pathogenesis of neurodegenerative, skin, cardiovascular, and tumoral diseases [27]. In these pathologies, Cath-D is aberrantly produced and processed in malignancy and over-secreted to the cell microenvironment where it acts as tumor and stromal cells mitogen, also its hyper secretion leads to excessive degradation of the extracellular matrix, which contributes to tumor progression and metastases [28].

The current results found no correlation between Cath-D concentrations and ER (r = -0.1, P= 0.35) and PR status(r = -.17, P = 0.2). A lack of correlation between ER and Cath-D in breast tumors was previously reported and the number of tumors with high concentrations of cathepsin D was not significantly different in ER-positive and ER-negative samples [29]. In contrast, NIKOLIÆ-VUKOSAVLJEVIÆ *et al*., (2002) [30] found a direct association between cathepsin D and ER and PR status.

Even though cath-D has initially shown to be induced by estrogen in ER-positive breast cancer cell lines, it is also constitutively overexpressed in ER-negative tumors (by an unknown mechanism) as shown in cell lines and patients [31], this may interpret the lack of correlation between its expression and ER status.

GP88 and Cath-D are representatives of cytokines, growth factors, and proteases. Stromal and tumor cells exchange enzymes, growth factors, and cytokines that modify the local extracellular matrix, stimulate migration and invasion, and promote the proliferation and survival of stromal and tumor cells [32], this may interpret their association in IDC in the present study.

Conclusion:

From the results of the present study, it could be concluded that:

- The marked immunohistochemical expression of GP88 and Cath-D in the malignant group versus control and benign groups indicates that these biomarkers might be potential tumor antigens and valuable therapeutic targets in breast cancer.

- The strong statistical correlation between the immunohistochemical expression of GP88 with the large tumor size, high tumor grade, and LNM, and the lack of correlation between its expression and ER and HER2/neu indicates that GP88 might be an independent prognostic marker for poor prognosis in breast cancer.

Competing interests:

Authors declare that they have no competing interests, financials, or others.

REFERENCES

1. Khan HM, Saxena A, Vera V, Abdool-Ghany F, Gabbidon K, Perea N, et al. Black Hispanic and Black non-Hispanic breast cancer survival data analysis with half-normal model application. Asian Pac J Cancer Prev 2014; 15: 9453-8.

2. Dorling L, Carvalho S et al. Breast Cancer Risk Genes – Association Analysis in More than 113,000 Women. N Engl J Med 2021; 384: 428–439.

3. Lehner J., Stötzer O.J., Fersching D., Nagel D. and Holdenrieder S., Circulating plasma DNA and

DNA integrity in breast cancer patients undergoing neoadjuvant chemotherapy, Clin. Chim. Acta., 425, 20611 (2013)

4. 11. Liu C-j, Bosch X. Progranulin: a growth factor, a novel TNFR ligand and a drug target. Pharmacol Ther. 2012;133(1):124–32.

5- Shulman LN, Willett W, Sievers A, Knaul FM. Breast cancer in developing countries: opportunities for improved survival. J Oncol. 2010; 2010: doi:10.1155/2010/595167.

6. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

7. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 2014; 64: 52-62.

8. Shulman LN, Willett W, Sievers A, Knaul FM. Breast cancer in developing countries: opportunities for improved survival. J Oncol. 2010; 2010: doi:10.1155/2010/595167.

9. Shi WB, Yang LJ, Hu X, Zhou J, Zhang Q, Shao ZM. Clinicopathological features and prognosis of invasive micropapillary carcinoma compared to invasive ductal carcinoma: a population-based study from China. PLoS One. 2014; 9: doi: 10.1371/journal.pone.0101390.

10. Bhikoo R, Srinivasa S, Yu TC, Moss D, Hill AG. Systematic review of breast cancer biology in developing countries (part 1): Africa , the Middle East, Eastern Europe, Mexico, the Caribbean and South America. Cancers (Basel). 2011; 3: 2358-81.

11. Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. Int J Cell Biol 2012; 2012: doi: 10.1155/2012/762825.

12. Tkaczuk KR, Yue B, Zhan M, Tait N, Yarlagadda L, Dai H, Serrero G. Increased Circulating Level of the Survival Factor GP88 (Progranulin) in the Serum of Breast Cancer Patients When Compared to Healthy Subjects. Breast Cancer 2011; 5:155-62.

13. Han JJ, Yu M, Houston N, Steinberg SM, Kohn EC.Progranulin is a potential prognostic biomarker in advanced epithelial ovarian cancers. Gynecol Oncol. 2011; 120: 5-10.

14. Serrero G, Ioffe OB. Expression of PC-cell-derived growth factor in benign and malignant human breast epithelium. Hum pathol 2003; 34: 1148-54.

15. Masson O, Bach AS, Derocq D, Prébois C, Laurent-Matha V, Pattingre S, et al. Pathophysiological functions of cathepsin D: Targeting its catalytic activity versus its protein binding activity? Biochimie 2010; 92:1635-43.

16. Liaudet-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, Laurent-Matha V, et al. Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. Cancer Lett 2006; 237:167-79.

17. Brujan I, Mărgăritescu C, Simionescu C, Pirici D, Fronie A, Foarfă C. Cathepsin-D expression in breast lesion: an immunohistochemical study. Rom J Morphol Embryol 2009; 50: 31-9.

18. Hu L, Roth JM, Brooks P, Luty J, Karpatkin S. Thrombin up-regulated cathepsin D which enhances angiogenesis, growth, and metastasis. Cancer Res 2008; 68:4666-73.

19. Gion M, Mione R, Dittadi R, Romanelli M, Pappagallo L, Capitanio G, et al. Relationship between cathepsin D and other pathol ogical and biological parameters in 1752 patients with primary breast cancer. Eur J Cancer. 1995; 31: 671-7.

20. Paksoy M, Hardal U, Caglar C. Expression of cathepsin D and E-cadherin in primary laryngeal cancers correlation with necklymph node involvement. J Cancer Res Clin Oncol 2011; 137: 1371-7.

21. Brujan I, Mărgăritescu C, Simionescu C, Pirici D, Fronie A, Foarfă C. Cathepsin-D expression in breast lesion:

an immunohistochemical study. Rom J Morphol Embryol 2009; 50: 31-9.

22. Paksoy M, Hardal U, Caglar C. Expression of cathepsin D and E-cadherin in primary laryngeal cancers correlation with necklymph node involvement. J Cancer Res Clin Oncol 2011; 137: 1371-7.

23. Ruibal A, Herranz M, Arias JI. Clinical and biological significance of Cathepsin D levels in breast cancer cytosol in women over 70 years. Biomark Cancer. 2012; 4:1-6.

24. Carrascosa Lloret C, Cremades Mira A, Sánchez Sanchis M, Rodrigo Guanter V, Beltrán Armada JR, Sala Aznar A, VacacelaCórdova R, et al. Study of cathepsin D levels in invasive bladder cancer and its stroma. Correlation with tumor stage, cytological grade, lymph node metastasis and survival. Acta Urol Esp 2002; 26: 335-8.

25. Huang L, Liu Z, Chen S, Liu Y, Shao Z. A prognostic model for triple-negative breast cancer patients based on node status, cathepsin-D and Ki-67 index. PLoS One. 2013; 8: doi: 10.1371/journal.pone.0083081.

26. Benes P, Vetvicka V, Fusek M. Cathepsin-D many functions of one aspartic protease. Crit Rev Oncol Hematol 2008; 68:12–28.

27. Abbott DE, Margaryan NV, Jeruss JS, Khan S, Kaklamani V, Winchester DJ,et al. Reevaluating cathepsin D as a biomarker for breast cancer: serum activity levels versus histopathology. Cancer Biol Ther. 2010; 9:23-30.

28. Duffy MJ, Brouillet JP, Reilly D, McDermott E, O'Higgins N, Fennelly JJ, et al. Cathepsin D concentration in breast cancer cytosols: correlation with biochemical, histological, and clinical findings. Clin Chem 1991; 37; 101-4.

29. Nikolić-Vukosavljević D, Markićević M, Kanjer K, Todorović-Raković N, Nesković-Konstantinović Z. Immunoradiometric assay of cathepsin D: Estrogen-regulated vs. nonestrogen-regulated cathepsin D expression in relation to clinico-patholoical features of breast cancer. Arch Oncol 2002; 10: 115-7.

30. Rochefort H, Glondu M, Sahla ME, Platet N, Garcia M. How to target estrogen receptor-negative breast cancer? Endocr Relat Cancer. 2003; 10; 261-6.

31. Liotta LA, Kohn EC. The microenvironment of the tumour-host interface. Nature. 2001; 411: 375-9.