Immunohistochemical Expression of Estrogen And Progesterone Receptors in Neuroendocrine Breast Carcinomas

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Abstract:

Neuroendocrine (NE) breast carcinomas are defined by the diffuse expression of NE-markers. They may coexpress NE and non-NE substances such as glycoproteins and apocrine protein "divergent differentiation". In the present study, twenty NE-breast carcinomas, expressing chromogranin A (CgA) and neurone specific enolase (NSE) were selected from a series of two hundreds retrospective breast carcinomas, in addition to ten normal breast tissue biopsies taken as control. The NE-tumors were graded into G1, G2 & G3 and were divided according to the mucin expression into group 1 (Mucinous NE-tumors) and group 2 (other variants that did not express mucin). Each group included 10 cases.

Immunohistochemistry was applied to paraffin sections for investigating CgA & NSE expression as well as estrogen and progesterone receptors (ER & PR) status. Group 1 tumors fell within lower grades than those of group 2. Moreover, ER & PR positivity rates were 75% & 65% respectively. Also, ER expression was not significantly correlated with NE phenotype (P = 0.14) or with grade (P = 0.24) while PR expression was significantly higher in group 1 than in group 2 (P = 0.02) and in G1, G2 than G3 (P = 0.04) Both steroid receptors co-expression was significantly higher in group 1 than in group 2 tumors (P = 0.05) and found in G1 and G2 but not in G3 tumors. Finally, ER and PR were coexpressed, although fewer & sporadic, in all control biopsies (100%) compared to its presence only in 45% of the studied NE tumors.

The study concluded that NE-breast tumors, producing high level of NE markers were specific entity and those coexpressing ER & PR might possess a multidirectional differentiation. Moreover, it may appear quite necessary to define estrogen & progesterone receptors status in NE breast carcinomas so as to decide, whether or not to try hormone therapy as adjuvant treatment modality in these tumors.

Key words: Immunohistochemistry, Estrogen Receptors, Progesterone Receptors, Neuroendocrine, Breast Carcinoma

Introduction:

Breast carcinomas are a multifaceted entity that may show various types of differentiation. In breast carcinomas of usual type, focal neuroendocrine and apocrine differentiations are occasionally detected in scattered cells by the immun-ocytochemical expression of NE and apocrine markers[1-3]. In addition, scattered hormone-producing cells have been descrybed in two cases of apocrine breast carcinoma[4].

The term "neuroendocrine" was applied to a heterogenous series of breast carcinomas while in other non-endocrine organs, such as lung[5] and prostate[6], this term defined tumors uniformly and homogenously positive for NE markers and correlated to a specific clinical evolution[7]. As a consequence, the NE-differentiated breast carcinomas were confined to those with a relevant percent-age ($\geq$ 50%) of cells expressing NE-markers[8]. Moreover, all the existing studies on the clinical evolution of NE-breast carcinomas regarded them as a single entity[1,3,7,8]. Small cell carcinomas

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are considered as undifferentiated variety of the NE-breast carcinomas [9].

A relatively common phenomenon of diagnostic interest in NE breast carcinomas, is the presence of a divergent differentiation which indicates the ability of a tumor to produce both NE and non-NE substances such as glycoproteins. Mucin production is generally indeed a common feature in NE-breast carcinomas and has been correlated to a low aggressiveness of tumors[10].

Normally, the ER is expressed in less than 20% of the luminal epithelial and myoepithelial cells as well as being detectable in fibroblasts and other stromal cells[11]. Also, the PR is expressed in less than 20% of luminal epithelial cells but not elsewhere in the human breast[12]. All cells normally expressing the PR have been found to contain also the ER, however, these steroid receptor-expressing cells are separate from, but often adjacent to, those labelled with markers of proliferation[13].

The sex steroid hormones are assumed normally to control the proliferative activity of the luminal epithelial cells indirectly in a mechanism that the receptor-containing cells act as sensors which secrete positive or negative paracrine and /or juxtacrine growth factors, according to the prevailing estradiol/progesterone concentrations, to influence the activity of nearly division-competent cells. This would attenuate the sensitivity of the mammary epithelium to steroid hormones such that proliferation will occur only when a sufficient concentration of positive growth factors has accumulated[12].

Since the breast is one of the target tissues of sex steroid hormones and since the presence of ER & PR within a tissue generally considered a prerequisite for this tissue to manifest long term response to hormonal therapy [7], this study evaluates the steroid receptor levels in NE-breast carcinomas and investigates the influences of the histologic grade as well as ER & PR status on the biological evolution of such tumors.

Material and Methods:

The material of this work consisted of a sum of twenty NE-breast carcinoma cases selected from a series of two hundreds retrospective breast carcinoma biopsies obtained from Al-Azhar University Hospitals during the peroid (2000-2003). The NE-tumors were graded using the Elston and Ellis grading system[14], which considered the nuclear pleomorphism, the number of mitoses and the formation of tubules within the tumor. In addition, ten biopsies of normal non-neoplastic breast tissues were taken as control. Expression of CgA and NSE as well as estrogen and progesterone receptors (ER & PR) status were investigated in all studied cases.

Formalin-fixed, paraffin-embedded blocks were sectioned at 5μ thickness. One section was stained with Hematoxylin and Eosin, for routine histopathologic study. Additional four sections were used for immunohistochemical (IHC) study to investigate the expression of NE-markers (namely CgA & NSE) as well as ER & PR immunoreactivity. Mouse monoclonal primary antibodies for CgA (LVRB 9003 R1- Dako), ER (A 589-Dako), ER (1D5-N 1595-Dako) and PR (1A6-N 1595-Dako) were applied, each one on a section. Antigen retrieval was done by microwave heating in citrate solution (Biogenex-Neufahrn, Germany). Secondary antimouse antibodies using peroxidase labelled biotin streptavidin complex detection system (Dako, Copenhagen, Denmark) for CgA, NSE, ER and PR were applied. Counterstaining was performed using Mayer's Hematoxylin (Biogenex, Cat, No. 94583). The age as well as other clinicopathologic data were obtained from the patients' files.

Positive reaction for CgA & NSE appeared as brownish cytoplasmic granular staining of the tumors cells while positive reaction for ER & PR appeared as brownish nuclear staining of these cells. For evaluation of IHC results, the following limits were used in consideration of positive reaction: $\geq$ 50% for each of CgA & NSE, $\geq$ 20% for each of ER & PR. The intensity of nuclear staining for ER & PR were scored as: 0(-) negative, 1(+): Weakly positive (presence of few weakly stained positive cells), 2(++): Moderately positive (numerous positive cells and occasionally positive cell clusters with moderate intensity) & 3(+++)

Strongly positive (multiple clusters or diffuse intense positive staining).

Statistical Analysis:

The association of ER and PR with the histological type and grade were analyzed using a X2 test. All data were analyzed for statistical significance by the Pearson's Chi-square tests. A *P* value $\leq$ 0.05 was considered a statistically significant, $\leq$ 0.01 was a highly significant and $\leq$ 0.001 was a very highly significant one.

Results:

This study examined 20 NE-breast carcinoma cases showing immunoreactivity for CgA (Fig. 1) & NSE (Fig. 2) in 50-75% of its tumor cells, appearing as brownish cytoplasmic immunostaining. They were divided morphologically into two groups; group 1: Mucinous carcinomas (10/20-50%) showing a variable amount of extracellular mucin ranged from small pools (6 cases, Fig. 3) to large lakes (4 cases) and group 2: Other NE-variants which showed no mucin secretion (10/20 – 50%) including solid cohesive (4 cases), alveolar (3 cases, Fig.4) & poorly differentiated (3 cases) (Table 1). In addition, 10 normal breast tissue biopsies were used as control. The patient's age ranged from 52 into 72 years (mean 62.3 years). Regarding the Elston and Ellis grading system[14], the NE-tumors showed no tubular formation and their histologic grade was significantly influenced by the number of mitoses (P = 0.0039) that ranged from 4/10 high power fields (HPF) in well differentiated (G1) to 25/10 HPF in poorly differentiated (G3) tumors. In the intermediate differentiated (G2) tumors, the mitotic index was 10/10 HPF. Accordingly, six (6/20-30%) were G1, ten (10/20-50%) were G2 and four cases (4/20-20%) were G3. The patients with poorly differentiated tumors didn't have non-mammary carcinomas. Also, the small cell variety, considered as undiffer-

entiated, was excluded in the present study. All group 1 cases fell within G1 and G2 while groups 2 cases fell within G2 and G3 (Table 2).

Positive immunoreactivity for ER & PR appeared as brownish nuclear staining in 45-60% of the malignant cells. Positive ER immunoreactivity appeared in fifteen out of the NE- carcinomas cases (15/20-75%) including eight group 1 cases (8/10-80%), five of which were G1 & three were G2 (Fig. 5) as well as seven group 2 cases (7/10-70%), three of which were solid cohesive (G2), two were alveolar (G2) (Fig. 6) and two were poorly differentiated (G3) variant. Among the fifteen ER positive NE tumors; six were strongly positive (3 +), four were moderately positive (2 +) and five were weakly positive (1+) (Table 3).

Positive PR immunoreactivity appeared in thirteen out of the NE-cases (13/20-65%) including eight group 1 cases (8/10-80%), five of which were G1 & three were G2 in addition to five group 2 cases (5/10-50%), two of which were solid cohesive (G2), two were alveolar (G2, Fig. 7) and one was poorly differentiated (G3) variant. Among the thirteen PR positive NE-tumors, six were strongly positive (3+), six were moderately positive (2+) and one was weakly positive (1+) (Table 4).

ER+/PR+ phenotype was noticed in nine cases (9/20-45%); seven of which were group 1 including four G1 & three G2 cases and two were group 2 including one solid cohesive (G2) & one alveolar (G2) case. None of the G3 NE tumors in the present study expressed ER+/PR+ phenotype (Table 5).

All control cases showed few insignificant CgA-or NSE-positive cells and also revealed disregarded, sporadic ER-& PR- positive cells.

Table (1): Histotypes of the studied NE-breast carcinomas

His to type	No.	%
Group 1: Mucinous	10	50
Group 2: Others		
▪ Solid cohesive	4	20
▪ Alveolar	3	15
▪ Poorly differentiated	3	15
Total	**20**	**100**

Table (2): Histologic types & grades of the studied NE breast carcinomas (n = 20):

Histo type	Grades (G)		
	G1	G2	G3
Group 1: Mucinous	6	4	0
Group 2: Others:			
Solid cohesive	0	3	1
Alveolar	0	3	0
Poorly differentiated	0	0	3
Total	**6 (30%)**	**10 (50%)**	**4 (20%)**

Table (3): ER expression in the studied NE-breast carcinomas   (n = 20):

Histotype and Grade (G)	ER-positive cases (No; = 15/20 – 75%)				
	No	%	Weak (1+)	Mod. (2+)	Strong (3+)
Group 1: Mucinous (n =10):					
G1	5/6	83.3	1	1	3
G2	3/4	75	1	1	1
Total	**8/10**	**80**	**2**	**2**	**4**
Group 2: Others (n =10):					
Solid cohesive (G2)	3/4	75	1	1	1
Alveolar (G2)	2/3	66.7	0	1	1
Poorly differentiated (G3)	2/3	66.7	2	0	0
Total	**7/10**	**70**	**3**	**2**	**2**

Mod. = Moderate;   ER = Estrogen Receptors;
No; = Number of immunoreactive tumors / examined tumors.

**Table (4): PR expression in the studied NE breast carcinomas (n = 20):**

Histotype and Grade (G)	PR - positive cases (No; = 13/20 – 65%)				
	No	%	Weak (1+)	Mod. (2+)	Strong (3+)
Group 1: Mucinous (n =10);					
G1	5/6	83.3	0	3	2
G2	3/4	75	0	2	1
Total	**8/10**	**80**	**0**	**5**	**3**
Group 2: Others (n =10):					
Solid cohesive (G2)	2/4	50	0	1	1
Alveolar (G2)	2/3	66.7	0	0	2
Poorly differentiated (G3)	1/3	33.3	1	0	0
Total	**5/10**	**50**	**1**	**1**	**3**

PR = Progesterone Receptors;   Mod = Moderate;
No; = Number of immunoreactive tumors / examined tumors.

**Table (5): ER+ /PR+ phenotype in the studied NE-breast carcinomas (n = 20)**

| Histotype	ER+/PR+ Phenotype (No; 9/20-45%)	
	No;	%
Group 1: Mucinous		
G1	4	40
G2	3	30
Total	**7**	**70**
Group 2: Others (n = 10)		
Solid cohesive (G2)	1	10
Alveolar (G2)	1	10
Total	**2**	**20**

ER+/PR+ phenotype = Estrogen and progesterone positive coexpression;
No; = Number of immunoreactive tumors /examined tumors.
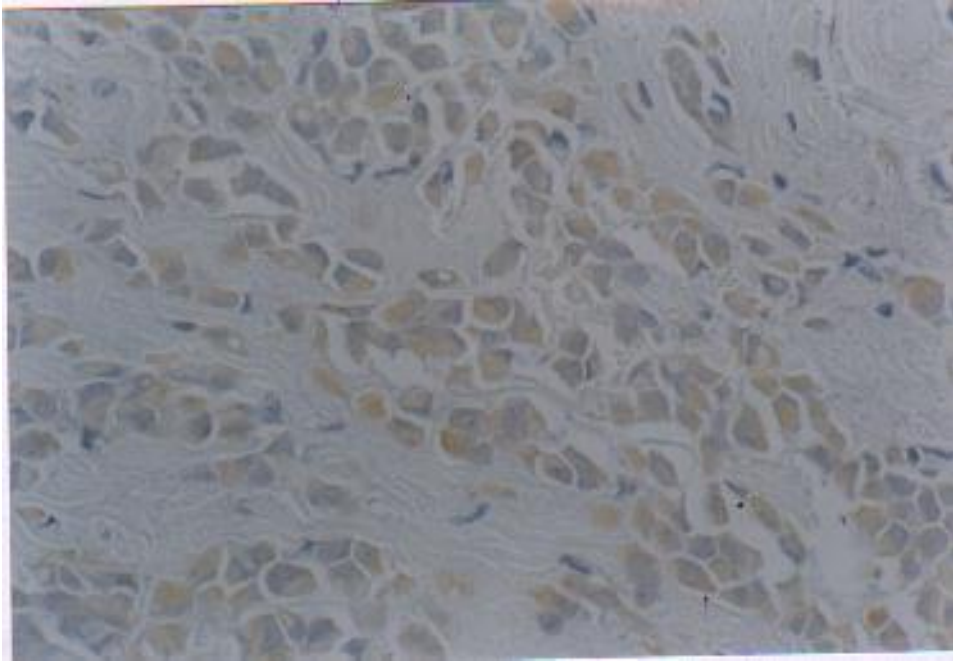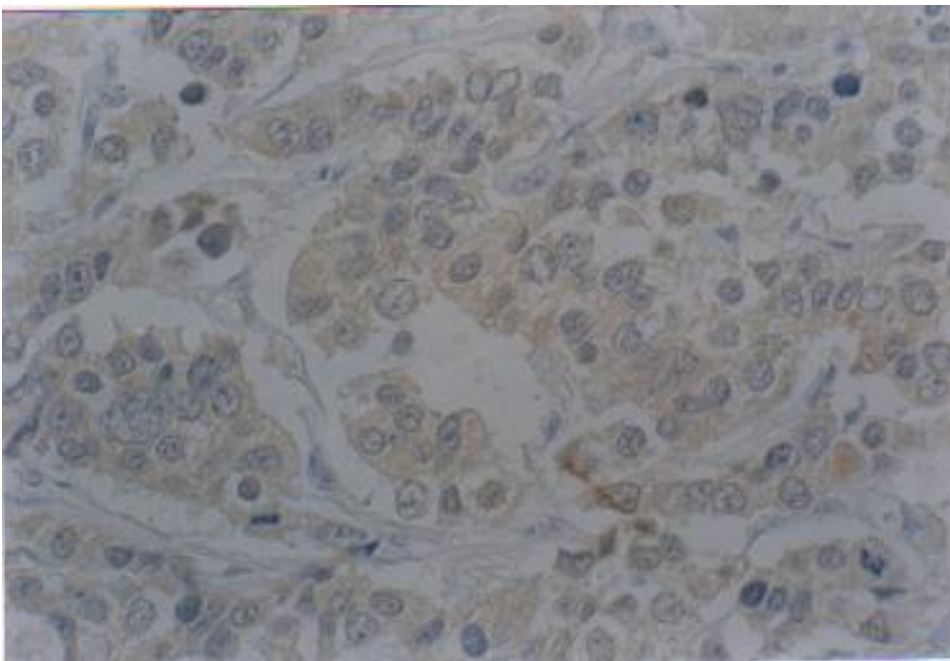
Fig. (1): Alveolar NE-breast carcinoma, grade 2, showing CgA-positive tumor cells
(brownish granular cytoplasm)   (Immunoperoxidase [IP] X 400).
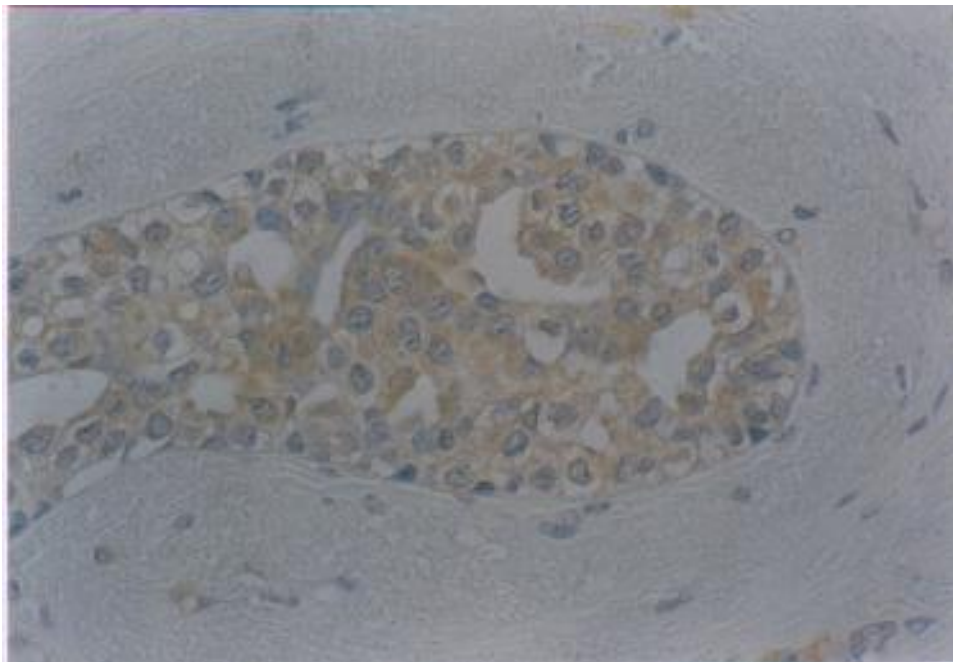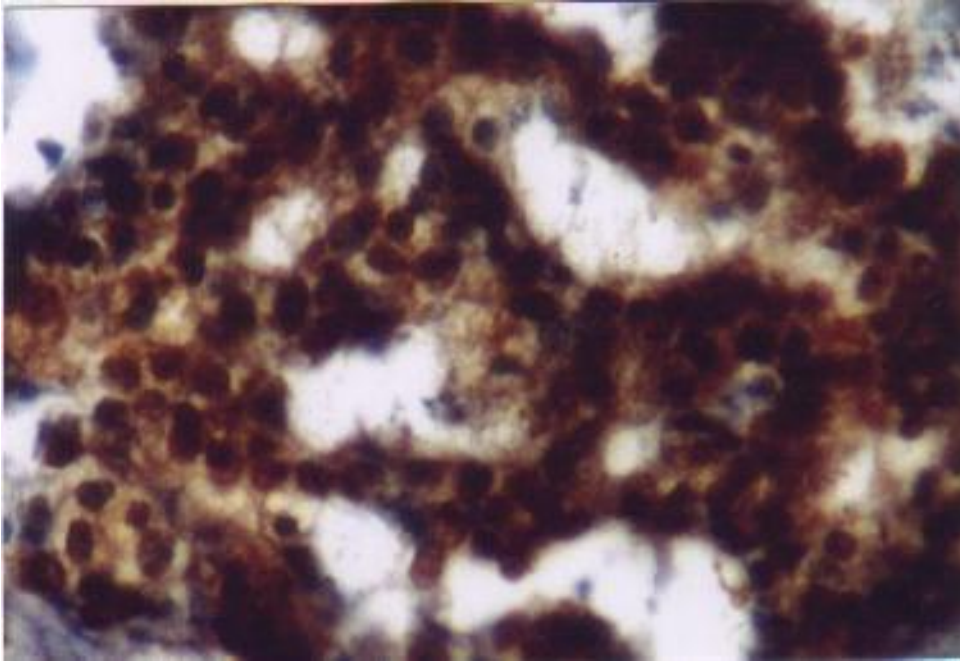

Fig. (2): Mucinous NE-breast carcinoma, grade 2, showing NSE-positive neoplastic cells
(brownish granular cytoplasm)   (IP X 200).
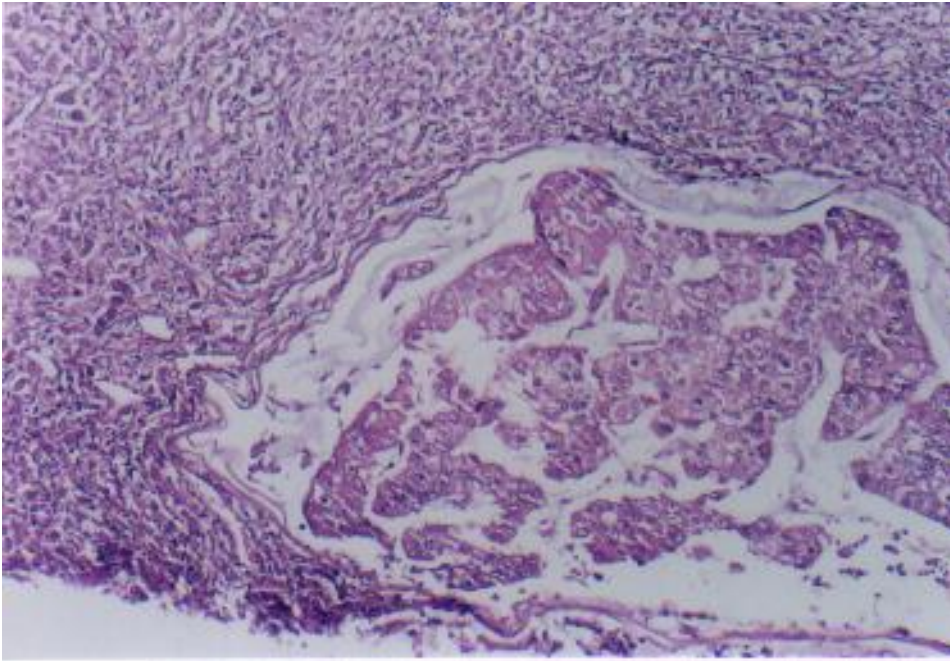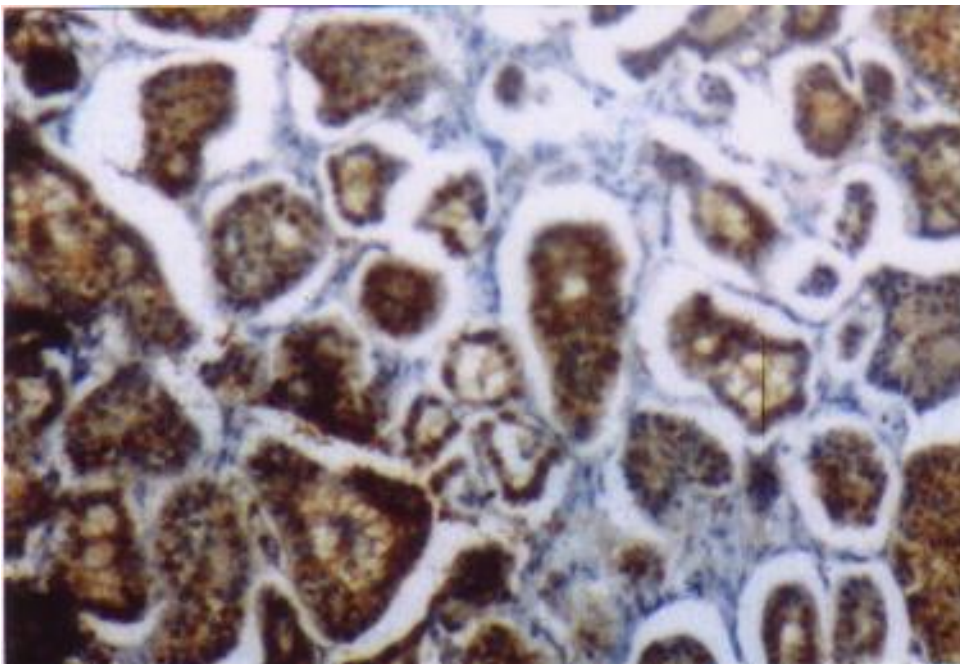
Fig. (3): Mucinous NE-breast carcinoma, grade 2, showing a cribriform sheet of malignant cells floating in a pool of mucin   (H x E x 100)
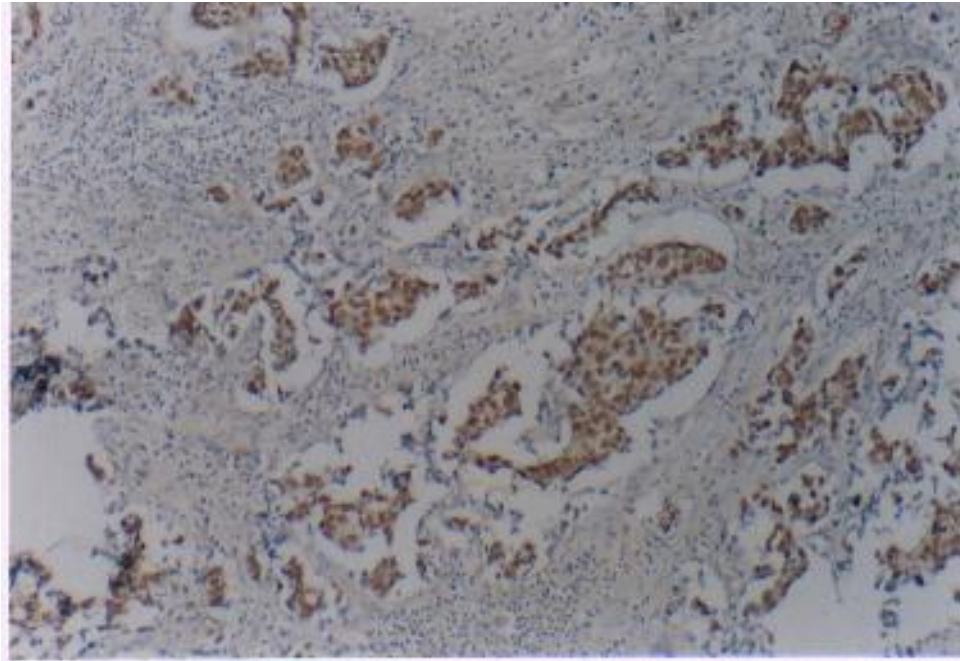

Fig. (4): Alveolar NE-breast carcinoma, grade 2, showing NSE - positive tumor cells
(IP X 400).

Fig. (5): Mucinous NE-breast carcinoma, grade 2, strongly positive for ER immunoreactivity.
(IP X 400).

Fig. (6): Alveolar NE- breast carcinoma, grade 2, strongly positive for ER immunoreactivity
(IP X 100).

Fig. (7): Alveolar NE-breast carcinoma, grade 2, strongly positive for PR immunoreactivity
(IP X 100).

Discussion and Conclusion:

Neuroendocrine (NE) differentiation in breast carcinomas has been considered as a peculiar feature of minimal clinical significance [10].

The immunohistochemical technique was applied in this study to demonstrate the NE-differentiation in breast carcinomas as the identification by Hematoxylin and Eosin alone was highly subjective and unreliable [3,15].

In this work, the tumors showed immunoreactivity for CgA and NSE in 50-75% of its cells coinciding with Sapino et al.,[8] who stated that the NE-breast carcinomas are only defined when expressing NE-markers in, at least, 50% of their cells. A clinical significance of such definition as reported by Giovanella et al.,[16] relies on the fact that a marked elevation of serum CgA has been detected in patients affected by small (< 2cm) but intensely immunoreactive NE-tumors.

Our study showed that the NE-phenotype of tumors was correlated with grades as the mucinous NE-tumors has lower grades than other NE-variants. This finding agreed with those of Sapino et al.,[7] who mentioned that the mucinous differentiation was a favourable prognostic factor and the patients with mucinous NE-

carcinomas has a significantly longer survival time than did patients with tumors of the other histologic types (P = 0.05). They, as well, stated that the histological grading was another important prognostic parameter in NE-breast carcinomas (P = 0.007) as all G1 cases, with a low proliferative activity, survived at more than 10-years of follow-up while poorly differentiated (G3) NE-tumors, with a high proliferative activity, were very aggressive.

Also, the study showed ER & PR positivity rates of 75% & 65% respectively. These figures were close to those stated by other investigators who found that ER & PR positivity rates were 76.1% & 64.4% respectively[7] and 74.8% & 65.3% respectively[10]. Such variability in the rates may be explained by the difference in methods applied.

In the present study, ER expression was not significantly correlated with NE-phenotype (P = 0.13) or with grade (P = 0.24) while PR expression was significantly higher in group 1 than in group 2 cases (P = 0.02) and in lower grades (G1 & G2) than in grade 3 tumors (P = 0.04). Moreover, ER & PR coexpression was significantly found more in group 1 than in group 2 cases (P = 0.05) and in lower

grades (G1 & G2) but not in grade 3 tumors (P = 0.05). These data were mimicing those mentioned by Sapino et al.,[10], who, as well, significantly correlated the tumor divergent differentiation with the clinical follow-up. Such multidirectional capacity, detected in the other organs[6.17], is maintained in case of NE-breast carcinomas by cells of well differentiated tumors and being greatly minimized by the poorly differentiated cells. In addition, they found that one of the most important parameters for a favourable prognosis was the ER expression (P = 0.001), being highly correlated with a long overall survival, followed by PR expression (P = 0.02).

Finally, ER & PR coexpressed only in 45% of the studied NE-tumors compared to their coexpression in all control cases (100%), although in the latter, the ER+/PR+ immunoreactive cells were sporadic and smaller in number. This finding was approximated that reported by Clarke et al.,[12] and Birsak et al.,[18] suggesting that the tumor cells escape the normal growth control by sex steroid hormones.

In conclusions, the present NE cases coexpressing ER & PR may be a good example of multidirectional differentiation of the breast neoplasms since this differentiation with the production of NE- and exocrine protein is rather a frequent phenomenon in tumors of endocrine organs [6, 17,19].

In addition, our results indicate that NE-breast carcinomas, recognized as tumors producing high level of NE-markers, are specific entity and demonstrate that the pathologists should specify the histologic grade as well as the ER & PR expression, which had been established to be important parameters in the evolution of these tumors. Moreover, since the patients with NE-tumors containing both the ER and the PR had the greatest probability of responding to endocrine therapy and had a better prognosis than those having tumors without these steroid receptors[20], the presence of these receptors and their responsiveness to hormonal treatment might be a guide for therapeutic measures. Further extensive studies are recommended together with attempts at correlation with survival, before trying hormonal therapy as adjuvant treatment modality in NE-breast carcinoma patients with high receptor levels.

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إظهار مستقبلات الاستروجين والبروجستيرون بالكيمياء النسيجية المناعية فى سرطانات الثدى العصبية الصماء

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تعرف سرطانات الثدى العصبية الصماء بإظهارها الواسع للدلالات العصبية الصماء كما أنها قد تظهر أيضا مواد اخرى غير عصبية صماء "التمييز المتشتت" وقد تم دراسة 20 حالة من هذه السرطانات اختيرت من نحو 200 حالة من سرطانات الثدى المختلفة بالإضافة الى 10 عينات ضابطة من نسيج الثدى الطبيعى وقد قسمت الحالات السرطانية فى هذا البحث الى ثلاث درجات حسب مقياس اليستون وإليس وهى الدرجة الأولى "عالية التمييز" والدرجة الثانية "متوسطة التمييز" والدرجة الثالثة "ضعيفة التمييز" كما قسمت هذه الحالات الى مجموعتين حسب نسبة افراز الميوسين . وقد تم دراسة اظهار الدلالات العصبية الصماء (NSE, CgA   ) مع مستقبلات الاستروجين والبروجستيرون (PR, ER) ووجد الأتى:

وجود حالات المجموعة الأولى فى درجة أقل من نظيرتها فى المجموعة الثانية.

كانت نسب إظهار مستقبلات الاستروجين والبروجستيرون 75% و 65% على الترتيب.

وجد أن إظهار مستقبلات الاستروجين لا ترتبط بنوع او درجة الورم بينما وجد أن مستقبلات البروجستيرون أعلى فى المجموعة الأولى منه فى الثانية .

كان إظهار تلك المستقبلات معاً أعلى فى المجموعة الأولى عنه فى المجموعة الثانية وكذلك أعلى فى الدرجة الأولى والثانية دون الثالثة .

أخيرا كان إظهار تلك المستقبلات معاً فى جميع العينات الضابطة (100%) -وإن كانت من خلايا الإيجابية قليلة ومتناثرة- بالمقارنة بإظهارها فقد فى 45% من الأورام العصبية الصماء.

ونستنتج من هذه الدراسة أن أورام الثدى العصبية الصماء هى نوعية خاصة فالأورام التى تظهر مستقبلات الاستروجين والبروجستيرون معاً قد تمتلك تمييز فى عدة اتجاهات ولذلك فمن الضرورى تحديد تلك المستقبلات فى هذه الأورام كى نحاول بدء العلاج الهرمونى.