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Original Research Article

Assessment of neuroendocrine markers in different molecular subtypes of invasive breast carcinoma and its impact on prognosis

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Abstract

Neuroendocrine differentiation has been detected in many histologic types of breast carcinoma, including invasive ductal, lobular, colloid, or papillary carcinoma and even insitu carcinoma. This study detected the immunohistochemical expression of neuroendocrine markers in invasive breast carcinoma molecular subtypes and its impact on prognosis. Also their relation to other clinicopathological factors was also analyzed. 242 cases of invasive breast carcinoma were assembled retrospectively from Mansoura University, Faculty of Medicine, Oncology Center, Egypt between 2010 and 2012. IHC FOR ER, PR, Her2neu, ki67 were done for molecular subtyping. Synaptophysin, chromogranin and CD56 were stained and recorded then assessment of the relationship between them and different clinicopathological parameters was done. Chromogranin A and synaptophysin, positivity were statistically associated with invasive breast carcinoma molecular subtypes (P value 0.007, 0.01 respectively) while CD56 was not (p value 0.9). Chromogranin A positivity showed significant association with tumor grade, histological subtype, molecular subtype, patient outcome death, and recurrence or metastasis (P values of 0.04, 0.00, 0.007, 0.005, 0.009 respectively). Synaptophysin positivity showed significant association with tumor grade, molecular subtype, patient outcome (death, and recurrence or metastasis)(P values of 0.04, 0.01, 0.01, 0.002 respectively). Neuroendocrine differentiation is more commonly associated with luminal B subtype which usually has poorer prognosis, higher tumor grades and better chemotherapeutic response than luminal A. More studies are required to understand the relationship of luminal B subtype and neuroendocrine differentiation.

Keywords: Neuroendocrine markers, molecular subtypes, breast carcinoma, prognosis

INTRODUCTION

Primary neuroendocrine tumors usually are not found in the breast because breast lacks endocrine cells. This fact confirms the hypothesis that origin of neuroendocrine breast carcinomas is not preexisting endocrine cells, but are mainly due to process of differentiation within breast carcinoma (Maluf and Koerner, 1994; Wachter et al., 2014).

Lakhani et al. (2012) and Banu et al. (2015) reported that WHO 2003 included a subtype of invasive mammary carcinomas with neuroendocrine features. The current

WHO defines them as carcinomas exhibit expression of chromogranin and/or synaptophysin except for small cell carcinoma which is diagnosed mainly by morphology but show weak specific neuroendocrine markers expression and more frequent expression of NSE.

The WHO classification of breast tumor divides carcinomas of the breast with neuroendocrine features into well differentiated neuroendocrine tumor, poorly differentiated neuroendocrine tumor/small cell carcinoma, and invasive breast carcinoma with neuroendocrine differentiation (Rosen and Gattuso, 2017).

Neuroendocrine differentiation may be usually seen more frequent in invasive carcinoma (NOS) and certain low grade subtypes as cellular invasive mucinous carcinoma and solid papillary carcinoma (Lakhani et al., 2012; Banu et al., 2015; Rosen and Gattuso, 2017). In a study conducted by Wei et al had shown that carcinomas with neuroendocrine differentiation had a more worse prognosis than carcinoma of no histological special type (Wei et al., 2010).

Wachter et al concluded that some poorly differentiated luminal B carcinomas may show neuroendocrine differentiation (Banu et al., 2015) low-grade carcinomas as invasive mucinous carcinomas included in the luminal A molecular subgroup (Weigelt et al., 2009) and solid papillary carcinoma which mostly represent an intraductal carcinoma with possible invasive component (Nassar et al., 2006), these tumors usually have a very good prognosis. Otherwise small cell carcinoma of the breast is a high-grade neuroendocrine carcinoma, but less aggressive than small cell carcinoma of the lung (Shin et al., 2001). There is confusion about the prognosis or therapeutic options of invasive breast cancer with neuroendocrine differentiation.

The key for neuroendocrine breast carcinoma diagnosis depends usually on histopathological morphology and immunohistochemical markers (Wachter et al., 2014). Microscopic features of neuroendocrine differentiation include the arrangement of tumor cells in nest and solid structures. Tumor cells are usually more or less uniform and nuclei are round to oval, vesicular with stippled chromatin and granular eosinophilic cytoplasm. Neuroendocrine differentiation is considered when at least 50% of tumor cells with moderate to strong immunohistochemical expression of chromogranin A or synaptophysin (Wachter et al., 2014; Banu et al., 2015; Adegbola et al., 2005).

Chromogranin and synaptophysin are the most specific marker of neuroendocrine differentiation. CD56 and NSE can be more sensitive but less specific and used only for screening not for definite diagnosis of neuroendocrine differentiation (Dabbs, 2014).

On clinical point of view, the importance of neuroendocrine differentiation in invasive breast carcinoma is not clear, as some studies declared that no prognostic value for its identification (Sawaki et al., 2010). Others showed that it is associated with better prognosis (Righi et al., 2010; Rovera et al., 2008) on the contrary to another study indicated that it was associated with a worse prognosis (Kwon et al., 2014). This vague prognostic impact can be explained by the heterogenous nature of the tumor that fall under the category of neuroendocrine differentiation of breast cancer as these tumors include both low grade slowly progressing special subtypes and aggressive high grade carcinomas (Tan et al., 2015). In addition, metastatic breast carcinomas with diffuse neuroendocrine differentiation were associated with high grade morphology but also estrogen receptor positive (Shin et al., 2000).

The main purpose of the study is to detect neuroendocrine expression in different breast carcinoma molecular subtypes using immunohistochemical markers, then test the prognostic value of neuroendocrine expression in breast carcinoma.

MATERIALS AND METHODS

Patient selection: 242 cases of invasive breast carcinoma were assembled retrospectively from Mansoura University, Faculty of Medicine and Oncology Center, Egypt between 2010 and 2012. Those cases underwent modified radical mastectomy operations and received postoperative hormonal, chemotherapy, or radiotherapy. Postoperative follow-up for those cases and data were obtained until 2015. Median follow-up period was 28.1 months with ± 17.4 SD. Follow-up was performed within arrange of 2–68 months. Approval of the ethics committee of Mansoura University was obtained.

Tumor blocks which were formalin fixed then paraffin wax-embedded collected and cut for Hematoxylin and eosin-stained slides. Revision of diagnosis was done according to the WHO classification 2012(17). A total of 215 (88.8%) cases were invasive ductal carcinoma (NOS). 24 (9.9%) cases were invasive lobular carcinoma. 3 cases (1.2%) were mucinous carcinoma. Nottingham Grading System was a guide for tumor grading (Elston and Ellis, 1991). A manual tissue microarray (TMA) was performed using a mechanical pencil tip (Shebl et al., 2011; Soliman and Yussif, 2016).

Immunohistochemistry (IHC)

The TMA blocks were recut at 3–4 µm thick on coated slides, deparaffinized, and rehydrated in descending grades of alcohol into water. Antigen retrieval by citrate buffer at pH suitable for primary antibody and by microwave heating for 10 min. Next, incubation of sections in 3% H2O2 blocking medium for 5 min, followed by washing with distilled water, then incubation for 60 min at room temperature with mouse monoclonal primary antibodies against the following antigens: ER (1D5, 1:50; pH, 7.3; Dako, San Jose, USA), PR (PR 636,

1:50; pH, 7.3; Dako, San Jose, USA), HER2/neu (CB11, 1:50; pH, 7.3; Novocastra, Newcastle, U.K), and cell marque Ki-67 (sp6) rabbit monoclonal antibody (REF275R-18). Chromogranin (68-75kDa; ph 6.0; NeoMarkers for Lab Vision Corporation). Synaptophysin (Ab-2 clone SYP02; ph6.0; NeoMarkers for Lab Vision Corporation). Prediluted CD56 ((MRQ-42); rabbit monoclonal antibody; ph7.3-7.7; Rocklin, CA95677 USA). Immunodetection was performed using Dako RealTM En Vision TM system, peroxidase/DAB+, Rabbit/Mouse for 3 min (Code: K5007, Dako, Glostrup, Denmark). Slides were stained with hematoxylin for 1 min. check for accuracy through positive internal controls for ER and PR in normal epithelial cells of breast ducts. Positive external controls from breast carcinomas positive for ER, PR, and HER2/neu were used. Negative controls were also prepared by PBS instead of primary antibody.

IHC evaluation

ASCO/CAP guidelines recommended that ER and PR are positive when at least 1% of the tumor cells showed nuclear staining (Deyarmin et al., 2013). HER2/neu was scored as follows: 0, no staining or faint incomplete membranous staining in < 10% of cells; 1, faint incomplete membranous staining in > 10% of cells; 2, weak to moderate complete membranous staining in > 10% of cells; and 3, strong complete membranous staining in > 10% of cells. Only score 3 was considered positive (Varadharajan et al., 2015). Cut off value of ki67 was 14% (Soliman and Yussif, 2016). Molecular subtyping of cases was determined through evaluation of ER, PR, HER2/neu and ki67 by IHC.

Assessment of positivity for chromogranin, synaptophysin and CD56 depend on detection of at least 50% of tumor cells show moderate to strong expression of chromogranin A, synaptophysin and CD56 was considered as indicative of neuroendocrine differentiation (Wachter et al., 2014; Banu et al., 2015; Adegbola et al., 2005).

Statistical analysis

Data of all cases were arranged, coded, and analyzed using SPSS version 20 (IBM). Descriptive statistics was presented as mean±standard deviation and frequency (number-percent). Chi square test (χ2-value) was used for intergroup comparison of categorical data. Kaplan-Meier test was used to determine the equality of survival distribution among breast carcinoma with or without neuroendocrine features. The IHC expression of neuroendocrine markers was correlated with clinical and histopathological parameters of breast carcinoma, including age, size of tumor, histological type, grade of tumor, lymph node status, and patient outcome.

RESULTS

The study included 242 patients with infilterating breast carcinomas. The mean age of the patients was 55 ±12 year, from 31 to 88 years. The clinicopathological data of cases are shown in Table 1.

Table 1 shows that among the 242 cases, 215 cases were IDC NOS (88.8%), 24 cases were invasive lobular carcinoma (ILC) (9.9%) cases, and only 3 cases were mucinous carcinoma (1.2%). Approximately 25.6%, 46.7%, 27.7% of the cases were grade 1, 2 and 3 respectively. And 21% of the cases displayed tumor size of more than 2 cm. Approximately 76% of the patients exhibited pathologically positive lymph nodes, and 58.3%, 38.8%, 2.9% of the cases were in stage III, II, and I respectively. Additionally, 28.8% of the cases developed distant metastasis and recurrence, and 22% of the cases were dead. As for ER+, PR+, and HER2+ (score, 3+) cases were 61.6%, 57.4%, and 20.7% of cases respectively. Chromogranin, synaptophysin, and CD56 positivity were detected in 15.3%, 9%, and 9.5% of the cases respectively (Figure 1). According to this immunophenotyping, the cases used in this study were classified as luminal A, luminal B, HER2, and triple-negative in 43.8%, 21.9%, 12%, and 21.9% of the cases respectively.

Table 2 shows the immunohistochemical results of specific (chromogranin A and synaptophysin) and non-specific (CD56) neuroendocrine markers in cases in correlation with molecular subtypes: Chromogranin A, synaptophysin, positivity were significantly associated with the molecular subtypes (P value 0.007, 0.01 respectively) while CD56 was not (p value 0.9). In luminal A carcinoma: chromogranin A was negative in 96/ 106 cases (91%). 10 cases (4 diffuse, 5 focal) were positive (9%). 8 of them were IDC NOS and 2 cases were mucinous. Synaptophysin was negative in 100 / 106 cases (94.3%). 6 cases (4 diffuse, 2 focal) were positive. All of them were IDC NOS. CD56 was negative in 96 cases out of 106 cases (91.6%). 10 cases were focally positive (9.4%). 9 of them were IDC NOS and one case was mucinous carcinoma.

In luminal B carcinoma: chromogranin A was negative in 37 / 53 cases (70%). 16 cases (11 diffuse &5 focal) were positive (9%). 9 of them were IDC NOS and 7 cases were ILC. Synaptophysin was negative in 43 / 53 cases (81%). 10 cases were positive (5 diffuse, 5 focal). 4 of them were IDC NOS and 2 cases were ILC. CD56 was negative in 48 / 53 cases (90.6%).5 cases were positive (diffuse) (9.4%). All of them were IDC NOS.

In HER2 enriched carcinoma: chromogranin A was negative in 25 / 29 cases (86%). 4 cases were focally expressed (14%). All of them were IDC NOS. Synaptophysin was negative in all 29 cases (100%). CD56 was negative in 27 / 29 cases (93.1%). 2 cases were focally positive (6.9%). All of them were IDC NOS.

In Triple –ve carcinoma: chromogranin A was negative

Table 1. The Clinicopathological features of the studied cases

Tumor characteristic		**N**	**%**
Tumor grade	**G1**	**62**	**25.6%**
	G2	113	46.7%
	G3	67	27.7%
Tumor size	≤ 2cm	191	78.9%
	>2cm	51	21.1%
Lymph node	N	58	24%
	P	184	76%
Tumor stage	Stage I	7	2.9%
	Stage II	94	38.8%
	Stage III	141	58.3%
Live or dead	Live	175	72.3%
	dead	50	22.2%
Metastasis or recurrence	N	163	71.2%
	P	66	28.8%
Histological type	IDC	215	88.8%
	ILC	24	9.9%
	MUCINOUS	3	1.2%
chromogranin	N	205	84.7%
	P	37	15.3%
Synaptophysin	N	220	91%
	P	22	9%
CD56	N	219	90.5%
	P	23	9.5%
ER	N	93	38.4%
	P	149	61.6%
PR	N	103	42.6%
	P	139	57.4%
Her 2	N	192	79.3%
	P	50	20.7%
Molecular type	Her2	29	12.0%
	Luminal A	106	43.8%
	Luminal B	53	21.9%
	triple –ve	54	21.9%

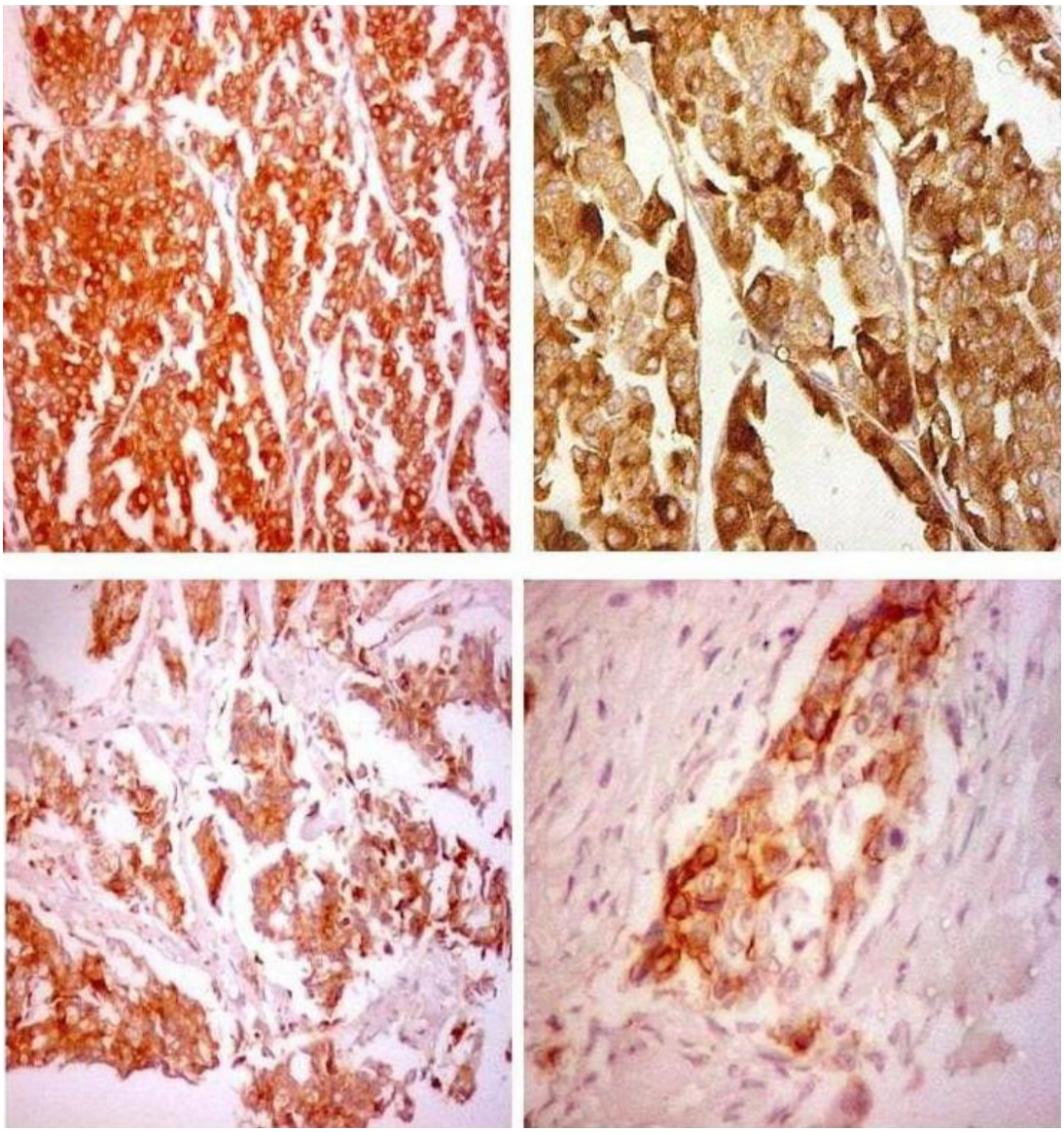

Figure 1. A case of infilterating duct carcinoma show diffuse positivity for synaptophysin in tumor cells x200 (top left). The same case also show diffuse positivity for chromograninx200 (top right). Another case of infiltrating duct carcinoma show positive reaction for CD56 in >50% of tumor cells x200 (bottom left). Another case of infiltrating duct carcinoma show focal positivity for CD56 x400 (bottom right).

Table 2. Expression of specific (chromogranin A and synaptophysin) and non-specific (CD56) neuroendocrine markers in different molecular subtypes of breast carcinoma

		Chromogranin		synaptophysin		CD56	
		-ve	+ve	-ve	+ve	-ve	+ve
Luminal A	n	96	10	100	6	96	10
	%	91%	9%	94.3%	5.7%	91.6%	9.4%
Luminal B	N	37	16	43	10	48	5
	%	70%	30 %	81%	19%	91.6%	9.4%
Her 2	N	25	4	29	0	27	2
	%	86%	14%	100%	0%	93.1%	6.9%
Triple –ve	N	47	7	48	6	48	6
	%	87%	13%	89%	11%	88.9%	11.1%
p		0.007		0.01		0.9	

Table 3. The relationship of chromogranin expression with the clinicopathological, molecular subtypes, immunohistochemical characteristics and patient survival of breast carcinoma

		Chromogranin A				P	
		-ve		+ve			
Age	< 55	34	64%	14	36%	0.6	
	> 55	37	69%	17	31%		
Tumor Grade	G1	57	92%	5	8%	0.04	*
	G2	97	85%	16	14%		
	G3	51	76%	16	23%		
Tumor Size	≤ 2	162	85%	29	15%	0.5	
	> 2	43	84%	8	16%		
Lymph node	N	49	85%	9	15%	0.5	
	P	156	85%	28	15%		
Stage	I	7	100%	0	0%	0.3	
	II	82	87%	12	13%		
	III	116	82%	25	18%		
Histological Type	IDC	187	87%	28	13%	0.00	*
	ILC	18	75%	6	25%		
	Mucinous	0	0%	3	100%		
ER	N	81	87%	12	13%	0.2	
	P	124	83%	25	17%		
PR	N	89	86%	14	14%	0.3	
	P	116	83.5%	23	16.5%		
Her2	N	163	85%	29	15%	0.5	
	P	42	84%	8	16%		
Molecular subtypes	Luminal A	96	91%	10	9%	0.007	*
	Luminal B	37	70%	16	30%		
	HER2	25	86%	4	14%		
	Triple -ve	47	87%	7	13%		
Live or dead	live	153	87%	22	13%	0.005	*
	dead	35	70%	15	30%		
Recurence& Metastasis	no	144	88%	19	12%	0.009	*
	yes	49	74%	17	26%		

in 47 / 54 cases (87%). 7 cases (2 diffuse, 5 focal) were positive (13%). 4 of them were IDC NOS, 2 cases were ILC and one case was mucinous. Synaptophysin was negative in 48 / 54 cases (89%). 6 cases (4 focal, 2 diffuse) were positive (6.9%). 5 of them were IDC NOS and one case was mucinous. CD56 was negative in 48/54 cases (88.9%). 6 cases (2 diffuse, 4 focal) were positive (11.1%). 5 of them were IDC NOS and one case was ILC.

Table 3 demonstrates the relationship of chromogranin A with the clinicopathological data, molecular subtypes, immunohistochemical characteristics of breast carcinoma: Chromogranin A positivity was significantly associated with tumor grade, histological subtype, molecular subtype, patient outcome death, and recurrence or metastasis (P values of 0.04, 0.00, 0.007, 0.005, 0.009 respectively). Chromogranin A positivity was present in 8%, 14%, 23% of G1, G2, and G3 carcinoma respectively. Chromogranin A positivity was present in 13%, 25% and 100 % of IDC, ILC, and mucinous carcinoma respectively. Chromogranin A positivity was present in 9%, 30%, 14%, 13% of Luminal A, Luminal B, Her2, and Triple -ve carcinoma respectively. As for overall survival chromogranin A positivity was present in 13% and 30% of live and dead patients respectively. Chromogranin A positivity was present in 26% of cases complicated with recurrent carcinoma.

Table 4 demonstrates the relationship of synaptophysin expression with the clinicopathological data, molecular subtypes, immunohistochemical characteristics of breast carcinoma cases: Synaptophysin positivity was significantly associated with tumor grade, molecular subtype, patient outcome (death, and recurrence or metastasis) (P values of 0.04, 0.01, 0.01, 0.002 respectively). Synaptophysin positivity was present in 2%, 11% and 13% of G1, G2, and G3 carcinoma respectively. Synaptophysin positivity was present in 6%, 19%, 0%, 11% of Luminal A, Luminal B, Her2, and Triple -ve carcinoma subtype respectively. Synaptophysin positivity was present in 7% and 20% of live and dead patient respectively. Synaptophysin positivity was present in 20% of cases complicated by recurrent

Table 4. The relationship of synaptophysin expression with the clinicopathological characteristics, molecular subtypes, immunohistochemical characteristics and patient survival of breast carcinoma.

		Synaptophysin				P	
		-ve		+ve			
Age	< 55	121	94.5%	7	5.5%	0.03	
	> 55	99	87%	15	13%		
Tumor Grade	G1	61	98%	1	2%	0.04	*
	G2	101	89%	12	11%		
	G3	58	87%	9	13%		
Tumor Size	≤ 2	175	92%	16	8%	0.3	
	> 2	45	88%	6	12%		
Lymph node	N	54	93%	4	7%	0.3	
	P	166	90%	18	10%		
Stage	I	7	100%	0	0%	0.1	
	II	89	95%	5	5%		
	III	124	88%	17	12%		
Histological Type	IDC	196	91%	19	9%	0.3	
	ILC	22	92%	2	8%		
	Mucinous	2	67%	1	33%		
ER	N	86	92.5%	7	7.5%	0.3	
	P	134	90%	15	10%		
PR	N	94	91%	9	9%	0.5	
	P	126	91%	13	9%		
Her2	N	170	88.5%	22	11.5%	0.0 5	
	P	50	100%	0	0%		
Molecular subtypes	Luminal A	100	94%	6	6%	0.01	*
	Luminal B	43	81%	10	19%		
	HER2	29	100%	0	0%		
	Triple -ve	48	89%	6	11%		
Live or dead	live	163	93%	12	7%	0.01	*
	dead	40	80%	10	20%		
Recurence& Metastasis	No	154	94.5%	9	5.5%	0.002	*
	Yes	53	80%	13	20%		

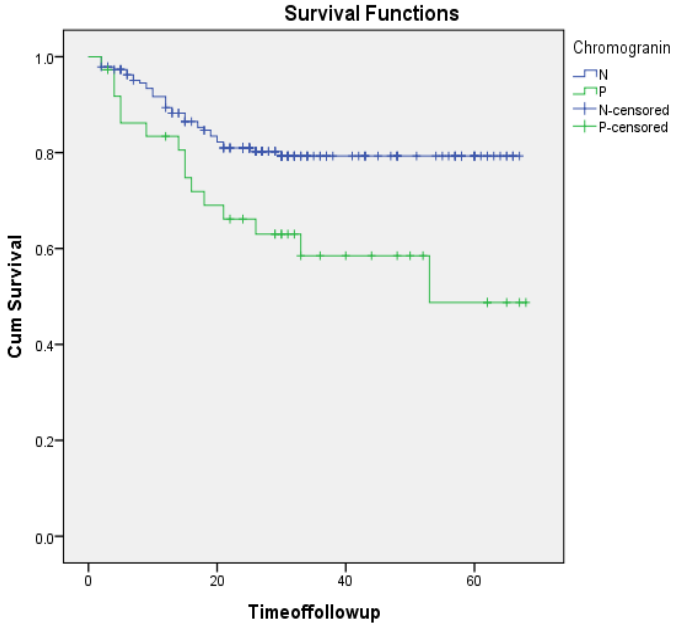

Figure 2. Survival curves of breast cancer patients. Patients with Negative chromogranin have better OS than those with positive one (P =0.006)

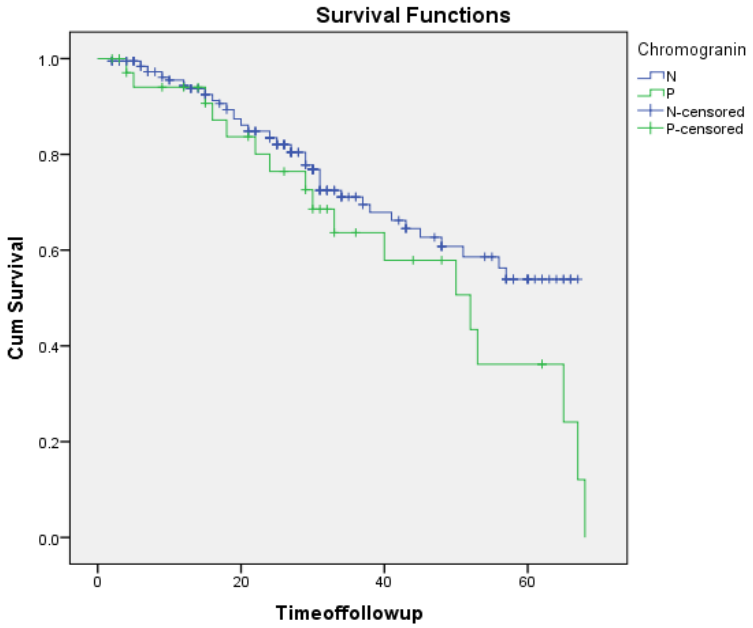

Figure 3. Disease free survival of breast cancer patients. Patients with Negative chromogranin are have a better or a greater disease-free survival (P =0.015)

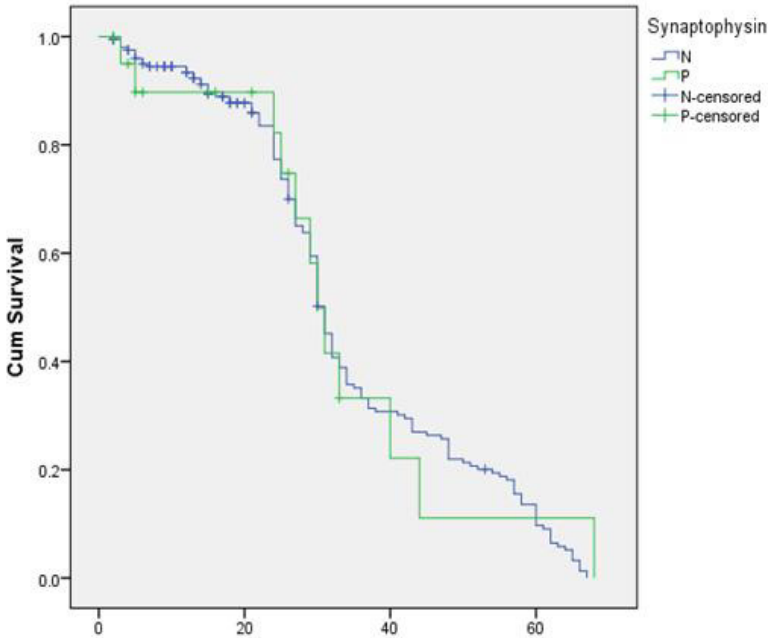

Figure 4. Survival curves of breast cancer patients. Synaptophysin is not significantly correlated with the overall survival of the patient (P =0.3)

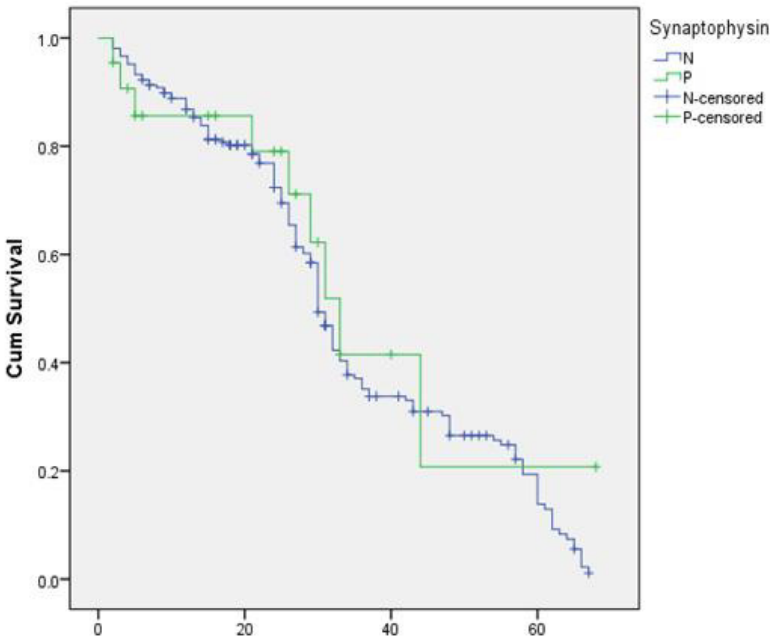

Figure 5. Disease free survival of breast cancer patients. Synaptophysin is not significantly correlated with the disease free survival of the patient (P =0.6)

carcinoma. Correlation of CD56 positivity with different clinicopathological parameters, immunohistochemical results and patient survival revealed no statistically significant association with any of these different parameters

DISCUSSION

The actual incidence of neuroendocrine carcinoma is not accurate because neuroendocrine markers are not usually stained in breast carcinoma. Prognosis of neuroendocrine carcinoma of the breast is variable from good to bad prognosis (Inno et al., 2016). The presence of some of neuroendocrine features was detected in low-grade histological special types as mucinous and invasive papillary carcinomas, that are usually included in the category of luminal A group which has a good prognosis and retain the estrogen receptor (Weigelt et al., 2009). This study is directed to detect neuroendocrine expression in different breast carcinomas molecular subtypes by using specific and nonspecific neuroendocrine markers. Then test the importance of neuroendocrine expression in relation to breast cancer molecular subtypes of 242 breast cancer cases. Also in our study we focus on the relationship of neuroendocrine expression and clinicopathological characteristics of breast carcinoma.

In the present study we depend on histopatho-logical features of neuroendocrine differentiation in addition to immunohistochemical markers. Histopatho-logical features of neuroendocrine differentiation include arrangement of tumor cells in nest and solid structures which are groups of uniform cells with round to oval vesicular nuclei, stippled chromatin and granular eosinophilic cytoplasm.

Diagnosis of neuroendocrine differentiation is confirmed when at least 50% of tumor cells are showing moderate to strong cytoplasmic staining for chromogranin A or synaptophysin (Wachter et al., 2014; Banu et al., 2015; Adegbola et al., 2005).

In this study we found a significant association of neuroendocrine expression of breast carcinoma by specific neuroendocrine markers (chromogranin and synaptophysin) and the molecular subtypes of breast carcinoma (P value 0.007, 0.01 respectively), however non specific neuroendocrine marker as CD56 was not significantly associated with the molecular subtypes of breast carcinoma (P value 0.9). Chromogranin positivity was found in 9% of luminal A subtype, 30% of luminal B subtype and 14% of HER2 enriched type. These results were in accordance with Wachter et al. (2014). Neuroendocrine expression was significantly associated with high grade breast carcinoma and this result was similar to that study done by Wachter et al. In our study, Mucinous carcinomas and ILC had the greatest association with neuroendocrine differentiation. These results confirmed that documented by Lakhani et al.2012, Khamar et al. 2015, and Varadharajan et al. 2015. Neuroendocrine differentiation was significantly correlated with tumor grade, it was present in 8% of grade 1 and 23% of Grade 3 as mentioned in other

studies that cocluded association of neuroendocrine differentiation with higher tumor grade (Wang et al., 2014). Neuroendocrine expression tested by chromogranin was significantly associated with more recurrence and metastasis. These results run in parallel to what documented in Kwon et al. (2015). Another study also concluded its association with poorer clinical outcome when compared with invasive ductal carcinoma (NOS) (Zhang et al., 2013; Tian et al., 2011). On the contrary previous studies declare that there is no difference in prognosis between either subtypes (Makretsov et al., 2003; Sapino et al., 2001).

CONCLUSION

Since the diagnosis of neuroendocrine differentiation is confirmed when at least 50% of tumor cells are showing moderate to strong cytoplasmic staining for chromogranin A or synaptophysin. In addition, neuroendocrine markers are not usually done for diagnosis and immunohistologic subtyping of cancer breast. These two factors lead to the rarity of the diagnosed cases. That's why prognosis is still unknown because number of reported cases is limited. So, further complementary studies are helpful to assess the importance of routine testing for neuroendocrine differentiation in breast carcinomas. Neuroendocrine differentiation is more commonly associated with luminal B subtype that is usually have poorer prognosis, higher tumor grades and better response to chemotherapy than luminal A. Additional studies are required to explain the relationship of luminal B subtype and neuroendocrine differentiation.

Conflict of interest statement

No potential conflicts of interest are disclosed.

REFERENCES

Adegbola T, Connolly CE, Mortimer G (2005). Small cell neuroendocrine carcinoma of the breast: a report of three cases and review of the literature. *J Clin Pathol, 58*(7), 775-778.

Banu, Khamar Jaha, Ballal C, Rajesh, Pai, Narendra, & Permi, Harish (2015). Primary Neuroendocrine Carcinoma of Breast. *Indian Journal of Surgical Oncology, 6*(2), 110-112. doi: 10.1007/s13193-014-0370-z

Dabbs DJ (2014). Diagnostic immunohistochemistry: theranostic and genomic applications. 4th ed. Philadelphia, PA: Elsevier Saunders,; 264.

Deyarmin B, Kane JL, Valente AL, van Laar R, Gallagher C, Shriver CD, Ellsworth RE (2013). Effect of ASCO/CAP guidelines for determining ER status on molecular subtype. *Ann Surg Oncol, 20*(1), 87-93.

Elston CW, Ellis IO (1991). Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology, 19*(5), 403-410.

Inno A, Bogina G, Turazza M, Bortesi L, Duranti S, Massocco A, Gori S (2016). Neuroendocrine Carcinoma of the Breast: Current Evidence and Future Perspectives. *Oncologist, 21*(1), 28-32.

Kwon SY, Bae YK, Gu MJ, Choi JE, Kang SH, Lee SJ, Park JY (2014). Neuroendocrine differentiation correlates with hormone receptor expression and decreased survival in patients with invasive breast carcinoma. *Histopathology, 64*(5), 647-659.

Lakhani SR, Ellis I, Schnitt S, Tan PH, van de Vijver MJ (2012). WHO Classification of Tumors of the Breast. International Agency For Research on Cancer, IARC Press, Lyon, France

Makretsov, Nikita, Gilks, C. Blake, Coldman, Andrew J., Hayes, Malcolm, & Huntsman, David (2003). Tissue microarray analysis of neuroendocrine differentiation and its prognostic significance in breast cancer. *Human Pathology, 34*(10), 1001-1008.

Maluf HM, Koerner FC (1994). Carcinomas of the breast with endocrine differentiation: a review. *Virchows Archiv, 425*(5), 449-457. doi: 10.1007/bf00197547

Nassar, Hind, Qureshi, Hina, VolkanAdsay, N., & Visscher, Daniel. (2006). Clinicopathologic Analysis of Solid Papillary Carcinoma of the Breast and Associated Invasive Carcinomas. *The Ame. J. Surg. Pathol. 30*(4), 501-507.

Righi, L., Sapino, A., Marchio, C., Papotti, M., & Bussolati, G. (2010). Neuroendocrine differentiation in breast cancer: established facts and unresolved problems. *Semin Diagn Pathol, 27*(1), 69-76.

Rosen LE, P. Gattuso (2017). Neuroendocrine Tumors of the Breast. Arch Pathol Lab Med, 141(11): p. 1577-1581.

Rovera, Francesca, Masciocchi, Patrizia, Coglitore, Alessandra, La Rosa, Stefano, Dionigi, Gianlorenzo, Marelli, Marina,. . Dionigi, Renzo (2008). Neuroendocrine carcinomas of the breast. *Int. J. Surg. 6*, S113-S115.

Sapino A, Papotti, M., Righi, L., Cassoni, P., Chiusa, L., & Bussolati, G. (2001). Clinical significance of neuroendocrine carcinoma of the breast. *Annals of Oncology, 12*(suppl_2), S115-S1

Sawaki, Masataka, Yokoi, Kazuki, Nagasaka, Tetsuro, Watanabe, Reiko, Kagawa, Chikara, Takada, Hideki, Nakao, Akimasa. (2010). Prognostic importance of neuroendocrine differentiation in Japanese breast cancer patients. *Surgery Today, 40*(9), 831-835.

Shebl AM, Zalata KR, Amin MM, El-Hawary AK (2011). An inexpensive method of small paraffin tissue microarrays using mechanical pencil tips. *Diagn Pathol, 6*, 117.

Shin SJ, DeLellis RA, Ying L, Rosen PP (2000). Small cell carcinoma of the breast: a clinicopathologic and immunohistochemical study of nine patients. Am J Surg Pathol, 24(9), 1231-1238.

Shin, Sandra J., DeLellis, Ronald A., & Rosen, Paul Peter. (2001). Small Cell Carcinoma of the Breast—Additional Immunohistochemical Studies. *The Ame. J. Surg. Pathol. 25*(6), 831-832.

Soliman NA, Yussif SM (2016). Ki-67 as a prognostic marker according to breast cancer molecular subtype. *Cancer Biology & Medicine, 13*(4), 496-504.

Tan PH, Schnitt SJ, van de Vijver, M. J., Ellis, I. O., & Lakhani, S. R. (2015). Papillary and neuroendocrine breast lesions: the WHO stance. *Histopathology, 66*(6), 761-770.

Tian, Zhen, Wei, Bing, Tang, Feng, Wei, Wei, Gilcrease, Michael Z., Huo, Lei, Wu, Yun (2011). Prognostic significance of tumor grading and staging in mammary carcinomas with neuroendocrine differentiation. *Human Pathology, 42*(8), 1169-1177.

Varadharajan, Eswari, Priya, Shanmuga, Prakash, Geetha, Mugundan, Archana, & Easwaramurthi, Praveen. (2015). Mucinous Carcinoma of the Breast with Neuroendocrine Differentiation. *Iran. J. Pathol. 10*(3), 231-236.

Wachter DL, Hartmann A, Beckmann MW, Fasching PA, Hein AB, Christian M, Agaimy A (2014). Expression of Neuroendocrine Markers in Different Molecular Subtypes of Breast Carcinoma. *BioMed Research International, 2014*, 9.

Wang, Jun, Wei, Bing, Albarracin, Constance T., Hu, Jianhua, Abraham, Susan C., & Wu, Yun. (2014). Invasive neuroendocrine carcinoma of the breast: a population-based study from the surveillance, epidemiology and end results (SEER) database. *BMC Cancer, 14*(1), 147.

Wei, Bing, Ding, Tian, Xing, Yan, Wei, Wei, Tian, Zhen, Tang, Feng, Wu, Yun. (2010). Invasive neuroendocrine carcinoma of the breast. *Cancer, 116*(19), 4463-4473. doi: 10.1002/cncr.25352

Weigelt, Britta, Geyer, Felipe C., Horlings, Hugo M., Kreike, Bas, Halfwerk, Hans, & Reis-Filho, Jorge S. (2009). Mucinous and neuroendocrine breast carcinomas are transcriptionally distinct from invasive ductal carcinomas of no special type. *Modern Pathology, 22*, 1401. doi: 10.1038/modpathol.2009.112

Zhang, Y., Chen, Z., Bao, Y., Du, Z., Li, Q., Zhao, Y., & Tang, F. (2013). Invasive neuroendocrine carcinoma of the breast: a prognostic research of 107 Chinese patients. Neoplasma, 60(2), 215-222.