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γ-amino butyric acid (GABA) level as an overall survival risk factor in breast cancer

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Abstract

Introduction and objective. The γ-amino butyric acid (GABA) plays important role in the proliferation and migration of cancer cells. The aim of the study was to evaluate the level of GABA in breast cancer, in relation to clinical and epidemiological data.

Materials and method. The study was conducted on 89 patients with breast cancer in stage I-II. GABA level was assessed using spectrofluorometric method in tumour homogenates. Immunoexpression of E-cadherin was evaluated histologically on paraffin fixed specimens. Overall and disease-free survival was assessed for a 15-year interval period.

Results. Median overall survival was significantly longer (127.2 months) in patients with a high level of GABA (>89.3 μg/1), compared with a group with a low level of the amino acid (106.4 months). Disease-free survival was insignificantly different – 99 and 109 months, respectively. A significantly longer overall survival (131.2 months) was seen among patients with a high level of GABA and positive E-cadherin immunoexpression, compared with a group characterized by a low level of GABA and lack of E-cadherin immunorectivity (98.1 months). The co-existence of negative immunoexpression of E-cadherin and low GABA concentration resulted in a six-fold increase in the risk of death (HR=6.03).

Conclusion. GABA has a significant prognostic value in breast cancer. Co-existence of a low level of GABA and loss of E-cadherin immune-expression seems to be a new, independent, and negative prognostic marker of the neoplasm.

Key words

breast cancer, E-cadherin, GABA/ γ -amino butyric acid/, prognostic factors, prognosis

INTRODUCTION

Breast cancer is the most common cancer among women worldwide. Although in Poland an average incidence is observed, the scale of the problem is still enormous. This is proved by the fact that each year there are more than 12,000 registered new cases. Despite sophisticated treatments, including chemotherapy, hormone therapy, radiation therapy or surgery, more than 5,000 women die each year from breast cancer. Optimal treatment of malignances is based on evaluation of the risk of cancer progression. Unfortunately, despite early detection of cancer and application of modern treatment, progression of the neoplastic disease occurs even in some patients with good prognosis. Prognostic factors applied in everyday practice are still inefficient for selecting the group with an early breast cancer with the worst prognosis [1,2]. For this reason, various new biological and epidemiological factors have been evaluated. One of the most interesting and promising markers is γ-aminobutyric acid (GABA), which is the major inhibitory neurotransmitter in the central nervous system. It is synthesized primarily from glutamate by glutamate decarboxylase (GAD 65 and GAD 67) and acts on ionotropic (GABA-A or GABA-C) and metabotropic (GABA-B) receptors. GABA and its receptors are mostly expressed in various brain structures, as well as in many peripheral organs such as pancreas, kidney, intestine, prostate, testis, ovary and liver, where they are responsible for neuronal stimulation and hormonal secretion. Generally, GABAergic signaling is altered in cancer cells. It was proved that GABA and its receptors are often increased and up-regulated in many types of cancers, involving various internal organs, e.g. pancreatic, gastric and breast cancer [3–5].

Recent studies using human cancer cell lines, animal models, and human tissue have suggested a close correlation between the GABAergic system and tumour development [6, 7]. Generally, most studies confirm that activation of GABA receptors suppress tumour cell proliferation [8–11] and inhibits migration [8, 9, 12, 13]. These data suggests that the GABAergic system plays a significant role in the pathology of cells, and it cannot be ruled out that GABA is a significant factor affecting the survival time of patients with breast cancer.

The aim of this study was to evaluate the level of GABA in relation to clinical and epidemiological data in patients with breast cancer, and confirm its relationship with 15 years survival.

MATERIALS AND METHOD

89 Caucasian women radically treated for breast cancer in 1995–1996 were selected. According to the national law, no written permission was obtained from the patients since personal data was not presented and analyzed throughout the study and in the final report. All patients underwent radical mastectomy. In 83 patients, adjuvant treatment was applied.

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30 patients were treated with chemotherapy according to the CMF scheme (cyclophosphamide, methotrexate and fluorouracil 5FU) and 6 according to the FAC scheme (cyclophosphamide, doxorubicin and fluorouracil 5FU). After chemotherapy, 16 of those patients were additionally treated with antiestrogens. 47 patients underwent only hormonotherapy with tamoxifen. Adjuvant radiotherapy was applied in 15 patients. Follow-up lasted from 6 months to 15 years. Complete characteristics of the examined population is presented in Table 1. Biological subtypes in the group of 74 cases of invasive breast carcinoma of no special type (ductal and lobular types), based on occurrence of estrogen receptor (ER), progesterone receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER2), is presented in Table 2.

Table 1. Characteristic of examined population

Factors	No. of patients
Age (Mena ± SD, years)	58.1 ±12.5
Menopausal status	
Pre-menopausal	15
Post-menopausal	74
Histopathological subtype	
ductal cancer	66
lobular cancer	8
other	15[a]
Histological grade	
G1	10
G2	52
G3	27
Tumour size	
T1	10
T2	72
T3	7
Lymph node status	
positive	35
negative	54
Estrogen receptor status (ER)	
positive	68
negative	21
Progesterone receptor status (PR)	
positive	51
negative	38
E-cadherin status	
positive >70%	67
negative <70%	22
HER-2 overexpression	
positive	18
negative	71
GABA	
< (89.3 μg/l)	45
> (89.3 μg/l)	44
Ki 67	
<14%	52
>14%	37

a – other histological types: mucinous 6, tubular 4, medullary 5

GABA concentration in the tumour was measured, as previously reported [11], using the spectrofluorometric method by Lowe et al. [14], with Sutton et al. [15] modification. Cut-off concentration value for GABA was established at 89.3 μg/1. Such a value was the 95th percentile of GABA concentration in normal breast tissue removed during excision of benign lesions of the breast, as described previously [16]. GABA level below or above 89.3 μg/1 was considered as low and high, respectively.

Table 2. Incidence of low and high GABA concentration in relation to biological subtypes in the group of 74 cases of invasive breast carcinoma of no special type (ductal and lobular types), based on occurrence of ER, PR and HER2 and Ki 67

	No. of patients	% of patients	Low GABA	High GABA
Luminal A (HER2-, ER+, PR+, Ki-67<14%)	23	31	13	10
Luminal B (HER2-, ER+, PR+, Ki-67>14%)	26	35	9	17
Luminal B (HER2+ ER+, PR+, Ki-67 – any)	12	16	9	3
HER2 (HER2+, ER-, PR-, Ki-67 – any)	3	4	2	1
Triple negative (HER2-, ER-, PR-, Ki-67 – any)	10	14	4	6

Immunoexpression of E-cadherin was evaluated histologically on paraffin fixed specimens, as previously reported [17].

Statistical analysis. Distribution of examined variables was estimated with the D'Agostino-Pearson test. The correlation between the GABA concentration and the remaining risk factors was estimated with cross tables analysis. Prognostic value of determining the GABA content and the expression of E-cadherin in prediction of asymptomatic and overall survival time of patients with breast cancer was evaluated using the survival analysis method by Kaplan-Meier. The influence of GABA and E-cadherin values on overall survival time was estimated by the method of proportional hazard regression analysis by Cox. Gehan's generalized Wilcoxon test was used for comparison of survival between patients' group.

Disease-free survival time was estimated concerning the date of surgical procedure and date of breast cancer relapse. Overall survival time was calculated as the number of months passed since the surgery to the day of the patient's death. Overall follow-up time was 15 years.

The 0.05 level ($p<0.05$) of probability was used as the criterion of significance.

RESULTS

Overall survival (OS) was from 7–180 months; mean 102 months, median 119 months. In the course of 15 year follow-up, disease progression was observed in 32 patients. Local relapse occurred in 2 patients and distant metastasis in the other 30. 32 patients died within the study period. Disease-free survival (DFS) was from 1–180 months; mean 87 months and median 103 months.

Insignificant correlations between the GABA level and tumour size, lymph node metastatic spreading, tumour grading (G) and the expression of ER and HER2 receptors were revealed. However, a statistically significant relationship with PR receptor status was found (Tab. 3).

Significant differences between the GABA level in correspondence of biological subtypes was revealed only in Luminal A vs. Luminal B (HER2-) ($p = 0.028$) and in Luminal B (HER2-) vs. Luminal B (HER2+) ($p = 0.02$) groups (Tab. 2).

The median overall survival in patients with high GABA level was 127.2 months and was significantly longer ($p=0.048$) compared to the other patients, in whom it was 106.4 months. The difference in the probability of surviving 15 years between the patients with high and low GABA level was about 23%. The probability of 15-year overall survival in patients with

Table 3. Incidence of low and high GABA concentration in relation to selected molecular and clinical risk factors

Risk factors		GABA		p
		<89.3 µg/l	>89.3 µg/l	
Estrogen receptor status /ER/	Negative	11	10	NS
	Positive	31	37	
Progesterone receptor status /PR/	Negative	15	23	<0.05
	Positive	31	20	
HER-2 overexpression	Negative	45	26	NS
	positive	10	8	
E-cadherin status	negative	10	12	NS
	positive	38	29	
Lymph node status	negative	29	25	NS
	positive	15	20	
Histological grade	1+2	27	35	NS
	3	15	12	
Ki 67	<14%	23	29	<0.05
	>14%	16	21	
Histopathological subtype	Ductal cancer	29	37	NS
	Lobular cancer	5	3	
	other	7	8	
Tumour size	≤ 2	4	6	NS
	2–5	44	33	
	>5	3	4	

Table 4. Hazard ratio for E-cadherin and GABA values in the examined population

		Hazard ratio	p
E-cadherin/GABA	positive/high	-	-
	positive/low	3.12	0,0930
	negative/high	3.72	0,1506
	negative/low	6.03	0.01

low GABA was 60%, and among patients with high GABA was 83%.

The disease-free survival time did not differ significantly in relation to the GABA level. However, it was slightly longer in patients with lower GABA level (median, Me=109.2 months), compared to the other patients (Me = 99 months).

Analyzing the overall survival of particular patient subgroups in relation to GABA level and the studied prognostic factors, a difference in the group of patients with varied E-cadherin expression and GABA level was found. Insignificant and comparable differences of overall survival and disease free survival were observed.

The longest survival time was revealed in patients with a high level of GABA and positive E-cadherin expression (Me = 131.2 months). The shortest survival time occurred in patients with a low level of GABA and loss of E-cadherin expression (Me = 98.1 months). The difference in 15 years survival probability between the two groups was about 38%. Patients with either positive E-cadherin expression and low GABA level or lack of E-cadherin expression and high GABA level had a median overall survival time of 116.2 or 119.3 months, respectively.

DISCUUSSION

Most studies have concentrated only the mechanism of GABA activity and expression of GABA receptors in the neoplastic process [3–13]. No direct data were available regarding survival time of breast cancer patients depending on GABA level. Unlike previously published data, the current study demonstrates considerable differences in the overall and disease-free survival time correlated with the GABA level in neoplastic tumor. However, the authors' preliminary report indicated that 10-year survival depends on the GABA level [16]. Additionally, the most currently observed significant correlations were confirmed among patients with different E-cadherin immunoexpression. Observations by the authors prove a significant role of GABA in the development of breast cancer, and proves it potential role as a new prognostic factor.

The presence of GABA in neoplastic cells and its large concentration differences in respective patients, with values both below and above the established norm, was revealed. However, it should be stressed that the GABA level did not correlate with any of the studied clinical (i.e., tumour size, lymph nodes metastatic spreading) and histological (immunoexpression of ER, HER2) classical prognostic factors. However, significant correlation was found only between GABA level and biological subtypes, as well as expression of PR and proliferation factor Ki67. A similar relationship was described by Galiègue et al. [18] who revealed that increased immuneexpression of Ki67 was related to high expression of peripheral benzodiazepine receptor for GABA.

According to some studies, it is assumed that GABA may be a strong inhibitor of cell proliferation and migration [6,7]. This may explain the significantly shorter overall and disease-free survival time among current evaluated patients with a low level of GABA. It has been demonstrated that the activation of GABA B receptor by baclofen – agonist of peripheral benzodiazepine GABA – inhibit the proliferation of human pancreatic ductal adenocarcinoma (PDAC) cells PANC-1 and BXPC-3, human pulmonary adenocarcinoma (PAC) cells NCI-H322, immortalized human pancreatic duct epithelial cells HPDE6-C7, and small airway epithelial cells HPL1D [9, 10]. Moreover, Wang et al. [10] and Opolski at al. [11] described how hepatocarcinoma cells Bel-7402 and Huh-7 and mammary cancer 16/C in mice were also inhibited by baclofen. It has also been proved that inhibition of cancer cell migration of, respectively, human colon carcinoma cells SW480 and breast carcinoma cells MDA-MB-468 was diminished by the drug [12, 13].

The reason for the low level of GABA, specific for shorter survival of breast cancer patients, may be caused by differences in oxygenation in the tumour parenchyma. It is well known that GABA is decomposed by GABA-T transaminase operating mainly in oxygen conditions [19]. Augmented neoangiogenesis may be a factor causing increased oxygenation and transaminase activity, thus leading to the decrease in the GABA level. A secondary, reduced level of GABA may, in turn, lead to increased cell proliferation and migration and finally affect survival.

On the other hand, the current data facilitated the selection of patients dependent on prognosis. The best prognosis was observed in patients with high GABA level and positive E-cadherin immunoexpression, and the worst in patients with an opposite value of both factors. However, correlation between aberrant E-cadherin immunoexpression and higher

histological tumour grading, high risk of distant metastasis, as well as worse prognosis among breast cancer patients, have already been proved [20]. E-cadherin influence on the neoplastic cell migration and its prognostic value for overall survival and disease survival were also stressed [21]. Gould Rothberg and Bracken [22], based on meta-analysis, revealed that loss of E-cadherin immunoexpression may be an independent negative prognostic indicator for an infiltrating ductal breast carcinoma. It is also worth pointing out that both GABA and E-cadherin take part in the regulation of cellular migration and metastasis formation, which may partly explain the results of the current study.

Moreover, a relationship between the GABAergic system and cadherins has been demonstrated in some recent studies. Fiederlink et al. [23] indicate that E-cadherin signaling importantly contributes to the regulation of GABAergic synapses in cortical neurons. Also, Li et al. [27], in the analysis of protocadherins, which are generally involved in the pre- and postsynaptic contacts, described a relationship between them and GABAergic synapses. Both studies concerned nervous system physiology, but the mechanism of metastasis formation is a complex process, and despite the lack of similar data in literature the mutual influence of the two studied factors cannot be ruled out.

The presented results confirm that the decreased GABA level, as well as lower immunoexpression of E-cadherin, influence the disease progression and shorter survival time in breast cancer patients. Both factors influenced the survival time independently from the tumour size, the degree of malignancy and presence of metastasis in the axillary lymph nodes. The co-existence of both studied parameters representing different stages of carcinogenesis is especially important as it allows for the selection of breast cancer patients with bad prognosis.

The main limitation of presented study is the relatively low number of study subjects. Nevertheless, it is the first such prospective analysis in which the level of GABA was established in neoplastic tissue with 15 years follow-up. Due to the long observation time, patients were treated using various methods, including chemotherapy schemes that are rarely used today, such as CMF. However, as almost all patients were treated using the same protocol, it can be certain that the type of systemic treatment did not interfere with the prognostic value of GABA and E-cadherin. Furthermore, the GABAergic system was found to increase the therapeutic efficiency of some cytostatic drugs by reverse apoptosis resistance of cancer cells *in vitro* and *in vivo* [25].

CONCLUSIONS

The presence of GABA seems to be a useful prognostic factor in breast cancer. Patients with a high level of GABA were characterized with better prognosis concerning their disease-free survival and overall survival, compared to patients with a low GABA level. The difference in prognosis is especially visible in patients with various E-cadherin immunoexpression. The best prognosis occurs in patients with a high level of GABA and E-cadherin immunoreacivity, while the worst is in patients with a low level of GABA and loss of E-cadherin immonoexpression. Both parameters have prognostic value and may be used to select patients with an especially bad prognosis. However, the current observations have to be confirmed in a larger multicentre study, using a bigger and more representative population, before they can be incorporated into daily clinical practice.

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