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Concentration of serotonin in perimenopausal women with ductal breast cancer and polycystic ovary syndrome

S. Stanosz[1], J. von Mach Szczypiński[1], A. Fogel[2], E. Sowińska-Przepiera[3], J. Kościuszkiewicz[1], E. Żochowska[3], A. Syrenicz[3]

[1]Laboratory of Menopause and Andropause, Pomeranian Medical University, Szczecin
[2]Department of Biogenic Amines, Polish Academy of Sciences, Lodz
[3]Department of Endocrinology, Metabolic and Internal Diseases, Pomeranian Medical University, Szczecin (Poland)

Summary

Purpose of Investigation: To analyze a relationship between histological grade of ductal breast cancer (BC) and intratumor concentration of serotonin in women with polycystic ovarian syndrome (PCOS), and to study the long-term effects of innovative neurohormonal therapy in this group. *Materials and Methods:* The study included 65 women (28 premenopausal and 37 postmenopausal, age 43-69 years) with PCOS and BC. Aside from mastectomy, chemotherapy and menopausal status-specific hormonal treatment, all subjects received metformin, quinagolide, melatonin, and methysergide maleate. Serum concentrations of sex hormones were determined post-mastectomy and after five and ten years. *Results:* Intratumoral concentrations of serotonin in grade II and III BC were higher than in grade I tumors. After ten years, all subjects had normal concentrations of estrogens, prolactin, and serotonin, and showed no signs of BC recurrence. *Conclusion:* Intratumor level of serotonin may serve as a marker of BC aggressiveness. Neurohormonal therapy may prevent progression of BC.

Key words: Serotonin; Sex hormones; Breast cancer; Menopause; Polycystic ovary syndrome.

Introduction

Serotonin is an indole monoamine synthesized from tryptophan in enterochromaffin cells of the digestive tract, and further metabolized in the liver and lungs [1-3]. The majority of serotonin undergoes oxidative deamination by monoamine oxidases to 5-hydroxyindole acetaldehyde which is then oxidized by aldehyde dehydrogenase to 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophol, both excreted in urine in conjugation with glucuronic or sulfuric acid. Serotonin serves as a neurotransmitter and neurohormone in the central and peripheral nervous system [4-6], and participates in the regulation of sleep, blood pressure, body temperature, and hypothalamic-pituitary system function [7, 8]. However, serotonin has been also implicated in the pathogenesis of premenstrual syndrome [9] and polycystic ovary syndrome (PCOS) [10].

The aims of the study were: 1) to analyze a relationship between the histological grade of ductal carcinoma of the breast and the concentration of serotonin in cancer tissue in a group of women with PCOS, and 2) to analyze the long-term effects of an innovative neurohormonal therapy in this group of patients.

Materials and Methods

The study included a total of 65 women with breast cancer (BC) (43-69 years of age), subjected to mastectomy and chemotherapy at the Regional Comprehensive Cancer Center in Szczecin. Prior to BC detection, all the study participants were diagnosed with PCOS on the basis of biochemical, sonographic, and clinical findings. The patients were divided into two groups based on their gonadotropin and estrogen concentrations and clinical characteristics. Group 1 (mean age 42.1±5.2 years) included 28 premenopausal women, and group 2 (mean age 53.1±4.6 years) 37 postmenopausal women.

The vast majority of the study subjects (87%) lived in urban agglomerations. Tobacco smoking was declared by 39.6% of women from group 1 and 27.4% form group 2. Mean BMI was 25.4 ± 2.1 kg/m^2 for group 1 and 27.6 ± 4.1 kg/m^2 for group 2.

After mastectomy, combination pharmacotherapy according to Stanosz *et al.* [11] was continued for six months on an outpatient basis to normalize sex hormone and serotonin levels. Premenopausal women from group 1 received intravaginal micronized progesterone during the second phase of their menstrual cycle (50 mg/day for six days and then 100 mg/day for another six days). Postmenopausal women from group 2 were subjected to a modified transdermal hormone replacement therapy [12] with a transdermal 17-beta estradiol given for 22 days in increasing and decreasing doses to mimic a natural rhythm, along with micronized progesterone administered intravaginally

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Table 1. — *Baseline serum concentrations of gonadotropins, estrogens, progesterone, PRL, and serotonin in pre- and BC and PCOS.*

Group	Age (years)	Gonadotropins (U/L)		Estrogens (pg/mL)		Progesterone (ng/mL)	Prolactin (ng/mL)			Serotonin (nmol/L)
		FSH	LH	E1	E2		PRL1	PRL2	%PRL	
1	42.1	19.1	24.7	89.4***	81.2**	7.4***	26.4*	301.7**	1150	502.7**
(n=28)	±5.2	±9.7	±7.1	±11.6	±9.4	±1.2	±4.7	±45.5		±131.4
2	53.1	25.4	27.1	22.4	14.6	0.4	19.1	286.4	1505	685.1
(n=37)	±4.6	±11	±3.1	±7.4	±7.1	±1.2	±7.8	±97.2		±112.3

Group 1: premenopausal women, group 2: postmenopausal women; statistically significant intergroup differences at *p <0.05, **p<0.01 and***p<0.001.

Table 2. — *Concentration of serotonin in cancer tissue depending on Bloom-Richardson grade.*

Bloom-Richardson grade	n	Tumor diameter		Number of lymph nodes involved					Serotonin in cancer tissue (nmol/L)	p
		<1 cm	>2 cm	Thoracic (n)		Axillary (n)		Supraclavicular (n)		
				<3 cm	>3 cm	<3 cm	>3 cm			
I	11	9	2	5	10	1	4	1	563.8 ±206.3	
II	29	19	10	16	2	5	2	2	755.6 ±328.8	
III	25	3	22	9	11	2	5	3	822.6 ±313.6	

I vs. II p = 0.08; I vs. III p = 0.02; II vs. III p = 0.45.

Table 3. — *Serum concentrations of gonadotropins, estrogens, progesterone, prolactin and serotonin in 56 women with complete 10-year follow-up*

Hormone	Time from mastectomy		
	Baseline	5 years	10 years
FSH (μ/L)	22.3±4.7	21.3±4.7	18.7±3.4
LH (μ/L)	25.9±5.4	19.7±7.1*	23.4±5.2*
E1 (pg/mL)	104±12.3	53.4±9.3***	56.5±4.1***
E2 (pg/mL)	97±8.2	49.7±6.7***	44.7±3.9***
P (ng/mL)	1.2±0.9	2.7±1.9***	1.9±1.1***
PRL (ng/mL)	44.2±7.1	15.4±2.3***	17.1±1.9***
Serotonin (mmol/L)	591±81.4	385±11.4***	371±91.4***

Significantly different from the baseline at *p <0.05, **p<0.01 and ***p<0.001

for 12 days beginning from day 12 (50 mg/day for six days and then 100 mg/day for another six days). Additionally, patients from both groups received metformin (850 mg/day), quinagolide (75 mg/day for 12 days), melatonin (5 mg/day melatonin), and methysergide maleate (3 mg/day every second day).

Surgical BC specimens were subjected to histopathological examination at the Department of Pathomorphology and Genetics, Pomeranian Medical University in Szczecin. Histological grade was determined according to Bloom-Richardson.

Fasting blood samples for biochemical tests were obtained from the cubital vein, 24 hours post-surgery and during control visits at five and ten years thereafter. Serum concentrations of FSH, LH, estrogens (E1 and E2), prolactin (PRL1 and PRL2), progesterone, and serotonin were determined immunoenzymatically (IBL). BC tissue was obtained intraoperatively, frozen in liquid nitrogen and stored at -83°C until analysis. Concentrations of serotonin in cancer tissue were measured at the Department of Biogenic Amines, Polish Academy of Sciences in Lodz, with a radioimmunoassay and 9483 E gamma counter.

Statistical analysis was conducted with Statistica PL v. 7.1

package. Normal distribution of the study variables was verified with Shapiro-Wilk test. Intergroup comparisons were conducted with Student *t*-test and Mann-Whitney U-test for independent variables. An association between serotonin concentration in cancer tissue and histological grade was determined on the basis of Spearman's rank correlation coefficient (R). The threshold of statistical significance for all the tests was set at $p \leq 0.05$.

The protocol of the study was approved by the Local Bioethics Committee at the Pomeranian Medical University in Szczecin (decision no. BN001/55/2005), and a written informed consent was sought from all the study participants prior to any procedure.

Results

At the baseline, women from group 1 presented with significantly lower concentrations of gonadotropins and serotonin, and showed significantly higher levels of estrogens, progesterone, and PRL than patients from group 2 (Table 1).

Patients with grades II and III ductal BC presented with markedly higher intratumoral concentrations of serotonin than women with grade I cancer. Furthermore, a significant correlation was found between the concentration of serotonin in cancer tissue and histological grade according to Bloom-Richardson (R = 0.35, p = 0.01) Table 2).

Five years after the surgery, serum concentrations of LH, E1, E2, PRL, and serotonin were significantly lower than at the baseline, whereas the level of progesterone increased significantly (Table 3). After ten years of follow-up, all subjects presented with normal concentrations of estrogens, PRL, and serotonin, and none of them showed any pathologic lesions, relapse or metastases. Three (4.6%) women from group 2 died during the follow-up period due to a circulatory failure.

Discussion

Ductal carcinoma is the most common type of BC. Due to wide availability of mammographic screening, this malignancy is detected with increasing frequency nowadays. A number of risk factors for BC have been identified to date, among them hereditary predisposition, genetic factors (e.g. mutations of the *BRCA1* and *BRCA2* genes), benign mammary hyperplasia with proliferation, hormonal factors, ionizing radiation, obesity, alcohol consumption, and tobacco smoking. Although an array of carcinogenesis markers, cytokines, and growth factors were implied to be useful for detection and monitoring of BC [13], still there is a need for novel accurate methods that would facilitate early diagnosis and further monitoring of this malignancy.

The present longitudinal study identified hyperserotonemia as a factor involved in the progression and aggressiveness of ductal carcinoma. Furthermore, the authors found a significant correlation between the concentration of serotonin in cancer tissue and histological grade. Women with grade II and III tumors presented with markedly higher serum concentrations of serotonin than patients with grade I malignancies. These findings are consistent with the data published by Frobe *et al.* [14] who also observed higher serum concentrations of serotonin in patients with advanced BC rather than in those with localized form of this malignancy. Therefore, these authors concluded that concentration of serotonin may serve as an early marker of relapse or metastasis in breast cancer [14].

Mitogenic role of serotonin in BC cells was previously documented in a study analyzing expression of serotoninergic receptors (5-HT2A) in this malignancy [15]. Furthermore, previous studies revealed enhanced expression of other serotoninergic receptors in the blood of BC patients (5HT2AR, 5HT3AR) [16], and in cancer tissue (5HTR1A, 5HTR1B, 5HTR2B, 5HTR4) [17]. Consequently, serotonin emerged as a local regulator of epithelial homeostasis in the breast [18-20]. According to Pai *et al.* [18], enhanced synthesis of serotonin is accompanied by altered expression of 5-HT receptors; this results in a dysregulation: an overexpression of some isoforms and a suppression of the others may promote growth of malignant cells in the breast, e.g. stimulating their proliferation or abnormal survival patterns. As a result, serotonin is deprived of its regulatory role in the homeostasis of mammary epithelium [18].

Although it was postulated that widespread use of selective serotonin reuptake inhibitors (SSRIs) for the treatment of depression may result in higher synaptic levels of this neurotransmitter, and thus promote breast carcinogenesis, this hypothesis was not confirmed during the course of long-term clinical observations [21, 22]. However, Kelly *et al.* [23] found that administration of a SSRI (paroxetine) increased mortality risk in postmenopausal BC patients treated with tamoxifen; this implies that SSRIs may interfere with the beneficial effects of the latter agent. Furthermore, SSRIs were implicated in hyperprolactinemia [24]. Secretion of PRL is inhibited by dopamine and stimulated by thyrotropin releasing hormone, vasoactive intestinal peptide, and serotonin [4]. Previous studies confirmed a link between increased concentrations of PRL and BC risk in pre- and postmenopausal women [25, 26]. In the present study, pretreatment hyperserotonemia resulted to be associated with higher baseline levels of PRL. Consequently, the present patients were administered bromocriptine, an agonist of dopaminergic receptors which effectively reduces concentrations of PRL [27], as well as a modified hormone replacement therapy [11] consisting of melatonin, metformin, and methysergide maleate to reduce serotonin level. Such treatment resulted in normalization of gonadotropin, PRL, and serotonin concentrations.

None of the present patients showed a relapse or metastases during a ten-year follow-up, which implies that normalization of serotonin and PRL concentrations may reduce the risk of recurrence in BC patients. Hyperserotonemia was previously shown to correlate with the expression of 5-HT receptor subtypes [15, 16, 20] and aggressiveness of ductal carcinoma. Clinical and biochemical observations showed that cyclic parenteral administration of micronized estradiol and progesterone may restore a physiological menstrual cycle, normalize concentrations of gonadotropins, estrogens, progesterone, and serotonin in BC patients [11].

Conclusion

In conclusion, this study showed that concentration of serotonin in cancer tissue may serve as a marker of ductal carcinoma aggressiveness. An innovative neurohormonal therapy may prevent progression of BC and normalize the levels of sex hormones and serotonin.

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Corresponding Author:
E. SOWIŃSKA-PRZEPIERA, M.D.
Department of Endocrinology, Metabolic
and Internal Diseases, Pomeranian Medical University
1 Unii Lubelskiej Str.
71-252 Szczecin (Poland)
e-mail: elasowprzep@wp.pl