Influence of chemotherapy to hormonal levels in postmenopausal breast cancer patients

O. PRIBYLOVA[1*], D. SPRINGER[2], A. SVOBODNIK[3], M. KYR[3,] T. ZIMA[2], L. PETRUZELKA[1]

[1]Department of Oncology, 1st Faculty of Medicine, Charles University and General Teaching Hospital, Prague, Czech Republic e-mail: olga.pribylova@vfn.cz *[2]Institute of Clinical Biochemistry and Laboratory Diagnostics 1st Faculty of Medicine, [3]Department of Biostatistic and analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

Received Janury 1, 2008

Targeting and tailoring of therapy is the latest trend in breast cancer treatment. The efficacy of the available treatment must be estimated and the probable benefit for the patient determined.

The aim of this project was to find out wether also in postmenopausal women chemotherapy can affect hormonal levels in serum and if even the levels of IGF-1 and IGFBP-3 can be changed. In the group of 72 postmenopausal breast cancer patients blood samples were taken before, during and after adjuvant chemotherapy and levels of estradiol, progesterone, LH, FSH, IGF-1 and IGFBP-3 were evaluated. We did not find any statistically significant dependence on tumor stage, expression of hormonal receptors or HER-2 and treatment regimen with studied hormones.Serum levels before treatment in comparison with status during treatment were significantly different in LH, FSH and progesterone value.

Hormone levels after the treatment in comparison with status during treatment were significantly different only in levels of estradiol. Significant differences in all parameters were found except IGF-1. There was not any statistical dependence on the menopausal gap, age, weight or type of chemotherapy. We can conclude, that also in postmenopausal women hormonal changes can take part in the final effect of adjuvant treatment.

Key words: *breast cancer / postmenopausal/ chemotherapy/ hormonal levels / IGF-1/ IGFBP-3)*

The diagnosis of breast cancer presents several dilemmas for the patients and the physicians. The efficacy of the available treatment must be estimated and the probable benefit for the patient determined. Targeting and tailoring of therapy is the latest trend in breast cancer treatment.

The variability of survival is explained by differences in tumor invasiveness, growth rate, metastatic potential and other mechanisms that are not fully understood. To optimise treatment for each patient several measurable factors available at the time of diagnosis were determined, so called prognostic and predictive factors. The expression of oestrogen receptors is one of most important biomarker [1].

Systemic adjuvant therapy with hormonal treatment, chemotherapy and targeted biological treatment are undoubtedly related to the improvement of mortality from breast cancer. Effective adjuvant systemic treatment improves relapse-free and overall survival [2, 3]. It is known that chemotherapy in premenopausal woman leads to hormonal deprivation, which takes part on final effect of adjuvant treatment [2]. There is not precise knowledge wether also in postmenopausal women chemotherapy affects hormonal levels.

Epithelial breast cells are under the influence of a variety of hormones and growth factor. However breast cancer cells acquired the phenotype characteristic for malignancy (unregulated proliferation, protection from cell death, and metastasis), they continue to respond to extracellular signals. In addition to estrogen, breast cancer responds to various stimuli including growth factors and cytokines.

Estrogen receptors (ER) and progesterone receptors (PR) are gene regulatory proteins and their stimulation by oestrogen and progesterone plays a well-established role in mammary epithelial growth, differentiation and pathology. Both steroid hormones are well known for their abilities to modulate directly the expression of growth factor receptor pathways. Receptor activation is regulated through its phosphorylation. Signal transduction pathways induced by growth

* Corresponding author

factors and hormones may directly or indirectly regulate steroid receptor function that can be affected also by epidermal growth factors, heregulin and insuline-like growth factors (IGF). IGF-1 and IGF-2 system performs multiple functions, comprises a complex network of ligands, their cognate receptors and binding proteins (IGFBP). IGF performs endocrine, paracrine and autocrine roles mediated through the IGF receptor [4, 5, 6].

IGF receptor has although a structure of heterodimeric tyrosine kinase. Cellular responsiveness to IGFs is stimulated by oestrogen. IGF has also been reported to activate ER. There could be a cross talk between their pathways with mechanisms not yet fully understood [7, 8].

In premenopausal patients chemotherapy can induce amenorrhoea by causing ovarian failure. The raise of survival is partly caused by additional endocrine effect [9]. In postmenopausal patients is depression of level of circulating oestrogen the most important feature of aromatase inhibitors [10].

Measurement of serum levels of estrogen, progesterone, FSH, LH, IGF-1 and IGFBP-3 could clarify the indirect effect of chemotherapy through the mediation of hormonal action also in postmenopausal patients. Aim of our study is to elucidate still unknown influence of chemotherapy on the estrogen synthesis in this group.

Material and methods

Seventy-two postmenopausal patients after surgery for breast cancer who has been designed to be treated by chemotherapy were asked to take part in this study. Age of them was 50-84 years with median 59 years. Patients were 2-34 years after menopause, median 12 years. Characteristic of the group based on TNM classification is in table 1., all patients were M0.

All patients had infiltrating ductal carcinoma of different grade: G1: 11, G2: 36 and G3: 25 patients.

All patients had ER, PR and HER-2 expression measured in tissue samples taken during surgery. Tissue HER-2 expression was assayed with the DAKO Company Herceptest, expression of ER and PR was assayed imunohistochemically with the DAKO murine antihuman antibody CLONE 1D5 and CLONE PgR 636. The cut-off for positivity of ER and PR is 10%. Results are summarised in tables 2. and 3.

All patients treated by chemotherapy were of higher risk, it means ER-, N+, T3, HER-2 3+. They were treated mostly with antracycline containing regimens - doxorubicin plus cyclophosphamide 4 cycles, followed by docetaxel 4 cycles (AC-T) or 5FU, doxorubicin, cyclophosphamide (FAC) 6 cycles or doxorubicin, cyclophosphamide (AC) 4 cycles (table 4.).

Blood sample of 5 ml was taken before chemotherapy, after the second or the third cycle and after finishing the treatment.

Table 1. Characteristic of the group according to T and N

T and N	Number of patients (N=72)
T1c, N0	11
T1 any, N1	16
T2, N0	13
T2, N1	15
T3, N0	8
T3, N1	9

Table 2. Expression of hormonal receptors

receptor	ER+	ER-
PR+	29	2
PR-	5	36

Table 3. HER-2 tissue expression

HER-2	number
+	22
++	27
+++	23

Table 4. Characteristic of the group according to chemotherapy regimens

regimen	Number of patient
AC-T	19
FAC	6
AC	47

The level of estradiol, progesterone, FSH, IGF-1 and IGFBP-3 has been measured. FSH, estradiol and progesterone are determined by ARCHITECT Chemiluminiscent Microparticle Immunoassay (ABBOTT USA) , LH was measured by ADVIA, CENTAUR Bayer system. The IGF-1 was measured by immunoradiometric assay kit – IMMUNOTECH, France. The DSL Insuline-like Growth Factor Binding Protein-3 (IGFBP-3) Immunoradiometric Assay Kit provided materials for the quantitative measurement of IGFBP-3 in serum**.**

Results were statistically analysed using either unpaired or paired t-test or their non-parametric alternatives (Mann-Whitney, Wicoxon). Spearman's rank correlation coefficient was used in correlation analyses.

Results

We compared levels of studied parameters before the treatment with levels during (after 2nd cycle in patients treated by AC regimen and after 3rd cycle in the rest of patients) and after end of the treatment.

Results of serum hormonal levels in the treated group are summarised in table 5.

Table 5. Descriptive analysis: monitored hormones serum levels

	Before treatment(B) mean ± SD	During treatment(D) mean ± SD	After treatment(A) mean ± SD
LH [U/l]	31,15 ± 14,1	#27,88 ± 13,29	30,62 ± 17,24
FSH [U/l]	63,47 ± 30,48	#56,31 ± 23,14	57,6 ± 31,13
Progesterone[nmol/l]	1,62 ± 1,21	#1,34 ± 1,14	1,48 ± 1,59
Estradiol [nmol/l]	0,12 ± 0,08	0,14 ± 0,25	*0,08 ± 0,06
IGF-1 [μg/l]	161,75 ± 107,95	161,07 ± 85,28	158,9 ± 78,62
]=gfe1051IGFBP-3 [mg/l]	4,15 ± 1,1	4,19 ± 1,09	*4,69 ± 1,12

statistically significant ($p < 0,05$) B versus D
* statistically significant ($p < 0,05$) D versus A

We did not find any statistically significance between tumor stage, expression of hormonal receptors or HER-2 and treatment regimen with studied hormones. Serum levels before treatment in comparison with status during treatment were significantly different in LH, FSH and progesterone values.

Hormone levels after the treatment in comparison with status during treatment were significantly different only in levels of estradiol – decrease -0,04 nmol/l ($p < 0,05$) and IGFBP-3 where increase of average 0,46 mg/l ($p < 0,05$) was found.

These results suggest that decrease in LH, FSH, and progesterone begins during treatment period and further lowering is not significant. In contrast to this finding decrease of estradiol level and increase of IGFBP-3 was evident after therapy. Levels of IGF-1 did not alter significantly, but the variance is really wide and is not in association with the treatment. This finding was confirmed by a non-parametric test.

Relationship between levels of monitored parameters before and during treatment to chemotherapy is from statistical point of view positive in all active substances, the strongest linkage was found in LH, FSH and IGF-1. The higher were the values before treatment the higher the values during the treatment were found (positive correlation). On the other hand, negative correlation was found between IGFBP-3 values before treatment and their changes during the treatment. Thus, the higher was the value before treatment the deeper decrease during the treatment was found. There was not any linkage between levels before and after treatment.

Spearman's correlation coefficient of our results is summarised in table 6. All values were statistically significant ($p<0,05$).

Tab. 6. Spearman's correlation coefficients (R)

Parameter hormone	Before vs. during the treatment	Changes during the treatment vs. before treatment
LH	0,83	-0,35
FSH	0,88	-0,57
Progesterone	0,59	-0,53
Estradiol	0,45	-0,48
IGF-1	0,72	-0,27
IGFBP-3	0,58	-0,25

We also evaluated relationship of hormonal levels to menopausal gap. Significantly negative correlation was discovered between this gap and LH (Spearman's correlation coefficient R=-0,27, R=-0,35, R=-0,39) and IGF (R=-0,35, R=-0,29, R=-0,43) before during and also after chemotherapy. This means that the larger the menopausal gap the lower the hormones levels were found. The correlation increases as the period between the evaluated values extends. Thus, the negative association is stronger between values before and after the treatment. Less evident is negative correlation between the menopausal gap and FSH levels. Significant negative correlation was identified only between the gap and hormonal levels after the treatment (R=-0,38). We did not found any relationship in other monitored parameters.

We focused as well on relations between hormone levels and patients weigh. Patients weight negatively correlated with progesterone levels before the treatment (R=-0,32) and positively correlated with progesterone levels after the treatment (R=-0,56). The levels of progesterone before treatment are lower and after treatment levels are higher in patients with higher weigh. It means that decrease of progesterone value during therapy is smaller in patients with higher weigh. The correlation of weigh and progesterone changes is statistically significant (R=0,27).

Discussion

The IGF-1 plays a fundamental role in the development of the breast cancer. The IGF system has been implicated in breast cancer progression because of its mitogenic and anti-apoptotic influence on the breast epithelial cells [5]. Increasing interest is focused on its role as a major determinant of breast cancer and its involvement in the development of resistance to tamoxifen and trastuzumab [11]. IGF-1 plays an important role in breast cancer risk [12, 13]. IGFs are important mediators of growth, development, and survival, are synthesised by almost any tissue in the body, and a complex network of molecules, including binding proteins, proteases and receptors modulates their action. IGF-1 receptor by the cancer cells may play a significant role in a transformed phenotype of malignant cells [14,15]. In this process IGFs are interacting with

other molecular systems, such as steroid hormones etc. The functions of IGF-1 are mainly mediated through its receptors. The availability of free IGF-1 for interaction with receptor is modulated by IGF binding proteins IGFBP 1-6. IGFBP-3 limits the IGF-1 binding potential to its receptors. Interactions with other receptors, including oestrogen and growth factors were proven [16]. Data in the literature show that a growth hormone, prolactin, estradiol, progesterone, cortisol could be responsible for regulation of IGF-1 activity [17].

In premenopausal women is the main action of chemotherapy to changing of hormonal levels well established [18]. However little is still known about the influence of chemotherapy on growth factors which regulates neoplasm growth and participate a the disease progression – i.e. IGF-1 and its binding protein. The effect of chemotherapy on hormone levels is not well identified in postmenopausal patients. In some studies [19, 20, 21] significant decrease of IGF was found after chemotherapy in premenopausal patients, however other authors [22] did not find any changes in serum IGF-1 concentration in patients with early stage of breast cancer, but observed a significant increase of serum IGF-1 concentration in women with an advanced stage. That may suggest that IGF-1 plays a part in breast cancer progression. We did not find statistically significant changes in this parameter.

In the light of the latest reports on the role of IGF-1 in breast cancer pathogenesis it is essential to understand the effect of currently used therapy on plasma IGF-1 levels. In vitro studies show that IGF-1 protects breast cancer cell from anticancer drug-induced cell death by reducing apoptosis [23].

In postmenopausal breast cancer patients mainly antiestrogen therapy influence on IGF-1 levels was studied. In these patients, IGF-1 levels significantly decreased while IGFBP-3 did not change [24, 13]. No statistically significant correlation was found between mean plasma concentration of IGF-1 and serum estradiol, prolactin, progesterone, cortisol and other hormonal levels [17].

Influence of tamoxifen, chemotherapy and combination of both to selected hormonal levels was studied in pre- and postmenopausal breast cancer patient [25]. In postmenopausal patient no significant difference in pre- and post-treatment levels of estradiol was found. The levels of LH and FSH in patient treated with tamoxifen alone or in combination were significantly lower than those treated with chemotherapy. Premenopausal women treated with combination of chemotherapy and tamoxifen had significantly lower gonadotropin levels and higher estradiol and progesterone levels in comparison with patient treated with chemotherapy alone. The increase of hormonal levels was reversible, induced by tamoxifen.

It could be concluded that hypothalamo-pituitary-ovarian axis is profoundly alliterated by chemo- and hormonal therapy, especially in premenopausal women [26, 27, 28, 29].

In conclusion, we have found that also in postmenopausal women hormonal levels in serum can be affected by chemotherapy, mainly the level of estrogen, and this mechanism of action might be a part of final result of chemotherapy. It could be important mainly in patients with hormonal dependent tumors who need chemotherapy with respect to other evaluated risk factors. Because of negative statistical correlation of estrogen levels to the menopausal gap it could be in consequence of chemotherapy influence to the residual ovarian production of estradiol. The mechanism of changes in levels of monitored substances could consist also in impact of cytostatic drugs on the production in alternative tissues i.e. fat, breast and other or on process of regulation (FSH, LH). The problem needs further investigation of possible mechanisms of described changes.

This work was supported by the Research Project Ministry of Education, Youth and Sports of the Czech Republic 002162 0808.

Refrences

[1] De VITA VT, HELLMAN S, ROSENBERG SA. Cancer principles § practice of oncology. 7th edition, Lippincott Williams § Wilkins, Philadelphia, 2005;1399–1471.

[2] POWELS TJ. Prognostic impact of amenorrhoea after adjuvant chemotherapy. European Journal of Cancer 1998; 34: 603–605.

[3] Early Breast cancer Collaborative Group Ovarian ablation in early breast cancer: overview of randomised trails. Lancet 2000; 348: 1189–1196.

[4] CLARK GM. Prognostic and predictive factors, disease of the breast, 2nd edition, Lippincott Williams § Wilkins, Philadelphia, 2000: 489–513.

[5] SACHDEV D, YEE D. The IGF system and breast cancer, Endocrine Related Cancer 2001; 8: 197–209.

[6] CULLEN KJ, YEE D, SLY WS et al. Insulin like growth factor receptor expression and function in human breast cancer. Cancer Research 1990; 50: 48–53.

[7] CHO H, ARONICA SM, KATZENELLENBOGEN BS. Regulation of progesterone receptor gene expression in MCF-7 breast cancer cells. Endocrinology 1994; 134: 658–664

[8] LEE AV, YEE D. Insuline-like growth factors and breast cancer. Biomedicine and Pharmacotherapy 1995; 49: 415–421.

[9] Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. A Engl J Med 2001; 34: 1997.

[10] GEISLER J, KING N, DOWSETT M et al. Influence of anastrozol, a non steroidal aromatase inhibitor, on in vivo aromatisation and plasma estrogen levels in postmenopausal women with breast cancer. Br. J. Cancer 1996; 74: 1286-1291.

[11] LABAN C, BUSTIN SA, JENKINS PJ. The GH-IGF-1 axis and breast cancer. Trends Endocrinol. Meta. 2003; 14: 28–34.

[12] GIOVANUCCI E. Insulin like growth factor-I and binding protein-3 and risk of cancer. Horm. Res. 1999; 51: Suppl 3, 34–41.

[13] POLLAK M. IGF-I physiology and breast cancer. Recent Results Cancer Res. 1998, 152: 63–70.

[14] ELLIS MJ, JENKINS S, HANFELT J et al. Insulin-like growth factors in human breast cancer. Breast Cancer Res. Treat. 1998; 52: 175–184.

[15] MOSCHOS SJ, MANTZOROS CS. The role of IGF system in cancer: from basic to clinical studies and clinical applications. Oncology 2002; 63: 317–332.

[16] FURSTENBERGER G, MORANT R, SENN HJ. Insuline-like growth factors and breast cancer. Onkologie 2003; 26: 290–294.

[17] KAJDANIUK D, MAREK B. Influence of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil on plasma insulin-like growth factor-I and chosen hormones in breast cancer in pre-menopausal patients. Journal of Clinical Pharmacy § Therapeutics 2000; 25: 67–70.

[18] KUTLUK O, OZGUR O. Measuring the impact of chemotherapy on fertility in women with breast cancer. Journal of clinical oncology 2006, 24: 4044-4046.

[19] PEYRAT JP, REVILLION F, BONNETERRE J. Plasma insuline-like growth factor in primary breast cancer patients treated with adjuvant chemotherapy. British Journal of Cancer 1998; 77: 1669–1671.

[20] BARNI S, LISSONI P, BIVIO F. serum levels of insuline-like growth factor-I in operable breast cancer in relation to main prognostic variables and their perioperative changes in relation to those of prolactin. Tumori 1994; 80: 212–215.

[21] BRUNING PF, VAN DOORN J, BONFRER JM. Insulin-like growth-factor-binding protein 3 is decreased in early-stage operable pre-menopausal breast cancer. International Journal of Cancer 1995; 62: 266–270.

[22] FAVONI RE, CUPIS A, PERROTTA A. Insulin-like growth factor-I and IGF-binding proteins blood serum levels in women with early- and late-stage breast cancer: mutual relationship and possible correlation with patientś hormonal status. Journal of Cancer Research and Clinical Oncology 1995; 121: 674–682.

[23] DUNN SE, HARDMAN RA, KARI FW et al. Insuline-like growth factor-I alters drug sensitivity of HBL 100 human breast cancer cells by inhibition of apoptosis induced by diverse anticancer drugs. Cancer Research 1997; 57: 2687–2693.

[24] HELLE SI, LONNING PE. Insulin-like growth factors in breast cancer. Oncologica 1996; 35: 19–22.

[25] YASUMURA T, AKAMI T, MITSUO M et al. The effect of adjuvant therapy with or without tamoxifen on the endocrine function of patients with breast cancer. Jpn. J. Surg 1990; 20: 369–375.

[26] ANJUM S, KHAN S, BAIG et al. Effect of chemotherapy on circulating steroid hormone levels in postoperative premenopausal breast cancer patients. J. Pak. Med. Assoc. 1991; 41: 296–298.

[27] POIKONEN P, SAARTO T, ELOMAA I et al. Prognostic effect of amenorrhoea and elevated serum gonadotropin levels induced by adjuvant chemotherapy in premenopausal node-positive breast cancer patients. Eur. J. Cancer 2000; 36: 43–48.

[28] JORDAN VC, FRITZ NF, LANGAN-FAHEY S et al. Alteration of endocrine parameters in premenopausal women with breast cancer during long-term adjuvant therapy with tamoxifen as the single agent. J. Natl. Cancer Inst. 1991; 16: 1488–1491.

[29] STURGEON SR, POTISCHMAN N, MALONE KE et al. Serum levels of sex hormones and breast cancer risk in premenopausal women. Cancer Causes Control 2004; 15: 45–53.