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Urinary steroids at time of surgery in postmenopausal women with breast cancer

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Summary

Urinary steroid metabolites were measured by capillary gas chromatography in 22 postmenopausal women with operable breast cancer on day before the tumour excision and in 20 hospitalised control who were before an operation from other cause than cancer. Serum dehydroepiandrosterone-sulphat (DHEAS) and testosterone (T) level were measured by radioimmunassay in the same groups and same time. There was no significant difference in the level of urinary androgen metabolites. Pregnanediol level was significantly lower ($P < 0.05$) in cancer patients. In the 5 patients with positive axillary nodes the tetrahydrocortisol and α-cortolone levels were significantly ($P < 0.05$) higher than in node negative ones. There was no significant differences in the serum DHEAS and T levels. These results indicate that metabolic changes are existing in postmenopausal patients which may be a cause or a consequence of the disease.

Introduction

Breast cancer is one of the leading cause of death among women. About one third of the total breast cancers is hormone dependent [1] and oestradiol has the main role in this type of tumour development [2, 3]. High serum oestrogen levels in postmenopausal women are associated with an increase in breast cancer risk [4]. Other hormones may play an important role in breast cancer development as well. Experimental data also support the role of adrenal androgens [5–8], progesterone [9] and glucocorticoids [10] in the proliferation of breast cancer.

The supposed effects of androgens in breast cancer are: risk enhancing role as their function as precursors of oestrogens [7, 8] or risk factor of recurrence in operable breast cancer [11, 12].

On the role of progesterone and oestradiol in breast cancer more theories are existing [9]. By the 'unopposed oestradiol' theory the ratio of oestradiol to progesterone is determining in the breast cancer risk; 'oestradiol plus progesterone' theory supposes that the progesterone increases the effect of oestradiol. The third theory is the 'oestrogen alone hypothesis' which supposes that oestrogens without progesterone have a role in breast cancer.

Y.S. López-Boado [10] suggested a possible role of glucocorticoids in breast cancer as they found that dexamethasone and 5α-dihydrotestoterone strongly induced the accumulation of Zn-α_2-gp mRNA, a human protein that is produced by a specific subset of breast carcinomas.

In the present study urinary steroid excretion and serum DHEAS and T levels have been compared in

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postmenopausal women with breast cancer and matched control women to assess possible metabolic changes in postmenopausal breast cancer patients.

Materials and methods

Subjects. 24-h urine samples were collected from 22 women with primary operable breast cancer and 20 control women on the day before the surgery. The patients were considered in postmenopausal status if more than 2 years elapsed since their last menstrual period. The control women were aged matched patients of the surgical department who were undergoing operation from other causes: bone fracture (5 patients), gall bladder stone (3 patients), benign diseases of the breast (5 patients), goiter (1 patients), incisional hernia (1 patients), with no endocrine source. Mean ages were 61.8 ± 7.9 and 59.8 ± 8.2 years, respectively.

Classification of the tumours. The size of the tumor and the status of the axillary nodes were measured during the histological examination. Distant metastasis were examined by bone scintigraphy, by abdominal ultrasonography and by chest X-ray examination. The patients were classified into 3 groups according to Bloom and Richardson [13] on the basis of their tumour size, of the presence of axillary node involvement and of the presence of distant metastasis.

Radioimmunoassay. DHEAS and T from serum were measured by radioimmunoassay using commercial kits purchased from Merck KGaA (Darmstadt, Germany).

Extraction of urine. Solid phase extraction on Sep-Pak C18 cartridges, enzymatic hydrolysis and methoxime-silyl derivatization were applied according to the method of Shackleton and Honour [14].

Chromatography. Gas chromatographic analysis was carried out on Hewlett-Packard 5890 Series II. gas chromatograph equipped with flame ionisation detector, on an ULTRA-1 column (25 m × 0.2 mm × 0.33 μm) according to Homoki et al. [15]. The temperature program was as follows: initial temperature of 50 °C was hold for 2 min, then increased to 180 °C at 30 °C/min. After a 4-min isotherm period the temperature was then increased to 300 °C by 2.1 °C/min, and held for 8 min. The splitless injection mode was employed.

Evaluation. Student's t-probe was applied to evaluation of the results.

Results

The mean values of age, weight, height, the date of first and last menses are shown in Table 1. There were no significant differences in the mentioned parameters between the breast cancer patients and the control group. There were 5 node positive patients among the breast cancer patients.

The distribution of the patient according to their tumour status are displayed on the Table 2.

The separation of the urine steroid metabolites is shown in Figure 1.

The mean values of the daily urinary excretion of androgen and progesterone metabolites are displayed in Figure 2. There are no significant differences in the excreted steroid levels, except for preg-

Table 1. Distribution of patients and controls according to their age, height, weight and time of first or last menses

	Breast cancer	Control
No of cases	22	20
Age (yr)	61.2 ± 8.0	60.4 ± 8.1
Weight (kg)	72.3 ± 12.3	69.5 ± 9.1
Height (m)	1.62 ± 0.061	1.61 ± 0.063
Time of first menses (yr)	13.9 ± 1.7	13.9 ± 2.4
Time of last menses (yr)	50.5 ± 2.6	47.8 ± 4.2

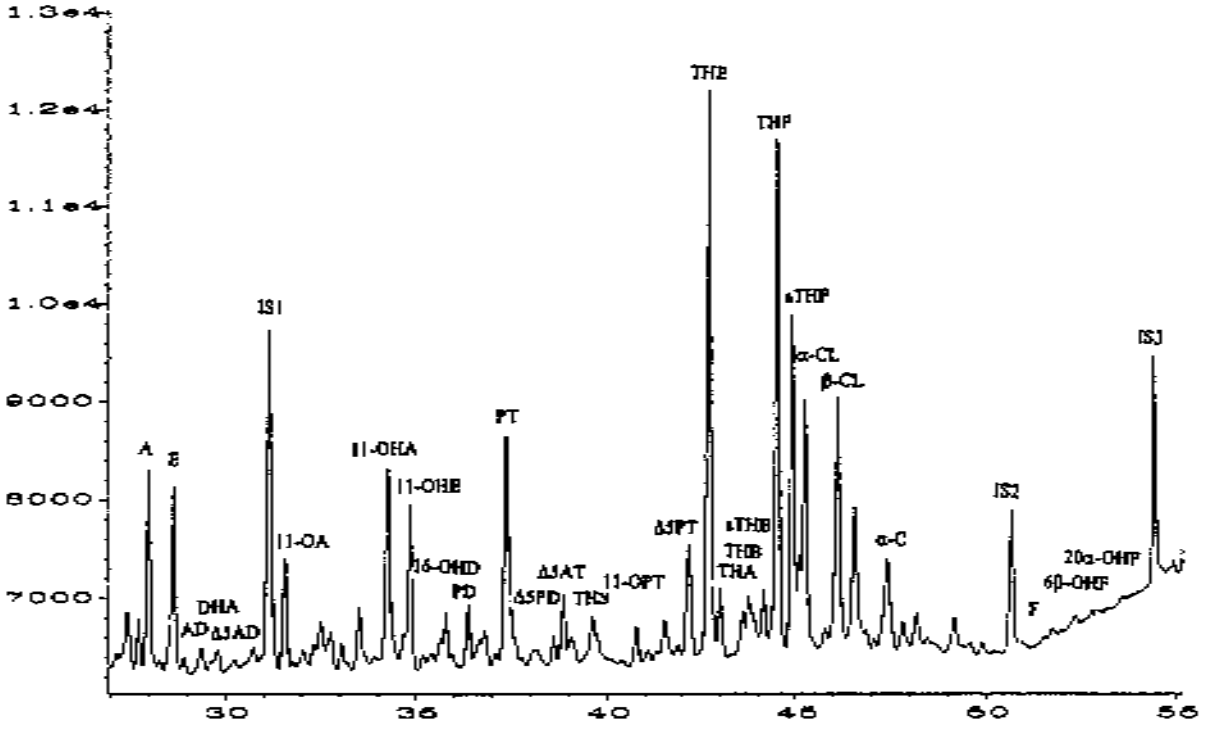

Figure 1. Gas chromatographic separation of urinary steroids in a 80 year-old woman with breast cancer Abbreviations: A: Androsterone (3α-hydroxy-5α-androstan-17-one); E: Etiocholanolone (3α-hydroxy-5β-androstan-17-one); AD: Androstanediol (3α, 17β-dihydroxy-5α-androstane); DHEA: Dehydroepiandrosterone (5-androsten-3β-ol-17-one); Δ5AD: Androstenediol (3β, 17β-dihydroxy-5-androstene); 11O–A: 11-keto-androsterone (3α-hydroxy-5α-androstan-11, 17-dione); 11-OHA: 11-hydroxy-androsterone (3α, 11β-dihydroxy-5α-androstan-17-one); 11-OHE: 11-hydroxy-etiocholanolone (3α, 11β-dihydroxy-5β-androstan-17-one); 16OHD: 16-hydroxy-DHEA (3β, 16α-dihydroxy-5-androsten-17-one); PD: Pregnanediol (3α, 20α-dihydroxy-5β-pregnane); PT: Pregnanetriol (3α, 17α, 20α-trihydroxy-5β-pregnane); Δ5PD: Pregnenediol (3β, 20α-dihydroxy-5-pregnene); Δ5AT: Androstenetriol (3β, 16α, 17β-trihydroxy-5-androsten); THS: Tetrahydro-11-deoxycortisol (3α, 17α, 21-trihydroxy-5β-pregnane-20-one); 11OPT: 11-keto-pregnaneteriol (3α, 17α, 20α-trihydroxy-5β-pregnane-11-one); Δ5PT: Pregnenetriol (3β, 17α, 20α-trihydroxy-5-pregnene); THE: Tetrahydrocortisol (3α, 17α, 21-trihydroxy-5β-pregnane-11, 20-dione); THA: Tetrahydro-11-dehydrocorticosterone (3α, 21-dihydroxy-5β-pegnan-11, 20-dione); THB: Tetrahydro-corticosterone (3α, 11β, 21-trihydroxy-5β-pregnan-20-one); aTHB: Allo-tetrahydrocorticosterone (3α, 11β, 21-trihydroxy-5α-pregnan-20-one); THF: Tetrahydro-cortisol (3α, 11β, 17α, 21-tetrahydroxy-5β-pregnane-20-one); aTHF: Allo-tetrahydrocortisone (3α, 11β, 17α, 21-tetrahydroxy-5α-pregnane-20-one); α-CL: α-cortolone (3α, 17α, 20α, 21-tetrahydroxy-5-β-pregnane-11-one); βCL: β-cortolone (3α, 17α, 20β, 21-tetrahydroxy-5β-pregnane-11-one); αC: α-cortol (3α, 11β, 17α, 20α, 21-pentahydroxy-5β-pregnane); F: Cortisol (11β, 17α, 21-trihydroxy-4-pregnene-3,20-dione); 6β-OHF: 6β-hydroxy-cortisol (6β, 11β, 17α, 21-tetrahydroxy-4-pregnene-3,20-dione); 20α-OHF: 20α-hydroxy-cortisol (11β, 17α, 20α, 21-tetrahydroxy-4-pregnene-3,20 dione).

nanediol between patients and controls. The level of pregnanediol was significantly ($P < 0.05$) lower in patients.

The mean values of daily urinary excretion of cortisol metabolites are shown in Figure 3.

Comparing the axillary node positive patients with the negative ones the THE and β-cortolone level were significantly ($P < 0.05$) higher in the axillary node positive patients. Figure 4 shows the mean values for cortisol metabolites of the axillary

Table 2. Distribution of the patients according their tumor stages

	Tumour stages			
	I.	II.A	II.B	III.
Number of patients	7	10	4	1

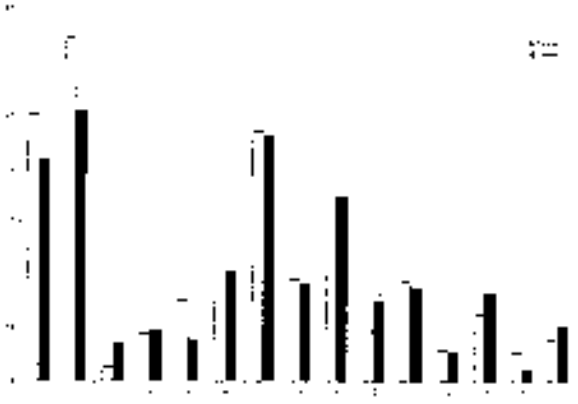

Figure 2. Mean values of urinary excretion of androgen and pregnane metabolites in patients and control women. 1, A; 2, E; 3, AD; 4, DHEA; 5, n5AD; 6, 11-OA; 7, 11-OHA, 8, 11-OHE; 9, 16-OHD; 10, PD; 11, PT; 12, n5PD; 13, n5-AT; 14, 11OPT; 15, n5-PT; * $P < 0.05$.

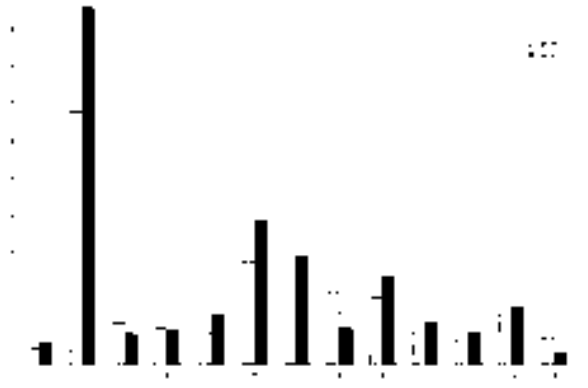

Figure 3. Mean values of urinary excretion of cortisol metabolites in patients and control women. 1, THS; 2, THE; 3, THA; 4, THB; 5, aTHB; 6, THF; 7, aTHF; 8, α-CL; 9, β-CL; 10, α-C; 11, F; 12, 6β-OHF; 13, 20α-OHF; * $P < 0.05$.

node positive and the axillary node negative patients.

The levels of serum DHEAS and T are summarised in the Table 3. No significant difference were in the serum DHEAS and T levels between the patients and control women.

The mean values and standard error of means of urinary steroid levels are indicated in Table 4.

Discussion

The hypothetized effect of androgens, especially DHEA or DHEAS, on the proliferation of breast cancer is supported by experimental data in cell culture [6, 16–18] and in rats [19].

Studies on adrenal androgens in women with breast cancer led to contradicting results. Positive association between serum level of testosterone [12, 20–22], androstanedione [22], DHT [22], DHEAS [22] DHEA [7, 23] and breast cancer risk was reported. In postmenopausal patients supranormal serum level of DHEA and DHEAS was displayed by Zumoff [8]. Ballerini et al. [11] found correlation in node-positive patients between the relapse-free survival rate and the serum testosterone level. Opposing of these results Barrett-Conor et al. [24] reported absence of association of plasma DHEA-S and breast cancer and they concluded that the plasma DHEAS-S has no protective effect in breast cancer risk in postmenopausal women.

As for urinary steroid levels Bulbrook et al. [25] found that women who later developed breast cancer, excreted significantly less androsterone and etiocholanolone, the two major metabolites of DHEA. Higher urinary T level [21, 26] and androstanedione level was reported by Secreto [27].

The present study on urinary excretion did not find any difference in urinary androgen levels and in serum DHEAS and T levels between breast cancer patients and controls in postmenopausal women.

Our findings on the significantly ($P < 0.05$) lower

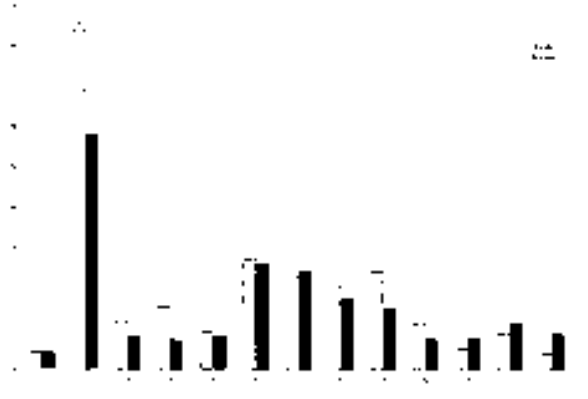

Figure 4. Mean values of urinary excretion of cortisol metabolites in axillary node positive and negative women. Steroids and P values as in Figure 3.

Table 3. Mean values and standard error of means (S.E.M.) of serum DHEAS and T in patients and control women

	DHEAS		T	
	Mean (μmol/L)	S.E.M.	Mean (μmol/L)	S.E.M.
patients	4.1	0.9	1.1	0.8
control women	3.6	0.8	1.1	0.2

PD level in patients compared with controls directed our interest to the role of progesterone in breast cancer. Key and Pike [9] discussed the three possible theories of the hormonal control of breast cell division and the role of oestradiol and progesterone balance on the risk of breast cancer.

The majority of the studies dealt with measuring progesterone or pregnanediol in premenopausal patients and we could not find any data on the postmenopausal serum progesterone or urinary pregnanediol levels in breast cancer patients. In women with premenopausal operable breast cancer lower progesterone level was found by Bernstein et al. [4] but another study [22] found no difference in the progesterone level of patients and controls. Badwe et al. [28] found in patients with node positive premenopausal operable breast cancer that women with high progesterone levels have a survival advantage over those with low levels. The value in the luteal phase is hardly measurable as breast cancer is often associated with irregular menstrual cycle. Plu-Bureau's et al. [29] results in premenopausal women with benign breast disease did not support the hypothesis that progesterone might increase the breast cancer risk (Oestrogene plus progesterone hypothesis). Our findings on the lower level of pregnanediol in postmenopausal breast cancer patients suggest the possibility that the balance between the oestradiol and progesterone level has a role in postmenopausal patients 'unopposed oestradiol hypothesis' too, or it can be consider that low progesterone levels in patients is an early consequence of breast cancer.

Our other finding that the level of THE, a principle urinary metabolite of cortisol is increased in node positive patients supports the possibility that glucocorticods are involved in metabolic changes in node positive breast cancer patients, too. This possibility was proposed by López-Boado et al. [10], when they found in T-47/D cells that the Zn-α_2-glycoprotein, a major breast cyst fluid protein belonging to the immunoglobulin superfamily and secret-

Table 4. Mean values and standard error of mean of the steroid levels in urine in patients and control women

Steroid	Mean (μmol/24 h) ± S.E.M.		Steroid	Mean (μmol/24 h) ± S.E.M.	
	Patient	Control		Patient	Control
A	2.50 ± 0.49	2.08 ± 0.43	11OPT	0.26 ± 0.12	0.10 ± 0.02
E	3.20 ± 0.65	2.54 ± 0.44	n 5PT	0.38 ± 0.08	0.51 ± 0.10
AD	0.14 ± 0.03	0.35 ± 0.12	THE	6.79 ± 0.82	9.58 ± 1.51
DHEA	0.45 ± 0.08	0.49 ± 0.09	THA	1.11 ± 0.24	0.82 ± 0.19
n 5AD	0.76 ± 0.55	0.39 ± 0.28	THB	0.96 ± 0.21	0.94 ± 0.18
11-OA	0.94 ± 0.22	1.04 ± 0.30	aTHB	0.86 ± 0.09	1.32 ± 0.20
11-OHA	2.35 ± 0.37	2.30 ± 0.42	THF	2.74 ± 0.34	3.86 ± 0.66
11-OHE	0.96 ± 0.20	0.92 ± 0.19	aTHF	2.45 ± 0.57	2.91 ± 0.50
16-OHD	1.14 ± 0.15	1.74 ± 0.32	αCL	1.94 ± 0.25	2.98 ± 0.55
PD	0.47 ± 0.06	0.74 ± 0.12	βCL	1.79 ± 0.25	2.39 ± 0.36
PT	0.83 ± 0.13	0.88 ± 0.15	αC	0.87 ± 0.14	1.12 ± 0.19
n 5PD	0.29 ± 0.06	0.27 ± 0.05	F	0.66 ± 0.11	0.88 ± 0.16
n 5AT	0.62 ± 0.11	0.83 ± 0.15	6βF	1.36 ± 0.32	1.57 ± 0.31
THS	0.43 ± 0.07	0.60 ± 0.10	20αF	0.71 ± 0.26	0.30 ± 0.07

ed by some breast carcinomas, was strongly induced by glucocorticoids and androgens in T-47/D breast cancer cells.

This study gives some information on the metabolic behaviour of breast cancer patients, but gives no answer whether the changes are a cause or a consequence of breast cancer.

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