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Urinary Epidermal Growth Factor Excretion and Breast Cancer Risk

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Abstract—*The amount of urinary epidermal growth factor (EGF) excreted was determined in 350 normal women of whom 37 subsequently developed breast cancer. These were a group of women selected on a case-control basis from 5000 volunteers who had participated in a prospective epidemiological study.*

Urinary EGF excretion was not correlated with known risk factors such as age at menarche or menopause, age at first or last full-term child or parity. Neither was it associated with day or length of menstrual cycle, breast mammographic parenchymal pattern or the blood concentration of prolactin, dehydroepiandrosterone or its sulphate ester.

Univariate analysis indicated that the amount of urinary EGF was significantly correlated with urinary creatinine ($P < 0.001$), *age* ($P < 0.001$), *urinary androsterone* ($P < 0.02$) *or aetiocholanolone* ($P < 0.02$), *height* ($P < 0.05$) *and weight* ($P < 0.05$). *However, multivariate analysis showed that the amount of urinary EGF was correlated only with creatinine excretion* ($P < 0.001$) *and age* ($P < 0.001$) *and that the significance of the other correlations were probably due to the confounding influence of creatinine.*

INTRODUCTION

THE ROLE of autocrine or paracrine secretion of growth factors in the aetiology of breast cancer is unknown. Epidermal growth factor (EGF) is a potent mitogen and its receptor has been implicated as a possible mediating factor in the autocrine or paracrine control of tumour progression [1]. The amount of EGF-receptor has also been shown to be related to the clinical course of breast cancer [2, 3]. Both EGF and transforming growth factor alpha (TGF-α) share the same receptor and both have similar, although not identical, spectra of biological activities [4, 5]. However, the fact that EGF is found in relatively large quantities in breast cyst fluid (250 ng/ml) and human milk (100 ng/ml) [6] makes it reasonable to assume that EGF is an important ligand for breast EGF receptors. Such considerations have led to the suggestion that EGF could be involved in paracrine or autocrine regulation of cellular proliferation [6].

Although it has been reported that urinary EGF levels are elevated in women with breast cancer [7] there is no information regarding EGF and breast cancer risk. EGF is present in measurable quantities in human urine [8, 9], although its origin is uncertain, and in view of this paucity of information it seems worthwhile to examine the relationship between urinary EGF levels and breast cancer risk. The purpose of this study was, therefore, to determine the amounts of urinary EGF in normal women for whom risk factors, such as reproductive history [10], urinary androgen metabolite excretion [11] and prolactin [12], were known and also in a group of women who developed breast cancer subsequent to their urine being collected.

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MATERIALS AND METHODS

Subjects

The subjects were 350 normal female volunteers living on the island of Guernsey and were a subset from a 5000-woman cohort (see [13]). All had collected a 24 h urine specimen and answered a detailed questionnaire. All urine specimens were stored at −20°C until analysis. Of these 350 women, 37 had breast cancer diagnosed 1–105 months after collection of their urine specimens. The women were selected on a case-control basis with about eight

Table 1. *Characteristics of subjects*

	All volunteers $n = 350$			Pre-cancers $n = 37$		
	Mean	Median	Range	Mean	Median	Range
Age (years)	47.2	45.5	34–76	49.6	51.0	35–66
Weight (kg)	64.7	64	44–102	66.5	65	52–94
Height (cm)	160.7	161	145–179	161.9	163	149–173
Parity	2.25	2	0–8	1.92	2	0–8

controls per case. Details of both of these groups of women are given in Table 1.

Assay of EGF

EGF concentration was determined using a double-antibody radioimmunoassay method described by Gregory *et al.* [8] except the antibody-bound EGF was separated from unbound EGF using a solid-phase second antibody (Sac-Cel, Wellcome Reagents Ltd). The inhibition curves for urine specimens were parallel to those obtained for standard EGF. The specificity of the antibody was satisfactory, there being no cross-reaction with human growth hormone or insulin-like growth factor-1 [6]. The cross-reaction with TGF-α was negligible (<0.001%; personal communication from Dr. H. Gregory). The inter- and intra-assay coefficients of variations were less than 10%.

The results of urinary EGF excretion obtained in this study generally agree with those in the literature. Thus Gregory *et al.* [8] reported a mean figure of 782 ± 253 (S.D.) ng EGF/kg body wt/24 h compared with an average in this study of 761 ± 340. Relative to creatinine excretion we found an average of 57.6 ± 18.2 (S.D.) μg EGF/g creatinine compared with reported mean values of 70 [14], 34 [15] and 29.7 [9]. In terms of ng EGF excreted per ml of urine there have been reports of 30 [8], 32 [15], 47 [6] and 83 [14] compared to 34.8 in this report. In the last two comparison (i.e. μg EGF/g creatinine and ng EGF/ml urine) some of these figures were obtained on spot urine specimens.

Hormone assays

Blood prolactin levels were assayed using a double-antibody radioimmunoassay [16]. Urinary androsterone and aetiocholanolone were measured by gas-liquid chromatography [17] and blood dehydroepiandrosterone and its sulphate ester levels were determined by radioimmunoassay [18]. Urinary androsterone and aetiocholanolone levels were measured in 172 of the volunteers. Blood steroid levels were known for 155 subjects.

Wolfe grade

The parenchymal pattern of the individual mammograms was categorised as being either N1, P1, P2 or DY according to the criteria of Wolfe [19, 20].

Statistics

Data were examined firstly using univariate analysis of each of the observed factors and, secondly, by a multivariate approach. In the former case the relation of EGF and these variables were based upon either linear regression (e.g. age and EGF excretion) or, where appropriate, rank correlation (e.g. parity and EGF excretion).

RESULTS

Univariate analysis

There was a highly significant positive correlation between urinary EGF excretion and creatinine or age (Table 2) and a highly significant negative correlation between EGF and age. A significant positive correlation between the levels of excretion of the androgen metabolite androsterone (or aetiocholanolone) and EGF was also found. The correlations between EGF excretion and height, or weight, reached formal significance (Table 2).

No significant correlations were found between EGF excretion and the reproductive variables age at menarche or menopause, parity and menstrual cycle status (Table 2). The average excretion of EGF was similar for parous and nulliparous women. Parenchymal mammographic patterns as defined by Wolfe [19, 20] were not related to EGF excretion. There was no correlation between EGF and blood levels of prolactin, dehydroepiandrosterone or its sulphate ester, even though there is a highly significant correlation between these latter blood steroids and their urinary metabolites, androsterone and aetiocholanolone.

Multivariate analysis

Most of the significant correlations found by univariate analysis were almost certainly the result of the confounding influence of urinary creatinine

Table 2. Univariate correlations of urinary EGF

Variable	r	P
Creatinine (mg/24 h)	0.73	<0.001
Age (years)	−0.37	<0.001
Urinary androsterone (mg/24 h)*	0.36	<0.02
Urinary aetiocholanolone (mg/24 h)*	0.31	<0.02
Weight (kg)	0.18	<0.05
Height (cm)	0.17	<0.05
Parity	Non-significant	
Age at menarche	Non-significant	
Age of menopause	Non-significant	
Age at first baby	Non-significant	
Age at last baby	Non-significant	
Day of menstrual cycle	Non-significant	
Length of menstrual cycle	Non-significant	
Wolfe grade pattern	Non-significant	
Dehydroepiandrosterone (ng/ml blood)†	Non-significant	
Dehydroepiandrosterone sulphate (μg/ml blood)†	Non-significant	
Prolactin (ng/ml)	Non-significant	

The number of observations was 350 except for variables denoted *(n = 172) and †(n = 155).

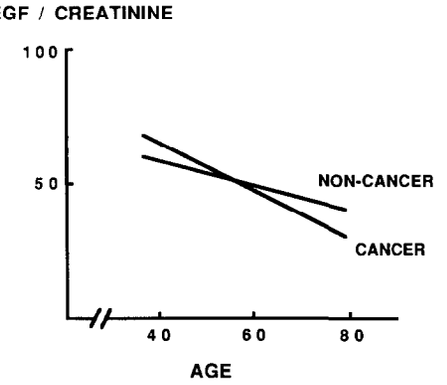

Fig. 1. Linear correlation of the ratio of EGF to creatinine excretion and age. The linear regression equation (regression coefficient and significance) for the non-cancer group (n = 313) *was* y = 81.3−0.495x (r = −0.231, P < 0.02) *and for the pre-cancer group* (n = 37) *was* y = 105−1.030x (r = −0.525, P < 0.001), *where* y *is the ratio of EGF* (μg/24 h) *to creatinine* (g/24 h) *excretion and* x *is age* (*years*).

excretion and that the only significant correlation was between the excretion levels of urinary EGF, creatinine and age ($\chi^2 = 50.1$; $P \ll 0.001$; $\chi^2 = 8.65$; $P \ll 0.001$, respectively).

The linear regression curves for the ratio EGF/creatinine excretion on age for women who were diagnosed as having breast cancer subsequent to urine collection was similar to that for the unaffected population ($\chi^2 = 0.22$, N.S.) (Fig. 1).

DISCUSSION

Studies on human breast cancer cell lines have shown that EGF is a powerful factor in influencing the proliferation of these cells [21–23]. Studies by Sainsbury *et al.* [2, 3, 24] have shown that the presence of EGF-receptors in the tumours of breast cancer patients was related to prognosis. In spite of the possible importance of EGF in the aetiology and clinical course of breast cancer there appears to be no relationship between the urinary excretion of EGF and any breast cancer risk factor. Furthermore, women who subsequently developed breast cancer had normal levels of urinary EGF. The positive correlation with androsterone and aetiocholanolone was of particular interest since EGF synthesis by the mouse submaxillary gland is stimulated by androgens [25]. However, the correlation was completely explicable as being due to the confounding effect of creatinine. The only significant correlation was between the amounts of urinary EGF, creatinine and age, confirming the results of others [9, 26].

Whether the amounts of urinary EGF reflect circulating levels of this growth factor in humans is unclear. Evidence from the mouse indicates that most, if not all, urinary EGF originates from synthesis in the distal tubules of the kidneys [27–29] and that negligible amounts of intravenously administered radioactively labelled EGF are found in the urine [30]. However, Gregory *et al.* [8] reported that in human blood-borne radioactive EGF passes very rapidly into urine. How good the mouse is as a model for man is difficult to assess especially if one considers that the concentration in EGF in human submandibular gland is 0.0002% of that found in mouse [31]. Mattila *et al.* [14] reported that the amount of urinary EGF in children rises to a peak in their second year; an age which does not coincide with any known changes in renal development. However, it has been reported that subnormal concentrations of urinary EGF are found in patients with kidney disease [15].

The results indicate that urinary EGF levels are not related to many of the known risk factors for breast cancer. Furthermore, pre-cancer patients

did not display any differences in EGF excretion compared to controls. Thus the determination of urinary EGF excretion is unlikely to be of value as a measure of breast cancer risk.

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