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Breast Duct Fluid Dehydroepiandrosterone Sulphate in Fibrocystic Disease

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Abstract—*We studied the DHAS concentrations in breast duct fluids obtained by nipple aspiration from 73 healthy non-lactating women and 23 women with gross cystic disease (GCD) of the breast. The presence of DHAS in breast fluid is age-related: no DHAS could be detected in breast fluid in 50% of healthy subjects aged 20–30 years and in 29% of healthy subjects aged 31–40 years. All healthy subjects aged 41–50 years showed DHAS in breast fluid. All but two fluids obtained from GCD patients (age range 20–40 years) contain DHAS. Moreover the DHAS concentration in this group was higher than in age-matched controls* ($P < 0.01$). *Higher DHAS levels in GCD patients may thus be regarded as a factor modifying the mammary hormonal environment, possibly involved in the clinical course of the disease.*

INTRODUCTION

BREAST secretions [1] and breast cyst fluid [2, 3] have already been found to contain remarkably high levels of dehydroepiandrosterone sulphate (DHAS). Interestingly, fluids obtained from human breast cysts lined by apocrine epithelium had higher DHAS levels than those lined by flattened epithelium [4]. It has also been stated that dehydroepiandrosterone (DHA) is secreted as a sulphate by the axillary apocrine glands [5], while the ultrastructural evidence makes it clear that metaplastic breast apocrine epithelium is similar to normal axillary apocrine tissue [6]. DHAS levels have thus been proposed as an index of breast apocrine activity [4]. Aprocrine metaplasia has been suspected to be related to factors which predispose towards breast cancer, since apocrine metaplastic transformation of the columnar epithelium is more common in Western than Japanese women (with less frequent breast cancer) [7], and apocrine cysts are more common in breasts of high-risk cancer patients [8, 9].

Breast tissue may carry out metabolic transformation of adrenal androgens [10, 11] and hypotheses involving DHAS and its metabolites to explain the development of breast cancer have been proposed [12–14].

This study was undertaken to determine if the DHAS content of breast secretions and apocrine metaplasia are related to fibrocystic disease.

MATERIALS AND METHODS

We studied 138 healthy non-lactating women, aged 20–50 years, with no history of breast disease, and 45 women aged 21–40 years, with gross cystic disease (GCD) of the breast [15]. GCD was diagnosed on the basis of clinical examination (two or more palpable cysts), Rx mammographic and echotomographic features (cyst dia. ≥ 3 mm). In the majority of cases, the diagnosis was confirmed by microscopic examination of the aspirate.

Breast duct fluid was obtained by nipple aspiration [1, 16] from 53% of subjects who entered this study (73 healthy and 23 patients with GCD). None of these subjects had received oral contraceptives or other hormone preparations for at least 9 months. In 42 subjects it was obtained in the follicular phase of the cycle, in the remaining 54 in the luteal phase.

The volume of secretions obtained ranged from 5 to 30 μl and was measured by collection in a calibrated capillary tube. The samples were stored in phosphate buffer at −20° C until assayed. In 30% of subjects, bilateral secretions were obtained and in these cases, since DHAS concentrations were similar in the fluids from the two breasts ($r = 0.882$; $P < 0.001$), the mean value was considered.

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DHAS was determined directly in diluted fluid by RIA using an antibody supplied by BioMerieux (Charbonnières-les-Bains, France). Unconjugated DHA cross-reacted completely (100%), androsterone sulphate cross-reacted appreciably (22%), whereas a number of other steroids and sulphate conjugates showed minimal or no detectable cross-reactivity (< 0.02%). The intra-assay error, measured as the coefficient of variation, was 11%.

In five breast secretions, DHAS was determined as DHA after solvolysis and liquid chromatography. Samples were heated to 120° C for 90 min at pH 4.5 to hydrolyse DHAS [17]. After cooling, the hydrolysate was extracted with ethyl ether. The residue after evaporation of ethyl ether was redissolved with 0.3 ml of isooctane–ethylacetate (96 : 4) and chomatographed on celite–ethyleneglycol 2 : 1 (w/v) microcolumns, using isooctane–benzene (96 : 4) as mobile phase for the DHA fraction [18, 19]. RIA was performed on DHA chromatographic fraction using the same antiserum employed for DHAS determination and DHA to construct the standard curve. In each case, if consideration is given to the losses during solvolysis and the different molecular weight of the two steroids, levels detected were about 25% lower and directly correlated to DHAS RIA values ($r = 0.988$).

To rule out the possible presence of agents in breast secretions interfering with the assay by masking detectable levels of DHAS, we added the steroid in amounts equivalent to 5000 μg/dl in five samples without detectable DHAS and 20,000 μg/dl in another five samples. The concentrations measured by RIA were 4825 ± 290 μg/dl and 19,500 ± 1200 μg/dl, respectively (mean ± S.D.).

Statistical analyses were performed with the chi-square test, the non-parametric Mann–Whitney *U*-test and, for the correlation, the usual least squares method.

RESULTS

In 30% of normal subjects, no DHAS could be detected in breast secretion, while in the remaining 70% its concentrations were distributed in a wide range from 1 to 300 times that of plasma (mean ± S.D. = 7332 ± 7026 μg/dl; median value = 5400 μg/dl). No correlation was found between DHAS concentration and the volume of secretion. On dividing the normal subjects into age groups, we observed that the presence of DHAS in breast fluid is age-related. No DHAS could be detected in breast fluid from 50% of healthy subjects aged 20–30 years and from 29% of subjects aged 31–40 years. All normal subjects aged 41–50 years showed DHAS in breast fluid (Fig. 1). The chi-square test showed a significant difference between age groups 20–30 and 41–50 years ($P < 0.005$) and between age groups 31–40 years and 41–50 years

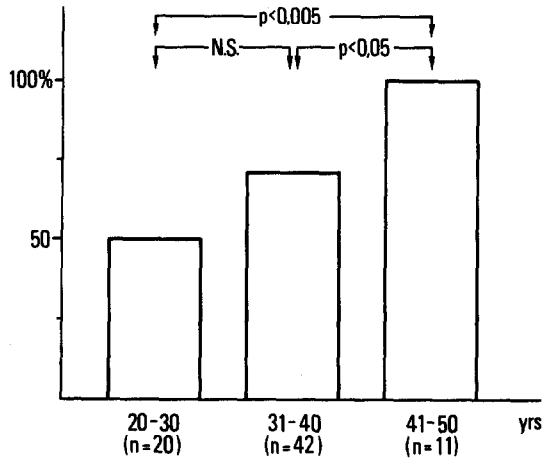

Fig. 1. Percentage of normal subjects with detectable levels of DHAS in breast fluid in different age groups.

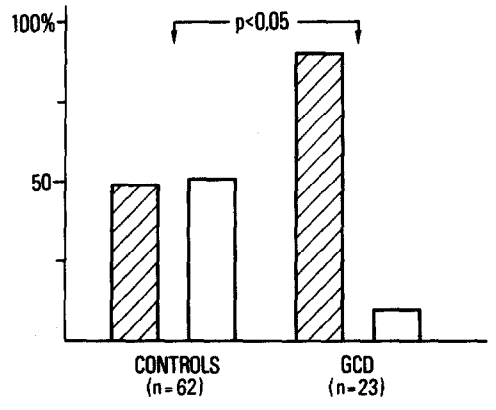

Fig. 2. Percentage of subjects with detectable (shaded bars) and undetectable (outlined bars) levels of DHAS in breast fluid in GCD and age-matched controls (age range 20–40 years).

($P < 0.05$), whereas the difference was not significant between the 20–30 and 31–40 years groups. No difference was found when the subjects were clustered by parity, lactation or menstrual cycle phase.

DHAS was detectable in all but two fluids from GCD patients (age range = 20–40 years; mean ± S.D. = 34 ± 5 years). The percentage of subjects with detectable levels of DHAS in breast fluid was higher in patients with GCD than in age-matched controls (age range = 20–40 years; mean ± S.D. = 33 ± 5 years) ($P < 0.05$) (Fig. 2). DHAS values in GCD patients from this age group (median = 15,447 μg/dl; mean ± S.D. = 20,407 ± 16,590 μg/dl) were also higher than in age-matched controls (median = 4800 μg/dl; mean ± S.D. = 6941 ± 6794 μg/dl) ($P < 0.01$) (Fig. 3).

DISCUSSION

Our mean DHAS concentration in breast fluid is similar to that found by Miller and Forest [20]. Nevertheless, in our study, 50% of the normal subjects in the 20–30 year age group presented

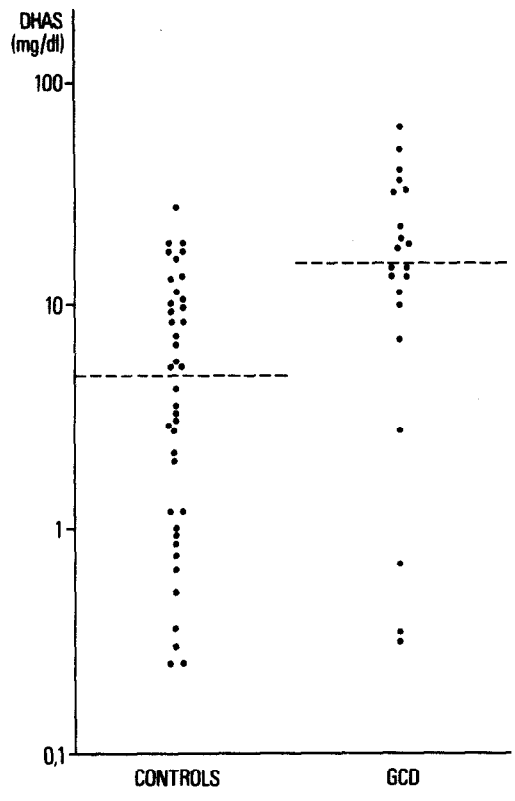

Fig. 3. DHAS concentrations in breast fluid of patients with GCD and age-matched controls (age range 20–40 years; P < 0.01).

undetectable levels. This is not surprising, if DHAS levels are an index of breast apocrine activity [4], because it is conceivable that the majority of young subjects lack this differentiation of mammary epithelium. On the other hand the finding that the number of subjects with no DHAS in breast fluid decreases with age suggests that apocrine metaplasia is a very common age-related phenomenon in breast tissue. Thus, it seems unlikely that apocrine metasplasia *per se* is a risk factor for breast cancer.

Nevertheless, the higher incidence of apocrine metaplasia in GCD cannot be neglected. Our data on GCD patients extend our previous observations [21] and demonstrate that the percentage of subjects with detectable levels of DHAS in breast fluid is higher in patients with GCD than in age-matched controls and that these patients also have higher values, despite the high variability of this parameter. This finding may be an expression of the greater extent of apocrine metaplasia in women with GCD.

Recent studies have demonstrated that the histopathologic findings, because of the high degree of heterogeneity seen in benign breast diseases, do not tell the whole story of risk for breast cancer [22]. In addition to the histological findings, the evaluation of intramammary steroid hormones may yield more meaningful information. Higher DHAS levels in GCD patients may thus be regarded as a factor modifying the mammary hormonal environment. Breast tissue undergoes marked changes in growth as a response to hormone stimulation. Evidence has been accumulated that it can convert DHAS into physiologically active metabolites [23, 24] and molecules that act as regulators of oestrogen–receptor interaction [25] or may influence the action of oestrogen by modifying oestradiol metabolism [26].

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