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Levels of Eighteen Non-conjugated and Conjugated Steroids in Human Breast Cyst Fluid: Relationships with Cyst Type

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Abstract—*The present study investigates the levels of a large series of* 18 *non-conjugated or conjugated steroids in* 71 *samples of human breast cyst fluid (BCF) as divided into three groups corresponding to different electrolyte composition. In the type* 1 *group, the* K^+/Na^+ *ratio was higher than* 1.5, *while in type* 2 *it was lower than* 0.66 *and finally type* 3 *had an intermediate ratio. Pregnenolone (PREG) and progesterone (PROG) levels were approximately* 2*-fold higher* ($P < 0.05$) *in the type* 2 *than in the type* 1 *group while both* 17*-OH-pregnenolone (17-OH-PREG) and* 17*-OH-progesterone (17-OH-PROG) concentrations were similar in these two groups. Most of the C-*19 *steroids analyzed, namely dehydroepiandrosterone sulfate (DHEAS), androst-5-ene-*3β,17β*-diol (5-ene-DIOL), testosterone (TESTO), dihydrotestosterone (DHT), androstane-*3α,17β*-diol (*3α*-DIOL), androsterone (ADT), androstane-*3α,17β*-diol glucuronide (*3α*-DIOL-G) and androsterone glucuronide (ADT-G) were* 180–360% ($P < 0.05$) *higher in type* 1 *than in type* 2 *cysts while no difference in C-*18 *steroid and C-*18 *steroid glucuronide levels was observed. A small or no difference was seen in steroid levels between types* 2 *and* 3. *We conclude that the arbitrary division according to the electrolyte composition of BCF permits identification of different patterns of steroid concentrations in BCF. However, the mechanism responsible for both sets of parameters remain unclear.*

INTRODUCTION

While breast cysts by themselves are not considered to be precancerous lesions, they have been associated with a higher risk for bearers of developing breast cancer [1–3]. With the hypothesis that benign and malignant breast disease could have some common pathogenic factors, the analysis of breast cyst fluid (BCF) could well provide information about the environment of the breast tissue regarding focal lesions prone to malignant transformation.

Among the major observations so far available from BCF analysis, the classification of cysts on the basis of electrolyte composition has received particular attention [4–6]. Indeed, the BCF levels of K^+, which are inversely correlated with those of Na^+, have been found to be asymmetrically distributed from the mean values, thus suggesting that different populations of cysts exist. Furthermore, it has been pointed out that the two major subgroups of cyst fluid may reflect different sources of constituents or differences in the secretory activity of the epithelium lining the cysts [6, 7].

With respect to the steroid in BCF, only a few reports have so far appeared despite the presence of estrogen, progesterone, and androgen receptors in breast tissue and the major importance of steroids in the development and growth of this tissue. High intracystic concentrations of androgens and estrogens suggest that BCF may be able to accumulate steroids and conjugated steroids [2, 5, 8, 9]. The observation that dehydroepiandrosterone sulfate (DHEAS) levels were strongly correlated with K^+ concentrations [6] further supports the interest of the examination of the steroidal composition of this cyst. In order to achieve a better knowledge in this regard, we have analyzed a large number of steroids and conjugates in BCF and pointed out the differences in their levels between groups categorized according to the electrolyte composition.

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MATERIALS AND METHODS

The subjects included 71 women, aged 29–50, who had undergone needle aspiration of breast cysts, according to the technique of Zajicek [10]. The possibility of cancer was excluded by echography, mammography and repeated clinical examination. In order to gain homogeneity in terms of the hormonal status, aspiration was carried out during the early and mid-follicular phases of the menstrual cycle. After aspiration, gross cyst disease fluid was stored at −20°C until assayed. The volume of BCF specimens varied from 2 to 30 ml.

Sodium and potassium levels were measured by flame photometry as previously reported [6]. The steroids were determined by procedures originally devised for the analysis of steroids in plasma [11–13]. Intra-assay variation analysis was less than 16% and to minimize inter-assay variation, all breast cyst samples were assayed simultaneously. DHEAS was measured using a procedure orginally devised for the determination in plasma [11] while steroid glucuronide analysis involved an extraction followed by treatment with β-glucuronidase and column chromatography [11]. All radioimmunoassay results are shown as the means ± S.E.M. of triplicate determinations on individual samples. Radioimmunoassays were calculated according to Rodbard and Lewald [14] and statistical significance was measured according to the multiple-range test of Duncan-Kramer [15].

RESULTS

As shown in Fig. 1, the cysts were divided into three groups, depending upon the ratio of K^+/Na^+ in BCF. In the type 1 group, the ratios K^+/Na^+ were higher than 1.5, while in type 2, values were lower than 0.66. According to this classification [6], 32 cysts were classified as type 1, 27 as type 2 and, finally, seven women who had an electrolyte ratio between 0.66 and 1.5 were classified as members of the type 3 group.

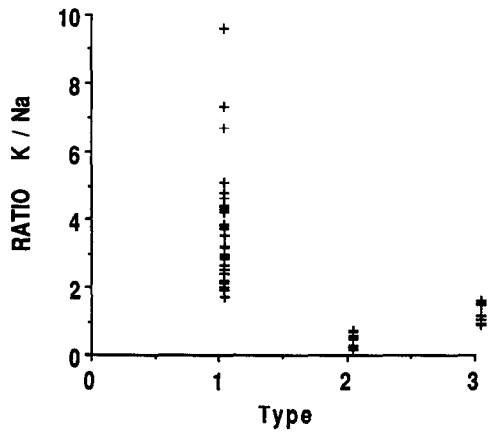

Fig 1. *Ratio of* K^+/Na^+ *in human breast cyst fluid. Cyst divided in three types according to their cationic concentration: type* 1 ($K^+/Na^+ > 1.5$), *type* 2 ($K^+/Na^+ < 0.66$) *and type* 3 ($K^+/Na^+ > 0.66 < 1.5$).

Table 1 illustrates the levels in BCF of a series of 18 non-conjugated steroids, steroid sulfates and steroid glucuronides in the three groups of cysts. It can be seen that PREG and PROG levels were approximately 2-fold higher ($P < 0.05$) in the type 2 than in the type 1 group while both 17-OH-PREG and 17-OH-PROG concentrations were in the same range for these two groups. However, in type 1, DHEAS levels exceeded, by approximately 3-fold, those found in type 2 while intermediate values were found in type 3. It can also be seen that the type 1 also showed a general trend toward an elevation of all C-19 steroids measured. In fact, 5-ene-DIOL, TESTO, DHT, 3α-DIOL, ADT, 3α-DIOL-G and ADT-G concentrations were 180–360% ($P < 0.05$) more elevated in type 1 than in type 2 cysts while DHEA and 4-ene-dione were in the same range for both groups. There were minor or no differences in steroid levels between groups 2 and 3.

The correlations between the concentrations of some steroids in BCF of type 1 and type 2 were next examined. There was no correlation between the C-21 steroids themselves nor between the C-21 steroids and the C-19 steroids (data not shown). However, the C-19 steroids in BCF were, for the large majority, significantly correlated between themselves in both groups. This is well illustrated by the statistical relationship betwen DHEAS and ADT-G in both type groups (Fig. 2). The correlation between C-19 steroids is further illustrated by the relationship between 3α-DIOL-G and DHT as well as ADT-G and 3α-DIOL-G which are expressed by linear regressions as shown in Figs. 3 and 4. While estrone and its glucuronide as well as E_2 and its glucuronide were significantly correlated (data not shown), there was no relationship between C-18 steroids and C-19 steroids nor between C-18 steroids and C-21 steroids.

DISCUSSION

Based on the observation that the levels of electrolytes in the BCF are not symmetrically distributed, several authors have suggested different subtypes of breast cyst fluid. According to Dogliotti's classification [6], we have been able to identify two main groups of cysts, namely, type 1 and type 2, with respectively a high and low K^+/Na^+ ratio, with a small number of intermediate values (type 3). Furthermore, our data extend the observations of Dogliotti *et al.* [6] demonstrating a clear separation of DHEAS levels between type 1 and 2. In addition, the present study includes a determination of a much larger series of steroids and indicates that BCF accumulates a large number of non-conjugated and conjugated steroids. From the present results,

Table 1. *Distribution of steroid concentrations* (nM ± *S.E.M.*) *in breast cyst fluid according to* K^+/Na^+ ratio

Steroids	K^+/Na^+ ratio Type 1	Type 2	Type 3
Pregnenolone	24.8 ± 2.1	55.1 ± 9.2*	29.1 ± 3.5
17-OH-Pregnenolone	1.1 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
Progesterone	25.0 ± 3.2	44.2 ± 3.8*	36.7 ± 7.2
17-OH-Progesterone	1.3 ± 0.1	1.5 ± 0.1	1.2 ± 0.2
Dehydroepiandrosterone sulfate	12,555 ± 1667	4747 ± 870*	10,992 ± 4356
Dehydroepiandrosterone	36.9 ± 4.2	29.4 ± 2.8	35.6 ± 9.3
Androst-5-ene-3β,17β-diol	7.6 ± 0.9	4.2 ± 0.6*	5.9 ± 1.6
Androstenedione	7.5 ± 0.9	6.0 ± 0.6	8.9 ± 1.8
Testosterone	3.6 ± 0.2	2.5 ± 0.2*	3.0 ± 0.4
Dihydrotestosterone	3.1 ± 0.3	1.9 ± 0.2*	2.5 ± 0.4
Androstane-3α,17β-diol	3.9 ± 0.5	2.3 ± 0.3*	2.6 ± 0.3
Androsterone	9.3 ± 1.2	3.5 ± 0.4*	6.1 ± 1.5
Androsterone-3α,17β-diol glucuronide	15.8 ± 1.8	7.5 ± 1.6*	8.4 ± 1.6
Androsterone glucuronide	235 ± 37	65 ± 11*	66 ± 15*
Estradiol	0.18 ± 0.02	0.16 ± 0.01	0.22 ± 0.04
Estrone	0.68 ± 0.10	0.50 ± 0.09	0.71 ± 0.02
Estradiol glucuronide	0.07 ± 0.09	0.11 ± 0.02	0.11 ± 0.08
Estrone glucuronide	0.17 ± 0.01	0.17 ± 0.02	0.09 ± 0.02

*$P < 0.05$.

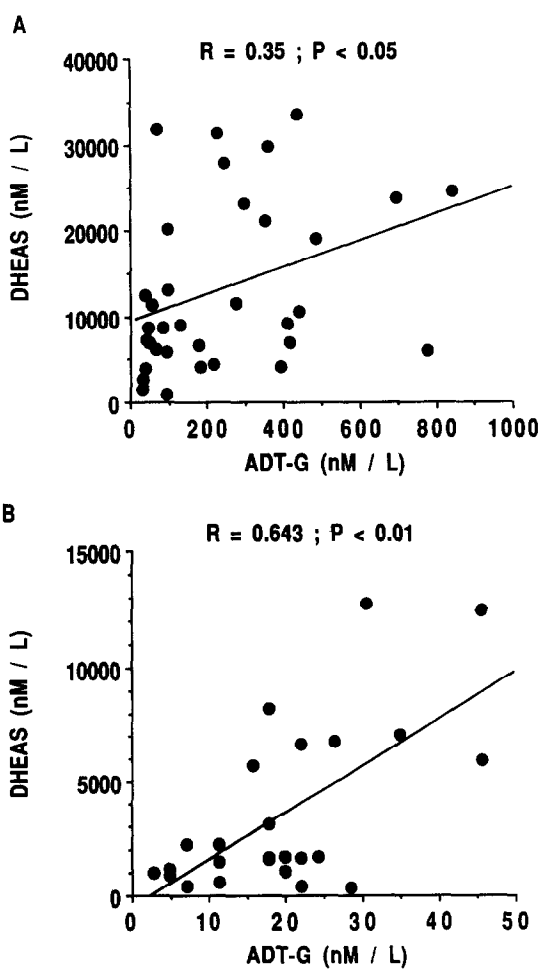

Fig. 2. *Correlation between DHEAS and ADT-G levels in breast cyst fluid. A, Type* 1. *B, Type* 2.

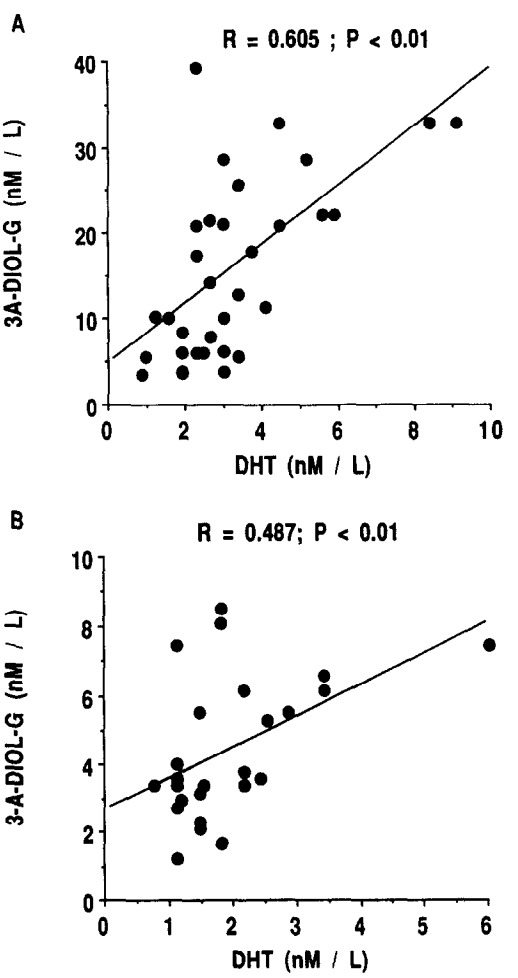

Fig. 3. *Correlation between* 3α-*DIOL-G and DHT levels in breast cyst fluid. A, Type* 1. *B, Type* 2.

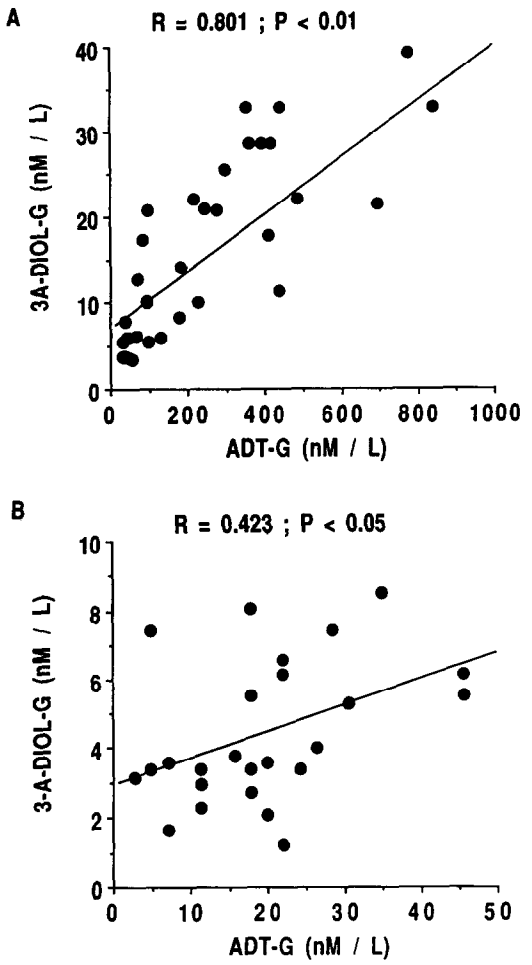

Fig. 4. Correlation between 3α-DIOL-G and ADT-G levels in breast cyst fluid. A, Type 1. B, Type 2.

it appears that the C-21 steroids, namely PROG and PREG, can be selectively elevated in type 2 while C-19 steroids are at higher concentrations in type 1 BCF. With regard to C-18 steroids, we did not find significant differences between the two major types of cysts. Due to the differences in the molecules examined, our data cannot be viewed as conflicting with those of recent reports which have focused on the occurrence of higher concentrations of estriol-3-sulfate and estrone-3-sulfate in type 1 cysts [9, 16]. Unfortunately, except for DHEAS, steroid sulfates were not analyzed in our study due to the technical procedure used.

The accumulation of PREG and PROG in cyst fluid has been reported previously [8] and it was suggested that this phenomenon was due to protein binding permitting specific accumulation of these two C-21 steroids. In the present study, we have found the PREG and PROG levels are much higher in type 2 than in type 1 BCF, thus indicating that the progestin binding protein might be more elevated in the type 2. Such an observation is in agreement with the report by Rosner *et al.* [17] who found that proteins are more elevated in cyst fluid with high levels of Na^+ and, more specifically, they observed that the steroid binding proteins such as corticosteroid binding globulin (CBG) and sex hormone-binding globulin (SHBG) were of higher concentrations in those fluids. The presence of low concentrations of 17-OH-PREG as well as 17-OH-PROG in the three types of BCF suggests that C-21 steroid accumulation is limited to PREG and PROG, further supporting the presence of specific progestin binding components, as demonstrated by Pearlman *et al.* [18] and by Hagensen *et al.* [19].

Another interesting finding was the presence of high concentrations of ADT-G and 3α-DIOL-G in BCF and the relationship between these steroids and their C-19 steroid precursors, namely DHEAS and DHT. The concept of DHT formation in breast from DHEAS and the DHT conversion into the C-19 steroid glucuronides is in agreement with our previous observations [11–13] and those of others [8, 20, 21]. The significant relationship observed between DHEAS and ADT-G as well as the high levels of these conjugates strongly suggest that DHEAS is converted by the breast tissue into potent androgens. Indeed, it has recently been proposed by several authors [11–13, 20, 21] that circulating C-19 steroid glucuronides, namely ADT-G and 3α-DIOL-G, can be used as markers of peripheral androgen conversion.

Classification by electrolyte composition has also shown marked differences in C-19 steroid levels between groups. In contrast with the higher values of C-21 steroids in type 2, our data clearly demonstrate that type 1 cyst fluid can accumulate selectively C-19 steroids while the main androgen metabolite, ADT-G, is found 3.5-fold more elevated in type 1 than in type 2. The relatively small increase of the non-conjugated C-19 steroids in type 1 is thus amplified by the higher difference in ADT-G concentrations. Taken together, our data thus suggest that, in type 1 cyst fluid, high levels of androgens and metabolites are associated with the presence of high levels of K^+. While there may be separate mechanisms, the well known action of androgens in the breast [22] makes the present observation highly interesting and the relationship between these two parameters requires further exploration.

It is, however, important to note that despite the high levels of C-19 steroids in the type 1 BCF, the C-18 steroids measured by us were relatively unaffected in terms of non-conjugated as well as steroid glucuronides. While the presence of aromatase in the breast tissue is well known, the origin of estrogens in this tissue is, however, not well established. The present data suggest a low aromatase activity in the environment of the BCF and the

possibility of a major contribution of estrogens in the cyst fluid from the circulating blood cannot be excluded.

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