Increased Androgenic Activity and Breast Cancer Risk in Premenopausal Women

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ABSTRACT

Blood and urine specimens from 27 premenopausal breast cancer patients and 62 healthy controls have been compared with respect to concentration of testosterone and progesterone in blood and of testosterone and androstanediol in urine, measured in the luteal phase of the menstrual cycle. There was a strong positive association between the concentration of the two androgens, either in blood or urine, and breast cancer risk. A strong association was also observed with decreasing levels of progesterone. The association was statistically significant (p for trend less than 0.01) for each hormone; the rate ratios were 10.2 for serum testosterone (highest category), 5.6 for serum progesterone (lowest category), 8.4 for urinary testosterone (highest category), and 5.2 for androstanediol (highest category). The rate ratio for women presenting both high serum testosterone and low progesterone was 21.8 (4.1 to 116.1). Considering the exposure to at least one of three androgens at the highest level and low progesterone, the rate ratio was as high as 90.2 (8.2 to 959.7). This study provides evidence for the hypothesis that increased androgenic activity is an important risk indicator for breast cancer, particularly when associated with anovulation, as indicated by low serum progesterone level.

INTRODUCTION

Data on hormonal measurements on a group of breast cancer patients and healthy controls were published recently by some of us (11, 12). The results of these studies support the hypothesis, originally proposed by Grattarola (3, 5) and Grattarola et al. (6, 7), that anovulation and increased ovarian androgen secretion are a predisposing factor toward the development of breast cancer. This set of data was available for further elaboration, and a more detailed epidemiological analysis is now presented.

MATERIALS AND METHODS

Our case series comprises 27 premenopausal, newly diagnosed and histologically confirmed breast cancer patients who were admitted between January 1980 and December 1981 to the Istituto Nazionale Tumori, Milano, Italy. The controls were 62 premenopausal healthy women who voluntarily underwent a physical examination of the breast at the same hospital and during the same period of time. The series include 4 cases and 7 controls not comprised in the previously published reports. Information on constitutional and reproductive factors, surgical operations, drug use, and previous diseases were collected on each patient and control by the physicians attending the Outpatient Department. Only persons who did not receive digital, antihypertensive, psychotropic, or hormonal therapy (including oral contraceptives) during the preceding 6 months and who were not bilaterally oophorectomized were requested to enter the study. None of them had ever had malignant neoplasms outside the breast or severe endocrine or metabolic diseases or had received antineoplastic chemotherapy. All of the potential controls affected by a benign mammary condition were excluded. No information was recorded on women who were excluded or who refused to participate. A sample of 20 ml of peripheral venous blood and a complete 24-h urine collection were obtained during the luteal phase of the cycle, namely, between the 20th and 23rd day after the beginning of the last menstrual period and, for cases, always before mastectomy.

A detailed description of the laboratory techniques for hormonal analyses has been published elsewhere (11, 12). TS, PG, TU, and adiol are considered in the present paper. For 4 cases and 15 controls, urine samples were not available. We estimated the incidence rate of breast cancer among women exposed to a specified risk indicator relative to the rate among nonexposed. The RRs associated with hormonal levels were computed on the assumption that the measurements in our specimens were a reliable index of an extended risk period preceding the illness. Statistical analysis was initially performed using classic methods for stratified data (9). Subsequently, a multiple logistic regression approach was used to compensate for the lack in statistical power due to the small size of the study and to simultaneously control for several potentially confounding factors and to evaluate interaction (2). The program package GLIM (1) has been used for this purpose. The contribution of each variable or interaction item included in the model was assessed by evaluating the maximum of the likelihood function in the presence and in the absence of the index item. The RRs are those expected on the basis of the logistic model. In all of the analyses presented, account has been taken of year of birth (by single year). Linear trends were computed on continuous variables. Statistical tests reflect 2-tailed probabilities.

RESULTS

Cases and controls did not significantly differ in age, age at menarche, age at first pregnancy, number of pregnancies, height, and weight.

Table 1 summarizes the results of the hormonal measurements on blood and urine. The limits of the 3 discrete categories were obtained from the frequency distribution of controls. As for androgens, the reference category corresponds to the distribution’s 3 lower quartiles; the lower limit of the 2 other categories are the 75th and 90th percentiles. For PG, the reference category corresponds to the 3 upper quartiles, while the 25th and the 10th percentiles are taken as the upper bounds of the 2 other categories. The estimated age-standardized RR for intermediate levels of TS was 2.6 (95% confidence interval, 0.8 to 10.9) and,

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The abbreviations used are: TS, serum testosterone; TU, urinary testosterone; adiol, 5a-androstan-3o,17|a-diol; PG, progesterone; IAA, increased androgenic activity; RR, rate ratio.
The RRs for 2 increasing levels of TU are 2.3 (0.5 to 9.6) and which, again, correspond to a highly significant trend (p = 0.003). The RRs for 2 increasing levels of TU are 2.3 (0.5 to 9.6) and 5.6 (1.6 to 20.0), and, for 2 increasing levels of adiol, the RRs are 2.0 (0.4 to 10.0) and 5.2 (1.4 to 19.9); these trends are both significant (p = 0.002 and 0.013, respectively).

Age was a substantial negative confounder of TS and TU. The prevalences of high (≥90th percentile) TS and of high TU among cases were nearly stable with increasing age, while the same figures were clearly decreasing for controls. Accordingly, the RRs for TS and TU were progressively greater toward menopause. Age was therefore always kept in the logistic equation. By contrast, age was neither a major confounder nor a modifier of the effect of PG and adiol. Adjusting for other possible confounders (age at first pregnancy, number of children, age at menarche, weight, and height) did not materially affect our estimates, and they were not further considered in the analysis.

Table 2 examines the relationship between TS and PG. Keeping the interaction factor in the equation, the age-standardized RR for exposure to low PG alone (≤25th percentile) is 4.2 (1.1 to 15.8); for exposure to high TS (≥75th percentile), it is 4.9 (1.0 to 23.6) and, for the 2 exposures combined, it is 21.8 (4.1 to 116.1). Thus, high TS levels appear to be associated with a substantially higher incidence of breast cancer in the presence of low levels of PG. The effect of standardizing each hormone by the other is shown on the margins: the RRs, 4.3 for PG and 5.1 for TS, did not appreciably differ from the corresponding crude ones (i.e., simply age-standardized). The RR expected, assuming a noninteractive (i.e., simply additive) effect of the combined exposures, is 8.4 (4.3 + 5.1 − 1.0); it is evident that our finding much more closely resembles the value expected on the basis of an interactive effect (i.e., at least multiplicative = 4.3 · 5.1 = 21.9).

Table 3 examines the relationship between serum and urinary levels of testosterone. The RR for high TS alone (≥90th percentile) is 9.9 (1.7 to 56.3); for high TU alone (≥90th percentile), it is 11.0 (1.8 to 66.5); and, for the combined exposures, it is 13.3 (1.8 to 100.9). Cross-standardization decreases the crude RR of TS from 8.0 to 5.0 (1.2 to 20.7) and of TU from 7.2 to 5.4 (1.3 to 22.8), which corresponds to about a 30% decrease in both cases. No interaction can be suggested; the RR expected on the assumption of a multiplicative effect (5.0 · 5.4 = 27.0) is much higher than the observed value. Similar results were obtained adjusting TS or TU for dichotomous levels of adiol and vice versa. A weak positive, although not always significant, correlation coefficient (R) was observed between the 3 variables on controls only, while no correlation was evident on cases. Namely, R was 0.23 (p = 0.13) on controls and 0.02 (p = 0.93) on cases between TS and TU; 0.29 (p = 0.06) and -0.09 (p = 0.70) between TU and adiol. A weak positive, although not always significant, correlation coefficient (R) was observed between the 3 variables on controls only, while no correlation was evident on cases.

Since most of the effect associated with each of the 3 androgenic hormones was still apparent after adjustment by each other, we examined the relationship between an increase in the IAA, taken as at least one value of TS, TU, or adiol equal or higher than the 90th percentile of controls distributions, and the
levels of PG (Table 4). In this case, the RR for subjects characterized by both risk factors, IAA and low PG, with reference to those who do not have any of them, is as high as 90.2 (8.2 to 989.7), substantially higher than when only TS was considered (Table 2); the RR for low PG alone is 2.2 (0.3 to 15.7) and, for IAA, it is 7.6 (1.5 to 38.6). Thus, a low level of PG appears to be significantly associated with a high risk of breast cancer only in the presence of IAA. Adjusting for each other, the RR for low PG is 4.8 (1.2 to 19.1) and, for IAA, it is 13.5 (3.4 to 53.6).

**DISCUSSION**

Our data suggest that high androgen concentrations, either in blood or in urine, are associated with high risk for breast cancer and that the risk increases when anovulatory menstrual cycles are also present, as indicated by the combination of high levels of androgens with low levels of progesterone. These results raise the question of whether the observed association has to be regarded as noncausal, i.e., the consequence of confounding or selection bias or rather a marker of an endocrine imbalance caused by the tumor itself, or whether it should be interpreted as causally related; i.e., androgen overproduction is an endocrine determinant of breast cancer occurrence.

The study has indubitable limitations. In the study design, the question of a differential selection of women with and without breast cancer was not thoroughly addressed. We know from previous experience that the women who attend our Outpatient Department tend to be self-selected on the basis of the known constitutional or reproductive risk factors for breast cancer. Familiarity for breast cancer and nulliparity, for example, are overrepresented among them. Familiarity, however, was not associated with an increased androgenic activity in our data (11), and nulliparity was taken into account in the analysis without detecting any confounding effect. Even if some residual effect of selecting high-risk controls persisted in the data after adjustment, one would expect our estimates to be biased toward the null, instead of toward an overestimation of the effect. It must also be considered that information was missing on sociological indicators, such as social class, education, and place of birth, which might have been associated with a differential selection of cases and controls. Furthermore, women with benign mammary conditions have been excluded from the controls. Since there are indications that women with hyperplastic alterations of breast epithelium have endocrine abnormalities similar to breast cancer patients (5), their exclusion from the control series might therefore have caused an overestimation of the effect. However, this limitation does not alter the validity of the observed differences between cancer patients and normal women.

It can be argued that the endocrine imbalance might be the consequence of the tumor itself. Although the design of our case-control study does not allow us to draw conclusions on the temporal sequence of the events, there is some evidence suggesting that this is unlikely. In particular, testosterone excretion higher than in healthy controls was found in mastectomized patients in one study (13). Other studies (14, 15) have reported, in breast cancer patients, a high frequency of hyperplasia of ovarian interstitial cells, i.e., of the cells deputed to the production of androgens (10). Interstitial cell hyperplasia has been associated with increased urinary excretion of testosterone (3, 4). The ovarian origin of hyperandrogenism is also suggested by the observation that, in metastasized breast cancer patients with increased urinary testosterone, oophorectomy is followed by a significant reduction in the urinary levels of the hormone, irrespective of remission status (13). Finally, increased urinary excretion (5, 11), as well as high circulating levels (9), of testosterone were found in women with epithelial breast hyperplasia, a well-known risk factor for breast cancer (8).

Notwithstanding the aforementioned limitations and the small size of the study, our data provide substantial evidence that increased androgenic activity should be regarded as an important risk indicator for breast cancer. This evidence rests on the following points: (a) the very high RRs associated with increased androgenic activity; (b) the highly significant concentration-effect trends; (c) the even higher effect among women with low progesterone levels; and (d) the increasing risk for high testosterone concentrations, either in blood or in urine, with advancing age, which might suggest that the risk is a function of cumulative or chronic exposure to androgen overproduction. The risk for women with both high testosterone and low progesterone in serum was more than 4-fold higher than was the risk estimated on each one taken independently. This result perfectly fits the value expected on the basis of a multiplicative (biological interactive) model.

High levels of serum testosterone, urinary testosterone, and androstenediol suggest that increased androgenic activity plays an important role in breast cancer. It is not clear, however, whether altered levels of any of them should be considered as indicative of the presence of an underlying common disturbance. Strictly speaking, our data do not encourage this conclusion, since the 3 metabolites do not seem to be replaceable by each other. In fact, a residual, although lower, risk is still evident after...
cross-standardization. Besides, they are weakly correlated among controls and totally uncorrelated among cases (a difference which is, so far, not easily interpretable). This apparent independency can be partially explained by considering that a change in their levels is the final and measurable consequence of a complex chain of events involving ovarian, adrenal, and peripheral productions, hepatic metabolism, peripheral utilization, transport, and excretion. Thus, any detectable increase in circulating or urinary levels may reflect a disturbance involving one or several of these events, so that no definite answer can be obtained on a study based on a single measurement. A study design involving serial specimens might have led to a more precise definition of exposure and, conceivably, to a more accurate estimate of RRs. However, whether increased androgen levels are expression of the same event or not, their grouping within a single index of IAA maximizes the RR estimate (RR = 13.5), probably through a more sensitive categorization of hyperandrogenism. A further increase in the RR, of about 7 times higher, was observed when PG also was taken into account. Indeed, women with IAA and low PG showed the highest RR (90.2).

Our findings show that low progesterone levels, which suggest a decreased corpus luteum function, occur more frequently among breast cancer patients than among healthy controls. It is not clear, however, whether low progesterone has to be considered a risk factor in the absence of increased androgenic activity. Low levels of this hormone, when combined with high levels of androgens, might be interpreted as an indicator of a profound anatomi cal and/or functional lesion of the ovary.

These results are in agreement with the hypothesis originally described by Grattarola (3, 5) and Grattarola et al. (6, 7) and recently referred to as "the ovarian androgen excess hypothesis" (16). Grattarola suggested, on the basis of some pathophysiological evidence, that increased androgenic activity, anovulation, and insufficient luteal phase are all aspects of the same lesion of the ovary, namely, the hyperplasia of interstitial cells. Reduced fertility, a well-known risk indicator for breast cancer (8), can be a result of the same alteration of the ovary.

Our findings, if confirmed, will have important implications in better understanding the hormonal etiology of breast cancer, possibly opening some perspectives on the primary prevention of the disease.

REFERENCES

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