Androgens and Breast Cancer in Premenopausal Women

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ABSTRACT

We investigated the role of androgens in premenopausal breast cancer by comparing serum testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone sulfate, progesterone, sex-hormone-binding globulin-binding capacity, and urinary testosterone and androstenediol in 63 women with breast adenocarcinoma and 70 healthy controls of similar age. With variables dichotomized at the 75th percentile, the age-adjusted relative risk was 3.4 (95% confidence interval, 1.6–7.3) for high versus low levels of serum testosterone, 2.1 (0.9–4.8) for urinary testosterone, and 2.5 (1.1–5.9) for serum dihydrotestosterone. We observed no differences in other hormones. The strength of the associations changed markedly with increasing time to the onset of the next menses. The risk for testosterone and dihydrotestosterone, which was negligible in women with onset within 5 days of sampling, increased progressively to nearly 10-fold higher than in unstratified data in women with onset 10 days or more after sampling. This study provides arguments in favor of a role for increased androgenic activity in premenopausal breast cancer. It also suggests that unknown factors related to cycle length may be important in modulating the strength of the association with testosterone. The results are discussed also in reference to possible biases and inadequacies in study design.

INTRODUCTION

In a series of articles, Grattarola et al. (1–4) reported that pre- and postmenopausal breast cancer patients had urinary testosterone levels higher than normal. They also showed that androgen excess originated from the hyperplastic interstitial cells of the ovary and suggested that ovarian androgens may play an important role in the development of malignant breast disease (1, 4). A few case-control studies (5–7) supported Grattarola’s findings and the “ovarian androgen excess hypothesis,” as has been referred to in the literature (8).

More recently, plasma testosterone levels were found to be higher in premenopausal Caucasian women than in Japanese and South African black women (9), who are at considerably lower risk for breast cancer. Plasma testosterone levels were found to be elevated also in Japanese breast cancer patients compared to Japanese healthy women (10) and in Chinese cases compared to Chinese controls (11). A prospective study, based on only 17 pre- and 39 postmenopausal cases, did not show differences in serum testosterone levels between breast cancer cases and non-cases (12). In a case-control study from our group (13), a strong association was found between high testosterone levels in blood and urine and breast cancer, the effect being maximum in women with high testosterone and low progesterone levels.

We report here the results of a case-control study in which we further explored Grattarola’s hypothesis that increased androgenic activity (as indicated by elevated circulating and urinary testosterone), anovulation, insufficient luteal phase, and reduced fertility are all aspects of the same, relatively common ovarian condition, the hyperplasia of interstitial cells. To improve our understanding of a scenario that may involve complex interrelations between various hormones, we decided to measure levels of testosterone along with those of its immediate metabolic precursor, androstenedione, and of its powerful effector at the cellular level, DHT, in all study subjects. For a more complete evaluation, serum levels of dehydroepiandrosterone sulfate (DHEAS), a major adrenal androgen, the binding capacity of SHBG, and the urinary levels of the androgenic metabolic androstenediol were also measured. To confirm our previous findings, progesterone assays were performed in all serum specimens.

MATERIALS AND METHODS

Study Population. The study population consisted of women 30 to 49 years of age reporting: (a) at least one intact ovary; (b) at least 10 menstrual cycles during the preceding 12 months; (c) a medical history negative for cancer and major endocrinological conditions; and (d) residence within the greater Milan area. Carefully excluded were all women reporting the use of hormonal drugs of any kind during the previous 3 months and those whose specimen collection could not be arranged as required (see below).

Eligible cases were patients consecutively listed for admission to the Italian National Cancer Institute Hospital in Milan for suspicious primary breast cancer, between October 1983 and December 1985. Only patients presenting with tumors smaller than 4 cm in diameter at clinical inspection, with no clinical symptoms other than a lump, with no evidence of metastases other than to the regional lymph nodes, and who could be recruited before undergoing surgical, medical, or radiological treatments for the condition were accepted. Enrollment was conditional to the pathological confirmation of an invasive carcinoma. A total of 63 eligible cases were recruited.

Eligible controls were women reporting the use of hormonal drugs of any kind during the previous 3 months and those whose specimen collection could not be arranged as required (see below).

Controls were contacted by asking each potential case to list non-blood-related members of the family, or acquaintances or, in absence, sisters, who were close in age to them (within 5 years). Most patients provided only one name. When more than one was available, the closest in age was contacted first. After checking for eligibility, 70 controls were recruited. Of these, 23 were relatives or friends of patients recruited as cases, and 47 were related to patients eventually excluded because of lack of histological confirmation, or because their samples had not been collected at the specified time of the cycle, or because they had failed to comply with sample collection procedures. Of the 70 controls, 47 were acquaintances, 13 were in-laws, and 10 were sisters.

Cases and controls were given an interview questionnaire which focused on demographic, socioeconomic, reproductive, and medical information and were asked to report the exact first day of the last menses. Counting back from that day, all were instructed to collect a complete 24-h sample of urine, starting with the second urine of any morning between the 18th and the 21st day of the cycle. This time, which in a 28-day ovulatory cycle corresponds to the midluteal phase, was chosen arbitrarily, irrespective of total cycle length and whether or not...
not ovulation had occurred. Women were asked to return to the hospital between 9 a.m. and 12 noon the following morning to deliver the urine and to donate 20 ml of blood from an antecubital vein. The exact time at blood sampling was recorded to adjust for circadian effects. Each woman was contacted at a later time to record the date of the following menses. Urine and blood samples were quickly delivered to the institute’s laboratory of endocrinology. Hormonal determinations were carried out blindly.

Laboratory Methods. Hormones in urine were measured by gas chromatography according to the method of Mauvais-Jarvis et al. (14), as reported previously (15). Circulating hormones were measured by radioimmunoassay using commercial kits purchased from Biomerieux (Charbonnier les Bains, France) for urinary testosterone, DHT, and androstenedione; from Sciavo (Milan, Italy) for DHEAS; and from Diagnostic Product Co. (Los Angeles, CA) for progesterone. SHBG was quantitated by its ability to bind DHT. The binding protein saturated with [1,2-3H]DHT (Biodata, Milan, Italy) was separated by the ammonium sulfate technique of Rosner (16).

Serum testosterone, DHT, and androstenedione were measured after ether extraction followed by partition chromatography on a Celite column. Elution was carried out stepwise using 5 ml of isooctane (androstenedione fraction), followed by 6 ml of 6% ethyl acetate in isooctane (DHT fraction), then 6 ml of 20% ethyl acetate in isooctane (testosterone fraction).

The coefficients of intra- and interassay variation were 9.7 and 11.0% for testosterone, 7.9 and 12.3% for DHT, 6.1 and 13.5% for androstenedione, 6.3 and 6.8% for DHEAS, 6.0 and 13.0% for progesterone, and 3.5 and 5.2% for SHBG, respectively.

Statistical Analyses. Unconditional logistic regression was used to obtain estimates of RR (17). Maximum likelihood estimates of the regression parameters and their estimated standard errors were computed using the statistical package GLIM (18). Continuous exposure variables were categorized using the percentiles of the frequency distributions of the controls to select appropriate cutoff points. Given the small sample size and following the criteria used in a previous study (13), basic results are presented dichotomizing androgens at the 75th percentile, progesterone and SHBG at the 25th percentile. Adjustment for confounders, such as age and other covariates, was achieved by including linear or higher powers of the continuous variables in the model, when appropriate. Significance tests of the parameters and approximate 95% CIs are computed based on the gaussian distribution. χ² values for trend of change in RRs per unit change of hormones were computed on continuous variables as the ratio between the estimated coefficient and its standard error. Tests for linear trend of the log RR by categories of a risk modifier were carried out on the regression coefficient of the interaction term between indicator variables of exposure and modifier. The Mantel-Haenszel (19) and Mantel extension (20) procedures were used to assess significance of association and trend in 2 x k tables, when appropriate.

RESULTS

Table 1 shows the distribution of cases and controls according to demographic and social characteristics and known risk indicators for breast cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
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<td></td>
</tr>
<tr>
<td>30-39</td>
<td>21</td>
<td>33.3</td>
</tr>
<tr>
<td>40-44</td>
<td>19</td>
<td>30.2</td>
</tr>
<tr>
<td>45-49</td>
<td>23</td>
<td>36.5</td>
</tr>
<tr>
<td>Place of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milan region</td>
<td>40</td>
<td>63.5</td>
</tr>
<tr>
<td>North Italy</td>
<td>10</td>
<td>15.9</td>
</tr>
<tr>
<td>Rest of Italy</td>
<td>13</td>
<td>20.6</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue collar</td>
<td>14</td>
<td>22.2</td>
</tr>
<tr>
<td>White collar</td>
<td>31</td>
<td>49.2</td>
</tr>
<tr>
<td>Professional</td>
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<td>28.6</td>
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<tr>
<td>Parity</td>
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<td></td>
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<tr>
<td>0</td>
<td>13</td>
<td>20.6</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>23.8</td>
</tr>
<tr>
<td>2+</td>
<td>35</td>
<td>55.6</td>
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<td>Age at first child</td>
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<td>26-30</td>
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</tr>
<tr>
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<td>14.0</td>
</tr>
<tr>
<td>Age at menarche</td>
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<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>7</td>
<td>11.1</td>
</tr>
<tr>
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<td>61.9</td>
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<td>27.0</td>
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<tr>
<td>Body mass index*</td>
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<td>&lt;21.4</td>
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<td>44.4</td>
</tr>
<tr>
<td>21.4-23.5</td>
<td>11</td>
<td>17.5</td>
</tr>
<tr>
<td>23.6+</td>
<td>24</td>
<td>38.1</td>
</tr>
</tbody>
</table>

* Weight (kg)/height (m²).

Table 2 shows the median values and ranges of all hormonal measurements, separately by cases and controls. The table also includes median and range for total cycle duration. Median values for serum and urinary testosterone and DHT were appreciably higher among cases than among controls, whereas no marked differences were observed for other hormones. Cases and controls had comparable total cycle lengths. Since cases were slightly older than controls and since longer cycles may be more frequent among older women, we compared the median of cycle length between cases and controls within categories of age. Median duration of cycles were homogeneous across ages within both groups and closely comparable between groups.

Table 3 shows the RRs associated with hormonal variables. The age-adjusted RR for high levels of serum testosterone was 3.4 (95% CI, 1.6–7.3). The χ² for trend for unit increase of serum testosterone was 1.95, which is of borderline statistical significance (P = 0.05). Since serum testosterone was highly correlated with most other androgens and was strongly associated with breast cancer, it was kept in all subsequent models. The age-adjusted RR for high levels of urinary testosterone was 2.3. When adjusting for serum testosterone, the RR decreased to 2.1 (95% CI, 0.9–4.8). The age-adjusted RR for DHT was 2.6, which decreased to 2.5 (95% CI, 1.1–5.9) after adjustment for serum testosterone. No significant associations were found for androstenedione, androstenediol, DHEAS, SHBG, and progesterone, and, aside from serum testosterone, no statistically significant trends of variation per unit change were observed.

Further statistical analyses, presented in Table 4, dealt with time of onset of the next menses. No associations with breast cancer were observed among women whose specimens were collected within 4 days of the next menses and who thus had cycles lasting 26 days or less. In women who donated 5 to 9
days prior to the next menses (a period corresponding to the midluteal phase when the follicular lasts about 14 days) the RRs were 4.8 for serum testosterone (95% CI, 1.6–13.7), 2.1 for urinary testosterone (95% CI, 0.7–6.2), 3.2 for DHT (95% CI, 1.1–9.5), 2.2 for androstenedione (95% CI, 0.8–6.3), and 2.0 for DHEAS (95% CI, 0.6–6.5). The RRs for androstenedione and progesterone were close to 1.0. Among women who donated 10 or more days before the next menses, the RRs associated with high levels of all androgens but DHEAS increased dramatically. The RR for serum testosterone was 32.1 (95% CI, 6.8–153.0), for urinary testosterone 14.5 (95% CI, 2.0–27.0), for DHT 14.7 (95% CI, 1.8–122.5), for androstenedione 5.2 (95% CI, 0.6–41.7), and for androstenedione 4.6 (95% CI, 0.5–39.3). There was no evident pattern of change in the RRs for progesterone, which were low in both extreme categories, but not significantly so. The RRs for low serum SHBG levels revealed a decreasing risk by increasing time to the next menses.

The RR was 3.8 (95% CI, 0.6–25.5) in the 0–4 days category, 0.7 (95% CI, 0.2–2.8) in the middle category, and 0.1 (95% CI, 0.0–1.6) in the 10+ category.

**DISCUSSION**

This study provides further evidence that an important association exists between increased circulating levels of androgenic hormones and breast cancer in premenopausal women. In our data, serum testosterone was strongly associated with breast cancer. Serum DHT and urinary testosterone also indicated a relevant, although not as important, relationship. Overall, these findings indirectly support Grattarola's hypothesis that increased ovarian androgenic activity plays an important role in the development of female breast cancer (1–3,8).

Contrary to our previous findings (13), we found no difference between cases and controls in serum progesterone. Such discrepancy between the two studies may be attributable in part to drastic changes in the modalities for the recruitment of the controls, but not of the cases. In fact, when comparing the frequency distributions of hormones between the studies, those for progesterone were overlapping among cases but quite apart among controls, with that of the new study uniformly shifted toward lower values. Furthermore, in studies based on a single donation of biological specimens, if the fluctuations of a given hormone are ample, different results from different studies, even when laboratory methods are comparable, are plausible. Intraindividual variability of single measurements of steroid hormones has rarely been evaluated. In a recent study (21), the correlation was good for serum testosterone (r = 0.73), DHT (r = 0.80), and androstenedione (r = 0.70) but was poorer for urinary androgens. Unlike androgens, serum progesterone appears to be subject to fluctuations so dramatic that a single measurement cannot be used to estimate corpus luteum activity (22).

Friends and relatives of cases served as controls, a choice dictated by two essentially practical reasons. One was that the study was set in a highly specialized hospital attended exclusively by cancer patients and with limited access to alternative sources of non-cancer controls. The second was that we knew from previous experience that breast cancer patients treated in our institute are not good representatives of incident cases from the general population. For instance, they tend to be of higher socioeconomic status. We therefore tried to recruit a reference group of women who, when developing a breast lump, would be as likely as the cases to consult our institute. As indicated in a recent report, however, studies using friend controls may be
flawed by selection bias if any of the exposures under study is also a determinant of friendship (23). In our case, since we focused exclusively on biological measurements which are not likely to be greatly influenced by social determinants, selection bias may have played a negligible role. Although one cannot exclude that unknown selection forces had operated so that cases with abnormally high androgenic levels (or, which is equivalent, controls with abnormally low ones) were recruited, it is unlikely that such bias could entirely explain the strong effects observed.

In the retrospective design of the study, the results presented here are compatible with the noncausal hypothesis that high androgen levels in breast cancer patients are contributed entirely or in part by the presence of the tumor or are a consequence of the disease. Although we have been careful to exclude all cases with large or metastasized tumors, the possibility that the tumor itself produces and releases steroid hormones in the bloodstream cannot be ruled out. Contrary to this view, recent data suggest that the concentration in human breast cancer tissue of testosterone and other androgens, such as androstenedione, the immediate metabolic precursor of testosterone, and DHEAS, is normal or even reduced (24). In our data, tumor size at pathology and levels of serum testosterone and other hormones were not correlated.

Another possibility is that the secretion of steroid hormones in breast cancer is increased due to the psychological stress induced by the combined effect of the presence of cancer and the fear of imminent surgery. In this case, the responsibility for the observed increase in androgenic levels should be of the adrenal. Unfortunately, we did not measure cortisol. Although recent data in men suggest that stress causes a drastic fall rather than an increase in testosterone levels (25) and although we would have expected a stronger association with DHEAS and androstenedione, the major androgens secreted by the adrenal (26), this possibility is legitimate. Our data seem to suggest that androstenedione levels may be slightly lower among cases than among controls (Tables 2 and 3). Table 5 indicates that androstenedione levels could be even lower among cases after adjustment for serum testosterone. In Table 5, study subjects are classified according to three levels of androstenedione and serum testosterone, cutoff points being the median and the 75% percentile. The increased risk with high testosterone levels and the strong correlation between the two hormones (see Pearson’s correlation coefficients at the bottom of the table) mask the inverse association between androstenedione and breast cancer. Upon adjustment for age and serum testosterone, the RR for women in the upper quartile of androstenedione versus those under the median is 0.4 (95% CI, 0.1-1.2). Although the association is not statistically significant (P for trend = 0.09), this finding is interesting because it appears consistent with previous observations in which adrenal androgens were protective for breast cancer (27).

When time to the next rather than time since the previous menstrual cycle was considered, the association between high androgen levels and breast cancer increased progressively and sharply with increasing cycle duration. While there was no apparent association in women whose cycle began within 5 days of blood donation, women with delays ranging between 5 and 9 days, which roughly corresponds to a 28-day cycle, had intermediate risks of breast cancer for serum testosterone and

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