The Relation of Hormone Discriminants in Plasma and Breast Cancer

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ABSTRACT

Changes in both the urinary estrogens and androgens have been implicated as discriminants for risk of breast cancer. Since few studies of the plasma hormone profile have been reported, an investigation of the hormone profile of two populations, one with a high incidence, Caucasian North American, and the other with a low incidence, Bantu, of breast cancer was carried out. Initial results show an elevated testosterone level prior to puberty and higher estrone and estradiol levels in young Bantu women. The dehydroepiandrosterone (DHEA) sulphate and androsterone sulphate levels were comparable in the two populations. Both luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels are elevated in pre-menopausal Bantu women with breast cancer. Data suggest considerable differences in the hormone profiles between the two populations. Whether or not these changes result from differences in production or clearance is under investigation.

Introduction

Carcinoma of the breast has been reported to account for over 20 percent of all the cancer mortality of American women. It has been estimated that approximately 69,000 new cases of breast cancer are diagnosed each year in the United States. Epidemiological studies show that the incidence of breast cancer is low in Japanese and in African women compared to Caucasian women in North America and England.

A comparison of the annual incidence rate per 100,000 women, with age between two populations, one with a high incidence rate (North America) and the other with a low (Japan), is shown in figure 1. Both populations show a comparable increased incidence with age. The incidence in Japanese women plateaus around 45 years of age, while the incidence in American women continues to increase. As shown by epidemiological data, the risk of breast cancer can be altered by an early preg-
It is apparent, therefore, that although breast cancer may be of multi-factorial origin, changes in the hormone balance can alter the risk of breast cancer. In the normal life cycle, the hormone profiles change at set points: birth, puberty and menopause, and also gradually with age with the establishment of the hormone profile being dependent on the hypothalamic pituitary axis, ovaries, adrenals and thyroid gland. In regard to the relation of breast cancer and these hormone abnormalities, both altered estrogen excretion\(^{15}\) and depressed androgen excretion\(^{5}\) have been postulated as discriminant factors.

Although the estriol ratio is higher in Asians than North Americans, the changes in the estriol ratio in pre-menopausal women with breast cancer are ambiguous as shown in table I. Furthermore, Swain et al\(^{23}\) have reported comparable serum estrogen levels in healthy and pre-menopausal women with benign breast cancer and are uncertain as to whether or not changes in hormone profiles related to benign and metastatic breast cancer are the same.

Depressed androgen excretion and an altered ratio of excretion of androsterone to etiocholanolone (5α/5β ratio)\(^5\) have been questioned\(^9\) and may occur only in advanced cases.\(^{11}\) The ratio may also be specific for the individual with the value being determined at puberty.\(^{24}\)

It was, therefore, of interest to compare the plasma hormone profiles of two populations, one at high risk, Caucasian North Americans, and the other at low risk, Bantu women, for breast cancer. This investigation reports the levels of gonadotrophins, estrogens and androgens in the two populations.

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**TABLE I**

**Urinary Estrogens in Premenopausal Women µg per 24 Hours**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number</th>
<th>Estrone</th>
<th>Estradiol</th>
<th>Estriol</th>
<th>Estradiol + Estriol</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Premenopausal</td>
<td>15</td>
<td>5.6±1.1</td>
<td>5.3±1.5</td>
<td>10.0±0.3</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>*Premenopausal</td>
<td>6</td>
<td>2.8</td>
<td>4.1</td>
<td>7.3</td>
<td>0.6±0.4</td>
</tr>
<tr>
<td>+ Breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>†Premenopausal</td>
<td>8</td>
<td>16.2±4.7</td>
<td>4.5±1.7</td>
<td>20.8±4.0</td>
<td>1.0±0.14</td>
</tr>
<tr>
<td>†Premenopausal</td>
<td>47</td>
<td>10.1±4.3</td>
<td>3.6±2.0</td>
<td>17.2±8.3</td>
<td>1.2±0.25</td>
</tr>
<tr>
<td>+ Breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>/Asians</td>
<td>29</td>
<td>5.3, 7.7(^*)</td>
<td>2.5, 3.6</td>
<td>8.2, 15.1</td>
<td>1.27, 1.64</td>
</tr>
<tr>
<td>/North Americans</td>
<td>30</td>
<td>7.0, 13.3(^*)</td>
<td>3.4, 7.6</td>
<td>8.3, 14.3</td>
<td>0.83, 0.69</td>
</tr>
</tbody>
</table>

\(^*\)Lemon et al\(^ {16}\)  †Arguelles et al\(^ 1\) (on 14th day of menstrual cycle)  /MacMahon et al\(^ {17}\)

\(^*\)Follicular and luteal mean values  \(^\#\)P<0.01
Methods

Peripheral blood was obtained from an antecubital vein using a heparinized syringe, while blood from the umbilical vein was obtained under free circulation before severance. The plasma was immediately separated by centrifugation at 4° and frozen at —20°. The samples from Cape Town were flown in a frozen state to our laboratory.*

The amounts of plasma analyzed varied with the source of the sample. Details of the separation of the individual hormones are shown in figure 2.

Estradiol, estrone and estriol were separated on Sephadex LH.20 column according to Hertogh et al.13 and were assayed by the cytosol protein binding method of Nagai and Longcope.21 Each assay was determined in quadruplicate, using duplicate samples at two dilutions. The standard curve for estradiol and estrone for eleven experimental runs is shown in figure 3. Testosterone was determined in duplicate samples by radio-immune assay (RIA) as described by Chen et al.8 while androstenedione and dehydroepiandrosterone (DHEA) were assayed by RIA after Furuya et al.10 Analysis of the androsterone sulphate and DHEA sulphate was carried out by a modification of the method of Wang et al.25 as described by Bulbrock.4

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by the double antibody radioimmune assay after Midgeley.18,19 Purified LH and FSH were iodinated with I125† as described by Greenwood et al.12†

Results

Comparison of the gonadotrophin levels in umbilical cord vein blood from Caucasians and Bantu showed an increase in the FSH in cord blood from Bantu women. Although the estradiol and estrone levels did not differ, the testosterone level was higher in cord blood from Bantu women (table II). Considerably higher levels of LH than FSH were present in cord blood from both populations. It is of interest that the testosterone level is also higher in nine year old Bantu girls (Table III) while the plasma

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† Cambridge Nuclear.

‡ The purified LH, FSH and antibodies, except antibody II, anti-rabbit gamma globulin (Antibodies Incorporated, Davis, California) were obtained from the Hormone Distribution Division, Endocrinology Section of the National Institute of Arthritic and Metabolic Diseases, Bethesda, MD.
estradiol level, but not the estrone, was also higher than in Caucasian nine year old girls.

In Caucasians, blood samples were taken on the ninth day of the follicular phase and the 20th day of the luteal phase as well as samples on two menstrual cycles. Similar samples of Bantu to date have been taken only on the 20th day of the luteal phase.

Comparison of the estrone and estradiol levels show the level of both estrogens to be significantly higher in Bantu women (table IV). Although the estradiol level increased after puberty, it was of interest that the estrone level, which was high in both populations of nine year old girls, increased further in young Bantu women but not in Caucasian women. The plasma testosterone levels were comparable in both populations of young women.

Preliminary analysis of the androgen content was carried out by gas chromatographic analysis of the androsterone and DHEA sulphate in the plasma. As shown in table V, the DHEA sulphate and androsterone sulphate were comparable in the Caucasian and Bantu women.

Higher serum values of LH were present in young Bantu women than in Caucasian women. Both the LH and FSH increased in premenopausal Bantu women with breast cancer (table VI), to values higher than those found in perimenopausal Caucasian women, while further increases in LH and FSH were evident in postmenopausal Bantu women with breast cancer.

**Discussion**

Comparative studies of British and Japanese women indicate a lower excretion of androgens in young Japanese women and a significantly higher urinary excretion of androsterone to etiocholanolone in older Japanese women versus Caucasian women. However, Kumaoka et al reported comparable levels of urinary 11-deoxy 17-ketosteroids in Japanese and American breast cancer patients, although both pre- and postmenopausal patients were taken as a group.

Preliminary analysis of the plasma androsterone sulphate and DHEA sulphate in this study, which are comparable to those

**TABLE II**

<table>
<thead>
<tr>
<th>Concentration of Unconjugated Estrone (E₁), Estradiol (E₂) and Testosterone (T) and of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) in Umbilical Cord Plasma in Female Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E₁</strong> (ng per ml)</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td><strong>North America</strong></td>
</tr>
<tr>
<td>Caucasian</td>
</tr>
<tr>
<td><strong>Bantu</strong></td>
</tr>
</tbody>
</table>

*Number of samples †Mean and standard error ‡Range of values $P<0.05 ^{*}P<0.01
TABLE III
CONCENTRATION OF UNCONJUGATED ESTRONE (E₁), ESTRADIOL (E₂) AND TESTOSTERONE (T) AND OF LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH) IN NINE TO TEN YEAR OLD GIRLS

<table>
<thead>
<tr>
<th></th>
<th>E₁</th>
<th>E₂</th>
<th>T</th>
<th>LH</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng per dl</td>
<td>ng per dl</td>
<td>ng per dl</td>
<td>MIU per ml</td>
<td>MIU per ml</td>
</tr>
<tr>
<td>North American</td>
<td>20*</td>
<td>24.5±3.1†</td>
<td>5.7±0.7</td>
<td>8.6±2.0</td>
<td>1.4±0.1 (60*)</td>
</tr>
<tr>
<td>(Caucasian)</td>
<td></td>
<td>(10.1-48)</td>
<td>(2.1-12.0)</td>
<td>(0-27)</td>
<td>(0.6-2.0)</td>
</tr>
<tr>
<td>Bantu</td>
<td>26*</td>
<td>21.9±1.4</td>
<td>8.6±0.9§</td>
<td>19.3±2.2X</td>
<td>1.2±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10.6-42)</td>
<td>(4.2-19)</td>
<td>(0-38)</td>
<td>(0.7-2.9)</td>
</tr>
</tbody>
</table>

*Number of samples †Mean and standard error §Range of values  
Number of samples  
Samples taken on 20th day of menstrual cycle /Mean and standard error ×Range of values  

reported by Bulbrook et al⁷ show no difference in the levels between Bantu and Caucasian women.

Comparison of the testosterone in umbilical cord blood and in nine year old Bantu girls suggests a different androgen balance during growth in Bantu women. Changes in LH and FSH were evident with age. After birth, the LH concentration fell and then increased to comparable levels in nine year old Bantu and Caucasian women.

In Bantu women with breast cancer a more marked elevation of the FSH occurred in both pre- and post menopausal patients compared to young healthy Bantu women, while the serum LH in premenopausal Bantu women with breast cancer was comparable to that found in perimenopausal Caucasian women. The effect of elevated gonadotrophins on the serum estrogen and androgen levels is still under analysis.

Although MacMahon and Cole¹⁷ reported lower urinary estriol ratio, especially in young Asian women, and Briggs³ reported less urinary estrone and estradiol in Asians versus Europeans and Africans, comparison of the levels of urinary estrogens in Africans and Europeans is of interest since the latter have a higher incidence of breast cancer. The finding, however, may be explained by the fact that the African women were obtained from different ethnic groups.

In regard to the plasma estrogen levels, both the estradiol and estrone content was significantly higher in young Bantu women.

TABLE IV
CONCENTRATION OF UNCONJUGATED ESTRONE (E₁), ESTRADIOL (E₂) AND TESTOSTERONE (T) IN YOUNG WOMEN 20 TO 30 YEARS OF AGE

<table>
<thead>
<tr>
<th></th>
<th>E₁</th>
<th>E₂</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng per dl</td>
<td>ng per dl</td>
<td>ng per dl</td>
</tr>
<tr>
<td>North American</td>
<td>25.8±2.4f</td>
<td>24.3±2.1</td>
<td>42.6±3.7</td>
</tr>
<tr>
<td>(Caucasian)</td>
<td>(18-36)</td>
<td>(20-38)</td>
<td>(30-64)</td>
</tr>
<tr>
<td>Bantu</td>
<td>59*</td>
<td>32.4±1.38</td>
<td>31.5±1.68*</td>
</tr>
<tr>
<td></td>
<td>(18-57)</td>
<td>(13-54)</td>
<td>(24-82)</td>
</tr>
</tbody>
</table>

*Number of samples †Mean and standard error /Range of values  
Number of samples  
Samples taken on 20th day of menstrual cycle /Mean and standard error ×Range of values  

TABLE V
CONCENTRATION OF ANDROSTERONE SULPHATE AND DEHYDROEPIANDROSTERONE (DHEA) SULPHATE IN µG PER 100 DL OF PLASMA IN YOUNG WOMEN 20 TO 30 YEARS OF AGE

<table>
<thead>
<tr>
<th></th>
<th>Androsterone</th>
<th>DHEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sulfate</td>
<td>Sulfate</td>
</tr>
<tr>
<td></td>
<td>µg per dl</td>
<td>µg per dl</td>
</tr>
<tr>
<td>North American</td>
<td>48.7±8.07f</td>
<td>161.6±25.2</td>
</tr>
<tr>
<td>(Caucasian)</td>
<td>(13.7-109.8)</td>
<td>(38.8-383.4)</td>
</tr>
<tr>
<td>Bantu</td>
<td>107±32.1</td>
<td>144.4±23.2</td>
</tr>
<tr>
<td></td>
<td>(12.2-229.6)</td>
<td>(47.6-299.0)</td>
</tr>
</tbody>
</table>

*Number of samples †Mean and standard error /Range of values  
Number of samples  
Range of values
Whether or not the higher level of estradiol in nine year old Bantu girls and the subsequent higher levels of estrogens in young Bantu women compared with Caucasian women reflects a basic difference is estrogen metabolism is unknown. To investigate these differences further, the analysis of the plasma androgen and estrogen levels and rates of production and peripheral interconversion of labeled precursors in normal premenopausal Bantu and Caucasian women and in premenopausal women with breast cancer is in progress.

Acknowledgments

The authors wish to thank Ms. L. Garbaczewski, Ms. A. DePace, Ms. R. Neil, Dr. R. Khalid and Mr. H. Kumar for their skilled assistance.

References


### TABLE VI

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>FSH</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MIU per ml</td>
<td>MIU per ml</td>
</tr>
<tr>
<td><strong>North American</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Caucasian) 26*</td>
<td>3.1±0.3†</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>Age 20-30 years</td>
<td>(1.1-6.2)/</td>
<td>(1.9-8.6)/</td>
</tr>
<tr>
<td><strong>Perimenopausal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28* (Over 40 years)</td>
<td>8.2±1.5§</td>
<td>9.1±1.3§</td>
</tr>
<tr>
<td>Age 20-30 years</td>
<td>(1.4-31.7)</td>
<td>(2.1-34.4)</td>
</tr>
<tr>
<td><strong>Bantu Women</strong> 42*</td>
<td>5.8±1.5</td>
<td>4.8±0.3</td>
</tr>
<tr>
<td>Age 20-30 years</td>
<td>(0.7-91.7)</td>
<td>(1.2-12.1)</td>
</tr>
<tr>
<td><strong>Premenopausal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with breast cancer 9*</td>
<td>11.5±3.8</td>
<td>27.4±7.0</td>
</tr>
<tr>
<td>(1.9-31.2)</td>
<td>(5.0-70.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Postmenopausal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with breast cancer 14*</td>
<td>21.0±4.5</td>
<td>37.4±5.6</td>
</tr>
<tr>
<td></td>
<td>(4.2-60.0)</td>
<td>(9.0-93.6)</td>
</tr>
</tbody>
</table>

*Number of samples
†Mean standard error
§P<0.01 perimenopausal versus premenopausal
‡P<0.01 premenopausal women with breast cancer versus young Bantu women

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>FSH</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MIU per ml</td>
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<td><strong>North American</strong></td>
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<td>5.3±0.3</td>
</tr>
<tr>
<td>Age 20-30 years</td>
<td>(1.1-6.2)/</td>
<td>(1.9-8.6)/</td>
</tr>
<tr>
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<td>(5.0-70.8)</td>
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<td><strong>Postmenopausal</strong></td>
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<td>37.4±5.6</td>
</tr>
<tr>
<td></td>
<td>(4.2-60.0)</td>
<td>(9.0-93.6)</td>
</tr>
</tbody>
</table>

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