Serum Lipids and Tissue DNA Content in Egyptian Female Breast Cancer Patients

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Background: Several clinical studies suggest the prognostic significance of serum lipid levels and tissue DNA content in breast cancer. In the course of investigating the biological features of this disease among Egyptian female patients, we examined the serum lipid levels and tissue DNA content of premenopausal and postmenopausal breast cancer patients.

Methods: Levels of total lipid, total cholesterol, and triglycerides were measured in the sera of women with breast cancer and compared with those of the control women. The DNA content in breast cancer tissue was also measured in these patients.

Results: Total lipid levels showed a significant increase in both premenopausal (follicular and luteal) and postmenopausal patients. Total cholesterol levels significantly increased in premenopausal (follicular and luteal) patients with no significant change in postmenopausal women. Triglyceride levels showed a significant increase in postmenopausal women, whereas no significant differences were observed in premenopausal patients. Tumors of premenopausal patients, in both follicular and luteal phases, showed a higher DNA content as compared with those of postmenopausal patients. Breast cancer tissues of grade III showed significantly higher DNA content than those of grade I and grade II.

Conclusions: This study suggests an association between high levels of serum, total lipid and total cholesterol, and increased breast cancer risk in premenopausal women. Such an association is also suggested for the high total serum lipid and triglyceride levels in postmenopausal women. The DNA content in breast cancer tissue might be useful in determining a suitable therapy for individual cases, based on the malignancy grade.

Key words: breast cancer – serum lipids – tumor DNA content

INTRODUCTION

Breast cancer is the most common malignancy (34.8%) among Egyptian females (1). The etiology of this carcinoma is attributed to several factors. These include the history of breast cancer in the family, menarche at an early age, first full-term pregnancy at a late age, any delivery at a late age, menopause and parity at a late age (2–5), as well as endogenous and exogenous hormones (6–8). Levels of circulating lipids and lipoproteins have also been associated with breast cancer risk, though published results have been inconsistent (9–15).

The DNA content of breast tumor cells reflects the biological properties associated with the malignancy of the tumor (16) and might be useful in determining the therapy suitable for individual patients, based on the malignancy grade (17). The DNA ploidy might be regarded as a prognostic predictor for the survival of breast carcinoma patients (18). Aneuploid tumors tend to be more anaplastic and estrogen receptor (ER) poor which are more common in premenopausal than in postmenopausal women (19,20).

In a previous study (6), we reported the hormonal profiles and ERs in Egyptian female breast cancer patients in an attempt to investigate the biological features of this disease among these women. In the present study, this subject is further investigated by examining the possible association between serum lipids levels, tumor DNA content, and breast cancer risk in premenopausal and postmenopausal patients.

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PATIENTS AND METHODS

REAGENTS

All chemicals were of analytical-grade and were purchased from Merck (Germany) and BDH (England).

SUBJECTS

Serum and tumor samples were collected from females treated at the National Cancer Institute in Cairo (Egypt).

LIPID PATTERN STUDIES

Lipid analyses were carried out on serum samples from 119 breast cancer patients and 50 control women, the latter had a mean age of 39 years (range: 25–62 years). The control group included 30 premenopausal women (15 in the follicular phase and 15 in the luteal phase) and 20 postmenopausal women. These women were apparently healthy volunteers who were not taking oral contraceptives or any form of hormonal medication.

The group of patients constituted 119 women with primary breast carcinoma and had a mean age of 40 years (range: 28–63 years). Lipid pattern studies were carried out before they received any treatment. This group included 60 premenopausal patients (35 in the follicular phase and 25 in the luteal phase) and 59 postmenopausal patients.

Women were classified as postmenopausal if they had not had naturally occurring menstrual cycles during the preceding three years, or, if they had undergone a hysterectomy without complete oophorectomy before menopause and were 48 years of age or older (six cases and three control women). All subjects answered a questionnaire which contained details on age, height, weight, age at menarche, age at first delivery and age at menopause.

DNA CONTENT STUDIES

DNA content was examined in 71 tumor tissue samples from primary breast carcinoma patients. Histopathological typing of the primary tumors showed that 82% of the breast carcinomas were of the invasive ductal type, 11% were of the invasive lobular type and 7% were medullary carcinomas. Thirty-one percent (22/71) of the primary tumors were grade I, 48% (34/71) were grade II and 21% (15/71) were grade III. Sixty-five percent (46/71) of the primary tumors were ER positive and 35% (25/71) were ER negative.

SERUM SAMPLES

Blood samples were drawn from cases and control women between 8 a.m. and 9 a.m. After centrifugation, serum samples were immediately stored at –20°C for future analysis.

TISSUE HOMOGENATE

Specimens were cleared of surrounding fat, cut into small pieces and weighed. Each sample was pulverized into a powder in a prechilled thermovac tissue pulverizer. Samples were kept frozen at all times and maintained at –70°C for future analysis.

METHODS

Total serum lipid levels were measured using a spectrophotometric method described by Knight et al. (21). Total serum cholesterol was measured according to the method described by Allain et al. (22). Serum triglyceride levels were determined by a spectrophotometric kit supplied by Boehringer Mannheim, according to the protocol specified in the manufacturer’s manual, based on the method described by McGowan et al. (23).

The DNA content of the carcinoma tissues was extracted and determined according to the method described by Melmed et al. (24).

All results were expressed as mean ± S.E. The differences between the two means were analyzed using the Student’s t-test. Correlation between two different parameters was examined using a linear regression analysis. (P ≤ 0.05) were considered significant.

RESULTS

There were no significant differences in age and BMI of the cases and control women. The premenopausal (follicular and luteal) and postmenopausal patients had higher mean total serum lipid level (P < 0.01, < 0.02 and < 0.001) than the control women, by 18, 16 and 17%, respectively (Table 1). Mean total cholesterol levels showed a 13 and 21% increase in premenopausal patients in follicular and luteal phases (P < 0.005 and < 0.001), respectively, compared to the control women, whereas no significant changes were observed in postmenopausal patients (Table 1). The mean triglycerides level in postmenopausal patients was 31% higher than that of the control women (P < 0.001), whereas no significant differences were observed in premenopausal cases (Table 1).

Table 2 shows that the DNA content in tumors of premenopausal patients in both follicular and luteal phases was higher than that in postmenopausal patients, (P < 0.05 and P < 0.05), by 17 and 19.5%, respectively.

As shown in Table 3, the DNA content in grade III breast cancer tissues was higher than that in grade I (P < 0.01) and grade II (P < 0.05) by 29 and 18%, respectively.

DISCUSSION

The results of the present study have demonstrated a 17% increase in the total serum lipid levels of premenopausal (in both follicular and luteal phases) and postmenopausal breast cancer patients compared to the control women. This is in agreement with results of other studies (25,26), although the percentage increase in our study was lower than that reported by a previous study (25) which showed a 22% increase for...
premenopausal patients and 49% increase for postmenopausal patients.

We observed a significant increase in total serum cholesterol levels of premenopausal patients in follicular (13%) and in luteal (21.4%) phases, which is in agreement with a 15% increase for premenopausal patients as reported by other studies (25). However, some researchers have found no association between the total serum cholesterol levels of premenopausal women and breast cancer risk (27). The non-significant change in total serum cholesterol levels of postmenopausal cases reported in the present study is consistent with reports of other studies (9,27,28). Several other case-control and prospective studies found that elevated total serum cholesterol levels is associated with increased breast cancer risk (25,26,29–32). Hence, the association between total serum cholesterol levels and breast cancer risk still seems to be controversial, and the published results are inconsistent.

Our results show a significant increase in serum triglyceride levels in postmenopausal breast cancer patients as compared with the control women, which is consistent with results reported by other studies (25,26). However, the percentage increase of triglyceride levels (31%) reported in our study is higher than that reported in a previous study (22%) (25). On the other hand, we found no significant change in serum triglyceride levels of premenopausal patients, which is in agreement with results of other studies (27), although some researchers reported elevated serum triglyceride levels in premenopausal breast cancer patients (12). High serum triglycerides levels were observed in breast cancer patients (28). Further, the serum triglyceride levels were higher in patients who were at a more advanced stage IV breast cancer compared with those in stage I (28). In a case-control study (9), women with the highest serum triglyceride levels had an increased breast cancer risk and it was suggested that this risk may be modified by apo-E4 genotype. Luo et al. (10,11) showed that DHEA and the anti-estrogen EM-800 suppressed the development of DMBA-induced mammary carcinoma in rats, which was accompanied by decreased levels of serum triglycerides.

Several studies have investigated the role of diet, especially dietary fat, in the etiology of breast carcinoma, but its significance has remained controversial (33,34). Although the relationship between diet and plasma lipid levels is complex, diets containing a large amount of saturated fats may lead to higher plasma lipid levels, particularly cholesterol (25). Namura et al. (34) suggested that elevated lipid levels precede the development of the disease and thus may have an etiological or predic-

### Table 1. Serum levels (mean ± SE) of total lipid, total cholesterol and triglycerides in cases and controls

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (mean ± SD)</th>
<th>BMI (mean ± SD)</th>
<th>Total lipid (mg%)</th>
<th>Total cholesterol (mg%)</th>
<th>Triglycerides (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular (n = 35)</td>
<td>32.9 ± 1.6</td>
<td>24 ± 0.6</td>
<td>715 ± 24.9</td>
<td>234 ± 5.6</td>
<td>107 ± 6.5</td>
</tr>
<tr>
<td>Luteal (n = 25)</td>
<td>33.1 ± 1.9</td>
<td>24 ± 0.8</td>
<td>692 ± 29.6</td>
<td>244 ± 8.5</td>
<td>116 ± 6.8</td>
</tr>
<tr>
<td>Postmenopausal cases (n = 59)</td>
<td>47.0 ± 3.1</td>
<td>24.6 ± 0.7</td>
<td>781 ± 19.2</td>
<td>250 ± 4.9</td>
<td>159 ± 5.1</td>
</tr>
<tr>
<td>Premenopausal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular (n = 15)</td>
<td>31.6 ± 1.2</td>
<td>24.9 ± 0.8</td>
<td>606 ± 22.2</td>
<td>207 ± 6.8</td>
<td>94 ± 6.7</td>
</tr>
<tr>
<td>Luteal (n = 15)</td>
<td>32.0 ± 1.6</td>
<td>25.0 ± 0.9</td>
<td>598 ± 23.9</td>
<td>201 ± 6.6</td>
<td>103 ± 8.6</td>
</tr>
<tr>
<td>Postmenopausal controls (n = 20)</td>
<td>46.7 ± 3.9</td>
<td>25.2 ± 0.7</td>
<td>665 ± 27.7</td>
<td>236 ± 6.9</td>
<td>121 ± 6.8</td>
</tr>
</tbody>
</table>

P values between premenopausal follicular cases and controls: NS*
P values between premenopausal luteal cases and controls: NS NS 0.02 0.001 NS
P values between postmenopausal cases and controls: NS NS 0.01 0.005 NS

* NS, not significant.

### Table 2. DNA content (mean ± SE) in breast cancer tissues in relation to menstrual status

<table>
<thead>
<tr>
<th>Menstrual status</th>
<th>DNA content (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal</td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>2.46 ± 0.1 * (26)</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>2.51 ± 0.1 ** (17)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>2.1 ± 0.1 (28)</td>
</tr>
</tbody>
</table>

Number of patients in parenthesis. *P < 0.05 for premenopausal patients (follicular phase) vs. postmenopausal patients. **P < 0.05 for premenopausal patients (luteal phase) vs. postmenopausal patients.

### Table 3. DNA content (mean ± SE) in breast cancer tissues in relation with histopathological grade

<table>
<thead>
<tr>
<th>Histopathological grade</th>
<th>DNA content (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>2.1 ± 0.14 * (22)</td>
</tr>
<tr>
<td>Grade II</td>
<td>2.3 ± 0.07 ** (34)</td>
</tr>
<tr>
<td>Grade III</td>
<td>2.71 ± 0.16 (15)</td>
</tr>
</tbody>
</table>

Number of patients in parenthesis. *P < 0.01 for grade I vs grade III. **P < 0.05 for grade II vs grade III.
tive significance. A possible influence of hyperlipidemia on the prognosis of breast cancer might be explained by several mechanisms. Firstly, hyperlipidemia is more likely to occur in obese patients, a condition which may affect prognosis. Secondly, disturbances in lipid metabolism might be associated with immunosuppression (35). Thirdly, since cholesterol is a precursor of steroid hormones, an increased steroid hormone production, including estrogen, might promote the growth of hormone-dependent tumors (36). Finally, it should be noted that although the etiological role of hyperlipidemia in breast cancer remains unclear and controversial, high serum lipid levels may be associated with a less favorable prognosis.

The present study shows a higher DNA content in premenopausal breast cancer patients, in both follicular (17%) and luteal (19.5%) phases, as compared with postmenopausal breast cancer patients. This is in agreement with previously reported results (37). The proliferating activity of a tumor is reflected in the rate of DNA synthesis, which is found to be higher in premenopausal than in postmenopausal women (20) and in patients under 50 years (38). Aneuploid tumors, which are more common in premenopausal than in postmenopausal women (37), tend to be more anaplastic and poorer in ERs (19). We had earlier reported that the content of ERs in the tumors of premenopausal patients was lower than that of postmenopausal patients, based on the data from the same breast cancer patient’s groups examined in the present study for the determination of DNA content (6).

According to the findings of the current study, the DNA content in grade III breast cancer tissues was higher than those of grade I and grade II, by 29 and 18%, respectively. Thus, DNA content appears to gradually increase in proportion with the LC grade I and grade II, by 29 and 18%, respectively. DNA content in grade III breast cancer tissues was higher than those of grade I and grade II, by 29 and 18%, respectively. Thus, DNA content appears to gradually increase in proportion with the malignancy grade of the carcinomas. These findings are in agreement with those reported by other studies (16) who indicated that the DNA content of breast cancer tissue reflects the biological properties associated with the malignancy of the tumor.

In conclusion, the present study suggests an association between high total serum lipid and cholesterol levels, and an increased breast cancer risk in premenopausal women. Such an association is also suggested for the high levels of total serum lipids and triglycerides in postmenopausal women. Evaluation of DNA content in breast cancer tissue might be useful in determining suitable therapy for individual cases based on the malignancy grade.

References


