TO STUDY THE SIGNIFICANCE OF APOPTOTIC ENZYME GRANZYME H IN BREAST CANCER PATIENTS

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Background: Breast cancer is the most common type of cancer and the most common cause of cancer-related mortality among women worldwide. The rising global incidence, morbidity and mortality from breast cancer have led to intensified efforts in search for etiological factors of the disease. Present study tried to find out the significance of apoptotic enzyme granzyme H in breast cancer patients. Methods: It was a cross sectional study. Fifty women with pre/post menopausal status were included in the study. Their physiological characteristics including age at menarche, menopause, life style and receptor status including oestrogen, progesterone and HER 2-neu were recorded. Level of serum granzyme H was estimated. Results: It was observed that there is a variation in physiological characteristics. Level of serum granzyme was increased in patients before chemotherapy that may be decreased after treatment but not as much decreased as in normal control subjects. Conclusion: Increased level of granzyme H after chemotherapy may indicate the response of treatment. A direct relationship of oestrogen with granzyme H was also observed. However further study is suggested to reach a better conclusion.

Key Words: Granzyme H, Apoptosis, Breast cancer.

INTRODUCTION

Breast cancer is the most common type of cancer and the most common cause of cancer-related mortality among women worldwide. The rising global incidence, morbidity and mortality from breast cancer have led to intensified efforts in search for etiological factors of the disease. Risk factors that modulate the development of breast cancer are age at menarche, age at menopause, race, socio-economic status, never married reproductive events, use of oral contraceptive pills, lifestyle risk factors (diet, obesity and physical activity) and familial history of breast cancer. Standard prognostic factors include clinical and pathological staging, especially lymph node status and tumour size. Tumour grade and estimates of lymphatic invasion appear to be moderately strong predictive factors, but reproducibility is poor, especially for grade 2 tumours.

Data from the Surveillance Epidemiology and End Results Program indicate that the incidence of breast cancer increases up to 80 years of age and plateaus between 80 and 85 years of age. It is also reported that more than 50% of breast cancers occur in women 65 years of age and older. With increasing age, the risk of co-morbid conditions and cancer-related death also increases. Conversely a study reported that younger age predict higher breast cancer worry. The role of hormone receptor status as a prognostic factor is less clear, but most studies have found that hormone receptor positivity is associated with a longer survival time. It is found that oestrogen receptor positivity predicted a significantly longer disease-free interval and overall survival, but only in the subset of patients with operable breast cancer. Another study reported that with locally advanced breast tumours found that ER and PR negativity was associated with shorter overall survival times in univariate analyses. However according to multivariate analysis, PR status was still significantly associated with survival.

Other variables that have been investigated as possible prognostic markers in locally advanced breast cancer include measures of proliferation, p53, HER-2, and nuclear grade. Another study found that standard predictive factors that indicate breast cancer metastasis are hormone receptor status and HER-2 amplification. It is indicated that induction of apoptosis in immune cells is yet another mechanism used by tumours to evade immune recognition. Granzymes (serine esterase) are major components of the granules of cytolytic lymphocytes, natural killer and cytotoxic T cells. Upon interaction with target cells, cytotoxic T lymphocytes and natural killer cells vectorially secrete highly specialized cytoplasmic granules containing perforin and a family of 11 serine proteases or granzymes. The exocytosis of death-inducing granymes stored in granules of cytotoxic lymphocytes allows the immune system to rapidly eliminate intracellular pathogens and transformed cells. The membrane-disrupting protein perforin allows entry of granzymes into the target cell where they induce apoptosis by cleaving target substrates in the cytoplasm and nucleus. Objectives of the study were to find out the level of granzyme H in pre/post menopausal patients with breast cancer before and after chemotherapeutic treatment and compared to normal control subjects; and to propose its tumour marker role in breast cancer.

MATERIALS AND METHOD

Fifty female patients with breast cancer stage-2, 25 pre- and 25 postmenopausal, were taken from Oncology out door of SRGH Lahore. Level of serum
granzyme was estimated by using by control Poly, ADP, ribose polymerase and inhibitor 3,4-Dichloroisocoumarin. Receptor status of oestrogen, progesterone and HER 2-neu was estimated by using the staining procedure.

Table-1: Physiological variables in pre and postmenopausal women

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Premenopausal Women (n=25)</th>
<th>Postmenopausal Women (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche</td>
<td>13.18±0.98</td>
<td>12.50±1.07</td>
</tr>
<tr>
<td>Age at postmenopausal</td>
<td>50.89±2.76</td>
<td>50.92±0.92</td>
</tr>
<tr>
<td>Profession</td>
<td>All</td>
<td>13 non-professional</td>
</tr>
<tr>
<td>Life style</td>
<td>Active (5)</td>
<td>Active (2)</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>married</td>
</tr>
<tr>
<td>Parity</td>
<td>3.25±1.67</td>
<td>2.5±1.04</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>10±15±7.6</td>
<td>10±10±8.0</td>
</tr>
<tr>
<td>Oestrogen receptor status</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Progesterone receptor status</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>HER 2-neu receptor status</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

RESULTS

Physiological characteristics of pre and postmenopausal women were tabulated. Mean age at menarche was 13 years in pre-menopausal women while in postmenopausal women it was 12 years old. Mean menopausal age in postmenopausal women was 50 years. All pre-menopausal women were professional while in case of post menopausal women 50% were professional and 50% were non-professional. Life style data and marital status showed that most of the women of both group were unmarried, having a sedentary lifestyle. Parital status showed that mean number of children in pre-menopausal women was 3.25±1.67 while in postmenopausal women it was 2.5±1.04. Blood pressure of both groups seems to be normal. Level at menarche was not significant risk factors. It is observed that premenopausal women have a high risk of breast cancer as compared to pre-menopausal women. Blood pressure of both groups seems to be normal. It is observed that premenopausal women having more oestrogen, progesterone and HER 2-neu receptor positive status as compared to postmenopausal women. Level of granzyme H in pre/post menopausal women and control subjects were also tabulated (Table-2).

Table-2: Level of Granzyme H before and after chemotherapeutic treatment in pre/postmenopausal women and controls. (Mean±SD)

<table>
<thead>
<tr>
<th>Level of granzyme H (pg/ml)</th>
<th>Premenopausal Women (n=25)</th>
<th>Postmenopausal Women (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>0.38±0.04</td>
<td>0.48±0.03*</td>
</tr>
<tr>
<td>After treatment</td>
<td>0.46±0.13</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>Controls</td>
<td>0.50±0.02</td>
<td>0.58±0.03</td>
</tr>
</tbody>
</table>

*p<0.001 = highly significant

It is observed that the level of granzyme H in both group of patient before starting their treatment was decreased as compared to normal control subjects. This showed a highly significant difference (p<0.001). After taking chemotherapeutic treatment that depend on receptor status of oestrogen, progesterone and HER 2-neu, the level of granzyme H was increased significantly (p<0.01) as compared to level of granzyme H before chemotherapeutic treatment.

DISCUSSION

Granzymes are major components of granules of cytolytic lymphocytes, natural killer and cytotoxic T cells. Their generally accepted mode of action consists of their directed secretion towards a neoplastic target cell and perforin-dependent delivery to target cell cytosol, where they engage in various actions resulting in target cell apoptosis.16

We observed that mean age of menarche, profession and parietal status does not relate to breast cancer. Multiple analysis of a group of workers12 also indicated that variables such as higher education, early age at menarche were not significant risk factors. It is observed that premenopausal women have a high risk of breast cancer as compared to pre-menopausal women. However, present data is in contrast to the study13 who found that more than 50% of breast cancers occur in women 65 years of age and older. A study explained that the reason for more indolent course of breast cancer in older women is that prevalence of hormone-receptor rich, well differentiated and slowly proliferating neoplasms increases with age. Ability of the host to support tumour growth may also decrease with age.18

We observed a sedentary lifestyle in both groups of patients. Many reports suggest that physically active women have a somewhat lower breast cancer incidence than physicially inactive women.19 A study10 recommended adoption of dietary patterns emphasizing regular physical activity to all people at risk for cancer and cardiovascular disease. It is observed that progesterone receptor status was more positive in premenopausal women than postmenopausal women. However oestrogen receptor status was more positive in premenopausal women that post menopausal women. Receptor status of HER 2-neu was positive in a few women of both groups. It is also observed that sedentary lifestyle leads to obesity (data not shown). A study reported that obesity was more consistently associated with increased risk of hormone receptor-positive than hormone receptor-negative tumours, possibly reflecting increased oestrogen synthesis in adipose stores and greater bioavailability.21

It was reported22 that oestrogen stimulated certain malignant tumours derived from postmenopausal women. Study also observed inhibitory effect of 3H-thymidine incorporation into DNA by the effect of


85
progestrone on malignant lesions. When menstrual status was considered, it was found that DNA synthesis was significantly higher in presence of insulin and hydrocortisone in malignant tumours derived from premenopausal women than from postmenopausal women. On the other hand, Autoradiographic studies indicated that the hormones shortened the length of cell cycle of normal breast tissue. Oestrogen can alter the S phase duration with a consequent increase in the rate of DNA synthesis.

We observed that level of granzyme H was more in post menopausal women before and after treatment than premenopausal women. Data indicate that high apoptotic rates in cancer tissues are indicative of a favourable patient outcome. The study provides indirect evidence that this process may involve cell cycle inhibitors physiologically. It was observed that the positive hormonal receptor is directly related with the level of granzyme H. A study proposed that estrogens can act on target cells to control their destruction by immune system cells and shows that induction of PI-9 expression can inhibit both CTL and NK cell-mediated apoptosis. It is found that oestrogen induction of PI-9 may reduce the ability of cytolytic lymphocytes-mediated immune surveillance to destroy newly transformed cells, thus providing a novel mechanism for an oestrogen-mediated increase in tumour incidence.

CONCLUSION
An increased level of granzyme H after chemotherapy may indicate the response of treatment. A direct relationship of oestrogen with granzyme H was also observed. However further study is suggested to reach a better conclusion.

REFERENCES