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.[1] The rising global incidence, morbidity and mortality from breast cancer have led to intensified efforts in the 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.[1,2] 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.[3]

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.[4] It is also reported that more than 50% of breast cancers occur in women 65 years of age and older.[5] With increasing age, the risk of co-morbid conditions and cancer-related death also increases.[6] Conversely a study reported that younger age predict higher breast cancer worry.[7]

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.[8] Another study reported[9] 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.[10] Another study found that standard predictive factors that indicate breast cancer metastasis are hormone receptor status and HER-2 amplification.[11]

It is indicated that induction of apoptosis in immune cells is yet another mechanism used by tumours to evade immune recognition.[12] 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.[13] The exocytosis of death-inducing granzymes 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.[14,15]

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

Parameters	Premenopausal Women (n=25)	Postmenopausal Women (n=25)
Age at menarche	13.18±0.98 SE=0.30	12.50±1.07 SE=0.38
Age at postmenopausal	-	50.89±2.76 SE=0.92
Profession	All	13 non-professional 12 professional
Life style	Active (5) Sedentary (20)	Active (2) Sedentary (23)
Marital status	Married	one unmarried
Parity	3.25±1.67 SE=0.59	2.55±1.04 SE=0.31
Blood pressure	110±15.4/70±7.8	100±10.4/60±4.8
Oestrogen receptor status (positive)	15	10
Progesterone receptor status (positive)	18	5
HER 2-neu receptor (positive)	5	2

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 life style. Parietal status showed that mean number of children in pre-menopausal women was more that postmenopausal women. Blood pressure of both groups seems to be normal. Receptor status was also estimated. It is found that pre menopausal women having more oestrogen, progesterone and HER 2-neu receptor positive status as compared to post menopausal women. Level of granzyme H in pre/post menopausal women and normal 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)

Level of granzyme H (pg/ml)	Premenopausal Women (n=25)	Postmenopausal Women (n=25)
Before treatment	0.38±0.04*	0.48±0.03*
After treatment	0.46±0.13	0.56±0.02
Controls	0.50±0.02	0.58±0.03

*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 workers[12] 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. Our study is in accordance with a study,[13] which reported, that younger age predicts higher breast cancer worry. However, present data is in contrast to the study[17] 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 life style in both groups of patents. Many reports suggest that physically active women have a somewhat lower breast cancer incidence than physically inactive women.[19] A study[20] 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 reported[22] that oestrogen stimulated certain malignant tumours derived from postmenopausal women. Study also observed inhibitory effect of ^{3}H-thymidine incorporation into DNA by the effect of

progesterone on malignant lesions. When menopausal 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.[22] 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.[23]

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.[24] 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.[25]

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

1. Hortobagyi GN, de la Garza Salazar J, Pritchard K, Amadori D, Haidinger R, Hudis CA, et al. The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer. 2005;6(5):391–401.
2. Dumitrescu RG, Cotarla I. Understanding breast cancer risk–where do we stand in 2005? J Cell Mol Med. 2005;9(1):208–21.
3. Hayes DF. Prognostic and predictive factors revisited. Breast. 2005;14(6):493–9. Epub: 2005 Oct 18.
4. Yancik R, Ries LA. Cancer in older persons. Magnitude of the problem--how do we apply what we know? Cancer 1994;74(7 Suppl):1995–2003.
5. Silliman RA, Baeke P. Breast cancer in the older woman. In: Balducci L, Ersher WB, Lyman GH, ed. Comprehensive geriatric oncology. Amsterdam: Harwood, 1998.
6. Holmes FF. Clinical course of cancer in the elderly. Cancer Control 1994;1:108–14.
7. Loescher LJ. Cancer worry in women with hereditary risk factors for breast cancer. Oncol Nurs Forum. 2003;30(5):767–72.
8. Stewart JF, King RJ & Winter PJ. Oestrogen receptors, clinical features and prognosis in stage III breast cancer. Eur J Cancer Clin Oncol 1982;18:1315–20.
9. Bonnefoi H, Diebold-Berger S & Therasse P. Locally advanced/inflammatory breast cancers treated with intensive epirubicin-based neoadjuvant chemotherapy: are there molecular markers in the primary tumour that predict for 5-year clinical outcome? Ann Oncol 2003;14:406–13.
10. Silvestrini R, Daidone MG, Valagussa P. Cell kinetics as a prognostic marker in locally advanced breast cancer. Cancer Treat Rep 1987;71:375–9.
11. Hayes DF.Prognostic and predictive factors revisited. Breast. 2005;14(6):493–9. Epub 2005 Oct 18.
12. Whiteside TL: Apoptosis of immune cells in the tumour microenvironment and peripheral circulation of patients with cancer: implications for immunotherapy. Vaccine 2002, 20(Suppl 4):A46–51.
13. Salcedo, TW, Azzoni L, Wolf SF, Perussia, BF. Modulation of perforin and granzyme messenger RNA expression in human natural killer cells J Immunol. 1993, 151:52511–20.
14. Ashton-Rickardt PG. The granule pathway of programmed cell death. Crit Rev Immunol. 2005;25(3):161–82.
15. Shi L, Keefe D, Durand E, Feng H, Zhang D, Lieberman J. Granzyme B binds to target cells mostly by charge and must be added at the same time as perforin to trigger apoptosis. J Immunol. 2005;174(9):5456–61.
16. Regner M, Mullbacher A. Granzymes in cytolytic lymphocytes--to kill a killer? Immunol Cell Biol. 2004;82(2):161–9.
17. Yavari P, Mosavizadeh M, Sadrol-Hefazi B, Mehrabi Y. Reproductive characteristics and the risk of breast cancer-a case-control study in Iran. Asian Pac J Cancer Prev. 2005;6(3):370–5.
18. Loescher LJ. Cancer worry in women with hereditary risk factors for breast cancer. Oncol Nurs Forum. 2003 Sep-Oct;30(5):767–72.
19. Silliman RA, Baeke P. Breast cancer in the older woman. In: Balducci L, Ersher WB, Lyman GH, ed. Comprehensive geriatric oncology. Amsterdam: Harwood, 1998.
20. Nixon Stanford JL, Weiss NS, Voigt LF, Daling JR, Habel LA, Rossing MA. Combined estrogen and progestin hormone replacement therapy in relation to risk of breast cancer in middle-aged women. JAMA 1995;274:137–42.
21. Bernstein L, Patel AV, Ursin G, Sullivan-Halley J, Press MF, Deapen D, et al. Lifetime recreational exercise activity and breast cancer risk among black women and white women. J Natl Cancer Inst. 2005;97(22):1671–9.
22. Williams MT, Hord NG. The role of dietary factors in cancer prevention: beyond fruits and vegetables. Nutr Clin Pract. 2005;20(4):451–9.
23. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature**.** Cancer Epidemiol Biomarkers Prev. 2004;13(10):1558–68.
24. Calaf G, Russo IH, Roi LD, Russo J. Effects of peptides and steroid hormones on cell kinetic parameters of normal human breast tissue in organ culture In Vitro Cell Dev Biol. 1986;22(3 Pt 1):135–40.
25. Gloria Calaf, Fernando Garrido, Carlos Moyano, Roberto Rodríguez. Influence of hormones on DNA synthesis of breast tumors in culture. Breast Cancer Research and Treatment 1986;8(3):233–40.
26. Thomas Schöndorf, Uwe-Jochen Göhring, Martina Becker, Markus Hoopmann, Torsten Schmidt, Sabine Rützel, et al. High Apoptotic Index Correlates to p21 and p27 Expression Indicating a Favorable Outcome of Primary Breast Cancer Patients, but Lacking Prognostic Significance in Multivariate Analysis Pathobiology 2004;71:217–22.
27. Xinguo Jiang, Brent A. Orr, David M. Kranz, David J. Shapiro**.** Estrogen Induction of the Granzyme B Inhibitor, Proteinase Inhibitor 9 Protects Cells against Apoptosis Mediated by Cytotoxic T Lymphocytes and Natural Killer Cells. 2006 by The Endocrine Society by American Association of Immunologist.

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