

# Granzyme H Serum Levels Variations with Both Reproductive Hormone Receptors, and Related Hormone Receptors in Breast Cancer Patients

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## Abstract

**Background:** Breast Cancer (BC) is the most common cancer in Iranian women, meanwhile the Iranian patients are relatively young. Granzyme H (GZMH) is a functional cytotoxic serine protease of NK cell granules, which expands the cell death-inducing repertoire of innate immune system. GZMH is constitutively and highly expressed in human NK cells, in order to possess chymotrypsin-like (chymase) enzymatic activity. The purpose of this study was to determine GZMH level, in BC and healthy women.

**Methods:** 30 breast cancer patients, and 30 control women in premenopausal status, have participated in this study. GZMH, Estrogen levels, and ER, PR have been measured in cancer and healthy women subsequently, as using ELISA, Radioimmunoassay, and Immunohistochemistry methods.

**Results:** Mean GZMH value was lower in BC than healthy women ( $p < 0.0001$ ).

**Conclusion:** Our study has implicated existence of suppressor or problem for producing of GZMH in patients group and levels of estrogen couldn't effect on making positive ER, PR.

**Keywords:** Breast Cancer; Estrogen; Granzyme H

**Please cite this article as:** Tahbaz-lahafi B, Akbari ME, Amir-rassouli H, Rahimipour A, Azargashb E, Nafissi N, Jahani F. Granzyme H Serum Levels Variations with Both Reproductive Hormone receptors, and Related Hormone Receptors in Breast Cancer Patients. *Iran J Cancer Prev.* 2014; 7(1):48-52.

## Introduction

Iran, which is located in southwest Asia, located in an epidemiologic transition and faces the double burden of diseases. In addition, cancer is a major public health problem in Iran [1]. Breast Cancer is the most common cancer in Iranian women; meanwhile the Iranian patients are relatively young. Given that Iran has a female population of about 38 million, this corresponds to a total number of 8500 new cases of breast cancer annually [2].

Breast cancer has more frequently occurred in wealthy countries due to a higher prevalence of BC risk factors, such as aged population, older age at first pregnancy, nulliparity, inappropriate breastfeeding, high-calorie intake, sedentary occupation and steroid hormonal consumption.

On the other hand, BC survival would be lower in less affluent countries, and in women with low income, or educational level.

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Received: 2 Nov. 2013

Accepted: 21 Dec. 2013

*Iran J Cancer Prev.* 2014; 1:48-52

Based on the report from the Ministry of Health and Medical Education (MOHME); Cancer is the third cause of death in Iran after cardiovascular heart disease, then the injuries [3, 4].

Experimental data has strongly suggested that estrogens have a role in the development and growth of breast cancer [5].

Tumor formation may also result from excessive hormonal stimulation of an organ in which normal growth and function would be under endocrine control. The response of an organ to the proliferative effects of a hormone might be a progression from normal growth, to hyperplasia, then to neoplasia.

Indirect evidence of this sequence has included the increased risk of breast cancer associated with early menarche, late first full-term pregnancy, and late menopause as well as the reduced risk associated with early menopause [5].

Estrogens promote cell proliferation and metastases in several human cancers. Different actions of estrogens have likely contributed to tumor development-blocking immune surveillance [6].

Immune surveillance has described the process whereby precancerous and malignant cells have recognized by the immune system as damaged, and then would be consequently targeted for elimination [7].

Natural Killer (NK) cells and Cytotoxic T Lymphocytes (CTLs) both have played important roles in the innate and adaptive immunity against intracellular pathogens and tumor cells [8].

Granule exocytosis is the main pathway for immune elimination of virus-infected cells and tumor cells by CTLs and NK cells. After target-cell recognition, Release of the cytotoxic granule contents in to the immunological synapse, have formed between the killer cell and its target induces apoptosis. The granules have contained perforin and a family of serine proteases known as granzymes [9]. The perforin/granzyme (Gzm) pathway is a major mechanism for these cytotoxic lymphocytes to kill their targets. The Gzms are a highly conserved set of serine proteases existing in humans [8].

Granzyme H (GZM H) has regarded as an orphan granzyme with unknown biologic functions in immune defense cells.

GZM H, which expands the cell death-inducing repertoire of innate immune system, was a functional cytotoxic serine protease of NK cell granule [10].

Several studies have recently shown GzmH to be constitutively and highly expressed in human NK cells and to possess chymotrypsin-like (chymase) enzymatic activity [11].

Human GZMH triggers rapid cell death of target tumor cells that is characteristic of DNA fragmentation and mitochondrial damage and generation of reactive oxygen species [8, 12].

The steroid hormones, estradiol, plays an important role in the progression of breast cancer, and a majority of the human breast cancers start out as estrogen dependent and express the Estrogen Receptor (ER). The biological effects of estrogen have mediated by its binding to one of the structurally and functionally distinct ERs (ER $\alpha$  and ER $\beta$ ) [13]. The action of progesterone has mediated through its intracellular cognate receptor, the Progesterone Receptor (PR), which functions as a transcription factor that regulates gene expression. Mutation or aberrant expression of the coregulators might thus affect the normal function of the PR, and hence disrupt the normal development of the

mammary gland, which might lead to breast cancer [14].

## Materials and Methods

30 breast cancer patients who were under study at Cancer Research Center, Shahid Beheshti University of Medical Sciences (CRC, SBUIMS), and 30 control women in premenopausal status, have participated in this study. A total four ml blood has drawn from patients and control women with a 21-gauge needle by syringe. Blood sample have centrifuged immediately (3000 $\times$ g for 5 min) to obtain serum. Serum has kept in -80°C for future analysis. Radioimmunoassay (RIA) has applied for measurement of serum estrogen (Bio source, KIP0629). A sandwich enzyme-linked immunosorbent Assay (ELISA) has applied for measurement of granzyme H in serum (CUSABIO, GZMH-E17366h). Immunohistochemistry (IHC) for assaying estrogen and progesterone receptors (Novocastra, Product Code: RTU-CB11, RTU-PCR-312) Result have expressed as mean $\pm$ SD of experiments. Student's t-test has used for comparison the means between two groups, Logistic Regression for BC risk. To assess correlation between GranzymeH and Estrogen, correlation bivariate test has performed. For all tests, P<0.05 has considered statistically significant. Data have analyzed using SPSS software version 16.

## Results

Mean of serum levels of Granzyme H patients was 71.6 $\pm$ 12.2 and 96.16 $\pm$ 13.4 in control group (p<0.0001). Mean of serum Estrogen in patients group was 107.5 $\pm$ 23.8 and in control group was 90.5 $\pm$ 14.8 (p<0.003) (Table1). There was a weak inverse correlation between GZMH and estrogen levels in patient group (r=-0.3 p<0.02). Spearman correlation analysis between Estrogen, ER, and PR has indicated a weak positive non-significant correlation in patient group. (r=0.10, r=0.04).

## Discussion

Granzymes are components of granules in natural killer cells and cytotoxic T cells. They are secreted towards a neoplastic target cell subsequent to perforin-dependent delivery to target cell cytosol where they have caused apoptosis in target cell (tumor) [15]. According to our finding, levels of Granzyme H in patients group were lower than controls group. Razavi et al. have shown that the level of GZMH before starting chemotherapeutic treatment has decreased [15]. Experimental data

**Table 1.** Demographic characteristics and some matched confounding variables.

Variables	Case	Control
Age (mean±SD) (year)	38.0±6.8	34.4±8.9
Granzyme H (mean±SD) (pg/ml)	71.6±12.2	96.16±13.4
Estrogen (mean±SD) (pg/ml)	107.5±23.8	90.5±14.8
BMI(mean±SD)	41.1±6.5	39.3±5.5
Age at monarch (mean±SD) (year)	13.6±1.5	13.1±1.3
Number of delivery (mean±SD)	1.6±1.5	0.7±1.1
Breastfeeding (mean±SD) (month)	13.8±14.5	9.2±16.0
Single, nulliparity (%)	26.6%	66.6%
Hormones consumption (%)	50%	16.7%
Smoking (%)	6.7%	0
Family history (%)	36.7%	6.7%

have strongly suggested that estrogens play a great role in development and growth of breast cancer [5]. Mean levels of serum estrogen in patients group were statistically higher than controls group ( $p<0.003$ ). There was a weak correlation between GZMH and estrogen levels in patient group ( $r=-0.3$ ). According to study of Jiang X et al., estrogen has induced increasing levels of Granzyme B inhibitor, and caused blocking cell death induced by natural killer cells [6].

**Table 2.** Confounding factors in breast patients

Variables	P value	OR	CI(95%)
BMI	0.4	1.1	0.7-1.6
hormones consumption	0.01	32.6	1.8-57.3
Granzyme H	0.009	0.8	0.8-0.9
Number of delivery	0.4	1.9	0.3-10.7
Breastfeeding	0.9	0.9	0.9-1.0
Age at monarch	0.8	1.0	0.5-2.0
Family history	0.6	2.1	0.07-7.6

So our study has implicated existence of suppressor or problem for producing of GZMH in patients group, but it has seemed to be, that contrary to Granzyme B. According to our finding levels of estrogen couldn't affect on making positive ER, PR. Some factors like hormone consumption could increase the risk of BC. Logistic regression analysis between hormone consumption with breast cancer, have indicated significant statistically relationship ( $p<0.01$ , OR=32.6, CI: 1.8-57.3) (Table 2). Epidemiologic data have provided strong evidence for an association between plasma estrogens and breast cancer risk. The 1996, large meta-analysis of the relationship between oral contraceptive use and breast cancer risk, have shown that a history of recent oral contraceptive use, rather than duration of use, was a better predictor of breast cancer risk. These data have based primarily on older high-dose and moderate-dose oral contraceptive pills and not the recently introduced low-dose pills. It is, therefore, likely that the small increase in breast cancer risk associated with the early formulations of oral contraceptives would diminish with the new low-dose pills [16]. Human GZMH triggers rapid cell death of target tumor cells that is characteristic of DNA fragmentation and mitochondrial damage and generation of reactive oxygen species [8,12]. We have observed that decreasing level of Granzyme H has increased the risk of breast cancer ( $p<0.009$ , OR=0.8, CI: 0.8-0.9) (Table 2).

But our study has shown that BMI, number of delivery or breastfeeding, and age at monarch couldn't affect on risk of BC. Early age at menarche has been found to be one of the important determinants in etiology of breast cancer. Many epidemiologic studies have suggested that as younger a woman's age at monarch, would be the higher her risk of breast cancer. Since monarch at a young age has associated with earlier onset of regular menstrual cycles, early exposure to hormonal milieu associated with regular ovulatory menstrual cycles might be an important etiologic factor [17]. One case-control study has conducted in Brazil, has shown that breastfeeding could not have a protective effect against breast cancer. Some researchers have believed that the number of children would not make sense for avoiding breast cancers. But many other studies have revealed that null parity is a great risk for breast cancer and the number of children is a protective factor against breast cancer. Another study, which has conducted in Nigeria, has shown that parity and breastfeeding are protective against breast cancer. Parity and lactation period have reduced the lifetime number of ovulatory cycles,

reducing the risk of breast cancer particularly in young mothers. This also possibly has reduced estrogen and increased prolactin production, which might decrease women's cumulative exposure to estrogen, thereby inhibiting the initiation or growth of breast cancer cells [4]. A lower risk of breast cancer in women with high body mass index has reported among premenopausal woman by London et al. (1989), Ursin et al. (1995) Franceschi et al. (1996) whereas some studies have found no association [17].

In our study family history couldn't have significant effect on BC but in patients group percentage of family history is higher than controls group. In any case, many epidemiologic studies have suggested that a family history of breast cancer increases a woman's risk of developing breast cancer and the extent of risk varies according to the nature of the family history, type of relative affected, age at which relative developed breast cancer the number of relatives affected and might vary according to the age of the individual [17]. There are not any similar studies in Iranian populations so further study has suggested to reach a better conclusion.

## Conclusion

Our study has implicated existence of suppressor or problem for producing of GZMH in patients group and levels of estrogen couldn't effect on making positive ER, PR.

## Acknowledgment

The authors have especial thanks for Faculty of Paramedical Sciences and Cancer Research Center of Shohada Hospital in Shahid Beheshti University of Medical Sciences staff for managing administrative issues. The authors have declared that the approval has not required.

## Conflict of Interest

There is no conflict of interest in this study.

## Authors' Contribution

Behnoosh Tahbaz-Lahafi has designed and written this article, Eznollah Azargashb and Houshang Amir-Rassouli and Ali Rahimipour have analyzed the data, Behnoosh Tahbaz Lahafi and Nahid Nafissi and Farida Jahani have collected the data. All authors have read and approved the final manuscript.

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