

## Comparative Study of Serum levels of Granzyme H & Estrogen In Patients Suffering From Breast Cancer

Behnoosh Tahbaz lahafi<sup>1</sup>, Houshang Amirrassouli\*<sup>1</sup>, Mohammad-Esmaeil Akbari<sup>2</sup>, Saeed Namaki<sup>1</sup>, Faranak Kazerouni<sup>1</sup>, Ali Rahimipour<sup>1</sup>, Jahangir Mohammadzade<sup>1</sup>

<sup>1</sup> Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

<sup>2</sup>Cancer Research Center, Shohada Hospital, Shahid Behesht University of Medical Sciences, Tajrish, Tehran, Iran.

\*Corresponding Author: email address: houshangan@sbmu.ac.ir (H. Amirrassouli)

### ABSTRACT

GranzymeH is a functional cytotoxic serine protease of NK cell granules, which expands the cell death-inducing repertoire of innate immune system. The purpose of this study was to determine GranzymeH (GZMH) level in breast cancer (BC) and healthy women. This study was performed on 30 patients with BC and 30 healthy women. GZMH and Estrogen levels were measured in cancer patients and healthy women subsequently using ELISA and Radioimmunoassay (RIA) methods. Mean GZMH value was lower in BC than healthy women ( $p < 0.0001$ ) and mean Estrogen level was higher in BC patients in comparison to healthy women ( $p < 0.003$ ). Our finding indicates probability of existence of suppressor or a problem in production of GZMH in cancer patients.

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

### INTRODUCTION

Breast cancer is fairly common, because of its well publicized nature, and potential for lethality, breast cancer is arguably the most frightening type of cancer diagnosis someone can receive [1]. Breast cancer (BC) is the most prevalent cancer in Iranian women and the fifth most common cause of cancer-related death in Iran [2]. Experimental data strongly suggest that estrogen has a role in the development and growth of breast cancer [3]. Estrogen may be implicated in breast cancer risk because of 1) its role in stimulating breast cell division, 2) its work during the critical period of breast growth and development, 3) its effect on other hormones that stimulate breast cell division and 4) its support of the growth of estrogen-responsive [4]. Cytotoxic T cells (CTL) and natural killer (NK) cells are indispensable factors in the body's ongoing defence against viral infection and tumor development. CTL/NK cells recognize and kill infected or aberrant target cells by two major pathways: either through introduction of a battery of proteases—called granzymes—to the target cell cytosol, or through TNF superfamily-dependent

killing [5]. Granzymes are chymotrypsin (CHT)-like serine proteases expressed in the secretory granules of CTLs and NK cells. Upon the encounter of diseased cells, granzymes are released by exocytosis, along with other granular proteins, into the immunological synapse where they are subsequently taken up to target cells via the actions of the pore-forming protein perforin (PFN). Once in the target cell cytosol, granzymes proteolytically cleave a variety of death substrates that, in turn, lead to eventual cell suicide [6]. The granule-mediated pathway is often the predominant pathway for CTL and NK cell-induced cell death [7]. GZMH is constitutively expressed in human resting NK cells. This suggests GZMH may play an important role in cytotoxicity induced by human NK cells [8]. GZMH is thus a functional cytotoxic serine protease of NK cell granules, which expands the cell death-inducing repertoire of innate immune system [9]. The death induced by GZMH displays many features of apoptosis, including DNA degradation, chromatin condensation, mitochondrial depolarization, and generation of reactive oxygen species [10]. The

purpose of this study was to determine GranzymeH level in breast cancer(BC) and healthy women.

### MATERIALS AND METHODS

In this study 30 patients with BC who referred to Cancer Research Center, Shahid Beheshti University of Medical Sciences and 30 healthy women as the control group were recruited. In our study clinical staging of the breast cancer patients was based on the American Joint Committee on Cancer (AJCC) classification, with reference to which 10% of the patients were in stage I, 73.3% were in stage II, 16.7% were in stage III [11].

The exclusion criteria for all subjects enrolled in this study were presence of immunological disorder, acute / chronic inflammatory disease and history of immunosuppressive or radiation therapy [12]. All participants gave their written informed consent and agreed to proceed with study protocol. 4 cc blood was collected from the subjects, serum was isolated and stored at -80°C. The concentration of serum estrogen of all study subjects was measured using RIA method (Biosource, KIP0629). Serum GZMH was measured using the ELISA method (CUSABIO, GZMH-E17366h).

#### Statistical Analysis

Results were expressed as mean  $\pm$  SD of experiments. Student's *t*-test was used for comparison of the means between two groups, and one-way ANOVA for comparison between multiple groups. To assess correlation between GranzymeH and Estrogen, age, cancer stage correlation bivariate test was performed. For all tests,  $P < 0.05$  was considered statistically significant. Data were analyzed using SPSS software version 16.

### RESULTS

As shown in table 1 in this study the mean age of subjects was  $36.2 \pm 8.12$  (ages 24-54 years).

**Table 1:** age range of participated women

Age	Min	Max	Mean	SD
	24	54	36.2	8.12

Based on comparing the obtained results with the control group GZMH was significantly lower in the cancer patients ( $71.6 \pm 12.2$  vs  $96.16 \pm 13.14$  pg/ml  $p < 0.0001$ ). Estrogen level was statistically

higher in patients than the control group ( $107.5 \pm 23.8$  vs  $90.5 \pm 14.8$  pg/ml  $p < 0.003$ ). There was no significant correlation between cancer stage and Estrogen level in patient group. A weak significant inverse correlation was seen between estrogen and GZMH ( $r = -0.3$   $p < 0.02$ ).

### DISCUSSION

NK cells express the strongly proapoptotic GZMA and GZMB along with perforin. However since other granzymes of NK cell like GZMH has so far received little attention. We decided to determine GZMH level in breast cancer patients. [9] According to our finding GZMH level was significantly lower in patients than the control group this result is consistent with the observation of Razavi NZ et al who reported lower GZMH level in cancer patients before and after chemotherapy as compared to normal control subjects. [13] Our finding can be an indication of the importance of granzymes; a major component of granules of cytolytic lymphocyte, NK and cytotoxic T, and its possible contribution as an alternative cytotoxic effector protease to NK cell function in tumor elimination [13]. Razavi NZ et al showed that level of GZMH was increased in BC patients after chemotherapy which is probably result of high apoptotic ratio in cancer tissues indicative of a favourable patient outcome [13]. In consistence with previous research works in our study estrogen level was higher in BC patients in comparison to the control group. ( $p < 0.003$ ) It is found that estrogen induction of PI-9 (Proteinase inhibitor 9) may reduce the ability of cytolytic lymphocyte mediated immune surveillance to destroy newly transformed cells [14].

### CONCLUSION

We found a weak inverse correlation between GZMH and estrogen which can be indicative of inductive effect of estrogen on GZMH. Further studies are required for understanding the exact mechanism related to decreased level of GZMH in BC patients. In conclusion our finding indicates probability of existence of suppressor or a problem in production of GZMH in cancer patients.

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