

Estimating CD56 and Perforin in Rats Primed by Breast Cancer Cell Line T47D

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Abstract

Breast cancer is the most common disease in females and it has become a major health problem affecting women worldwide. This study was carry out on different groups of female albino rats *Rattus rattus*, that primed by different forms of antigens prepared from cell line of human breast cancer T47d. The current study has been designed to determine the immune response of rats for breast cancer cells antigens. The immune parameters CD56 and perforin have been estimated. Significant increase ($P<0.05$) is found in the level of expression of CD56 on the surfaces of natural killer cells of rats in all treated groups, but higher level in group that injected by mixture of freezing killed antigen with adjuvant that reached to 83.164 ± 7.616 ng/ml is compared with the control groups. The results have indicated the presence of significant increase in the level of cellular toxin perforin in all treatment groups especially the rats that immunized by freezing killed antigen with adjuvant which reached to 113.45 ± 15.925 ng/ml compared with the healthy rats.

Key words: breast cancer, T47D, CD56 and perforin.

الخلاصة

سرطان الثدي هو الورم الخبيث الأكثر شيوعاً في الإناث إذ أصبح مشكلة صحية كبيرة تؤثر على النساء في جميع أنحاء العالم. أجريت تجارب هذه الدراسة على مجاميع مختلفة من إناث الجرذان البيض *Rattus rattus* المحقونة بالأشكال المختلفة من مستضدات خلايا خط سرطان الثدي البشري T47d. وقد صممت الدراسة الحالية لتحديد الاستجابة المناعية لإناث الجرذان المجرعة بأشكال مختلفة من تلك المستضدات. حيث تم تقدير المؤشرات المناعية والمتمثلة بـ CD56 و البيرفورين ($P<0.05$) وجد زيادة معنوية ($P<0.05$) في مستوى تعبير المؤشر CD56 على سطوح الخلايا القاتلة الطبيعية للجرذان المجرعة بالأشكال المختلفة من المستضدات مقارنة مع مجموعتي السيطرة. ولكن المستوى الأعلى كان في مجموعتي الجرذان المجرعة بالمستضد المقتول بالتجميد والممزوج بالمساعد المناعي إذ بلغت 83.164 ± 7.616 (نانوغرام/مل) على التوالي. أشارت نتائج الدراسة الى وجود ارتفاع معنوي ($P<0.05$) في مستوى السم الخلوي البيرفورين Perforin في المجاميع المعاملة مقارنة مع مجموعتي السيطرة، ولوحظ ارتفاع ذلك المستوى في مجموعة الجرذان الممنعة بالمستضد المقتول بالتجميد الممزوج مع المساعد المناعي إذ بلغ 113.45 ± 15.925 (نانوغرام/مل).

كلمات مفتاحية: سرطان الثدي، خط سرطان الثدي T47D، الـ CD56 و البيرفورين.

Introduction

Breast cancer is the greatest frequently cancer that occur in women, and constitutes nearly one third of all cancers (Zhao *et al.*, 2009), furthermore, it is the most common female malignant disease, and the second leading cause of cancer related death in the United States (Yang *et al.*, 2014). It is not a single disease, it is a heterogeneous disease in terms of gene expression, morphology, clinical course, and response to treatment (Sendur *et al.*, 2012; Edge 2013).

Human cancer cell lines represent a foundation for tumor biology and drug discovery by facile experimental manipulation, general and detailed mechanical studies, and various high productivity applications. Cell line panels are explained with both genetic and pharmacological data, either within a tumor lineage or across multiple cancer types (Lin *et al.*, 2008; Sos *et al.*, 2009; Dry *et al.*, 2010).

Natural killer cells are the most important cell type in the human body and it is recognized by the surface marker CD56. They play a crucial role in the immune system's host response in destroying or lysing the cells of infectious and malignant. These cells receive their name from the fact that they are produced naturally by the

body, and their sole aim is to search and destroy harmful cells (**Pribylova and Malickova, 2008**). Perforin (PRF) (which is encoded by the *PRF1* gene) is a pore-forming toxin (**Law et al., 2010**). It is a glycoprotein responsible for the formation of pore in the cell membranes of target cells. Perforin is capable of polymerize and form a channel in the membrane of target cell, act in the delivery of the contents of cytotoxic granules (granzymes and granulysin) to target cell membranes (**Osińska et al., 2014**). It is produced mainly from natural killer (NK) cells and CD8-positive T-cells (**Voskoboinik et al., 2010**). Nevertheless, CD4-positive T-cells are also capable of express a little amount of perforin, when classic cytotoxicity is ineffective or disturbed (**Osińska et al., 2014**). Therefore the aim of this study is to evaluate the immune response of rats immunized by breast cancer cell line (alive breast cancer cell line T47d, killed breast cancer cell line T47d) with and without adjuvant using the following immune function tests:

- 1- Estimating the level of CD-56 on NK cells.
- 2- Estimating the level of perforin.

Materials and Methods

Preparation of Breast Cancer T47D Cell Line

Five ml volume of fresh growth media had been used to suspend cells via tapping the tube to free cells from tube wall. Then the suspension was transferred to 25 ml cell culture flask. The flask cover was closed and then the cells were checked under inverted microscope to insure the presence of appropriate amount of cells in flask and then incubated at temperature 37 °C. After that the falcons were checked daily to detect the changes in the media color and monolayer formation (**Darling and Morgan, 1995**).

Laboratory Animals

Rats that were used in this study were female albino rats adult (White Albino rats *Rattus rattus*). The total number was 50 rats, divided in to 10 groups, each group consisted of 5 rats. The ages of this rats ranged from (8 to 12) week and weights ranged from 250-300 mg.

Preparation of Antigen

Different forms of breast cancer cell line antigens were prepared according to **Bradshaw (1996)**.

Immunization of Laboratory Animals

Immunization of animals (rats) occurred according to **Hay and Westwood, (2002)**.

Collection of Samples

The anatomy of rats occurred after two weeks of the last injection, in order to obtain the blood. The sera had been separated and then placed in appendroff tubes and store in freeze until used.

Immunological Assay

ELISA Protocol

The levels of CD56 and perforin were estimated by ELISA manual procedure according to Creative Diagnostics Company (USA).

Statistical Analysis

The result of this study was presented as mean \pm SD of the collected data. Statistical analysis of mean value was performed through ANOVA and L.S.D. by using the statistical software package SPSS. The difference was considered to be significant at 0.05 level (Susan, *et al.*, 1997).

Results

1. The level of CD56:- The level of adhesion molecule CD56 (ng/ml) expression by NK cells were shown in **figures (1 and 2)** in sera of rats treated by different types of antigen with or without of adjuvant. It was clear that the level of CD56 had been significantly increased ($P<0.05$) in the sera of treatment groups in comparison with control group, but this level was higher in treatment groups. The level of CD56 was higher in treatment groups injected by freezing, heating and chemical killed antigen with adjuvant in comparison with treatment group primed by alive antigen and with control group.

The results showed that there was significantly increase in the sera of all treatment groups in comparison with control groups. The level of CD56 was 35 ± 4.652 and 36.662 ± 4.431 in control and control with adjuvant group respectively, 67 ± 8.125 and 55.664 ± 5.02 in group that injected by alive antigen and by mixture of alive antigen with adjuvant, 78.998 ± 8.004 and 83.164 ± 7.616 in group that injected by freezing killed antigen and by mixture of freezing killed antigen with adjuvant, 77.997 ± 6.47 and 67.665 ± 7.23 in group that injected by heating killed antigen and by mixture of heating killed antigen with adjuvant, 78.5 ± 6.105 and 77.832 ± 8.192 in group that injected by chemical killed antigen and by mixture of chemical killed antigen with adjuvant, but the highest level of CD56 shown in group that injected by mixture of freezing killed antigen and adjuvant that reached to 83.164 ng/ml.

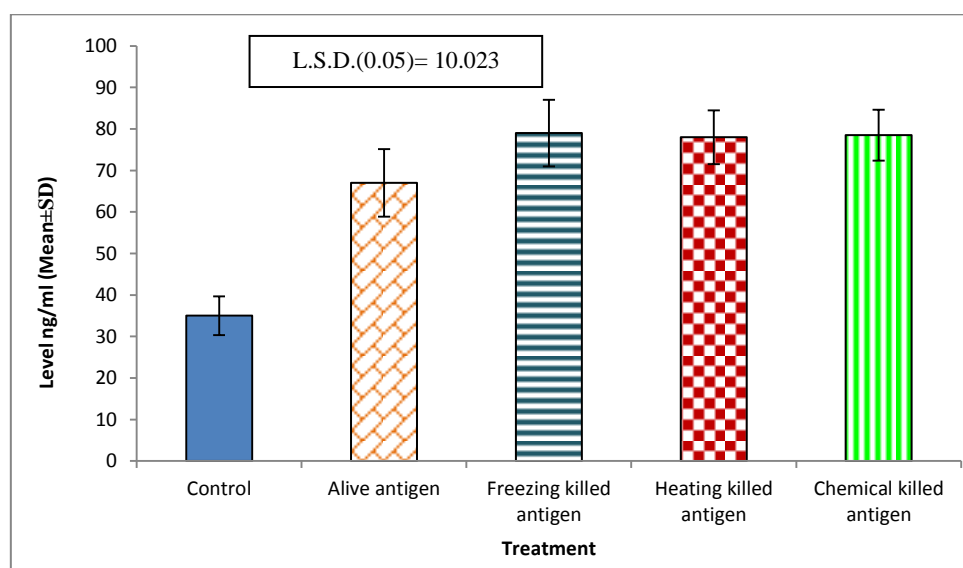


Fig. 1:- The level of CD56 in sera of rats injected by different forms of breast cancer cell line antigens.

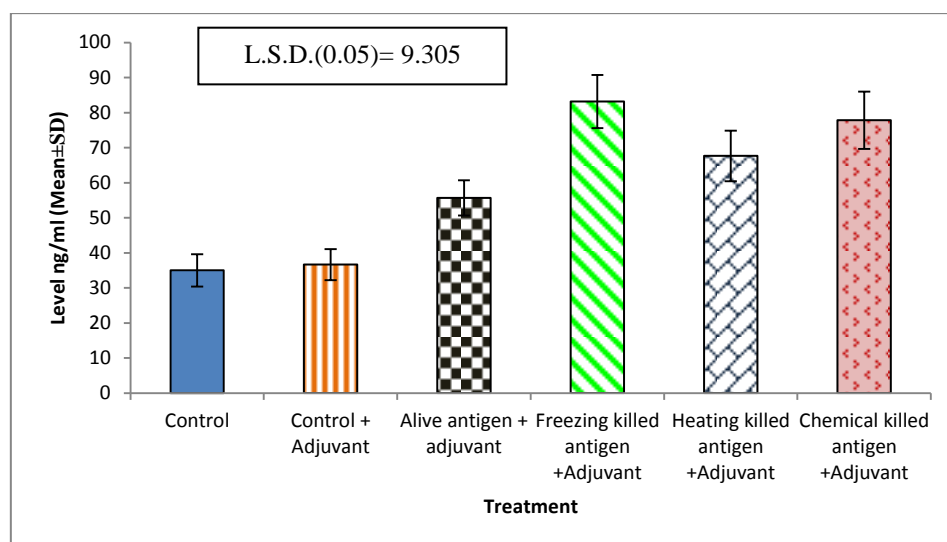


Fig. 2:- The level of CD56 in sera of rats that injected by mixture of breast cancer antigen with adjuvant.

2. The level of perforin:- The results indicated that there was a significant increase ($P < 0.05$) in the level of perforin in treatment groups in contrast with healthy control group as revealed in **figures 3 and 4**. The level of perforin in control and control with adjuvant group was 20.937 ± 5.312 and 24.687 ± 3.437 (ng/ml) respectively, while it was 64.375 ± 11.875 and 70.625 ± 11.25 (ng/ml) in group that injected by alive antigen and by mixture of alive antigen with adjuvant respectively, 44.375 ± 4.875 and 113.45 ± 15.925 (ng/ml) in group that injected by freezing killed antigen and by mixture of freezing killed antigen with adjuvant, 94.687 ± 12.562 and 76.875 ± 10.625 (ng/ml) in group that injected heating killed antigen and by mixture of heating killed antigen with adjuvant, 65.625 ± 9.975 and 40.625 ± 5.255 (ng/ml) in group that injected by chemical killed antigen and by mixture of chemical killed antigen and adjuvant.

The data pointed out that the level of perforin was higher in treatment group immunized by mixture of freezing killed antigen with adjuvant in comparison with other primed groups which reached 113.45 ng/ml. Furthermore the results showed that the level of perforin was higher in treatment group immunized by heating killed antigen in contrast with other groups of treatments and control which reached to 94.68 ng/ml.

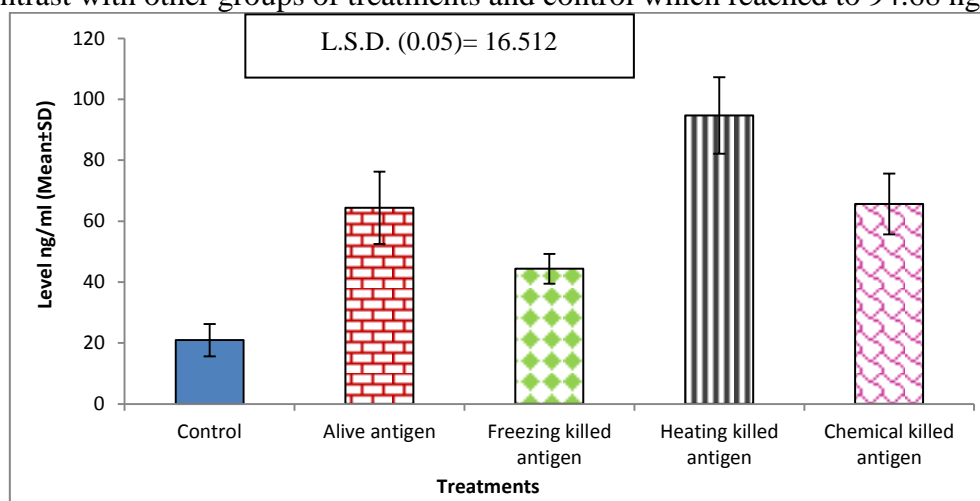


Fig. 3:- Perforin levels in rats injected by breast cancer cell antigen.

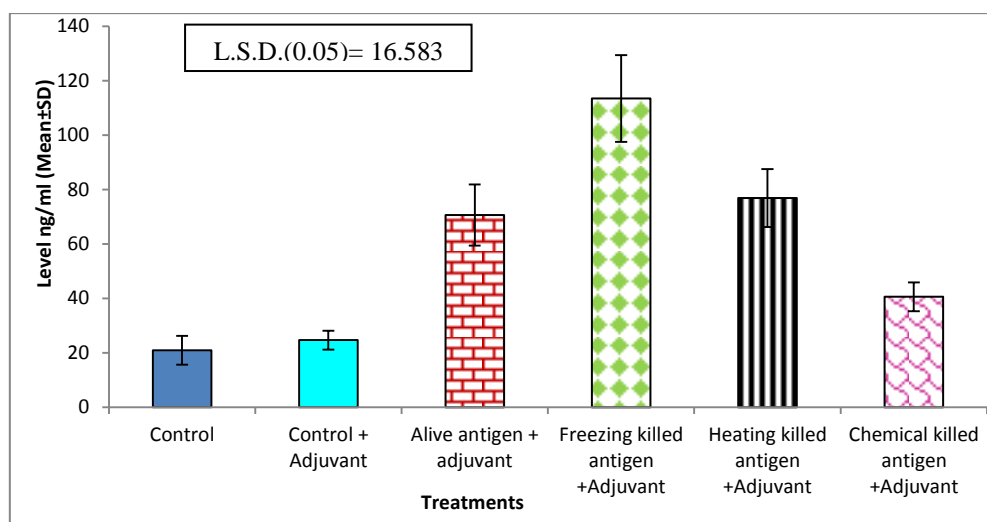


Fig. 4:- Perforin level in sera of rats primed by mixture of breast cancer cell antigen with adjuvant.

Discussion

Breast cancer is the most important cause of mortality in women through the world (yang, *et al.*, 2012). In the our study, the results demonstrate that there is significantly elevation in the level of the adhesion molecules CD56 of natural killer cells (NK) in all treated groups in contrast to negative control group and control group injected by adjuvant group, as it is show in **Figures (1 and 2)**. Natural killer (NK) cells are not found in large numbers in advanced human neoplasms, indicating that they do not normally home efficiently to malignant tissues. For instance, a low prevalence of gastric and colorectal tumor-infiltrating CD56+ cells in liver with multiple metastases is detected. Moreover, the percentage of intrahepatic NK (CD56+) cells is also decreased in patients with metastases compared to those without, being almost twice lower than CD8+ and CD4+. This suggests that low number of NK cell can be due to the escape of metastatic cells from the mechanisms of liver immune control (Gulubova, *et al.*, 2009). Natural killer (NK) cells can control on both local tumor growth and metastasis due to their ability to exert direct cellular cytotoxicity without prior sensitization and to secrete immunostimulatory cytokines like IFN- γ , ant the latter participates in cancer elimination by inhibiting cellular proliferation and angiogenesis, promoting apoptosis, and stimulating the adaptive immune system, and it is instrumental for enhancing Ag processing and presentation (Levy, *et al.*, 2011). Mordoh, *et al.*, 2011 illustrates that there is an association between the decreased activity or low numbers of circulating NK cells with progression of cancers and correlation between an absolute decrease in the activity of the NK cells and an absolute decrease in the lytic potential of these cells. The elevated levels of the adhesion molecules CD56 that expressed by natural killer cells in the sera may be due to the activity of the NK cells against tumors. These cells have critical roles in resistance and maintain the growth of breast cancer and then destruction of cancerous cells.

The results of this study reveal that there is clearly increased in the level of perforin in treated groups in contrast with control groups, and the highest levels of perforin appear in group that immunized by mixture of freezing killed antigen with adjuvant, as shown in the **Figures (3 and 4)**. Perforin and granzymes have a possible role of in tumor apoptosis, growth, or both processes in prostate cancer pateints (Fang, *et al.*, 2012). The level of perforin expression is decreased in pancreatic cancer patients versus healthy donors (Yu, *et al.*, 2012). Also Mohammadzade, *et al.*, (2013)

finds in their study on prostate cancer patients and benign prostatic hyperplasia patients that serum levels of perforin are significantly decreased in prostate cancer patients in comparison to benign prostatic hyperplasia patients. Insignificant expression of perforin in T lymphocytes, NKT, NK cells may be result of tumor activity leading to the development of a chemical barrier around the tumor that possibly prevents infiltration and activation of this cells, in other words, their finding may indicates the possibility of problem existing in expression of perforin in and around the tumor. In addition **Tokmadzic, et al., (2011)** shows that the low frequency of perforin secreted by lymphocytes in prostate tissue of patients with benign prostatic hyperplasia and, particularly, prostate cancer can be the consequence of local tissue microenvironment and one of the mechanisms involved in the pathogenesis of prostate hyperplasia following malignant alteration. The high levels of perforin in the sera of rats injected by breast cancer antigen, are the result of important antitumor role for this marker in resistance of breast cancer and the defense of the body.

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