# Estimation of Interferon-α (IFN-α) and Tumor Necrosis Factor-α (TNF-α) in Female Rats Immunized by Human Breast Cancer Cell Line T47D

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## Abstract

Breast cancer is the greatest frequently cancer that occurs in women. This study was carried out on different groups of female albino rats *Rattus rattus*, that are immunized by different forms of antigens prepared from a 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 IFN-α and TNF-α have been estimated. The results have shown the existence of a significant increase in the level of IFN-α in all treated groups compared with two control groups, and higher level shown in groups of rats that are injected by freezing antigen mixed with adjuvant, heating killed antigen and chemical killed antigen compared with other groups, which reached to 1.783±0.143,1.766±0.0.183 and 1.708±0.175 (ng/ml) respectively. The level of TNF-α reveals a significant increase in the treatment groups compared with control groups, and the highest level has been in groups of rats injected by freezing killed antigen mixed with an adjuvant and also by chemical killed antigen with an adjuvant which reached to 0.265±0.045 and 0.242±0.067 (ng/ml) respectively.

**Keywords:** Human breast cancer, T47D, IFN-α, and TNF-α.

## الخلاصة

سرطان الثدي هو الورم الخبيث الأكثر شيوعا في الإناث. 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 بتلك المستضدات. حيث تم تقدير المؤشرات المناعية والمتمثلة بـ IFN–α و TNF–α.بينت النتائج وجود ارتفاع معنوي ($P<0.05$) في مستوى الحركي الخلوي الأنترفيرون–الفا (IFN–α) في مجاميع المعاملة مقارنة مع مجموعتي السيطرة، وتبين أن مجاميع الجرذان الممنعة بالمستضد المقتول بالتجميد الممزوج مع المساعد المناعي ، المستضد المقتول حراريا و المستضد المقتول كيميائيا هي الاعلى تركيزا" في مستوى تلك الحركيات الخلوية مقارنة مع المجاميع الأخرى ، إذ بلغت المستويات في تلك المجاميع1.783±0.143، 1.766±0.0.183 و 1.708±0.175 (نانوغرام / مل) على التوالي. لوحظ وجود زيادة معنوية ($P<0.05$) في مستوى الحركي الخلوي عامل تنخر الورم–الفا (TNF–α) في مجاميع المعاملة مقارنة مع مجموعتي السيطرة، ولوحظت أعلى المستويات في مجاميع الجرذان الممنعة بالمستضد المقتول بالتجميد الممزوج مع المساعد المناعي والمستضد المقتول كيميائيا والممزوج بالمساعد المناعي ايضا إذ بلغت 0.265±0.045 و 0.242±0.067 (نانوغرام/مل) على التوالي.

**الكلمات المفتاحية:** سرطان الثدي، مستضدات خلايا خط سرطان الثدي البشري، الأنترفيرون–الفا، عامل تنخر الورم–الفا.

## Introduction

Cancer is a broad group of diseases involving unregulated cell growth. Cancer is characterized by out-of-control cell growth to become immortal,forming malignant tumors, and invading nearby parts of the body **(Maity, 2013)**.Breast cancer in women is a major health problem worldwide, with approximately 1.3 million women was diagnosed with breast cancer in 2008 **(Smith *et al.*, 2012; Bray, *et al.*, 2013)**. 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).**

Interferon-alpha (IFN-α) is a type I interferon that plays an important role in the defense of the host **(Zaritsky *et al*., 2013)**. Interferon-α mainly produced by leukocytes, plasmacytoid and conventional dendritic cells **(Theofilopoulos *et al*., 2005).** Interferon-α has anticancer effects both in the preclinical models and in the clinical setting, also this cytokine increased *MHC* class I expression **(Moschos and Kirkwood, 2007).**

Tumor necrosis factor (TNF) is an inflammatory cytokine that is synthesized and secreted from several cell types especially activated macrophage, natural killer cells, and T-cells, which promotes inflammation and endothelial activation **(Harris and Keane, 2010).** In addition to that, it has significant roles in cancer pathogenesis**(Aydin *et al.*, 2012)**.

The aims of this study are 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- Estimation the level of rat cytokine IFN-α.
2- Estimation the level of rat cytokine TNF-α.

## Materials and Methods

### Breast cancer T47D cell line culture

T47D (Human ductal breast epithelial tumor cell line) was cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% Fetal Bovine Serum, 1 mg/ml of amphotericin B, 1ml of gentamycin,0.5 ml Streptomycin. Cell lines were maintained at 37°C in an incubator. After that the falcons were checked daily to detect the changes in the media color and monolayer formation **(Darling and Morgan, 1995)**.

## Antigen Preparation

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

### Laboratory Animals

Laboratory animals used in this study are adult females of White albino rats *Rattus rattus*. The total number was 50 rats, divided into 10 groups, each group consist of 5 rats. The ages of this rats ranged from (8 to 12) week and their weights ranged from 250-300 mg.

### Immunization of laboratory animals

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

### Collection of samples

The anatomy of rats occurs after two weeks of the last injection, in order to obtain the blood. The sera have been separated and then placed in appendroff tubes and store in the freezer until use.

### Immunological Assay

### ELISA Protocol

The levels of IFN-α and TNF-α were estimated by ELISA according to the manual procedure of Creative Diagnostics Company (USA).

## Statistical Analysis

The result of this study presented as mean±standard deviation (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. A difference was considered to be significant at 0.05 level**(Susan, *et al.*, 1997).**

## Results

**1. Interferon-α (IFN-α) Level:- Figures (1 and 2)** described the levels of cytokine IFN-α in sera of rats immunized with antigens and by a mixture of antigen with an adjuvant. The results illustrated that IFN-α level was higher in the sera of rats injected by heating killed antigen with adjuvant, chemical killed antigen in comparison with control group (0.641±0.071 ng/ml), which reached to 1.708±0.175 and 1.708±0.175 (ng/ml) respectively,while in group immunized by mixture of freezing antigen with adjuvant revealed a significant increase (P<0.05) which reached 1.783 ng/ml in contrast to control groups 0.641±0.071 and 0.724±0.091 (ng/ml) respectively.

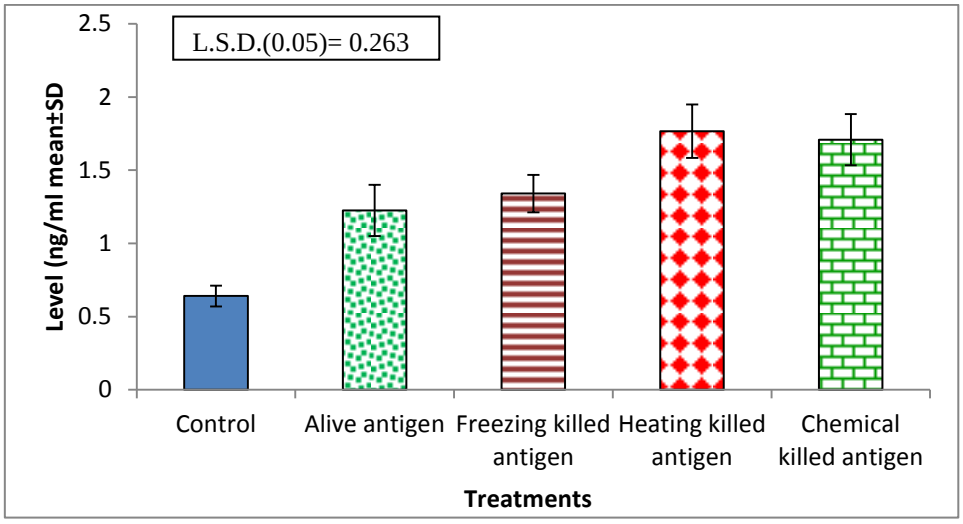


**Fig. 1:-** The IFN-α level in rats immunized by breast cancer cell antigen.

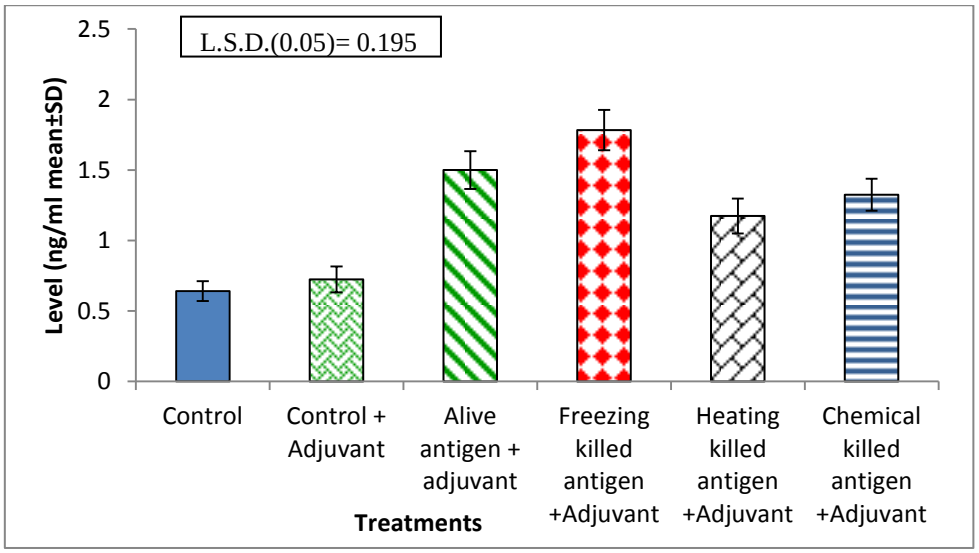


**Fig. 2:-** The level of IFN-α in sera of rats immunized by a mixture of breast cancer antigen with an adjuvant.

**2. Tumor necrosis factor-α (TNF-α) Level:-Figures (3 and 4)** showed that the mean distribution of TNF-α level (ng/ml) among treatment groups and control group. The results illustrated that there was an increase in the level of this cytokine in rats primed by chemical killed antigen 0.21±0.065 ng/ml in comparison with other groups of treatments and control.The levels of IFN-α were higher in both rat groups primed by a mixture of freezing antigen with adjuvant and mixture of chemical killed antigen with adjuvant 0.265±0.045 and 0.242±0.067 (ng/ml) in contrast with other treatment groups.

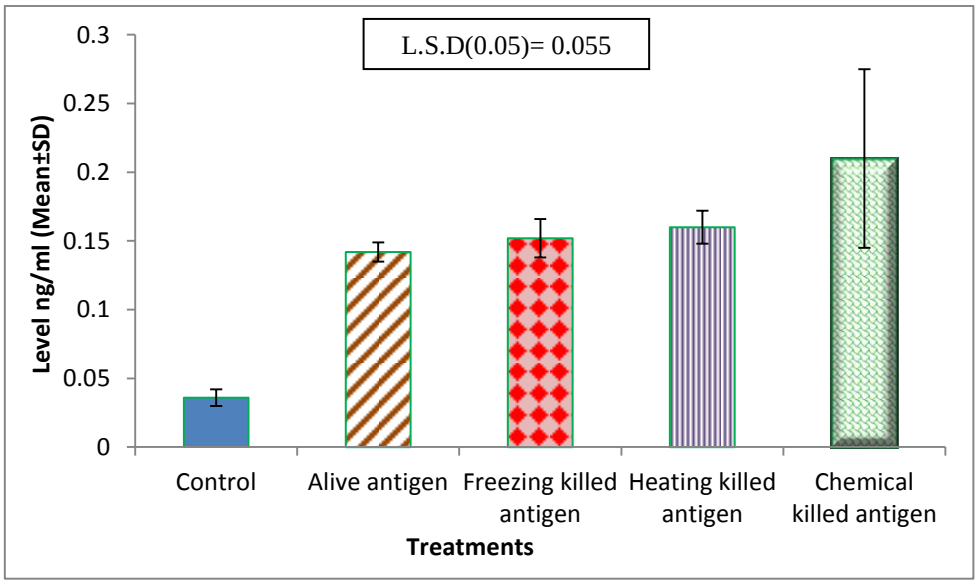


**Fig. 3:** Tumor necrosis factor-α (TNF-α) level in rat immunized by different forms of breast cancer cell antigen.

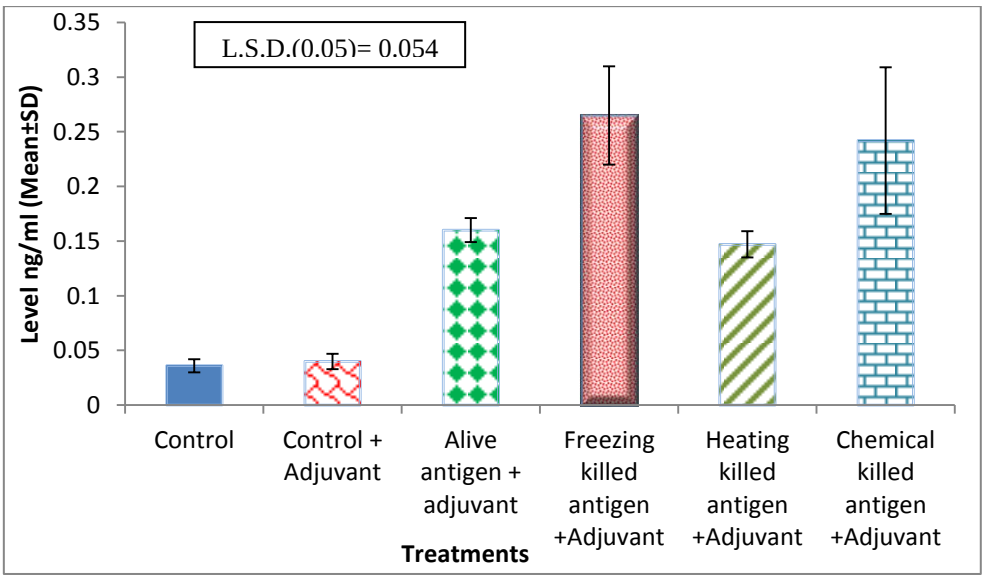


**Fig. 4:-** Tumor necrosis factor-α (TNF-α) level in rat injected with a mixture of antigen with an adjuvant.

## Discussion

Breast cancer is the most vital cause of mortality in women over the world **(Haghighat*et al.*2012 ; yang *et al.*, 2012)**.From the results of this study, we found that there were a significant increase in the level of the cytokine IFN-α in sera of all treated group in comparison with the control groups, and the highest level appears in rats group immunized by mixture of freezing killed antigen with adjuvant as shown in **Figures (1** and **2).**

Interferon-α (IFN-α) has the anti-tumor effect against pancreatic cancer in the experimental models *in vitro* and *in vivo* **(Jost *et al.*, 2010**; **Morak*et al.*, 2011)**. Also, **Zaidi *et al.*, (2012)** has been reported that IFN-α possesses anti-tumor activity on pancreatic cancer.

Interferon-α (IFN-α) level is significantly higher in patients with resected high-risk melanoma compared with healthy controls, and they demonstrated that IFN-α promotes a Th1 cells shift in host against tumors, increasing cell mediated cytotoxicity, and it has a role in attracting Th1 lymphocyte traffic to the tumor site **(Yurkovetsky*et al.*, 2007)**. Furthermore, there was a significant decrease in the serum level of IFN-α in patients with colorectal cancer at all stages compared with healthy persons (0.026±0.13 pg/ml vs 3.33±5.3 pg/ml), and they believed that these observations indicate decreased preoperative serum levels of IFN-α could be caused by cancer cells which developed a variety of cellular and molecular mechanisms to escape from immune surveillance**(Stanilov *et al.*, 2010)**.

The high levels of interferon-α (IFN-α) in this study may be due to the fact that it has anti-tumor activity and it mediated the mechanisms that fight and destroy tumor cells.

The results of this study, showed that there was a clear increase in the level of TNF-α in the sera of rats in treated groups compared with the control groups, as shown in (**Figures 3** and **4**). These results agreed with **Sharma *et al.*, 2014**, who found that there was a significant increase in the plasma level of TNF-α in patients with breast cancer in contrast to healthy subjects which reached 181.7±56 pg/ml, while its levels decreased after chemotherapy for patients that reached 147.0±57 pg/ml. So TNF-α can be used as a replacement marker for determining disease progress or efficacy of treatment. Since, the elevated levels of TNF-α in sera of patients with esophageal cancer in contrast with the control group, they were 12.35±9.69 and 4.62±3.06 pg/mL, respectively, and so it may be utilized as tumor markers for the diagnosis of esophageal cancer **(Aydin *et al.*, 2012)**. Meantime, **Yurkovetsky*et al.*, (2007)** reported that TNF-α level is significantly higher in patients with resected high-risk melanoma compared with healthy controls.So that this cytokine is multifunctional, and is one of the most commonly studied Cytokines. It is associated with various cancer types and enables the destruction of cancer cells **(Konukoglu and Turhan, 2005).** It is involved in the inflammatory responses promotion and plays an important role in the pathogenesis of autoimmune, inflammatory and malignant diseases. So high plasma TNF levels in cancer patients are associated with a poor disease outcome **(Sharm, *et al.*, 2014)**.

The elevated levels of TNF-α in the sera of treated groups in this study may be due to the anti-tumor activity of TNF-α and it plays an important role in the destruction of cancer cells.

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