

## Estimation of Serum Levels of Visfatin and Tumor Necrosis Factor-alpha in Iraqi Women with Breast Cancer

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### ABSTRACT

**Background:** Breast cancer is the most prevalent kind of female cancer among women today affects both emerging and industrialized nations.

**Objective:** This study intended to assess the levels of Visfatin and pro-inflammatory cytokines (TNF- $\alpha$ ) in Iraqi women who had breast cancer.

**Patients and methods:** In this study, serum concentrations of Visfatin and TNF- $\alpha$  were determined using samples from 90 Iraqi women (50 patients and 40 healthy volunteers).

**Results:** The current study's findings demonstrated that both Visfatin and TNF- $\alpha$  were highly elevated in patients with breast cancer ( $P > 0.05$ ), and they were also strongly associated.

**Conclusions:** This study has found an association between increased levels of serum Visfatin and TNF- $\alpha$  and breast cancer.

**Keywords:** Breast cancer, Visfatin, TNF- $\alpha$ .

### INTRODUCTION

The leading cause of mortality for women worldwide, breast cancer is currently the most prevalent female malignancy in both developing and industrialized nations<sup>(1)</sup>. Breast cancer is a molecularly diverse disease that arises from specific genetic abnormalities in the breast epithelial cells and causes a range of clinical symptoms in each patient<sup>(2)</sup>. The number of new cases of breast cancer in 2020 is expected to be 2.26 million (95% UI: 2.24-2.28). Female breast cancer is the most prevalent malignancy worldwide<sup>(3)</sup>. Approximately 685,000 people will lose their lives due to breast cancer in 2020<sup>(4)</sup>.

The World Health Organization reports that breast cancer is the most common malignant tumor in women in Iraq<sup>(5)</sup>. In 2016, breast cancer was the second leading cause of cancer-related mortality in Iraqi women (23.6%) killing 897 women<sup>(6)</sup>. Since breast cancer is easier to treat when it is little and has not spread, early identification can increase the likelihood that the patient will survive<sup>(7)</sup>.

A subclass of cytokines known as adipocytokines is mostly, though not always, released by adipose tissue<sup>(8)</sup>. It is an adipocytokine that is present in visceral fat and works in mammalian cells as both an intracellular enzyme and an external secretory factor. It functions as an inflammatory cytokine and plays a significant part in cellular energy and stress responses<sup>(9)</sup> and increases inflammation by synthesis of visfatin<sup>(10)</sup>. Adipocytes and macrophages in adipose tissue are responsible for its secretion<sup>(11)</sup>. It was discovered that it plays a role in breast cancer because a study found that the serum level of visfatin was significantly higher in patients with various stages of breast cancer than in benign and control groups. Additionally, the concentration of visfatin gradually rose in proportion to the tumor's growth<sup>(12)</sup>.

When postmenopausal status is further examined, it is discovered that postmenopausal breast cancer patients have consistently higher serum visfatin levels than the control group<sup>(13,14)</sup>, whereas premenopausal breast cancer patients have higher serum visfatin levels. Contrarily, menstruation was either found to be higher or lower<sup>(14)</sup> or slightly different from controls<sup>(13)</sup>.

Macrophages, neutrophils, fibroblasts, natural killer cells, astrocytes, T and B cells, Kupffer cells, smooth muscle cells, and keratinocytes are only some of the cells that generate the highly multidirectional inflammatory cytokine tumor necrosis factor-alpha (TNF- $\alpha$ ). Furthermore, cancer cells release TNF- $\alpha$ , which may serve as an intrinsic tumor promoter<sup>(15)</sup>. TNF- $\alpha$  has been shown to be a major mediator of inflammation<sup>(15)</sup> and employs a number of signaling pathways to control the expression of growth factors and other cytokines<sup>(16)</sup>. Present study goaled to assess of the level of adipocytokine (Visfatin) and TNF- $\alpha$  in breast cancer patients and a control group of healthy women.

### SUBJECTS AND METHODS

Ninety women were involved in the study, 50 of them were breast cancer patients who visited the Iraqi German Centre for Functional Diagnostics and Oncology in Baghdad, and the remaining 40 were female volunteers who seemed to be in good health.

**Ethical consent:** Written informed consent was obtained from each patient to participate in the current study. The Central Scientific Research Ethics Committee at Tikrit University approved this research. This work was carried out in accordance with the World Medical Association (Declaration of

## Helsinki) Code of Ethics for Studies Involving Humans.

**Inclusion criteria:** Patients with malignant or invasive breast tumors who were diagnosed with breast cancer, prior to partial mastectomy or mastectomy and who did not undergo any type of cancer treatment such as chemotherapy, immunotherapy or radiotherapy, and patients who agreed to participate in this study.

**Exclusion criteria:** Cases that had mastectomy, cases that underwent chemotherapy, radiotherapy, immunotherapy and other types of cancer treatment. Cases that refused consent.

**Sampling:** Five milliliters of blood were collected from the patients included in the study, placed in gel tubes and left to clot for 20 minutes and then centrifuged for fifteen minutes at a speed of three thousand revolutions per minute to collect serum. The serum was then placed in three Eppendorf tubes and stored in a deep freezer at  $-20^{\circ}\text{C}$ , samples were brought to room temperature again before these tests were performed.

### Evaluation of visfatin and TNF-alpha serum concentrations:

Commercial kits were used to evaluate the concentrations of Visfatin and TNF- $\alpha$  using Enzyme-Linked Immunosorbent Assay technique.

### Study enrollment procedures:

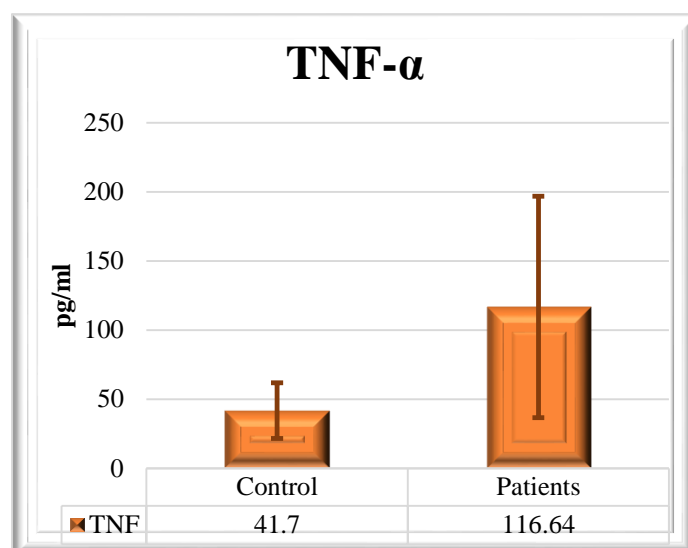
Detailed information was recorded for all cases, including age, gender, weight, genetic factor for breast cancer, and others. The presence of breast cancer was confirmed through the medical history taken from the patients and the tests they performed, such as imaging and tissue biopsy.

### Statistical analysis

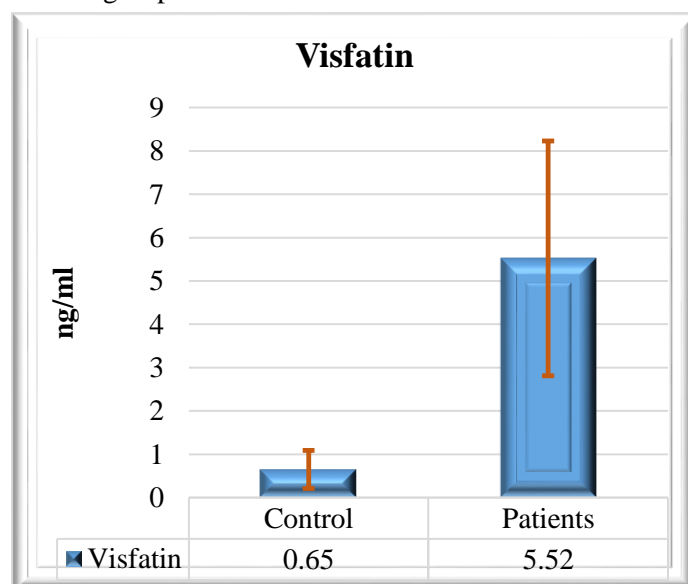
The Duncan test implemented in SPSS V. 23 was used to find significant differences between the groups, and the data were expressed as mean  $\pm$  Standard Deviation.

## RESULTS

Figure (1) and table (1) showed a significant increase ( $P=0.000$ ) in serum level of visfatin hormone in the women patients with breast cancer, as its level was  $5.52 \pm 2.71$  ng/ml compared to  $0.65 \pm 0.44$  ng/ml healthy women subjects. Also, the present study showed a significant increase ( $P=0.000$ ) in serum concentration of TNF- $\alpha$ , as its level reached  $116.64 \pm 80.11$  pg/ml in women patients with breast cancer compared to  $41.7 \pm 20.05$  pg/ml in healthy subjects as shown in figure (2) and table (1).



**Figure (1):** Assessment Serum Visfatin (ng/ml) in studied groups.



**Figure (2):** Assessment Serum TNF- $\alpha$  (pg/ml) in studied groups.

**Table (1):** Concentration of serum Visfatin and TNF- $\alpha$  in Women with breast cancer compared to healthy women subject.

Group	Visfatin (ng/ml)   TNF- $\alpha$ (pg/ml)	
	Mean $\pm$ SD	
Healthy women	$0.65 \pm 0.44$	$41.7 \pm 20.05$
Women with breast cancer	$5.52 \pm 2.71$	$116.64 \pm 80.11$
P-value	0.000	0.000

$P \leq 0.05$

## DISCUSSION

A subset of cytokines known as visfatin, sometimes known as adipocytokines, is mostly but not solely released by adipose tissue <sup>(17)</sup>. Secreted adipocytokines,

which are similar to cytokines, have autocrine, paracrine, and endocrine actions in both normal and abnormal states, such as breast cancer<sup>(18)</sup>. So the present study aimed to evaluate serum visfatin among Iraqi females with breast cancer.

Statistical analysis in the current study as showed in figure (1) revealed significant increases in serum visfatin among patients group, this results are in agreement with several previous studies<sup>(19, 9, 13)</sup>. Some studies findings all point to the same observations that there is a significant increase in serum visfatin among Saudi women with cancer<sup>(20, 21, m14)</sup>.

The present study's elevated visfatin levels in breast cancer patients are explained by a number of factors, including the fact that extracellular visfatin encourages the cell cycle's G1 to S transition, which boosts the proliferation of breast cancer cells<sup>(22)</sup> and activation of survival signaling<sup>(23)</sup>. Extracellular visfatin has an impact on the development of breast tumors in xenograft mice, according to in vivo studies<sup>(21, 24)</sup>. According to a study, mice treated with breast cancer cells are shielded from tumor necrosis factor by visfatin. Tumor necrosis factor-alpha causes apoptosis by preventing caspase-mediated activation of a protein called PARP. This is accomplished by increasing survivin expression and decreasing caspase activity. Visfatin protect breast cancer cells from tumor necrosis factor-alpha-induced apoptosis through up-regulation of survivin and inhibition of caspase-mediated PARP activation. This is another explanation for the increase in visfatin<sup>(23)</sup>.

In xenograft models using zebrafish and mice, extracellular visfatin has been shown to promote the metastasis of breast cancer cells in vivo.<sup>(21)</sup> An increase in the levels of MMP-2 and MMP-9 that are released into the bloodstream may be linked to this mediation<sup>(22)</sup>, and stimulation of TGF-1 receptor tyrosine kinase-mediated signaling to promote by increasing the expression of mesenchymal markers, epithelial cells that are able to undergo a process called EMT (N-cadherin, ZEB1, and vimentin)<sup>(25)</sup>. Increased mRNA expression of vascular endothelial growth factor is also seen in breast cancer cells treated with extracellular visfatin (VEGF)<sup>(22)</sup> and hormone receptor phosphorylation<sup>(24)</sup>.

Obesity, inflammation, and insulin resistance are all linked to visfatin's role as an immunomodulatory cytokine in the inflammatory reactions<sup>(26)</sup>. Inflammatory cytokine upregulation, such as that seen with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), plays a significant role in the initiation of insulin resistance<sup>(27)</sup>. While a study found that inflammatory cytokines inhibited visfatin mRNA production in adipocytes<sup>(28)</sup>, the opposite was true for human adipocytes and amniotic epithelial cells. Pro-inflammatory cytokines significantly promoted visfatin protein expression<sup>(27)</sup>, and these findings may explain the

increased visfatin levels, as the serum level of TNF- $\alpha$  was increased significantly in patients with breast cancer.

From above findings the present study can conclude that there are significant correlation between visfatin and other immunological parameters like TNF- $\alpha$ .

Strongly inducible secretory proteins called cytokines help cells in the immune system communicate with one another. Interleukins, tumor necrosis factors, and interferons are a few of the protein families into which they are subdivided. Several cytokines are responsible for controlling microclimate of inflammation inside a tumor. Interleukin (IL)-1, IL-6, and TNF- $\alpha$  are inflammatory cytokines that promote cancer cell proliferation and invasion<sup>(29)</sup>. Activation of cytokine receptors and intracellular signaling by nuclear factor (NF)- $\kappa$ B also accelerate tumor development<sup>(30)</sup>.

Statistical analysis of present study also showed a significant increase ( $P < 0.05$ ) in serum level of TNF- $\alpha$  in Iraqi females with breast cancer, this result is confirmed by several previous studies, which conclude that serum levels of TNF- $\alpha$  were increased in case of breast cancer<sup>(31, 32, 33)</sup>. In agreement with the positive connection we found only for big tumors, a study reported specifically that stage III breast cancer patients had significantly higher TNF- concentrations than controls<sup>(31)</sup>.

Studies using breast cancer-derived cell lines suggest that TNF- $\alpha$  may trigger either cell death or growth, depending on the specifics of the biological setting, but it is unclear how this pro-inflammatory cytokine affects tumor growth and metastasis in breast cancer<sup>(33, 34)</sup>.

T17 cell-produced IL-17 not only increases the production of chemokines like CXCL-2 and CXCL-8 but also significantly increases the secretion of intestinal stimuli like GM-CSF, which in turn increases the secretion of inflammatory cytokines like IL-6 and TNF- $\alpha$ . In general, increased TNF- $\alpha$  may be due to the increase in serum levels of IL-17. GM-CSF, which also causes inflammation, causes an increase in granulocytes and granulopoiesis in inflammatory tissue<sup>(35)</sup>.

## CONCLUSION

Present study concluded that both visfatin and TNF- $\alpha$  are increased and highly correlated during breast cancer.

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**Author contribution:** Authors contributed equally in the study.

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