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Inflammatory and metabolic synergy Investigating the role of IL-33 with the adipokines resistin and visfatin in promoting the growth and invasion of breast cancer cells.

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ABSTRACT

The present study was designed to assess and evaluate specificimmunological (IL-33) and physiological (Resistin and Visfatin) parameters among a selected cohort of Iraqi women diagnosed with breast cancer in Baghdad city. A total of 60 whole blood samples were collected and categorized into two groups: the first group consisted of healthy individuals serving as the control group, while the second group comprised clinically confirmed breast cancer patients. The findings of the current study demonstrated highly significant elevations ($P \leq 0.05$) in the concentration levels of the pro-inflammatory cytokine IL-33 in the patient group compared with the control group. Moreover, the results revealed a marked and statistically significant increase in both Resistin and Visfatin molecules among breast cancer patients. These findings indicate that elevated levels of specific biomarkers may play a key role in the immuno-metabolic regulation underlying breast cancer development and progression. The study suggests that these biomarkers influence tumor growth, immune response, and the tumor microenvironment. Furthermore, they may serve as valuable diagnostic tools for early detection and as prognostic indicators to predict disease severity, treatment response, and risk of recurrence. Overall, these results highlight their potential application in personalized medicine strategies for breast cancer management..

Keywords: Breast Cancer, IL-33, Resistin, Visfatin

Introduction

Breast cancer is a heterogeneous disorder characterized by the uncontrolled proliferation of mammary epithelial cells, arising from a complex interplay of genetic mutations, hormonal influences, and microenvironmental alterations. At the cellular level, tumorigenesis is driven by aberrant activation of signaling pathways such as PI3K/AKT, MAPK, and JAK/STAT, which promote unchecked cell growth, survival, and angiogenesis. These disruptions also enable cancer cells to evade apoptosis and immune surveillance, supporting tumor progression. Collectively, these mechanisms highlight the multifactorial nature of breast cancer and the challenges in developing targeted therapies. [1-3]. Endogenous hormones, particularly estrogen and progesterone, are believed to be important factors in proliferating breast tissue while mutations such as BRCA1/2 and TP53 also predispose to neoplastic transformation [3].

The TME involving fibroblasts, endothelial cells and immune infiltrates supports cancer progression by secreting cytokines, remodeling ECM and promoting EMT. [4,5].

Interleukin-33 (IL-33) is a pleiotropic IL-1 family cytokine functioning as an alarmin released upon cellular damage and after binding to receptor ST2. IL-33 has been reported to be upregulated in tumor tissues and correlated with advanced stage and poor prognosis in breast cancer [6]. Functionally, IL-33 drives breast cancer development by increasing the traits of cancer stem cells, activating the Wnt/β-catenin pathway and maintaining an epithelial–mesenchymal transition to regulate proliferation and metastasis [6,7].

IL-33 also remodels the tumor microenvironment by recruiting regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), creating an immunosuppressive niche that dampens anti-cancer immune responses. This immunosuppressive milieu facilitates tumor growth by inhibiting cytotoxic T cell activity and reducing the effectiveness of natural killer (NK) cells. Additionally, IL-33 signaling can promote the secretion of pro-tumorigenic cytokines and chemokines, further enhancing angiogenesis and tissue remodeling. Overall, these effects highlight IL-33's dual role in supporting both immune evasion and tumor progression [7,8]

Resistin is a cysteine-rich secretory protein first described as an adipocyte-derived hormone and expressed mainly by immune cells, including macrophages and monocytes in humans. It is a member of the resistin-like molecule (RELM) family and possesses pleiotropic biological activities. At the structural level, resistin is produced by the RETN gene and present as oligomeric complexes in circulation, whereas it engages toll-like receptor 4 (TLR4) and adenylyl cyclase-associated protein 1 (CAP1) to induce cytoplasmic inflammatory signaling pathways [9]. Resistin as a functional link between metabolic disorders and inflammation Resistin has been implicated to mediate the metabolic dysfunction-inflammation axis that leads to insulin resistance, endothelial damage, and tumor-favorable microenvironment. Increasing evidence indicates an involvement of resistin in breast cancer pathobiology. In the clinical context, resistin is significantly elevated in circulation of women with breast cancer relative to healthy controls and obese patients have higher circulating levels compared to nonobese individuals highlighting the connection between obesity and cancer via this factor [8-10]. At the cellular level, resistin promotes oxidative stress by reprogramming/redox adaptation of breast cancer cells leading to a pro-oxidative state conducive for carcinogenesis and survival. [11]

Visfatin, also known as pre-B-cell colony-enhancing factor (PBEF), is a multifunctional adipocytokine with both enzymatic and cytokine-like activities. ,visfatin is secreted by adipocytes, macrophages, and other immune cells, where it exerts pro-inflammatory effects by activating NF-κB and TLR4 pathways [12]. Elevated visfatin levels are associated with metabolic disorders such as obesity, insulin resistance, and cardiovascular dysfunction, positioning it as a mediator between metabolism and inflammation [13]. Its dual role as an enzyme and adipokine allows visfatin to integrate cellular energy status with inflammatory signaling. Furthermore, visfatin regulates oxidative stress responses, endothelial activity, and immune cell recruitment [12,13]. These characteristics highlight visfatin as a key link between adipose biology, immunity, and systemic homeostasis.

Visfatin has been referred to as a biomarker and tumor promoter in recent studies of breast cancer. Clinical researches have shown that plasma visfatin is obviously elevated in breast cancer patients compared to healthy controls and associated with tumor size,progress TNM category,and shorter overall survival [14]. Molecularly, visfatin activates cell oncogenic signalling pathways such as PI3K/AKT, STAT3 and NF-kappaB to promote proliferation, EMT process, invasion and metastasis [15]. It also regulates the tumor microenvironment by promoting secretion of pro-inflammatory cytokines (IL-6, TNF-α) and angiogenic factors like VEGF, which help in the progression of cancer [13].

Materials and Methods

Patients and Blood Collection

This study was conducted between January 2025 and July 2025 and involved a total of 60 blood samples. The samples were divided into two groups: the first group consisted of 30 samples collected from adult Iraqi women diagnosed with breast cancer, while the second group included 30 samples from healthy individuals who served as the control group. All blood samples were obtained from various external clinics and laboratories located in Baghdad. For each participant, approximately 5 ml of venous blood was collected using a sterile disposable syringe and transferred into clean, dry plain tubes without anticoagulants. The samples were then left at room temperature to allow clotting, followed by centrifugation at 4000 rpm for 10 minutes to separate the serum, ensuring no hemolysis was present.

Determination of Parameters

The levels of IL-33, resistin, and visfatin were measured using commercially available ELISA kits supplied by MyBioSource (USA), following the manufacturer's instructions.

Statistical Analysis

The analysis of the data was conducted via the SAS programme (version 2001). A one-way analysis of variance (ANOVA) was utilised to evaluate distinctions between groups, succeeded by Duncan's varied range test to determine variations that were statistically significant. A p-value of < 0.05 was deemed supportive of statistical significance.

Results and Discussion

This study demonstrated a significant increase in serum levels of the pro-inflammatory cytokine IL-33 ($p \leq 0.05$) in the breast cancer group (622.89 ± 31.46 pg/ml) compared to the control group, as shown in Figure 1.

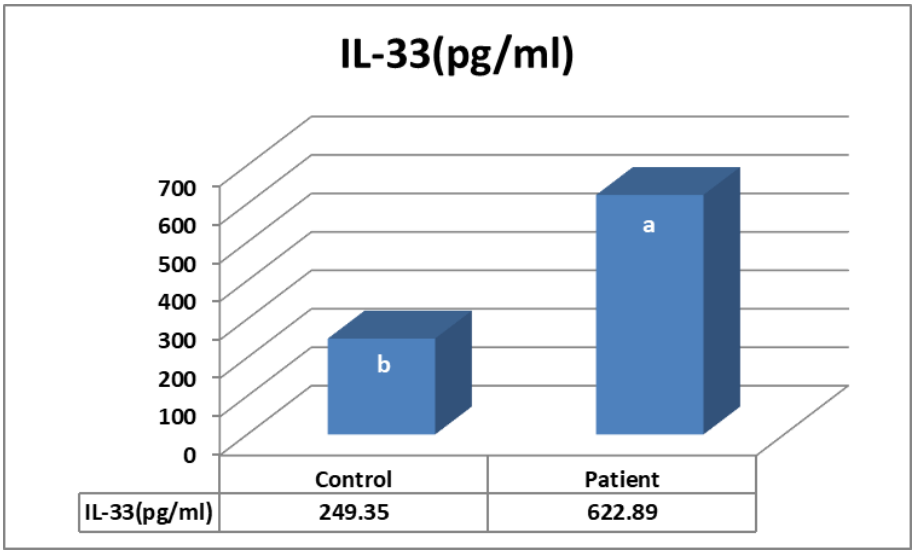


Fig. 1. Concentrations of IL-33 (pg/ml) in the study groups. The distinct letters indicate substantial variations among them at a significance level of $P < 0.05$ (**)

These findings are consistent with the results reported by Haghbin et al. (2023) [16], who observed a significant elevation in serum IL-33 levels among breast cancer patients compared to the control group.

IL-33 is a cytokine belonging to the IL-1 family, produced upon tissue injury. It is mostly localized in the nuclei of epithelial and endothelial cells and retained until cellular stress or damage prompts its release. Upon release, IL-33 attaches to the ST2 receptor, therefore activating the NF-κB and MAPK signaling pathways, which subsequently stimulates cytokine production from Th2 cells, mast cells, innate lymphoid cells, and

regulatory T cells. Recent data also associates IL-33 with angiogenesis, epithelial–mesenchymal transition (EMT), and immune suppression, all of which are pivotal to tumor growth [17].

In breast tumors, hypoxia, necrosis, and therapy-induced stress cause nuclear IL-33 to be passively released into the microenvironment. Unlike apoptotic death (where caspase cleavage inactivates IL-33), necrotic tumor tissue sustains high extracellular IL-33 levels increasing systemic concentrations [17]. Additionally IL-33 is highly expressed in breast tumor epithelial cells and stromal compartments, where it activates the Wnt/β-catenin axis, enhances stemness, and facilitates metastatic spread. This local overexpression correlates with elevated serum IL-33 and sST2 in breast cancer patients compared to benign lesions [6]. Furthermore IL-33/ST2 signaling cascade expands $ST2^{+}$ regulatory T cells, polarizes macrophages toward the M2 phenotype, and promotes TGF-β secretion. These changes sustain a low-grade inflammatory loop that continuously feeds IL-33 production and release. [18]. Additionally, analyses of patient serum reveal that both IL-33 and its soluble decoy receptor sST2 are significantly increased in individuals with breast cancer, showing a positive correlation with VEGF levels and indicators of tumor aggressiveness. These observations point to a systemic activation of the IL-33/ST2 signaling pathway, which likely supports tumor development by enhancing angiogenesis and altering the tumor microenvironment. Elevated circulating IL-33 may explain the increased recruitment of immunosuppressive cells and the higher release of pro-tumorigenic cytokines. Overall, these results suggest that IL-33 and sST2 could serve as valuable biomarkers for assessing disease severity and may represent promising targets for therapeutic strategies [18,19].

This study demonstrated a notable elevation in serum resistin levels ($P \leq 0.05$)in the breast cancer cohort (7.41 ± 1.82 ng/ml) relative to the control group throughout the study duration, as seen in Figure 2.

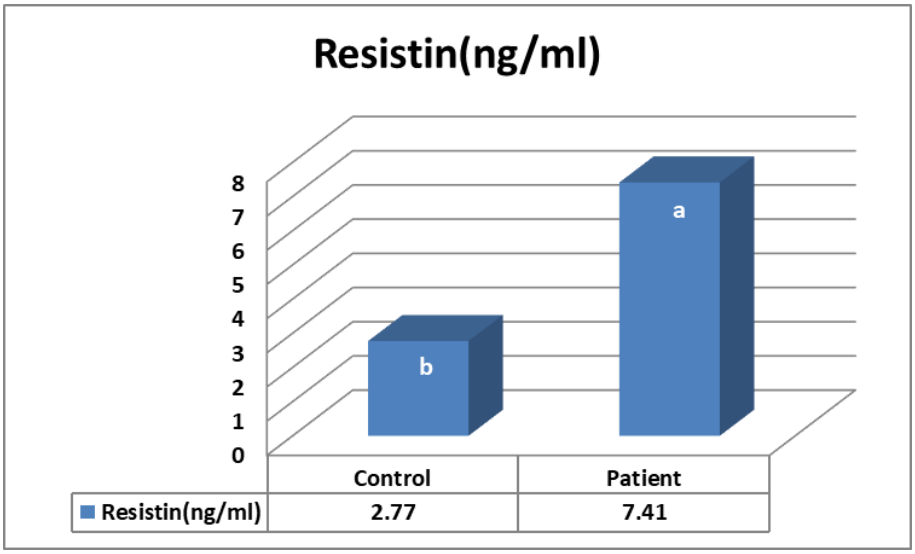


Fig. 2. Concentrations of resistin (ng/ml) in the study groups. The distinct letters indicate substantial variations among them at a significance level of $P < 0.05$ (**)

The results align with the findings of El-Benhawy et al. (2015) [20], which indicated a notable elevation in serum resistin levels in breast cancer patients relative to the control group.

Resistin is a cysteine-enriched adipokine mostly released by adipocytes and immune cells. It facilitates inflammation by stimulating pro-inflammatory cytokines, including IL-6 and TNF-α, and enhancing the expression of endothelial adhesion molecules such as ICAM-1 and VCAM-1. These findings indicate a possible involvement of resistin in tumorigenesis by promoting chronic inflammation and a pro-tumorigenic vascular milieu [21].

Serum resistin levels are markedly increased in breast cancer patients relative to healthy individuals. Mechanistically, this rise reflects multiple oncogenic pathways, obesity-related adipose tissue expansion increases resistin secretion, establishing a systemic proinflammatory milieu that promotes carcinogenesis, also resistin activates NF-κB and STAT3 signaling, which induce proliferation, survival, and angiogenesis of breast tumor cells [22]. Additionally, resistin enhances epithelial–mesenchymal transition (EMT), increasing tumor invasiveness and metastatic potential [14]. Moreover, resistin influences the tumor microenvironment by attracting tumor-associated macrophages (TAMs) and stimulating the production of immunosuppressive cytokines like IL-10, which helps cancer cells evade immune detection. The presence of TAMs strengthens the pro-tumor environment by promoting angiogenesis, remodeling the extracellular matrix, and supporting tumor cell survival. Furthermore, resistin-mediated signaling can trigger pathways that drive chronic inflammation and metabolic changes, thereby enhancing tumor growth and contributing to therapy resistance [21,22].

This investigation revealed a notable elevation in serum visfatin levels ($P \leq 0.05$) in the breast cancer cohort (10.31 ± 1.29 ng/ml) relative to the control group throughout the study duration, as seen in Figure 3.

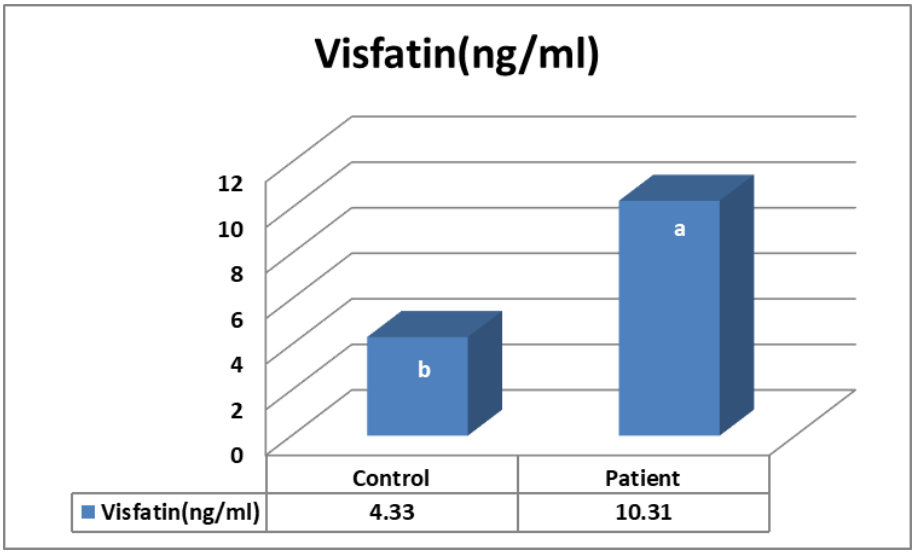


Fig. 3. Concentrations of visfatin (ng/ml) in the study groups. The distinct letters indicate substantial variations among them at a significance level of $P < 0.05$ (**)

The results align with the observations made by Ascar and Fadhel (2024) [23] and El-Benhawy et al. (2015) [20], all of which noted a substantial increase in serum visfatin levels in breast cancer patients relative to the control group.

Visfatin is an adipocytokine primarily released by visceral fat, macrophages, and neoplastic cells. It is essential in cellular energy metabolism by facilitating the creation of nicotinamide adenine dinucleotide (NAD+), a vital coenzyme in redox processes. In addition to its metabolic roles, visfatin has pro-inflammatory characteristics, affecting immunological responses and perhaps leading to chronic inflammation. Increased visfatin expression has been linked to tumor advancement and worse prognosis in many cancers. In breast cancer, elevated visfatin levels are associated with larger tumor size, negative oestrogen and progesterone receptor status, and reduced survival rates. [24].

Serum visfatin levels are elevated in breast cancer patients relative to healthy individuals, indicating its role in metabolic and oncogenic pathways where obesity and metabolic dysregulation augment visfatin secretion, creating a systemic proinflammatory milieu that predisposes to breast tumorigenesis [14]. Additionally visfatin activates signaling cascades including PI3K/Akt, NF-κB, and STAT3, which promote cell

proliferation, angiogenesis, and resistance to apoptosis; Furthermore visfatin enhances epithelial–mesenchymal transition (EMT), thereby increasing tumor invasiveness and metastatic potential [25]. In addition, visfatin shapes the tumor microenvironment by attracting immunosuppressive cells and enhancing the production of vascular endothelial growth factor (VEGF), thereby creating conditions that support tumor progression. The infiltration of these suppressive immune cells compromises anti-tumor immune responses, enabling cancer cells to escape immune surveillance. Moreover, VEGF-driven angiogenesis provides a continuous supply of oxygen and nutrients to the tumor, while also facilitating its spread to distant tissues [25,26].

Conclusion

In conclusion, our findings demonstrate that serum levels of IL-33, resistin, and visfatin are significantly elevated in patients with breast cancer compared with healthy controls. These biomarkers collectively reflect a complex interplay between chronic inflammation, adipokine dysregulation, and tumor-promoting signaling. Elevated IL-33 may reshape the tumor microenvironment by enhancing immunosuppressive pathways, while resistin contributes to insulin resistance, endothelial dysfunction, and pro-inflammatory cytokine release. Likewise, visfatin exerts both enzymatic and cytokine-like functions, linking metabolic disturbances to oncogenic processes. Together, these factors highlight the dual roles of immune modulation and metabolic dysfunction in breast cancer progression. Importantly, their combined assessment may improve risk stratification and provide insights into disease prognosis. Moreover, exploring the dynamic interplay among these biomarkers may uncover new mechanisms underlying the crosstalk between tumor progression, immune regulation, and metabolic dysfunction. Targeting these interconnected pathways could therefore provide promising opportunities for developing comprehensive and integrated therapeutic approaches in the management of breast cancer

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