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Research Article

Assessment of Serum Soluble Toll-like Receptor-4 and Interleukin-8 as Biomarkers in Patients with Breast Cancer

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Abstract

Background: Chemokines and toll-like receptors (TLR) have a synergistic role in the initiation and progression of breast cancer. TLR-4 is involved in the invasiveness of cancerous cells. Interleukin-8 (IL-8) has a significant role in boosting the angiogenesis and growth of cancer cells. ***Objective***: To measure the level of soluble TLR-4 and interleukin-8 in patients with breast cancer and to study the possibility of using them as diagnostic and prognostic biomarkers in these patients. ***Methods***: The study enrolled a total of 200 female subjects: 100 patients who were diagnosed with breast cancer and 100 healthy controls. Soluble TLR-4 and IL-8 levels in the blood were evaluated by the enzyme-linked immunosorbent assay. ***Results***: upregulated levels of soluble TLR-4 as well as interleukin-8 in individuals with breast cancer compared to controls (5.79 *vs.* 1.01 ng/ml for TLR-4 and 29.1 *vs.* 7.1 pg/ml for IL-8). The concentration of soluble Toll-like receptor-4 besides interleukin-8 is significantly higher in stage IV than in other stages. A substantial increase in these biomarkers in grade 3 was detected compared to other grades. ROC curves show that both biomarkers were useful in differentiating between healthy females and those who have breast cancer. ***Conclusions***: High concentrations of soluble TLR-4 as well as IL-8 have been linked to advanced stages of breast cancer, and these markers were effective in detecting the disease and predicting its prognosis.

Keywords: Biomarker, Breast cancer, Interleukin-8, Toll-like receptors.

تقييم المستقبلات شبيهة التول القابلة للذوبان-4 والإنترلوكين-8 في المصل كمؤشرات حيوية في مرضى سرطان الثدي

الخلاصة

الخلفية: تلعب الكيموكينات والمستقبلات الشبيهة بالتول (TLR) دورا تآزريا في بدء سرطان الثدي وتطوره. يشارك TLR-4 في غزو الخلايا السرطانية. يلعب Interleukin-8 دورا مهما في تعزيز تكوين الأوعية الدموية ونمو الخلايا السرطانية. **الهدف**: قياس مستوى TLR-4 و IL-8 القابل للذوبان في المصل لدى مريضات سرطان الثدي ودراسة إمكانية استخدامهما كمؤشرات حيوية تشخيصية وتنبؤية في هؤلاء المرضى. **الطريقة**: سجلت الدراسة ما مجموعه 200 امرأة: 100 مريضة تم تشخيصهن بسرطان الثدي و 100 عنصر تحكم صحي. تم تقييم مستويات TLR-4 و IL-8 القابلة للذوبان في الدم بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم. **النتائج**: تم تسجيل مستويات أعلى من TLR-4 القابل للذوبان وكذلك إنترلوكين -8 في النساء المصابات بسرطان الثدي مقارنة بالضوابط (5.79مقابل 1.01 نانوغرام / مل ل TLR-4 و 29.1 مقابل 7.1 بيكوغرام / مل ل IL-8). تركيز مستقبلات تشبه Toll-4 القابلة للذوبان إلى جانب interleukin-8 أعلى بكثير في المرحلة الرابعة منه في المراحل الأخرى. تم الكشف عن زيادة كبيرة في هذه المؤشرات الحيوية في الدرجة 3 مقارنة بالدرجات الأخرى. تظهر منحنيات ROC أن كلا المؤشرات الحيوية كانت مفيدة في التمييز بين الإناث الأصحاء والمصابات بسرطان الثدي. **الاستنتاجات**: تم ربط تركيزات عالية من TLR-4 القابل للذوبان وكذلك IL-8 بمراحل متقدمة من سرطان الثدي، وكانت هذه العلامات فعالة في الكشف عن المرض والتنبؤ بتشخيصه.

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INTRODUCTION

Breast tumor is the principal etiology of mortality owing to malignancy in females universally, and it is the most common etiology of tumor death in Iraq owing to its high metastatic capability [1]. The type I transmembrane protein family known as toll-like receptors, or TLRs, was initially discovered in mammalian immune cells [2]. The TLRs are expressed by several immune cells, like macrophages, in addition to cancerous cells [3]. When internal tissue damage is recognized by TLRs, certain transcriptional responses are triggered, such as NF-κB, which results in inflammation [4]. The TLRs were expressed in many malignancies involving breast cancer; their expression in tumor cells can enhance both cell survival and inflammation inside the tumor microenvironment [5, 6]. *Soluble* Toll-like receptor-4 (sTLR-4) was the first TLR to be identified in humans and was expressed by non-immune as well as immune cells [7]. A family of soluble mediators called cytokines is known to control the human immune system and may be involved in the onset of breast tumors [8]. Tumor and endothelial cells generate chemotactic cytokines, which may be essential in the development of cancer by encouraging angiogenesis in addition to immune surveillance evasion [9]. The cytokine interleukin-8 (IL-8), which is coded by the CXCL8 gene, belongs to the CXC family of chemokines, which is linked to inflammation and angiogenesis. It draws and activates neutrophils and a variety of other immune cells, which initiates an inflammatory response. Interleukin-8 was released by both cancerous and stromal cells, where endothelial cells and monocytes emit it in response [10]. IL-8 also has indirect consequences due to chemotactic infiltration and immune cell activation that release angiogenic and growth factors [11]. According to previous studies, IL-8 accelerates the advancement of breast tumors by enhancing invasion of cells, metastasis and angiogenesis [12, 13]. Research goals are to analyze the values of serum sTLR-4 as well as IL-8 in breast tumor subjects, in addition to studying their association with the disease's stage and grade. Finally, we will investigate their role as diagnostic and prognostic biomarkers in breast carcinoma.

METHODS

The study is case-control research that recruited a total of 200 females, which were divided into two groups: 100 breast cancer female patients and 100 healthy individuals whose age and sex matched those of the patients as controls. The patients were diagnosed with breast cancer and they were recruited between May 2023 and December 2023 by senior specialist doctors by clinical, radiological, and cytological analysis at private clinics. The ages of the female participants ranged from 23 to 65 years, with a mean age of 29.2±10.5 years. Patients were divided according to cancer grade into three grades (I = 32, II =34, and III = 34 patients) as well as the cancer stage into four stages (I = 19, II =25, III = 30, and stage IV = 26 patients) by the senior specialist doctors in accordance with Union International Convention on Cancer's (UICC/AJCC) standards for the American Joint Committee on Cancer [14]. Individuals with autoimmune diseases, liver, kidney, or other tumor types were not allowed to participate in the study. Prior to enrollment, all participants in the research were assigned written permission. Five milliliters of blood were obtained from each patient in a gel tube with no anti-coagulant, kept at ambient temperature to clot, then a 10-minute centrifugation of the blood at 2000 x was performed. After that, the resulting serum samples were kept at -20°C until use. Soluble TLR-4 and IL-8 were analyzed by using the *ELISA* test (Shanghai YL Biont, China, Catalog No. YLA1587HU for TLR-4 and YLA1210HU for IL-8) according to the kit instructions.

Ethical approval

The University of Mosul's College of Medicine Ethical Committee authorized this research (number UOM/COM/MREC/22-23) on May 5, 2023.

Statistical analysis

The SPSS (IBM Corp.) program (version 24.0) and GraphPad (version 9.0.0) were used to statistically analyze the study's findings. The data was shown as mean ± SD. The Z-test was employed to assess variations between the two categories. Using ANOVA, or univariate analysis of variance, the statistical significance of the mean difference when there are more than two categories was evaluated. To determine the ideal cut-off for sTLR-4 and IL-8, the ROC curve was employed. A *p*-value less than 0.05 was used to establish the significance threshold.

RESULTS

The demographic features of the studied patient group are summarized in Table 1.

Table 1: demography of breast cancer patients

Parameter	*n*(%)
Age = (29.2±10.5) years	
Age group	
< 30 years	35(35)
31-49 years	38(38)
≥ 50 years	27(27
Cancer stage	
I	19(19)
II	25(25)
III	30(30)
IV	26(26)
Cancer grades	
I	32(32)
II	34(34)
III	34(34)
Total	100(100)

The results showed a statistically significant upregulation of both sTLR-4 (5.79±1.32 ng/ml *versus* 1.01±0.03 ng/ml) and IL-8 (29.1±3.6 pg/mL *vs.* 7.1±1.5 pg/mL) levels when comparing patients with breast

cancer to controls (p=0.0021, p=0.010, respectively). The results are shown in Table 2.

Table 2: sTLR-4 and IL-8 mean in patients with breast tumor *versus* controls

Markers	Patients	Control	*p-Value*
sTLR-4 (ng/ml)	5.79±1.32	1.01±0.03	0.0021
Interleukin-8 (pg/ml)	29.1±3.6	7.1±1.5	0.010

Values were expressed as mean±SD.

Figure 1 displays the distribution of serum mean levels of sTLR-4 (ng/ml) and interleukin-8 (pg/ml) by the patients' clinical stages of breast cancer.

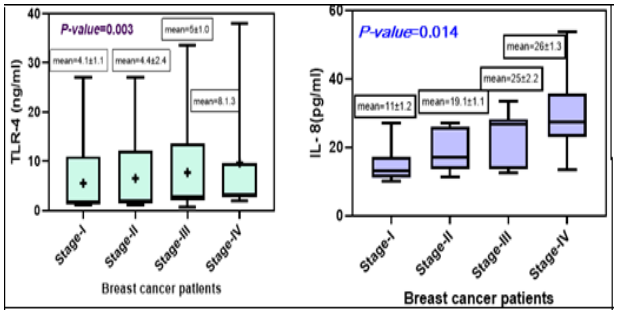

Figure 1: serum sTLR-4 and IL-8 concentrations in breast tumor patients based on tumor stage

The highest mean levels of sTLR-4 values were in stage IV (8±1.3 ng/ml), and the lowest values were in stage I (4.1±1.1 ng/ml). The variances in between stages were statistically substantial (p=0.003). Additionally, the highest mean concentrations of IL-8 values have been found in stage IV (26±1.3 pg/ml), while the lowest values were in stage I (11±1.2 pg/ml). The variances in-between stages were statistically significant (p= 0.014). The mean values of sTLR-4 and IL-8 in breast tumors exhibited statistically significant differences when comparing between grades (I, II and III) (p= 0.015, (p= 0.015, p= 0.022 correspondingly), as illustrated in Figure 2.

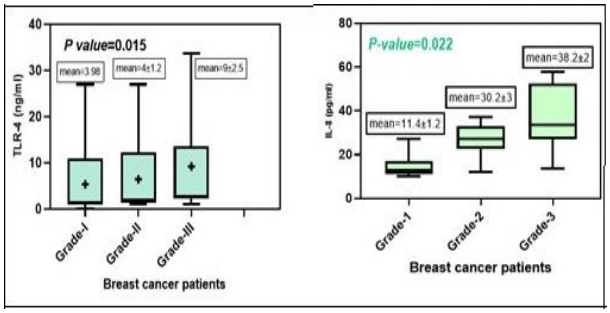

Figure 2: Comparison of serum sTLR-4 and IL-8 concentrations in breast tumor patients based on cancer grade.

The sTLR-4 mean levels in grade-III are the highest (9±1.2 ng/ml), while the levels in grade-I and II are the lowest (3.98±1.0 ng/ml and 4±1.2 ng/ml, respectively). The mean IL-8 levels in grade III are the greatest (38.2±2 pg/ml), while the levels in grades I and II are the lowest (11.4±1.2 pg/ml and 30.2±3 pg/ml, respectively). This study shows that serum sTLR-4 is positively associated with IL-8 (r-value = 0.21; p-value= 0.031) (Figure 3).

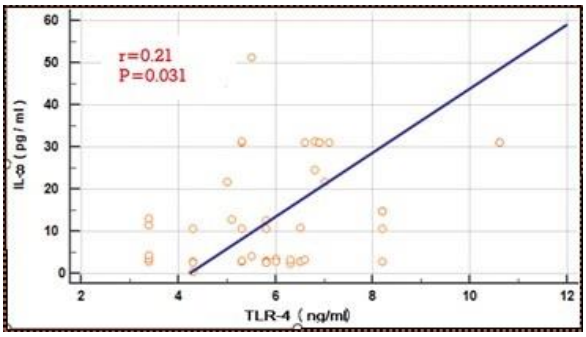

Figure 3: Correlation between serum soluble TLR-4 and IL-8 concentrations in breast cancer patients.

For sTLR-4, the portion below the curve was 0.85, while the corresponding specificity and sensitivity were 90.0% and 86.7% at a threshold of 4.2 ng/ml, respectively. sTLR-4 was used to differentiate between breast tumor patients and healthy people. When IL-8 was used in distinguishing between patients with breast tumors and healthy individuals, the portion below the curve was 0.72; specificity and sensitivity were 89.6% and 75.4%, respectively, at a threshold of 28.8 pg/ml (Figure 4).

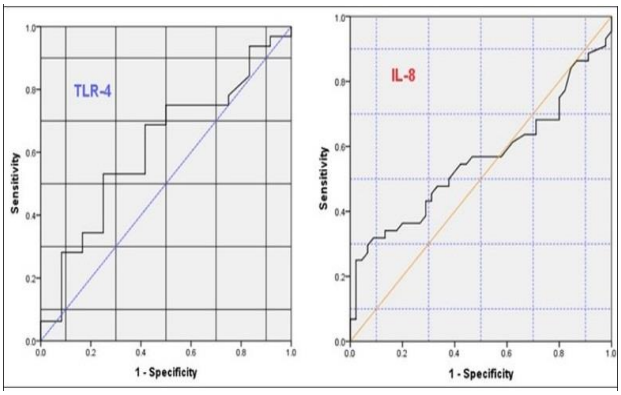

Figure 4: ROC curve of serum soluble TLR-4 besides IL-8 level in breast tumor patients, The space beneath the curve (AUC) was 0.85 for sTLR-4 while 0.72 for IL-8.

DISCUSSION

Since it is well recognized that Toll-like receptors and inflammatory cytokines have an impact on the growth of tumors, they have undergone extensive research as possible prognostic and diagnostic biomarkers in a variety of human malignancies [15]. The current study shows upregulation of sTLR-4 when comparing breast tumor patients to controls (p= 0.0021); these findings were similar to those found by Wang *et al.* (2017), *who* reported a rise in sTLR-4 value in breast cancer tissues in contrast to tissues that are usual [16]. Also, this finding is consistent with other studies that show that sTLR-4 knockdown might actively prevent breast cancer cell growth and survival [17]. Soluble TLR-4 serves as a transcription-related factor that promotes the production of several proteins linked to cell

development as well as apoptosis [18]. It interacts with a number of cytokines to mediate MyD88-dependent pathways that encourage carcinogenesis [18]. This research found that sTLR-4 is correlated with tumor stage (p-value= 0.003) and grade (p-value= 0.015), which coincides with other studies [16,19,20]. A study performed by Yang *et al.* shows that decreased expression of sTLR-4 prevents breast cancerous cell development; this result coincides with our findings [17]. The positive correlation of sTLR-4 with tumor grade and stage is due to the fact that one of the pathways for persistent inflammation in carcinogenesis and progression is thought to be TLR-4 signaling inside cancerous cells, which may facilitate cancerous cell immune evasion and development [17]. Interleukin-8 (CXCL-8) is a pleiotropic proinflammatory chemokine associated with inflammation and impacts several cellular processes, such as angiogenesis and cancer growth. This chemokine also acts as an autocrine tumor cytokine [21]. The current study's findings demonstrated a substantial increase in IL-8 level in tumor patients' serum in contrast to the healthy group (p= 0.010), a result that goes with several other studies [22–24]. According to published research, IL-8 is overexpressed in various tumor cell types, including those that cause prostate and stomach cancers, and it contributes to metastasis and invasion [25,26]. Numerous cell types, including monocytes and neutrophils, naturally generate IL-8. Tumor cells also release IL-8 under certain aberrant circumstances, leading to the promotion of angiogenesis and multiplication of cancer cells, both of which are pro-tumorigenic activities [27]. The current analysis also reveals a substantial rise in IL-8 levels in the advanced stages (p-value = 0.014) as well as grades (p-value = 0.022) of tumors. These findings imply that circulating levels of IL-8 increase with advanced illness, which coincides with other studies [28,29]. Serum IL-8 appears to reflect tumor aggressiveness due to its progressive increase with tumor stage and grade; additionally, there is proof that IL-8 directly promotes tumor growth through mitogenic and angiogenic actions, which have been linked to tumor aggressiveness [30]. The information demonstrates the variety of interleukin-8's function inside the microenvironment of cancer, which also supports the designation of this molecule as a possible biomarker. This further supports the classification of this molecule as a potential biomarker for breast carcinoma diagnosis and prognosis [31]. The process of epithelial-into-mesenchymal transition (EMT) in breast cancer augments the cytokine signaling of IL-8 and IL-8R activity in cancerous cells. Moreover, mesenchymal cells' production of IL-8 was able to cause the surrounding epithelial cells to undergo EMT, which was required for breast cancerous cells to obtain and preserve their aggressive characteristics. Furthermore, the capability of cancerous cells to invade adjacent tissues was significantly reduced when IL-8 receptors were blocked [32]. Prior research indicates a correlation between IL-8 expression and late disease stage as well as poor outcome in individuals suffering from breast cancer. As a result, metastatic invasiveness is linked to increased IL-8 levels [10, 33]; thus, IL-8 appears to have significant potential as a cancer biomarker for prognosis and/or prediction of the disease [34]. Finally, this study also demonstrates a positive relationship between sTLR-4 and IL-8, which increases the possibility of using both biomarkers in the diagnosis of this cancer. There are no previous studies that found a correlation between these two markers in breast cancer patients.

Conclusion

The findings indicate that sTLR-4 and IL-8 play a substantial role in the development of breast cancer, as well as function as critical biomarkers for prognosis and diagnosis. Given the high expression levels of sTLR-4 and IL-8 on malignant cells and their pivotal roles in the development of cancer, these molecules could potentially serve as promising targets for novel therapeutic strategies targeting breast carcinomas.

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Conflict of interests

No conflict of interests was declared by the author.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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