



## Research Article

# Evaluation of Interleukin-22 and Toll-like receptor-2 as an Immunological Biomarkers in Breast Cancer Patients

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## ABSTRACT

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**Keywords:** Breast cancer; Cytokines; Toll-like receptor-2; Biomarker



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**Background:** Interleukin-22 is an effector cytokine belongs to the IL-10 family. Signaling of this cytokine control tumor development by boosting cancerous cells growth. Toll-like receptor-2 act key function in activation of tumor cell proliferation besides increasing tumor cell invasion.

**Objectives:** to assess the level and reliability of IL-22 and TLR-2 in patients with breast cancer as a particular diagnostic and prognostic biomarkers in these patients

**Subjects and Methods:** Ninety breast cancer and sixty healthy control women participated in the current study. ELISA was utilized to measure IL-22 and TLR-2 in the serum of all participating individuals.

**Results:** The results revealed a significantly higher IL-22 concentrations in patients with breast cancer compared to controls (169.1±43.6 ng/L vs. 82.1±19.5 ng/L (P = 0.010)). Patients in Stage-IV and Grades -2 and -3 demonstrated elevated IL-22 concentrations (239±65.1 ng/L besides 201.2±36 ng/L and 321.2±62.6 ng/L respectively), while there is no significant differences in the concentrations of TLR-2 between patients (for different stages and grades) and control (39.1±11.2 ng/ml vs. 32.3±10.7 ng/ml (P = 0.062)).

**Conclusions:** Increased serum concentrations of IL-22 in patients with breast cancer with higher concentration in advanced stages. This might confirm a potential value of this cytokine as a marker in the pathogenesis of the disease and its promising role as new diagnostic tools of this cancer. TLR-2 shows non-significant difference in the concentration in patients with breast cancer as compared to control group.

## Introduction

The most frequent occurring malignancy is breast cancer and the main reason for women death in Iraq (1-3). Cytokines are biomolecules whose biological characteristics point to a critical involvement in infections, hematopoiesis, and homeostasis demonstrating their multifunctional role that regulates the body's response to viral disorders and even cancer by regulating tissue renewal (4,5). According to several studies, cytokines are essential in the growth of breast cancer (6,7). Some of these mediators, promote breast tumor growth as well as metastasis, whereas others, have the opposite effect.

Interleukin-22 (IL-22), a member of the family of IL-10, is generated

via T-helper (Th)-17 lymphocytes, innate lymphoid (ILCs) and NKT lymphocytes (8). IL-22/IL-22RA1 axis has complicated and context-specific function in cancer. Cancer patients were shown to exhibit elevated amounts of IL-22, associated with a poor prognosis (9). Transmembrane receptors, also known as Toll-like receptors (TLRs), serve as a crucial link between innate and adaptive immune responses, and are essential parts of the host's innate immune system. Immune-related cells often include TLRs, however epithelial cells can also express them, also, they are widely expressed on tumor cells, and have been involved in the development and spread of cancer (10). TLR signaling activation in tumor cells causes the production of immunosuppressive and proinflammatory cytokines, which promote tumor cell resistance to cytotoxic lymphocytes and result in immune evasion (11).

The research objective was to measure the level of IL-22 as well as TLR-2 in breast tumor patients' serum at various clinical stages and grades and to discover the possibility of using them as diagnostic biomarkers.

### Subjects and Methods

A whole sample including 150 females were enrolled in this case-control research. ninety patients with breast cancer who were diagnosed clinically, radiologically and cytologically by specialist senior doctors in private clinics during the period from, beside 60 healthy women as controls, all participating individuals were females whose age range from (23-67) years with mean age of (29.2 years ± 10.5) and they classified into four stages (I-IV) based on the suggestions of the American Joint Committee on Cancer (12). These patients were all chosen for this research prior to surgery, and they were all receive no treatment. Three mL of each participant's entire blood were drawn, the samples of clotted blood were centrifuged promptly. at 4000×g for ten minutes, the separation and preservation of serum samples at -20°C till the immunological examination. ELISA assay was done by using a kit from (Shanghai YL Biont, China, Catalog No: YLA0682HU for IL-22 and YLA1588HU for TLR-2). IL-22 and TLR-2 concentrations were determined using the method recommended by the kit's manufacturer. This study was authorized by the Ethical Committee of University of Mosul-College of Medicine.

#### Statistical analysis

The results of this study were statistically evaluated using the Statistical Software for the Social Sciences (version 24). The information was presented as mean ± SD. Z-test was used to assess the variations between the two groups. Statistical significance of the mean difference when there are more than two groupings was assessed utilizing analysis of variance test (ANOVA). Receiver operating characteristic (ROC) curve was used to determine the ideal cut-off for IL-22 beside TLR-2. Significance level was defined by a P-value that is under 0.05.

### Results

It was found that breast tumor patients have substantial difference in the mean of IL-22 than controls (P = 0.010), while there is no substantial difference in TLR-2 level in-between patients and controls (P = 0.062), as presented in table 1.

**Table 1:** means of IL-22 and TLR-2 between breast cancer patients and controls

	Breast-cancer (Mean± SD)	Control (Mean± SD)	P-Value
<b>Interleukin-22 (ng/L)</b>	169.1±43.6	82.1±19.5	0.010
<b>TLR-2 (ng/ml)</b>	39.1±11.2	32.3±10.7	0.062

Table (2) summarizes the four groups of breast cancer patients according to cancer stage and the mean values for IL-22 and TLR-2 for each stage, it was found that stage (IV) had the highest mean value of IL-22 (239±65.1 ng/L), followed by stage- III which has a mean value of (211.3±52.2 ng/L), while stage (I) had the lowest value (105.6±13.2 ng/L). Furthermore, a substantial difference between each group was found statistically (P = 0.014).

Regarding TLR-2, results of the current study show no discernible difference in the level of this chemokine among patients' groups regarding stages of breast cancer (P =0.087).

**Table 2:** Assessment of serum IL-22 and TLR-2 concentrations in breast cancer patients and controls according on tumor stage.

Stage	Sample number	Interleukin-22 (ng/L) Mean± SD	TLR-2 (ng/ml) Mean± SD
<b>Stage - I</b>	21	105.6±13.2	26.9±11.3
<b>Stage - II</b>	30	198.1±41.1	30.0±9.2
<b>Stage - III</b>	23	211.3±52.2	31.1±10.1
<b>Stage - IV</b>	16	239±65.1	28.2±14.0
<b>P-value</b>		0.014	0.087

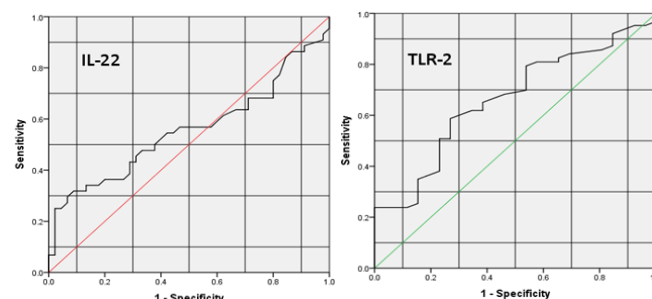
When compare between grades (1, 2 and 3), average IL-22 levels in breast tumor showed statistically significant differences (P = 0.028), as presented in table 3. Grade-2 and -3 have the highest mean value of IL-22 (201.2±36 ng/L and 321.2±62.6 ng/L) while grade -1 has the lowest level (102.4±45.2 ng/L).

Regarding TLR-2, results of the present study show no substantial difference in the level of this chemokine among patients' groups regarding grades of breast cancer (P = 0.064).

**Table 3:** Assessment of serum IL-22 and TLR-2 concentrations in breast cancer patients and controls according on tumor grade.

Grade	Sample number	Interleukin-22 (ng/L) Mean± SD	TLR-2 (ng/ml) Mean± SD
<b>Grade-1</b>	21	102.4±45.2	29.7±7.5
<b>Grade-2</b>	38	201.2±36	27.9±6.4
<b>Grade-3</b>	31	321.2±62.6	31.1±1.6
<b>P-value</b>		0.028	0.064

By using ROC curve to investigate the accuracy of serum IL-22 and TLR-2 concentrations in the diagnosis of breast malignancy patients in comparison to controls, the space beneath the curvature for IL-22 is 0.72, while specificity and sensitivity are 89.6% and 75.4%, correspondingly, at a threshold of < 188.8 ng/L in which IL-22 was used to distinguish between breast cancer and healthy peoples while the ROC curve of TLR-2, the area under the curve is 0.841, the specificity and sensitivity were 90.2% and 87.7%, consistently, at a threshold of < 34.1 ng/L (figure 1).



**Figure 1:** serum IL-22 and TLR-2 ROC curve in breast cancer patients, the area under the curve (AUC) for IL-22 was 0.72 while it was 0.841 for TLR-2.

## Discussion

Tumor initiation, angiogenesis, and metastasis have all been implicated as being mediated by inflammation. Oncogenic alterations, hypoxia, cytokines, and chemokines can attract inflammatory cells. Inflammation in a tumor's microenvironment consists of immune cells that have infiltrated and activated fibroblasts that release cytokines, in addition to chemokines, and growth factors that the tumor reacts to (13).

One important effector cytokine, interleukin-22 (IL-22), is included in the development of various illnesses as well as cancer. According to several lines of study, IL-22 signaling may inhibit the development of malignancies by boosting cell survival and proliferation (14, 15). Notably, in a genetic model, cancer was reduced when the IL-22 pathway was shut down using an IL-22-binding protein, an internal antagonist (16). The effector pathway of IL-22 is unique since it is the only immune cell released cytokine that is known to operate predominantly on non-immune epithelial cells that have their specific receptor (IL-22-R) (17).

In this research, the mean concentrations of IL-22 between the group with breast carcinoma and the healthy control group displayed substantial differences ( $P < 0.05$ ). This study findings, were consistent with several other studies (18, 19). Findings from research on humans and mice link dysregulation of IL-22 and IL-22R1 signal pathway to elevated risk besides a bad prognosis in a number of malignancies (20). The findings of the study showed a statistically significant difference between IL-22 values and tumor stages in breast malignancy patients ( $P < 0.05$ ), it appears in the initial stages of malignancy besides greatly elevated as the tumor progressed to advanced stage, so its level is elevated in advanced stages (stage IV) Another studies found that stage III-IV patients' IL-22 serum levels were considerably greater than those of stage I-II patients (19). By promoting migration, survival, and proliferation of endothelial cells, IL-22 aids in tumor angiogenesis and growth. Additionally, the use of antibodies that neutralize IL-22 prevents microvascular density, angiogenesis and tumor development (21). The findings of the study also showed detectable correlation between IL-22 serum levels and cancer grades ( $P < 0.05$ ) and showed that grades II and III was substantially correlated with high IL-22 levels, which agreed with other studies findings (17). IL-22 has been recognized as an important epithelial homeostasis regulator. These characters depict IL-22's role in epithelial malignancies as one that promotes cancer (18).

Endothelial, immune as well as epithelial cells all express TLR2. The broad definition of TLR2 in line with its wide variety of duties and purposes (23). TLR2 is essential for the initiation of innate immune lymphocytes and could help in the stimulation of anti-tumor responses due to its capability to excite antigen-presenting lymphocytes and, in turn, tumor-specific T lymphocytes. TLR2 is displayed on immune cells for instance macrophages and neutrophils as well, and this might encourage the formation of the metastatic niche and an immunosuppressive environment, which in turn promotes the growth of tumors and the metastatic dissemination (24). However, the current study also reveals a non-significant level in TLR-2 in breast tumor patients compared to controls ( $P > 0.05$ ) similarly, it shows no significant difference in TLR-2 mean level according to different stages and grades of patient's groups. According to ROC curve study, IL-22 and TLR-2 has elevated specificity and sensitivity in the differentiation of breast cancer patients, hence they can be used as diagnostic markers of this tumor with high reliability.

## Conclusion

There is a relevance of IL-22 cytokine in the pathophysiology of the illness and the likelihood of employing it as diagnostic biomarkers for the disease are highlighted by the considerable increase in IL-22 concentrations in breast cancer patients as opposed to controls.

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This research did not receive any specific fund.

## Conflict of Interest

The authors declare that they have no competing interests.

## Data availability

Data are available upon reasonable request.

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