Case Study

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Estimation of Toll-like Receptors- 9 Levels in Iraqi Breast Cancer Patients

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تقدير تركيز TLR-9 لمرضى الثدي العراقيين

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ABSTRACT

Background:

Innate receptor called toll-like receptor 9 is capable of identifying both microbial and vertebrate DNA.It has been found in both normal mammary gland epithelial cells and breast milk cells.TLR-9was estimated in serum of patients and breast tissue for breast disease.

Methods:

A 100 women (17–60 years old) were determined undergoing breast surgery at Babylon Province's Al-Fayhaa Al Ahly Hospital and AL-Hilla Teaching Hospital provided blood and breast tissue samples. Hospital histology lab and clinical surgery performed histological confirmation of breast diseases(benign and malignant tumors). Twenty blood samples were taken as controls from women who appeared to be in good health. TLR9 was measured in the serum of patients with breast diseases as well as the control group using ELIZA.

Results:

The mean level of TLR9 in serum of patient was 339.4667 ng/l while control was 316.9929 ng/l with found significant differences where P_value was (0.05). while tissue of patient was 276.1064 ng/l. Concentrations of TLR9 were significantly higher in serum compared with tissues at (P≥0.05).The results found no significantly differences in concentrations of TLR9 among types of diseases in sera of patients. This study found concentration of TLR9 increased with primary breast diseases and it might be prognostic marker

Conclusion :

This study found concentration of TLR 9 increased with primary breast diseases and it might be prognostic marker

Keywords: breast patient; ELIZA ;TLR-9;CpG DNA; breast Patients and control.

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INTRODUCTION

One class of pattern recognition receptors (PRR) that recognizes different kinds of microbial pathogen-related pattern molecules (PAMP) is the toll-like receptor (TLR) [1]. PRRs are highly conserved**.** Ten members of the Toll-like receptor family (TLR1–10) have been found in humans, while 12 members (TLR1–9 and TLR11–13) have been found in mice [2].

The intracellular toll-like receptors, such as TLR3, TLR7, TLR8, TLR9, and TLR10, are dispersed in the endosome, while TLR1-6 are found on the cell surface [3].TLR9 is an innate immune system cellular DNA receptor. When TLR9 recognizes DNA, it triggers an inflammatory response. TLR9 expression has been demonstrated to have prognostic significance only in patients with triple-negative breast cancer (TNBC). TLR9 mRNA and protein have also been found in breast milk cells and also found in normal mammary gland epithelial cells [4, 5]. TLR9 mRNA and protein are also widely expressed in breast cancer cell lines and clinical breast cancer specimens tumor. low tumor TLR9 expression upon diagnosis was associated with a significantly shortened disease-free-specific survival [6, 7].

Similar to pathogenic microbes, tumor cells have high levels of unmethylated CpG-DNA. Hypomethylation is an early event in breast cancer linked to a poor prognosis, and circulating tumor-specific serum DNA in breast cancer has been shown to be a marker for a poor prognosis recently [8, 9]. The present study aims to evaluate the concentrations of TLR 9 in sera and breast tissues.

MATERIALS AND METHODS

Subjects

A 100 specimens (95 female and 5 male) (blood and tissue) were enrolled in this study, age between 17 to 60 years, among male and female with breast surgery during July 2023 to November 2023 at AL_Hilla Teaching Hospital and AL_Fayhaa Al_Ahly Hospital ,Babylon Province, Iraq .for either malignant or benign tumors, the women and men underwent lumpectomy breast procedures.

Tissue collection

Tissue was taken for analysis thirty minutes after pickup , The fresh tissue was placed in a normal sodium chloride-filled, sterile plane tube. They were homogenized with a sterile surgical scalpel and wooden sticks.

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Blood collection

Twenty blood samples—five men and fifteen women—were taken as controls. Five milliliters of blood were drawn by venipuncture using disposable syringes, and the blood was then transferred into a disposable tube (a gel tube) and centrifuged for ten minutes at 3000 revolutions per minute. Sera samples were meticulously put into Eppendorf tubes and stored at a deep freeze prior to use.

Estimation of Toll Like receptor 9

Toll like receptor 9 concentrations in sera and tissues were determined, as per the manufacturing company (BT LAB, China), that uses the assay for enzyme-linked immunosorbent. Kits were used for the quantification of human TLR-9 . An equation that fits the standard curve was used to calculate the test's results.

STATISTICAL ANALYSIS:

The analysis of the current study was performed by using SPSS (Statistical Process for Social Sciences) version 23 ,. Results are expressed as (mean±SD) paired t-test was used to analyze the differences between systemic and mucosal immune response of the breast tumors. Independent – samples T test was used to compare patient and control systemic . ANOVA test between groups .Correlation test between immunological markers .P-value below 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

An essential family of receptors known as toll-like receptors (TLRs) serves as the body's first line of defense against microorganisms. TLR9 is an intracellular TLR or nucleic acid sensor, similar to other TLRs (TLR3, TLR7, and TLR8) that are restricted to the endoplasmic reticulum (ER), endosomes, and lysosomes [10]. Nucleic acids from bacteria and viruses, such as ssRNA and the unmethylated CpG DNA motif, are recognized by TLR 9. The expression of some TLRs has been found to be upregulated in a variety of tumor cells, tissues, and tumor cell lines. TLR2, 3, 4, 6, and 9 have been linked to hepatocellular carcinoma; TLR1, 2, 3, and 4 have been linked to colon cancer; and TLR3, 4, and 9 are highly expressed in breast cancer [11].

The concentrations of TLR9 were non significantly increased in serum of patients(339.4667) compared with control (316.9929) as the table (1). The concentrations of TLR 9 in serum were significantly increased in serum (339.4667) compared with tissue(276.1064) at $p \geq 0.05$. The reduction of breast tissue could be Oxygen concentration in the tumor microenvironment is a key TLR regulator. Similar to how hypoxia affects other TLRs in various cell types, it increases the expression of TLR9 in orthotopic breast tumors in vivo and in breast cancer cells in vitro, as shown in table (2).

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Table 1 concentrations of Toll like receptors in serum of breast patients and control

Parameter TLR_9	M±SD ng |L	P-value
Serum of patient	339.4667±38.99831	0.00
Tissue of patient	276.1064±78.46002	

Table 2: comparative between serum in patient and tissue in patient

Parameter TLR_9 serum	M±SD ng |L	P- value
Patients	339.4667±38.99831	0.321
Control	316.9929±89.23364	

This study's groupings of breast diseases include fibroadenoma (33%), ductal invasive (22%), fibrocystic change (26%), and granulomatous mastitis (10%). Gynecomastia was found to be 6% and fat necrosis to be 3%. These findings corroborated a study that found a higher percentage of fibrocystic change (44.82%) than fibroadenoma (34.48%), granulomatous mastitis (13.79%), and other conditions (lipoma, fat necrosis) (6.89%). [12].

The concentrations of TLR 9 were appeared non significantly difference among type of breast diseases. The results agreed with an investigation conducted in 2023 which demonstrated non-significant differences TLR4 concentrations among groups of diseases [13] In Table 3 and table 4.

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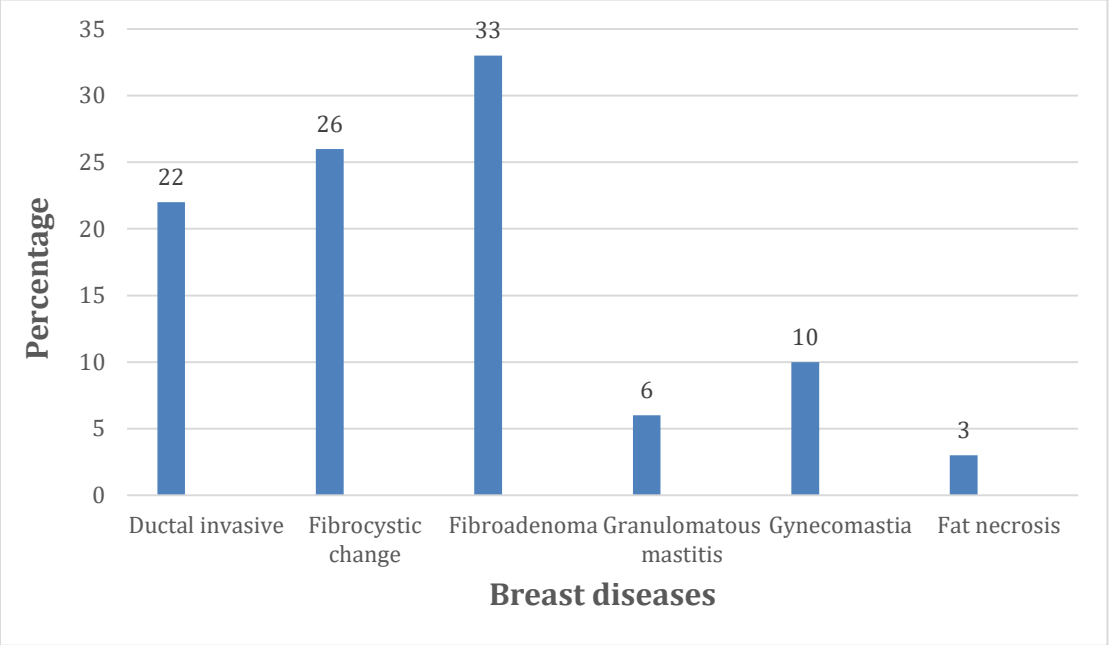

Figure 1: percentage of breast diseases

Table(3) : Concentration of serum of TLR_9 in patients with breast tumors according type tumors

Types of breast diseases	TLR_9 serum ng/l M±SD
Invasive carcinoma	328.487±47.950 (a)
Fibroadenoma	326.781±42.575 (a)
Fat necrosis	367.135±25.172 (a)
Fibrocystic change	359.288±22.656 (a)
gynecomastia	346.417±31.572 (a)
Granulomatous mastitis	338.523±32.185 (a)

Similar letters in the same column indicate that there is no significant difference (P>0.02).(ANOVA_Duncan).

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Table (4):Concentration of tissue of TLR 9 in patient according type of disease

Types of breast diseases	TLR_9 tissue N/ml M±SD
Invasive carcinoma	294.765±124.315 (a)
Fibroadenoma	245.915±55.465 (a)
Fat necrosis	220.935±9.095 (a)
Fibrocystic change	315.365±29.756 (a)
gynecomastia	269.042±83.052 (a)
Granulomatous mastia	233.950±58.457 (a)

Similar letters in the same column indicate that there is no significant difference (P>0.02).(ANOVA_Duncan).

Table (5): correlation between serum and tissue in TLR_9 in patients

TLR 9	serum	Tissue
serum	1	0.187 .298
Tissue	0.187 .298	1

The correlation between concentrations of TLR9 in serum and tissue table 5 the results appeared non significantly positive correlate between them.

CONCLUSIONS

This study found concentration of TLR 9 increased with primary breast diseases and it might be prognostic marker.

Ethical approval

On July 15, 2023, The Babil Health Directorate's Ethical Committee approved the study protocol. Additionally, prior to taking the sample, verbal consent from the patients was obtained. To guarantee the participants' safety, safety precautions were implemented during the sampling process. The Ethics Committee of the Iraqi Ministry of Health also completed this work, adhering to all national regulations.

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Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

مستقبل الحمض النووي المسمى بالمستقبل الشبيه 9 قادر على تحديد كل من الحمض النووي الميكروبي والفقاري، والمستلم لهذا المستقبل هو مكون CpG في الحمض النووي ، وهذا المستلم يتواجد في كل من الخلايا الظهارية الطبيعية للغدة اللبنية وخلايا حليب الثدي .

الهدف: تقدير تركيز TLR 9 في مصل وانسجة الثدي للمرضى الذين يعانون من أمراض الثدي .

الطرق: تضمنت الدراسة 100 أمرأة ,اعمارهن يتراوح بين (17–60 سنة) خضعن لعملية استئصال للثدي في مستشفى الفيحاء الاهلي ومستشفى الحلة التعليمي بمحافظة بابل وتم جمع عينات من الدم وأنسجة الثدي المستأصل وتم تأكيد الورم بالمستشفى من خلال التشخيص النسيجي لأمراض الثدي (الاورام الحميدة والخبيثة) .تم أخذ عشرين عينة دم كمجموعة سيطرة من نساء غير مصابات . استخدمت مقايسة الممتز المناعي المرتبط بالإنزيم لتحديد التراكيز المستقبل TLR–9

النتائج : أظهرت النتائج زيادة معنوية في متوسط مستوى TLR –9 في مصل المرضى (339.4667) نانوجرام/لتر مقارنة بالسيطرة (316.9929) نانوجرام/ لتر عند مستوى معنوية ($P \leq 0.05$)_ . و أظهرت النتائج ان تراكيز TLR–9 كانت أعلى معنوياً في المصل مقارنة مع الانسجة 276.1064عند ($P0.05 \leq$) . لم تجد النتائج اختلافات كبيرة في تركيز TLR–9 بين أنواع الامراض في أمصال المرضى ربما نتيجة لصغر العينة لدينا .

الاستنتاج : وجدت هذه الدراسة أن تركيز TLR–9يزداد مع أمراض الثدي الاولية وقد يكون علامة تشخيصية للمرض .

الكلمات المفتاحية: مريضة الثدي; مقايسة الامتصاص المناعي المرتبط بالإنزيم; TLR ; تسلسل CpG ; مرضى الثدي والسيطرة.