Original Article

Validity of serum Toll-like receptor-2 (TLR-2) in women with breast tumor

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Summary:

Background: Toll-like receptor -2 (TLR-2)play important roles in tumor biology; by activation and promotion of tumor cell proliferation, resistance to apoptosis andalso, enhancement of tumor cell invasion and metastasis by regulating metalloproteinase and integrin's.As toll-like receptors are widely expressed on tumor cells and participatein the initiation and progression of cancer, they may thus serve an important target and have an effective perspective on breast cancer treatment.

Objectives: The aims of the present study was to determine the levels of TLR-2 in the sera of healthy people and patients with benign and malignant breast tumors and also to investigate the validity of using TLR-2 as specific diagnostic markers of breast cancer. It`sdetection at early stage of disease could identify those patients with a high risk of progression to aggressive cancers.

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Patients and methods: Thirty breast cancer femaleswith age range from 27-76 years were included in this study and they were among patients who attending the National Center For Early Detection Of Cancer - medical city complex / Ministry of Health,during the period from October 2011 to February 2012.Thirty clinically diagnosed patients with benign fibro adenoma and twenty apparently healthy women were chosen as a case control and healthy control groups respectively. For all these study groups, serum level of TLR-2 using sandwich ELISA technique was carried out.

Results: There was a statically significant difference in the serum level of TLR-2in blood of breast cancer patients and case control group in comparison to healthy controls ($p > 0.001$),however there was no significant differences in such a level between the first two groups ($p=0.44$); therefore we were dealing with breast tumor cases in general regarding serum TLR-2.By using Receiver operating characteristic curve (ROC) area, in order to study the validity value of serum Toll like receptor-2 in differentiated breast tumor patients from healthy controls, serum TLR-2has the highest area under the curve (0.930) with cut off value associated with highest (perfect) sensitivity (100%) was equal to or above 0.14ng/ml.

Conclusion: The current study showed that serum levels of TLR-2were significantly higher in patients with benign and malignant breast tumors which may confirm a possible role of this marker in the pathogenesis of the disease, furthermore the best sensitivity and highest accuracy obtained from serum toll-like receptor-2 was by using a cut off values equal to or above 0.14ng/ml; Therefore, TLR-2 may be promising new diagnostic tools especially at early stages and among patients at high risk.

Key words: Toll like receptor-2, Breast cancer, Validity.

Introduction:

Breast cancer is the most common type of malignancy recorded in the cancer registries of almost all countries within the Eastern Mediterranean Region. In Iraq, the continuous rise in the incidence rate is associated with an obvious trend to affect pre-menopausal women (1). The Irish National Cancer Registry predicts that by 2020, the number of cases in this country could increase from the current yearly average of 1,895 to almost 5,000cases per annum (2).Toll-like receptors (TLRs) are a group of glycoproteins located mostly in the cellular membranes and represent a sense danger signals, acting as a key molecules in bridging innate and adaptive immune responses and play a significant role in cancer immunosurveillance(3,4,5)Toll like receptors are widely expressed on tumor cells and playan important roles in the initiation and progression of cancer, Their expression on breast cancer cells and mononuclear inflammatory cells can promote inflammation and cell survival in tumor microenvironment, in addition, they can play a crucial role in promoting angiogenesis, proliferation, invasion, resistant to apoptosisand metastasis by regulating metalloproteinase and integrin's(6, 7, 8).Moreover, the activation of TLR signaling in tumor cells induces the synthesis of proinflammatory factors and immunosuppressive molecules, which enhance the resistance of tumor cells to cytotoxic lymphocyte attack and lead to immune evasion. Thus, the neoplastic process may usurp TLR signaling pathways to advance cancer progression (6). However, TLRs activation may be a two-edged sword, with both antitumor andprotumor consequences (8,9).A soluble form of human Toll-like receptor-2 has also been identified. LeBouder and his colleagues in 2003 found that blood monocytes release a constant amount of soluble Toll-like receptor-2(sTLR-2) that might result from the post-translational modification of the transmembrane receptor protein. SixsTLR-2 isoforms, are naturally present in human

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milk and plasma (10, 11, 12).Naturally soluble TLRs (sTLRs) might serve as an important first- line negative regulatory mechanism; preventing the excessive initial triggering of the membrane- bound TLR and subsequent over activation, however it has a physiological feature that contributes to controlled, yet efficient, host innate immune response(13, 14, 15, 16).

Patients and methods:

Patients study groups

Thirty breast cancer females with age range from 27-76 years were included in this study. These patients were diagnosed clinically, radiologically and cytologically by specialists, and they were among patients who attending the National Center For Early Detection Of Cancer - medical city complex / Ministry of Health,during the period of October 2011 through February 2012.Thirty clinically diagnosed patients with benign fibroadenoma and twenty apparently healthy women were chosen as a case control and healthy control groups respectively. For all these study groups, serum level of Toll like receptor-2 using sandwich ELISA technique was carried out.

Kits And Reagents:

Toll Like Receptor-2 Elisa Kit (Antibodies-Online.Com):The Kit Was A Sandwich Enzyme Immunoassay For In Vitro quantitative measurement of TLR-2 in human serum, plasma and other biological fluids (24).

Statistical analysis:These were done using SPSS version 20 computer software (Statistical Package for Social Sciences). The majority of the outcome quantitative variables were non-normally distributed. Such variables are described by median and interquartile range. Statistical significance of differences between averages for parameters of normal distribution was assessed using the Student's t-test, and the difference in median of a quantitative non-normally distributed variable between 2 groups was assessed by nonparametric Mann-Whitney test. The statistical significance, direction and strength of linear correlation between two quantitative variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient. P value less than the 0.05 level of significance were considered statistically significant (17).

Results:

The ranges, median, and mean values of serum Toll-like receptor-2 in blood of breast cancer patient and control groups are shown in table1. There was a statically significant difference in the serum level of toll like receptor-2 between breast cancer patients and healthy controls (p 0.001<) and even between patient control group and healthy control (p0.001<), however, there was no statistical differences between patients and case control group (p=0.44).

Since no statistical significant differences in serum Toll like receptor-2 median values between breast cancer patient (malignant tumors) and case control group (benign tumors), therefore we can deal with breast tumor cases in general regarding Toll like receptor-2 (Table 1, Figure 1).

Table 1: The ranges, median, and mean values of serum Toll-like receptor-2 in blood of breast cancer patients and control groups.

toll like receptor-2 (ng/ml)	Breast Cancer Group (A)	Patient control group (benign fibroadenoma) (B)	Healthy Control Group (C)
Range	(0.178-27.601)	(0.178 - 26.499)	(0.1 - 4.693)
Median	.288	.288	.100
Inter-quartile rang	(0.178 - 0.729)	(0.288 - 0.729)	(0.1 - 0.1)
Number	(*N=30)	(N=30)	(N=20)
Mean rank	47.4	50.9	14.7
**P value A vs. C			<0.001
P value B vs. C			<0.001
P value A vs. B		***Ns (0.44)	

***N.** = Number

**P (Mann-Whitney) For Difference In Median

*** Ns= non significant

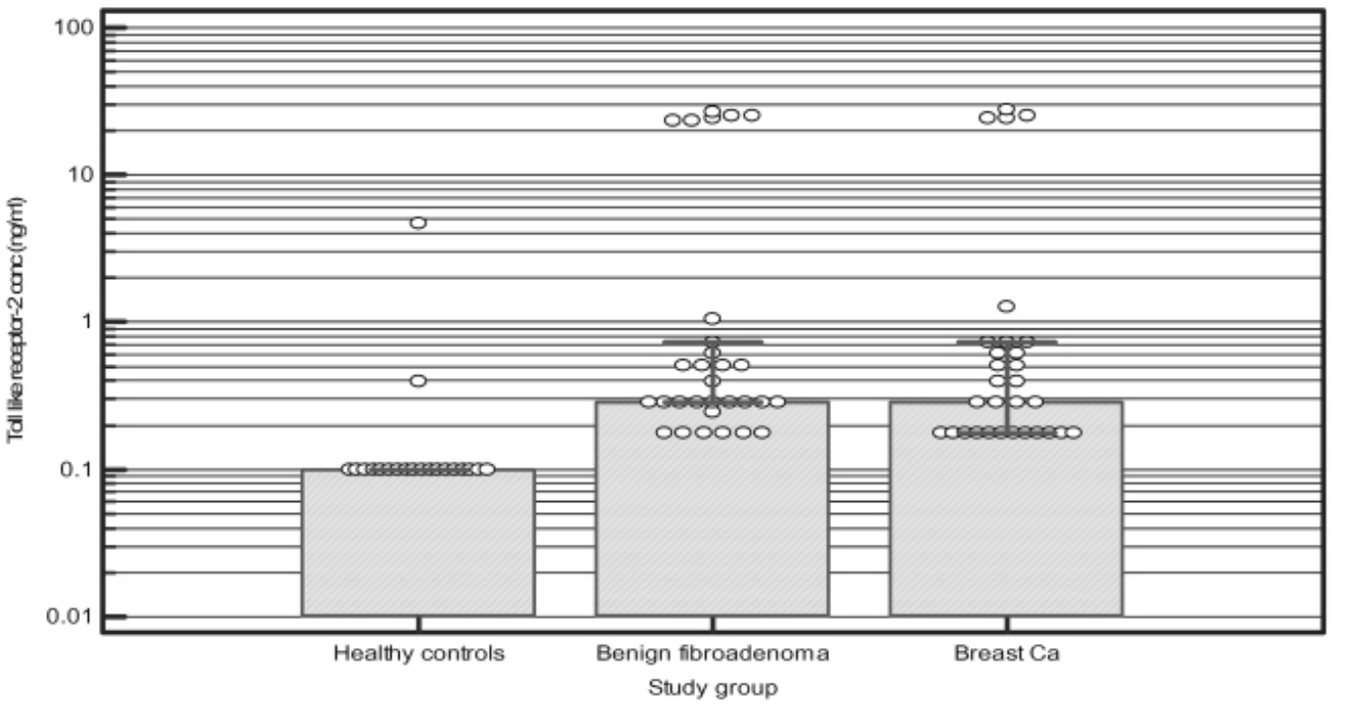

Figure 1: The differences in median of serum Toll like receptor-2 between the 3 study groups.

➢ Receiver operating characteristic curve (ROC) area and validity parameters of Toll-like receptors-2 in diagnosis of breast cancer:

A- ROC area in differentiation between breast cancer, case control, and healthy control groups:

A.1. ROC areafor breast cancer patients and case control group :Table 2 and Figure 2Showed that area under the curve for serum toll like receptor-2 was 0.55 (p value 0.46) Since no statistical significant differences in serum toll like receptor-2 between breast cancer patient (malignant tumors) and case control group (benign tumors), therefore we can deal with breast tumor cases in general regarding toll like receptor-2.

A.2. ROC area for breast tumor patients and healthy control group: In order to study the validity value of Toll-like receptor-2 in differentiated breast tumor patients from healthy controls, table 3 and figure 3Showed that ROC areas(0.930) for serum Toll-like receptor-2 in general was obviously higher than those in (table 2 and Figure 2) Therefore we can deal with breast tumor cases in general.

B- Validity parameters in differentiation between breast tumor patients and healthy control.

The validity parameters of TLR-2 in differentiating breast tumor patients from healthy control were determined according to sensitivity, specificity, accuracy, PPV and NPV.

B.1. Validity parameters for TLR-2 in Table 4The cut off value associated with highest (perfect) sensitivity (100%) was equal or above0.14ng/ml. This cut-off value qualifies as the optimum (typical) cut-off value associated with highest accuracy, being able to classify a tested individual into tumor or non-tumor with 97.5% accuracy.Testing positive at this cut-off value will establish a possible diagnosis of tumor with 90.9% confidence in a clinical setting where the primary diagnosis of tumor has equal odds probability (50% pretest probability), and with 98.9% confidence in a clinical setting where the primary diagnosis (based on history and examination) of tumor has a high probability (90% pretest probability).The highest specificity cut-off value is equal or above13.94 ng /ml (100% specificity) Testing positive at this cut –off value will establish tumor diagnosis of breast tumor with 100% confidence.

Table 2: ROC area for serum Toll-like receptor -2 for differentiating between breast cancer patients group and case control group.

	ROC area	P
Toll like receptor-2 conc. (ng/ml)	0.55	0.46[NS]

Table3 :Rocarea from Serum Toll-Like Receptor -2 in differentiating between breast tumor patient(whether benign or malignant) from healthy control.

	ROC area	P
Toll like receptor-2 conc (ng/ml)	0.930	<0.001

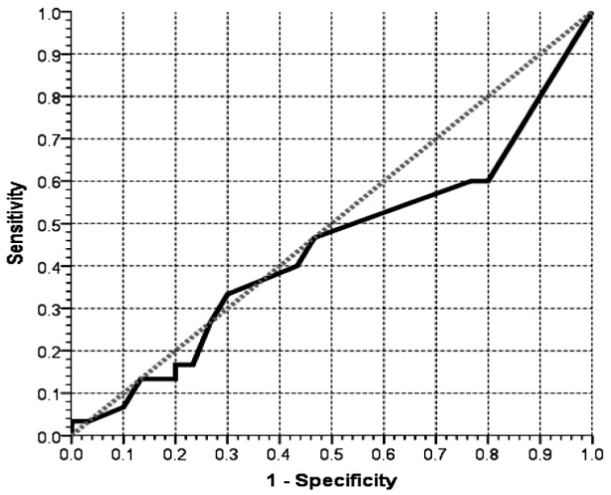

Figure 2:ROC curve showing the trade- off between sensitivity (rate of true positive) and 1-specificity (rate of false positive) for serum toll-like receptor-2.

- ROC curve showing the differentiation between benign and malignant tumors.

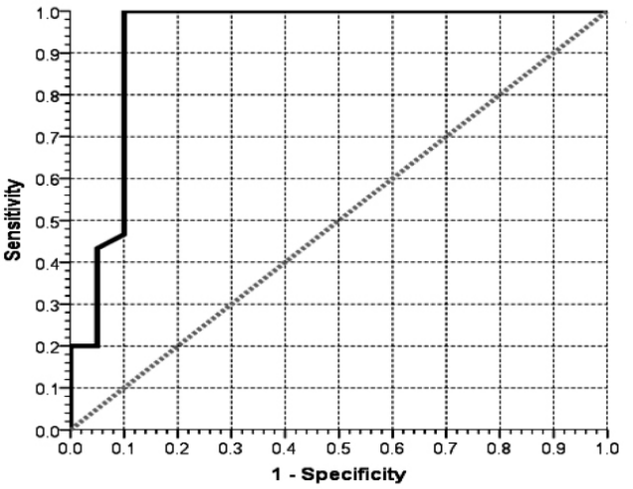

Figure 3: the ROC curve showing the trade- off between sensitivity (rate of true positive) and 1-specificity (rate of false positive) for serum Toll-like receptor-2.

- ROC cure showing the differentiation between breast tumors (whether benign or malignant) and no tumor (healthy control).

Table 4. Validity parameters for the studied serum Toll like receptor-2 in differentiating between breast tumor patients and healthy controls.

Positive if ≥ cut-off value				PPV at pretest probability =		NPV at pretest probability =
	Sensitivity	Specificity	Accuracy	50%	90%	10%
Toll like receptor-2 conc (ng/ml)						
0.14 highest sensitivity optimum	100.0	90.0	97.5	90.9	98.9	100.0
0.21	70.0	90.0	75.0	87.5	98.4	96.4
0.27	68.3	90.0	73.8	87.2	98.4	96.2
0.34	46.7	90.0	57.5	82.4	97.7	93.8
0.45	41.7	95.0	55.0	89.3	98.7	93.6
0.56	31.7	95.0	47.5	86.4	98.3	92.6
0.67	26.7	95.0	43.8	84.2	98.0	92.1
0.89	20.0	95.0	38.8	80.0	97.3	91.4
1.17	18.3	95.0	37.5	78.6	97.1	91.3
2.99	16.7	95.0	36.3	76.9	96.8	91.1
13.94 the highest specificity optimum	16.7	100.0	37.5	100.0	100.0	91.5

Discussion:

The data of the present study revealed a statistical significant difference in serum level of TLR-2 in blood of breast cancer patients and case control groups in comparison to healthy control group (p0.001<), however there was no significant differences in such a level between the first two groups (p=0.44); therefore we was dealing with breast tumor cases in general regarding serum TLR-2.Serum TLR-2 is the only soluble form of mammalian TLR to date identified that occurs naturally, because it is constitutively released by normal monocytes and present in normal human plasma, breast milk and saliva(10,11,15, 12).It has been proposed that serum TLRs may protect the host from excessive initial triggering of TLR-2; which may result in deleterious TLR-2 mediated innate responses. The full extent of serum TLR-2 `s regulators capacity, the mechanism(s) underlying it, and its biological relevance in vivo have not, however, been addressed to date (10, 18, 13, 14, 15,16).A significant relationship between breast tumors and TLR-2 was reported by Xie et al (2010)who found that TLR-2 was differentially expressed in breast epithelial cancer cell lines and homogenous untransformed breast cell lines, TLR-2 expression levels in these cell lines were consistent with their differential metastatic activities (19).It is well known that TLRs are expressed on cells of immune system, but there is growing evidence that TLRs are also expressed on tumor cells, when they may influence tumor growth and host immune response (20,8). Activation of TLRs expressed on tumor cells may have profound consequences for tumor growth by factors released after TLR activation (8).Xie et al., 2009 demonstrated that TLR-2 is a critical receptor responsible for NF-KB signaling activating and highly invasive capacity of MDA-MB-231 breast cancer cells (21).Transcription factor NF-KB plays a crucial role in human breast cancer cells, and its constitutive activity has been found in cancer cell invasion and metastasis (22, 23). Also , In order to study the validity value of serum Toll-like receptor-2 in differentiating between breast tumor patients and healthy controls, the present study showed that in a patient with serum TLR-2 equal to or above 0.14ng/ml (cut off value)one can establish the diagnosis of breast tumor with 90.9% confidence (PPV) in a clinical situation where the present probability of tumor is 50%, or 98.9% confidence (PPV) in a clinical situation with a high pretest probability (90%). Testing negative at this cut off value would exclude a diagnosis of breast tumor with 100% confidence (NPV) in a clinical setting were the primary diagnosis of tumor is of small probability (10%pretest probability).In another words, if a physician has a (50%) clinical suspicion about a patients as a case of breast mass (breast tumor) and send her for testing serum TLR-2 and the result was positive, this test gives 90.9% confidence for physician to establish the diagnosis, but if that physician has 90% clinical suspicion about the patient to be a case of breast mass(breast tumor) and the result of serum level of TLR-2 was positive, these result gives 98.9% confidence for physician to establish the diagnosis; while if a physician suspicion was 10% for a patient to be a case of breast tumor and want to exclude the diagnosis for the tumor, serum TLR-2 negativity can give 100% confidence for the physician to exclude the diagnosis.Since their discovery a decade ago, TLRs have been shown to be critical for efficient innate and adaptive immunity;in addition the framework of TLR-mediated signaling pathway has been explained; however, TLR activation may be a two-edged sword, with both antitumor and protumor consequences (8).

Authors' contribution:

-Hind H. Al-Ammiri / study conception, data collection & analysis design and interpretation of results.

- Aida R. Al-Derzi / study conception, data analysis & critical revision.

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