Assessment of P53 and soluble FasL (sFasL) serum concentration in females with benign & malignant breast tumors

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Abstract

Background: During Embryonal development and morphogenesis apoptosis may be induced by two pathways. The first is an external P53 protein signal originating from other cell-also named as death signal another is specific cell reaction to external stress factors. Serum concentration of proteins regulating external pathway may be useful in early diagnosis and staging of breast tumors.

Objective: The aim of the study was to evaluate P-53 and sFasL serum concentrations in patients with benign and malignant breast tumors.

Methods: The study population was composed of 69 patients [33 patients with newly diagnosed breast carcinoma, 6 patients with recurrent carcinoma and 30 patients with fibroadenoma (tumor types were verified by fine –needle biopsy)], ELISA Technique was applied for estimation of both P-53 & sFasL levels as well as apparently healthy 50 volunteer's women.

Results: p53 and sFasL levels were evaluated before surgery. The results revealed high p53 serum concentration in patients with recurrent breast cancer in comparison with other groups which may be considered as a prognostic marker; while, sFasL revealed no significant differences between its levels among the studied groups though it was elevated among benign breast tumor subjects.

Conclusion: Estimation of P-53 level showed significantly elevation of the concentration among the sera of recurrent breast cancer in comparison with other groups which may be considered as a prognostic marker.

Key words: p53, sFasL, breast cancer.

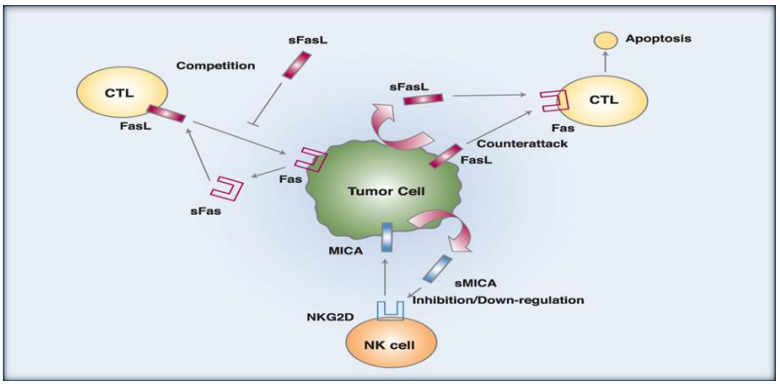
Introduction

Breast cancer is the most common cancer diagnosed and the second leading cause of cancer death among United State (US) women[(1)]. Also breast carcinoma is the most common malignant tumor in Iraqi women[(2)] .

Cancer is caused in all or almost all instances by mutation or by some other abnormal activation of cellular genes that control cell growth and cell mitosis. The abnormal genes are called oncogenes. As many as 100 different oncogenes have been discovered[(3)]. Protein 53 (p53) or Tumor protein 53(Tp53) was originally defined as an oncogenic protein. However, in the late 1980s; several critical discoveries recognized p53 as an anti-oncogensis[(4)]. It was denoted that p53 is subjected to tight regulation at multiple levels. In cancer cells, its function can be compromised by various mechanisms: 1- mutations of Tp53 2- alteration of p53 regulators and 3- alteration of p53 target gene[(5)]. P53 protein is regarded as a valuable prognostic marker in cancer with potential use as a molecular target[(6)]. Defective P53 could allow abnormal cells to proliferate, resulting in cancer. As many as 50% of all human tumors contain p53 mutants[(7)]

There is another factor which was observed to play a crucial role in cancer development; it is the apoptosis (programmed death of cells) appears as the critical functioning of p53 in tumor suppression[(8)]. Fatty acid synthase (Fas) receptor (death receptor) [belongs to the family of tumor necrosis factor TNF-α related death receptors] and Fatty acid synthase Ligand (FasL) is its corresponding ligand, activates the other major apoptotic pathway shown (Figure. 1)[(910,)] . Down regulation of Fas has been shown in some carcinomas including breast cancer[(9)]. Some pre-clinical studies suggest that classic anticancer agents also require Fas. Recently, presence of death receptor and its corresponding ligand (FasL) induces cell death[(9)] .

The aim of this study was to evaluate sFasL and P53 levels in patients with benign and malignant breast tumors that may be predictors, diagnostic and prognostic factors in breast cancer.



Subjects, Materials & Methods:

It is a case control study involved 69 serum samples were collected from Iraqi women patients suffering from breast tumors and stored frozen at (-80ºC) until assayed. These samples included 39 malignant cases and 30 fibroadenoma benign breast

tumors. Benign cases were considered as a disease control group [or case control] for malignant breast tumors. The patients' age ranged from (17-76) years [with (52.8 ± 12.3, 26.9 ± 8.3, 43.1 ± 12.9) Mean ± SD for malignant, benign breast tumors and apparently healthy control group respectively].

Patients' diagnosis depended on the following steps at the same sequences (physical examination, ultrasound and or/ mammography, fine needle biopsy (for cytological test) and finally confirmed in postoperative histopathology (for malignant cases only); which have been performed under the supervision of consultant physician. According to the postoperative diagnosis, patients were divided into three groups as shown in Table (1)

Table 1: Patients distribution in groups according to their histo-pathological diagnosis

Group	No. of patients	Histopathological diagnosis
I	31	Mammary ductal carcinoma
II	2	Mammary lobular carcinoma
III	6	Recurrence ductal carcinoma

Sample collection has been performed during the period between September/2011 and May/2012 at The National Center for Early Detection of Cancer in Medical City Complex.

The ELISA kit (creative-biomart researcher tests) was used for the quantitative determinations of sFasL and p53 serum level. The non parametric Mann- Whitney U test was chosen as the sure most appropriate means to statistically compare the central measures (medians & mean rank).

The median was accepted as the measure of centralization due to its statistical resistant to extreme measures as well as its relation to the non-parametric test. In both analyses, the significant coefficient was set at $p< 0.05$[11].

Results

1. antitation of P53 concentration (Pg/ µL) among the sera of the studied groups

The concentrations of P53 levels among the different studied groups were listed in Table 1 and Figure 1. It is clear from this table that there were highly significant differences between mean rank levels of P53 concentration among recurrent cases (106.75Pg/µL) in comparison with its corresponding levels in the other groups (P<0.001). However, there was no significant difference between P53 level among primary cancer (46.68 pg/µL) and benign cases (50.22pg/µL) (P =0.62). Moreover, both primary breast cancer and Fibroadenoma cases showed highly significant difference in P53 level in comparison with apparently healthy control group (69.05pg/µL) (P = 0.002, 0.008 for primary and benign cases respectively).

2. Estimation of sFasL level (pg/ µL) among the sera of the studied groups

Level of sFasL in the sera of the different studied groups in Table 3 and Figure 2 showed that apoptosis was non-significantly higher among benign tumors' subjects than the other forms of breast cancers (either primary or recurrent) (P =0.16).

3. Evaluation of p53 level in discriminating between recurrent & primary breast cancer cases

By application of reciprocal of (ROC) program in order to distinguish between recurrent and primary breast cancer; it appeared that sFasL concentration doesn't discriminate between recurrent and primary breast cancer significantly (P= 0.83). However, P53 level showed highly significant action in discriminating between both recurrent and primary breast cancer cases (P=0.004) [see Table 4 and Figure 3].

4. Determination the optimum significant value of P53 for discrimination between recurrent & primary breast cancer

Table 4 revealed that the positive optimum cut-off value of P53 was 77.9 pg/ µL which yielded 87.9% specificity and 66.7% sensitivity with 84.6% accuracy.

Table 1: Level of P53 in the sera of different studied groups

P53 level (pg/µL)	Recurrent breast Ca	Primary breast Ca	Fibroadenoma (Benign tumor)	Healthy controls	P (Kruskal-Wallis)
Range	(64 - 491.6)	(33 - 161.2)	(30.9 - 289.1)	(39.5 - 65.7)	<0.001
Median	81.6	44.0	48.6	61.3	
Interquartile range	(76.3 - 97.7)	(41.7 - 69.8)	(41.7 - 64)	(59.5 - 63.2)	
N	6	33	30	50	
Mean rank	106.75	46.68	50.22	69.05	
Recurrent breast Ca Vs. Primary breast Ca					0.004
Recurrent breast Ca vs. Fibroadenoma (Benign tumor)					0.008
Recurrent breast Ca vs. Healthy controls <0.001					<0.001
Primary breast Ca vs. Fibroadenoma (Benign tumor)					0.62 NS
Primary breast Ca vs. Healthy controls					0.002
Fibroadenoma (Benign tumor) vs. Healthy controls					0.008

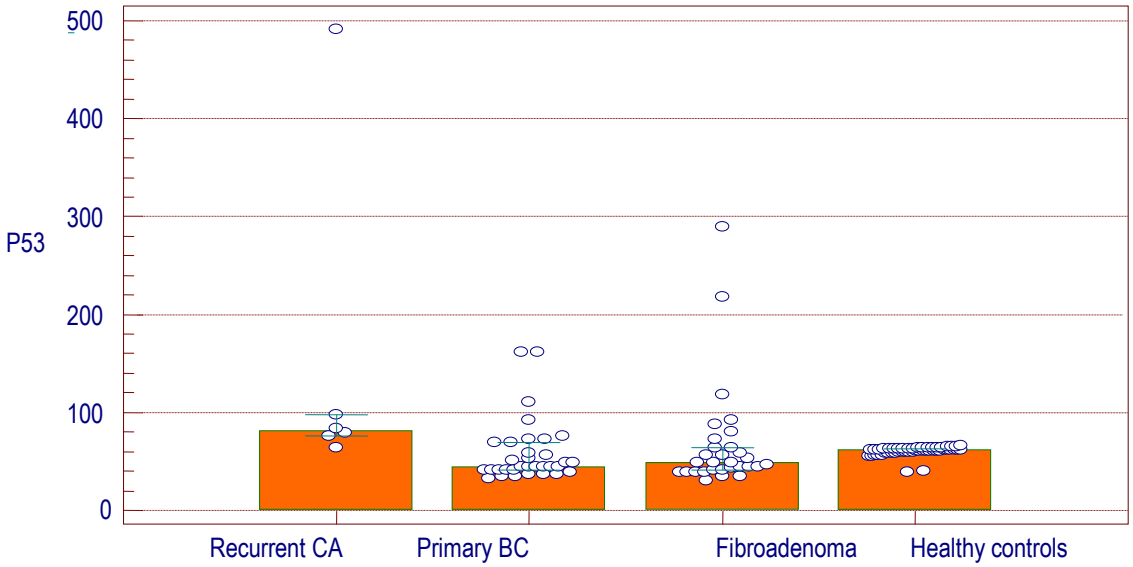


Figure 1: Mean and distribution of different P53 value among the different studied groups

Table 2: Levels of sFasL (pg/ µL) in the sera of the studied groups

sFasL level (pg/µL)	Recurrent breast Ca	Primary breast Ca	Fibroadenoma (Benign tumor)	Healthy controls	P (Kruskal-Wallis)
Range	(4.2 - 71.1)	(1.3 - 394.6)	(3.2 - 176.8)	(6.5 - 13.5)	0.16 [NS]
Median	6.7	9.1	13.5	10.2	
Interquartile range	(4.3 - 29.9)	(6.7 - 17.8)	(6.7 - 15.8)	(6.7 - 11.3)	
N	6	33	30	50	
Mean rank	51.50	59.68	71.63	54.25	

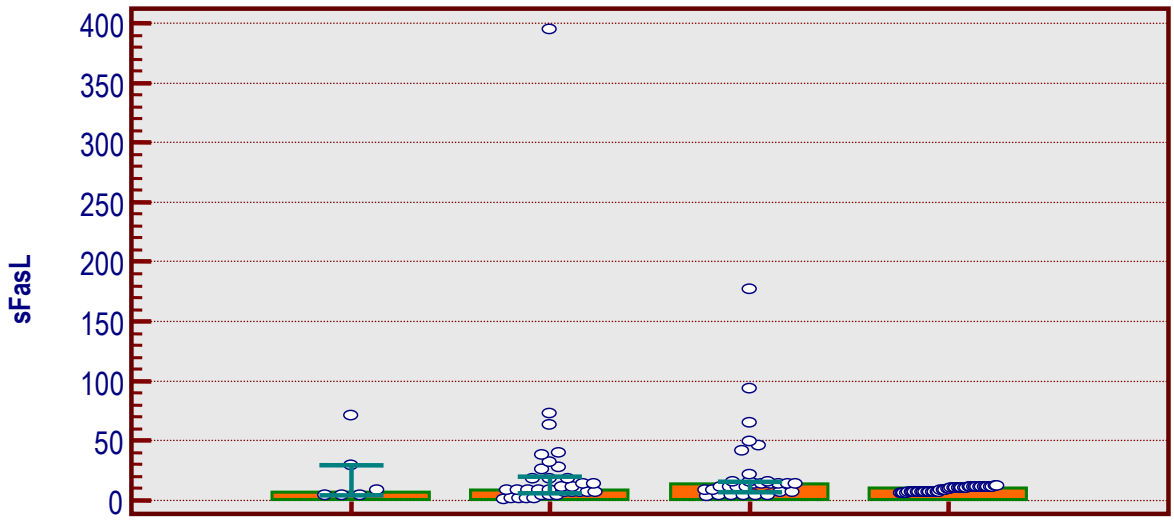


Figure 2: Distribution of values with the mean of sFasL among the sera of different groups

Table 3: Comparison between the recurrent and primary breast cancer ROC area with immunological parameters

Immunological parameters	ROC area	P
sFasL	.528	0.83[NS]
P53	.876	0.004

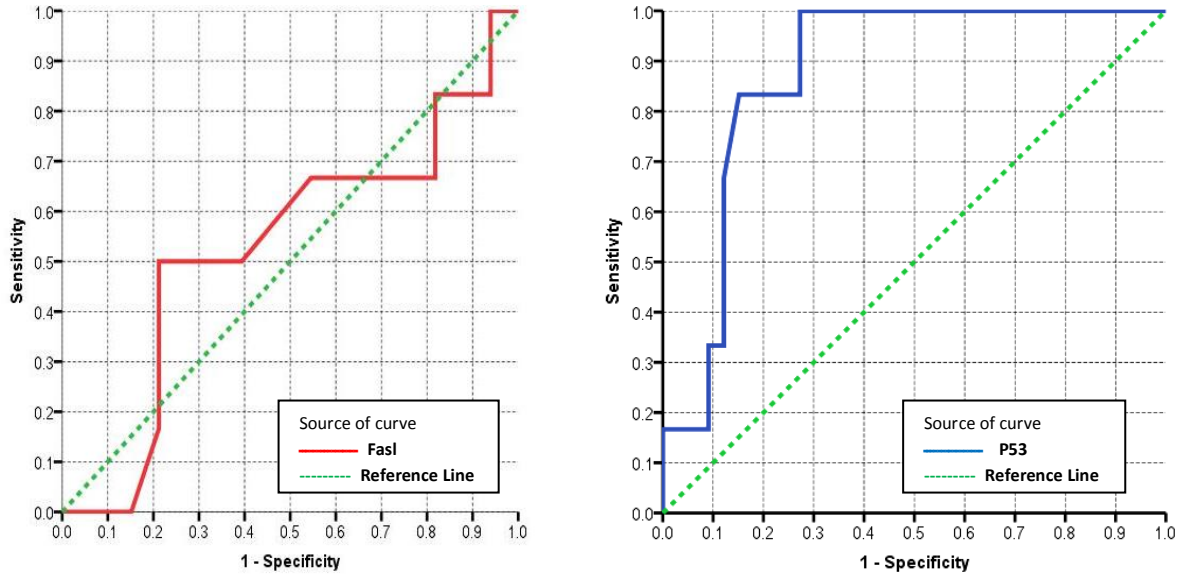


Figure 3: ROC curve of sFasL and sIL-17 BR level (Left) and P53 concentration (Right) to differentiate between recurrent and primary cancer.

Table 4: Optimum cut-off value of P53, -sFasL &-sIL-17BR with the highest percentage of sensitivity, specificity and accuracy for each.

Positive if ≥ cut-off value P53(pg/ µL)	Sensitivity	Specificity	Accuracy	PPV at pretest probability =		NPV at pretest probability = 10%
				50%	90%	
61.3 (Highest sensitivity)	100.0	72.7	76.9	78.6	97.1	100.0
77.9 (Optimum cut-off)	66.7	87.9	84.6	84.6	98.0	96.0
326.4 (Highest specificity)	16.7	100.0	87.2	100.0	100.0	91.5
sFasL (pg/ µL)						
4.25 (Highest specificity)	16.7	78.8	69.2	44.0	87.6	89.5
4.4 (Optimum cut-off)	50.0	78.8	74.4	70.2	95.5	93.4
72.1 (Highest sensitivity)	100.0	6.1	20.5	51.6	90.5	100.0

Discussion

Breast cancer is a complex disease. Its etiology is multifactorial, its period of development can span decades, and its clinical course is highly variable.

The present study was dealt with the detection of ***P53*** and sFasL levels in patients with benign and malignant breast tumors and their usefulness as diagnostic and prognostic factors in breast cancer.

Regarding ***P53 protein*** this study revealed that there was an elevation in its level among recurrent breast cancer group with highly significant differences with primary BC or benign breast tumors. These results were in consistent with the others studies [6,7]; which referred to P53 as a valuable prognostic marker in cancer with a potential use as a molecular target[6]. The interpretation of this results related to the fact that defective p53 could allow abnormal cells to proliferate, resulting in cancer , which may be explain that as many as 50% of all human tumors contain p53 mutants[7]. Here, it was sought to determine the prognostic value of p53 in breast cancer.

The interpretations of the present results can be summarized in the following aspects which reveal the highest significance of p53 level in recurrent group in comparison with the other studied groups. This result is similar to the corresponding one of a

recent study performed in Egypt by Taha I. Hewala and his colleagues[12] .

In order to select the best optimum concentration of P53 marker, the specificity, sensitivity, and accuracy must be considered before making such decision.

The level of ***apoptosis*** was represented by the level of ***sFasL***. The present results referred to decline in sFasL among recurrent BC. On the contrary, it was found to be elevated among Fibroadenoma breast tumor. This result (although it was statistically non-significant) was similar to that of a group of Japanese researchers studied the use of sFasL concentration as a marker of neoplasm in patients with gastric carcinoma[13]. In comparison with the current study the statistically increment in level of sFasL attributed to those patients (group of men over 50) compared with a group of healthy individuals. In comparisons with other investigations which did not show statistically significant difference[12]; and quietly resembled the present results. In 2009, Nilay Kavathia and his colleagues studied the use of sFasL concentration as serum markers of apoptosis decrement with age and different cancers' stages. Their study reflected a function sFasL as (stimulator of apoptosis).

Their studied reflected also that serum sFasL levels in breast cancer decreased with increasing stage[14]. The above mentioned results were in concomitant with the current result which revealed a reduction in sFasL level in recurrent patients compared with elevated level among primary breast cancer and healthy control group. There are obvious low median levels of sFasL among recurrent group in comparison with other studied groups. However, no statistically significant difference were noticed which may be due to the small number of patients who participated in the study that limits the breadth of this result.

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