



## Serum survivin levels and their relationship to histological parameters in breast cancer patients

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### ABSTRACT

*This study was conducted to investigate the serum levels of survivin and the relationship with known histological parameters in breast cancer patients. Fifty-four patients with newly diagnosed breast cancer and 25 healthy volunteers were enrolled. Serum samples were collected from the patients before Chemotherapy. Serum survivin concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Serum survivin levels in patients were not significantly different from controls ( $p=0.204$ ). Also, survivin concentrations were not significantly related to age and histological parameters in patients with breast cancer except for nodal involvement. Serum survivin levels were significantly higher in patients with nodal involvement compared with node negatives ( $p=0.03$ ). However, serum survivin levels were not influenced by the number of involved nodes ( $p=0.78$ ). In conclusion, our study suggests that serum survivin levels could be a sensitive marker for detecting metastases in lymph nodes in breast cancer patients by ELISA technique.*

**Keywords:** breast cancer, anti-apoptosis, survivin, histological parameters, ELISA.

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### INTRODUCTION

Breast cancer is the second most common cancer in the world, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Also, breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths) [1].

Clinical parameters, such as tumor size and lymph node status have been used as prognostic factors in breast cancer. However, it has been necessary to establish additional factors in order to attempt to classify patients according to indicators such as the recurrence risk and the type of therapy to administer [2]

Survivin, a unique member of the inhibitor of apoptosis protein (IAP) family, is one of the most extensively studied molecules. A 16.5 KDa intracellular protein and is expressed in G2/M phase of cell cycle. It plays central roles in regulation of cell division, inhibition of apoptosis and promotion of angiogenesis [3].

Survivin, unlike other IAPs, is undetectable in terminally differentiated adult tissues, but is prominently expressed in the vast majority of neoplasms [4]. Survivin has been demonstrated to be over expressed and associated with poor clinical outcomes in various cancers, including carcinomas of ovarian [5], lung [6], colon [7], bladder [8], endometrial [9] and prostate [10]

In breast cancer, high survivin expression found to be related to poor survival in breast cancer patients [11]. But conflicting data have been published about the relationship of survivin with several histopathological and clinical parameters in breast carcinomas. Some studies who used immunohistochemistry (IHC) or reverse transcription polymerase chain reaction (RT-PCR) to determine survivin expression failed to find a significant association between

survivin and various clinicopathological factors[12] [13] [14]. However other studies who used ELISA to measure the absolute protein concentration of survivin found a relationship with prognostic parameters[15] [16].

This study was conducted to investigate the survivin level in the circulation of breast cancer patients before chemotherapy in order to find out its implication in the pathogenesis of breast cancer and its relationship with prognostic parameters.

We used a methodologically simple ELISA.

## EXPERIMENTAL SECTION

### Patients

The study was performed at Breast Cancer Unit ( al Bairouni University hospital ) and the faculty of pharmacy in Damascus (SYRIA) and was approved by the scientific research ethics committee of Damascus University, Damascus, Syria.

All subjects gave written informed consent to participation in the study.

This study was carried out on 79 individuals: 54 breast cancer female patients . The characteristics of patients are listed in (Table 1). A number of clinical pathological parameters were shown including details on age ,tumor stage, grade, tumor size, histology type, nodal status and hormonal analysis. Staging was performed according to the TNM system of the American Joint Commission on Cancer (AJCC). And 25 healthy female volunteers as control group.

Characteristic	No. of patients	%
Age		
≤35	5	9.26
36-50	29	53.70
51-62	14	25.92
63-74	4	7.40
≥75	2	3.70
Histology type		
Invasive ductal carcinoma	48	88.89
Invasive lobular carcinoma	6	11.11
Tumor size(cm)		
≤2	14	25.92
2.1-4.9	27	50
≥5	13	24.07
Lymph node status		
Negative/positive	20/34	37.03/62.96
Number of involved nodes		
≤4	16	29.62
5-10	10	18.51
>11	8	14.81
Metastasis		
Negative/positive	48/6	88.88/11.11
Tumor grade		
Grade I-II	34	62.96
Grade III	17	31.48
Unknown	3 <sup>a</sup>	
ER status		
Negative/positive	24/27	44.44/50
Unknown	3 <sup>a</sup>	
PR status		
Negative/positive	23/28	42.59/51.85
Unknown	3 <sup>a</sup>	
Her2-neu		
Negative/positive	24/27	44.44/50
Unknown	3 <sup>a</sup>	
Stage		
I	7	12.96
II	22	40.74
III	20	37.03
IV	5	9.2

<sup>a</sup> Patients were not included in the statistical comparisons because of insufficient patient numbers.

### Measurement of serum survivin levels

Blood samples (5 to 10 ml) were drawn from all subjects into tubes without anticoagulant. The sera were collected following centrifugation and frozen immediately at  $-20^{\circ}\text{C}$  until analysis. Total human serum survivin concentrations were analyzed by an enzyme-linked immunosorbent assay (ELISA) method using (Human (surv) ELISA kit, Sun red.). The assay was carried out exactly as recommended by the manufacture.

### Statistical Analyses

Data were analyzed using SPSS program (version 20, IBM SPSS). The results were expressed as mean  $\pm$ SD. For quantitative data, comparison between two groups was done using Student's t-test. Comparison between three or more groups was done using ANOVA test. And p-value  $< 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Serum survivin in breast cancer patients and control subjects

The levels of serum survivin in breast cancer patients and control subjects are shown in (Table 2). There was no significant difference in the serum survivin levels between patients with breast cancer and control groups ( $p=0.204$ ).

**Table 2 Distribution of serum survivin values in patients with breast cancer and healthy controls**

	Serum survivin level (pg/l) Mean $\pm$ SD	P
Breast cancer patients (n=54)	145.43 $\pm$ 98.48	0.204
Controls (n=25)	184.16 $\pm$ 133.9	

### Relation of serum survivin levels with different prognostic parameters

Relation of serum survivin was tested with age, tumor size, nodal status, grade, stage, metastasis and ER, PR, Her2-neu status in our patients with breast cancer.

None of these parameters analyzed correlated significantly with the serum survivin levels, except for nodal involvement. The mean serum survivin levels was significantly higher in patients with nodal involvement (163.47 $\pm$ 118.4 pg/l) than the mean in the node negatives (114.75 $\pm$ 33.28 pg/l),  $p=0.03$ . However, serum survivin levels were not influenced by the number of involved nodes (mean values for less than or equal to four nodes, mean values for 5-10 nodes and more than or equal to 11 nodes were 170.25 $\pm$ 132.83 pg/l, 151.10 $\pm$ 89.74 pg/l and 165.38 $\pm$ 132.89 pg/l, respectively;  $p=0.78$ ).

(Table 3).

**Table 3 Associations between serum survivin levels with age and histological parameters in breast cancer patients**

Parameter	p value
Age	0.72
Tumor size	0.72
Node involvement	0.03*
Metastasis	0.19
Histology	0.19
Grade	0.94
ER status	0.36
PR status	0.36
Her-2 status	0.96
Stage	0.09

\* $p < 0.05$ , Statistically Significant.

Survivin is one of the inhibitors of apoptosis proteins (IAPs) family. It plays important roles in regulating both apoptosis and cell division, which are associated with carcinogenesis. Considering its differentiated expression between normal and malignant tissues, it has become an attractive molecule for early detection and prognosis in a wide variety of solid tumors including breast cancer [17].

In this study, we investigated the serum survivin levels using monoclonal antibodies in patients with breast cancer. We found that survivin concentrations were the same in patients and healthy controls. These results were in agreement with Guney et al [16] that studied serum and urine survivin in 43 patients with breast cancer and 21 healthy volunteers and studies among patients with other cancers who measured survivin in the serum [18] [19]. Also with Goksel et al [20] who used monoclonal antibodies in patients with early-stage breast cancer.

Previous studies using tissue cultures showed that survivin overexpression was found in breast cancer [12] [14]. This discrepancy between our results and data in literature may be due to the different material in which we assessed

survivin. We investigated survivin levels in serum whereas all other studies assessed it in tumor tissues. Also this finding may be explained by different techniques that have used to evaluate survivin expression.

Also our study aimed to investigate the relationship between prognostic factors and survivin levels. We found a statistically significant relationship between nodal status and serum levels of survivin ( $p=0.03$ ). these results were in agreement with Guney et al [16]. In a Previous published study using tissue cultures, found a significant correlation between survivin expression and the tumor size and lymph node involvement [15]. According to other studies, no significant correlations were found between survivin and the tumor size, tumor grade, nodal status, histology type and hormone receptors status [12] [14].

In this study serum survivin was measured by ELISA technique, so by simple method with less cost, higher throughput, more flexibility and small volume. We can measure this marker than other sophisticated method and can be used as a sensitive marker for detecting metastasis in lymph nodes in breast cancer patients.

### CONCLUSION

We suggest that serum survivin could be a sensitive marker for detecting metastasis in lymph nodes in breast cancer patients. Therefore may be a new indicator of prognosis in breast cancer.

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