

Assessment of Serum IL-6 Level in Breast Cancer and Its Correlation with Tumor Size

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ABSTRACT

Background: Breast cancer is a leading cancer among women worldwide. The present study was conducted to assess serum IL-6 level in breast cancer and its correlation with tumor size.

Materials & Methods: 51 FNAC proven cases of breast cancer and 30 FNAC proven cases of benign breast disease were included in the study. Patient presented with lump first assessed for the size of tumor using a Vernier caliper. FNAC was done under full aseptic precaution, to detect the cases of breast cancer patients and the cases of benign breast diseases. Trucut biopsy was performed in the cases, that were not diagnosed on FNAC. 3 ml of blood sample was taken by peripheral venepuncture. The sample was collected in pyrogen or endotoxin free collecting tube. After clot formation, centrifugation of sample was done at 1000g for 10 min. Serum removed and stored in aliquot. Interleukin-6 was assessed using ELISA technique.

Results: The age group of breast carcinoma patients ranged from 25 to 77 years with a mean age of 42.7 years. The maximum number of cases 24/51 (47.1%) were between 40-50 years of age. Twenty seven out of 51 patients of breast carcinoma (52.94 %) in our study were post-menopausal, while 16.66% cases (5/30) of benign breast disease and 50% of controls i.e. 15/30 were post- menopausal. 11 out of 51 breast cancer patients (21.5%) had tumor size less than 2cm, whereas maximum number of patients i.e. 26/51 (50.9 %) had tumor size between 2 to 5 cm. 14 out of 51 breast cancer patients (27.4 %) were found to have tumor size more than 5 cm. 28 cases of breast cancer cases (52.94%) with her2neu negative expression (0 and 1+) were found to have mean concentration of 16.22pg/ml (6.25 -32.5). Mean serum interleukin-6 concentration were found to be higher in 21 cases (41.17 %) of breast cancer patients with 3+ her2neu expression on IHC (mean 22.49 pg/ml).

Conclusion: Serum IL-6 levels in breast cancer patients are significantly higher than in patients of benign breast disease and normal controls. This increased levels in carcinoma breast patients correlates significantly and directly with tumor size, lymph node metastasis and distant metastasis. As all patients with IL-6 values of more than 12 pg/ml had confirmed breast cancer in our study, IL-

6 values higher than 12 pg/ml in a patient with an indeterminate non-inflammatory breast lump could be taken as highly suggestive of malignancy.

Key words: breast lump, lymph node metastasis, tumor size

Introduction

Breast cancer is a leading cancer among women worldwide. It has an age-standardized incidence rate (ASR) of 39.0 and 12.5 age-standardized death rate per 100,000 worldwide.¹ Due to changed life style in India increase incidence of breast cancer is quite alarming. Now it is second most common malignancy in Indian women. The age-standardized incidence rate for breast cancer in India is 22.9 per 100,000, one-third that of Western countries.²

The established risk factors for the breast cancer are female gender (20 times more common than males), increasing age, past history of breast cancer, nulliparity, early menarche, late menopause, positive family history of breast cancer in first degree relative and hormone replacement therapy.³ Prognostic factors for breast cancer are tumor type, grade, size, lymph node status and lymphovascular invasion, over expression of her2/neu, estrogenreceptor, progesterone receptor, p53 over expression, etc. Other prognostic factors included are Oncogene as c-myc proto-oncogene, tumor suppressor gene like p53, cell adhesion molecules 1- cadherin /catenin complex, transcription factors like NF-Kb which initiates the transcription of wide variety of genes IL-1, IL-6, IL-8 and TNF-alfa.^{4,5}

The biology of breast cancer is complex involving oncogenesis, evasion of host immune defense mechanism, angiogenesis, invasion and metastasis. It often progresses from a benign, non- invasive growth phenotype to a malignant, invasive, and metastatic growth phenotype. This progress is usually accompanied by expression of various types of genes that promote invasion and metastasis.⁶ The present study was conducted to assess serum IL-6 level in breast cancer and its correlation with tumor size.

Materials & Methods

The study was conducted in Department of Pathology in collaboration with Department of Surgery, Lady Hardinge Medical College and Associated Hospitals, New Delhi from November 2010 to March 2012. 51 FNAC proven cases of breast cancer and 30 FNAC proven cases of benign breast disease were included in the study.

Inclusion criteria was age group: 25-80 years. -Patient with breast lump - FNAC proven cases of breast cancer - FNAC proven cases of benign breast disease. Exclusion criteria was cases of control presented with any Acute or chronic infection, patient undergoing glucocorticoid therapy, hyperthyroidism, multiple myeloma.

Patient presented with lump first assessed for the size of tumor using a Vernier caliper. FNAC was done under full aseptic precaution, to detect the cases of breast cancer patients and the cases of benign breast diseases. Trucut biopsy was performed in the cases, that were not diagnosed on FNAC. 3 ml of blood sample was taken by peripheral venepuncture. The sample was collected in pyrogen or endotoxin free collecting tube. After clot formation, centrifugation of sample was done at 1000g for 10 min. Serum removed and stored in aliquot. Interleukin-6 was assessed using ELISA technique. Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

Results

Table 1: Age distribution in Carcinoma breast, Benign breast disease and Controls

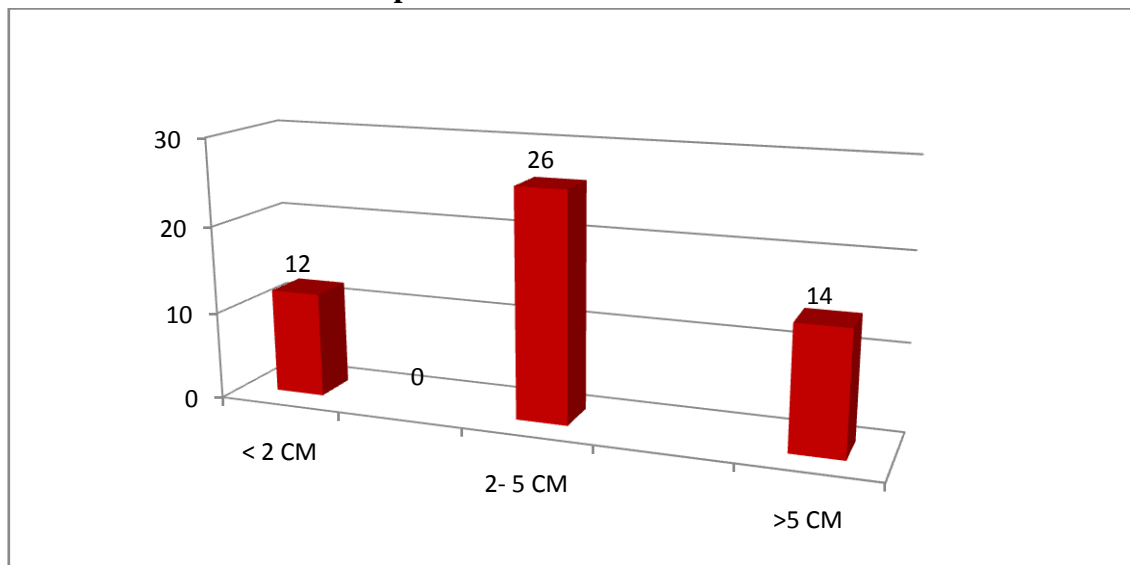
AGE (years)	Carcinoma breast	Benign breast disease	Controls
< 30	6 (11.8%)	21 (70%)	7 (23.3%)
31-40	11 (21.6%)	6 (20%)	10 (33.3%)
41-50	24 (47.1%)	3 (10%)	5 (16.66%)
51-60	6 (11.8%)	0 (0%)	4 (13.33%)
61-70	3 (5.9%)	0 (0%)	2 (6.66%)
71-80	1 (2.0%)	0 (0%)	2 (6.66%)
TOTAL	51	30	30

The age group of breast carcinoma patients ranged from 25 to 77 years with a mean age of 42.7 years. The maximum number of cases 24/51 (47.1%) were between 40-50 years of age. The age of patients with benign breast diseases varied from 25 to 45 years of age with a mean of 36 years. The age group in controls ranged from 25 to 66 years of age with a mean age of 41.2 years. All of the cases (carcinoma breast and benign breast disease) and controls included in the study were females.

Table 2: Menopausal status in cases of breast cancer, benign breast disease and controls

Menopausal status	Number/Percentage of Breast cancer patients	Number/Percentage of Benign Breast disease patients	CONTROLS
Pre-menopausal	24 / 51 (47.05%)	25 / 30 (83.33%)	15 /30 (50%)
Post-menopausal	27 / 51 (52.94 %)	5 /30 (16.66%)	15 /30 (50%)
TOTAL	51	30	30

Twenty seven out of 51 patients of breast carcinoma (52.94 %) in our study were post-menopausal, while 16.66% cases (5/30) of benign breast disease and 50/% of controls i.e. 15/30 were post-menopausal.

Graph I Distribution based on size

Graph I shows that 11 out of 51 breast cancer patients (21.5%) had tumor size less than 2cm, whereas maximum number of patients i.e. 26/51 (50.9 %) had tumor size between 2 to 5 cm. 14 out of 51 breast cancer patients (27.4 %) were found to have tumor size more than 5 cm.

TABLE 4: Distribution of IL-6 values in Carcinoma breast, Benign breast disease and Controls

IL-6 (pg/ml)	Diagnosis	No of cases	Mean $\pm$ SD (Range)
<12	Cancer Breast	15	8.9 $\pm$ 1.6 (25-11.45)
	BBD	30	6.34 $\pm$ 0.863 (3.19-11.25)
	Controls	30	3.625 $\pm$ 0.81 (1.5-7.75)
>12	Cancer Breast	36	22.55 $\pm$ 7.22 (12.25-38.75)
	BBD	Nil	-
	Controls	Nil	-

15 out of the 51 breast cancer patients were found to have IL-6 levels <12pg/ml (8.9 $\pm$ 1.6), while 36 breast cancer patients had IL-6 values >12pg/ml (22.55 $\pm$ 7.22). However all cases of BBD and controls had IL-6 values less than 12 pg/ml (BBD: 6.34 $\pm$ 0.86, and Controls: 3.625 $\pm$ 0.81).

Table 5: Correlations of S.IL-6 with tumor size in carcinoma breast and benign breast disease

Tumor size (cm)	Carcinoma breast		Benign breast disease	
	Number	S. IL-6 (pg) Mean/range	Number	S.IL-6 (pg)
< 2	11	9.3 (6.25 – 16.5)	16	6.51 (3.45-1.25)

2-5	26	16.53 (8.75 – 37.5)	12	5.56 (3.19-7.5)
>5	14	27.4 (16.2 – 38.75)	2	4.3 (3.5-5.25)
Total	51	Mean= 17.3353	30	Mean= 5.4

Mean concentration of serum IL-6 in breast cancer patients with tumor size less than 2 cm was 9.3 pg/ml (range =6.25 – 16.5). Gradually increased concentration of serum IL-6 was noticed as the tumor size increased i.e. mean concentration of serum IL-6 for tumor size between 2 to 5 cm and more than 5 cm had 16.53 pg/ml and 27.4 pg/ml (16.2 – 38.75) respectively.

Statistically significant correlation was found between the IL-6 concentration of tumor size >5 cm and <2cm (p value < 0.001). Increased concentration of serum IL-6 was noticed in patients with tumor size more than 5 cm compare to patients with tumor size 2-5 cm and it was also statistically significant (p value- 0.016). In case of benign breast disease serum IL-6 in patient with tumor size less than 2 cm, 2 to 5 cm and more than 5 cm were 6.51pg/ml (mean 3.45-11.25) 5.56pg/ml (mean 3.19-7.5) and 4.3pg/ml (mean 3.5-5.25) respectively.

Table 6: Correlation of S.IL-6 with in Her2 neu expression carcinoma breast

Her2 neu expression on IHC	No. of cases	FISH	S. IL-6 values (pg/ml) Range/Mean
0 and 1+	28 (20+7)		(6.25 -32.5) ;16.225
2+	2	Amplified	10.25
		Not amplified	15.25
3+	21		(9.50-38.7); 22.49
Total	51		

28 cases of breast cancer cases (52.94%) with her2neu negative expression (0 and 1+) were found to have mean concentration of 16.22pg/ml (6.25 -32.5). Mean serum interleukin-6 concentration were found to be higher in 21 cases (41.17 %) of breast cancer patients with 3+ her2neu expression on IHC (mean 22.49 pg/ml). Out of 51 cases of breast carcinoma 2 showed 2+ her2neu expression on IHC. FISH was put on these 2 cases. One of these case was amplified, while other one was not amplified. Serum interleukin-6 concentration in these cases were 15.25 pg/ml (FISH-amplified) and 10.25 pg/ml (FISH- non amplified). However, no statistically significant correlation was found between serum IL-6 concentration in breast cancer cases with Her2neu negative, Her2neu 1+, Her2neu 2+, Her2neu 3+ immune expression.

Discussion

Breast cancer is the most commonly occurred female cancer in the world. It is the most frequent cancer in both developed and developed regions as well as the most frequent cause of cancer death in these regions of the world.⁷ There are several treatment options for women diagnosed with breast cancer that include surgery, chemotherapy, radiation therapy, hormonal therapy and targeted therapies.^{8,9} The most appropriate treatment depends on the woman's risk profile and stage of disease, based on the tumor size, location, involvement of lymph nodes and whether or not tumor has spread to surrounded tissue or distant organs.¹⁰

The present study, conducted in Department of Pathology in collaboration with Department of Surgery, Lady Hardede Medical College and Associated Hospitals, on 51 cytologically/ trucut biopsy proven cases of breast cancer, 30 cases of benign breast disease and thirty age matched healthy females as a control. Serum of all the above patients was collected and subjected for detection of IL-6 levels by used solid phase ELISA.

The age group of breast carcinoma patients ranged from 25 to 77 years with a mean age of 42.7 years. The maximum number of breast cancer cases i.e. 24/51(47.1%) were between 40-50 years. In our study twenty seven out of 51 patients (52.94%) of breast carcinoma were post menopausal, while 24 patients were premenopausal. Older age increases the risk of breast cancer and most women are over the age of 60 when they are diagnosed although there is evidence that Indian women are more likely to develop breast cancer at earlier ages than their Western counterparts and that breast cancer peaks from ages 45-50 years in India. In our study highest number of breast cancer cases i.e. 24/51(47.1%) were between 40-50 years of age. Fourty six out of 51 patients of breast carcinoma (90.10 %) in our study were parous. Women who have never had children or those who are more than 30 years at the time of their first child's birth are twice as likely to developed breast cancer than women who had their first child before the age of 20 years. Maximum number of cases 26/51 (50.9 %) were found to have tumor size between 2 to 5cm. Accorded to Lai H¹¹if the tumor can be detected and treated before it has reached a size of 2 cm then the overall survival rate is around 75% in 10 years.

Out of total 51 cases of carcinoma breast 24 cases showed enlarged ipsilateral axillary lymph nodes. Accorded to Dimitriu¹², If the tumor can be detected before it is seen to be affected the lymph nodes, then the overall survival rate is very good, at over 75% in 10 years. 7 out of 51 (15.69%) cases of breast cancer on USG / CT scan were found to had lesions suggestive of distant metastasis, however none of these cases were confirmed on biopsy / FNAC. Out of total 51 patients 11 (21.56%) patients belonged to T1 stage with no lymph node or distant metastasis. 25 patients (49.01 %) were in 12 stage. Correlation of IL-6 values in Carcinoma breast, Benign breast disease and Controls: IL-6 values in breast cancer patients ranged from 6.25 to 38.75 pg/ml with a mean value of 17.83 pg/ml while in, benign breast disease and controls, mean levels of IL-6 was 6.34 (3.19 - 11.25) pg/ml and 3.62 (2.25-7.75) pg/ml respectively. Serum interleukin-6 levels were found to be higher in breast cancer patients compare to patients of benign breast disease (p-value 0.000) and controls (p-value 0.000) which was statistically significant, however no statistically significant correlation was found between IL6 values of patients of BBD and controls. Similar findings were reported by Fong¹³ as they also observed statistically significant higher IL-6 values in breast cancer patients compare to controls. 15 out of the 51 breast cancer patients were found to have IL-6 levels 12pg/ml (22.55 + 7.22).

Statistically significant correlation was found between the IL-6 levels in breast cancer patients with tumor size >5 cm and < 0.001). Increased levels of serum IL-6 were seen in patients with tumor size more than 5 cm as compare to patients with tumor size 2-5 cm. Similar findings have also been observed by Fong¹³ and Noguchi et al.¹⁴

Serum interleukin-6 levels found to be higher in breast cancer patients with lymph node metastasis (mean 21.84 pg/ml) compared to breast cancer patients without lymph node metastasis (14.02pg/ml). Ahmad et al found statistically significant correlation between serum IL-6 values in patients with more than 3 positive lymph nodes than those with less than 3 positive lymph nodes.

Conclusion

Authors found that serum IL-6 levels in breast cancer patients are significantly higher than in patients of benign breast disease and normal controls. This increased levels in carcinoma breast patients correlates significantly and directly with tumor size, lymph node metastasis and distant metastasis. As all patients with IL-6 values of more than 12 pg/ml had confirmed breast cancer in our study, IL-6 values higher than 12 pg/ml in a patient with an indeterminate non-inflammatory breast lump could be taken as highly suggestive of malignancy. However, in view of the relatively small size of our study, a much larger study would be required to validate this observation before it can be used as a diagnostic test for malignancy.

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