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Hormonal receptors and Her2 neu expression in patients with breast cancer: A cross sectional study in a tertiary care centre in Mysore

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Abstract

Background and objectives: Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast. Vascular Endothelial Growth Factor (VEGF) is most potent angiogenic factor and Interleukin-6 (IL-6) is a multifunctional cytokine. The hormone receptors and Human epidermal growth factor receptor 2 (HER2 Neu) expressions in breast cancer patient's forms an important indicator for the prognosis and treatment. This study was aimed to determine the hormone receptors and HER2 Neu expression in breast cancer patient and to correlating them with tumour markers like VEGF and IL-6.

Methodology: A total of 37 Confirmed subjects with breast cancer were analysed for serum levels of VEGF and IL-6. Immunohistochemical staining was done to evaluate Oestrogen Receptors (ER), Progesterone Receptors (PR) and HER-2/ neu status. The data collected was entered in Microsoft excel and analysed using SPSS Version 22. Associations and differences were interpreted as statistically significant at $p<0.05$.

Result: The mean value of IL-6 in Her2 neu and VEGF in Her2 neu positive tumours were 21.89 pg/ml and 193.94 pg/ml. The mean value of IL-6 in Her2 neu and VEGF in Her2 neu negative tumours were 20.72 pg/ml and 206.37 pg/ml respectively. There was no statistically significant difference in the mean values.

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Conclusion: The mean difference in values of VEGF and IL-6 among Her2 neu positive and negative was not significant statistically, but the subjects with positive status had a comparatively higher mean value of IL-6 and subjects with negative status had higher mean values of VEGF. Thus IL-6 and VEGF levels in serum can be used as a diagnostic tool and also as a prognostic factor in breast cancer.

Keywords: Breast cancer; VEGF; IL-6; HER2neu receptors; ER and PR.

INTRODUCTION

Worldwide, breast cancer is one of the most common cancers in women, in 2015 approximately 5,70,000 deaths are due to carcinoma of breast. Further, worldwide about 1.5 million women every year are diagnosed with breast cancer.[1,2] According to statistics, in India Breast cancer is now the most common cancer in women.[3]Breast cancer is the second cause of cancer death after Lung cancer in Asia (Kwong et al., 2015) and it's a multifactorial disease.[4]

Breast cancer is a complex disease with a large intertumoral and intratumoral heterogeneity. It is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast, based on that they are classified as ductal or lobular carcinoma. [5]

In breast carcinoma, number of tumour related features available to predict the prognosis of patients including histologic subtype, grade, lymph node states, Oestrogen Receptor (ER)/Progesterone Receptor (PR) status, /Human Epidermal Growth Factor Receptor-2/neu (her-2/neu) status, growth factors and its receptors, proliferative activity and DNA content, oncogenes, and tumour suppressor genes. [6]

Oestrogen and progesterone are important regulators of proliferation and differentiation of the mammary gland. These nuclear transcription factors exert their function by binding to their respective receptors: ER and PR. Two thirds of breast cancers express both ER and PR, 10% are ER-positive and PR-negative, 5% are ER negative and PR-positive and 20% of all breast cancers are negative for both ER and PR. [7]

The expression of PR is strongly dependent on oestrogen and is thus rarely seen in ER negative tumours. [8] ER and PR is a very powerful and useful predictor. In breast cancer, the response to hormonal therapy is quite good with higher content of ER and PR. [9]

Her2/neu is a biomarker and it is a transmembrane receptor that functions as a tyrosine kinase. Over expression occurs in about one third of breast cancers and are associated with high histologic grade, reduced survival, lower responsiveness to hormone receptor. [10]

Her2/neu has been shown to be a poor prognostic factor with over expression of the protein or amplification of the gene correlating with poor outcome in patients with axillary lymph node metastases but not in patients with tumour-negative lymph

nodes.[11] Her2/neu is thus a prognostic as well as predictive marker in breast cancer.[8]

The hormone receptors and HER2 Neu expression in breast cancer patients forms an important indicator of the prognosis and treatment options and thus this study was undertaken to assess the hormone receptors and HER2 Neu expression in breast cancer patient and to correlating them with tumour markers like vascular endothelial growth factor (VEGF) and Interleukin-6 (IL-6).

MATERIALS AND METHODS

A total of 37 Confirmed cases of breast cancer between 30 to 60 years of age were selected from JSS Medical College Hospital, Mysore. Study was conducted over a period of 1 year between January 2014 and August 2015. Under aseptic precautions 5ml of venous blood was drawn after obtaining written consent from the patients. Institutional scientific and Institutional Ethical clearance was taken from the committee before the commencement of the study.

The collected blood sample was allowed to stand for 30 minutes at room temperature in vacutainer and centrifuged at 3,000 x g for 5 min, and the supernatants were aliquoted and stored at -80°C until further analyses. The serum samples were thawed shortly before determination of VEGF and IL-6 using Enzyme-linked immunosorbent assay (ELISA).

Serum levels of IL-6 and VEGF were determined with an enzyme-linked immunosorbent assay (ELISA) from PEPROTECH USA. Both assays employ the quantitative sandwich enzyme immunoassay technique. The colour intensity in each well was measured at 450 nm for VEGF and IL-6 using an automated iMark ELISA reader from Biorad laboratories. The detection limits of the immunoassay were 16-1000 pg/ml for VEGF and 24-1500 pg/ml for IL-6. Immunohistochemical staining was done to evaluate ER, PR and HER-2/ neu status and Serum level determinations were performed.

Patients of breast cancer who are under chemotherapy or Radiation and Patients of Breast cancer who has underwent previous mastectomy, were excluded from the study.

Descriptive statistical analysis:

The data collected was entered in Microsoft excel and analysed using SPSS Version 22. Descriptive statistical measures like percentage, mean and standard deviation were applied. Inferential statistical tests wherever required were used. Associations and differences were interpreted as statistically significant at $p<0.05$.

RESULTS

In the present study, a total of 37 breast cancer subjects aged between 30 to 60 years, were participated in the study. Two patients (5.4%) tumours were in stage IA, fourteen (37.8%) were stage IIA, four (10.8%) were in stage IIB, nine (24.3%) were in stage IIIA, and eight (21.6%) were in stage IIIC. **(Table -1)**

Table 1: Distribution of the Subjects by the stage of breast cancer

Stage of breast cancer	Number of patients	Percent
IA	2	5.4
IIA	14	37.8
IIB	4	10.8
IIIA	9	24.3
IIIC	8	21.6

The Mean value IL-6 in oestrogen positive tumour was higher as compared to oestrogen negative tumour and there was no statistically significant difference in the mean values. Hence no correlation was noted between the two. **(Table -2)**

Table 2: Distribution of subjects with Oestrogen receptor status and IL-6

Estrogen Receptor	No of Cases	Mean IL-6(pg/ml)	p value
Positive	16	21.38	
Negative	21	21.33	0.64
Total	37		

The Mean value VEGF in oestrogen positive tumour was higher as compared to oestrogen negative tumour. Mean values of both did into show any statistical difference and hence no correlation was noted between the two. **(Table -3)**

Table 3: Distribution of subjects with Oestrogen receptor status and VEGF

Oestrogen Receptor	No of Cases	Mean VEGF (pg/ml)	p value
Positive	16	199.73	0.12
Negative	21	199.59	
Total	37		

The Mean value IL-6 in progesterone positive tumour was 22.20 pg/ml while it was 20.78 pg/ml in oestrogen negative tumours. There was no statistically significant difference in the mean values. Hence no correlation was noted between the two. **(Table -4)**

Table 4: Distribution of subjects with Progesterone receptor status and IL-6

Progesterone Receptor	No of Cases	Mean IL-6 (pg/ml)	p value
Positive	15	22.20	0.60
Negative	22	20.78	
Total	37		

The Mean value VEGF in progesterone positive tumour was 193.39pg/ml while it was 203.92 pg/ml in oestrogen negative tumours. There was no statistically significant difference in the mean values. Hence no correlation was noted between the two. **(Table -5)**

Table 5: Distribution of subjects with Progesterone receptor status and VEGF

Progesterone Receptor	No of Cases	Mean VEGF (pg/ml)	p value
Positive	15	193.39	0.84
Negative	22	203.92	
Total	37		

Even though the mean value of IL-6 in Her2 neu positive tumours was higher as compared to her2 neu negative tumours, but it was found to be statistically insignificant. **(Table -6)**

Table 6: Distribution of subjects by Her2 neu and IL-6

Her2 neu Expression	No of Cases	Mean IL-6(pg/ml)	P value
Positive	20	21.89	0.95
Negative	17	20.72	
Total	37		

The mean value of VEGF in Her2 neu positive tumour was 193.94 pg/ml and 206.37 pg/ml in her2 neu negative tumours. There was no statistically significant difference in the mean values. Hence no correlation was noted between the two. **(Table -7)**

Table 7: Distribution of subjects by Her2 neu and VEGF

Her2 neu Expression	No of Cases	Mean VEGF (pg/ml)	P value
Positive	20	193.94	0.83
Negative	17	206.37	
Total	37		

DISCUSSION

Breast cancer affects women in both developed and developing countries. It is the most common non-cutaneous malignancy, according to statistics in USA nearly one in three cancer diagnosed in women is a breast cancer.[12] Around 6.6% of all breast cancer cases are diagnosed in women less than 40 of age.[13] Breast cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast. Inflammatory cells existing in the tumour microenvironment play an important role in cancer progression. Many cancers arise from sites of chronic inflammation.

Hormone receptor studies such as ER, PR and HER2/neu are routinely done in breast carcinoma. Angiogenesis play an essential role in tumour growth, invasion and metastasis. This process is mainly regulated by increased activity of angiogenic factors such as vascular endothelial growth factor (VEGF), Interleukin-6 (IL-6) and other inflammatory markers.

Several studies have investigated the prognostic significance of VEGF and IL-6 in breast cancer. The present study was also done to evaluate the prognostic significance of VEGF and IL-6 in breast cancer by assessing the relation if it exists with hormonal receptors and Her2 neu expression. Several studies have reported that metastatic breast cancer patients have elevated serum levels of VEGF and IL-6 when compared with controls.

In the present study, 37 cases of histo-pathologically proven breast carcinomas were studied. VEGF and IL-6 assay was done in all the 37 cases and were also studied for hormonal receptor status and Her2/neu expression.

Tumours with low grade are well differentiated and predict a more favourable prognosis for the patient than poorly differentiated tumours with a high grade is 90-95% as opposed to 30-80% for patients with the highest grade.[14,15] Additionally, higher grade is associated with negative hormone receptor status and low grade with positive hormone receptor status. Therefore, histological grade is correlated with response to either endocrine therapy (low grade) or chemotherapy (high grade).[16]

The tumour size, condition of regional lymph nodes, cancer stage in accordance with the pTNM classification, histological type, grading, status of oestrogen and

progesterone receptors, form a group whose prognostic value for breast cancer has been fully documented. A high expression of VEGF is connected in most cases with poor prognosis.[17]

Various studies have been shown that IL-6 was shown to be an independent prognostic factor after multivariate analysis and was also associated with disease progression under therapy. [18] Although IL-6 expression in early-stage breast carcinomas has been correlated with low grade, oestrogen receptor status and good prognosis [19], studies have shown that IL-6 may contribute to disease progression, particularly in advanced breast cancer patients.

In our study nearly 16 subjects were positive for Oestrogen receptor and 15 were positive for progesterone receptors and the rest negative, but the difference in mean values of IL-6 among positive and negative cases were not statistically significant. This may be because the variation in IL-6 value is independent of the oestrogen and progesterone receptor expression status in patients with breast cancer.

The difference in Mean value of VEGF among subjects with their Hormone receptor status was also not statistically significant, reiterating the fact that it is independent of the hormone receptor expression.

A total of 20 subjects were positive for HER2neu receptors. The mean difference in values of VEGF and IL-6 among them was not significant statistically, but the subjects with positive status had a comparatively higher mean of IL-6 and subjects with negative status had a higher VEGF values.

Our study showed an overall increase in VEGF and IL-6 values among subjects with breast cancer which is a poor prognostic factor, which is similar to other studies done by Blay JY et al and Nakashima J et al. [20,21] But, is independent of the Hormone receptors and HER2 neu receptor expression.

CONCLUSION

The presence of the hormone receptors ER, PR in a patient's breast cancer is an example of a weak prognostic but strong predictive biomarker. The management and prognosis of breast cancer require the evaluation of ER PR and HER2/neu.

In our study the mean difference in values of VEGF and IL-6 among Her2 neu positive and negative was not significant statistically, but the subjects with positive status had a comparatively higher mean value of IL-6 and subjects with negative status had higher mean values of VEGF. Thus IL-6 and VEGF levels in serum can be used as a diagnostic tool and also as a prognostic factor in breast cancer.

Further studies can be done with these markers for early detection of recurrence of breast cancer. The small sample size is one limitation of our study; however, this may be the basis for large scale multicentric studies

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