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Interleukin-10 Gene Expression and Haematoxylin and Eosin staining in Breast Carcinoma

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Introduction: In India breast cancer is the 2nd most common cancer in women after cervical cancer and is associated with high mortality and morbidity. Role of IL-10 in cancer though, well accepted, is vaguely understood. IL-10 is known to exhibit both pro and anti-tumour activities. Polymorphism in interleukin genes are thought to influence the expression or function of these proteins. Hence our study was designed to evaluate the role of low penetrant interleukin-10 (IL-10) gene and its influence on tumorigenesis.

Material and Methods: This is a prospective and observational study conducted at the Department of Pathology in a Tertiary care teaching Hospital over a period of 1 year. All mastectomy specimens clinically diagnosed as breast carcinoma received at the department were included in the study. Two to five grams of tumour tissue and another sample from the normal peri-tumoural breast tissue were taken from the resected breast immediately after surgery and preserved in RNAlater solution. IL-10 mRNA expression was detected and quantified by RT-PCR analysis through TaqMan chemistry on 30 samples and 30 controls. After fixation with formalin, two tissue sections of 4-5micron thickness were taken from the tumour area and the corresponding paraffin blocks prepared, mounted on albumin coated slide for H & E staining.

Results: The mean age of the study is 50 years, the maximum and minimum age being 65 years and 32 years respectively. Even though either side of the breast can be affected in Ca. Breast, for reasons not known there is slight preponderance to left side breast. Most of the gross tumours were of size 4-5cms (n=12 i.e, 40%) followed by 5- 6cm (n=8 i.e, 28.5%). Majority of the patients are categorized as grade-II i.e. 53.5% of cases and next is grade-I tumours i.e. 26.5% cases. Metastasis Positive lymph nodes were present in 66.5% of cases; and were negative in 33.5% of cases. All grade 3 tumours expressed strong expression of IL-10 indicating that probably IL-10 expression is more in high grade tumours.

Conclusion: From this study, we conclude that IL-10 may serve as a useful biomarker with potential prognostic value as there is statistically significant association of high IL-10 mRNA levels in the breast tumour tissue when compared with peri-tumoral tissue.
Keywords: Breast Carcinoma, Interleukin-10, Gene Expression, H & E staining

Introduction

Worldwide, breast cancer is the most common invasive cancer in women. In India breast cancer is the 2nd most common cancer in women after cervical cancer and is associated with high mortality and morbidity. Several parameters have been investigated to predict the prognosis in breast cancer.

The etiology of cancer has been multi-factorial with various epidemiological attributes in combination with genetic factors[1]. The role of high penetrant genes like BRCA1, BRCA2, PTEN etc. are well established in etiology of breast cancer[2]. However, the role of low penetrant gene variations are a much less investigated and less established topic. Hence our study was designed to evaluate the role of low penetrant interleukin-10 (IL-10) gene expression and its influence on tumorigenesis.

Role of IL-10 in cancer, though well accepted, is vaguely understood. IL-10 is known to exhibit both pro and anti-tumour activities. Polymorphism in interleukin genes are thought to influence the expression or function of these proteins[3]. IL-10 produced by tumour cells and immune cells (mononuclear phagocytes) play an important role in tumour cell growth and proliferation[4].High levels of increased IL-10 mRNA can be detected in tumour cells when compared to peritumoral tissue of breast carcinoma patients. Stem cells isolated from breast cancer patients show significantly high mRNA expression of IL-10 and TGFβ1 when compared to normal individuals[5]. Increased IL-10 concentration is also frequently detected in serum of breast cancer patients. It is proposed that IL-10 is secreted at a higher rate by metastatic cancer cells for down-regulating inflammatory response of cell-mediated immunity[6].

IL-10 is over expressed in oestrogen receptor (ER)-negative breast tumours in comparison to ER-positive tumours. Breast cancer patients with progesterone receptor (PR) positive tumour have lower IL-10 level[7]. Majority of IL-10 polymorphism studies find significant association of IL-10 with breast cancer.IL-10 predominantly exerts tumour inhibiting action on breast cancer, however, it also has potential to promote tumour. This dual nature of IL-10 may be dependent on the IL-10 level available as well as temporal-spatial nature of their expression. However, IL-10 may serve as a crucial biomarker with a certain amount of prognostic significance.

Evading immune destruction is one of the hallmarks of cancer[8] and Interleukin-10 (IL-10) is well documented for its immune suppression. Normally T cells, B cells, dendritic cells and monocytes/macrophages express IL-10 during inflammation[9]. IL-10 is abundantly produced by tumour associated macrophages (TAMs) which form a major component of tumour tissue[10].

Hence it is hypothesized that IL-10 might facilitate tumour cells to escape immune surveillance. The immunosuppression of IL-10 is through inhibition of cytokine synthesis (TNF, IL-1, chemokine, and IL-12) by the macrophages needed for T cell activation. However, the exact role of IL-10 is controversial as there is growing evidence of anti-tumorigenic activity of IL-10. It is also known that IL-10 down regulates the synthesis of VEGF, IL-1b, TNF-α, IL-6, and MMP-9 needed for angiogenesis during tumour progression, exhibiting anti-tumorigenic properties[11]. TAMs which produce high levels of IL-10 play an essential role in the complex process of tumour-microenvironment co-evolution and tumorigenesis[12].

In our study, we are going to evaluate the expression of IL-10 mRNA in the tumour tissue and compare it with surrounding normal tissue.

Material and Methods

This is a prospective and observational study conducted at the Department of Pathology in a Tertiary care teaching Hospital over a period of 1 year. All mastectomy specimens clinically diagnosed as breast carcinoma received at the department were included in the study.

Inclusion Criteria:

- Properly labelled mastectomy specimens
- Fresh unfixed tissue samples
- Tumour tissue and adjacent normal tissue for analysis
- Complete clinicopathological data

Two to five grams of tumour tissue and another sample from the normal peri-tumoural breast tissue were taken from the resected breast immediately after surgery and preserved in RNAlater solution.

IL-10 mRNA expression was detected and quantified by RT-PCR analysis through TaqMan chemistry on 30 samples and 30 controls. After fixation with formalin, two tissue sections of 4-5 micron thickness were taken from the tumour area and the corresponding paraffin blocks prepared, mounted on albumin coated slide for H & E staining.

Results

The mean patient age was 50 years (range 30-70 years). Majority of cases were seen in 4^{th} to 6^{th} decade. Out of 30 cases 4 cases (14%) were premenopausal and 26 cases (86%) were post-menopausal.

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Table 1: Showing Age Wise Distribution of Cases

AGE GROUP OF STUDY	NUMBER	PERCENT
30-40 YEARS OF AGE	02	6.5%
41-50 YEARS OF AGE	10	33%
51-60 YEARS OF AGE	13	44%
61-70 YEARS OF AGE	05	16.5%
TOTAL	30	100%

The age group distribution of the subjects in the present sample is depicted in Table No.1. Accordingly, the commonly affected age group 51-60yrs (44%) followed closely by 41-50yrs (33%). The mean age is 50 years, the maximum and minimum age being 65 years and 32 years respectively.

Graph No 1: Pie Diagram Showing Pre and Post-Menopausal Status Of Cases Included In Our Study.

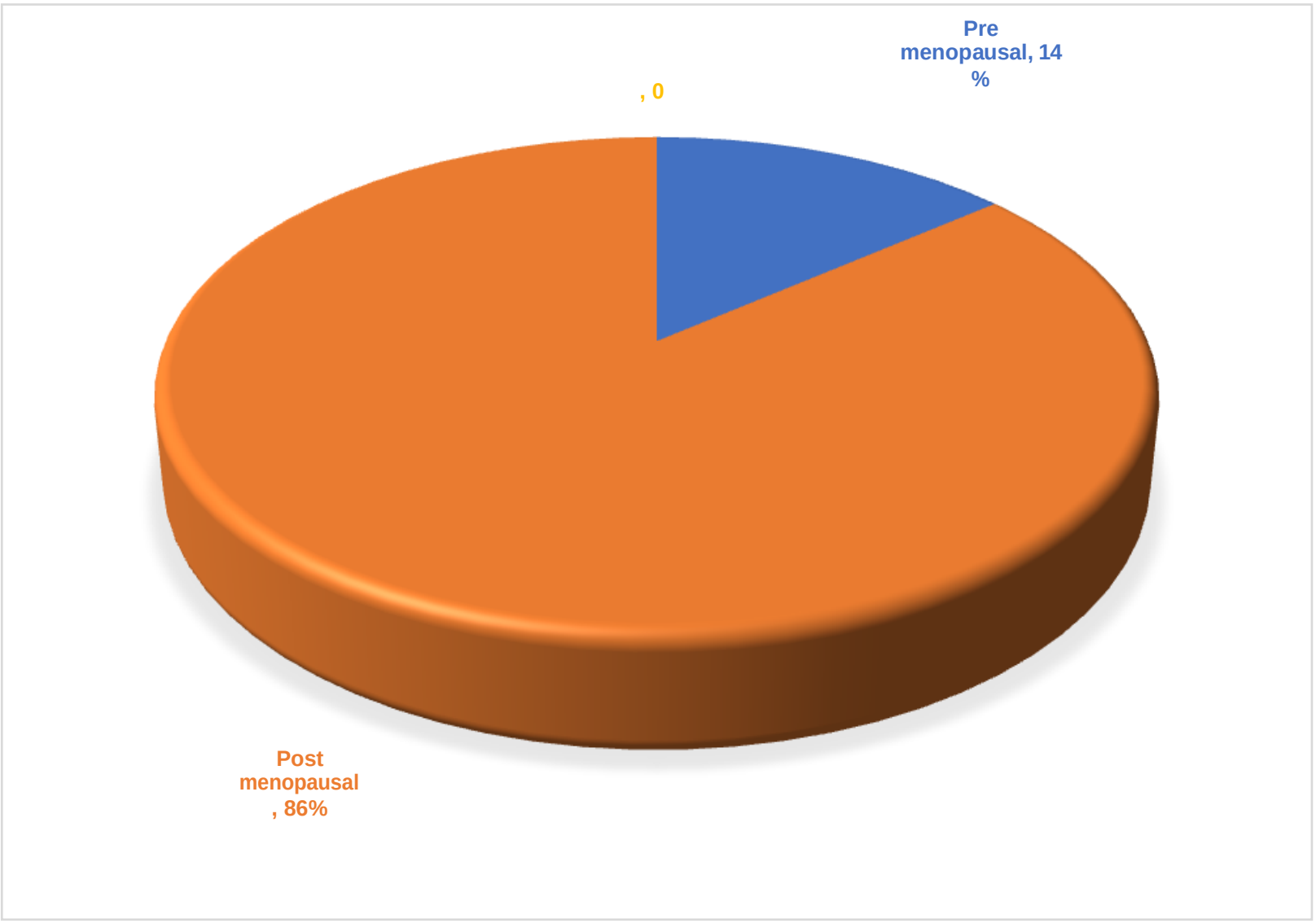
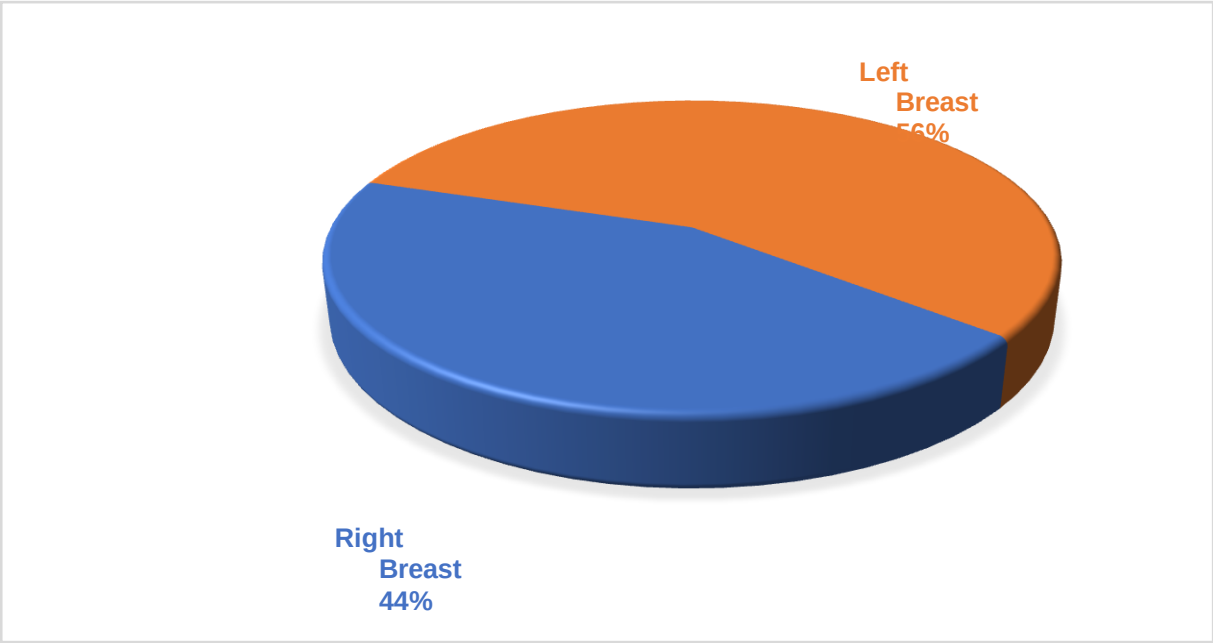


Table No: 2 Distribution Of Lesions According To Side Affected

LATERALITY OF TUMOUR	NUMBER	PERCENT
RIGHT BREAST	13	44%
LEFT BREAST	17	56%
TOTAL CASES	30	100%

Graph.No.2 Pie Diagram Showing Side Distribution of Tumour



Even though either side of the breast can be affected in Ca. Breast, for reasons not known there is slight preponderance to left side breast. Most of the gross tumours were of size 4-5cms (n=12 i.e, 40%) followed by 5- 6cm (n=8 i.e, 26.5%).

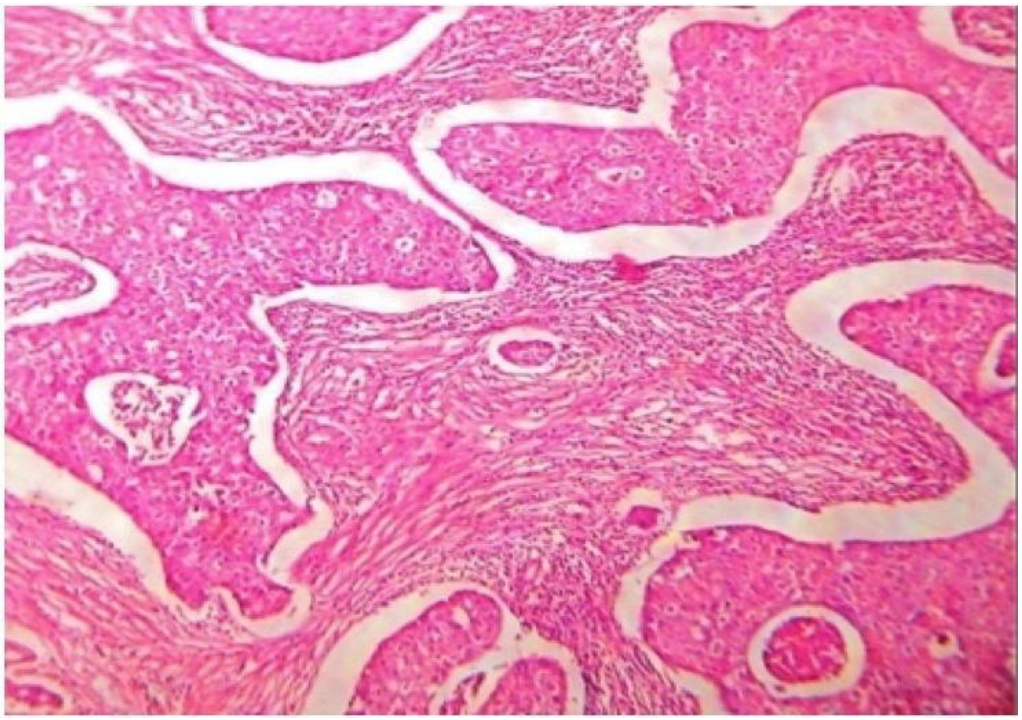


Figure 1: INVASIVE DUCTAL CARCINOMA (GRADE – 1)- H&E 10x

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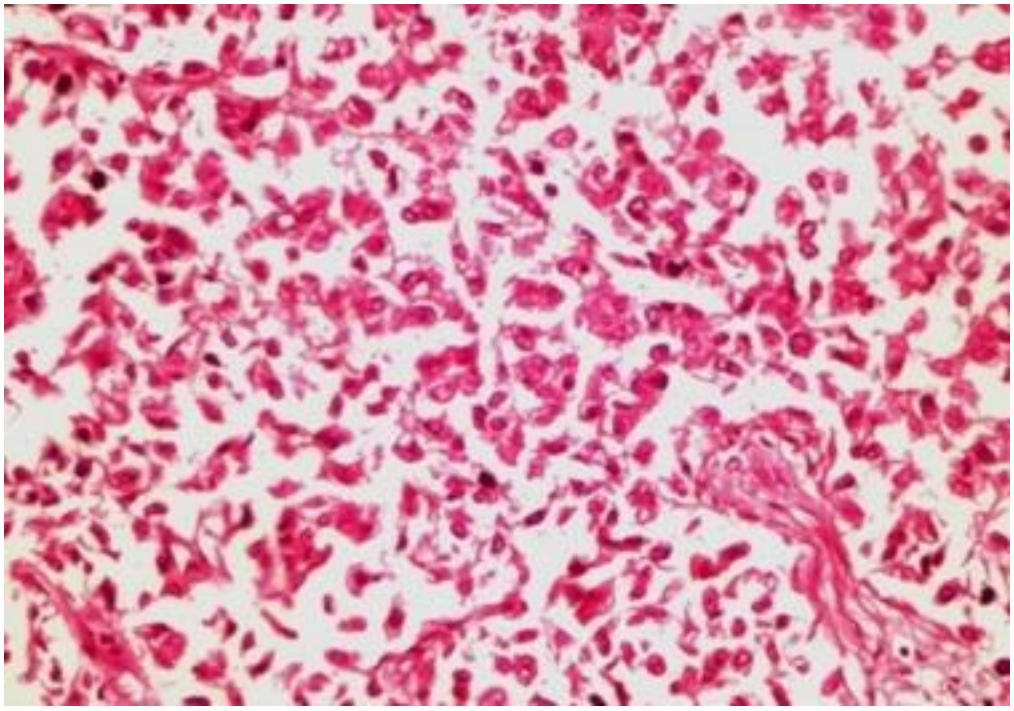

Figure 2: IDC-MODERATELY DIFFERENTIATED (GRADE- 2) H&E 40x

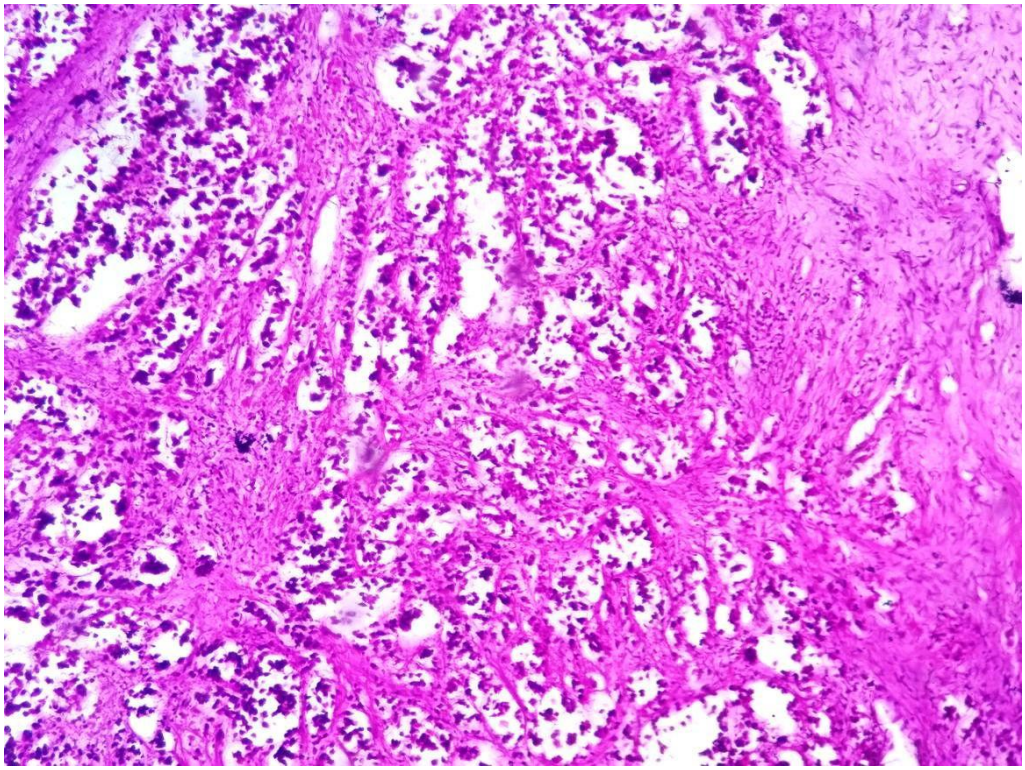

Figure 3: IDC-POORLY DIFFERENTIATED (GRADE-3) H&E 40x

Table No.3. Size Distribution of Tumour in Our Study sample

SIZE	NUMBER	PERCENT
2 x 3 cm	2	6.5%
3 x 4 cm	4	13.5%
4 x 5 cm	12	40%
5 x 6 cm	8	26.5%
6 x 7 cm	4	13.5%
TOTAL	30	100%

The tumour size distribution in the present sample is indicated in table No.3

Table 4: Distribution of Cases Acc. To Histological Grade by Bloom Richardson Scoring System

HISTOLOGICAL GRADE	NUMBER OF CASES	PERCENT
GRADE-I	08	26.5%
GRADE-II	16	53.5%
GRADE-III	06	20%
TOTAL CASES	30	100%

Majority of the patients are categorized as grade-II i.e. 53.5% of cases and next is grade-I tumours i.e. 26.5% cases.

Metastasis positive lymph nodes were present in 66.5% of cases; and were negative in 33.5% of cases.

Table No 5: Distribution of Lymph Node Metastasis In Study Subjects

Lymph Node spread	Number of cases	Percent
Reactive Lymph Nodes	10	33.5%
Metastatic Lymph Nodes	20	66.5%

Table No 6: Mean Relative quantification (RQ) Values for Il-10

	Neoplastic tissue	**Surrounding peritumoral tissue**
No of cases showing strong expression	**26 (86.5%)**	**02 (6.5%)**
No of cases showing weak expression	**04 (13.5%)**	**28 (93.5%)**

All grade 3 tumours expressed strong expression of IL-10 indicating that probably IL-10 expression is more in high grade tumours.

Discussion

We have conducted a study on 30 patients of clinically diagnosed breast cancer to determine the expression of interleukin-10 mRNA and, we have quantified it using RT-PCR analysis by determining the mean Relative quantification (RQ) value. Samples from all 30 cases were also subjected to histopathological examination to confirm the clinical diagnosis.

Most common age group of our study was 51-60years and 86% of subjects were of postmenopausal status. Histopathological examination revealed most of our tumours (53.5%) as grade 2 according to Bloom Richardson scoring system. Lymph node metastases were noted in 66.5% of cases.

86.5%(n=26) of tumour tissue samples showed strong expression of IL-10 whereas only 6.5% (n=2) of peritumoral tissue showed higher expression of IL-10. P value was derived from the student-t test and it was statistically significant (p value < 0.001). We did not correlate the IL-10 expression with other clinicopathological data like age, tumour size, tumour grade, lymph

nodal metastasis, menopausal status and hormonal status.

In a study by **E. Venetsanakos et al[13], 1997**, cytokine mRNA was detected by RT-PCR method in 26 breast tumour tissues and 11 normal breast tissues. 23 cases were histologically diagnosed as infiltrative ductal carcinoma while 3 cases were infiltrative lobular carcinoma. Heterogeneous mRNA profiles were observed within the tumours. A consistent feature was that, IL-10 mRNA was detected in over 50% of the tumours, IL-10 mRNA was detected in only two of the normal tissues. They concluded that IL-10 expression is associated with aggressively growing tumours and probably has an inhibitory role on T- cell function.

In **Chavey C. et al[14], 2007** study of 105 breast cancer and 13 healthy breast tissue samples, analysis was done by flow cytometry technology. Multiple cytokines were over expressed (IL-10, IL-6, IL-8, TNFα) in breast cancer tissue and none of the normal healthy tissue had IL10 expression. IL-10 was over expressed in ER-negative tumours compared with ER-positive ones indicating an association with high grade tumours. This study did not correlate IL-10 expression with age, tumour size, histological grade and lymph node status.

Hemanga Kumar Bhattacharjee et al[15], 2011 study was conducted on 60 breast cancer patients of which 54 had invasive ductal carcinoma and 6 patients had invasive lobular carcinoma. IL-10 expression was determined in tissue samples taken by immunohistochemistry. The results revealed IL-10 expression in 60% of tumour tissue samples while none of the peri-tumoral tissue showed IL-10 expression. Statistically significant correlation was observed with locally advanced disease, high tumour grade, receptor status (ER-, PR-, HER2+). Histological type showed no correlation with IL-10 levels, Thus high levels of IL-10 within the tumour tissue is a poor prognostic factor. This is augmented by few studies done in serum of breast cancer patients which also revealed high IL-10 levels.

Various other studies have been performed to detect single nucleotide polymorphisms in interleukin 10 gene promoter region. GCC haplotype or -1082AA and -592CA are associated with higher IL-10 expression in tumour tissue[16]. It is possible that genetic variation affects IL10 production and might determine the susceptibility to breast cancer. IL-10 promoter polymorphism was significantly associated with prognostic and predictive factors of breast cancer. Therefore, further studies on genetic polymorphisms may help us understand progression and evolution of breast cancer.

Conclusion

From this study, we conclude that IL-10 may serve as a useful biomarker with potential prognostic value as there is statistically significant association of high IL-10 mRNA levels and the breast tumour tissue when compared with peri-tumoral tissue.

Evidence from various studies suggest that IL-10 within tumour tissue has an important role in initiation and progression of breast carcinoma. This is also supported by high serum IL-10 levels noticed in such patients, although the mechanisms involved in the process are not exactly known.

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