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The Utility of Pro-inflammatory Cytokines-TNF Alpha and CRP as Indicators of Response to Chemotherapy in Patients with Breast Carcinoma

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Abstract

Objective: Breast cancer is the most commonly occurring female cancer in the world the incidence of which is more than double that of the second ranked cancer (cervical cancer). Not many studies have assessed the relationship between biomarkers of inflammation and long-term survival in breast cancer patients. The aim of this study was to determine whether circulating markers of inflammation (CRP and TNF-α), measured before and after chemotherapy, predict response to therapy.

Material: A total of 30 histological confirmed cases of breast cancer were enrolled for study. Total duration of study was two years. HsCRP was determined by solid phase direct sandwich ELISA method (Diaclore, France). Similarly TNF-α was also determined by Enzyme-linked immunosorbent method. Three-dimensional tumor size was determined radiologically through mammography. CT scan and MRI scan were taken at the time of diagnosis to detect metastasis.

Result: The mean values of hsCRP and TNF-α in patients of breast tumor decreased after three cycles of chemotherapy and this decrease was highly significant in patients with partial/complete response to chemotherapy. Similarly, levels of hsCRP and TNF-α were high in patients with estrogen receptor positive status than in estrogen receptor negative status. No significant correlation was observed between levels of hsCRP and TNF-α with progesterone receptor status and Her 2 neu status.

Conclusion: This study shows serum hsCRP and TNF-α levels were significantly elevated in confirmed cases of breast cancer and levels decreased after chemotherapy in patients showing response to it. So hsCRP and TNF-α can be used as a surrogate.

Keywords: TNF alpha; CRP; Breast cancer; Chemotherapy

Introduction

Breast cancer is the most commonly occurring female cancer in the world the incidence of which is more than double that of the second ranked cancer (cervical cancer). Breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths. It is the most frequent cancer in both developed and developing regions as well as the most frequent cause of cancer death in these regions of the world [1,2].

Although the incidence of breast cancers has increased globally over the last several decades [3]. The greatest increase has been in Asian countries. In Asia, breast cancer incidence peaks among women in their forties, whereas in the United States and Europe, it peaks among women in their sixties. In India premenopausal patients constituting about 50% of all patients. It is expected that in the coming decades, Asian countries would account for majority of new breast cancer patients diagnosed globally. Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India [4,5]. Breast cancer cases are expected to increase by 26% by 2020 and most of these will be seen in developing countries [6].

Chronic inflammation is a key contributor to cancer development and progression. Cancer survivors with chronic inflammation may have an elevated risk of recurrence as a result of the effects of inflammatory processes on cell growth or the presence of cancer cells that induce inflammation [7].

A pathogenic link has been identified between inflammatory mediators, inflammation related gene polymorphisms and carcinogenesis [8]. A substantial number of epidemiologic, gene association and molecular studies have now confirmed this important association. With better characterization of infiltrating immune cells, the precise role of inflammation in cancer has begun to be elucidated leading to a resolution of the initial contradiction that inflammation is protective in certain tumors [9,10], yet detrimental in others [11].

Overall, it appears that chronic inflammation more often stimulates then inhibits tumor development [12]. The persistence of chronic inflammation plays a critical role in initiating, sustaining and advancing tumor growth [13] and thus modulating the immune response may still be an alluring goal for therapeutic intervention [14].

The multifunctional cytokine, Tumour Necrosis Factor (TNF), is involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases [15]. Initially proposed to have anti-carcinogenic effects [16,17], TNF was later shown to be tumourigenic in both *in vitro* studies [18] and *in vivo* studies [19]. High plasma TNF levels

in cancer patients are associated with a poor disease outcome [20,21]. TNF is also a key angiogenic molecule that may promote angiogenesis directly by stimulating endothelial cell proliferation and indirectly by modulating expression of other proangiogenic factors [22]. Moreover, TNF is known to induce expression of adhesion molecules thought to be involved in the increased motility and invasive/metastatic behaviour of tumour cells [23].

TNF-alpha is also produced by tumors and can act as an endogenous tumor promoter. The role of TNF-alpha has been linked to all steps involved in tumor genesis including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis and metastasis [24].

High sensitivity C-reactive protein (hsCRP) is an acute phase protein found in the blood, the levels of which rise in response to inflammation [25]. It is a member of the pentraxin family of proteins [26]. The acute phase response develops in a wide range of acute and chronic inflammatory conditions. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. Rapid, marked increases in CRP occur with inflammation, infection, trauma and tissue necrosis, malignancies, and autoimmune disorders [26].

CRP is a measure of low-grade chronic inflammation and potential predictor of cancer risk and/or survival. Elevated CRP has been associated with poor survival in metastatic prostate [27]. (CRP measured during treatment), gastro esophageal [28] (measured before resection), colorectal (measured before and after resection) [29,30], inoperable non–small-cell lung [31] (measured before treatment) and pancreatic (measured at diagnosis) cancers [32].

Up to date no many studied have assessed the relationship between

biomarkers of inflammation and long-term survival in breast cancer patients. The aim of this study was to determine whether circulating markers of inflammation (CRP and TNF-α), measured before and after chemotherapy, predict response to therapy.

Objective

1. To evaluate the value of measuring inflammatory cytokines in prediction in response to therapy.

2. To measure the level of TNF Alpha and CRP in patients with breast cancer before and after treatment.

3. To correlate the level of TNF Alpha and CRP with the response to chemotherapy.

Material and Method

The present study was conducted in the department of Biochemistry, G.B Pant hospital in collaboration with department of surgery, Maulana Azad Medical College.

A total of 30 histological confirmed cases of breast cancer were enrolled for study. Total duration of study was two years. The study was commenced after institutional ethical clearance. An informed consent was taken from all the patients. Initial sample was collected before starting chemotherapy and final sample was collected after two weeks of three cycles of chemotherapy from antecubital vein under all aseptic conditions. Serum was separated by centrifugation and the sample was analyzed on the same day (and stored at -20ºC if storage was required for more than 1 day).

hsCRP was determined by solid phase direct sandwich ELISA method (Diaclore, France). Similarly TNF-α was determined by Enzyme-linked immunosorbent method. Three-dimensional tumour size was determined radiologically through mammography. CT scan and MRI scan were taken at the time of diagnosis to detect metastasis.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20 for windows. Values shown in the text, tables and figures are Mean ± SD. Student t test were applied for comparison of means of study groups. P value < 0.05 were considered significant(S) and P value <0.001 were considered highly significant (HS). Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

Result

Biochemical parameters of patients are given in Table 1 and Figure 1. The mean values of hsCRP in patients of breast tumor before chemotherapy were 283.7 ± 56.0 ng/ml which decreased to 258.0 ± 62 ng/ml after 3 cycles of chemotherapy. The difference was significant (p<0.05). Levels of TNF-α level before chemotherapy were 181.7 ± 56 pg/ml which after three cycles of chemotherapy decreased to 147.0 ± 57 pg/ml and the difference was highly significant (p<0.001).

As shown in Table 2 and Figure 2, the levels of hsCRP before chemotherapy was 263 ± 37 ng/ml which decreased to 224 ± 57 ng/

	Pre chemotherapy	Post chemotherapy (3 Cycles)	P value*	Significance
TNF-α(pg /ml)	181.7 ± 56	147.0 ± 57	0.001	HS
hsCRP (ng/ml)	283.7 ± 56.0	258.0 ± 62	0.012	S

HS: Highly Significant P Value
S: Significant P Value

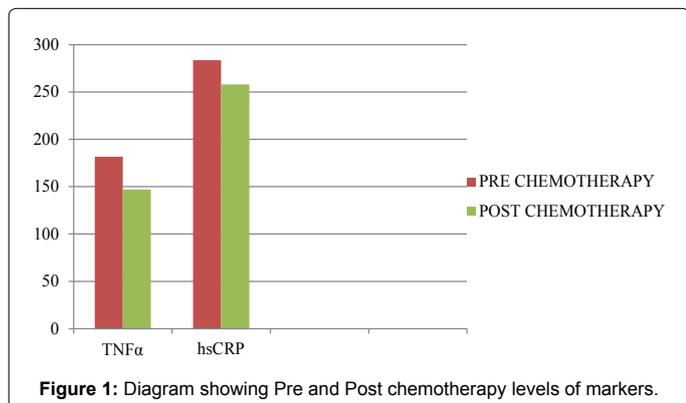**Table 1:** Biochemical parameters in patients of Breast tumor.

Figure 1: Diagram showing Pre and Post chemotherapy levels of markers.

	Partial/complete response (n=19)	P value		Stable/progressive disease (n=11)	P value	
TNF-α (Pre chemotherapy)	161 ± 44	0.00	HS	216 ± 59	0.58	NS
TNF-α (Post chemotherapy)	113 ± 27			204 ± 66		
hsCRP (Pre chemotherapy)	263 ± 37	0.00	HS	318 ± 47	00.8	NS
hsCRP (Post chemotherapy)	224 ± 57			315 ± 69		

Table 2: Pre and post chemotherapy levels of markers in patients with complete response and stable/progressive disease.

ml after chemotherapy in patients with partial/complete response. It clearly shows that after treatment mean values decreased significantly and were found to be statistically highly significant (p<0.001). TNF α levels before chemotherapy was 318 ± 47 ng/dl which decreased to 315 ± 69 ng/dl in patients with stable/progressive disease and this decrease was not found to be statistically significant (p<0.8).

Similarly, level of TNF-α done before chemotherapy was 161 ± 44 pg/dl which decreased to 113 ± 27 pg/dl after giving chemotherapy in patients with partial/complete response. The difference was highly significant (p<0.001). In patients with stable/progressive disease levels of TNF-α before chemotherapy was 216 ± 59 pg/dl which decreased to 204 ± 66 pg/dl and this decrease was found to be non-significant (p<0.58).

Levels of hsCRP were 314 ± 56 ng/dl in patients with estrogen receptor positive status and 270 ± 39 ng/dl in estrogen receptor negative status. The difference was significant with p value<0.05. Similarly levels of TNF-α was 212 ± 63 pg/dl in patient with estrogen receptor positive status and 128 ± 47 pg/dl in estrogen receptor negative status. The difference was significant with p value<0.05. No significant correlation

was observed between levels of hsCRP and TNF-α with progesterone receptor status and Her 2 neu status (Table 3 and Figure 3).

Discussion

Chronic inflammation represents a major risk factor for many cancer types, including liver, breast, prostate, pancreas, ovary, skin, gastric, colorectal and pulmonary carcinomas [33]. The raised levels of inflammatory cytokines like TNF-α and hs CRP before chemotherapy indicates that cytokines play a key role in linking chronic inflammation and cancer. They are activated by both inflammatory and tumor cells and their effect is similarly important for both sustaining chronic inflammation, promoting progression of tumor cells and micro environment proliferation and inhibiting immune mediated tumor surveillance. In general, cytokines can be divided into those that are pro-inflammatory (i.e., IL-4,10, IFNα, TNFα and MIF) and anti-inflammatory (i.e.,IL-4,10, IFNα and β); however, it has become clear that many of these molecules can have dual roles [8].

Chronic inflammation not only alters the tumor microenvironment by soluble mediators but also through recruitment of cells that differentiate into the tumor and its microenvironment. Recent studies has shown that tumor genesis is characterized by important differences in the genetic and epigenetic transformation of epithelium, stroma, vascular structures and immune cells. It has also been observed that when comparing each cellular component to its normal counterpart a large number of genes showing expression differences are soluble mediators or their receptors. Thus, inflammation may not only be important in the initiation of DNA damage but through the increased release of cytokines, reactive oxygen species and relative hypoxia, may also lead to a viscous cycle of increasing epigenetic alterations [34].

The hypothesis that TNF, produced in the tumor microenvironment, may promote cancer development and dissemination is supported by evidence from a range of animal experiments [24]. Ovarian cancer xenografts treated with TNF showed evidence of increased peritoneal adhesion and solid tumor formation and over expression of TNF confers metastatic properties on transplantable tumors in nude mice [35,36].

Numerous studies have linked TNF-α to breast cancer progression. As a result, the mechanisms by which TNF-α promotes breast cancer have been recently explored using both *in vitro* and *in vivo* models. Similarly a prospective study was conducted on forty patients with invasive breast cancer and their serum concentration of TNF-α were found to be elevated. Ren-Nan Feng et al. studied the role of TNF-α polymorphism and its levels in breast cancer [37,38]. Number of studies have explores the role of TNF-α in relation to an increased risk for cancer. Among them, most case-control studies have shown a higher cancer risk in people with elevated TNF-α levels.

C-reactive protein (CRP) is a nonspecific marker of systemic inflammation. Most studies suggested that CRP levels were higher in cancer cases than healthy subjects, and CRP levels for prediction of
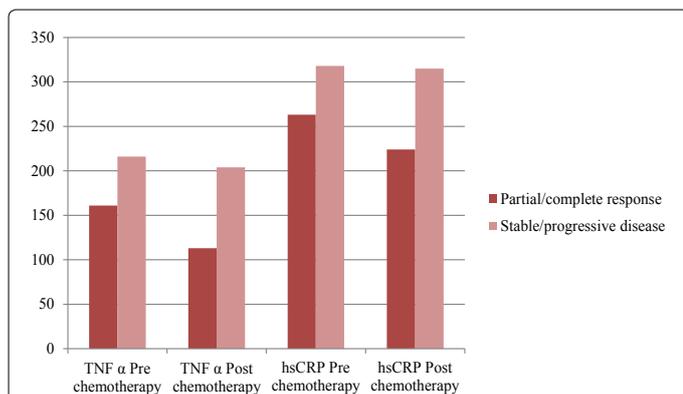

Figure 2: Diagram showing Pre and post chemotherapy levels of markers in patients with complete response and stable/progressive disease.
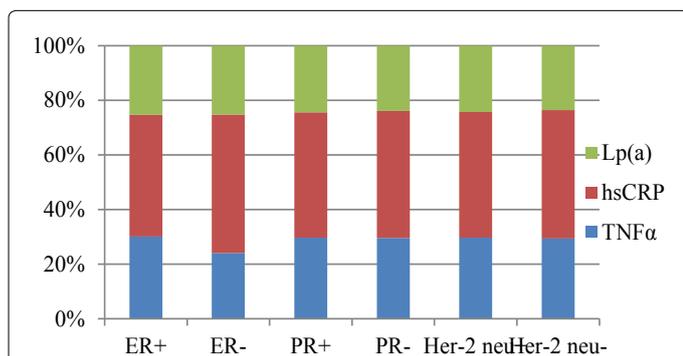

Figure 3: Diagram showing Variation in markers with receptor status.

Markers			Receptor status					
	Estrogen receptor status			Progesterone receptor status			Her-2 neu status		
	ER+(9)	ER-(21)	P value	PR+(9)	PR-(21)	P value	Her-2 neu +(21)	Her-2 neu-(9)	P value
TNF-α	212 ± 63	128 ± 47	0.049	188 ± 66	178 ± 49	0.65	186 ± 55	171 ± 60	0.5
hsCRP	314 ± 56	270 ± 39	0.04	290 ± 37	280 ± 29	0.8	288 ± 53	273 ± 62	0.6

Table 3: Variation in markers with receptor status.

treatment efficacy and patients mortality with various types of cancer have been extensively reported. Serum hs-CRP was positively associated with the risk of cancer. The results also support the hypothesis that chronic inflammation plays a role in cancer [39].

Breast cancer patients have elevated concentrations of CRP before surgery, more so in women with advanced disease [40] suggesting that CRP may be related to tumor burden or progression. Elevated pretreatment CRP and low albumin were associated with decreased cancer-specific survival in a study of 96 breast cancer patients presenting with metastatic relapse [41]. Similarly, in a study of 85 metastatic breast cancer patients, elevated pretreatment CRP was associated with decreased survival [42]. However, in a study of 300 patients with invasive primary operable breast cancer, CRP was not associated with survival [43]. CRP is also a risk factor for cardiovascular disease, which is more common among breast cancer patients after radiation treatment [44].

A Meta-analysis of Prospective Cohort Studies was conducted to study association Between C - reactive protein and Risk of Cancer by Yong-Zhong Guo et al., which showed that the elevated levels of CRP are associated with an increased risk of all-cancer, lung cancer, and possibly breast, prostate and colorectal cancer. The result supports a role of chronic inflammation in carcinogenesis [45].

Prognosis

Inflammatory status may also be a prognostic factor for breast cancer. Clinical and experimental data suggest that chronic inflammation promotes mammary tumor development through mechanisms involving chronic activation of humoral immunity and infiltration of Th2 cells and polarized innate inflammatory cells. Breast cancer patients have elevated concentrations of inflammatory cytokines before surgery, more so in women with advanced disease suggesting that levels of inflammatory cytokines may be related to tumor burden or progression [46,47].

Conclusion

This study shows serum hsCRP and TNF-α levels were significantly elevated in confirmed cases of breast cancer and levels decreased after chemotherapy in patients showing response to it. So hsCRP and TNF-α can be used as a surrogate marker for determining disease progress or efficacy of treatment. It is clear that inflammation may contribute to the genetic and epigenetic changes that can be observed in cells of the tumor microenvironment and this molecular link between inflammation and carcinogenesis.

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