

# Cathepsin D: A Marker of Metastasis in Breast Cancerous Tissue

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## ABSTRACT

The level of cathepsin D and other blood parameters in 50 metastatic breast cancer patients before any treatment or surgery were evaluated for metastasis. It was found that the total leukocyte counts, platelet, red blood cells and neutrophil counts and level of haemoglobin were significantly decreased in patients as compared to control subjects. The lymphocytes, monocyte and eosinophil counts were also found decreased in patients as compared to their controls. The level of cathepsin D was markedly increased in the patients confirming biopsy observations of metastasis in breast cancers. It is concluded that there is an association of cathepsin D with tumor invasion and metastasis.

**Key Words:** Cathepsin D; Hematology; Metastasis; Breast

## INTRODUCTION

Metastasis may be present in more than 70% of patients at the time of their initial diagnosis (Sugarbaker, 1981). There is an increasing evidence for the role of cysteine proteases cathepsin B, D, L and S in cancer progression. Cathepsin D, an aspartyl protease, is a lysosomal acid protease. It has documented mitogenic activity on estrogen depleted MCF-7 human adenocarcinoma cells in culture, and its secretion is constitutive in hormone induced breast cancer cell lines (Rochefort *et al.*, 1990). It was reported that cathepsin D over expression may stimulate cell proliferation of micrometastasis in nude mice by inactivating a secreted growth factor (Liaudet *et al.*, 1995). In contrast, *in vitro* studies have shown no correlation of cathepsin D levels and invasive behavior in MCF-7 cell line subclones expressing different level of the protease (Johnson *et al.*, 1993). Cathepsin D in erythrocytes was found to be localized mainly in membrane (Leto *et al.*, 1992). The reported study is to evaluate the level of cathepsin D and other blood parameters in metastatic breast cancer patient before any treatment or surgery.

## MATERIALS AND METHODS

Fifty patients with verified breast neoplastic disease (by needle biopsy) and twenty-five age and sex matched control with no history of breast cancer or with any disease participated in the investigation. The patients were selected from those admitted to cancer ward or those attending as out patients of Oncology Department, Sir Ganga Ram Hospital, Lahore.

**Biochemical parameters.** Blood samples were taken in fasting state. The serum was kept at  $-20^{\circ}\text{C}$  until use. Concentration of blood hemoglobin, platelets, total lymphocyte and eosinophil count (TLC), red blood cell count, polymorph neutrophil, lymphocytes, monocyte and eosinophil were determined by standard automated method

using Autoanalyzer (Hitachi 705, 1986). Cathepsin D was analysed using hemoglobin as substrate (Maciewicz & Ethrington, 1986).

**Statistics.** In statistical analysis, significant control test (t-test) of the difference between average values was used. P values  $<0.01$  were taken as significant difference (Steel & Torrie, 1984).

## RESULTS AND DISCUSSION

The level of hemoglobin reported in Table I have been found significantly decreased in patients (10.22 g/d) in comparison to the control subjects (12.83 g/d). This trend has been reported in earlier studies explaining that the late development of anemia in neoplastic state is a common part of the clinical pattern. It has been reported that if the supply of nutrients to the marrow is insufficient because of inability of the cells of the marrow to compete for either small or large precursor of protein, the bio-synthesis of hemoglobin and stored proteins of RBC can be suppressed resulting in anemia (Radock *et al.*, 1971). It is evident from Table I that platelets count was decreased in patients (177.60/cm) in comparison to the control subjects (253.2/cm) but the difference was non significant statistically. Similarly, the level of TLC counts and red blood cell counts was lower in patients ( $4.450 \times 10^3$  and  $3.40 \times 10^6$ ) as compared with controls ( $6.25 \times 10^3$  and  $4.0 \times 10^6$ ). Riott *et al.* (1993) stated that homeostatic mechanisms are significantly altered in malignant patients. They observed that about 50% of all patients and 95% of those with metastatic disease showed abnormality of homeostatic parameters such as abnormal activator of coagulation pathway, platelet aggregation and activation etc.

The differential counts have also been performed on both patients and control subjects and reported in Table I. It has been observed that although the level of polymorphs decreased in patients as compared to the control subjects but showed no significant difference. On the other hand, the

**Table I. Variation of parameters in breast cancer patients and control subjects**

Parameters	Patients (n=50)	Control subjects (n=25)	P value
Hemoglobin (g/dL)	10.22+ 0.58*	12.38+0.42	P<0.001
Platelets count (cm <sup>-1</sup> )	177.60+ 28.80	235.20+13.08	P<0.001
Total leukocyte count (cm <sup>-1</sup> )	4450+ 588.33	6250+500.20	P<0.01
Red blood cell count (cm <sup>-1</sup> )	3.40+ 0.01x10	4.00+02x10	P<0.001
Polymerph neutrophil	52.50+ 4.55	60+4.00	N.S.
Lymphocytes (5%)	35.83+ 3.35	30+ 340	N.S.
Monocytes (%)	6.00+ 1.97	5.00+1.84	N.S.
Eosinophils (%)	6.00+ 1.19	2.00+ 1.87	N.S.
Cathepsin D (Units)	8.58+ 0.75	2.90+ 0.21	P<0.001

\* Values left to right are the + sign are mean and S.E., respectively; P< -0.001 = Highly significant difference; P< -0.01 = Significant difference; N.S.= Non significant difference

percentage of lymphocytes, monocytes and eosinophil were decreased in the patients as compared to the subject controls but no significant difference was observed. The alteration of leukocyte counts and functions have been reported in a wide variety of hematologic infections, inflammatory metabolic and neoplastic disease. The present study indicated that TLC counts were decreased in patients as compared to the controls. Earlier workers reported that in the patients suffering with infections and inflammatory diseases, the leukocyte counts usually serve as a useful guide to the severity of the disease process (Maciewiaz & Ethrington, 1988). Percentage of neutrophils was also found decreased in the patients in comparison to the controls. It can be explained that neutrophils help to maintain the normal defensive barrier to microbial invasion and their decrease results in an increase in risk of infection (Dale, 1999). In the present study, an increase in the lymphocytes and eosinophil counts in the patients as compared to the controls was found. These observations are in agreement to the findings of Dale (1999), who reported an increase in lymphocyte, monocyte and eosinophil counts in certain infections such as lymphocytosis and in malignancy. The chief functions of lymphocytes are production of immunoglobins and expression of cellular immunity. On the other hand, monocytes are phagocytic cells with bactericidal capacities. The lymphocytes possess microbial potencies too and Eosinophils are irregularly present in allergic, collagen vascular disease and malignancies. The level of cathepsin D was increased significantly in breast cancer patients as compared to the control subjects (8.58 vs 2.90). These findings support the earlier work that cathepsin D over expression may facilitate the growth of tumor cells in secondary sites through the inactivation of growth factors inhibition suggesting further investigations to define the exact involvement of the process (Nand, 1963; Jiang *et al.*, 1994; Garcia *et al.*, 1996).

## CONCLUSION

It is concluded that there is an association of Cathepsin D with human invasion and metastasis. Further

research is needed to find out the effect of chemotherapy on Cathepsin D.

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