ORIGINAL ARTICLE

Role of Cathepsin D in Monitoring Breast Cancer Treatment Using Doxorubicin in Combinations with Cyclophosphamide and 5-Fluorouracil

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ABSTRACT

Background: Over-expression of cathepsin D by reactive stromal cells leads to an adverse clinical course in breast cancer. Its elevated level in breast cancer tissue is highly significant indicator of the potential for recurrence.

Aim: To find out the role of Cathepsin D in monitoring breast cancer using Doxorubicin in combinations with Cyclophosphamide and 5-fluorouracil (FAC).

Methods: Fifty female patients with breast cancer visited department of Oncology, Sir Ganga Ram Hospital and INMOL Hospital Lahore were selected in this study. Women with estrogen receptor positive breast cancer stage II and III and not received any radiotherapy as yet were included in the study. Estrogen receptor negative patients and Patients who have already received radiotherapy were excluded from the study. Patients are divided into two groups. Group –A including 50 Patients receiving Inj 5flourouracil 500mg/ m^2 I/V Day 1 and Day 8. Inj Doxorubicin 50 mg/m^2 I/V Day1. Inj Cyclophosphamide 500mg/m^2 I/V Day1. Course is to be repeated every 21 days x 6 courses. A detailed proforma regarding brief history and examination was filled. In Group B, 20 normal subjects (controls) with no history of any disease were taken. Out of 50 females, 12 were menstruating, 18 were peri-menopausal and 20 were post menopausal. Level of cathepsin D was estimated in three stages. Before starting chemotherapy, in the middle of chemotherapy and at the end of chemotherapy. Cathepsin D was assessed by a standard kit method ("BioVision" company).

Results: Level of cathepsin D in breast cancer women with different menstrual status was increased as compared to their controls. This showed highly significant difference ($P<0.001$). It was observed that the level of cathepsin D was high in menstruating women as compared to perimenopausal and postmenopausal women. In mid of treatment in women with different menstrual status, the levels of cathepsin D were significantly decreased ($P<0.001$) when compared with initial levels. At the end of treatment levels of cathepsin D were significantly decreased ($P<0.001$) when compared with initial and mid levels of cathepsin D. We observed that the reduction in the level of cathepsin D in menstruating women was 80.28% and at the end of treatment the reduction was 60.09%.

Conclusion: It is concluded that cathepsin D is a good prognostic marker in receptor positive breast cancer women treated with FAC. However, there is a need of more efficacious adjuvant treatments for declining breast cancer mortality especially in developing countries.

Keywords: Breast cancer, Cathepsin D, FAC treatment.

INTRODUCTION

Breast cancer is the most dreaded cancer among women due to its frequency and psychological impacts. This corresponds to a lifetime risk for a woman of about 6.2% in developed countries and 2.2% in developing countries[1]. There has been an increase in the incidence of breast cancer in developing countries[2]. In Pakistan, nearly one out of five females has a risk of developing cancer of breast[3]. The epidemiology of Breast Cancer in Pakistan is difficult to depict mainly due to a lack of tumor registry system in Pakistan[4].

Several factors are currently employed for prognosis, assessment and determination in breast cancer. It has been proposed that proteases secreted by cancer cells facilitate metastasis by degrading extra-cellular matrix[5]. Cathepsins, the lysosomal proteases have received more attention. Since the elevated expressions of Cathepsins and diminished levels of their inhibitors have been observed especially in aggressive cancer cells. Cathepsins have been suggested to be biological markers of malignant tumors and are useful for prognosis of the disease[6].

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Over-expression of cathepsin D by reactive stromal cells leads to an adverse clinical course in breast cancer[7]. Cathepsin D over-expression stimulates tumor formation and metastasis. Indeed, it plays an essential role in the different steps of tumor progression, in stimulating cancer cell proliferation, fibroblast outgrowth and angiogenesis, as well as in inhibiting tumor apoptosis[8]. However the general consensus is that the elevated concentrations of Cathepsin D in breast cancer tissue are highly significant indicators of the potential for recurrence[9].

Breast cancer is usually treated with surgery and then possibly with chemotherapy or radiation, or both. The cancer's stage, menopausal status, hormone-receptor status, HER2 status, and lymph node status will influence the chemotherapy regimen. Use of combination therapy is benefited as it has no overlapping of side effects[10,11].

Treatments are given with increasing aggressiveness according to the prognosis and risk of occurrence. Prognosis is important for treatment decisions because patients with a good prognosis are usually offered less invasive treatments, such as lumpectomy and radiation or hormone therapy, while patients with poor prognosis are usually offered more aggressive treatment, such as more extensive mastectomy and one or more chemotherapy drugs[12]. Prognostic factors are reflected in the classification scheme for breast cancer including stage, grade, recurrence of the disease, and the age and health of the patient[13].

Many combinations with endocrine therapy have been employed. Combination therapy with 5-fluorouracil (5-FU), Doxorubicin (Adriamycin) and Cyclophosphamide (Cytoxan) or FAC is one of the standard chemotherapies for advanced and recurrent breast cancer. Doxorubicin slows or stops the growth of cancer cells by preventing cell replication and protein synthesis[14]. Cyclophosphamide forms DNA crosslinks at guanine N-7 positions due to its metabolite phosphoramide mustard. This may lead to cell death[15]. 5-Fluorouracil is pyrimidine analog. It inhibits DNA synthesis by blocking the conversion of Dexoyuridilic acid to Deoxythymidylic acid. Thus DNA cannot be synthesized[16].

Over the past few years, particular attention has been paid to the fact that the high activity of Cathepsin D is connected with the increased cancer invasiveness. Adjuvant chemotherapy has established its beneficial effect in prolonging the disease-free interval and survival of patients with stage II and III breast cancer.

Present study was therefore tried to find out the role of Cathepsin D in monitoring breast cancer using Doxorubicin in combinations with Cyclophosphamide and 5-fluorouracil (FAC)

MATERIALS & METHODS

Fifty female patients with breast cancer visited department of Oncology ,Sir Ganga Ram Hospital and INMOL Hospital Lahore were included in this study. Women with estrogen receptor positive breast cancer stage II and III, not receiving any radiotherapy as yet were included in the study. Estrogen receptor negative patients and patients who have already received radiotherapy were excluded from the study.

Patients are divided into two groups. Group –A including 50 Patients receiving Inj 5flourouracil 500mg/ m^2 I/v Day 1 and Day 8. Inj Doxorubicin 50 mg/m^2 I/V Day1. Inj Cyclophosphamide 500mg/m^2 I/V Day1. Course of injection be repeated every 21 days x 6 courses. A detailed proforma regarding brief history and examination was filled. Group B were included age matched 20 normal subjects (controls) with no history of any disease. Out of 50 females, 12 were menstruating, 18 were peri-menopausal and 20 were post menopausal.

Five ml of blood was drawn from patients in three stages. Before starting chemotherapy first sample was taken, second sample was taken in the middle of chemotherapy i.e. after 3rd course and third blood sample at the end of chemotherapy i.e. after 6th course. Cathepsin D was assessed by a standard kit method ("BioVision" company).

Statistical Analysis: Data was analyzed by SPSS 18.0. Variables were expressed as mean±SD. Variables were compared by using Student 't' test. $P<0.05$ was considered as significance.

RESULTS

Level of Cathepsin D in breast cancer women and their comparison to controls with different menstrual status is tabulated as Table 1. It was observed that the level of cathepsin D in breast cancer women with different menstrual status was increased as compared to their controls. This showed highly significant different ($P<0.001$). It was observed that the level of cathepsin D was high in menstruating women as compared to perimenopausal and postmenopausal women.

Distribution of Cathepsin D levels among breast cancer women treated with FAC as per their menstruating status is tabulated as Table 2. In mid of treatment of menstruating women, the levels of cathepsin D were significantly decreased ($P<0.001$) when compared with initial levels. At the end of treatment levels of cathepsin D was significantly decreased ($P<0.001$) when compared with initial and mid levels of cathepsin D.

It was observed that in the mid treatment of menstruating women with combination chemotherapy

FAC the reduction in the level of cathepsin D was 80.28% and in the end of treatment the reduction was 60.09%. On the other hand in the mid treatment of perimenopausal women with combination chemotherapy FAC the reduction in the level of cathepsin D was 74.95% and in the end of treatment the reduction was 40%. In the mid treatment with combination therapy FAC of postmenopausal women, the reduction in the level of cathepsin D was 68.95% and in the end of treatment the reduction was 45% (Table 3).

Table 1: Level of Cathepsin D in breast cancer women and their comparison with controls with different menstrual status

Subjects	Cathepsin D
Menstruating women	14.7±3.0**
Peri menopausal women	9.9±0.8**
Post menopausal women	10.3±2.1**
Menstruating controls	2.3±0.6
Peri menopausal controls	2.5±0.4
Post menopausal controls	2.7±0.4

**P<0.001= Highly significant difference

Table-2: Variation in the level of Cathepsin D levels among women treated with FAC as per their menstruating status

Patients	Level of cathepsin D Before treatment	Level of cathepsin D in Mid of treatment	Level of cathepsin D at the end of treatment	Total reduction of cathepsin D in %
Menstruating women	14.7±3.0	11.9±3.2	8.8±2.1**	20%
Perimenopausal women	9.9±0.8	7.4±1.3	4.0±1.3**	35%
Postmenopausal women	10.3±2.1	7.0±1.3	4.6±1.3**	24%

**P<0.001= Highly significant difference.

Table 3: Comparison of % Cathepsin D levels of baseline for 3 menstruating statuses at mid treatment & end treatment time

		Group	FAC
		Mean	S.D. ±
Menstruating women	Mid treatment	80.28	8.43
	End treatment	60.09	12.26
Perimenopausal women	Mid treatment	74.95	10.04
	End treatment	40.00	10.91
Post menopausal women	Mid treatment	68.95	9.30
	End treatment	45.95	16.63

DISCUSSION

Ongoing investigation of combination chemotherapy should continue in the metastatic setting for many reasons that is to facilitate treatment, to develop combination chemotherapeutic regimens that improve efficacy with the goal of applying these regimens in the treatment of early stage disease and goal of enhancing the efficacy of conventional chemotherapy and applying such regimens to earlier stage disease[17,18]. Fluorouracil, Doxorubicin, and Cyclophosphamide (FAC) have been used in combination for node positive breast cancer patients[19].

It was observed that the level of cathepsin D in breast cancer women with different menstrual status was significantly increased (P<0.001) as compared to their normal controls. Our study is in accord with a study who found a modulatory role of cathepsin D in cell response in breast cancer treatment. Their study along with other studies reported that cathepsin D acts as key mediator of apoptosis induced by various chemotherapeutics[20]. According to a study increased cathepsin D expression correlates with more aggressive tumors and poorer prognosis for patients. A study reported that reduced level of Cathepsin D reduction with FAC chemotherapy regimen result in significant tumor regression and increased overall survival[21].

Present study was observed that the level of cathepsin D was high in menstruating women as compared to peri and postmenopausal women. This is consistent with a study showing that cathepsin D level is higher in menstruating patients than in postmenopausal patients[22]. These values are high because cathepsin D is an estrogen induced lysosomal protease which is produced and secreted in excess by the breast cancer cells as compared with normal mammary cells[23]. However according to a study there is no differences was observed in level of cathepsin D between the different phases of the menstrual cycle[24].

According to our study, with the chemotherapy based on FAC treatment it was observed that the level of cathepsin was progressively decreased from start of treatment to end of treatment. We found that

there is a significant difference in cathepsin D level of all the treatment times. Our study is in line with a study who reported that reduced level of cathepsin D at different time of treatment showed the effectiveness of combination therapy of FAC. Study reported that decreased expression of cathepsin D with chemotherapy regimen result in significant tumor regression and increased overall survival[25,21].

We observed that among three status of women the good response of treatment was observed in the sequence of perimenopausal, post menstruating and menstruating women (35%, 24%, 20%). According to a study, there is higher response rate and longer time to progression for the anthracycline-containing regimens[26]. However according to a study there is sufficient effect of chemotherapy in ER/PR-positive patients in premenopausal age groups[27].

CONCLUSION

It is concluded that cathepsin D is a good prognostic marker in receptor positive breast cancer women treated with FAC. However, there is a need of more efficacious adjuvant treatments for declining breast cancer mortality especially in developing countries.

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