

# Evaluation of the Hemogram of Breast Cancer Patients Treated by Therapeutic Protocol Based on Immunohistochemical Analysis: A Retrospective Study

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## Abstract

To gauge the pervasiveness, verdict, and treatment protocols related to hormone receptor status effecting blood chemistry in breast cancer patients, a retrospective study was conducted at the Bahawalpur Institute of Nuclear and Oncology (BINO), Pakistan. 180 breast cancer patients were enrolled in the study on the basis of data availability. Data was collected about patient's demographics, site, stage and grade of tumour; hormonal status; treatment strategy; Estrogen (ER), Progesterone (PR) and Human Epidermal growth factor receptor 2 (Her-2/Neu) receptors; Blood chemistry reports including TLC (Total Leukocyte Count), TRC (Total RBC Count), Haemoglobin, Platelets and Creatinine; and ADRs due to chemotherapy. SPSS version 20 was used for statistical analysis of the data. Majority of patients become victim of breast cancer were belonging to age group of 41 to 60 years and half patients had carcinoma of the right breast. Results predict that females present at Stage III was 57%. Post-menopausal women were highly susceptible to disease (63.97%) than pre-menopausal (36.03%). ER/PR positive status was in 50% patients while 23% patients had triple positive status. Chemotherapy was prescribed for hormone negative status patients whereas hormone therapy was preferred for hormone responsible tumours. Her-2 positive status patients were given monoclonal antibody therapy. Treatment strategies directly affected the hemogram of patients while remain un-affected in some patients. Slight decline was observed in TLC, TRC, haemoglobin and platelet count that caused anaemia, poor immunity, anorexia, weight loss, neutropenia and thrombocytopenia whereas elevation in creatinine level resulted in nephrotoxicity. Patients reported with Adverse Drug Reactions i.e. pain, fever, vomiting, hair loss, anorexia and lethargy were counselled for the life style modifications with special emphasis on dietary recommendations for combating the problems. Breast cancer therapy caused disruption of the normal hemogram values and resulted in bone marrow suppression that was evident from side effects appearance in patients. Nutritional counseling of this fatal disease is recommended for improving their quality of life.

**Keywords:** Blood chemistry; Chemotherapy; Estrogens; Premenopausal; Hemogram; Immunohistochemical analysis; Thrombocytopenia

## Introduction

Cancer is potentially fatal group of diseases results because of uncontrolled proliferation of abnormal cells in certain tissues of human body. This prevailing theory underpins that some critically regulating proteins of cell genes get mutated probably by the external factors, ultimately this aggressive cell behavior leads to death [1]. Worldwide, cancer became cause of death of 7.6 million people out of approximately 12.7 million diagnosed in 2008. Mortality rate due to cancer estimated is approx 13% of all deaths each year, which is believed to continue rising with an estimated 11.5 million deaths in 2030 [2]. In 2011, 30% of all new cancer cases in women was Breast cancer which is the most common type of cancer diagnosed in the UK, accounting for in 2011 and considered heterogeneous disease increasingly [3]. In Pakistan, Punjab Cancer Registry reported 11,046 malignancies between 2008 and 2010. Collective cancer registry report depicts that breast cancer count 12,886 which is 21% of all the registered cases in Pakistan 1994 to 2013. Moreover; it is estimated that approximately 75% cancer cases will be prevalent among developing countries by 2020 [4].

Despite availability of multiple treatment choices of cancer like antiangiogenic targets, genetic pathways, cancer stem cells, chemotherapy, immunotherapy and much more, still there is

continuous rise in the magnitude as well as mortality rate of cancer. 100 different types of cancer are classified according to the initially affected type of cell. Malignant breast neoplasm, other name of breast cancer, originated either from milk ducts inner linings or the lobules that are responsible for supplying the milk to these ducts. Ductal and lobular carcinomas are major types of this cancer [5]. Spectrum of abnormal changes in the cells lining of the breast milk ducts is Ductal carcinoma in situ (DCIS), invasion that intrudes upon and destroys adjacent tissues & may spread via lymphatic system or blood stream to other parts of the body. Yet the growth of abnormal cells not reach beyond the layer of cells of their origination, DCIS is considered a noninvasive

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form of carcinoma while Lobular carcinoma in situ (LCIS, also known as lobular neoplasia) is not a true cancer, but indicate high prevalence of invasive cancer. According to the data of 2006-2010, DCIS accounts 83% as compared to LCIS which account 12% of diagnosed insitu breast cancers [2].

Average diagnostic age for breast cancer predicted is 63 years in the United States and Western Europe while in Iran its 51 years [6]. Studies revealed that patients in young age become victim of more advance and aggressive breast cancer than older one [7-11]. Comparison of all aged group women on the basis of stage of disease, surgery adopted, histopathologic type rate of survival can easily evaluated from The National Cancer Institute's Surveillance, Epidemiology, and End Results Program. Elderly women present distant disease initially [12]. Younger breast cancer patients show significantly higher grade of disease with negative hormone receptor status, greater invasive extent to other parts of body and HER-2 amplification than older breast neoplasms patients [13].

For the diagnosis of breast cancer, pathological examination considered as the gold standard still considering estrogen, progesterone receptor and Her2/neu expression essential component to decide the best suitable treatment [14]. Important prognostic variable and forecaster of response to endocrine therapy of primary tumor of breast are biologic markers i.e ER and PR status [15]. Indicator of the advanced breast cancer disease is metastasis which is the important prognostic factor to describe the probability to what extent disease spread to distant lymph nodes. 30% to 50% of all diagnosed breast cancers have metastasize to the sentinel lymph node [16-18].

In US, breast cancer survivors estimated are over 2 million out of which approx 75% diagnosed were hormone receptor positive [19]. Women diagnosed with positive estrogen receptor (ER) and/or progesterone receptor (PR) status breast tumor are at lower risks of mortality compared to negative ER- and /or PR status breast cancer [20-23]. Survival rate of breast cancer patients with positive hormone receptor status can be elevated by adjuvant endocrine therapy and chemotherapy while patients with ER negative undergo aggressive chemotherapeutic treatment which is evaluated by clinical trials [15,24,25].

Hormone therapy is resistant to tyrosine kinase encoded growth-promoting protein HER-2/neu rather than inverse relation between ER/PR expression and HER-2/neu amplification [26]. Overexpression of HER-2/neu in tumor of women demonstrated from studies are about 15% to 20% of breast cancers. In order to identify the best treatment the HER2 protein overexpression test must be conducted for metastatic cancer [2]. Treatment strategies adopted in breast cancer patients clearly affect their blood chemistry like total leukocyte count (TLC), total red blood cell count (TRC), platelets, haemoglobin, urea, and creatinine. Patients need counselling hand to cope up with disease first and then side effects of the medication they are consuming, which would be helpful in improving the quality of life of this life threatening patients.

Among Asian countries especially in Pakistani population there has been alarming increase in the incidence of breast cancer but there are scant reports especially in Southern Punjab, Pakistan about breast cancer incidence, mortality, or risk factors. Therefore current work retrospective study i.e effect of cancer therapy on blood chemistry in breast cancer patients in Southern Punjab, Pakistan was designed to counsel them for their complications. The purpose of this study is to relate the immunohistochemical variables with the appropriate therapy adopted which ultimately effect blood chemistry of the patient,

continuous variables of the study come into seen were ER, PR and HER-2/neu and deviation in blood chemistry of patients after chemotherapy analyzed and accordingly patients were counselled for their diet plan and physical activity according to guidelines in order to manage the side effects. Data of past 1 year (2012-2013) for the above mentioned objectives was collected from BINO hospital, Bahawalpur.

## Materials and Methods

### Patient selection

All patient's data was gathered from the files of the Bahawalpur Institute of Nuclear Medicine and Oncology (BINO) in the city of Bahawalpur. During this period, 180 patients were analyzed as enrolled cases for prognostic hormone receptor status i.e. ER, PR, Her-2 having profound ductal and lobular carcinoma of breast, taking therapy which alter normal physiologic blood chemistry reports. Out of 180 patients, only 136 patients of breast cancer were selected for evaluation whose blood chemistry tests were regularly conducted. Demographic data of the selected patients was collected from pathology reports. Points of focus for this study were patient age at diagnosis, tumor location, histopathological carcinoma type, tumor's grade and stage at the time of diagnosis, hormone receptor status, therapy adopted, hemograms, chemotherapeutic side effects and other socioeconomic factors. Recent enrolled cases of the women with breast cancer aged between 25 to 85 years were analyzed and counselled for their diet plan and life style modification for better outcomes.

### Immunohistochemical analysis

The inclusion criteria for the study was based on data of blood chemistry reports of patients of BINO. Classification of breast carcinoma included was according to WHO's breast tumor's classification. Archived cases studied at BINO predict that 123 cases were of DCIS while only 8 cases were of LCIS. Grading criteria of carcinoma include the modified Bloom and Richardson method while staging was according to TMN staging system. IHC (immunohistochemistry) and FISH (Fluorescence insitu hybridization) techniques were used for the determination of hormone receptor status. If the tumor cells nuclear staining is >1% then ER/PR were considered positive. Using the ASCO/CAP recommendation, 0+ or 1+ score of immunoreactivity result of HER-2 considered negative while 3+ score considered positive [27].

### Therapeutic evaluation

Therapy adopted was based on hormone receptor status along with chemotherapy. Recommended treatment for the patients with positive hormonal status include chemotherapy and antiestrogens like tamoxifen. On the other hand, only chemotherapeutic treatment was given for negative status patients and radiotherapy is also the option suggested for any status. Herceptin is the mostly prescribed medicine for HER-2 positive breast cancer patients. Besides all mastectomy was also the part of treatment depending upon the condition of patient. Blood chemistry of the patients after each chemotherapeutic cycle were observed and any elevation, depression and normal value of total leukocyte count, red blood cells, haemoglobin, platelets and creatinine was evaluated which are leading cause of the patients common ADR's. Patients were consoled and appropriately counselled for their possible side effects by improving their lifestyle and utilizing balanced diet plan.

### Statistical analysis

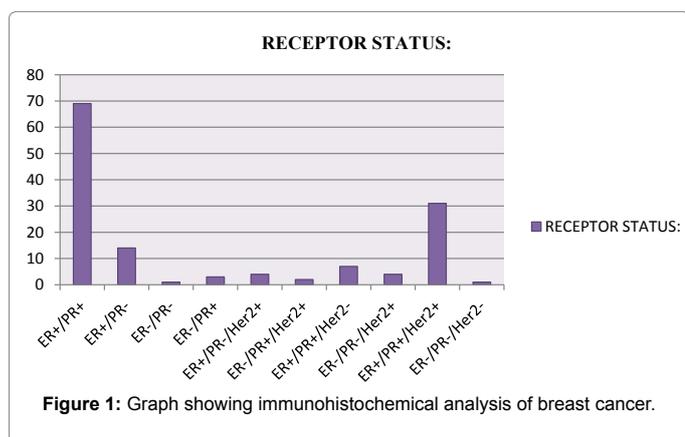
Data was statistically analyzed by Statistical Package for Social Sciences (SPSS, version 20). Descriptive statistics were performed and

the results were mentioned in the form of percentages and frequencies. Results were displayed in the form of tables and bar graphs.

## Results

Figure 1 shows that etiology of highest ratio of disease is possibly due to both ER+/PR+ followed by ER+/PR+/Her 2+ receptor status which predicts that the hormonal involvement is the developing cause of breast cancer.

Data reflects that profused type of breast carcinoma is invasive ductal carcinoma which is most appropriately diagnosed at stage III. Post-menopausal women become the victim of disease between the



age group of 41–60 years with the involvement of other hormonal aspects come into consideration. Table 2 predicts that chemotherapeutic treatment for breast carcinoma is chosen as effective one but render side effects. While on the other hand, hormonal treatment is in line for positive hormone receptor status.

**FAC/CAF:** Fluorouracil/Doxorubicin/Cyclophosphamide, **TAC:** Docetaxel/Doxorubicin/Cyclophosphamide, **FEC:** Fluorouracil/Epirubicin/Cyclophosphamide, **Antiestrogens (Tamoxifen)**

Medicines were prescribed in combinations for chemotherapeutic cycles. Preferred combinations are shown at the end of the table 2 which were used in the particular hospital in accordance with the American Cancer Guidelines. Females with hormone receptor positive switch to additional hormonal therapy antiestrogen for premenopausal women and aromatase inhibitors for postmenopausal diseased females.

Evaluated data shown in Table 3 concluded that after each successive chemotherapy cycle, patient profile confirmed that there is consecutive decline and reduction in leukocyte and erythrocyte count along with platelet and hemoglobin level where as moderate to slight elevation in the creatinine level.

## Discussion

Breast cancer is a potential life threatening disease worldwide. It is evident from researches that number of patients reported with breast cancer are increasing every year [28]. Data collected revealed that

Site of Tumor		Patients		Histology			
Right	69	Invasive ductal		Invasive lobular		Ductal carcinoma	Others
Left	67	123		8		4	1
Tumor grade							
G1		G2		G3		G4	
34		46		39		17	
AJCC Stage							
Stage 0		I	IIA	IIB	IIIA	IIIB	IIIC
0		1	21	29	9	52	17
Primary Tumor Size							
<2cm		2-5cm		>5cm		Any size and spread to chest	
6		40		38		35	
Unclassified							
17							
AGE							
<20		21-40		41-60		61-80	
0		14		93		29	
Premenopausal							
49							
Postmenopausal							
87							
Number of nodes positive							
0		1-9		10-18		19-27	
68		50		10		8	

**Table 1:** Epidemiology of breast cancer patients having all histopathology.

Recommended therapy									
Chemotherapy			Radiotherapy				Combined		
67			4				65		
Hormone receptor status									
ER+/PR+	ER+/PR-	ER-/PR-	ER-/PR+	ER+/PR-/Her2-neu+	ER-/PR+/Her2-neu+	ER+/PR+/Her2-neu-	ER-/PR-/Her2-neu+	ER+/PR+/Her2-neu+	
69	14	1	3	4	2	7	4	31	
Prescribed medications									
FAC or CAF	TAC	AC	Paclitaxel with carboplatin			Antiestrogens/Herceptin		Bonafos	
77	21	29	9			31		6	

**Table 2:** Therapeutic strategies adopted with relevance of hormone receptor status

	TLC	TRC	Hb	Platelets	Creatinine
<b>Severe increase</b>	--	--	--	--	<b>21</b>
<b>Moderate increase</b>	--	--	--	--	<b>45</b>
<b>Slight increase</b>	<b>8</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>49</b>
<b>Un-affected</b>	<b>14</b>	<b>42</b>	<b>31</b>	<b>26</b>	<b>21</b>
<b>Slight decrease</b>	<b>49</b>	<b>65</b>	<b>57</b>	<b>39</b>	<b>0</b>
<b>Moderate decrease</b>	<b>42</b>	<b>23</b>	<b>42</b>	<b>47</b>	--
<b>Severe decrease</b>	<b>23</b>	<b>6</b>	<b>6</b>	<b>23</b>	--

**Table 3:** Evaluation of treatment on the hemogram of patients after each chemotherapy cycle.

females of 41-60 years (68.38%) are more prone to disease whereas those with less than 20 years and greater than 80 years of age are least affected, which is in consonance with the study reported to The Cancer Registry of Norway on breast cancer [29]. A study conducted in Brazil observed higher prevalence among premenopausal women than postmenopausal Americans [27] which is opposing to our study but is consistent with the observation reported in breast cancer facts and figures [2]. Majority patients (90.44%) were having invasive/infiltrating ductal carcinoma [12]. 38.3% patients were diagnosed at stage IIIB followed by stage IIB (21.32%). Late diagnosis reported in Pakistan might be due ethical issues, urbanization, less awareness and other social- economic factors ultimately leads to increase rate of annual deaths [30].

On the basis of hormones receptor analysis, 50.73% patients were ER+/PR+ whereas remaining patients belong to other categories of immunohistochemistry. Her2 test was advised in most of the cases but it was not conducted by patients due to poor socio economic status [31]. 22.79% patients had triple positive receptor status and were greatest of total number of cases in which Her2 was performed which indicates that Her2 receptor test is useful for evaluating the treatment [15].

Treatment options are different in different health care settings which mainly depends upon patient disease status [2,9,28]. Improved prognosis reported in study on adopting radiotherapy following mastectomy, [32]. Chemotherapy was revealed as the treatment of choice given to 49.2% patients. Radiotherapy was adjuvant to chemotherapy in 47% patients. FAC/CAF was the most extensively used chemotherapeutic combination whereas TAC (15.44%), AC (21.34%), Antiestrogens/Herceptin (Hormonal therapy) 22.79% and Bonefos (4.41%) were other components of multidrug therapy. Similar studies were conducted for treatment evaluation in London population [28]. Due to higher incidence of recurrence in breast cancer patients, NICE guidelines state that radiotherapy should be offered in early and locally invasive breast cancer (National Institute for Health and Care Excellence 2010).

The evaluation of hemogram of cancer patients in this study is the first of its type in Pakistan so the point of focus was to investigate the effect of proposed treatment on normal physiology of patient. It helps in evaluating the adverse effects occurring after treatment and can guide the patients about life style modifications including nutritional recommendations and physical activity in relation to the severity and type of disease [33]. Chemotherapy causes bone marrow suppression which is evident from laboratory findings of the breast cancer patients receiving it. Patients become anaemic, feel anorexia and weight loss, disturbed protein and lipid profile dominant. Patients don't have proper counseling facilities about their nutrition during their therapeutic regimen to cope up with their side effects caused by treatment adopted rather it is chemotherapy, radiotherapy, mastectomy or hormonal/targeted therapy.

Highlighting red blood cells, hemoglobin and platelet count from laboratory findings give us clue to approach towards side effects management of cancer cachexia. Taking into account these realities patients were counseled according to American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention [34]. Crossing the margin line of normal creatinine level directly impact the normal functioning of kidney. Chemotherapy causes nephrotoxicity as evident from elevated creatinin level. Data reflects that this elevation is obvious cause of adverse effects patients experienced. Nutritional intervention studies on cancer patients conducted worldwide not only after chemotherapy but also for radiotherapy which have proven their efficient role in improving potential outcomes and quality of life of patients suffer from this fatal disease [33,35]. Generalizability of results is not possible due to insufficient sample size and single cancer setting inclusion in the study. No follow up of the patients was carried out to check the influence of life style modifications, for which the patients were counseled, due to lack of time and resources. After treatment, nutrition and life style modification are the only coping styles which can enhance their quality of life. So, such critical patients were routinely counseled for their diet plan to take food which improves their immunity and body physiology because they were already altered. This study can be a guideline for new treatment regimes in cancer patients so these drastic adverse effects could be managed appropriately. To overcome the constraints highlighted in the study, future research will be done on a larger scale to fill the gap which ultimately improve the quality of life of cancer patients.

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