ABSTRACT:
PURPOSE: To evaluate the non-hematological toxicities and liver enzymes alterations in breast cancer patients after chemotherapy treatment. To assess the role of liver enzymes in disease prognosis.

PATIENTS AND METHOD: 224 female breast cancer patients initially diagnosed with primary breast cancer in one breast, without metastasis to distant organs were selected from the oncology unit of the teaching hospital. Patients were divided in two groups on the basis of dissected lymph node(s) with and without metastasis. 224 female subjects without cancer history were selected as control. The blood samples were evaluated for liver function test. Breast cancer treatment was prescribed to breast cancer patients by the oncologist and included combinations of surgery, chemotherapy, radiotherapy and hormonal therapy. 201 patients were treated by chemotherapy included treatment and were evaluated for the non-hematological toxicities developed by the treatment. 23 patients included in the study were treated without chemotherapy and the treatment included surgery, radiotherapy and hormonal therapy. Blood samples of all the patients were evaluated for liver function, to assess the liver toxicity, 14 weeks after the last chemotherapy dose. During this period patients were treated for six weeks with radiotherapy and eight weeks with hormonal therapy, which later continued for five years.

RESULTS: Alopecia was experienced by all patients. High percentage of patients suffered weight loss, fatigue and nail changes. Toxicities experienced were grade I and II and were reversible. No incidence of death was reported on chemotherapy treatment. Evaluation of liver enzymes at disease presentation reported significantly high alanine transaminase level in patients with axillary lymph node metastasis as compared to control subjects and in patients treated without Hormonal therapy (p<0.05). In breast cancer patients alkaline phosphatase was significantly high after chemotherapy treatment as compared to that before treatment (p<0.05). No significant change in bilirubin levels was observed. Nolvodex was excluded as toxicity inducing factor since, exposure of patients with hormonal therapy was less than the period required to produce toxicity.

CONCLUSION: We conclude that toxicities experienced by the breast cancer patients after chemotherapy treatment were mild and reversible. Significantly raised alanine transaminase in patients with lymph node metastasis at disease presentation before starting the treatment is suggestive of its role in disease prognosis. Alanine transaminase level was significantly high in patients treated without hormonal therapy included treatment, thus indicating the protective effect of hormonal therapy on liver enzyme. Significantly high alkaline phosphatase level after chemotherapy treatment shows the relation between chemotherapy and hepatic toxicity development. Hormonal therapy does not participate in toxicity development.

KEY WORDS: Breast Cancer, Hepatic Toxicity, Liver Enzymes

INTRODUCTION:
Breast cancer is a most frequent cancer and expected annual prevalence is more than 4.4 millions in five years. Breast cancer treatment currently requires the joint efforts of a multi disciplinary team. The alternative treatment are constantly expending with the use of new effective chemotherapy, hormonal therapy and new ways to integrate systemic therapy, surgery, radiotherapy. Treatment plan should based on benefits and late toxic...
Affects. Therapy selection to increase efficacy and low toxicity based on tumor characteristic is necessary. The main prognostic factors associated with breast cancer are the number of involved lymph nodes, tumor size, histological grade and hormone receptor status (1). The most important prognostic factor affecting local control, disease free survival and overall survival was axillary lymph node metastasis (2). Studies have reported better disease free survival and overall survival for patients with estrogen receptor and progesterone receptor positive tumor (3). The importance of management of breast cancer is to provide local control and to reduce any potential for metastasis to local or distant sites. The management of breast cancer required a complex multidisciplinary approach involving surgeons, radiotherapist, medical oncologist and pathologist (4). Cytotoxic drugs (chemotherapy) may be used in two ways in cancer treatment, either they kill the cancer cells or modify their growth (5). Major groups of drugs used in cancer included alkylating agents, antimetabolites, plant alkaloids and antibiotics. The most commonly used regimen is the combination of cyclophosphamide (C), methotrexate (M) and 5-fluorouracil (F) in the form of CMF (6,4). Currently pre-operative chemotherapy is the standard of care in locally advanced breast cancer. It was reported to have association with few adverse effects and better disease free survival (7). In the treatment of breast cancer, the anti-estrogen effect is used (8). Tamoxifen is an important agent for the treatment of breast cancer (9). The treatment strategies commonly practiced in Pakistan include surgery, hormonal therapy, chemotherapy and radiotherapy. The risk for toxicity from adjuvant chemotherapy depend more on the type of regimen. Toxicity may distress patients and may delay or discontinue scheduled chemotherapy (10). The treatment strategies commonly practiced in Pakistan include surgery, hormonal therapy, chemotherapy and radiotherapy. The most serious toxicity is bone marrow depression resulting in granulocytopenia, thrombocytopenia and aplastic anemia. Alkylating agents damage the epithelial surfaces, the host defense mechanism is broken down and the susceptibility to all infections is increased. Drugs such as doxorubicin, 5-fluorouracil and methotrexate cause mucositis, decrease the rate of mucous lining renewal, causes stomatitis, diarrhoea and haemorrhages. Nausea, vomiting and dermatitis were reported as prominent complications of cytotoxic drugs (5). Increased exposure to toxic metabolites of cyclophosphamide leads to liver toxicity. The toxicity developed was related to how the regimen was metabolized after chemotherapy treatment (11). Toxic liver injury was reported to produce necrosis, fibrosis, cholestasis and vascular injury. Liver injury during cancer chemotherapy may not always due to anticancer drugs but may be due antibiotics, analgesics, antiemetics or other medications (12). Two pictures of hepatocellular injury was reported earlier one was hepatocellular injury with rises in aminotransferase, alkaline phosphotase and serum bilirubin. The other was stricture of intrahepatic or extrahepatic bile duct, accompanied by elevated alkaline phosphatase and bilirubin levels (13). Combination chemotherapy uses several

<table>
<thead>
<tr>
<th>Table 1</th>
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</thead>
<tbody>
<tr>
<td><strong>ADVERSE EVENTS EXPERIENCED BY BREAST CARCINOMA PATIENTS DURING CHEMOTHERAPY</strong></td>
</tr>
</tbody>
</table>

Adverse events including allergic reactions, cardiac symptoms and constitutional symptoms including, fatigue, insomnia, sweating and weight loss experienced by breast carcinoma patients after treatment with all types of chemotherapy was evaluated according to the common terminology criteria for adverse events (CTCAE) (20). Patients suffered drug fever for only three days on treatment by chemotherapy cycle; other symptoms were observed throughout the treatment period and were reversed after the completion of the treatment. Patients suffering adverse events are expressed as percentage according to adverse event Grades. Number of patients is given in parentheses. Total number of cases treated by chemotherapy (201).

<table>
<thead>
<tr>
<th>Adverse events type</th>
<th>Adverse events grade</th>
<th>Patients suffering adverse events after chemotherapy (Percentage)</th>
<th>Patients not suffering a particular adverse events after chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic Reaction/Drug Fever</td>
<td>Rash; Drug Fever &lt; 100.4°F</td>
<td>50 (100)</td>
<td>25 (50)</td>
</tr>
<tr>
<td></td>
<td>Dyspnea; Drug Fever &gt; 100.4°F</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Cardiac general hypotension</td>
<td>Brief (&lt;24 Hours) Fluid replacement or other therapy; No physiologic consequences</td>
<td>45 (91)</td>
<td>55 (110)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Mild Fatigue</td>
<td>75 (151)</td>
<td>25 (50)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Occasional, difficulty in sleeping not interfering with functions</td>
<td>49 (98)</td>
<td>51 (103)</td>
</tr>
<tr>
<td>Sweating</td>
<td>Mild and occasional</td>
<td>22 (45)</td>
<td>78 (156)</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>5 to ≤ 10% from base line</td>
<td>90 (180)</td>
<td>10 (21)</td>
</tr>
</tbody>
</table>
chemotherapeutic agents each with different mechanisms of action and toxicity profile.

AIM OF THE STUDY:
To evaluate the nonhematological and hepatic toxicities developed after chemotherapy included treatment in breast carcinoma patients and to assess the role of altered liver enzymes in disease prognosis.

MATERIAL AND METHOD:
Study was conducted on 224 non-pregnant female breast cancer patients with initially diagnosed primary infiltrating ductal carcinoma in one breast. Patients were selected from the Oncology clinic of a teaching hospital at Karachi in the years 2004-2008. Breast carcinoma of all the patients was proved by the biopsy. Metastasis to distant organs was excluded by ultrasound. Life history of the patients was recorded by personal interviews and included questions on their life history and family history. Patients were classified into groups on the basis of tumor grade, axillary lymph node metastasis and estrogen/progesterone receptor positivity as follows:

1. Histological Grade:
According to Bloom Richardson grading system, all tumors were divided into 3 grades, i.e. grades-I, II and III.

2. Axillary Lymph Node Metastases
Patients on the basis of Axillary Lymph Node Metastases were divided into three groups by numbers of positive nodes: negative axillary nodes, one to three positive axillary nodes and four or more positive axillary nodes.

3. Tumor size
The tumors were divided into three size ranges for analysis, T1, 0 to 2 cm; T2, 2.1 to 5.0 cm, and T3, equal or more than 5.1 cm.

4. Hormone Receptor
Patients were divided into two groups for treatment; hormone receptor positive tumor patients and hormone receptor negative tumor patients

Treatment for cancer patients was prescribed and monitored by the consultant oncologists. Treatment included combinations of surgery, chemotherapy, radiotherapy and hormonal therapy. Chemotherapy prescribed for the treatment included combinations of cyclophosphamide (C), adriamycin (A), 5-Flourouracil (F), methotrexate (M) adjusted by the Oncologist according to the body surface area (BSA) of the patients.

Sample Collection: Blood samples were collected by the patients consent at disease presentation and 14 weeks after last chemotherapy dose. Chemotherapy dose was given at the interval of three weeks. Liver changes during the treatment were monitored by sonography.

Control Subjects: Control subjects selected had no past cancer history. Blood samples were collected for liver function test evaluation by the control subjects consent.

Table- 2

<table>
<thead>
<tr>
<th>Adverse events type</th>
<th>Adverse events grade Patients suffering adverse events after chemotherapy (Percentage)</th>
<th>Patients not suffering a particular adverse events after chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>Localized or in a dependent area 05 (10)</td>
<td>95 (191)</td>
</tr>
<tr>
<td>Alopecia Hair Loss (Scalp and Body)</td>
<td>-</td>
<td>Complete 100 (201)</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>Slight/Localized 52 (105)</td>
<td>-</td>
</tr>
<tr>
<td>Nail Changes</td>
<td>Discoloration 87 (175)</td>
<td>-</td>
</tr>
</tbody>
</table>

Recommended Dose of Chemotherapy:
- F (5-Flourouracil): 500 mg/m2
- A (Adriamycin): 50 mg/m2
- C (Cyclophosphamide): 500 mg/m2
- M (Methotrexate): 40 mg/m2
- T (Taxol): 90 mg/m2

Dose adjustment of Chemotherapy:
- Body surface area (BSA) = \( \sqrt{\frac{\text{height} \times \text{weight}}{3600}} \)
- Required dose to be injected = \( \frac{\text{Recommended dose}}{\text{BSA}} \)
Hormonal therapy:
Recommended dose: Nolvodex provided by
the ICI company was given at the dose of
20 mg/day for 5 years.
Radiotherapy: Treatment was
completed in 6 weeks with 5 days/week
to boost the scar. Body parts exposed
to radiation were tumor bed, axillary
nodes and supra clavicular nodes. 2 grey
per day was given with photon and 2
grey per day was given with electron.
Total 60 grey per 30 fractions were
delivered.
Evaluation of non-hematological toxicity:
Toxicity experienced during and after the
treatment were observed after personal
examination and interview with the patients.
Toxicities were graded according to common
terminology criteria for adverse events. V
3.0 (15). A grading (severity) scale is provided
for each adverse events.
Evaluation of liver functions: Liver function
evaluation included total bilirubin and direct
bilirubin, Alanine transaminase (ALT) and
Alkaline phosphotase (ALP) estimation.
Chemical analyzer, Hitachi 912 provided
by Roche diagnostic Basil Germany was
used for the estimations. Kits for the
estimation were provided by Roche
diagnostic GmbH, D-68298 Mannheim,
Germany. Total bilirubin was estimated
by the method developed by Wahlefeld,
et al, 1972 (16). Direct bilirubin was estimated
by Gendrassik-Grof procedure (17) Alanine
Aminotransferase (ALT) was estimated by
the standard recommended method of
International federation of clinical chemistry
(IFCC) (18). Alkaline phosphatase (ALP)
was estimated by the method standardized
against International federation of clinical
chemistry (19).

STATISTICAL ANALYSIS:
Statistical package for social science (SPSS
version 11.0) was used for data feeding and
analysis. To compare proportion/percentage
of qualitative/categorical variables in the breast
cancer patients and control “Chi-square test”/
Test Proportion was applied. Means and
standard deviations of quantitative/continuous
variables in breast cancer cases and
control was analyzed by “Student’s t-
test” and analysis of variance (ANOVA).
In all statistical analysis only p-values <
0.05 was considered significant. BMI was
calculated by the following formula:
Weight (Kg)/[(Height (meter)2

DISCUSSION
Assessing chemotherapy toxicity is a good
opportunity for pharmacist to take part
and preventing toxicity in reducing patients’
discomfort (10). Toxicity level was high
when used with other treatment combination,
chemotherapy AC/CMF when given adjuvant
to radiotherapy in breast cancer treatment
then the toxicity level was increased as
compared to radiotherapy alone. Patients
after receiving six months treatment had
significantly higher incidence of skin toxicity,
oesophageitis, anorexia and nausea as
compared to radiotherapy alone (20). We
evaluated non-hematological toxicities
observed during chemotherapy treatment
including FAC, AC, AC-T and CMF. Criteria
for adverse event grading used were common
terminology criteria for adverse events (CTCAE). Adverse events experienced by
high percentage of patients were allergic
reactions, fatigue and weight loss (Table
1). Toxicity associated with taxane as
reported earlier included allergic reactions,
fatigue and weight loss (Table
1). In this study grade II toxicity (alopecia)
was experience by all the patients treated
by chemotherapy. Nail discoloration was
also experienced by high percentage of
patients, whereas bruising was occasionally
experienced by the patients (Table 2).
Toxicity depends upon the chemotherapy
combination and it was reported that FAC
treatment resulted in more alopecia as
compared to CMF (22), whereas tissue
necrosis takes place with Doxorubicin
extravasations and required skin grafting

<table>
<thead>
<tr>
<th>Table 3</th>
<th>GASTROINTESTINAL CHANGES EVALUATED DURING CHEMOTHERAPY OF BREAST CARCINOMA PATIENTS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse events type</td>
<td>Adverse events grade</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>Loss of appetite without alteration in eating habits</td>
</tr>
<tr>
<td>Constipation</td>
<td>Occasional use of Laxatives and dietary modification</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Increased oral fluid indicated; dry mucous membrane</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Increased of &lt; 4 stools per day over base line</td>
</tr>
</tbody>
</table>
We studied that chemotherapy produced Grade I toxicity on gastrointestinal system resulting in anorexia and dehydration (Table 3). Nausea and vomiting induced by cytotoxic drugs is the result of mechanism by which drugs stimulate the chemoreceptor trigger zone (in the floor of 4th ventricle) and stimulates peripheral receptors mediating cessation of peristalsis (6). With CMF worse toxicity profile was reported earlier including alopecia, nausea and vomiting (23). With Fluorouracil Grade III and IV gastrointestinal toxicity has been observed earlier. It was reported that the patients with low level of education, less social support, more severe life events and greater dissatisfaction with their medical care had worse post traumatic stress disorder (PTSD) including nausea, vomiting and distress (24). We conclude that the toxicities experienced by the patients after a combined treatment plan including surgery, chemotherapy, radiotherapy and hormonal therapy were Grade I and II. All the toxicities were reversible but they were experienced by the patients for at least six months after the completion of treatment. No incidence of death was reported due to chemotherapy in this study.

Hepato toxicity is seen chemically as increased serum bilirubin and alkaline phosphatase levels with moderate elevation in alanine aminotransferase. This pattern presents histologically, cholestasis with variable parenchymal necrosis (14). Drug induced hepatic injury is a frequent cause of hepatic dysfunction. Hepatic toxicity in clinical trials was correlated with increased alanine transaminase levels. Drugs toxicity is the most common cause of liver failure (25). Increased exposure to cyclophosphamide and its metabolite, 4-hydroxy cyclophosphamide leads to increased liver toxicity because they are converted to toxin acrolein and phosphorylamide mustard (11). We found a significantly high level of alkaline phosphatase in breast cancer patients with axillary lymph node metastasis after treatment plans including combinations of surgery, chemotherapy, radiotherapy and hormonal therapy, whereas alanine aminotransferase levels was significantly high after treatment plan without including hormonal therapy (Table 4). This study supports the previous studies reporting the pathogenesis of drug induced injury can be measured on the basis of two principal patterns of injury, the hepatocellular pattern characterized by predominant rise in transaminases resulting from the demise of hepatocyte due to necrosis. The cholestatic pattern is characterized by a predominant rise of serum alkaline phosphatase levels, that usually results from injury to the bile ductular cells either directly by the drug or its metabolite or indirectly by an adaptive immune response (25). This result supports the previously reported protective action of tamoxifen in reducing the liver enzymes increased by adjuvant chemotherapy (26). As previously reported that the concurrent administration of adjuvant chemotherapy and radiotherapy lead to unacceptable high level of acute toxicity (20). Increased organ toxicity was also reported due to increased exposure to bioactivated cyclophosphamide (27). Hepatic sinusoidal endothelial cells are injured by the cyclophosphamide metabolites that are generated within the hepatocyte. Cyclophosphamide alone is insufficient to produce the extensive sinusoidal injury that can be seen following cyclophosphamide and total body irradiation. Total body irradiation when used alone does not cause significant liver injury, however cyclophosphamide followed by total body irradiation is synergistic. Two potential mechanisms were reported, one involves a

### Table 4

**LIVER FUNCTION TESTS OF CONTROL AND BREAST CANCER PATIENTS WITH LYMPH NODE METASTASIS AT THE TIME OF DISEASE DIAGNOSIS.**

Variation in concentration of bilirubin total, bilirubin direct, bilirubin indirect, alanine transaminase level and alkaline phosphatase level of control and breast cancer patients with lymph node metastasis before and after different treatment plans including surgery (Sur), chemotherapy (Chem), hormonal therapy (HT) and radio therapy (RT). Adjuvant chemotherapy includes combinations of cyclophosphamide (C), Adriamycin (A), 5-Fluorouracil (F), Paclitaxel (T) and methotrexate (M) as FAC, CMF, AC and AC–T. Liver functions were evaluated, 14 weeks after last chemotherapy dose. The values are expressed as mean ± SEM and the numbers of cases are given in parenthesis.

<table>
<thead>
<tr>
<th>Liver function test</th>
<th>Control Subjects (224)</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Treatment</td>
<td>After Treatment</td>
</tr>
<tr>
<td><em>p &lt; 0.05 as compared to control subjects</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p &lt; 0.05 as compared to patients before starting any treatment</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (224)</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (Total) mg/dl</td>
<td>0.32±0.005</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td></td>
<td>0.62±0.02</td>
<td>0.51±0.01</td>
</tr>
<tr>
<td>Bilirubin (Direct) mg/dl</td>
<td>0.09±0.007</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td></td>
<td>0.12±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>Bilirubin (Indirect) mg/dL</td>
<td>0.24±0.005</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td></td>
<td>0.51±0.02</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Alanine Transaminase U/L</td>
<td>22.82±20.21</td>
<td>33.90±18.18</td>
</tr>
<tr>
<td></td>
<td>36.22±16.57</td>
<td>32.77±10.73</td>
</tr>
<tr>
<td>Alkaline Phosphatase U/L</td>
<td>68.49±7.74</td>
<td>81.38±1.43</td>
</tr>
<tr>
<td></td>
<td>115.38±1.50</td>
<td>82.81±1.33</td>
</tr>
</tbody>
</table>
* *p < 0.05 as compared to control subjects*
two step injury, sublethal damaged sinusoidal endothelial cells caused by cyclophosphamide metabolites followed by irradiation damage. An alternative mechanism involves depletion of reduced glutathione in hepatocytes and sinusoidal endothelial cells by cyclophosphamide leaving sinusoidal cells more vulnerable to damage by irradiation (11). Alanine transaminase was significantly high in patients with lymph node metastasis compared to control subjects (Table 4). This suggests the role of alanine aminotransferase in relation to axillary lymph node metastasis and disease prognosis. The pathogenesis of drug induced liver disease usually involve the participation of the parent drug or its metabolites that either directly effect the cell biochemistry or elicit an immune response. Susceptibility to drug induced hepatotoxicity is also influenced by genetic and environmental risk factors. Unpredictable, low frequency, idiosyncratic reactions often occur on a background of a higher rate of mild asymptomatic liver injury, it is very difficult to detect them but they may be detected by monitoring serum alanine aminotransferase levels (28). In patients without lymph node metastasis alkaline phosphatase level was significantly high after treatment as compared to the patients before starting the treatment. Alanine aminotransferase level was significantly high in patients treated without hormonal therapy (Table 5). Increased toxicity risk has been reported in patients with decline renal functions and increased creatinine level (30). Hepatotoxicity developed by Nolvodex therapy cannot be attributed to the raised liver enzymes after treatment since studies had reported that liver toxicity is developed by atleast three months following Nolvodex treatment and it is attributed to the fatty infiltration of liver (31). We estimated the liver enzymes eight weeks after Nolvodex treatment so the toxic effect of Nolvodex on elevation of liver enzymes can be excluded. We conclude that liver toxicity and elevation of liver enzymes was due to the chemotherapy treatment thus excluding the role of hormonal therapy in liver toxicity.

CONCLUSIONS:
Non hematological toxicities developed after chemotherapy included treatment were mild and reversible. Liver toxicity varies with the pattern of treatment. Nolvodex treatment exerted favorable protective effects on alanine aminotransferase. The hepatotoxicity development was attributed to chemotherapy treatment and hormonal therapy was excluded as toxicity developing. Reduced drug clearance may be a contributing factor for drug induced toxicity.

REFERENCES


