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Effect of doxorubicin and cyclophosphamide regimen versus taxane on liver enzymes in Iraqi women with breast cancer.

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Abstract

Objective: To investigate the effect of doxorubicin, cyclophosphamide and taxane chemotherapy on the liver enzymes in Iraqi women with breast cancer.
Methods: This cohort prospective study was carried out at the Biochemistry Department, College of Medicine, University of Baghdad and at the Oncology Clinic, Oncology Teaching Hospital, Baghdad, Iraq. It included 56 women with regular menstrual cycle (25-45 y) who were newly diagnosed with breast cancer. The women were classified into 3 groups: GI (pre-treatment): 29 women with breast cancer before starting chemotherapy and the post treatment group: the same 29 women of GI (pre-treatment) who finished 4 cycles of anthracycline chemotherapy (course 1), and GII: which involved another 27 women who had finished both courses of chemotherapy, (course 1) and 4 cycles of taxanes (course 2), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALK) and serum total proteins were measured using colorimetric methods.
Results: Concerning the liver biochemical parameters (ALT and AST); there was no significant difference between GII and both GI (pre-treatment and post treatment) groups. While the serum levels of these enzymes increased significantly in GI (post treatment) compared to the (pre-treatment) group. According to the serum ALP; there was no significant difference between all groups. While the serum TP shows a significant increase in GI (post treatment) compared to the (pre-treatment) group. And a highly significant increase in GII compared to both GI (pre-treatment and post treatment) groups.
Conclusion: Breast cancer chemotherapeutic agents' combinations cause hepatic toxicity indicated by changes in liver enzymes levels and activity.

Keywords: ALT, AST, ALK, Breast cancer, Hepatotoxicity, Total proteins.

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Introduction

Breast carcinoma is one of the most popular neoplasms in women and is a major cause of deaths in the world [1]. In Iraq, it is the first cancer in ranking diagnosed in women [2]. The rate of breast cancer increased between the age of 60 to 69 y [3]. Organ dysfunction is the most common in occurrence and the hepatic dysfunction is the most important one [4]. The liver damage occurred during chemotherapy may be due to antiemetics, antibiotics, analgesics used and not only due to anticancer therapy. Also, it may be caused by a previous condition such as infections (hepatitis), malnutrition, or suppression of immunity. So, it is not the toxic reaction the only cause [5,6]. Most of the hepatotoxic reactions are due to variation in the metabolism or due to immunologic reactions and therefore they are idiosyncratic and do not depend on the dose of the drug nor can be predictable [7]. Usually hepatotoxicity can produce serious reactions such as fibrosis, steatosis, cholestasis, necrosis and vascular injury [8]. Anthracycline (doxorubicin) is widely used to treat breast cancer. Although it can produce many side effects such as hepatotoxicity, cardiotoxicity, testicular toxicity and hematological toxicity [9]. Doxorubicin metabolism is extensively carried out in the liver through the antioxidant mechanisms mainly the glutathione pathway [10]. While cyclophosphamide metabolism is carried out through the cytochrome P450 system in the liver which converts cyclophosphamide to 4-hydroxy cyclophosphamide which is in equilibrium with aldophosphamide (the tautomeric form) of the drug. In cytolysis; it can be cleaved non-enzymatically into phosphoramide and acrolein. Which are both highly cytotoxic. In spite of its hepatic metabolism; only few studies reported elevated liver enzymes and thus its hepatotoxicity is due to an idiosyncratic reaction and not due to direct toxicity [11-13]. While paclitaxel (Taxol) and docetaxel (Taxotere) which are microtubule inhibiters are mainly metabolized by the liver and are restricted in patients with liver diseases [14].

Only few studies had investigated the associations of liver function tests with chemotherapy in breast cancer. The liver function tests measured the levels of important parameters including liver aminotransferases (ALT, AST), ALK and serum total proteins. Thus, the aim of the present study was to assess

the effect of chemotherapeutic agents on the liver enzymes in patients with breast cancer.

Subjects and Methods

This study was carried out at the Biochemistry Department, College of Medicine, University of Baghdad and at the Oncology Clinic at Oncology Teaching Hospital during the period from May 2018 to October 2018. It included 56 women who were already newly diagnosed by oncology group to have breast cancer. Their age range (25-45 years). The study design included; group I; pre-treatment (GI; pre-treatment); twenty nine women newly diagnosed with breast cancer before starting chemotherapy. Group I; posttreatment (GI; post treatment): the same twenty nine women of the pretreatment group who finished 4 cycles of anthracycline (Doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2) chemotherapy. And group II (GII): which involved another twenty seven women who were already finished both courses of chemotherapy, including (course 1 and 4 cycles of taxanes (Docetaxel) 100 mg/m^2 (course 2). All included women were subjected for imaging investigations including X-ray, ultrasound study, and if necessary computed tomography (C.T) scan and/or MRI in order to rule out invaded cancer in liver, bones and other sites of body. So, ensuring the absence of liver metastasis in all the involved women. Exclusion criteria included pregnant woman, chronic diseases (D.M, hypertension), alcoholic and smokers. Also, women consuming anti-inflammatory drugs. Formal consent was taken from each woman (verbal and written). We received ethical approval from the Scientific Committee of the Biochemistry Department, College of Medicine, University of Baghdad, Iraq. Five milliliters of venous blood were taken from each individual at time 8:00-11:00 a.m. Blood samples were collected in serum-separating tubes which allowed to clot at room temperature for thirty minutes, then the samples were centrifuged at (2000 Xg) for 10 minutes, the obtained serum was frozen at -20°C till the time of measurement. The level of enzymes (ALT, AST, ALK) and the total serum proteins were analysed using commercial kits. All material kits for the measured parameters were provided from Human GmbH. 65205 Wiesbaden, Germany. The Statistical Package for Social Sciences (SPSS) version 23 (SPSS Inc., Chicago, IL., USA), and Minitab analysis programs were used for all statistical studies. ANOVA and Student's t-tests were used to test for statistical significance. Linear regression was utilized to test for correlation between different studied parameters, and the significance of the r-value was assessed by related t-test. P-values of less than 0.05 were considered significant.

Results

Clinical

The clinical data in Table 1 shows that the difference in (mean ± SEM) value of the age was not significant between GI (38.79 ± 0.91) and GII (39.59 ± 0.95). Also, there was no significant difference in BMI values between GI (30.04 ± 0.94) and GII (31.78 ± 1.24).

Table 1. *The (mean ± SEM) values of the age and body mass index (BMI) between GI (pretreatment), GI (post treatment) and GII.*

	GI; pretreatment (n=29)	GI; post treatment (n=29)	GII (n=27)
Age (y)	38.79 ± 0.91	38.79 ± 0.91	39.59 ± 0.95
BMI (kg/m^2)	30.04 ± 0.94	30.04 ± 0.94	31.78 ± 1.24

ANOVA test reveals a non- significant difference between groups (NS).

Biochemical

Concerning the liver biochemical parameters (ALT and AST); there was no significant difference between GII and both GI (pretreatment and post treatment) groups. While the serum level of these enzymes increased significantly ($p<0.05$) in GI (post treatment) compared to GI (pretreatment). According to the serum ALP; there was no significant difference between all groups. While the serum TP shows a significant ($p<0.05$) increase in GI (post treatment) compared to GI (pretreatment). And a highly significant ($p<0.001$) increase in GII compared to both GI (pretreatment and post treatment) groups. The normal reference range for ALT and AST is (up to 12 IU/L). For ALK.is (32-92 IU/L) and for total proteins is (6.6-8.7 gm/dl) as shown in Table 2.

Table 2. *The (mean ± SEM) values of the serum AST, ALT, ALK and TP in GI (pretreatment), GI (post treatment) and GII.*

Parameter	GI (pretreatment)	GI (post treatment)	GII
ALT IU/L	6.68 ± 0.91	10.44 ± 1.00^{*a}	7.88 ± 2.25
AST IU/L	5.44 ± 0.76	11.41 ± 2.04^{*a}	8.77 ± 1.37
ALK IU/L	86.32 ±4.16	79.07 ± 3.48	83.25 ± 4.23
TP g/dl	6.48 ± 0.12	7.7 ± 0.3^{*a}	9.87 ± 0.4^{**a}

Data are expressed as (mean ± SEM). Values with different superscripts are significantly different: ^{*a}P<0.05 significant; ^{**a}p<0.001 highly significant.

The present study had shown that in GI (pretreatment); there was a significant positive correlation between BMI and serum ALK; (r=0.378), ($p<0.05$). Also, a significant positive correlation between age and serum ALK; (r=0.430), ($p<0.05$). Also, in GI (post treatment); there was a significant positive correlation between serum ALT and serum ALK; (r=0.440), ($p<0.05$). And, a highly significant positive correlation between serum AST and serum ALK; (r=0.561), ($p<0.01$). While in GII; there was a highly significant positive correlation between serum ALK and serum ALT; (r=0.554), ($p<0.01$).

Discussion

In the present study, there was no significant difference in the age and BMI between women´s group (Table 1). The (mean ± SEM) age of women of (G1; pretreatment) of the present study was (38.79 ± 0.91). Jemal et al. found that 25% of breast

cancer cases were before menopause [15]. This is in association with the present study as the age of patients was between 25-45 y. Also, the clinical characteristics of patients of the present study showed that the breast cancer women were overweight and obese. Several studies had concluded that the obesity is a risk factor to breast cancer and other types of cancer. To decrease the risk of breast cancer recurrence and death, the obese breast cancer patients need to lose their weight [16,17].

The present study shows an increase in the serum AST and ALT in GI (post treatment) compared to GI (pretreatment), (Table 2); these results were in congruence with Sathesh et al. who showed that doxorubicin administration cause tissue damage and an increase of enzymes membrane leakage of these enzymes [18]. Also, the present study was in agreement with Damodar et al. who revealed that the incidence of hepatotoxicity in patients treated with doxorubicin injection was 30.4% [19]. Yang et al. stated that about 40% of patients suffered liver damage after doxorubicin treatment [20]. This was supported with a study by Llesuy and Arnaiz who stated that doxorubicin administration produced increases of 51% and 53% in liver spontaneous chemiluminescence and malonaldehyde formation; respectively. The main characteristics of these processes were elevations in serum levels of ALT, AST and bilirubin [21]. The underlying mechanism is that doxorubicin causes an increase in the malonaldehyde levels together with a decrease in the serum levels of superoxide dismutase and catalase activity through the one-electron reduction of nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P-450 reductase enzyme [22,23]. Previous studies reported that administration of antioxidants such as vitamin E, C, and A could reduce the hepatotoxic effect of doxorubicin. And that both virgin olive oil and selenium could be given as dietary supplements with the appropriate concentration and dosage form to maximize the antitumor action of these agents and to minimize the hepatotoxic effect of doxorubicin [24,25].

Also the results of this study was consistent with that recorded by Tomoki et al., who found that doxorubicin elevates plasma AST and ALT activities in mice [26]. Other study established that there was close correlation between the administration of doxorubicin and the appearance of hepatic dysfunction [27]. Chauhan et al. found that the levels of these enzymes were increased but still within the normal reference ranges during the different courses of chemotherapy this was explained by the progressive liver damage caused by the chemotherapeutic drugs [28].

The present study showed that there was a non-significant difference in the serum levels of alkaline phosphatase breast cancer patients and patients treated with chemotherapy. These results were on the same line with a study done by Oluboyo et al. who stated that there was no statistically significant difference in the ALP activity between the breast cancer subjects on chemotherapy and those not on chemotherapy, It has been reported that chemotherapeutic agents can have both direct and indirect effects on the bone microenvironment ultimately leading to a decrease in the bone mineral density [29]. Also, the present study was in agreement with other studies in which they didn't find any significant difference in ALP levels in non-metastatic breast cancer [30,31]. On the other hand the present results were in contrary to previous studies in which they stated that the progressive increase in serum ALP was due to metastasis of breast cancer either to bone or liver [32,33]. Also, inconsistent with previous studies in which they stated that chemotherapy induce bone marrow density loss [34,35].

The results of the significant increase in the serum total proteins seen in the chemotherapy treated group rather than patients without chemotherapy were inconsistent with Chaun et al. results that showed that there was a non-significant change in the level of total proteins [28]. This fact can be explained by the decrease in the serum level of albumin through catabolism and increase in the serum level of globulins through synthesis [36]. And thus they had proposed that the serum levels of these proteins should be considered individually and not the amount of the total protein levels [37].

The significant positive correlation between BMI and serum ALK in GI (pretreatment) was in agreement with that observed by Khan et al. who stated that higher amounts of ALK were linked to obesity since adipocytes are good source for ALK isoenzymes [38]. Concerning the positive correlation between age and serum ALP; there was no previous studies explained this association in breast cancer. But, it was reported that increased overexpression of serum ALP was observed with increased aggressiveness of the disease [39].

Conclusion

The altered liver function tests caused by the chemotherapeutic agents used to treat breast cancer may cause an increased risk of hepatic toxicity.

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