Original Article

The Possible Cytoprotective Effects of Antioxidant Drugs (Vitamin E and C) Against the Toxicity of Doxorubicin in Breast Cancer Patients

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Summery:

Background: Breast cancer is the culmination of a multi-step process that occurs over a period of several years or decades and as a cause of death, is a salient "free radical" disease. Aim: The present study aims on investigating the possible protective role of antioxidant drugs (vitamins E and C) to cardiac cells against the oxidative stress induced damage during doxorubicin chemotherapy in patients with breast cancer.

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Patients and methods: Thirty two patients with different stages of breast carcinoma attending to Baghdad Teaching Hospital and ten healthy control subjects with age range between (29-61) years, mean (43.6±1.37) were included in this study. The patients were randomized into 3 groups, they were treated with either doxorubicin alone 60 mg/M² every 21 days, doxorubicin 60 mg/M² every 21 days +vitamin E 800IU/day for 42 days or a combination of vitamins E 800IU/day and vitamin C 1000mg/day for 42 days + doxorubicin 60 mg/M² every 21days. The oxidative stress and cardiac function parameters were evaluated before starting treatment and after 21 and 42 days respectively including assessment of serum levels of MDA, creatine kinase and lactate dehedrogenase activities.

Results: Using doxorubicin alone produce an elevation in the markers of oxidative stress and cardiac damage that can be reduced when antioxidant drugs vitamin E alone or a combination of vitamin E and vitamin C being added.

Conclusion: Antioxidant drugs, vitamin E or a combination of vitamins E and C when co-administered with the antineoplastic drug doxorubicin reduces its cytotoxicity on cardiac cells in breast cancer patients.

Key words: Breast cancer, Doxorubicin, Vitamin E, Vitamin C, MDA, Cardiotoxicity.

Introduction:

Breast cancer is the second most common malignant neoplasms after lung cancer in the world (1).The etiology of breast cancer is multifactoral. Hormonal, genetic and environmental factors appear to interplay in the pathogenesis of breast cancer(2).The underlying cause is thought to be DNA damage, which is oxidative in nature(3). Free radical reactions are expected to produce progressive adverse changes that accumulate with age throughout the body(4) in addition to alterations to metabolic pathways in tumor cells, an inadequate tumor vascular network, macrophage infiltration of the tumor and therapeutic interventions by some anticancer therapies that may add to the oxidative stress within breast carcinomas(5). The chemotherapeutic agents doxorubicin, mitomycin C, etoposide and cisplatin are superoxide generating agents (6). Doxorubicin is an anthracycline antibiotic and one of the most important anticancer agents used in the treatment of various neoplastic conditions including breast cancer (7). However, the clinical use of doxorubicin is associated with exerting toxic effects in several body organs including the heart through production of free radicals and reactive oxygen speices(8).Antioxidants include the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, as well as nonenzymic compounds such as α--tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), and glutathione(9) counteracting free radical damage. Ascorbate can reduce the initiating reactive oxygen species so that initial or continued lipid peroxidation is inhibited(10).

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Vitamin C (ascorbic acid) is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not by humans(11). It is an essential cofactor for eight mammalian enzymatic reactions and functions as an intra- and extracellular scavenger of free oxygen radicals(12). The relevant species, which receive electrons and are reduced by vitamin C include, reactive oxygen species, reactive nitrogen species and sulphur radicals, nonradical compounds, including hypochlorous acid, nitrosating compounds, and ozone(13), regeneration of the α-tocopherol, when exogenous radical oxidants interact with α-tocopherol and tocopheroxyl radical generatation can be reduced by ascorbate back to α-tocopherol(14)and transition metal-mediated reactions involving iron and copper(15). Vitamin E is the main lipid-soluble antioxidant and is thought to protect cells by its ability to quench free radicals (16).

Patients and methods:

This study was carried out on (42) females with age ranged between (29-61 years, mean 43.6±1.37). (10) Healthy subjects served as a control group and (32) females with different stages of breast cancer after mastectomy treated with either of the following systemic chemotherapeutic regimens: Regimen No. 1:- Doxorubicin 60 mg/M² (Ebewe Phrma Ges. m.b.H., Austria)+ Cyclophosphamide 600 mg/ M² in I.V. infusion once every 21 days. Regimen No. 2:- Doxorubicin 60 mg/M²+ Cyclophosphamide 600 mg/M² + 5- Florouracil 600 mg/M² in I.V. infusion once every 21 days.

These patients were diagnosed and treated in Baghdad Training Hospital under follow up of specialist physicians. The (32) treated patients were allocated in three subgroups as follow: Group (A): 10 breast cancer patients, who did not receive any antioxidant drugs during chemotherapy. Group (B):12 breast cancer patients, treated with a 800 IU / day vitamin E (Jamieson Lab., Torento,Canada) for 42 days during chemotherapy. Group (C):10 breast cancer patients, treated with a combination of 800 IU/day vitamin E and 1000 mg /day vitamin C (Al-shahba, Syria,Allepo) for 42 days during chemotherapy.

Venous blood (10 ml) was obtained from the forearm of each patient by vein puncture at a baseline before the initiation of therapy, after 21 days of treatment and at the end of 42 days for all patients' groups. Each blood sample was placed in EDTA-free tube to be centrifuged for 10 minutes at 3000rpm.Serum was then divided into several 1.5 ml eppendrof tubes and stored at (-20) until time for the assay.The serum were analyzed for Malondialdehyde (MDA), the end product of lipid peroxidation, and the activities of creatine kinase (CK) and lactate dehydrogenase (LDH) as markers of cardiac function according to standard methods. The mean values of all parameters were expressed with SEM; Student´s *t*-test and ANOVA were used to check their significance, and considered significantly different at *P* value < 0.05.

Results:

Table (1) showed the following: Breast cancer produces significant elevation (p <0.05) in serum MDA level (285%,348%,396%) compared to normal control group. Further significant elevation (p <0.05) in serum MDA level was observed as a result of therapy with doxorubicin (31%,74%) after 21 and 42 days respectively. Regarding therapy with vitamin E, there was a significant reduction (p <0.05) in serum MDA level (21%,39%) after 21 and 42 days of therapy respectively compared with baseline and also a significant (p <0.05) reduction (27%,59%) compared to doxorubicin therapy, but was higher than that observed for control group. Concerning treatment with a combination of vitamins E and C, a significant greater reduction was occurred (p <0.05) in serum MDA level (27%,52%) after 21 and 42 days respectively compared with baseline. There was also a significant (p <0.05) reduction (28%,65%) in serum MDA level compared to doxorubicin therapy, (3%) elevation and(13%) reduction after 21 and 42 days respectively compared to vitamin E therapy, but was also higher than that compared with controls.

Table (2) revealed that: The level of serum CPK level was significantly elevated (p <0.05) with doxorubicin therapy (17%,14%) after 21 and 42 days respectively compared with baseline value. Vitamin E therapy produced no significant change in serum CPK level after 21 days, but only a slight significant (p <0.05) elevation (4%) after 42 days compared to 21 days therapy. A significant (p <0.05) reduction in serum CPK level (16%) was observed after 21 days compared with doxorubicin therapy. Regarding therapy with a combination of vitamins E and C there was only a significant reduction (p <0.05) in serum CPK level (28%) after 21 days, but no significant change after 42 days of therapy compared to baseline. This combination produced a significant (p <0.05) reduction (38%) in comparison with doxorubicin therapy after 21 days, but no significant change after 42 days of therapy. Vitamin E therapy also significantly (p <0.05) reduced serum CPK level (26%) after 21 days of therapy, but with no significant change after 42 days of therapy. Table (3) showed the following: There was a significant (p <0.05) elevation in serum LDH level (9%,11%) after 21 and 42 days respectively with doxorubicin therapy compared to baseline. Concerning vitamin E therapy, there was no significant change in serum LDH level after 21 days while a slight significant (p <0.05) reduction (9%) after 42 days compared with baseline. Combination of vitamins E and C therapy also produced a slight

significant (p <0.05) reduction in serum LDH level (3%) after 21 and no significant change at the end of therapy compared to baseline. Therapy with vitamins E and C combination also produced a significant (p <0.05) reduction compared to doxorubicin therapy (14%,18%) after 21 and 42 days respectively and in comparison with vitamin E therapy, the combination significantly (p <0.05) reduced serum LDH (16%,11%) after 21 and 42 days respectively.

Discussion:

Breast cancer is one of the most common cancers in women of the developed and developing countries(17). With one million new cases in the world each year, it becomes the commonest malignancy in women and comprises 18% of all female cancers worldwide (18). The risk factors associated with breast cancer, may exert their effects via generation of reactive oxygen species such as superoxide radical ($O2^{\cdot-}$), hydroxyl radical (·OH), hydrogen peroxide(H2O2) which induce oxidative damage of DNA and neoplastic transformation(19). The data presented in this study show that serum MDA levels were significantly (p<0.05) increased in BC patients compared with normal control which may be attributed to over production of ROS, a state of systemic oxidative stress or a deficiency of antioxidant defense, table (1. Our results were also consistent with that recorded by Ray G. et al.(20) who study lipid peroxidation , free radical production and antioxidant status in BC patients, the rate of superoxide anion and hydrogen peroxide production was significantly higher in BC patients than normal. Free radical hypothesis has been obtained from *in vitro* studies that doxorubicin increases lipid peroxidation and free radical production in heart tissue(21). This hypothesis was supported by our study and consistent with that reported by Eser Öz and Mustafa N.İlhan et al.(22), who found that in doxorubicin-treated animals, the MDA levels of kidney, lung, liver and brain tissues were significantly increased compared to control rats. Veselina Gadjeva et al.(23)found plasma MDA level was significantly elevated in lymphoproliferative cancer patients treated with doxorubicin.

Vitamin C is able to diminish DNA damage by reducing radical species directly, decreasing formation of reactive species such as lipid hydroperoxides or preventing radical attack on proteins that repair DNA(24) whereas in many cell types, vitamin E accumulates primarily in lysosomes and mitochondria, where high levels of oxidative stress are presumed to exist and acts within these membranes by preventing propagated oxidation of saturated fatty acids because of its function to interrupt lipid peroxyl radicals((25,26,27). Thus vitamin E is an important structural component of biological membranes that contribute to their stability(28). Our results were consistent with that reported by K.Shahin et al.(29)who found that increased vitamin E supplementation decreased serum MDA level and also by T. Aghvami et al.(30)found that plasma vitamin E decreased significantly and MDA level increased significantly in BC patients as compared to the control. Vitamins E and C act potentially to prevent lipid peroxidation(31). The results of our study were consistent with that reported by Kazim Sahin et al.(32)who suggested that supplemental vitamin C and vitamin E reduce serum lipid peroxidation (MDA) level in laying hens. Another study by Antunes LM and Takahshi CS.(33)found that the efficiency of vitamin C and/or vitamin E in protection against doxorubicin-induced chromosomal damage due to free radicals production in rats was dose- dependent manner.

Treatment with doxorubicin is limited by its acute and chronic cardiotoxicity. The chronic toxicity is a cumulative, dose-dependent cardiomyopathy leads over time to a progressive left ventricular dilatation, loss of wall thickness, along with fibrosis, reduced contractility and insidiously evolves into congestive heart failure(34,35). Cardiotoxic effects of the drug have been demonstrated to increased oxidative stress caused by free radical over production and decrease in endogenous antioxidant reserve(36). Several mechanisms of doxorubicin's chronic cardiotoxicity which are not mutually exclusive, have been established and all contribute to the tissue selectively. First, the heart simply needs a high and continuous supply of ATP for its uninterrupted function and is thus extremely vulnerable. Second, the heart has a very low antioxidant capacity and is prone to the consequences of oxidative stress. For example, GP-peroxidase and catalase activity in heart muscle are very low. Third, doxorubicin can alter cardiac-specific transcription. Indeed, oxidative stress can alters the structure and function of lipid components that act as second messengers. Forth, disruption of the electron transport system and hydroxyl radical generation in mitochondria of cardiac cells by the formation of a semiqinone radical, which transfers its free electron rapidly to molecular oxygen, generating superoxide radicals, while the anthracycline is reoxidized to its original, quinone form(37). This mechanism of cardiotoxicity results in a significant increase in serum cardiac enzymes CPK and LDH as show in tables (2,3) which suggest an increase leakage from mitochondria due to generatation of free radicals that induce destructive myocardial injury leading to lysis of a number of myocytes, as well as loss of cytoplasmic membrane integrity(38).

The results in this study agree with those reported by Y. James Kang et al.(39)and Olson et al.(40)who

found that serum CPK and LDH levels were significantly increased by doxorubicin.

In this study serum LDH level was significantly elevated in baseline value before doxorubicin therapy is started as shown in table (2) which coincided with that reported by Seth PK et al.(41) and Sandhya Mishra et al.(42). This serum LDH level increment indicate that serum LDH might prove to be one of the most sensitive biomarker in carcinoma breast in early detection of the disease as diagnostic and prognostic.

Treatment with vitamin E produced a slight significant reduction in serum LDH level after 42 days compared with baseline. The combination of vitamins E and C produced a slight significant reduction in serum LDH after 21 days compared with baseline value. The reduction was significant after 21 and 42 days respectively in comparison with doxorubicin therapy and after 21 and 42 days respectively in comparison with vitamin E therapy that show a time and antioxidants potentiating-dependent manner as show in table (3).

Table (1): Effects of treatment with vitamin E and a combination of vitamins E and C on serum malonedialdehyde in breast cancer patients treated with doxorubicin

Group	Number of patients	Baseline serum MDA µmol/l	21 days post-treatment serum MDA µmol/l	42 days post-treatment serum MDA µmol/l
Control	10	0.633± 0.064	-	-
Doxorubicin	10	2.44±0.27a	3.2±0.22b	4.26±0.25c
Vitamin E+ Doxorubicin	12	2.84±0.18a	2.23±0.17 b†	1.73±0.17c†
VitaminE&C +Doxorubicin	10	3.14±0.28 a	2.29±0.24 b † *	1.51±0.18 c † *

Values are expressed as mean± standard error of mean
Results with non identical superscripts (a, b, c) within the same group were considered significantly different (P<0.05)
†= Significant at p<0.05 as compared with doxorubicin values
*= Significant at p<0.05 as compared with vitamin E and doxorubicin values
All MDA levels were significant at p<0.05 as compared with control values

Table (2): Effects of treatment with vitamin E and a combination of vitamins E and C on serum CPK level in breast cancer patients treated with doxorubicin

Group	Number of patients	Baseline serum CPK IU/l	21 days post-treatment serum CPK IU/l	42 days post-treatment serum CPK IU/l
Doxorubicin	10	86.20±12.63a	101.60±16.34 b	98.15±17.81 c
VitaminE+ Doxorubicin	12	89.70±15.16a	85.0±10.42a †	93.50±14.28b
Vitamin E&C + Doxorubicin	10	87.90±13.53a	62.90±7.19 b †*	87.20±16.15a

Values are expressed as mean± standard error of mean
Results with non identical superscripts (a, b, c) within the same group were considered significantly different (P<0.05)
†= Significant at p<0.05 as compared with doxorubicin values
*= Significant at p<0.05 as compared with vitamin E and doxorubicin values
Reference range: Men 24-195 IU/l, Women 24-170 IU/l

Table (3): Effects of treatment with vitamin E and a combination of vitamins E and C on serum LDH level in breast cancer patients treated with doxorubicin

Group	Number of patients	Baseline serum LDH IU/l	21 days post-treatment serum LDH IU/l	42 days post-treatment serum LDH IU/l
Doxorubicin	10	196.40±15.99 a	214.0±14.85 b	217.60±15.36 c
Vitamin E+ Doxorubicin	12	221.17±17.32 a	219.17±22.51 a	201.25±22.06 b
Vitamin E&C + Doxorubicin	10	191.00±18.18 a	185.20±14.00 b*†	178.60±13.64a *†

Values are expressed as mean± standard error of mean
Results with non identical superscripts (a, b, c) within the same group were considered significantly different (P<0.05)
†= Significant at p<0.05 as compared with doxorubicin values
*= Significant at p<0.05 as compared with vitamin E and doxorubicin values Reference range: 80-190 IU/l

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