Effect of tamoxifen on antioxidant vitamins, uric acid and gamma-glutamyl transpeptidase in breast cancer patients

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Abstract

Background: Many research works clearly indicate that free radical and reactive oxygen species play a major role in the etiology and development of breast cancer in postmenopausal women. Available literatures suggest that tamoxifen is a potent suppressor of lipid peroxidation in experimental animals**.**

Objectives: The objective of this study is to understand the antioxidant status and oxidative stress in breast malignancy of postmenopausal women before and after treatment with tamoxifen.

Methods: Eight to nine months' tamoxifen therapy (10 mg twice daily) in 19 postmenopausal women was conducted. Serum levels of vitamin A, E & C were determined; also uric acid& GGT were determined. The results were correlated with serum MDA levels. The results were compared with those in patients with breast benign tumors (N=21) and control group (N=23).

Results: A highly significant decrease in antioxidant vitamins levels in breast cancer patients were noticed (P<0.001) compared with those of benign tumor. Also a significant increase in uric acid, GGT, and MDA levels was observed in cancer patients (P<0.01). There was a significant increase in antioxidant vitamins (P<0.01) and significant decrease in uric acid, GGT and MDA levels in cancer patients after treatment with tamoxifen.

Conclusion: The results suggest that tamoxifen exerts a significant effect on the rate of lipid peroxidation and a major improvement in antioxidant status

Keywords: Breast cancer, Tamoxifen, Antioxidant Vitamins, GGT

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Introduction

Breast cancer is a complex and important malignancy among women in modern societies. In the year 2000 more than 500,000 death cases were attributed to worldwide breast cancer [(1)]. In Iraq this disease showed a multiple increases during the last decade according to the Ministry of Health [(2)]. Despite the importance of the disease, its etiology and pathogeneses have not been elucidated [(3)].

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury [(4, 5)]. The cellular oxidative damage is caused primarily by free radicals and reactive oxygen species. Free radicals have the ability to bind most normal cellular compounds; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids [(4)]. A disturbance of the balance between formation of active oxygen metabolites and the rate at which they are scavenged by different types of antioxidants is referred to as oxidative stress [(5)]. Prime targets of reactive oxygen species are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation, which may lead to damage of the cell structure and function [(6)]. Additionally, decomposition of lipid hydroperoxides yields a wide variety of

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end products, including malondialdehyde (MDA) [6]. MDA was considered as a marker of oxidative stress by many investigators [7].

Growing evidence has indicated the role of antioxidant vitamins in cancer prevention and treatment [8]. Antioxidant vitamins were known to prevent the cell damage caused by reactive oxygen species (ROS) and keep the immune system intact against the diverse effect of free radicals [9].

Tamoxifen, a nonsteroidal antiestrogenic drug is widely used in breast cancer cases. It induces tumor regression in women with advanced metastatic breast cancer [10]. The anti-tumor activity of tamoxifen is still uncertain. However, anti tumor activity of tamoxifen is largely believed to be due to occupying the intracellular estrogen receptor sites in target tissue thus, it's blocking the action of biologically active estrogen and estradiol. Recent reports demonstrate that tamoxifen exerts antiproliferative effects on estrogen receptor- positive breast cancer cells [11]. Additional anti proliferative effects of tamoxifen may be related to its inhibition of protein kinase [12] and it's binding to calmodulin, a protein that plays a role in DNA synthesis. Therefore, this drug acts as a suppressor of breast cancer not only through acting as a competitor to estrogen but also through mechanism. No previous studies had elucidated the relationship between antioxidant status and oxidative stress in breast cancer patients treated with tamoxifen.

In this study, the changes in serum levels of vitamin A, E & C, uric acid& GGT were determined in postmenopausal women before and after

treatment with tamoxifen. The results were correlated with those of MDA in the sera of the corresponding patients.

Patients and methods

Forty postmenopausal women inflicted with infiltrative ductal carcinoma were involved in this study. The patients were referred to Baghdad Teaching Hospital and the Hospital of Radiotherapy and Nuclear Medicine, Baghdad, Iraq, for the period starting from June 2004 to the end of July 2005. All cases were diagnosed by histopathological and radiological procedures provided in those medical centers. The mean age of the patients was 48±12 years with a range of 38-72 years. Body mass index of the corresponding patients was 21.9±7.5 Kg/m^2. Nineteen subjects of those cancer patients were treated with tamoxifen (10 mg twice daily) for eight to nine months by the same medical center.

Twenty-three patients with benign tumor (fibroadenoma) were used as pathological control. Healthy control group consisted of 21 postmenopausal women with comparable age and body mass index.

Ten milliliters samples of venous blood were taken from all groups. Those samples were taken in malignant cases before tamoxifen treatment (MBT group) and after treatment with the drug (MAT group). Blood samples were left for 20 minutes at room temperature. After blood coagulation, the sera were separated by centrifugation at 3000-x g for 15 minutes. Hemolyzed samples were discarded.

The concentration of vitamin A in the collected serum samples was determined according to the modified method of Neeld and Pearson [13]. Vitamin E was determined according to the method of Hashim & Schuttriger [14], while vitamin C was determined according to the method of Toronto [15]. Standard curves were established for each of the above determination using authentic samples of those vitamins. Serum uric acid was measured by enzymatic colorimetric assay using test kit supplied by uric acid Giesse diagnostics, Italy. Gamma glutamyl transpeptidase (GGT) activity was also

determined by the test kit of the above company. The method depends on the use of gamma glutamyl para anilide as substrate [16]. Malondialdehyde (MDA) levels were determined according to the method of Buege and Auts [17].

All results were expressed as mean ± SD. Descriptive and inferential statistic were used to describe the results and their interrelationship.

Results

Vitamin A shows a high significant decrease in cancer patients compared with that of patients with benign tumors as shown in table (2): the results show also a highly significant increase in the levels of this vitamin in cancer patients after treatment (MAT). A similar trend of significance was noticed in the serum level of vitamins E&C in different groups.

A significant decrease in serum GGT activity occurs in MAT group compared with MBT group. Similar changes were observed in the levels of serum uric acid of the corresponding groups. A gradual and significant decrease in serum MDA levels in MAT group compared with MBT group was seen.

(Table 3) shows ANOVA analysis and the results of correlation between oxidative stress index (represented by MDA level) and concentration of antioxidant vitamins, uric acid and GGT in MBT group and MAT group of breast cancer patients.

Highly significant correlations were noticed between MDA& vitamin A ($P<0.001$). Also a highly significant correlation was observed between MDA& vitamin C ($P<0.01$) and MDA& uric acid ($P<0.001$) while there is a significant correlation between MDA& vitamin E ($P<0.05$).

Table 1: Serum levels of antioxidant vitaminA,E &C, uric acid, gamma- glutamyl transpeptidase (GGT), and malondialdehyde (MDA) in different cases of breast tumors.

Gtoup type (No.of studies)	Component	Mean	SD	SE
(MBT)(N=40)	Vit.A (mg/dl) $x10^{-3}$	16.06	8.99	1.42
(MAT)(N=19)		48.31	17.60	4.01
(B)(N=23)		39.24	15.69	3.27
(C)(N=21)		51.49	21.74	4.74
MBT	Vit.E (mg/dl) $x10^{-2}$	36.68	18.67	2.90
MAT		77.70	20.75	4.64
B		82.93	22.78	4.75
C		102.74	27.96	6.01
MBT	Vit.C (mg/dl)	0.387	0.141	0.031
MAT		1.110	0.349	0.078
B		1.067	0.321	0.067
C		1.621	0.527	0.115
MBT	GGT (IU/L)	103.98	18.88	4.22
MAT		41.16	9.46	2.15
B		39.13	7.83	1.63
C		20.72	8.64	1.88

MBT	Uric Acid (mmol/L)	539.2	41.3	9.24
MAT		380.5	63.5	14.20
B		346.3	114.3	23.80
C		237.5	108.6	23.10
MBT	MDA (µmol/L)	2.211	0.312	0.061
MAT		0.988	0.323	0.072
B		0.949	0.315	0.065
C		0.701	0.179	0.039

MBT: Malignant before treatment
MAT: Malignant after treatment
B: Benign tumor group
C: healthy control

(Table 1) reveals the mean values of the sera levels of vitamin A, vitamin E, vitamin C, GGT activity, uric acid and MDA in women with breast malignancy before treatment (MBT group) and for those after treatment with tamoxifen (MAT group). The table shows also the mean values of the up-mentioned components in postmenopausal women with benign tumors (group B) and those for healthy women as control group (group C).

Table 2: The comparison of serum components in the different groups of breast tumors and healthy control.

	P-Value			
Component	**M vs. C**	**M vs. B**	**M vs. C**	**MAT vs. MBT**
Vit. A	0.001	0.001	0.01	0.001
Vit. E	0.01	0.01	0.01	0.001
Vit. C	0.01	0.02	0.04	0.001
GGT	0.01	0.01	0.05	0.01
Uric Acid	0.001	0.01	0.01	0.001
MDA	0.01	0.02	0.05	0.01

GGT: Gamma- glutamyltranspeptidase
MDA: Malondialdehyde

Table 3: Correlation coefficients and the significance levels of different serum chemical components in patients with breast tumors.

Component vs MDA	MBT					MAT				
	Slope	**Intercept**	**R^2**	**r**	**P**	**Slope**	**Intercept**	**R^2**	**r**	**P**
Vit. A	-67.4	2.28	0.372	0.160	<0.05	-13.86	1.66	0.659	0.754	<0.001
Vit. E	-2.25	2.75	0.432	0.658	<0.02	-0.73	1.55	0.220	0.466	<0.05
Vit, C	-0.002	0.98	0.151	0.386	<0.1	-0.003	0.32	0.46	0.676	<0.001
GGT	0.01	1.12	0.783	0.884	<0.001	0.02	0.12	0.380	0.616	<0.001
Uric acid	0.002	0.98	0.150	0.386	<0.1	0.003	0.03	0.460	0.676	<0.001

GGT: Gamma- glutamyltranspeptidase
MDA: Malondialdehyde

Discussion

There is a growing evidence for the role of free radicals and reactive oxygen species in the initiation and promotion of different kinds of malignant tumors [7]. Many investigators attributed such increased incidence of cancer with advancing age to the increasing level of free radicals reaction with age and to decreased ability of the immune system to detoxify those free radicals [8, 9]. A variety of pathological events such as diabetes, atherosclerosis and aging were also attributed to the enzymatic and non-enzymatic oxidation of biological molecules [7].

Many vitamins, enzymes, organic molecules and trace elements play a major role in scavenging those free radicals generated from food oxidation and many pollutants.

The results in (table 1) reveal a decrease in the levels of vitamins A, E&C in cancer patients. Such decreases may play a great role in the development of malignancy, since that antioxidant vitamins reveal great actions in the physical and chemical quenching of oxygen and superoxide radicals generated from oxidation processes inside the human cell. Vitamin A was shown to be involved in the stimulation of the immune system and cancer suppressor genes as well as deregulate of oncogenes and block tumorgenesis [18, 19]. Vitamin E was reported to be an important factor in the induction of apoptosis of cancer cells beside its action in quenching of free radicals and increase of the capability of the immune system [20]. Vitamin C is considered the most powerful natural antioxidant, which protects indispensable macromolecule in the human body from damage by free radicals.

The decrease in serum levels of antioxidant vitamins was greater in Iraqi women of breast cancer in comparison with western population. This can be attributed to nutritional differences among different societies and to the

difference in concentration of air, water and food pollutants among those populations [7].

The increase in serum uric acid levels in cancer patients can be referred to tissue hypoxia, which may lead to a concomitant increase in xanthine oxidase activity as reported by Moison et al. [21]. Uric acid is a well-known antioxidant in the human body and this phenomenon must lead to a decrease in its serum level with the development of malignancy. Such net increase in the concentration of uric acid in cancer patients can be attributed to the exceeding effect of increased xanthine oxidase activity (due to tissue hypoxia). This effect combined to increase cell damage, which is a recognized characterstic of cancer tissue. Our results are cosistent with those reported by Kolonel et al [22] and Hameed et al [23]. The significant increase in GGT activity in cancer patients can be attributed to the continuous release of this enzyme from the surface of cancer cells and its direct leakage to the blood circulation. GGT is a membrane-bound enzyme, which is affected significantly in cancer cell more than the normal cell due to changes of cell membrane accompanying carcinogenesis. It is noteworthy to consider this enzyme as tumor progression marker and can be used for monitoring of tumor regression also. Our results are more significant in comparison with those reported by Seth et al [24] and Mishra etal [25].

A highly significant change in MDA levels in cancer patients compared with other groups can be attributed to increase in the byproducts of lipid peroxidation due to transition to malignancy. (Table 3) shows a good negative correlation of this component with antioxidant vitamins while there was a significant positive correlation between MDA and each of uric acid &GGT.

The results of this study reveal the effect of tamoxifen treatment of antioxidant status and oxidative index (represented by serum MDA level) in the respective patients. The highly significant increase in the serum levels of antioxidant vitamins and the highly significant decrease in MDA&GGT after treatment reinforce the importance of using this drug for the recovery of oxidative status in breast cancer patients.

In fact the decrease in lipid peroxidation in the treated women is unexpected finding, because tamoxifen action was thought entirely to be due to its interference with biologically active estrogen and estradiol. Studies about estrogen and its metabolites show that estrogen is a natural antioxidant of membrane lipid peroxidation [26]. The inhibitory action of estrogen on lipid peroxidation remains obscure. However estrogens may donate hydrogen atoms from their phenolic hydroxyl groups to lipid peroxy radicals for the termination stage, moreover, the examination of the chemical structure of tamoxifen shows no apparent reason for why it acts as chain-breaking inhibitor of peroxidation, because there is no group bearing an easily donatable hydrogen atom. However antioxidant activity of tamoxifen may be explained in the following manner: during tamoxifen medication, tamoxifen is hydroxylated and demethylated in the liver under influnce of the enzyme cytochrome P-450 resulting in the formation of 4-hydroxy tamoxifen and N-desmethyl tamoxifen [27]. Like estrogen, 4-hydroxy tamoxifen has a more powerful inhibitory effect on lipid peroxidation than its parent compound. The phenolic hydroxyl group confers this chain breaking antioxidant property and the putative effects on membrane structure [11]. The clinical antiestrogenic activity of tamoxifen is likely because of the effect of the parent compound and its 4-hydroxy metabolite and its relative affinity for estrogen receptor sites [27].

The antioxidant vitamins in breast cancer patients before treatment (MBT group) were significantly lower than that of benign cases and control group as shown in (table 1). This may be due to the fact that in the cancerous stress condition, the requirements for vitamins increased progressively. Beside this, the increased levels of oxygen radicals in untreated patients themselves may reduce the availability of those vitamins in the blood of cancer patients. Both effects tend to reduce antioxidant vitamins level in the sera of cancer patients. In eight to nine months of treatment, the corresponding patients showed markedly elevated levels of antioxidant vitamins and concomitant decrease in uric acid and GGT levels. Reduction of serum MDA levels was noticed. These changes can be explained according to Jordan et al [28] observation who reported that tamoxifen tends to retard cell proliferation rather than kills such cells. The retarded cell proliferation leads to decrease in the utilization of antioxidant vitamins. This explanation can be reflected on the decrease in uric acid, GGT and MDA levels in the sera of MAT group in comparison with MBT group of breast cancer patients.

Finally, from all the above observations it can be concluded that tamoxifen inhibits the effects of free radicals and initiates the regeneration and recovery of antioxidant vitamins in women.

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