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ORIGINAL ARTICLE

Augmented antioxidant status in Tamoxifen treated postmenopausal women with breast cancer on co-administration with Coenzyme Q_{10}, Niacin and Riboflavin

Srinivasan Yuvaraj · Vummidi Giridhar Premkumar · Kothandaraman Vijayasarathy · Sitthu Govindaswamy Dinakaran Gangadaran · Panchanatham Sachdanandam

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Abstract
Background Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide (H_2O_2), hydroxyl radical have been implicated in pathogenesis of various diseases including cancer and metastasis. Tamoxifen (TAM) is a non-steroidal anti-estrogen drug most widely used as an adjuvant hormonal therapy in breast cancer. TAM also has estrogenic activity on liver and endometrium causing severe oxidative stress and hypertriglycerdemia. Coenzyme Q_{10} (CoQ_{10}), Niacin and Riboflavin are well-known potent antioxidants and protective agents against many diseases including cancer. In this context, this study was undertaken to find if co-administration of CoQ_{10}, Niacin and Riboflavin along with TAM could augment the antioxidant (AO) status in postmenopausal women with breast cancer.
Methods The vitamin supplementation with Tamoxifen was given for a period of 90 days. Blood samples were collected at the base line, 45th and 90th day during the course of treatment. Plasma lipids, lipid peroxides and various circulating enzymatic and non-enzymatic antioxidants were estimated in 78 untreated, sole TAM treated and combinatorial treated group along with 46 age- and sex-matched controls.
Results Enhanced oxidative stress as evidenced by increased lipids and lipid peroxides with decreased AO levels in untreated breast cancer patients was observed. Adjuvant TAM-treated group had a limited impact on the increased oxidative stress with decreased AO status. Severe hypertriglycerdemia was observed in TAM-treated group when compared to untreated and control subjects. Combinatorial therapy (CT) of CoQ_{10}, Niacin and Riboflavin along with TAM decreased the oxidative stress and increased the AO status.
Conclusion The antioxidant defense system is compromised in breast cancer patients. There is a shift in the oxidant / antioxidant balance in favor of lipid peroxidation (LPO), which could lead to tumour promotion observed in the disease. CT of CoQ_{10}, Niacin and Riboflavin along with TAM significantly increased the AO status, while decreasing lipid and lipid peroxides. The results suggest the necessity of therapeutic co-administration of antioxidants along with conventional drug to such patients. However, due to limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definitive conclusion, in terms of safety and efficacy of adding an AO therapy in treatment of breast cancer.

Keywords Breast cancer · Tamoxifen · Oxidative stress · Combinatorial therapy · CoQ_{10} · Niacin · Riboflavin

S. Yuvaraj · V. G. Premkumar · P. Sachdanandam (✉)
Department of Medical Biochemistry,
Dr. ALMP-GIBMS, University of Madras,
Taramani Campus, Chennai 600 113, Tamil Nadu, India
e-mail: psachdanandam@yahoo.co.in

K. Vijayasarathy · S. G. D. Gangadaran
Department of Medical Oncology,
Government Royapettah Hospital,
Kilpauk Medical College,
Chennai 600 014, Tamil Nadu, India

Introduction

An estimated 178,480 new cases of invasive breast cancer with 40,910 breast cancer deaths are expected to occur among women in US during 2007. Death rates from breast cancer in older women (age above 50 years) continuous to be higher than younger women (below 50 years) [1]. A further

problem is approximately 60% of patients with cancer are aged 65 years or over [2]. Age adjusted rates from breast cancer are 176 patients higher in developed than in developing nations [3]. In India, breast cancer is the second most common cancer in south India. According to a population-based study, the observed survival rate of breast cancer in Chennai is 48% [4].

During last two decades, a considerable attention has been focused on the involvement of free radicals in various diseases. ROS are produced by a number of processes in vivo is highly reactive and toxic. An imbalance between the production and detoxification of ROS results in oxidative stress [5]. ROS has been implicated in the pathogenesis of certain diseases, including cancer [6]. ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA), one of the end products of LPO. MDA may be involved in tumour promotion because it can interact with functional groups of a variety of cellular compounds [7]. However, biological system has evolved an array of enzymatic and non-enzymatic antioxidant defense mechanism to combat the deleterious effects of ROS. Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion into H_2O_2. Catalase and glutathione peroxidase (GPx) metabolizes H_2O_2 into water and molecular oxygen, while oxidizing glutathione which is further reduced back to its reduced state by glutathione reductase in presence of NADPH [8, 9].

The introduction of Tamoxifen, a non-steroidal anti-estrogen in early 1970s represented a landmark in the treatment of breast cancer. The anti tumor activity of TAM is largely believed to be due to its occupation of intracellular estrogen receptor sites in the target tissues and blocking the action of biologically active estrogen and estradiol. Since 1990, death rates from breast cancer have decreased by over 25% and this is at least part due to adjuvant treatment with TAM taken for a period of 5 years [10]. However, Adlard et al. found that patients experienced significant morbidity and toxicity while taking adjuvant TAM therapy [11]. TAM-induced fatty liver is observed in more than 30% of breast cancer patients who received adjuvant TAM treatment [12]. Studies from our lab and others have shown TAM-induced lipid abnormalities in breast cancer patients [13, 14]. Further, TAM leads to oxidative liver damage and it has been elucidated to be a hepato-carcinogen in rodents [15], which is due to ROS over-production that occurs during TAM metabolism. TAM forms DNA adducts and undergoes metabolic activation to an electrophillic species that binds to cellular macromolecules, which therefore become carcinogenic by a genotoxic mechanism [16]. Studies have suggested that oxidative stress might underline in the pathogenesis of TAM-induced toxicity [17]. Hence, this study is focused on reducing the contraindication of sole TAM treatment with an antioxidant vitamin combination of Coenzyme Q_{10} (CoQ_{10} 100 mg), Niacin (50 mg) and Riboflavin (10 mg).

CoQ_{10} is a lipid-soluble benzoquionone found in all tissues and membranes. CoQ_{10} is particularly high in the inner mitochondrial membrane, where it functions as an electron carrier in oxidative phosphorylation. CoQ_{10} is an endogenously synthesized lipid-soluble anti-oxidant and protects membrane phospholipids and serum LDL from lipid-peroxidation [18]. In the recent years, there is a growing body of evidence on protective role of CoQ_{10} in cardio-vascular diseases and cancer [19–21]. Riboflavin (vitamin B_2) a water soluble vitamin in its active coenzyme forms such as flavin mononucleotide (FMN^+) and flavin adenine dinucleotide (FAD^+), participates in redox processes involving one and two-electron transition and non-redox reactions such as photo-repair of thymidine dimers in photo-damaged DNA and the dehydration of non-activated organic substrates [22]. Niacin (nicotinic acid, Vitamin B_3) a water-soluble vitamin, serves as a precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both NAD and NADP can be reduced to NADH and NADPH, respectively and these coenzymes participate in oxidation-reduction reactions catalyzed by dehyrogenase and oxidoreductase enzymes. The NAD/NADP linked enzyme systems are involved in virtually every aspect of metabolic processes. Currently, Niacin is one of the premier lipid lowering agents available to treat various cardio-vascular diseases with its first clinical utility, which dates back to 1954 [23]. In this context, efforts were undertaken to understand if co-administration of CoQ_{10}, Niacin and Riboflavin along with TAM could augment the antioxidant status in postmenopausal women with breast cancer.

Materials and methods

Selection of patients

Seventy eight consecutively treated postmenopausal with resectable breast cancer were recruited from the Department of Medical Oncology, Government Royapettah Hospital, Chennai, India through their physicians according to the process approved by the institutional human ethical review board. Informed consent was obtained from all the subjects with due explanation before entering into the study. The patients characteristics are given in Table 1. Age younger than 70 years with potentially curable and histopathologically confirmed breast cancer patients were recruited. Patients with diabetes mellitus, renal and hepatic diseases were excluded from the study. Patients who were on vitamin supplementation, hypolipidimic or estrogen

Table 1 Characteristics of the patients and their disease ($N = 78$ patients)

Characteristics	*N*	%
Tumor size (T)		
T1	5	6.4
T2	31	39.7
T3	24	30.7
T4	18	23.0
Nodal status (N)		
N0	24	30.7
N1	41	52.5
N2	12	15.3
N3	1	1.2
N4	0	0
Metastasis (M)		
M0	78	100
M1	0	0
Histology		
Ductal invasive	76	97.4
Lobular invasive	2	2.5
Surgery		
Conservative	2	2.5
Mastectomy		
Simple	10	12.8
Radical	2	2.5
Modified radical	49	62.8
Patey's	9	11.5
Modified Patey's	2	2.5
No surgery	4	5.1
Family history of cancer		
Yes	9	11.5
No	69	88.5
Diet		
Mixed	73	93.5
Vegetarian	5	6.5

Age: median 57 years; range 43–70 years

N Number of patients; % Percentage

replacement therapeutic drugs were also excluded from the study. Age and socio-economically matched healthy controls were recruited from the hospital visitors, who were non-blood related to the individuals with disease and who have no family history of cancer.

Blood sampling and study design

Blood samples were drawn by venous arm puncture into EDTA tubes. The plasma was separated by centrifugation at 1500*g* for 15 min at 4°C. After aliquation of plasma the buffy coat was decanted and the packed red blood cells were washed thrice with physiologic saline. A known volume of erythrocytes was lysed with hypotonic phosphate buffer, pH 7.4. The hemolysate was separated by centrifugation at 2500*g* for 15 min at 4°C. The biochemical estimations were carried out immediately.

Blood samples were drawn from the disease-free, healthy, age-matched postmenopausal women (Group I), untreated breast cancer patients (Group II), breast cancer patients who were on Tamoxifen (20 mg a day, Nolvadex, AstraZeneca, India, 99.7% purity) therapy for more than a year (Group III), group III patients after blood sample collection were recruited into group IV and were supplemented with CoQ_{10} (100 mg Kaneka Q10, Kaneka Corporation, Japan, 99.5% purity), Riboflavin (10 mg, Madras Pharmaceuticals, India, 99.7% Purity) and Niacin (50 mg, Madras Pharmaceuticals, 99.7 % purity) along with Tamoxifen (20 mg/day) and blood samples were drawn at the end of 45th day (Group IV). Group IV patients continued the same protocol and blood samples were collected at the end of 90th day (Group V).

Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi [24]. The pink chromogen produced by the reaction of thiobarbituric acid with MDA, a secondary product of LPO was estimated at 532 nm. The plasma total cholesterol (TC) and triglycerides (TG) were assayed using commercial kit (Agappe Diagnostics, India). Plasma phospholipids (PL) were estimated by the method of Rouser et al., after digesting the lipid extract with perchloric acid [25]. Superoxide dismutase was assayed by the method of Marklund and Marklund based on the 50% inhibition of pyrogallol auto-oxidation at 420 nm [26]. The catalase activity was assayed according to the method of Sinha [27]. GPx and glutathione reductase (GRx) were assayed using the commercial kit (Randox Laboratories, UK) according to the manufactures instruction. The activity of glutathione-S-transferase was estimated by the method of Habig et al. by following the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenze (CDNB) as substrate [28]. The reduced glutathione (GSH) was determined by the method of Beutler et al. based on the development of a yellow color when 5,5′-dithiobio (2-nitrobenzoic acid) (DTNB) is added to compounds containing sulfhydryl groups [29]. Plasma ascorbic acid was estimated by the method Omaye et al., in which dehydro ascorbic acid is coupled with 2,4 dinitro phenyl hydrazine (DNPH) and when treated with sulfuric acid, it forms an orange red color compound, which was measured at 520 nm [30]. Vitamin A was measured according to method of Hansen by fluorometric analysis [31]. Plasma vitamin E was measured by the method of Barker and Frank [32]. The method involves the reduction of ferric ions to ferrous ions by α-tocopherol and the formation of a colored complex with 2, 2′-dipyridyl. Absorbance of the chromophore was measured at 520 nm. Hemoglobin in the hemolysate was measured according to the method of Darbkin and Austin [33]. Blood was diluted in an alkaline medium containing potassium cyanide and potassium ferricyanide. Hemoglobin oxidized

to methemoglobin combines with cyanide to form cyanmethemoglobin which was measured at 540 nm. Plasma was used for TC, PL, TG, LPO, vitamin A, vitamin E and vitamin C assays, while RBC were used for GSH, SOD, CAT, GPx, GRx and GST investigations.

Statistical analysis

The experimental data were analyzed for significant differences by independent student *t* test and paired *t* test using Statistical Package for Social Sciences (SPSS, version 11.0, Chicago, USA). Values were expressed as mean ± standard deviation (SD). *P* value < 0.05 was considered statistically significant.

Results

Table 1 depicts the patient's characteristics of TAM-treated patients. Group I comprised of healthy age and socio-economically matched post-menopausal women. Group II subjects were newly diagnosed and untreated post-menopausal women with breast cancer. Groups III–V are same patients followed on supplementation therapy for over a period of 90 days. Table 2 shows the levels of plasma lipids and lipid peroxides in control; TAM and CT breast cancer patients. The levels of plasma TC, TG and TBARS were significantly higher in untreated and TAM-treated patients when compared to the control subjects. Plasma TG levels were significantly higher in the TAM treated group as compared to untreated group ($P<0.001$). The levels of TC, PL and TBARS were non-significantly decreased in TAM treated group as compared to untreated subjects. On exogenous supplementation of AO along with TAM for 45 and 90 days, significant decrease in the lipid and lipid peroxide levels in groups IV and V subjects were observed. Figure 1 depicts the age associated TBARS levels in control, TAM and combinatorial treated group. A drastic age associated increase in TBARS levels were observed in all the groups.

Table 3 depicts the circulating antioxidant enzyme levels in control, TAM and CT group. There has been a significant decrease in all investigated AO enzymes in untreated and TAM treated group when compared to control subjects. These values were reverted back to near normal levels on vitamin supplementation for 45 days with CoQ_{10}, Niacin and Riboflavin. The AO levels between 45 and 90 days of CT showed no significant difference and the levels were comparable to that of control subjects. Table 4 depicts the circulating non-enzymatic anti-oxidant levels in control, TAM and CT group. The levels of all the non-enzymatic antioxidants (GSH, vitamin A, C and E) were found to be significantly decreased in untreated and TAM-treated group when compared to normal subjects. CT for 45 and 90 days

Table 2 Effect of Coenzyme Q_{10}, Niacin and Riboflavin on plasma lipid and lipid peroxide levels in postmenopausal breast cancer patients treated with Tamoxifen

Parameters	Group I control, normal age-matched, post menopausal women (46)	Group II Pretreatment untreated breast cancer women (78)	Group III Treatment with Tamoxifen (78)	Group IV 45 days after treatment with CoQ_{10}, Riboflavin and Niacin along with Tamoxifen (78)	Group V 90 days after treatment with CoQ_{10}, Riboflavin and Niacin along with Tamoxifen (78)
Total cholesterol (mg/dl)	199.47 ± 19	277.81 ± 26.17 ^{a***}	252.21 ± 25.96 $^{b***, c NS}$	209.50 ± 19.97 ^{d***}	202.09 ± 19.09 $^{e***, f NS}$
Phospholipids (mg/dl)	247.97 ± 21.70	294.89 ± 23.45 ^{a**}	270.36 ± 26.79 $^{b NS, c NS}$	258.77 ± 22.34 ^{d*}	250.03 ± 22.97 $^{e*, f NS}$
Triglycerides (mg/dl)	159.36 ± 12.09	186.99 ± 16.45 ^{a**}	225.93 ± 24.41 $^{b**, c***}$	189.09 ± 19.64 ^{d***}	166.73 ± 16.97 $^{e***, f NS}$
TBARS (nmoles/ml)	3.03 ± 0.41	5.12 ± 0.86 ^{a***}	4.51 ± 0.65 $^{b***, cNS}$	2.92 ± 0.37 ^{d***}	2.77 ± 0.31 $^{e**, f NS}$

Values are expressed as mean ± SD. Number of subjects are indicated in parentheses

Comparisons were made between: [a]Group I and Group II; [b]Group I and Group III; [c]Group II and III; [d]Group III and IV; [e]Group III and Group V; [f]Group I and Group V

Statistical significance expressed as; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant

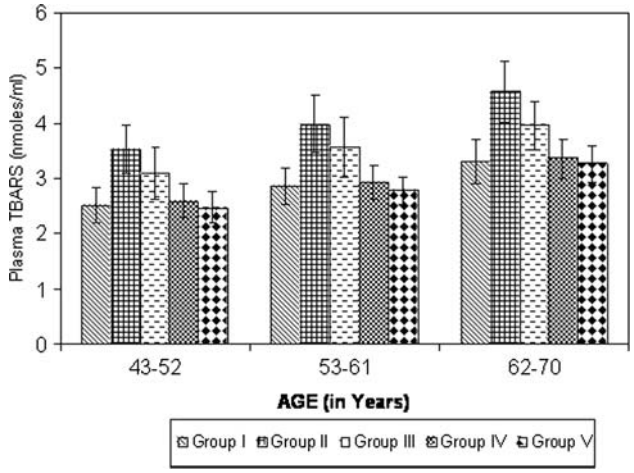

Fig. 1 Age associated lipid peroxidation in Control, TAM and CT subjects

has significantly increased the non-enzymatic AO status to normal levels comparable to the control subjects.

Discussion

The results of the study indicate that plasma lipids and lipid peroxides were higher in the untreated and TAM-treated postmenopausal breast cancer patient group than in the control group. In addition, concentration of several antioxidant vitamins and enzymes in the plasma and blood in untreated and TAM-treated group were lower than the control group. TAM treated patients on co-administration with $CoQ_{10,}$ Niacin and Riboflavin significantly increased the antioxidant concentration and decreased the plasma lipid and lipid peroxide levels as observed in the supplemented (group IV and V) patients.

Many studies have indicated the correlation of lipids and lipoproteins with the risk of breast cancer [34]. The exact mechanism by which lipid and lipoproteins contribute to carcinogenesis is not clearly understood. However, reports suggest that lipid peroxidation product; MDA may cross-link proteins and DNA on the same and opposite strands [35]. An earlier study reported that lipids might primarily affect the gonads, and subsequently higher estradiol secretion could influence the development of malignancies in the mammary glands and lymphoid system [36]. In the present study, higher levels of plasma lipid and lipid peroxides were found in untreated and TAM-treated patients. Severe hypertriglycerdemia was observed in the TAM-treated patients, when compared to untreated and control subjects as reported earlier from our laboratory [37]. A similar kind of lipid profile derangement with age related increase in MDA levels have been reported by several investigators [6, 34]. Reports also suggests that higher concentration of

Table 3 Effect of Coenzyme Q_{10}, Niacin and Riboflavin on blood antioxidant enzymes in postmenopausal breast cancer patients treated with Tamoxifen

Parameters	Group I: control, normal age-matched, Post menopausal women (46)	Group II: pretreatment, untreated breast cancer women (78)	Group III: treatment with Tamoxifen (78)	Group IV: 45 days after treatment with CoQ_{10}, Riboflavin and Niacin along with Tamoxifen (78)	Group V: 90 days after treatment with CoQ_{10}, Riboflavin and Niacin along with Tamoxifen (78)
SOD (U/g Hb)	820.24 ± 65.37	613.49 ± 58.14 [a***]	636.67 ± 67.24 [b**, c NS]	828.82 ± 90.28 [d **]	824.21 ± 86.58 [e**, f NS]
CAT (U/g Hb)	164.83 ± 17.51	119.78 ± 16.37 [a**]	137.66 ± 15.26 [b *, c NS]	159.48 ± 17.38 [d *]	162.59 ± 18.55 [e NS, f NS]
GPx (U/g Hb)	49.59 ± 7.31	36.21 ± 5.27 [a*]	38.17 ± 5.25 [b *, c NS]	53.81 ± 6.27 [d*]	56.29 ± 7.11 [e*, f NS]
GRx (U/g Hb)	8.21 ± 1.26	5.51 ± 0.86 [a*]	5.77 ± 0.67 [b *, c NS]	8.01 ± 1.86 [d *]	8.77 ± 1.67 [e*, f NS]
GST (U/g Hb)	2.21 ± 0.34	1.12 ± 0.24 [a*]	1.42 ± 0.21 [b**, c NS]	2.42 ± 0.26 [d*]	2.53 ± 0.21 [e*, f NS]

Values are expressed as mean ± SD. Number of subjects are indicated in parentheses

Comparisons were made between: [a]Group I and Group II; [b]Group I and Group III; [c]Group II and III; [d]Group III and IV; [e]Group III and Group V; [f]Group I and Group V

Statistical significance expressed as; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant

Table 4 Effect of Coenzyme Q_{10}, Niacin and Riboflavin on blood non-enzymatic antioxidants in postmenopausal breast cancer patients treated with Tamoxifen

Parameters	Group I: control, normal age-matched, Post menopausal women (46)	Group II: pretreatment, untreated breast cancer women (78)	Group III: treatment with Tamoxifen (78)	Group IV: 45 days after treatment with Co Q_{10}, Riboflavin and Niacin along with Tamoxifen (78)	Group V: 90 days after treatment with Co Q_{10}, Riboflavin and Niacin along with Tamoxifen (78)
Reduced glutathione (GSH) (μ mol/L)	1354.31 ± 150.66	538.81 ± 71.37 ^{a***}	702.17 ± 90.91 $^{b***, c NS}$	1362.29 ± 186.57 ^{d***}	1378.11 ± 170.12 $^{e***, f NS}$
Vitamin C (mg/L)	8.11 ± 1.14	5.22 ± 1.27 ^{a*}	5.81 ± 1.11 $^{b*, c NS}$	8.90 ± 1.57 ^{d*}	8.67 ± 1.67 $^{e*, f NS}$
Vitamin A (μg/dl)	260.12 ± 35.71	172.19 ± 25.81 ^{a**}	192.28 ± 26.92 $^{b*, c NS}$	275.75 ± 39.21 ^{d**}	270.21 ± 36.21 $^{e*, f NS}$
Vitamin E (μg/ml)	18.21 ± 2.12	11.21 ± 1.72 ^{a***}	12.91 ± 1.77 $^{b**, c NS}$	20.86 ± 2.41 ^{d***}	22.12 ± 2.36 $^{e***, f NS}$

Values are expressed as mean ± SD. Number of subjects are indicated in parentheses

Comparisons were made between: [a]Group I and Group II; [b]Group I and Group III; [c]Group II and III; [d]Group III and IV; [e]Group III and Group V; [f]Group I and Group V

Statistical significance expressed as; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant

TG may lead to decreased level of sex hormone-binding globulin, resulting in higher amount of free estradiol, which may likely to increase breast cancer risk [38]. In the current study, it has been observed that the CT reduces the lipid and lipid peroxide levels, which may also be due to reduced serum TNF-α level as observed in our earlier study [39]. TNF-α, a pro-inflammatory cytokine could exert a potential role in promoting loco-regional recurrence in breast cancer patients and in process of hypercholesterolemic atherosclerosis [40, 41]. CoQ_{10} is the only endogenously synthesized lipid-soluble antioxidant and protects membrane phospholipids and serum low density lipoprotein (LDL) from lipid peroxidation. Two different mechanisms of CoQ_{10} antioxidant function have been postulated: (1) it may act independently as a chain-breaking antioxidant, providing hydrogen atoms to reduce peroxyl and / or alkoxyl radical [42], or (2) it may form a redox interaction with other lipid-soluble antioxidants such as vitamin E by regenerating it from its phenoxyl radical form [43], which increases the LPL activity. Administration of different antioxidants quenches different free radicals: Riboflavin and Niacin neutralize hydroxyl and superoxide radicals [44], while CoQ_{10} quenches singlet oxygen and polyunsaturated fatty acid radicals [43]. The observed decrease in TAM-induced hypertriglycerdemia on CT might also be attributed to the hypolipidimic activity of Niacin, which lowers triglycerides and apolipoprotein-B containing lipoproteins (e.g. VLDL and LDL) mainly by decreasing fatty acid mobilization from adipose tissue triglycerides stores and by inhibiting hepatocyte diacylglycerol acyltransferase and triglycerides synthesis leading to increased intracellular apo-B degradation and subsequent decreased secretion of VLDL and LDL particles [45].

Antioxidant enzyme like SOD, CAT and GPx form the first line of defense against ROS and decrease in the activities contributes to the oxidant assault on cells. A significant decrease in all the circulating enzymatic antioxidants was found in untreated and TAM-treated patients, which was in line with a number of published reports [17, 46, 47]. However, contrary to our findings, increased levels of SOD, GPx and GRx in erythrocytes of the patients with breast cancer have also been reported [48]. Antioxidant depletion in circulation may be due to increased scavenging of lipid peroxides as well as sequestration by tumor cells. Tumor cells have been reported to sequester essential antioxidants, such as GSH to meet the demands of growing tumor [49]. TAM leads to oxidative liver damage by production of oxygen radical during its metabolism in liver. TAM has also shown to induce nitric oxide production in breast cancer patients through enhancement of nitric oxide synthase II expression [50], which may be reason for the observed decrease in antioxidant status in TAM treated patients. Supplementation of CoQ_{10}, Niacin and Riboflavin significantly

increased the circulating enzymatic AO in groups IV and V patients as compared to control subjects. GSH, an important non-protein thiol, in conjugation with GPx and GST, plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging ROS [51]. FAD, the flavocoenzyme form of Riboflavin is a coenzyme for GRx, which mediates regeneration of reduced glutathione, while niacin supplies electron for generation of NADPH and increases the GPx activity, which could attribute to the observed increase of these enzymatic AO in groups IV and V patients [52]. CoQ_{10} has been reported to increase the mRNA expression of GST and various DNA repair genes [53]. Recent in vitro studies on HL-60 cells have shown an increase in protein levels of MnSOD and catalase on supplementation with Coenzyme Q [54].

A uniform significant decrease in all the non-enzymatic AO (GSH, vitamin C, E and A) was found in the untreated and TAM-treated patients, as compared to the control subjects, the observed decrease in the non-enzymatic antioxidants was in line with several published reports [55, 56]. A decrease in blood GSH in circulation has been reported in several diseases including malignancies [57]. Vitamin C the major water-soluble AO, which acts as a free radical scavenger can react with a vitamin E radical to yield a vitamin C by GSH [58]. Since the regeneration of both vitamin E and C requires GSH, deficiency of GSH may be responsible for reduced levels of these AO in breast cancer patients [57]. A significant increase in all the circulating non-enzymatic AO parameters were observed after co-administration of TAM with CoQ_{10}, Niacin and Riboflavin in groups IV and V patients. The observed increase in the antioxidant status may be due to the individual or synergistic AO activity of the vitamin combination. CoQ_{10} can rescue tocopheryl radicals produced by reaction with lipid or oxygen radical by direct reduction back to vitamin E. Without CoQ_{10} in a membrane, regeneration of vitamin E is very slow. CoQ_{10} also regenerates vitamin C outside the cell from ascorbate radical, while vitamin C inside the cell can be regenerated by a GSH-based system, which is augmented by Riboflavin supplementation [59]. The enhanced vitamin C level in the supplemented group could also attribute to the observed decreased in phospholipids levels, as vitamin C activates the phospholipase A_2 which helps in degradation of PL. The coenzymes of Niacin (NAD^+/NADP) linked enzyme systems are virtually involved in every aspect of metabolic process, apart from its oxygen radical scavenging activity and genome stabilizing property [60].

In conclusion, untreated and sole TAM-treated postmenopausal breast cancer patients were found to be oxidatively stressed as evidenced by the decreased antioxidant levels with increased lipid and lipid peroxide levels. Oral supplementation of CoQ_{10}, Niacin and Riboflavin along with TAM significantly increased antioxidant levels and decreased in lipid and lipid peroxide levels significantly. Further specifically designed experiments are required to find, the exact mechanism of TAM-induced oxidative stress, and more clinical studies would be required to evaluate the safety and efficacy of adding an antioxidant supplementation with conventional therapy for treatment of breast cancer.

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