Effect of Coenzyme Q_{10}, Riboflavin and Niacin on Serum CEA and CA 15-3 Levels in Breast Cancer Patients Undergoing Tamoxifen Therapy

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In breast cancer patients, it is not the primary tumour, but its metastases at distant sites that are the main cause of death. Circulating breast cancer tumour markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) are reliable indicators of impending relapse, in which an increasing tumour marker level is associated with a very likelihood of developing recurrence. In the present study, 84 breast cancer patients were randomized to receive a daily supplement of 100 mg coenzyme Q_{10} (CoQ_{10}), 10 mg riboflavin and 50 mg niacin (CoRN) one dosage per day along with 10 mg tamoxifen (TAM) twice a day. Serum CEA and CA 15-3 levels were elevated in untreated breast cancer patients (group II) and their tumour marker levels significantly reduced upon tamoxifen therapy for more than 1 year (group III). Group III patients supplemented with CoRN for 45 d (group IV) and 90 d (group V) along with tamoxifen significantly reduced CEA and CA 15-3 levels. This study suggests supplementing CoRN to breast cancer patients along with tamoxifen reduces the serum tumour marker level and thereby reduce the risk of cancer recurrence and metastases.

Key words breast cancer; coenzyme Q_{10}; riboflavin; niacin; CEA; CA 15-3

Breast cancer is a major public-health issue worldwide and according to estimates in 2002, there were 1151298 new cases of breast cancer diagnosed and 410712 deaths caused by breast cancer and more than 4·4 million women living with breast cancer worldwide.[1] The antitumour activity of tamoxifen (TAM) is due to its occupation in the intracellular estrogen receptor sites of the target tissue and blocking the action of biologically active estrogen and estradiol.[2] In addition, the antiproliferative effects of TAM may relate to its inhibition of protein kinase C[3] and it's binding to calmodulin, a protein that plays a role in DNA synthesis.[4] A complicating factor is the relapse in breast cancer patients undergoing tamoxifen therapy. In this subset of patients, treatment is only palliative and recurrent breast cancer is incurable.[5]

CoQ_{10} or ubiquinone is a lipid-soluble component of virtually all cell membranes and has an important role in the respiratory metabolism as a mobile electron and proton carrier in the mitochondrial electron transport chain.[6] CoQ_{10} also functions as an antioxidant, which protects the cells both directly by preventing lipid peroxidation and indirectly by regenerating other antioxidants such as ascorbate and α-tocopherol.[7] CoQ_{10} increases the phagocytic activity in tumour induced mice and is found to increase the levels of IgG[8] and decrease the levels of circulating tumour necrosis factor-α and interleukin-6.[9] Riboflavin, in its active coenzymic forms like flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), participates in oxidation-reduction reactions in numerous metabolic pathways and in energy production *via* the respiratory chain.[10] Riboflavin captures reactive metabolites like tamoxifen and carcinogens to form a complex and thereby, prevents formation of DNA adducts,[11] prevents DNA methylation and maintains genomic stability.[12,13] Niacin and its co-factors, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), are essential for a variety of oxidation-reduction reactions that comprise tissue respiration.[14] The protective effect of nicotinamide may be due to its action as a free radical scavenger.[15] Niacin participates in DNA synthesis and acts as substrate for enzyme poly(ADP-ribose) polymerase-1 (PARP-1) which produces poly(ADP-ribose). The enzyme PARP-1 is associated with base excision repair, cellular differentiation, gene expression and plays a role in p53 expression and activation. Hence, riboflavin is important for protecting normal cell from genetic toxicity for maintaining genomic stability while remaining cytotoxic to neoplastic cells.[16,17]

Studies from our laboratory with CoRN supplementation to TAM in murine model have established antitumour activity of the drug by increasing the expression of tumour suppressor gene MnSOD. CoRN supplementation restored lipid peroxide levels and activities of enzymic and non-enzymic antioxidants to near normalcy in rat mammary carcinoma and thus proving its mitochondrial antioxidant activity.[18] In rat mammary carcinoma the net ATP production was diminished, which ultimately leads to cancer cachexia. Treatment with CoRN enhanced the activities of the Krebs cycle enzymes, and oxidative phosphorylation consequently enhanced ATP production. This increased amount of ATP was utilized by normal cells for their routine metabolism, reducing the cancer cachexia.[19]

CEA and CA 15-3 are serum tumour markers used for monitoring the clinical course of breast cancer patients. Estimation of these tumour marker changes during treatment have been analysed and applied as guidelines to measure the effectiveness of treatment.[20,21] In this study, the treatment prognosis of supplementing breast cancer patients undergoing tamoxifen therapy with CoRN were evaluated by measuring the tumour marker levels of CEA and CA 15-3.

MATERIALS AND METHODS

Subjects Subjects were recruited from the Medical On-

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Table 1. Characteristic of Breast Cancer Patient's in the Study Group

Characteristics		*n*	%
Age: Median:	47 years		
Range:	50—75 years		
Tumour size (T):	T1	4	5
	T2	33	39
	T3	26	31
	T4	21	25
Nodal status (N):	N0	26	30.9
	N1	44	52.4
	N2	13	15.5
	N3	1	1.2
	N4	0	0
Metastasis (M):	M0	84	100
	M1	0	0
Menopausal status:	Pre-menopausal	0	0
	Post-menopausal	84	100
Histology:	Ductal invasive	82	97.6
	Lobular invasive	2	2.4
Surgery:	Conservative	2	2.4
	Mastectomy		
	Simple	11	13
	Radical	2	2.4
	Modified radical	56	66.6
	Patey's	10	12
	Modified Patey's	1	1.2
	No surgery	2	2.4
Family history of cancer:	Yes	9	10.7
	No	75	89.3
Diet:	Mixed	80	95.2
	Vegetarian	4	4.8
Treatment:	Tamoxifen alone	8	9.5
	Chemotherapy+tamoxifen	40	47.6
	Radiation+tamoxifen	26	31
	Chemotherapy+radiation +tamoxifen	10	11.9

Patient characteristic of group III, IV and V breast cancer women. *n*=number of patients, %=percentage of patients.

cology Department of 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 of the study to them. The study recruits were younger than 75 years, with histopathologically confirmed breast cancer. The patient's characteristics are given in Table 1.

Study Design Group I: Age and socio-economically matched disease free, healthy women ($n=42$).

Group II: Breast cancer patients before the commencement of treatment ($n=84$).

Group III: Breast cancer patients undergoing tamoxifen therapy for more than 1 year (10 mg twice a day) ($n=84$).

Group IV: Group III patients after 45 d treatment with 100 mg CoQ_{10}, 10 mg riboflavin and 50 mg niacin along with tamoxifen.

Group V: Group III patients after 90 d treatment with 100 mg CoQ_{10}, 10 mg riboflavin and 50 mg niacin along with tamoxifen.

Patients were asked to take one capsule of CoQ_{10} (100 mg Kaneka Q10, Kaneka Corporation, Japan), one tablet of riboflavin and niacin (10 mg riboflavin and 50 mg niacin, Madras Pharmaceuticals, India) and two tablets of tamoxifen (10 mg Nolvadex, AstraZeneca, India) per day. All supplements were taken after breakfast, with a second tablet of tamoxifen being taken after dinner. Compliance was checked by counting the number of tablets handed out to the patients and recollected at the end of the study.

To reduce the within-subject variability, serum levels of CEA and CA 15-3 were measured in duplicates and the average of the measurements was used for data analysis. Measurement of tumour marker levels of CEA and CA 15-3 in one central laboratory eliminated the analytical variability. By way of precaution, the study subjects were enquired during every consultation whether they had experienced unusual symptoms.

Blood Collection Five milliliters of venous blood was collected in a serum separator tube (Vacutainer; Becton Dickinson) and immediately centrifuged after clotting at 3000 rpm for 10 min. Serum samples were aliquoted in 1.0 ml fractions and stored at −80 °C.

Estimation of CEA and CA 15-3 The CanAg CEA Enzyme Immunoassay (CanAg Diagnostics AB, Gothenburg, Sweden) is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique used to detect CEA in serum secreted from tumour cells. The Centocor CA 15-3 Radio Immunoassay (Fujirebio Diagnostics Inc., Malvera, PA, U.S.A.) utilizes two monoclonal antibodies (116D8 and DF3) which react with DF3-recombinant determinants expressed by human breast carcinoma cells. Both the tests were done following the procedure mentioned in the commercial kit. The test values above the standard curve were retested with appropriate dilutions.

Statistical Analysis Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test using statistical package for social science computer package version 10.0 (SPSS, Chicago, IL, U.S.A.). Values are expressed as mean±standard deviation (S.D.). The values were considered statistically significant if the p value was less than 0.05.

RESULTS AND DISCUSSION

The incidence of breast cancer is almost identical all over the world, but the incidence of its clinical malignant state is much higher in western countries.[22] Dietary and environmental factors are thought to play a role in the progression of a tumour from its latent into its clinical phase and if certain dietary factors and supplements delay tumour progression in the latent phase, it is not unlikely that they may also be effective in delaying tumour progression in an established tumour.[23]

Tamoxifen therapy for breast cancer has become more generally used and it remains a challenge to predict which patients are at greatest risk of relapse and thus, may benefit most from tamoxifen therapy. In these cases, measurement of serum tumour markers may be helpful in detecting the metastatic process, in the sub-clinical phase itself and assess the response to the treatment in breast cancer patients. Several studies have shown that, in approximately 90% of the metastatic patients, serum levels of CEA and CA 15-3 correlate directly with the response of the disease to the treatment.[20,21,24] Hence, elevations of tumour marker levels during the treatment phase could be a promising indicative of disease progression or regression.[24]

Several clinical trials administrating CoQ_{10} in cancer pa-

tients have indicated a tumour suppressive effect.[25—28] In breast cancer patients, administering 90 mg/d CoQ_{10} showed complete regression. An increased dosage of 390 mg/d of CoQ_{10} to breast cancer patients and a follow up study for five years showed complete regression of metastases in these patients.[27] Riboflavin influences epithelial integrity, tissue flavin concentrations, rate of prostaglandin biosynthesis and glutathione metabolism.[29] Riboflavin administration was found to decrease the risk of developing cancer by increasing the levels of total flavins, which has the capacity to capture reactive metabolites and thereby decrease the carcinogen binding to cellular macromolecules like DNA.[12] Riboflavin deficiency enhances the risk of esophagal cancer development at the initiation and promotion stages.[17] In addition to redox functions in energy metabolism, niacin, in the form of NAD^+ participates in a variety of ADP-ribosylation reactions, which is responsible for the majority of polymer synthesis. It plays an important role in DNA damage response including repair, maintenance of genomic stability and signaling events in apoptosis.[17,24]

The comparison of serum concentration of CEA and CA 15-3 between all the study groups were tabulated in Table 2. The cut-off value for CEA test was taken as 5 μg/l and for CA 15-3 test as 30 U/ml based on kit instructions as well as earlier reports.[5,30—32] Number of patients with CEA and CA 15-3 levels higher than the cut-off value were presented in Table 3. In group I control subjects, the tumour marker levels were significantly lower ($p<0.05$) than the other groups and none of the control subjects had tumour marker levels higher than the cut off value. Similar observation was reported by Robertson *et al.*, demonstrating high tumour marker levels in patients with breast carcinoma than in control subjects, pregnant women and in patients with benign disease.[24]

The mean CEA levels and CA 15-3 levels of group II patients were found to be higher than CEA and CA 15-3 levels of the other groups ($p<0.05$) and the number of patients with tumour marker levels higher than the cut off value was increased in this group. Earlier studies have reported elevated tumour marker levels of CEA and CA 15-3 in preoperative and untreated breast cancer patients when compared to treated patients.[33] CEA and CA 15-3 tumour marker levels above the cut off value is considered to yield poor prognosis to treatment and the risk of developing cancer recurrence and metastasis is increased in these patients.[21]

CEA and CA 15-3 levels of group III patients treated with tamoxifen for more than 1 year were found to be significantly lowered than group II patients ($p<0.05$) and the number of patients with tumour marker levels higher than the cut off value were also reduced. The results showed the beneficial effect of tamoxifen on breast cancer patients is in line with earlier studies.[34,35] The antitumour activity of tamoxifen is due to its antiestrogenic activity, mediated by competitive inhibition of estrogen binding to estrogen receptors.[34] Consequently, tamoxifen inhibits the expression of estrogen-regulated genes, including growth factors and angiogenic factors secreted by the tumour that may stimulate growth by autocrine or paracrine mechanisms.[35]

In group III patients treated with CoRN along with tamoxifen for 45 d (group IV) and 90 d (group V), there was a significant reduction ($p<0.05$) in tumour marker levels and the number of patients with tumour marker levels higher than the cut off value also reduced. Decrease in tumour marker levels prove the beneficial effect of CoRN supplementation to breast cancer patients, a decrease in marker level is indicative of a biochemical response offering better disease stabilization and survival as well as an improved quality of life.[21]

Based on collecting the remaining CoRN supplement tablets from the patients at the end of the study, compliance was estimated to be 100% in all the patients. The supplements were well tolerated and no side effects were reported by any of the participant. The study suggests that supplementation of CoRN along with tamoxifen to breast cancer patients reduces the serum tumour marker levels of CEA and CA 15-3, thereby offering better cancer prognosis by reducing the risk of developing cancer recurrence and metastasis.

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Table 2. Effect of CoRN Supplementation on Tumour Marker Levels in Breast Cancer Patients

Groups	CEA levels (μg/l)	CA 15-3 levels (U/ml)
Group I (46) Control, normal, age-matched women	1.07±0.18	5.59±1.36
Group II (84) Pre-treatment, untreated breast cancer women	18.45±4.13[a]*	50.33±12.75[a]*
Group III (84) Treatment with tamoxifen	7.8±3.2[b]*,c*	36.42±9.64[b]*,c*
Group IV (84) 45 d after treatment with CoRN along with tamoxifen	5.12±1.52[d]*	26.09±7.23[d]*
Group V (84) 90 d after treatment with CoRN along with tamoxifen	3.8±1.5 [e]*,f,NS	18.22±4.67[e]*,f,NS

Values are expressed as mean±S.D. Number of subjects are indicated in parentheses. Comparisons were made between: a, Group I and Group II; b, Group II and Group III; c, group I and III; d, Group III and IV; e, Group III and V; f, Group I and V. Statistical significance expressed as; $*p<0.05$; not significant as NS.

Table 3. Number of Patients with Serum CEA and CA 15-3 Levels Higher Than the Cut Off Value during CoRN Supplementation

Tumour markers	Number of patients with marker levels above the cut off value				
	Group I $n=42$	Group II $n=84$	Group III $n=84$	Group IV $n=84$	Group V $n=84$
CEA >5 μg/l	0	39 (46.43)	31 (36.9)	25 (29.76)	10 (11.9)
CA 15-3 >30 U/ml	0	32 (38.1)	28 (33.33)	15 (17.85)	0
CEA >5 μg/l+ CA 15-3 >30 U/ml	0	32 (38.1)	28 (33.33)	15 (17.85)	0

n=number of patients; number in parentheses represent percentages.

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