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EFFECTIVENESS OF PRANAYAMA ON THE LEVELS OF SERUM PROTEIN THIOLS AND GLUTATHIONE IN BREAST CANCER PATIENTS UNDERGOING RADIATION THERAPY : A RANDOMIZED CONTROLLED TRIAL

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Abstract : The effect of Pranayama on the levels of protein thiols and glutathione was studied among breast cancer patients receiving radiation therapy. 160 patients were randomised into experimental and control group using block randomisation. The experimental group received fractionated radiation for five days a week and performed Pranayama (Nadishodhana, Brahmari and Sheethali) for 30 minutes twice daily for five days a week. The control group received only radiation. Blood samples were collected from both the groups at the end of six weeks of radiation therapy and analysed for the levels of serum protein thiols and glutathione. An independent sample 't' test showed a significant difference in the level of serum protein thiols between the two groups (t = 4.43 p 0.001). A Mann-Whitney U test showed a significant difference (z = –3.07 p 0.002) in the

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level of glutathione as well. These Pranayama techniques improve the antioxidant status of breast cancer patients receiving radiation therapy.

Key words : protein thiols glutathione
pranayama radiation therapy

INTRODUCTION

Breast cancer is a very commonly occurring cancer among women in the metropolitan cities of India. It is fast replacing cervical cancer as the leading cause of cancer among women (1). Breast cancer is treated with surgery, chemotherapy, radiation therapy and hormone therapy depending on the histological stage and other particulars of the patient. Radiation therapy is employed usually to prevent local recurrence of cancer after doing surgery and chemotherapy. Radiation therapy causes DNA strand breakdown to kill the cancer cells. In the process, normal cells are also affected by DNA damage (2).

Radiation therapy is known to reduce antioxidants in the body. Protein thiols are believed to have a role in the DNA repair thus reducing the side effects of these therapies (3, 4). Glutathione is a tripeptide, L-γ-glutamyl-L-cysteinylglycine, present in high concentrations in most cell types. Glutathione donates hydrogen ion and unpaired electron to neutralize peroxides and free radicals. In this process, glutathione gets oxidised to glutathione disulphide (5).

Pranayama literally means the control of breath. Pranayama has been an ancient technique practiced by the Yogis for the spiritual growth. Many scientific studies have proven that these breathing techniques improve human heath by maintaining a physiological balance and it affects many systems of the body. Pranayama has also been shown to improve the antioxidant level in healthy people (6, 7).

This study assessed the effectiveness of Pranayama (Yogic breathing techniques) on the levels of protein thiols and glutathione among breast cancer patients receiving radiation therapy. In this study, by Pranayama we mean three breathing techniques called Nadishodhana Pranayama, Sheethali Pranayama and Brahmari Pranayama.

MATERIALS AND METHODS

Patients

The study was conducted after obtaining institutional ethical committee clearance. The study group consisted of a total of 160 patients which included both the control and the experimental group. The patients were allocated into experimental and control group using block randomization procedure (sixteen blocks of ten patients) after obtaining informed consent. Patients with any psychiatric disorder, who have not undergone any surgical treatment for their breast cancer, those with extreme mobility issues (e.g., unable to get in and out of a chair unassisted), those who have practiced yoga or taken yoga classes prior to diagnosis, those diagnosed with lymphedema at baseline and those with recurrent breast cancer were

excluded to ensure a more homogenous sample. Patients were not receiving any vitamin supplements or any other antioxidant supplements during the study period.

Procedure of pranayama

The patients in the experimental group performed Pranayama (Sheethali, Brahmari and Nadisodhana Pranayama) along with radiotherapy whereas patients in the control group received radiotherapy only. Experimental group of patients performed Pranayama, morning and evening for 5 days a week for 6 weeks (from the day of starting radiotherapy till the last day of radiotherapy). Patients performed Nadisodhana for approximately 5 minutes (21–25 cycles), Sheethali for approximately 5 minutes (50–60 cycles) and Brahmari for approximately 8 minutes (10 cycles). The initial sessions on Pranayama were given in the Yoga department for one week. The patients performed Pranayama morning and evening for the next 5 weeks in a separate room in the hospital under the supervision of a co-investigator.

Patients who were having locally advanced breast cancer and who underwent Modified radical mastectomy or Breast conserving surgery, followed by 8 cycles of chemotherapy [Doxorubicin 60 mg/m^2 IV d1 (Cyclophosphamide 600 mg/m^2 d1) 3 weekly * 4 cycles Followed by Paclitaxel 175 mg/m2 IV 3 weekly * 4 cycles] were enrolled in this study. After chemotherapy, patients were given radiation of 50 Gy in divided doses. Patients performed pranayama on same days when they came for radiation therapy. Blood was collected in red coloured vacoutainers for serum sample and purple vacoutainers with EDTA for packed cell, from both the group at the completion of radiation therapy and analysed for serum protein thiols and glutathione. Pre-test for these enzymes were done in a sample of 80 patients before starting radiation therapy.

Chemicals

5, 5'dithio-bis (2-nitrobenzoic acid) (DTNB) was obtained from Sigma chemicals, St. Louis, MO, USA. All other reagents were of chemical grade. Spectrophotometer Genesys 10 uv was used for analysis.

Procedure for serum anti-oxidant enzymes

Preparation of serum

Blood samples were collected in plain vacoutainers without EDTA and the sample was allowed to stand at room temperature for half an hour and centrifuged at 3000 rpm for 10 minutes in refrigerator centrifuge and serum was separated.

Protein thiols

Thiols are compounds that contain carbon bonded sulfhydryl group. Protein thiols in the plasma include the protein sulfhydryl groups and protein mixed disulphides with homocysteine, cysteinylglycine, cysteine and glutathione. Most of the cytosolic thiol groups are maintained in their reduced state by a variety of pathways. During oxidative stress, these protein thiols get oxidised mainly by the formation of disulphide bonds in the plasma. Thus protein thiols play a major role in antioxidant defences (8).

Protein thiols can be measured with a spectrophotometric method using dithionitrobenzene. The spectrophotometric assay for protein thiols is based on the reaction of 5,52-Dithiobis (2-nitrobenzoic acid) (DTNB) or Ellman's reagent with protein thiols. DTNB combines with accessible thiol group in proteins and reduces to stable intermediate compound of mixed disulfide, protein S-S aromatic compound. The reduced product of DTNB is 5 mercapto -2-nitrobenzoate. There is formation of yellow colour due to liberated p-nitrothiophenol anion, whose absorbance is measured at 412 nm after 5 mins.

Procedure for protein thiol estimation

Sample blank

In a test tube 920 µl of 0.2 M disodium hydrogen phosphate containing 2 mM disodium EDTA is taken and 100 µl of serum is added. The contents are mixed and optical density was taken at 412 nm wavelength exactly after 5 mins.

Test

In a test tube, 900 µl of 0.2 M disodium hydrogen phosphate containing 2 mM disodium EDTA, 100 µl of serum and 20 µl of 10 mM DTNB are added. The contents are mixed and optical density was taken at 412 nm wavelength exactly after 5 mins.

Reagent blank

In a test tube, 1000 µl of 0.2 M disodium hydrogen phosphate containing 2 mM disodium EDTA and 20 µl of 10 mM DTNB are added. The contents are mixed and optical density was taken at 412 nm wavelength exactly after 5 mins.

Sample blank was measured because bilirubin, β-carotene, and other plasma constituents that absorb at 412 nm can interfere with protein thiol measurement. The absorbance for sample and reagent blanks was subtracted from serum absorbance values to obtain the corrected values (9).

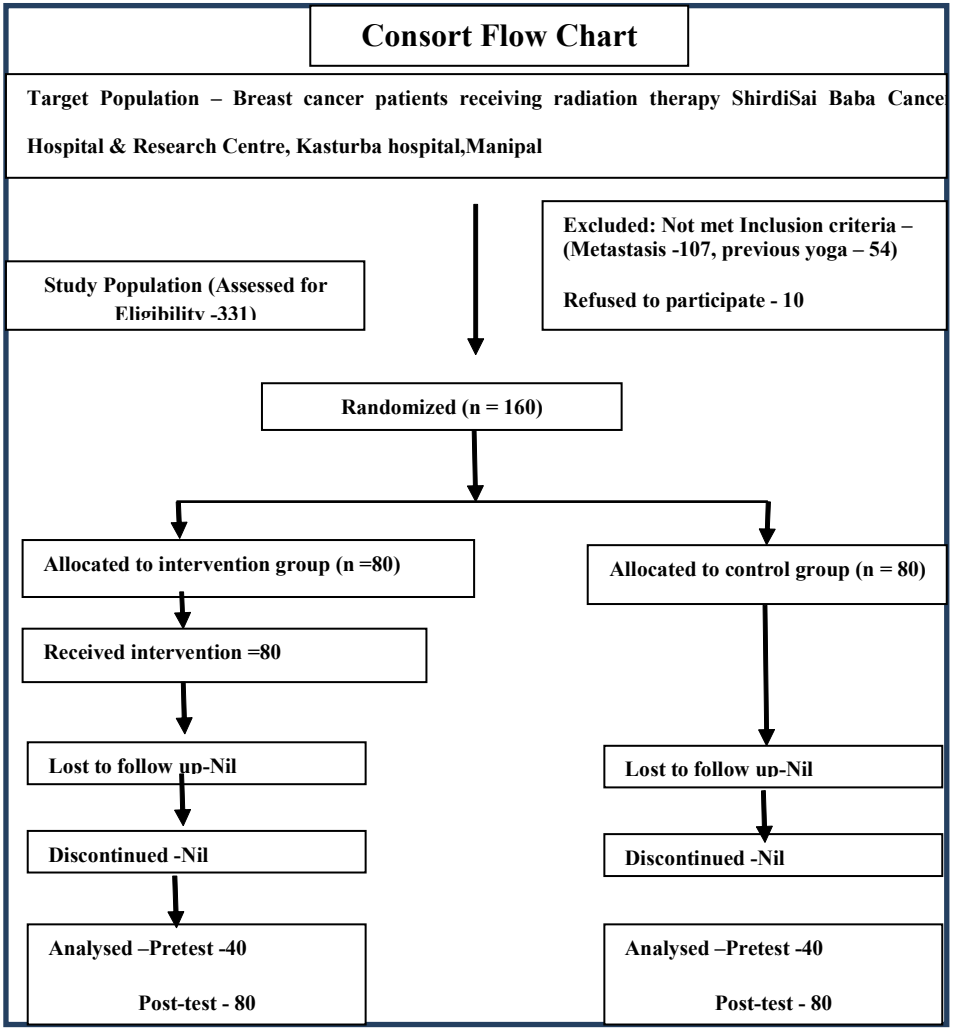
Preparation of packed cell

Blood samples were collected in EDTA bottle from patients. Blood was centrifuged at 3000 rpm for 10 minutes in refrigerator centrifuge without delay. Plasma was separated; buffy coat was carefully removed and separated, erythrocytes were washed thrice with cold normal saline. The haemoglobin content of the erythrocytes was determined by cyanmethemoglobin method. Erythrocytes enzymes were estimated in appropriately diluted haemolysates prepared by addition of distilled water.

Procedure for glutathione estimation

Glutathione, a tripeptide, γ-glutamylcysteinyl glycine, is the most abundant non-protein thiol in mammalian cells. Glutathione exists in two forms; the thiol-reduced (GSH) and disulphide oxidized (GSSG). Glutathione plays an important role in the detoxification of xenobiotic compounds and in the anti-oxidation of reactive oxygen species and free radicals (10).

1.810 ml of distilled water is added to 0.2 ml of the haemolysate. 0.01 ml of lysate is added to 3 ml of ferricyanide-cyanide



reagent (Drabkin's reagent). Reading is taken within 3 minutes at 540nm for hemoglobin estimation. Three ml of precipitating solution is added to the remaining haemolysate. After standing for 10 minutes, the mixture is filtered through a medium or coarse grade of filter paper. One milliliter of filtrate and 0.5 ml of DTNB is added to 4 ml of 0.3 M Na_2 HPO_4 solutions. It is read at 412 nm against a blank prepared by adding one ml of distilled water and 0.5 ml of DTNB to 4 ml of 0.3 M Na_2 HPO_4. (11).

Statistical analysis

Data were analyzed using SPSS package (version 16). Pretest and post test levels of serum protein thiols are expressed as mean and standard deviation. Since data were following normality, for comparison of serum protein thiol values between the experimental group and control group, an independent 't' test was used. The data for glutathione was compared using Mann Whitney U test as it was not following

normality. Statistical significance was fixed at p = 0.05.

RESULTS

Sample characteristics

The characteristics of the participants have been summarized in Table I. Data in the table show that majority of the women (140 out of 160) had locally advanced breast cancer and undergone modified radical mastectomy (123 out of 160) as the surgical treatment.

TABLE I: Sample Characteristics.

Sl. No.	*Experimental (n=80)*	*Control (n=80)*
Age in years		
≤ 45	46	35
> 45	34	45
Stages of breast cancer		
Stage 1	12	8
Stage 2	38	40
Stage3	30	32
Haemoglobin levels		
≤ 12	50	58
> 12	30	22
Surgery		
Modified radical mastectomy	63	60
Breast conservation	17	20

Comparison of serum protein thiols between the control group and the experimental group

There was no significant difference in the level of protein thiols between the experimental group and the control group at the beginning of radiation therapy. The serum concentration of protein thiols in the experimental group was 235.83±74.60 µmol/lit and in the control group was 213.20±88.03 µmol/lit with a p value of 0.206. Since the data were following normality, an independent 't' test was used to compare the mean difference of protein thiols between the control group and the experimental group. During the post test, i.e., at the end of radiation therapy, the serum concentration of protein thiols was significantly higher in the group who performed Pranayama (271.20 ±91.28 ìmol/lit) than in the control group (216.13±62.86 µmol/lit) (p 0.001). The patients who performed Pranayama along with radiation therapy had higher levels of serum protein thiols at the end of radiation therapy. As mentioned earlier, protein thiols get oxidised mainly by the formation of disulphide bonds in the plasma during oxidative stress. The elevated levels of protein thiols in the experimental group indicate that Pranayama influences the

TABLE II: Comparison of the mean values of serum protein thiols (µmol/lit) between experimental group and control group at the beginning of radiation therapy.

Groups (80)	*Mean±SD*	*'t' value*	*95% Confidence interval of the difference*	*P-value*
Experimental (40)	235.83±74.60	1.274	–12.70 to 57.95	0.206
Control (40)	213.20±88.03			

µmol/lit–micromoles per litre.

TABLE III: Comparison of the mean values of serum protein thiols (µmol/lit) between experimental group and control group at the end of radiation therapy.

Groups (160)	*Mean±SD*	*'t' value*	*95% Confidence interval of the difference*	*P-value*
Experimental (80)	271.20±91.28	4.43	30.53 to 79.60	0.001
Control (80)	216.13±62.86			

µmol/lit–micromoles per litre.

formation of serum protein thiols and reduces the toxicities to normal tissues related to radiation therapy.

Comparison of glutathione between the control group and the experimental group

With regard to glutathione, there was no statistically significant difference in the level of glutathione between the experimental group (26.61±15.99 mg/g of Hb) and the control group (27.03±11.63 mg/g of Hb) before starting the radiation therapy (p 0.857). Since the data were not following normality, a Mann Whitney U test was done to assess the difference between the two groups with regard to the level of glutathione. At the end of radiation therapy, the mean value of glutathione for the experimental group was (26.14±10.46 mg/g of Hb) which is higher than that of the control group (21.06±6.06 mg/g of Hb) (p 0.002). This also may probably indicate a less oxidative stress in breast cancer patients performing Pranayama when undergoing radiation therapy.

TABLE IV: Comparison of the median values of glutathione (mg/gHb) between experimental group and control group at the beginning of radiation therapy - Mann Whitney U test.

Groups (80)	*Inter quartile range*	*Median*	*Z value*	*p-value*
Experimental (40)	18.24 to 30.2	24.4	–0.18	0.857
Control (40)	19.89 to 33.97	22.38		

TABLE V: Comparison of the median values of glutathione (mg/gHb) between experimental group and control group at the end of radiation therapy - Mann Whitney U test.

Groups (160)	*Inter quartile range*	*Median*	*Z value*	*p-value*
Experimental (80)	18.31 to 30.55	24.21	–3.07	0.002
Control (80)	18.02 to 24.6	19.1		

DISCUSSION

Radiation therapy acts by two different methods to kill the cancer cells. Radiation causes DNA strand breakage either directly or indirectly by generating free radicals. One of the important radiation induced free radical species is the hydroxyl radical which results in the generation of other species of free radicals causing oxidative stress (12). Protein thiols are targets of oxidative stress. During oxidative stress, the protein thiols which are plasma sulfhydryl groups associated with protein get converted to disulphides resulting in a fall in their levels and antioxidant activity (8).

In the present study, experimental group of patients performed Pranayama, morning and evening for 5 days a week for 6 weeks (from the day of starting radiotherapy till the last day of radiotherapy). Patients performed Nadisodhana for approximately 5 minutes (21–25 cycles), Sheethali for approximately 5 minutes (50–60 cycles) and Brahmari for approximately 8 minutes (10 cycles). The levels of glutathione (GSH) and protein thiols two important antioxidants were significantly higher in patients with breast cancer undergoing radiation therapy and practising Pranayama, when compared to controls (breast cancer patients undergoing chemotherapy but not practising pranayama). The higher GSH and protein thiols may be protective for these patients in reducing the toxicities caused by radiation therapy.

Published studies to compare the antioxidant effects of Pranayama on breast cancer patients were not available. However, Sinha S et. al reported similar findings in a study conducted among the healthy male volunteers of Indian Navy. There were 30

volunteers in the experimental group who practised yoga, pranayama and meditation for one hour in the morning, five days a week for six months. There was a significant increase in the total antioxidant status and glutathione in healthy male volunteers from Navy who practised yoga and pranayama at the end of six months (13). Further Yadav et al. in their study concluded that yoga–based life style modification program was effective in reducing oxidative stress among a group of healthy volunteers and chronic patients (14). The present study confirms the results of these available studies.

As mentioned earlier, radiation therapy is known to induce free radicals in an effort to kill cancer cells. The exact physiological mechanism of Pranayama on antioxidants is not clear. It is encouraging to note that the level of protein thiols and glutathione, two important antioxidants have increased among this population in spite of the free radical injury. The elevated levels of protein thiols and glutathione may help in relieving the toxicities associated with radiation treatment among breast cancer patients. To understand the exact physiological mechanism of Pranayama on antioxidants, genetic studies on larger samples may be required. With the findings of the present study, it may be concluded that Pranayama may be employed as a supportive treatment to breast cancer patients undergoing radiation therapy.

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