Chapter IV

Analysis, Interpretation and Discussion
ANALYSIS, INTERPRETATION AND DISCUSSION

This chapter presents the analysis and interpretation of data collected to determine the effectiveness of Pranayama on cancer related fatigue and selected biochemical parameters among patients with breast cancer undergoing radiation therapy. The study was conducted among the breast cancer patients of Shirdi Sai Baba Cancer Hospital & Research Centre, Kasturba Hospital, Manipal. Breast cancer patients were randomized into experimental group (80) and control group (80) using block randomization technique. Experimental group of patients performed Pranayama along with radiation therapy whereas the control group received only radiation therapy. Data on cancer related fatigue and selected serum antioxidants and antioxidant enzymes were collected from the patients at the beginning and at the completion of six weeks of radiation therapy.

For interpretation, the data were categorized and analyzed based on the objectives of the study using descriptive and inferential statistics. The SPSS statistical package (16.0 version) was used for the analysis of the data.

The objectives of the study were to

1. determine the effectiveness of Pranayama on
   1.1 cancer related fatigue among breast cancer patients undergoing radiation therapy as measured by cancer fatigue scale.
   1.2 selected biochemical parameters among breast cancer patients undergoing radiation therapy
2. determine the relationship between cancer related fatigue and the level of glutathione(GSH) and its associated enzymes among breast cancer patients undergoing radiation therapy.
Organization of Study Findings

The data are presented under the following headings

Section 1: Sample characteristics

- Demographic characteristics of breast cancer patients in experimental and control groups.

Section 2: Comparison of pre-test values between the experimental and control group at the beginning of radiation therapy

- Comparison of demographic characteristics
- Comparison of pre-test scores of cancer related fatigue
- Comparison of pre-test levels of glutathione (GSH)
- Comparison of pre-test levels of serum protein thiols
- Comparison of pre-test levels of serum glutathione S transferase
- Comparison of pre-test levels of glutathione reductase
- Comparison of pre-test levels of glutathione peroxidase

Section 3: Description of scores of cancer related fatigue

- Classification of scores of cancer related fatigue at the beginning of radiation therapy based on the scoring of cancer fatigue scale.

Section 4: Effectiveness of Pranayama on cancer related fatigue

- Comparison of pretest and post-test scores of cancer related fatigue among the experimental group
Comparison of post-test scores of cancer related fatigue between the experimental and control group at the completion of radiation therapy

Comparison of breast cancer patients with mild fatigue among the experimental group and the control group

Comparison of breast cancer patients with moderate fatigue among the experimental group and the control group

Comparison of breast cancer patients with severe fatigue among the experimental group and the control group

Section 5: Effectiveness of Pranayama on biochemical parameters

Effectiveness of Pranayama on the levels of glutathione (GSH)

Comparison of pretest and post-test levels of glutathione among the experimental group

Comparison of post-test levels of glutathione between the experimental and control group at the completion of radiation therapy

Pre-test and post-test levels of glutathione among the experimental and control group.

Effectiveness of Pranayama on the levels of protein thiols

Comparison of pretest and post-test levels of protein thiols among the experimental group

Comparison of post-test levels of protein thiols between the experimental and control group at the completion of radiation therapy

Pre-test and post-test levels of protein thiols among the experimental and control group.

Effectiveness of Pranayama on the levels of glutathione S transferase

Comparison of pretest and post-test levels of glutathione S transferase among the experimental group
Chapter IV

**Analysis, Interpretation and Discussion**

- Comparison of post-test levels of glutathione S transferase between the experimental and control group at the completion of radiation therapy
- Pre-test and post-test levels of glutathione S transferase among the experimental and control group.

**Effectiveness of Pranayama on the levels of glutathione peroxidase**

- Comparison of pretest and post-test levels of glutathione peroxidase among the experimental group
- Comparison of post-test levels of glutathione peroxidase between the experimental and control group at the completion of radiation therapy
- Pre-test and post-test levels of glutathione peroxidase among the experimental and control group.

**Effectiveness of Pranayama on the levels of glutathione reductase**

- Comparison of pretest and post-test levels of glutathione reductase among the experimental group
- Comparison of post-test levels of glutathione reductase between the experimental and control group at the completion of radiation therapy
- Pre-test and post-test levels of glutathione reductase among the experimental and control group.
Section 6: Relationship between Cancer related fatigue and selected biochemical parameters

- Relationship between the pretest scores of Cancer related fatigue and pretest level of glutathione
- Relationship between pretest scores of Cancer related fatigue and pretest level of protein thiols
- Relationship between pretest scores of Cancer related fatigue and pretest level of glutathione S transferase
- Relationship between pretest scores of Cancer related fatigue and pretest level of glutathione peroxidase
- Relationship between pretest scores of Cancer related fatigue and pretest level of glutathione reductase
SECTION 1

Sample Characteristics

A sample of 160 breast cancer patients was randomized into experimental and control group when they came for radiation therapy to Shirdi Sai Baba Cancer Hospital & Research Centre, Kasturba Hospital, Manipal. These breast cancer patients have undergone surgical and chemotherapeutic treatments before starting radiation therapy. Radiation therapy was given to prevent the local recurrence of cancer. The experimental group of patients performed Pranayama along with radiation therapy whereas the control group of patients received only radiation therapy. Cancer related fatigue and selected biochemical parameters were assessed in these patients at the beginning and at the completion of radiation therapy.

Distribution of breast cancer patients according to age, stages of breast cancer, hemoglobin levels and surgery is given in table below as frequency and percentage.
Table 1: Demographic characteristics of breast cancer patients in experimental and control group.

<table>
<thead>
<tr>
<th></th>
<th>Experimental (80)</th>
<th>Control (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>1 Age in years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 45</td>
<td>46</td>
<td>57.5</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>34</td>
<td>42.5</td>
</tr>
<tr>
<td>2 Stages of breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Stage 2</td>
<td>38</td>
<td>47.5</td>
</tr>
<tr>
<td>Stage 3</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>3 Hemoglobin levels (g/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 12</td>
<td>50</td>
<td>62.5</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>30</td>
<td>37.5</td>
</tr>
<tr>
<td>4 Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified radical mastectomy</td>
<td>63</td>
<td>78.75</td>
</tr>
<tr>
<td>Breast conservation</td>
<td>17</td>
<td>21.25</td>
</tr>
</tbody>
</table>

NS - Not Significant

The data presented in table 1 show that majority of the breast cancer patients 140 (87.5%) out of 160 were in the stages of stage 2 and stage 3. Most of the patients 123 (76.87%) have undergone modified radical mastectomy as the surgical treatment and majority 118 (73.75%) had hemoglobin levels below 12g/dL.
SECTION 2

Comparison of pre-test values between the experimental and control group at the beginning of radiation therapy

The pre-test values for all the variables were statistically tested between the experimental group and control group at the beginning of radiation therapy to know whether the group differed significantly in their characteristics.

Table 2: Comparison of Demographic Characteristics (n=80+80)

<table>
<thead>
<tr>
<th></th>
<th>Experimental</th>
<th>Control</th>
<th>p value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=80)</td>
<td>(n=80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Age in years</td>
<td>43.57 ± 8.5</td>
<td>43.57 ± 8.5</td>
<td>0.632 t</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df 158</td>
</tr>
<tr>
<td></td>
<td>≤ 45</td>
<td>46</td>
<td>35</td>
<td>0.319#</td>
</tr>
<tr>
<td></td>
<td>&gt; 45</td>
<td>34</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Stages of breast cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage 1</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>38</td>
<td>40</td>
<td>0.456#</td>
</tr>
<tr>
<td></td>
<td>Stage 3</td>
<td>30</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hemoglobin levels (g/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.5 ± 0.9</td>
<td>11.5 ± 1.2</td>
<td></td>
<td>0.42 t</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df 158</td>
</tr>
<tr>
<td></td>
<td>≤ 12</td>
<td>50</td>
<td>58</td>
<td>0.740#</td>
</tr>
<tr>
<td></td>
<td>&gt; 12</td>
<td>30</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Modified radical mastectomy</td>
<td>63</td>
<td>60</td>
<td>0.722#</td>
</tr>
<tr>
<td></td>
<td>Breast conservation</td>
<td>17</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

# - Chi-square test, t – Independent t test

To determine whether the group differed significantly in their demographic characteristics, chi-square test and independent t test was done and p-value was computed.
at 0.05 level of significance. The p-values for all the demographic variables were greater than 0.05 which indicates that the experimental and control group did not differ significantly with regard to these variables at baseline.

**Comparison of pre-test scores of cancer related fatigue**

The scores of cancer related fatigue at the beginning of radiation therapy were compared between the experimental group and control group to check whether the groups differed at baseline. For comparison of cancer related fatigue scores between the two groups, normality was checked using Kolmogorov-Smirnov test for normality. Since the data were not following normality, a Mann-Whitney U test was done to compare the differences in scores of cancer related fatigue at the beginning of radiation therapy between the groups.

The following null hypothesis was formulated to compare the scores of cancer related fatigue at the beginning of radiation therapy between the experimental group and the control group at 0.05 level of significance.

H01: There will be no significant difference in the scores of cancer related fatigue at the beginning of radiation therapy between the experimental group and the control group

**Table 3: Difference between the pre-test scores of cancer related fatigue among the experimental group and control group using Mann-Whitney U test.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>Z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer related fatigue</td>
<td>Experimental</td>
<td>29</td>
<td>22 to 42</td>
<td>-0.475</td>
<td>0.635</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>32</td>
<td>21 to 43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data presented in table 3 show that there was no significant difference in the scores of cancer related fatigue between the control group and the experimental group at the beginning of radiation therapy (p = 0.635). Since the p value for comparing the cancer related fatigue scores between the two groups was greater than 0.05, it can be interpreted that there was no difference between the two groups at baseline with regard to cancer related fatigue scores. It may also be interpreted that if there is any difference in the scores of cancer related fatigue between the two groups during post test it may be due to the effect of intervention.

The findings of the study conducted by Moadel et al. are consistent with the present study findings. They assessed limitations in daily activity and energy level using ‘The Functional Assessment of Chronic Illness Therapy–Fatigue’ among one hundred and twenty eight breast cancer patients. The intervention group (n = 84) performed yoga as intervention for 12 weeks and control group (n = 44) did not perform yoga. The mean baseline fatigue scores for intervention group and control group were almost similar with scores being 34.27 ± 12.31 for the intervention group and 35.88 ± 14.42 for the control group. There was no significant difference in the scores of cancer related fatigue between the two groups at baseline.

Poirier, Lavdaniti and Donovan also observed that most of the patients experienced mild fatigue at baseline before radiation therapy and the fatigue severity of these patients was equivalent to their fatigue severity at the start of chemotherapy.

In the present study, all patients have undergone surgical treatment and chemotherapy before admitted for radiation therapy. There was a minimum gap of one month between the last cycle of chemotherapy and the start of radiation therapy. So most
patients experienced mild fatigue and there was no significant difference between the two groups with regard to the scores of cancer related fatigue at the beginning of radiation therapy.

**Comparison of pretest levels of biochemical parameters**

The biochemical parameters assessed in this study were few enzymatic and non-enzymatic antioxidants. An antioxidant is a substance that scavenges free radicals. The body endogenously produces non-enzymatic antioxidants such as glutathione (GSH) and enzymatic antioxidants such as glutathione reductase, glutathione S transferase and glutathione peroxidase. Balance between the levels of free radicals and antioxidant defence is very essential for a healthy human body. Imbalance between free radical production and their scavenging by antioxidants can lead to various diseases including cancer. Cancer and its treatments can also lead to an important phenomenon called oxidative stress. Oxidative stress is caused by an intracellular excess of reactive oxygen (ROS) free radical species over intracellular antioxidants.\(^9\)

The biochemical parameters assessed in this study were non-enzymatic antioxidants like glutathione (GSH) and protein thiols and enzymatic antioxidants such as glutathione reductase, glutathione S transferase and glutathione peroxidase.

Pretests of biochemical parameters to compare the experimental group and control group at baseline could be done only for a total of 80 patients. Due to delay in getting the reagents (since the procurement of reagents could only be done through the purchase department of the university, there were technical difficulties in getting the reagents on time) for the analysis of antioxidants and antioxidant enzymes, the reagents were saved for doing
the post-test and only 80 patients’ blood sample could be analyzed for pretest out of the total of 160 patients. The following tests describe the tests done to check whether the experimental and control group differed significantly at baseline with regard to these biochemical parameters.

**Comparison of pre-test levels of glutathione (GSH)**

Glutathione is a tripeptide, L-γ-glutamyl-L-cysteinylglycine, present in high concentrations in most cell types. By virtue of its reactive sulfhydryl group, glutathione is able to donate a hydrogen ion and unpaired electron and neutralize peroxides and free radicals.\(^{102}\)

Glutathione plays a major role in the physiological balance between pro-oxidants and antioxidants. Glutathione and its redox system enzymes, glutathione peroxidase and reductase, help in providing a widespread protection system from oxidative damage. As mentioned earlier, cancer and its treatments can lead to oxidative damage. Normal or increased levels of glutathione play an important role in these cellular defences against radiation.\(^{86}\)

The pre-test levels of glutathione (GSH) were compared between the experimental group and control group to check whether the groups differed at baseline. Normality of the data was checked using Kolmogorov-Smirnov test for normality. Since the data were not following normality, a Mann-Whitney U test was done to compare the differences in levels of glutathione (GSH) at the beginning of radiation therapy.

The following null hypothesis was formulated to compare the level of glutathione (GSH) at the beginning of radiation therapy between the experimental group and the control group at 0.05 level of significance.
H02  There will be no significant difference in the levels of glutathione (GSH) between the experimental group and control group at the beginning of radiation therapy.

Table 4: Difference between the pre-test levels of glutathione (GSH) among the experimental group and control group using Mann-Whitney U test. (n=40 +40)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td>Experimental</td>
<td>24.4</td>
<td>18.24 to 30.2</td>
<td>-0.180</td>
<td>0.857</td>
</tr>
<tr>
<td>(mg/gHb)</td>
<td>Control</td>
<td>22.38</td>
<td>19.89 to 33.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data in table 4 show that there was no statistically significant difference in the level of glutathione (GSH) between the experimental and control group at the beginning of radiation therapy. Hence the null hypothesis, H02 was accepted. Since the p value for comparing the level of glutathione (GSH) between the two groups was greater than 0.05, it can be interpreted that there was no difference between the two groups at baseline with regard to the level of glutathione (GSH). It may also be interpreted that if there is any difference in the levels of glutathione between the two groups during post test it may be due to the effect of intervention.

Glutathione\textsuperscript{103} (GSH) is the most prevalent non-protein thiols in mammalian cells. GSH acts as a reducing agent, antioxidant and a free radical scavenger. Due to these functions, alterations in GSH levels and metabolism have been associated with different human diseases including cancer.

Glutathione synthesis is generally decreased in the breast cancer patients. Glutathione is very important in maintaining the stability of erythrocyte membranes. A
decrease in blood glutathione in circulation has been reported in several diseases including malignancies. The lower glutathione levels seen in breast cancer patients support the hypothesis that the glutathione status is inversely related to malignant transformation.\textsuperscript{75,104} Reduced total serum antioxidant capacity and increased oxidative stress was seen in breast cancer patients undergoing chemotherapy as well.\textsuperscript{76} In the present study, it is difficult to say the breast cancer patients receiving radiation therapy had low levels of glutathione compared to the normal population. It would have been appropriate to check the standard normal levels of glutathione among women of the same age group without any other disease or breast cancer from the general population. Since it was not proposed in the beginning and due to ethical reasons later on a standard normal value for healthy women could not be set.

In another study, KASAPOVIĆ\textsuperscript{77,78} investigated the effects of breast cancer radiotherapy on the antioxidant enzyme activities of glutathione peroxidase and glutathione reductase as well as on the concentration of reduced glutathione in blood of patients aged 45-58 years and older than 60 years. Pre-test values of the level of glutathione was very low among patients older than 60 years (6.04±0.62 nmol/mg of prot) and in patients aged 45-58 years (5.67±0.58 nmol/mg of prot). In the present study, patients with age less than or equal to 45 years of age had an average pre-test value of glutathione of 26.43 ± 14.0 mg/gHb and patients with more than 45 years had an average pre-test value of glutathione of 27.16 ± 13.75 mg/gHb with a p value of 0.810 (using independent t test). This further shows that the group did not differ in baseline pre-test value of glutathione with regard to age. Hence it is concluded that the group of breast cancer patients were homogeneous with regard to the pre-test value of glutathione at the beginning of radiation therapy.
Comparison of pre-test levels of serum protein thiols

Thiols are compounds that contain carbon bonded sulphydryl group. Protein thiols in the plasma include the protein sulphydryl 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.\(^{105}\)

The levels of serum protein thiols were compared to see whether the group differed with regard to the level of protein thiols at baseline. Since the data were following normality, to compare the levels of protein thiols, between the groups, an independent \(t\) test was done. The following null hypothesis was stated to test the statistical significance.

\[ H_0 \text{ There will be no significant difference in the levels of serum protein thiols between the experimental group and control group at the beginning of radiation therapy.} \]

**Table 5: Difference between the pre-test levels of serum protein thiols among the experimental group and control group using Independent ‘t’ test.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean ± SD</th>
<th>‘t’ value</th>
<th>(p) value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Thiols</td>
<td>Experimental</td>
<td>235.83 ± 74.60</td>
<td>1.274</td>
<td>0.206</td>
<td>-12.70 to 57.95</td>
</tr>
<tr>
<td>(µmoles/lit)</td>
<td>Control</td>
<td>213.20 ± 88.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in the table 5 show that 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. Hence the null hypothesis, \(H_0\) was accepted. Thus it can be interpreted that the group did not differ significantly with regard to the level of serum protein thiols at baseline and the groups were homogenous.
All the patients in the study have undergone surgical treatment and chemotherapy for breast cancer which would more or less expose them to the same amount of oxidative stress. The similar levels of protein thiols in the experimental and control group may be attributed to these similarities between the two groups.

**Comparison of pre-test levels of serum glutathione S transferase**

Glutathione S transferases (GSTs) are a family of proteins that conjugate glutathione on the sulphur atom of cysteine to various electrophiles. GSTs play a vital role in protecting tissues against oxidative damage and oxidative stress.\(^{106}\)

To compare the difference between the levels of glutathione S transferase at baseline, a Mann - Whitney U test was done as the data were not following normality. The following null hypothesis was stated to test the statistical significance.

\[ H_0: \text{There will be no significant difference in the levels of glutathione S transferase between the experimental group and control group at the beginning of radiation therapy at 0.05 level of significance.} \]

**Table 6: Difference between the pre-test levels of serum glutathione S transferase among the experimental group and control group using Mann-Whitney U test.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione S transferase</td>
<td>Experimental</td>
<td>2.4</td>
<td>2.09 to 2.88</td>
<td>-2.86</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.78</td>
<td>2.4 to 3.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in the table 6 show that there was a significant difference in the levels of glutathione S transferase between the experimental group and control group at the beginning
of radiation therapy. Since the p value is less than 0.05, it can be interpreted that the group varied significantly at baseline with regard to the level of serum glutathione S transferase and the control group had a higher level. Hence the null hypothesis, H04 was rejected.

The exact reason for the increased level of glutathione S transferase among the control group is not clear. A study conducted by Prabasheela and Bhaskaran reports that the activity of glutathione – S – transferase in the serum of histopathologically proven breast cancer patients was higher (8.54 ± 0.67) compared to normal controls (2.52 ± 0.91); but it has decreased (5.37 ± 0.03) after the tumor has been removed surgically. As mentioned in the above study, glutathione – S – transferase may be elevated in patients with breast cancer. But why it was still elevated in a group of patients who have undergone surgery and chemotherapy for breast cancer is not explainable. Some polymorphisms of glutathione – S – transferase are known to increase the breast cancer susceptibility in older patients. Consistent with this notion, our report shows that the number of patients aged > 45 was slightly elevated in the control group. But the reason for the elevated level of glutathione – S – transferase in the control group cannot be attributed to the increased number of patients aged >45 without further studies on the polymorphisms of the enzyme among these patients.

Comparison of pre-test levels of glutathione reductase

Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSG) by NADPH to reduced glutathione. Glutathione reductase plays an important role in protecting the cells against oxidative damage. Assay of Glutathione Reductase has been used in the detection of hepatic and malignant disease, assessment of nutrition (riboflavin status) and detection of genetically determined deficiency states.
Since the data were not following normality, to compare the pretest values of glutathione reductase, between the groups, a Mann - Whitney U test was done. The following null hypothesis was stated to test the statistical significance.

\[ H_0 : \text{There will be no significant difference in the levels of glutathione reductase between the experimental group and control group at beginning of radiation therapy.} \]

Table 7: Difference between the pretest levels of glutathione reductase among the experimental and control group using Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td>Experimental</td>
<td>1.11</td>
<td>0.32 to 2.2</td>
<td>-1.425</td>
<td>0.154</td>
</tr>
<tr>
<td>reductase(IU/L)</td>
<td>Control</td>
<td>1.375</td>
<td>0.54 to 2.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no significant difference in the level of glutathione reductase between the experimental group and control group at the beginning of radiation therapy. The group did not differ significantly with regard to the level of glutathione reductase at baseline. In the study conducted by Prasasheela and Baskaran\(^{107}\) the level of glutathione reductase was higher among the breast cancer patients (1.78 ± 0.04) compared to normal controls (0.46 ± 0.02); but it has decreased (0.91 ± 0.05) after the tumor has been removed surgically.

In the present study patients were recruited after they have undergone surgical treatment and chemotherapy for breast cancer. Unlike the pre-test level of glutathione – S – transferase, the pre-test levels of glutathione reductase did not differ between the two groups in the present study. It may be interpreted that the groups were homogeneous with regard to the level of glutathione reductase at baseline.
Comparison of pre-test levels of glutathione peroxidase

Glutathione peroxidase is an enzyme found in cytoplasm and mitochondria of cells. Glutathione peroxidase acts on lipid hydroperoxide substrates that are released from membrane phospholipids and hydrolyzes H2O2 at low concentrations. Thus it catalyzes the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids, thereby effectively removing toxic peroxides from living cells. In the process, it catalyzes the oxidation of GSH to GSSG by hydrogen peroxides.\textsuperscript{109} It plays the important role of protecting cells from potential damage by free radicals, formed by peroxide decomposition.\textsuperscript{88,89}

To compare the difference between the levels of glutathione peroxidase at baseline, a Mann - Whitney U test was done as the data were not following normality. The following null hypothesis was stated to test the statistical significance.

\( H_0 \) There will be no significant difference in the levels of glutathione peroxidase between the experimental group and control group at the beginning of radiation therapy.

Table 8: Difference between the pretest levels of glutathione peroxidase among the experimental and control group using Mann-Whitney U test.

\( (n=40 +40) \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (IU/L)</td>
<td>Experimental</td>
<td>2.0</td>
<td>0.34 to 4.57</td>
<td>-0.510</td>
<td>0.610</td>
</tr>
<tr>
<td>Control</td>
<td>2.78</td>
<td>0.59 to 6.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no statistically significant difference in the level of glutathione peroxidase between the experimental and control group at the beginning of radiation therapy. Hence the null hypothesis, \( H_0 \) was accepted.
In the study conducted by Prabasheela and Baskaran the activity of glutathione peroxidase was assessed in the serum of 50 histopathologically proven breast cancer patients before and after surgery and compared with age matched control. The level of glutathione peroxidase was higher among the breast cancer patients (26.19 ± 1.3) compared to normal controls (15.83 ± 0.21); but it has decreased (23.71 ± 0.12) after the tumor has been removed surgically. Though the present study has not assessed patients immediately after breast cancer surgery, all the patients in the study, have undergone the surgical treatment as well as chemotherapy for breast cancer to ensure homogeneity. Thus it may be interpreted that the groups did not differ with regard to the level of glutathione peroxidase at baseline.
SECTION 3

Description of scores of cancer related fatigue

Assessment of cancer related fatigue was done using the Cancer fatigue scale. Cancer fatigue scale was developed after referring to the guidelines given by National Cancer Institute, ICD-10\(^1\)\(^{10,98}\) and NCCN\(^7\) (National Comprehensive Cancer network) on cancer related fatigue. The cancer fatigue scale had 18, eleven point scales to measure cancer related fatigue in physical and functional aspects, affective and cognitive areas.

The scoring was done based on the NCCN guidelines for age>12 years that fatigue will be measured on a 0 – 10 scale, and can be classified as:

None to mild : 0 - 3
Moderate fatigue : 4 - 6
Severe fatigue : 7 - 10

Classification of Scores of Cancer fatigue scale based on NCCN guidelines:

0 : No fatigue
1 - 54 : Mild fatigue
55 - 108 : Moderate fatigue
109 - 180 : Severe fatigue
Fig 4: Classification of scores of cancer related fatigue at the beginning of radiation therapy based on the scoring of cancer fatigue scale.

Majority of breast cancer patients in the experimental group 61(76.25%) and control group 70(87.5%) had only mild fatigue at the beginning of radiation therapy. Very few patients in the experimental group 9(11.25) and control group 7(8.75) had severe fatigue at the beginning of radiation therapy.

The breast cancer patients who were randomised for this study were selected after completion of surgical treatment and chemotherapy for breast cancer. The patients have completed several cycles of chemotherapy before starting radiation therapy. Surgical and chemotherapeutic treatments for breast cancer cause some amount of fatigue in patients. Findings of a study conducted by Williams and Schreier\textsuperscript{111} are consistent with this notion.
Williams and Schreier examined the effect of informational audiotapes on patients’ self-care behaviors (SCBs) to manage chemotherapy side effects of fatigue, anxiety, and sleep disturbance. Seventy-one women with breast cancer were randomly assigned to the control group (33) and to the experimental group (38). Fatigue on a scale ranging from 1 (not severe) to 5 (extremely severe) was measured. All the women reported some degree of fatigue at the beginning of chemotherapy. Fatigue increased in severity during the course of chemotherapy in both the groups. Though this study has been conducted in breast cancer patients receiving chemotherapy and may not be directly comparable to the present study, it may be interpreted that most of the breast cancer patients experience some amount of fatigue at the beginning of chemotherapy. Findings from the following study tell us that the fatigue escalates during chemotherapy and touches the baseline once the cycles of treatment are completed.

The study conducted by de Jong et al\textsuperscript{112} states that fatigue after completion of chemotherapy did not significantly differ from fatigue at baseline. They assessed the prevalence of fatigue and the course of fatigue as a function of chemotherapy in breast cancer patients undergoing adjuvant chemotherapy. Breast cancer patients were selected from six hospitals, mainly in the south of The Netherlands. 157 patients were interviewed four (n = 20) or five (n = 137) times on the five different dimensions of fatigue like general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue. A significant increase in the fatigue prevalence rates of all patients was seen after the start of chemotherapy, followed by stability in the prevalence rate during chemotherapy treatment and the prevalence declined after the chemotherapy. For all patients, the level of fatigue at the last measurement was not significantly different from the fatigue at baseline. Women with a mastectomy operation were significantly more fatigued during the whole observation...
period than women who underwent a lumpectomy. It is interesting to note that the fatigue touched the baseline in these patients after chemotherapy and the only factor which has influenced the level of fatigue was the type of surgery. Whereas further analysis of our study findings showed that there was no significant difference ($z=0.317$ p-value - $0.751$) in the pre-test scores of cancer related between the two groups with regard to the type of surgery.

Poirier\textsuperscript{100} examined the relationship among sick leave benefits, employment patterns, individual characteristics and fatigue among patients in a community hospital radiation oncology department. Seventy seven participants receiving radiation therapy to the breast, chest, head and neck, pelvis or prostate were recruited for the study. Out of the seventy seven participants, 34 were breast cancer patients. Cancer related fatigue was assessed using numeric scale, brief fatigue inventory and Piper Fatigue scale. Out of all the patients, 40(52\%) reported no fatigue, 36(47\%) reported mild fatigue (<4) and one participant reported moderate fatigue (4 to 6.9) at baseline before starting radiation therapy. Even though, the numbers of breast cancer patients were considerably less in this study, the findings of this study are consistent with our findings. Thus it can be interpreted that majority of the breast cancer patients experience only mild fatigue between the period from the completion of chemotherapy to the beginning of radiation therapy.

Another study conducted by Lavdaniti\textsuperscript{11} among Greek Patients with Breast Cancer (N=106) undergoing adjuvant radiotherapy in a major oncology center also found that most of the patients experienced low levels of fatigue with a mean fatigue score of 1.96 ($\pm$ 1.90) at baseline before starting radiation therapy. Data were collected with the Revised Piper Fatigue Scale (PFS) and the Short Form-36 (SF-36) Health Survey Scale in the first two
days of radiotherapy, during the third week, and during the last week of treatment. Ten women scored 4-6 on the Piper fatigue Scale and four women scored higher than seven. Approximately 13% of women experienced moderate to higher levels of fatigue at baseline. Findings of the above mentioned study are consistent with our study findings.

Donovan\(^{101}\) et al. assessed fatigue in 134 women receiving chemotherapy and radiotherapy or radiotherapy only for early stage breast cancer. Fatigue was assessed using the Fatigue Symptom Inventory (FSI). Findings suggested that fatigue severity decreased significantly during the period of time between the end of chemotherapy and the start of radiotherapy (\(p < 0.0005\)). Fatigue severity at the start of radiotherapy among these women was statistically equivalent to their fatigue severity at the start of chemotherapy. This is yet another study relevant to the findings of the present study.

In the present study, fatigue during the postoperative period and during and after chemotherapy was not assessed. However, the patients selected for the present study have undergone surgical treatment and chemotherapy before starting with radiation therapy. The present study findings supports the findings of all the above mentioned studies in that all the patients experienced some amount of fatigue when they came for radiation therapy. Although the breast cancer patients underwent surgery and chemotherapy before starting radiation therapy, majority of the patients had only mild fatigue at baseline before starting radiation therapy.
SECTION 4

Effectiveness of Pranayama on Cancer Related Fatigue

Comparison of pretest and post-test scores of cancer related fatigue among the experimental group

The following hypothesis was formulated to compare the pretest and posttest scores of cancer related fatigue among the experimental group at 0.05 level of significance.

H0: There will be no significant difference between the pretest and post test scores of cancer related fatigue among the experimental group

Table 9: Difference between the pretest and post-test scores of cancer related fatigue among the experimental group using Wilcoxon sign rank test

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>Pretest score</td>
<td>29</td>
<td>22 to 42</td>
<td>-6.338</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Posttest score</td>
<td>19</td>
<td>8 to 30.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 9 show that there was a significant difference in the pretest and post test scores of cancer related fatigue among the experimental group. The experimental group of patients experienced less amount of fatigue at the completion of radiation therapy.

Experimental group of patients performed pranayama along with radiation therapy. The amount of fatigue has decreased (from a median of 29 to 19) among the experimental group by the end of radiation therapy. Thus it can be interpreted that pranayama was effective in reducing cancer related fatigue among the experimental group of patients.
Comparison of post-test scores of cancer related fatigue between the experimental and control group at the completion of radiation therapy

The following null hypothesis was formulated to compare the scores of cancer related fatigue at the completion of radiation therapy between the experimental group and the control group at 0.05 level of significance.

H08 There will be no significant difference between the scores of cancer related fatigue among the experimental group and control group at the completion of radiation therapy.

Table 10: Difference between the post-test scores of cancer related fatigue among the experimental and control group using Mann-Whitney U test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer related fatigue</td>
<td>Experimental</td>
<td>19</td>
<td>8 to 30</td>
<td>-3.414</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>31</td>
<td>17 to 45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For comparison of post-test fatigue scores between the two groups, normality was checked using Kolmogorov-Smirnov test for normality. Since the data were not following normality, a Mann-Whitney U test was done to compare the differences in scores of cancer related fatigue at the completion of radiation therapy. Data presented in table 10 show that there was a significant difference in the cancer related fatigue experienced by the patients between the experimental group and control group at the completion of radiation therapy. Hence the null hypothesis H08 was rejected. The scores of the cancer related fatigue show that the symptom experienced by the breast cancer patients who received only radiation therapy was more severe compared to the patients who performed Pranayama while receiving radiation therapy.
We have seen in the previous discussions that breast cancer patients experience some amount of fatigue throughout the treatment. A study conducted by Goldstein et al\textsuperscript{113} among stage I or II breast cancer patients (n=252) reported that fatigue was prevalent at baseline affecting 51 (24\%) breast cancer patients. Fatigue was assessed at end of treatment and at 1, 3, 6, 9, and 12 months as well as 5 years later using Somatic and Psychological Health Report (SPHERE) questionnaire. The rates for fatigue was highest at end of treatment, affecting 90 (42\%) with a fatigue state, 46 (22\%) with mood disturbance, and 34 (16\%) with both. The rate of fatigue was lowest at 12 months (14\%; n=30). The rate for CRF was 24\% (n = 51) post-surgery and 31\% (n = 69) at end of treatment; it became persistent in 11\% (n = 24) at 6 months and 6\% (n = 12) at 12 months. So this study tells us that fatigue is a symptom consistently experienced by breast cancer patients at all phases of treatment.

Another study done by Servaes, Verhagen and Bleijenberg\textsuperscript{114} assessed the determinants of chronic fatigue in 150 disease free breast cancer patients from hospitals in Netherlands. Fatigue severity was measured by the fatigue severity subscale of checklist individual strength; a 20 item questionnaire to measure the four aspects of fatigue like severity, concentration, motivation and physical activity. Fifty seven patients (38\%) were severely fatigued, compared with 11\% in a matched sample of women without a history of cancer. This study was conducted after the breast cancer patients have completed all methods of treatment. The mean duration between the data collection period and the completion of treatment was 29 months. So this study further emphasises that fatigue is a very prevalent problem among breast cancer patients even when they are disease free.
Since the present study was conducted among breast cancer patients undergoing radiation therapy, further literature review was done among these populations. A study conducted by Manir et al\textsuperscript{24} among one hundred and ten stage I to stage III post-mastectomy breast cancer patients assessed the prevalence, course and degree of fatigue during adjuvant treatments. Fatigue was assessed using Functional Assessment of Illness Therapy - Fatigue subscale (FACIT-F) at Department of Radiotherapy, Medical College and Hospitals, Kolkata, West Bengal. Patients were treated with six cycles of 5-Flurouracil, Doxorubicin, and Cyclophosphamide with standard dose schedule in 21-28 days interval and those who had indication to radiotherapy were treated with 50Gy of external beam radiation in 25 fractions, 5 days a week with Cobalt 60 machine. During radiotherapy, patients were interviewed each week during radiotherapy, and second and fourth week after completion of therapy. Seventy-two patients (91%) had fatigue during chemotherapy. Pre-radiotherapy assessment showed lower scores of fatigue. Prevalence of fatigue increased significantly from second assessment onward. Fifty-two patients (77%) had fatigue during radiotherapy. Prevalence increased in the third week of radiotherapy and decreased subsequently. Findings of this study are consistent with our findings in that patients experienced minimal fatigue before radiotherapy.

Another study confirming the fact that patients experience fatigue during the radiation therapy was done by Whelan et al.\textsuperscript{115} Here they have compared the quality of life of 837 breast carcinoma patients treated by lumpectomy and axillary lymph node dissection and randomly allocated to receive either radiation (n = 416) or no radiation therapy (n =421). Patients assigned to radiation therapy received a dose of 40 Gy given by cobalt-60 over a period of 3 weeks with 16 daily fractions followed by a booster dose of 12.5 Gy in 5
daily fractions. Fatigue along with other symptoms was assessed using a modified version of
the breast cancer chemotherapy questionnaire (BCQ) 18. There was a significant difference
in the scores of fatigue at one month (p 0.005) and at two months (p 0.0001) post treatment
between the experimental and control group with the patients assigned to radiation therapy
experiencing more fatigue.

The above mentioned studies have shown that breast cancer patients experienced
fatigue during treatment for cancer. Patients receiving radiation therapy mostly experienced
increasing levels of fatigue during the course of treatment. The present study findings also
show that breast cancer patients experienced fatigue during the course of radiation therapy
with the exception that breast cancer patients who performed pranayama along with
radiation therapy experienced less amount of fatigue.

Further we have explored the different interventions used to treat cancer related
fatigue to compare findings with the present study findings. Many studies have attempted
various non-pharmacological approaches for treating cancer related fatigue. Wanchai, Armer
and Stewart116 in their systematic review have reported that the non-pharmacologic
supportive strategies for CRF are commonly exercise, education and counselling, sleep
therapy, and complementary therapy. These therapies are found to be helpful methods in
improving quality of life in patients with breast cancer experiencing CRF.

Barsevick, Newhall and Brown117 in the review, management of cancer related
fatigue emphasises that largest body of data support exercise as an effective measure in
reducing CRF followed by patient education and counselling.
McNeely et al\textsuperscript{118} summarized and reported the evidence concerning the effects of exercise on breast cancer patients and survivors referring many different databases. Randomised controlled trials comparing exercise with a placebo, controlled comparison or standard care were included for review. Six studies involving 319 patients assessed the effect of exercise on symptoms of fatigue. One study measured fatigue using the Functional Assessment of Cancer Therapy–Fatigue (FACT–F) quality-of-life scale, four studies used the revised Piper Fatigue Scale and one study used a visual analogue scale for measuring fatigue. The pooled results from all six studies showed that exercise significantly improved symptoms of fatigue (SMD 0.46, 95\% CI 0.23 to 0.70). So from this review it is clear that exercise is used as an intervention to combat fatigue.

Van Weert et al\textsuperscript{119} compared physical training combined with cognitive behavioral therapy (CBT) with physical training alone and with no intervention for the management of cancer related fatigue. One hundred and forty seven cancer survivors were randomly assigned to a group that received physical training combined with CBT or a group that received physical training alone and were scheduled for baseline measurements. Five dimensions of fatigue like general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue were measured before and after the intervention. There was no significant difference in fatigue among the 3 groups at baseline. At the post-intervention assessment, the non-intervention control group reported significantly more fatigue in all domains. Finally, the results showed that physical training combined with CBT and physical training alone was equally effective in reducing fatigue. This study further confirms that exercise used along with other behavioural interventions can reduce cancer related fatigue.
The abovementioned studies emphasized the usefulness of exercise as an intervention for managing cancer related fatigue. Like other forms of exercises, yoga and Pranayama are also physical exercises with mindfulness and with emphasis or attention to one’s own breathing. A few studies have shown that yoga was effective in reducing cancer related fatigue.

In the review by Wanchai, Armer and Stewart\textsuperscript{116} the study which used restorative yoga with pre-test and post-test design showed that patients with breast cancer who attended a yoga group reported a significant improvement in fatigue, whereas no significant difference was found in the control group. Findings of this study are consistent with our study findings.

Kirshbaum\textsuperscript{120} in their review states that five broad categories of interventions were identified for cancer related fatigue in terms of applicability to community practice: exercise, pharmacological approaches, adjustment strategies, complementary therapies and psycho-educational interventions. The author identifies that most complementary therapies are considered to be valuable in the management of fatigue to a certain extent since they tend to promote overall wellbeing. Small scale studies involving yoga (DiStasio, 2008), acupuncture and acupressure (Johnston et al. 2007; Molassiotis et al. 2007), massage (Cassileth and Vickers, 2004), healing touch (Post-White, 2003) and aromatherapy and foot soak with reflexology (Stasi et al. 2003) have all demonstrated promising results. Here again yoga has proved to be reducing cancer related fatigue. In the present study, we have found that pranayama; a part of yoga was effective in reducing cancer related fatigue.

In another review, Cramer et al\textsuperscript{121} assessed and analyzed the evidence for effects of yoga on health-related quality of life and psychological health in breast cancer patients and survivors. Twelve RCTs involving 742 patients were included in the systematic review and
ten studies were included for meta-analysis. This review found that there is a short term effect on health related quality of life of breast cancer patients who performed yoga. Yoga was also found to have short-term effects on anxiety, depression, perceived stress, and psychological distress. Authors recommend Yoga as an intervention to improve psychological health during active breast cancer treatment. Though the present study has not studied the psychological aspects of breast cancer patients separately, it was included in the cancer fatigue questionnaire.

In another study, Danhauer et al.\textsuperscript{122} determined the effectiveness of restorative yoga intervention as a supportive therapy for women diagnosed with ovarian or breast cancer on self-reported fatigue, psychological distress and well-being, and quality of life on 14 breast cancer patients. The restorative yoga intervention included combined physical postures, breathing, and deep relaxation given for 75 minutes for a period of 10 weeks. Fatigue was assessed at baseline, immediate post-intervention, and 2 months post-intervention. Fatigue improved significantly between pre- and post-intervention. Women with breast cancer reported a higher quality of life after the intervention. Although the restorative yoga intervention was not designed to provide group support, many of the participants noted the value of the social aspects of the yoga classes. The present study also found that there was a reduction in the level of fatigue experienced by the women who practised Pranayama than the women who has undergone radiation therapy only. The researcher has also noted group cohesion between the study participants and the easiness with which the participants discussed their problems with each other.

Bower et al.\textsuperscript{14} assessed “Subjective fatigue severity” with the Fatigue Symptom Inventory (FSI) and “vigor” with the vigor subscale of the Multidimensional Fatigue Symptom Inventory.
Inventory among breast cancer survivors with persistent post-treatment fatigue. There was a control group who received health education for 120 minutes once in a week for 12 weeks and an experimental group who performed Iyengar yoga for 90 minutes twice a week for 12 weeks. It was found that Yoga resulted in statistically significant improvements in fatigue severity and vigor. The present study findings also showed that there is a significant difference in the cancer related fatigue experienced by the control group and the experimental group. The group who performed pranayama reported less amount of fatigue compared to the control group.

Moadel et al.\textsuperscript{99} in another study reported that there was no significant difference in the levels of fatigue among breast cancer patients between the group who performed yoga (34.37 ± 11.26) and the waitlist control group (33.82 ± 12.97) at three months follow up. These findings are not consistent with our study findings.

Findings, contradictory to the present study findings were observed also by Chandwani et al.\textsuperscript{123} They assessed the effects of yoga on quality of life (QOL) and psychosocial outcomes in sixty-one women with breast cancer undergoing radiotherapy. Patients were randomly assigned to either yoga or a wait-list group. Experimental group received Yoga classes biweekly during the 6 weeks of radiotherapy. Participants completed questionnaires on Quality of Life, fatigue etc. before radiotherapy and then again 1 week, 1 month, and 3 months after the end of radiotherapy. There was no significant difference between the groups with regard to fatigue, depression or sleep scores at any observation point. Whereas in the present study even though sleep improvement was not the primary outcome, majority of patients who performed pranayama reported an improvement in sleep along with reduction in the scores of fatigue.
Another review contradicts the view that yoga is effective in reducing fatigue. Boehm, Ostermann, Milazzo and Bussing\textsuperscript{124} reviewed the effectiveness of yoga interventions for fatigue by searching the controlled clinical studies in Pubmed until January 2012. Out of the 19 studies, 18 were randomised and single blinded and one was non-randomised. Studies were done on participants suffering from cancer, multiple sclerosis, dialysis, chronic pancreatitis, fibromyalgia, asthma, or healthy people. The authors concluded that although yoga is in general a safe therapeutic intervention and effective to attenuate several other health-related symptoms, the meta-analysis was not able to define relevant effects of yoga on patients suffering from fatigue. Since this review included a mixture of patients it is difficult to conclude the result in terms of cancer patients especially in terms of breast cancer patients.

The discussion above has some studies supporting the findings of the present study and some studies contradicting the present study findings. To further analyse the effectiveness of pranayama on different aspects of fatigue, comparisons were made between the two groups regarding severity of cancer related fatigue, the affective and cognitive aspects. Since the data were not following normality, a Mann-Whitney U test was done to compare the different aspects of fatigue between the two groups. The severity of fatigue had a median score of 3, the physical aspects had a median score of 2 and the affective aspects had a median score of 5 and cognitive aspects had a median of one for the experimental group and the severity of fatigue had a median score of 8, the physical aspects had a median score of 6 and the affective aspects had a median score of 10 and cognitive aspects had a median of two for the control group. There was a significant difference between the two groups with regard to the severity of cancer related fatigue (Z score-4.092, p-value<0.001),
physical aspects of cancer related fatigue (Z score-3.158, p-value 0.002), affective aspects (Z score-3.410, p-value 0.001) and cognitive aspects (Z score-4.092, p-value 0.011). Thus it may be interpreted that pranayama was very effective in reducing the different aspects or domains of cancer related fatigue.

The data were further analysed based on stage of the disease, age, haemoglobin levels and type of surgery between the groups to check whether pranayama was effective in reducing cancer related fatigue with regard to these variables. Since the data were not following normality, a Mann-Whitney U test was used to compare the two groups.

Analysis showed that there was no significant difference in the scores of cancer related fatigue between the stage 1 breast cancer patients of the two groups (z-1.231, p-value 0.218) whereas there was a significant difference in the scores of cancer related fatigue between the stage II breast cancer patients of the two groups (z-2.185, p-value 0.029) and between the stage III breast cancer patients of the two groups (z-2.017, p-value 0.044).

A significant difference in the scores of cancer related fatigue could not be observed among patients with stage 1 breast cancer probably because of the less number of patients with stage 1 breast cancer in this study. The study needs to be repeated on a larger number of only stage 1 breast cancer patients to validate these findings.

Analysis showed that there was a significant difference in the scores of cancer related fatigue between the patients with more than 45 years of age of the two groups (z-2.092, p-value 0.036) and between the patients with less than or equal to 45 years of age of the two groups (z-2.595, p-value 0.009). There was no difference in the effectiveness of
pranayama in reducing cancer related fatigue with regard to the age of the patients. There was significant reduction in cancer related fatigue in patients below 45 and above 45 years of age and those who practiced pranayama.

Here again the analysis showed that there was a significant difference in the scores of cancer related fatigue between the patients with more haemoglobin of the two groups (z-3.53, p-value <0.001) whereas there was no significant difference in the scores of cancer related fatigue between the patients with less haemoglobin of the two groups (z-1.033, p-value 0.302). Low haemoglobin concentration and anaemia are known risk factors for cancer related fatigue. It may be interpreted that pranayama did not have any influence in reducing cancer related fatigue among breast cancer patients with low haemoglobin.

There was a significant difference in the scores of cancer related fatigue between the patients who have undergone modified radical mastectomy of the two groups (z-4.071, p-value <0.001) whereas there was no significant difference between the patients who have undergone breast conservation surgery of the two groups (z-0.246, p-value 0.806). There was no significant difference between the patients with breast conservation surgery probably because of the less number of patients in that group.
Pre-test and Post-test scores of cancer related fatigue among the experimental group and control group.

Fig 5: Comparison of breast cancer patients with mild fatigue among the experimental group and the control group

The number of patients with mild fatigue were more in the experimental group compared to control group during the post-test.
Pre-test and Post-test scores of cancer related fatigue among the experimental group and control group.

The number of patients with moderate fatigue were more in the control group during the post-test.

Fig 6: Comparison of breast cancer patients with moderate fatigue among the experimental group and the control group.
Pre-test and Post-test scores of cancer related fatigue among the experimental group and control group.

Fig 7: Comparison of breast cancer patients with severe fatigue among the experimental group and the control group

The number of patients with severe fatigue were more in the control group during the post-test.
SECTION 5

Effectiveness of Pranayama on biochemical parameters

In this study antioxidant enzymes like glutathione reductase, glutathione peroxidase, glutathione S transferase, and non-enzymatic antioxidants like protein thiols and glutathione were assessed at the beginning of radiation therapy and at the completion of radiation therapy. Effectiveness of Pranayama on each of the antioxidants and antioxidant enzymes are analysed and presented in the following tables.

Due to delay in getting the reagents for the analysis of antioxidants and antioxidant enzymes, the reagents were saved for doing the post-test and only 80 patients’ blood sample could be analyzed for pretest out of the total of 160 patients. The following statistical tests describe the tests done to check whether there was a difference in the pretest values and post-test values among the experimental group and between the post-test values of the experimental and control group. When comparing the pretest and posttest levels of biochemical parameters among the experimental group, the same 40 patients’ post-test tests results were considered whose pretest was done.

Effectiveness of Pranayama on the levels of glutathione (GSH)

Comparison of pretest and post-test levels of glutathione among the experimental group

The following hypothesis was formulated to compare the pretest and post-test levels of glutathione among the experimental group at 0.05 level of significance.

H09 There will be no significant difference between the pretest and post-test levels of glutathione among the experimental group.
Table 11: Difference between the pretest and post-test levels of glutathione among the experimental group using Wilcoxon sign rank test (n=40)

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest level</td>
<td>24.4</td>
<td>18.24 to 30.2</td>
<td>-2.796</td>
<td>0.005</td>
</tr>
<tr>
<td>Experimental</td>
<td>Posttest level</td>
<td>26.6</td>
<td>18.31 to 30.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 11 show that there was a significant difference in the pretest and post-test levels of glutathione among the experimental group. Thus H09 was rejected. It may be interpreted that pranayama was effective in improving the level of glutathione among the experimental group.

Comparison of post-test levels of glutathione between the experimental and control group at the completion of radiation therapy

To find out the effectiveness of Pranayama on the levels of glutathione (GSH) between the experimental and control group, a Mann – Whitney U test was done. The following null hypothesis was stated to test the statistical significance.

H010: There will be no significant difference between the pretest and post-test levels of glutathione among the experimental group

Table 12: Difference between the post-test levels of glutathione among the experimental group and control group using Mann-Whitney U test (n=80 +80)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Glutathione</td>
<td>Experimental</td>
<td>26.6</td>
<td>18.31 to 30.5</td>
<td>-3.07</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>19.1</td>
<td>18.02 to 24.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
There was a significant difference in the level of reduced glutathione between the experimental group and control group at the completion of radiation therapy. Thus the null hypothesis H01 was rejected. The level of reduced glutathione in the experimental group was higher than that of the control group which indicates a less oxidative stress in breast cancer patients performing Pranayama when undergoing radiation therapy.

There are no similar studies done on breast cancer patients finding the effectiveness of yoga/pranayama on the level of reduced glutathione; however Cheong and Lim\textsuperscript{125} assessed the properties of regular yoga training on oxidative stress and antioxidant components, including total glutathione (GSH) and its redox systems (GSH-peroxidase and reductase) in healthy university students in the Republic of Korea. Out of the 25 students who participated in the study, twelve students practised yoga 30 mins 5 times a week for 12 weeks whereas the control group practised aerobic exercise for 12 weeks. The total GSH content in the serum levels was significantly higher in the yoga training group (80.45 ± 21.89 μM) compared to the control group.

Sengar et al\textsuperscript{126} assessed the effect of Sudarshan Kriya and Pranayama on antioxidant status of two groups of police trainees measuring glutathione and antioxidant enzymes at the end of 5 months of practice. Glutathione levels were significantly higher in practitioners of Sudarshan Kriya and Pranayama compared to non-practitioners (t = 6.968, p = 2.038 e - 06).

Sinha et al\textsuperscript{115} reported similar findings in a study conducted among the healthy male volunteers of Indian Navy. There were 30 volunteers in the experimental group who performed yoga, pranayama and meditation for one hour in the morning, five days a week for six months. There was a significant increase in the level of glutathione in healthy male
volunteers from navy who performed yoga and pranayama at the end of six months. GSH level increased significantly ($p < 0.05$) from the baseline value of 235.3 _ 16.9 nmol/L to 331.7 _ 37.6 nmol/L in the yoga group.

Findings of these studies are consistent with the present study findings in that pranayama improved the level of glutathione in all these studies. But since the population under study were so different, it was not possible to validate the findings of the present study based on these studies.

Further analysis of the data was done to see whether the level of glutathione was different with regard to variables like age, stage of the disease, type of surgery and haemoglobin levels. There was no significant difference in the level of glutathione between the patients of the two groups who have stage I breast cancer ($z=-1.890$, $p$-value 0.069) and there was no significant difference in the level of glutathione between the patients of the two groups who have stage II breast cancer ($z=-1.092$, $p$-value 0.275) whereas there was a significant difference in the level of glutathione between the patients of the two groups for stage III breast cancer patients ($z=-2.470$, $p$-value 0.014).

There was no significant difference in the level of glutathione between the patients of the two groups who were less than or equal to 45 years of age of the two groups ($z=-1.115$, $p$-value 0.265) whereas there was a significant difference in the level of glutathione for breast cancer patients who were more than 45 years of age of the two groups ($z=-3.436$, $p$-value 0.001).

There was a significant difference in the level of glutathione between the patients of the two groups who had less than or equal to 12g/dL of haemoglobin ($z=-2.697.534$, $p$-value...
0.007) whereas there was no significant difference in the level of glutathione between the patients of the two groups who had more than 12g/dL haemoglobin ($z=1.564$, $p$-value 0.118). The exact reason for this difference is not clear. But it is interesting to note that this group of patients did not have a difference in the scores of cancer related fatigue as well.

There was a significant difference in the level of glutathione between the patients of the two groups who had modified radical mastectomy ($z=2.347$, $p$-value 0.019) whereas there was no significant difference in the level of glutathione between the patients of the two groups who had breast conservation surgery ($z=0.869$, $p$-value 0.390). There was no significant difference in the group who underwent breast conservation surgery probably because there was only less number of patients in this group.

**Pre-test and post-test levels of glutathione among the experimental and control group.**

![Fig 8: Bar diagram showing the Pre-test and Post-test levels of glutathione among the experimental and control group.](image)
Data in figure above show that the level of reduced glutathione in blood was maintained during the course of radiation therapy by the group who performed Pranayama. Post-test level of glutathione was higher in the experimental group than in the control group who received only radiation therapy.

Lamson and Brignall\textsuperscript{127} reviewed whether concurrent administration of antioxidants is contraindicated during cancer therapeutics. Glutathione is a tri-peptide thiol (sulphydryl-containing) compound which is the major intracellular antioxidant in the body. Oral glutathione is poorly absorbed, with negligible plasma concentrations found after oral administration. Aerosol administration of glutathione and intravenous administration are considered as effective means of delivery to the plasma. The authors further elaborated that in a randomized pilot trial involving 45 participants who received pelvic radiotherapy, intravenous administration of glutathione prior to radiotherapy resulted in less post-therapy diarrhoea (28%, compared to 52% of controls) and patients were found more likely to complete the treatment cycle (71% to 52%). The authors concluded that Glutathione is not thought to interfere with radiotherapy.

Conklin\textsuperscript{128} approves that although radiation kills cells by generating very high levels of free radicals, this does not necessarily mean that antioxidants are contraindicated in all cases. The author further explains that radiotherapy is most effective in well-oxygenated tissues, whereas the central portions of tumours are often hypoxic. So antioxidants may actually play a beneficial role in radiation therapy by improving blood flow within tumours and the surrounding tissues, thus rendering tumors more susceptible to radiation. Since free radical generation is proportional to the oxygen tension in the tissue, antioxidants given in
amounts that improve blood flow may have improved antineoplastic effect. Di Mascio\textsuperscript{129} emphasizes that Glutathione is the most important cellular thiol, acting as a substrate for several transferase, peroxidases, and other enzymes. Moss\textsuperscript{130} in his review commented that a blanket rejection of the concurrent use of antioxidants along with cancer treatment serves neither the scientific community nor the cancer patients.

**Effectiveness of Pranayama on levels of protein thiols**

**Comparison of pretest and post-test levels of protein thiols among the experimental group**

The following null hypothesis was formulated to compare the pre-test and post-test levels of protein thiols among the experimental group at 0.05 level of significance.

H011 There will be no significant difference between the pretest and post-test levels of protein thiols among the experimental group

Since the data were following normality, a paired ‘t’ test was done to compare the pretest and post-test levels of protein thiols among the experimental group.

**Table 13: Difference between the pretest and post-test levels of protein thiols among the experimental group using paired ‘t’ test**

\[(n= 40)\]

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Mean</th>
<th>Confidence Interval</th>
<th>‘t’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>Pretest level</td>
<td>235.8 ± 74.6</td>
<td>8.08 to 33.15</td>
<td>-0.382</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Posttest level</td>
<td>243.5 ± 106.48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 13 show that there was a significant difference in the pretest and post-test levels of protein thiols among the experimental group. The experimental group of
patients had increased levels of protein thiols at the completion of radiation therapy. Thus the null hypothesis H01 was rejected.

**Comparison of post-test levels of protein thiols between the experimental and control group at the completion of radiation therapy**

The following null hypothesis was formulated to compare the post-test levels of protein thiols among the experimental group and control group at 0.05 level of significance.

H012: There will be no significant difference between the post-test levels of protein thiols among the experimental group and control group at the completion of radiation therapy

Since the data were following normality, an independent ‘t’ test was done to compare the post-test levels of protein thiols among the experimental group and control group.

**Table 14: Difference between the post-test levels of protein thiols among the experimental group and control group using independent ‘t’ test**

(n=80 +80)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>Confidence Interval</th>
<th>‘t’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Thiols</td>
<td>Experimental</td>
<td>243.56 ± 106.4</td>
<td>30.53 to 79.60</td>
<td>4.43</td>
<td>0.001</td>
</tr>
<tr>
<td>(µmoles/lit)</td>
<td>Control</td>
<td>216.13 ± 62.86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in the table above suggests that there was a significant difference in the level of protein thiols between the experimental group and the control group at the completion of radiation therapy. There were significantly increased levels of protein thiols among patients who performed Pranayama along with radiation therapy. Thus the null hypothesis H012 was rejected.
There are no published studies available on the effectiveness of pranayama on the levels of protein thiols among breast cancer patients. In the study conducted by Unsal et al\textsuperscript{74} among cancer patients undergoing radiation therapy of different anatomic sites, a marked lipid peroxidation increase and significant decrease of plasma RSH level was noticed after irradiation.

Hall and Giaccia have highlighted the fact that\textsuperscript{79} radiation therapy causes DNA strand breakage in cells by the generation of free radicals. Radiation therapy aims to prevent the local recurrence of cancer and controls the growth of tumor locally by causing direct DNA strand breakage or by the liberation of free radicals. However when reactive oxygen species (ROS) are in excess of cellular antioxidants, oxidative damage can occur to surrounding cellular structures.

Thiols are compounds that contain carbon bonded sulphydryl 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 oxidized mainly by the formation of disulphide bonds in the plasma. Thus protein thiols play a major role in antioxidant defences.\textsuperscript{105}

Simone and Charles\textsuperscript{131} argued that increased level of antioxidants do not interfere with therapeutic modalities of cancer, decrease their side effects and protect normal tissue. The elevated levels of protein thiols in the experimental group may have beneficial effects against the side effects of radiation therapy.
Further analysis of the data was done to see whether the level of protein thiols was different with regard to variables like age, stage of the disease, type of surgery and haemoglobin levels. There was no significant difference in the level of protein thiols between the patients of the two groups who have stage 1 breast cancer ($z=1.701$, $p$-value $0.109$) whereas there was a significant difference in the level of protein thiols between the patients of the two groups who have stage II breast cancer ($z=2.811$, $p$-value $0.005$) and also for stage III breast cancer patients ($z=2.566$, $p$-value $0.010$). There was no significant difference between the patients with stage 1 breast cancer probably because of the less number of patients in that group.

There was a significant difference in the level of protein thiols between the patients of the two groups who were less than or equal to 45 years of age of the two groups ($z=3.584$, $p$-value $<0.001$) and for breast cancer patients who were more than 45 years of age of the two groups ($z=2.258$, $p$-value $0.024$).

There was a significant difference in the level of protein thiols between the patients of the two groups who had less than or equal to 12g/dL of haemoglobin ($z=3.064$, $p$-value $0.002$) and there was also significant difference in the level of protein thiols between the patients of the two groups who had more than 12g/dL haemoglobin ($z=3.257$, $p$-value $0.001$).

There was a significant difference in the level of protein thiols between the patients of the two groups who had modified radical mastectomy ($z=3.846$, $p$-value $<0.001$) whereas there was no significant difference in the level of protein thiols between the patients of the two groups who had breast conservation surgery ($z=1.549$, $p$-value $0.123$). There was no significant difference in the group who underwent breast conservation surgery probably because there was only less number of patients in this group.
Pre-test and post-test levels of protein thiols among the experimental and control group.

Fig 9: Bar diagram showing the pre-test and post-test levels of protein thiols among the experimental and control group.

Data in the figure above show that the post levels of protein thiols were higher in the experimental group compared to the control group.
Effectiveness of Pranayama on levels of glutathione S transferase

Comparison of pretest and post-test levels of glutathione S transferase among the experimental group

The following null hypothesis was formulated to compare the pretest and post-test levels of glutathione S transferase among the experimental group at 0.05 level of significance.

H013 There will be no significant difference between the pretest and post-test levels of glutathione S transferase among the experimental group

Since the data were not following normality, a Wilcoxon sign rank test was done to compare the pretest and post-test levels of glutathione S transferase among the experimental group.

Table 15: Difference between the pretest and post-test levels of glutathione S transferase among the experimental group using Wilcoxon sign rank test

(n=40)

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>Pretest level</td>
<td>2.4</td>
<td>2.09 to 2.88</td>
<td>-3.28</td>
<td>0.201</td>
</tr>
<tr>
<td></td>
<td>Posttest level</td>
<td>3.00</td>
<td>2.79 to 5.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 15 show that there was no significant difference in the pretest and post-test levels of glutathione S transferase among the experimental group. Thus the null hypothesis H013 was accepted.
Comparison of post-test levels of glutathione S transferase between the experimental and control group at the completion of radiation therapy

The following null hypothesis was formulated to compare the post-test levels of glutathione S transferase among the experimental group and control group at 0.05 level of significance.

H014: There will be no significant difference between the post-test levels of glutathione S transferase among the experimental group and control group at the completion of radiation therapy

Since the data were not following normality, a Mann Whitney U test was done to compare the post-test levels of glutathione S transferase among the experimental group and control group.

Table 16: Difference between the post-test levels of glutathione S transferase among the experimental and control group at the completion of radiation therapy using Mann Whitney U test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione S transferase</td>
<td>Experimental</td>
<td>3.0</td>
<td>2.79 to 5.06</td>
<td>-2.57</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.56</td>
<td>2.71 to 7.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a statistically significant difference in the level of Glutathione S transferase between the experimental and control group at the completion of radiation therapy. As in the pre-test, the level of glutathione S transferase was higher among the control group than in the experimental group. This means that Pranayama did not have any effect on the level of glutathione S transferase among breast cancer patients receiving
radiation therapy. Glutathione S transferase plays an important role in the regulation of glutathione levels in cells. This enzyme catalyzes the formation of glutathione conjugates with cytotoxic agents and thereby protects the cells against various cytotoxic effects.\textsuperscript{132}

Prabasheela et al\textsuperscript{133} assessed antioxidant enzymes in the blood samples of breast cancer patients from Kancheepuram and compared them with age matched control. The level of GST was reduced in breast cancer patients (1.12 ± 0.05) compared to controls (1.65 ± 0.02).

There are no studies available to compare the effect of Pranayama on glutathione S transferase. Woolston et al\textsuperscript{134} in their study has assessed the nuclear and cytoplasmic expression of glutathione S-transferase-θ, -π, and -α using conventional immunohistochemistry on a tissue microarray of 224 breast tumors. It was concluded that a high cytoplasmic expression of glutathione S-transferase-θ significantly correlated with a greater risk of local recurrence.

Ambrosone et al\textsuperscript{135} assessed the genetic and non-genetic predictors of skin toxicity associated with radiation therapy, among women with breast cancer in Germany from 1998 to 2001. These women received primary radiation therapy following breast conserving surgery and had not previously received chemotherapy. Side effects were monitored and recorded at cumulative doses of 36–42 Gy, 44–50 Gy, and 60–66 Gy. Reduced enzyme activity of GSTP1 genotypes was significantly associated with acute side effects of radiation therapy.

Ambrosone et al\textsuperscript{136} in another study, evaluated the role of GSTM1- and GSTT1-null genotypes on disease-free and overall survival. Out of the 251 women who participated in the study, 54 women received only radiation therapy for breast cancer. The study results showed that women with null genotypes for GSTM1 and GSTT1 had reduced hazard of death and recurrence of cancer.
Hashemi et al\textsuperscript{137} in a case-control study with 134 breast cancer patients and 152 healthy cancer free women in Iran observed that GSTM1 and GSTP1, but not GSTT1 genetic polymorphisms are associated with increased risk of breast cancer in our population.

In the present study it was shown that there was a consistently elevated level of glutathione S transferase in the control group. This means that pranayama did not have any effect on the level of glutathione S transferase. This may probably indicate that pranayama cannot influence gene coding and DNA transcription for the formation of new proteins of enzymes.

Further analysis of the data was done to see whether the level of glutathione S transferase was different with regard to variables like age, stage of the disease, type of surgery and haemoglobin levels. There was no significant difference in the post test level of glutathione S transferase between the patients of the two groups who have stage I breast cancer (\(z\)-0.189, p-value 0.92) and there was no significant difference in the post test level of glutathione S transferase between the patients of the two groups who have stage II breast cancer (\(z\)-1.828, p-value 0.068) and also for stage III breast cancer patients (\(z\)-1.728, p-value 0.084).

There was no significant difference in the post test level of glutathione S transferase between the patients of the two groups who were less than or equal to 45 years of age of the two groups (\(z\)-1.568, p-value 0.118) whereas there was a significant difference between the patients of the two groups who were more than 45 years of age (\(z\)-1.986.595, p-value 0.047).

There was a significant difference in the post test level of glutathione S transferase between the patients of the two groups who had less than or equal to 12g/dL of haemoglobin (\(z\)-1.963, p-value 0.050) whereas there was no significant difference in the post test level of
glutathione S transferase between the patients of the two groups who had more than 12g/dL haemoglobin (z-1.829, p- value 0.067).

There was a significant difference in the post test level of glutathione S transferase between the patients of the two groups who had modified radical mastectomy (z-2.736, p- value 0.006) whereas there was no significant difference in the post test level of glutathione S transferase between the patients of the two groups who had breast conservation surgery (z-0.944, p- value 0.345). There was no significant difference in the group who underwent breast conservation surgery probably because there was only less number of patients in this group.

**Pre-test and post-test levels of glutathione S transferase among the experimental and control group.**

![Bar diagram showing the pre-test and post-test levels of glutathione S transferase among the experimental and control group.](image)

Fig 10: Bar diagram showing the pre-test and post-test levels of glutathione S transferase among the experimental and control group.
Data in the figure above show that the post levels of glutathione S transferase were higher in the control group compared to the experimental group.

**Effectiveness of Pranayama on levels of glutathione peroxidase**

**Comparison of pretest and post-test levels of glutathione peroxidase among the experimental group**

The following null hypothesis was formulated to compare the pretest and posttest levels of glutathione peroxidase among the experimental group at 0.05 level of significance.

\[ H_0: \text{There will be no significant difference between the pretest and post-test levels of glutathione peroxidase among the experimental group} \]

Since the data were not following normality, a Wilcoxon sign rank test was done to compare the pretest and post-test levels of glutathione peroxidase among the experimental group.

**Table 17: Difference between the pretest and post-test levels of glutathione peroxidase among the experimental group using Wilcoxon sign rank test (n=40)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>Pretest level</td>
<td>2.0</td>
<td>0.34 to 4.57</td>
<td>-0.854</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>Posttest level</td>
<td>0.64</td>
<td>0.45 to 1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 17 show that there was no significant difference in the pretest and post-test levels of glutathione peroxidase among the experimental group. Hence the null hypothesis $H_{015}$ was accepted.
In KASAPOVIC’s study, radiotherapy in patients older than 60 years decreased the activity of GPx (17.46±0.50 vs. 14.65±0.93 mU/mg of prot., p<0.05) whereas in patients aged 45-58 years, the level of glutathione peroxidase remained unchanged after radiotherapy.

**Comparison of post-test levels of glutathione peroxidase between the experimental and control group at the completion of radiation therapy**

The following null hypothesis was formulated to compare the post-test levels of glutathione peroxidase among the experimental group and control group at 0.05 level of significance.

H0: There will be no significant difference between the post-test levels of glutathione peroxidase among the experimental group and control group at the completion of radiation therapy

Since the data were not following normality, a Mann Whitney U test was done to compare the post-test levels of glutathione peroxidase among the experimental group and control group.

**Table 18: Difference between the post-test levels of glutathione peroxidase among the experimental and control group at the completion of radiation therapy using Mann Whitney U test**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase(IU/L)</td>
<td>Experimental</td>
<td>0.64</td>
<td>0.45 to 1.7</td>
<td>-0.637</td>
<td>0.524</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.64</td>
<td>0.08 to 1.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
There was no statistically significant difference in the level of Glutathione peroxidase between the experimental and control group at the completion of radiation therapy. Glutathione Peroxidase catalyzes the reduction of organic peroxide (ROOH), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). Glutathione peroxidase detoxifies peroxides in living cells. Glutathione peroxidase thus helps in protecting the cells and tissues from damage by free radicals, which are released during the decomposition of peroxides. When these free radicals attack the lipid components of the cells, lipid peroxidation takes place. Glutathione peroxidases with the help of glutathione, reduce peroxides to alcohols thus preventing the formation of free radicals.\(^{90,88,89,138}\) Pranayama did not have any effect on the level of glutathione peroxidase. It may probably indicate that Pranayama does not have an effect at the gene coding and DNA transcription level and it is not capable of producing new protein molecules of this enzyme. There are no comparable studies done on breast cancer patients undergoing radiation therapy.

However, Nikam et al\(^{16}\) conducted a study in Belgaum, Karnataka, India to assess the effect of Pranayama practicing on lipid peroxidation and antioxidants in coronary artery disease (CAD). There were a total of 60 coronary artery disease patients in the age group 40-60 years. Out of these, 30 patients were practicing Bhasrika Pranayama, Kapalbhati Pranayama, Bhya Pranayama, Anulom-vilom Pranayama and Brahmari Pranayama and the other 30 were kept on drug therapy. Age and sex matched 60 normal healthy subjects from Anand Yoga Center, Nipani and Belgaum was used as controls. In CAD patients there was significantly decreased activity of Glutathione peroxidase in the beginning. This lowered activity of Glutathione peroxidase was significantly raised after practicing 4 and 6 weeks of Pranayama and it became within normal range after 8 weeks.
In the study conducted by Cheong and Lim\textsuperscript{125} assessing the properties of regular yoga training on oxidative stress and antioxidant components among healthy university students, there were no remarkable changes in the serum levels of glutathione peroxidase activities after 12-week intervention.

As discussed earlier, in this study Pranayama failed to have an influence on the level of glutathione peroxidase probably because of its lack of ability to act at the DNA transcription for the formation of new proteins.

Further analysis of the data was done to see whether the level of glutathione peroxidase was different with regard to variables like age, stage of the disease, type of surgery and haemoglobin levels. There was no significant difference in the level of glutathione peroxidase between the patients of the two groups who have stage 1 breast cancer (z-1.134, p- value 0.257) and there was no significant difference in the level of glutathione peroxidase between the patients of the two groups who have stage II breast cancer (z-0.466, p- value 0.641) and also for stage III breast cancer patients (z-.984, p- value 0.325).

There was no significant difference in the level of glutathione peroxidase between the patients of the two groups who were less than or equal to 45 years of age of the two groups (z-0.359, p- value 0.720) and for breast cancer patients who were more than 45 years of age of the two groups (z-0.584, p- value 0.559).

There was no significant difference in the level of glutathione peroxidase between the patients of the two groups who had less than or equal to 12g/dL of haemoglobin (z-1.114, p- value 0.265) and there was no significant difference in the level of glutathione peroxidase between the patients of the two groups who had more than 12g/dL haemoglobin (z-0.668, p- value 0.504).
There was no significant difference in the level of glutathione peroxidase between the patients of the two groups who had modified radical mastectomy ($z=-0.597$, $p$-value 0.550) and there was no significant difference in the level of glutathione peroxidase between the patients of the two groups who had breast conservation surgery ($z=-0.491$, $p$-value 0.628).

**Pre-test and post-test levels of glutathione peroxidase among the experimental and control group.**

![](image)

**Fig 11: Bar diagram showing the pre-test and post-test levels of glutathione peroxidase among the experimental and control group.**

Data in the figure above show that the post levels of glutathione peroxidase were lower in both the control group and experimental group.
Effectiveness of Pranayama on glutathione reductase

Comparison of pretest and post-test levels of glutathione reductase among the experimental group

The following null hypothesis was formulated to compare the pretest and post-test levels of glutathione reductase among the experimental group at 0.05 level of significance.

H017: There will be no significant difference between the pretest and post-test levels of glutathione reductase among the experimental group

Since the data were not following normality, a Wilcoxon sign rank test was done to compare the pretest and post-test levels of glutathione reductase among the experimental group.

Table 19: Difference between the pretest and post-test levels of glutathione reductase among the experimental group using Wilcoxon sign rank test

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest level</td>
<td>1.1</td>
<td>0.32 to 2.2</td>
<td>-0.9</td>
<td>0.368</td>
</tr>
<tr>
<td></td>
<td>Posttest level</td>
<td>1.0</td>
<td>0.165 to 1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data in table 19 show that there was no significant difference in the pretest and post-test levels of glutathione reductase among the experimental group.

Comparison of post-test levels of glutathione reductase between the experimental and control group at the completion of radiation therapy

The following null hypothesis was formulated to compare the post-test levels of glutathione reductase among the experimental group and control group at 0.05 level of significance.
H018: There will be no significant difference between the post-test levels of glutathione reductase among the experimental group and control group at the completion of radiation therapy.

Since the data were not following normality, a Mann Whitney U test was done to compare the post-test levels of glutathione reductase among the experimental group and control group.

Table 20: Difference between the post-test levels of glutathione reductase among the experimental and control group at the completion of radiation therapy using Mann Whitney U test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Median</th>
<th>IQR</th>
<th>‘z’ value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione reductase</td>
<td>Experimental</td>
<td>1.0</td>
<td>0.165 to 1.7</td>
<td>-1.449</td>
<td>0.147</td>
</tr>
<tr>
<td>(IU/L)</td>
<td>Control</td>
<td>1.29</td>
<td>0.4 to 1.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was no statistically significant difference in the level of Glutathione reductase between the experimental and control group. This again probably indicates that Pranayama did not have an effect at the DNA transcription level. Thus H018 was accepted. The role of glutathione reductase in antioxidant defense is to reduce glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. Sinha et al conducted a study\(^15\) in Delhi on healthy male volunteers (51) from the Indian Navy who were divided into two groups - a yoga group (n = 30) and a control group (n = 21). The yoga group performed yogasanas and pranayama for one hour in the morning, five days per week for six months whereas the control group practised routine physical exercises. After completion of six months of training, fasting blood samples were analysed for reduced glutathione (GSH), oxidized
glutathione (GSSG), glutathione reductase (GR), activity, and total antioxidant activity (TAS). Glutathione reductase activity increased significantly in the control group (p < 0.05).

In the study conducted by Cheong and Lim\textsuperscript{125} there were no remarkable changes in the serum levels of glutathione reductase activities at the completion of 12-week intervention.

Further analysis of the data was done to see whether the level of glutathione reductase was different with regard to variables like age, stage of the disease, type of surgery and haemoglobin levels. There was no significant difference in the level of glutathione reductase between the patients of the two groups who have stage I breast cancer (z-1.512, p-value 0.164) and there was no significant difference in the level of glutathione reductase between the patients of the two groups who have stage II breast cancer (z-0.197, p-value 0.844) and also for stage III breast cancer patients (z-1.805, p-value 0.071).

There was no significant difference in the level of glutathione reductase between the patients of the two groups who were less than or equal to 45 years of age of the two groups (z-0.790, p-value 0.430) and for breast cancer patients who were more than 45 years of age of the two groups (z-0.872, p-value 0.383).

There was no significant difference in the level of glutathione reductase between the patients of the two groups who had less than or equal to 12g/dL of haemoglobin (z-0.649, p-value 0.516) and there was no significant difference in the level of glutathione reductase between the patients of the two groups who had more than 12g/dL haemoglobin (z-1.519, p-value 0.129).
There was no significant difference in the level of glutathione reductase between the patients of the two groups who had modified radical mastectomy ($z=1.358$, $p$-value 0.174) and there was no significant difference in the level of glutathione reductase between the patients of the two groups who had breast conservation surgery ($z=0.718$, $p$-value 0.473).

**Pre-test and post-test levels of glutathione reductase among the experimental and control group.**

![Bar chart showing pre and post test levels of glutathione reductase](image)

**Fig 12:** Bar diagram showing the pre-test and post-test levels of glutathione reductase among the experimental and control group.

Data in the figure above show that the post levels of glutathione reductase were lower in both the control group and experimental group.
SECTION 6

Relationship between Cancer related fatigue and selected biochemical parameters

Relationship between Cancer Related Fatigue and the level of Glutathione

To find out the relationship between Cancer Related Fatigue and the level of reduced glutathione Spearman rho was done. The test statistic compared the pretest values of cancer related fatigue and the pretest level of glutathione to find out the relationship between them. The following null hypothesis was stated to test the statistical significance.

H0 19: There will be no significant relationship between the scores of cancer related fatigue and the level of glutathione (GSH)

Table 21: Relationship between Cancer Related Fatigue and Glutathione

<table>
<thead>
<tr>
<th>(n=40)</th>
<th>Spearman rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest scores of Cancer related fatigue vs. Pretest level of Reduced Glutathione (n=80)</td>
<td>-0.013</td>
<td>0.906</td>
</tr>
</tbody>
</table>

There was a very weak negative relationship between pre-test scores of cancer related fatigue and the pre-test level of reduced glutathione which was not significant statistically.

Relationship between Cancer Related Fatigue and the level of Protein thiols

To find out the relationship between Cancer Related Fatigue and the level of protein thiols Spearman rho was done. The test statistic compared the pretest values of cancer related fatigue and the pretest level of protein thiols to find out the relationship between them. The following null hypothesis was stated to test the statistical significance.
H0 20: There will be no significant relationship between the scores of cancer related fatigue and the level of protein thiols

Table 22: Relationship between Cancer Related Fatigue and Protein Thiols

<table>
<thead>
<tr>
<th>Pretest scores of Cancer related fatigue vs. Pretest level of Protein Thiols (n=80)</th>
<th>Spearman rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.161</td>
<td>0.141</td>
<td></td>
</tr>
</tbody>
</table>

There was a very weak negative relationship between pre-test scores of cancer related fatigue and the pre-test level of protein thiols which was not significant statistically.

Relationship between Cancer Related Fatigue and Glutathione S transferase

To find out the relationship between Cancer Related Fatigue and the level of glutathione S transferase, Spearman rho was done. The test statistic compared the pretest values of cancer related fatigue and the pretest level of glutathione S transferase to find out the relationship between them. The following null hypothesis was stated to test the statistical significance.

H0 21: There will be no significant relationship between the scores of cancer related fatigue and the level of glutathione S transferase
Table 23: Relationship between Cancer Related Fatigue and Glutathione S transferase

(n=40)

<table>
<thead>
<tr>
<th></th>
<th>Spearman rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest scores of Cancer related fatigue vs. Pretest level of Glutathione S transferase (n=80)</td>
<td>-0.105</td>
<td>0.337</td>
</tr>
</tbody>
</table>

There was a very weak negative relationship between pre-test scores of cancer related fatigue and the pre-test level of glutathione S transferase which was not significant statistically.

Relationship between Cancer Related Fatigue and Glutathione Peroxidase

To find out the relationship between Cancer Related Fatigue and glutathione peroxidase Spearman rho was done. The test statistic compared the pretest values of cancer related fatigue and glutathione peroxidase to find out the relationship between them. The following null hypothesis was stated to test the statistical significance.

H0 22 There will be no significant relationship between the scores of cancer related fatigue and the level of glutathione peroxidase

Table 24: Relationship between Cancer Related Fatigue and Glutathione peroxidase

(n=40)

<table>
<thead>
<tr>
<th></th>
<th>Spearman rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretest scores of Cancer related fatigue vs. Pretest level of Glutathione peroxidase (n=80)</td>
<td>-0.188</td>
<td>0.083</td>
</tr>
</tbody>
</table>

There was a very weak negative relationship between pre-test scores of cancer related fatigue and the pre-test level of glutathione peroxidase which was not significant statistically.
Relationship between Cancer Related Fatigue and the level of Glutathione Reductase

To find out the relationship between Cancer Related Fatigue and the level of glutathione reductase, Spearman rho was done. The test statistic compared the pretest values of cancer related fatigue and the pretest level of glutathione reductase to find out the relationship between them. The following null hypothesis was stated to test the statistical significance.

H0 23 There will be no significant relationship between the scores of cancer related fatigue and the level of glutathione reductase

Table 25: Relationship between Cancer Related Fatigue and the level of Glutathione reductase

<table>
<thead>
<tr>
<th>Pretest scores of Cancer related fatigue vs. Pretest level of Glutathione Reductase (n=80)</th>
<th>Spearman rho</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.151</td>
<td>0.167</td>
</tr>
</tbody>
</table>

There was a very weak negative relationship between pre-test scores of cancer related fatigue and the pre-test level of glutathione reductase which was not significant statistically.

Kidd\(^85\) highlights in his article that the antioxidant defence systems guard the body against oxidative stress by scavenging the free radicals and donating hydrogen atom. The endogenous cellular antioxidant defences are maintained by the glutathione system. GSH (reduced glutathione) is an important cell protectant because of its reducing power. GSH works along with its redox system enzymes, glutathione peroxidase and reductase and is a primary protectant of skin, lens, cornea, and retina against radiation damage, and the
biochemical foundation of P450 detoxication in the liver, kidneys, lungs, intestinal epithelia, and other organs. Harlan et al.\textsuperscript{80} identifies the major role glutathione plays in the physiological balance between pro-oxidants and antioxidants. Glutathione and its redox system enzymes, glutathione peroxidase and reductase, help in providing a widespread protection system from oxidative damage. Normal or increased levels of glutathione play an important role in the cellular defense against radiation.

Horneber et al\textsuperscript{139} explored the epidemiology, pathogenesis, diagnosis, and treatment of cancer related fatigue. The authors have done a selective review of the pertinent literature (retrieved by searches in the Cochrane Library, Embase, and Medline), and the guidelines of the National Comprehensive Cancer Network. The causes and mechanisms of CRF may be associated with the tumor itself or with its treatment, or indeed with a potential genetic predisposition, an accompanying physical or mental illness, or behavioral and environmental factors. The authors highlight that dysregulation of inflammatory cytokines, a disturbance of hypothalamic regulatory circuits and changes in the CNS serotonergic system, a disturbance of circadian melatonin secretion and the sleep-wake rhythm, and gene polymorphisms for regulatory proteins of oxidative phosphorylation, signal transduction in B cells, the expression of pro-inflammatory cytokines, and catecholamine metabolism are also considered as causes for cancer fatigue.

A link between oxidative stress and fatigue and fatiguing illnesses was proposed by Michael Maes.\textsuperscript{83,84} Fatiguing illnesses are associated with increased production of free radicals, decrease in the level of antioxidants and the damage caused by oxidative stress. Ferreira and Reid\textsuperscript{33} assert that the glutathione cycle regulates thiol redox status in muscle fibers and is the
biochemical gateway by which thiol donors inhibit fatigue. There is a relationship between reactive oxygen species and thiol chemistry in fatigue and thiol-based countermeasures to reduce fatigue. This may lead to new directions for mechanistic and translational research.\textsuperscript{140, 141}

The present study findings do not support the view that cancer related fatigue is linked to oxidative stress. There was only a very weak negative correlation between pre-test scores of cancer related fatigue and the pre-test level of reduced glutathione and its associated enzymes which was not significant statistically.