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Nonenzymatic Evaluation of Antioxidant and Lipid Peroxidation Level in Breast Cancer Patients Receiving Juice of Wheat Grass during Treatment

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Abstract

For breast cancer, chemotherapy is the most common treatment in the world. In breast cancer patients, oxidative stress leads to accumulation of free radicals, which generate more oxidative stress during chemotherapy. This chemotherapeutic approach also leads to enhanced generation of reactive oxygen species and increased oxidative stress as a result. Blood samples were collected from 30 subjects (15 patients who received wheat grass juice (WGJ) and 15 patients who were only on chemotherapy) in the age range 25-60. The goal of the present investigation was to study the relationship between oxidative stress and breast cancer by measuring the non-enzymatic antioxidant levels of Glutathione reductase (GSH) and Malondialdehyde (MDA), which are the markers of lipid peroxidation in breast cancer patients and effect of wheat grass juice on these markers. From the results obtained, it was clear that MDA levels were higher whereas GSH levels decreased in breast cancer patients compared with normal controls. Significant changes in the MDA and GSH values were observed between the group receiving WGJ and the group receiving only chemotherapy. The administration of WGJ along with the treatment reduces the extent of oxidative damage and related complications in breast cancer patients.

Keywords: Wheat grass juice; Breast cancer; MDA (Malondialdehyde); GSH (Reduced glutathione).

1. INTRODUCTION

Carcinoma of the breast is the most common malignancy in women [1]. The progress accomplished in the therapy of breast cancer has led to improved survival rate. To treat breast cancer, most often chemotherapy is used [2]. The administration of anticancer agents is associated with oxidative stress [3]. Oxidative stress is a condition that occurs when there is an imbalance between reactive oxygen species (ROS) and antioxidants reaction capacity, which stimulates the development of a disease, such as breast cancer [4]. Furthermore, ROS, such as superoxide anions, and hydrogen peroxide-induced lipid peroxidation [5, 6] play a major role in malignant transformation and tumor cell proliferation and invasion [7]. Biomarkers of oxidative stress include plasma malondialdehyde (MDA) and reduced glutathione (GSH) [8]. Antioxidants are protective agents that inactivate ROS and play an essential role in the protection of cells from oxidative damage [9]. This can be divided into two systems: enzymatic and nonenzymatic [10]. Glutathione is the most abundant low-molecular-mass molecule that provides reducing equivalents to protect cells against free radicals (oxidative stress). To overcome this oxidative stress and the side effects of chemotherapy, some kind of complementary and alternative medicines are used [11]. Fresh wheat grass juice (WGJ) is known to have all the nutrients necessary to sustain life [12]. It contains vitamins, minerals, fiber, and various cancer-fighting phytonutrients and enzymes; all occur in their simplest forms, require minimal digestive activity, and are balanced in nature [13, 14]. It is clear that patients with breast cancer suffer from chronic oxidative stress and have an altered redox state characterized by gross depletion of antioxidant nutrients [15, 16]. Clinical events in breast cancer patients precipitate directly by severe antioxidant depletion resulting in inadequate protection. Therefore, clinical trials of the use of WGJ involving the supplementation of combined antioxidant nutrients may generate useful information to control oxidative stress. The aim of the present investigation was to study the effect of WGJ on oxidative stress by measuring the MDA and GSH level.

2. MATERIALS AND METHODS

The study was performed on a total of 30 subjects, which included two groups, and all of them were women aged 25-60 with histopathologically confirmed breast cancer (15 received chemotherapy alone and the remaining freshly prepared WGJ in empty stomach as daily dietary supplement along with chemotherapy). This study was approved by the ethical committee of the Cancer Hospital and Research Institute. All the patients having a history of cardiovascular diseases and diabetes mellitus were excluded from the study. After obtaining a written consent, a total of 5 ml blood was withdrawn aseptically from the antecubital vein from each subject. Samples of blood were collected before every chemotherapy cycle till 6 cycles of treatment. The samples were centrifuged at 3,000 rpm for 10 min to separate serum and RBCs. The separated serum was collected for further analysis

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and stored at -20°C . Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (TCA) and boiling with thiobarbituric acid, which reacts with malondialdehyde to give a pink color, as per the method of [17].

Total GSH content was measured by the method of [18]. This method is based on the development of a yellow color when 5, 5-dithio bis-2-nitro benzoate (DTNB) is added to compounds containing sulfhydryl groups. All the experiments of samples from the breast cancer women were performed in triplicate and the mean value was taken.

2.1. Statistical Analysis

The statistical analysis was made by the SPSS statistics software, version 20 for Windows. A multivariate ANCOVA was applied to assess the difference between means of intervention of both groups. The results were expressed in mean \pm SD. p values ($p < 0.05$) were considered as significant.

3. RESULTS AND DISCUSSION

Our results showed significantly elevated ($p < 0.05$) levels of MDA while the mean value of GSH was significantly reduced ($p < 0.05$) in cancer patients only receiving chemotherapy than patients receiving WGJ during the treatment (Table 1, Figure 1). MDA is a good indicator of oxidative damage. In addition, MDA is a product of lipid peroxidation and is generated in excess amounts in breast cancer patients. Levels were higher in the study group than control group; these results agree with previous studies of Rajneesh *et al.* [19], which reported increased MDA levels, as measured by thiobarbituric acid reactive substance (TBARS) method, in breast cancer patients. The high levels of MDA in cancerous conditions could result from the deterioration of antioxidant defenses [20].

The present study reported a deficiency in levels of GSH in patients with breast cancer and was lower in healthy controls. A previous study [21] suggested that glutathione (GSH) is a major intracellular reducing agent, which is very sensitive to oxidative pressure and has several important functions, such as protection against oxidative stress, induction of apoptosis activation/regulation of gene expression, and proliferation of T lymphocytes [22]. It was found that the antioxidant level decreased in breast cancer patients due to increased ROS, as studied by Pande *et al.* [23]; in our study, GSH levels were significantly lower in the untreated model control group than in the normal one while patients receiving WGJ showed significantly increased level of GSH. The results obtained by us are similar to the results obtained by Rajneesh *et al.* [19], where they conducted a similar test in patients with breast cancer. It is concluded that the biochemical changes of MDA and GSH may be due to enzymes; also MDA

Table 1: Nonenzymatic antioxidant and lipid peroxidation level in breast cancer patients.

Parameter	Chemotherapy cycle 1	Chemotherapy cycle 2	Chemotherapy cycle 3	Chemotherapy cycle 4	Chemotherapy cycle 5	Chemotherapy cycle 6
GSH	1.82 ± 0.32	1.73 ± 0.28	1.66 ± 0.34	1.58 ± 0.26	1.52 ± 0.33	1.47 ± 0.29
GSH + WGJ	1.98 ± 0.34	2.03 ± 0.32	2.23 ± 0.27	2.35 ± 0.28	2.39 ± 0.22	2.43 ± 0.24
MDA	6.94 ± 2.11	7.78 ± 2.21	7.83 ± 2.02	8.05 ± 2.35	8.34 ± 2.13	8.67 ± 2.04
MDA + WGJ	6.62 ± 1.43	6.56 ± 1.23	6.43 ± 1.12	6.31 ± 1.21	6.22 ± 1.24	6.01 ± 1.14

Values are expressed in mean and standard deviation.

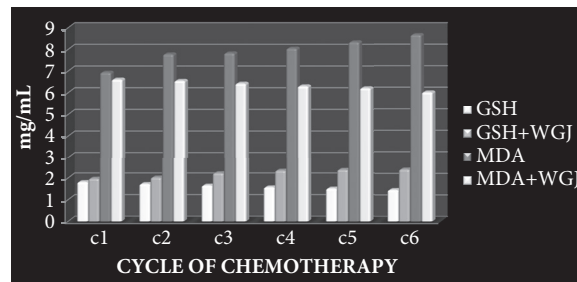
Except cycle 1, all are statistically significant values.

WGJ = wheat grass juice.

GSH (reduced glutathione): mg/mL, MDA (malondialdehyde): mg/mL.

Control value of GSH is 3.25 ± 0.65 and MDA 3.01 ± 2.11 .

Figure 1: Nonenzymatic antioxidant and lipid peroxidation level in breast cancer patients.



and GSH act as biomarkers for early detection of recurrent disease and also monitoring the effective therapeutic follow-up of the patients. These are the best biomarkers for diagnosis, prognosis, and treatment of breast cancer.

In conclusion, the administration of WGJ along with the chemotherapy helps reduce the extent of lipid peroxidation and increases the level of GSH and controls related complications in breast cancer patients and this aspect needs further evaluation.

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Author Contributions

Pragati Singh has done all the experimental work while Dr. B.R. Shrivastav and Dr. Archana Shrivastav advised her and provided their research facilities to carry out this work.

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Conflict of Interest

None.

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