International Journal of Innovative Studies in Medical Sciences (IJISMS)

ISSN 2457-063X (Online) **www.ijisms.com** *Volume: 2 Issue: 6 | 2018*

Measurement Of Serum Protein Carbonyl Group Levels In Breast Cancer Patients

Sibel Söylemez[1],Ayşe Banu Çaycı[1], Durmuş Ayan[2], Özlem Gülbahar[1], Deniz Yamaç[3]

[1]Department of Medical Biochemistry, School of Medicine, Gazi University, Ankara, Turkey

[2]Department of Medical Biochemistry, Amasya Central Public Health Laboratory, Amasya, Turkey

[3] Department of Medical Oncology, School of Medicine, Gazi University, Ankara, Turkey

Abstract:

Aim: *The aim of this study was to examine the changes in serum protein carbonyl group levels, which is an indicator of protein oxidation, in patients with breast cancer before and after 3 cycles of chemotherapy compared to healthy control groups.*

Methods: *Blood samples were obtained from 24 newly diagnosed patients with active tumor before treatment and after 3 cycles of chemotherapy, and from 10 patients in the control group. Serum protein carbonyl group levels were determined according to the spectrophotometric method based on the presence of 2,4-dinitrophenylhydrazone as a result of 2,4-dinitrophenylhydrazine reacting with the carbonyl group.*

Results: *A statistically significant difference was found between serum protein carbonyl group levels of breast cancer patients before and after chemotherapy ($p< 0.05$). A significant difference was also found between healthy controls and pre-chemotherapy levels ($p< 0.001$). However, there was no statistically significant difference between the serum protein carbonyl group levels of the control group and breast cancer patients after chemotherapy ($p> 0.05$).*

Conclusion: *Free radical accumulation and oxidation of proteins can cause damage in breast cancer. Serum protein carbonyl group levels may be associated with treatment of breast cancer.*

Keywords:*protein oxidation, protein carbonyl groups, breast cancer*

1. INTRODUCTION

Cancer is abnormal tissue growth. Furthermore, cancer cells also invade local tissues and metastasize to other tissues of the body. Cancer growth is not very different from normal tissues even in rapidly developing tumors. However, tumor development occurs due to uncontrolled growth and lack of apoptosis [1].

Although the cause of cancer is not exactly known, it is proposed that most of human tumors occur due to environmental factors. These factors are chemical agents, viruses and radiation. Genetic factors also play a role in cancer development [2].

The role of free radicals in carcinogenesis is a result of multiple biochemical interactions. The most important of these interactions is their damage to proteins because of their permanence. This is because the mitochondrial DNA is likely to be exposed to more mutation causing factors than normal somatic DNA since it is not surrounded by histones and is close to the electron transport system. This phenomenon will be exacerbated as the proteins in the electron transport system are permanently damaged by oxidation [3].

Carcinogenesis studies using experimental animal models highlight the relationship between protein oxidation and carcinogenesis. For example, in rats treated with carcinogen benzoapyrene, the elevation of 8-hydroxydeoxyguanosine levels indicating nucleotide damage in the DNA occurs simultaneously with the increase of protein carbonyl groups [4-6].

In addition, ferric nitriloacetate, which is a renal carcinogen as well as a renal and hepatic tumor promoter, reduces quinone reductase activity and significantly increases protein carbonyl levels [7]. These findings indicate that the formation of carbonyl groups may be involved in the process of carcinogenesis.

2. MATERIALS AND METHODS

This study was carried out in Gazi University Faculty of Medicine, Medical Biochemistry Research Laboratory.

After obtaining ethic committee approval and consents of the participants, patient blood samples used in the experiments was obtained from Medical Oncology Polyclinic of Gazi University Medical Faculty Hospital. Blood samples were collected from 24 newly diagnosed patients with active tumor who were not surgically treated (Group A). These patients were treated with 3 cycles of 5-fluorouracil, adriamicin, cyclophosphamide, and blood samples were collected again after chemotherapy (Group B). Blood samples collected from patients were separated into serum and stored at approximately -80°C until the experiment.

The control group was selected from healthy individuals who had no benign or malignant lesions in the breast detected by mammography, who had no disease history, medication use, smoking and alcohol use habits; and blood samples were collected.

Determination of Serum Protein Carbonyl Groups

Protein carbonyl group levels were determined according to the spectrophotometric method defined by Levine et al. [8], based on the presence of 2,4-dinitrophenylhydrazone as a result of 2,4-dinitrophenylhydrazine reacting with the carbonyl group. Measurements were performed at 380 nm with a Schimadzu UV 1601 spectrophotometer.

Determination of Protein Content in Supernatant

Quantification of protein content was performed by Lowry's method [9]. Ɛ was taken as 22000 M^{-1} cm^{-1}, and the results were expressed as nmol/mg protein.

Statistical Analysis

Statistical analyses were conducted with Statistical Package for Social Sciences (SPSS) Version 10.0 for Windows. Differences between the groups were evaluated by Kruskal-Wallis variance analysis. Binary group comparisons were performed by Mann-Whitney U analysis.

Table1: Serum protein carbonyl group levels (nmol/mg protein) of all groups subjects (mean±SD) and p values

Group	Protein Carbonyl nmol/mg protein	n	p value Control	p value Group A	p value Group B
Control	0,62± 0,06	10	-	<0.001	0.118
Group A	1,83± 0,87	24	<0.001	-	<0.05
Group B	1,11± 0,73	24	0.118	<0.05	-

3. RESULTS

There was a significant difference between serum protein carbonyl group levels before chemotherapy (Group A) and after chemotherapy (Group B) ($p< 0.05$). When Group A was compared with the control group consisting of healthy individuals, it was seen that there was a significant difference between serum protein carbonyl group levels ($p< 0.001$). When Group B was compared with the control group, no significant

difference was found between the serum protein carbonyl group levels ($p> 0.05$). (Table 1)

4. DISCUSSION

Protein carbonyl groups, which are formed at a relatively early stage of oxidative damage and remain stable in the circulation for longer periods of time compared to other damage parameters such as malondialdehyde and oxidized glutathione, are considered to be a reliable marker [10]. The oxidation of the side chains of amino acids in the protein structure or the oxidative degradation of the peptide bonds may form protein carbonyl groups. Another way how protein carbonyl groups are formed is the interaction of nucleophilic side chains of cysteine, histidine, and lysine amino acids with reactive carbonyl compounds derived from lipids or carbohydrates [11].

The first study investigating the relationship between cancer and protein carbonyl groups was made in Poland on pediatric cancers. Protein carbonyl group levels were measured in a total of 65 cases (25 brain tumors, 25 bone tumors, 5 liver tumors, 5 lymphomas, and 5 germ cell tumors) and were compared with data from 40 healthy children at the same age. Protein carbonyl group levels in the cancer group were twice as high as in the healthy group. This difference was found to be highly significant [12]. In our study, we measured protein carbonyl group levels in 24 patients with breast cancer and found that serum protein carbonyl group levels of the patient group were significantly higher than healthy individuals ($p< 0.001$). Serum protein carbonyl group levels of breast cancer patients after chemotherapy decreased significantly compared to the pre-treatment period ($p< 0.05$) but were still higher than the control group.

Oxidative damage to proteins results in decreased enzymatic functions and increased protein catabolism. In particular, protein oxidation in red spheres occurs at an earlier stage independent of membrane damage and is an earlier indicator of oxidative damage compared to lipid peroxidation [13]. An increase in protein carbonyl levels is not only indicative of oxidative stress but also indicates protein dysfunction [10].

In a study conducted on bladder cancer patients, protein-bound thiol groups were found to be higher in cancer patients compared to healthy individuals. In particular, glutathione levels involved in providing intracellular thiol activity were found to be low in many cancer patients. Reduction of serum proteins and thiol groups in these patients is thought to be the result of oxidative modifications in proteins caused by increased protein catabolism and degradation [14].

Ushmorov et al. analyzed the etiopathogenesis of cachexia in rats with fibrosarcoma. Reduction of albumin levels with the increase in protein catabolism is used to monitor cancer cachexia. Reduction of albumin levels correlates with decreased body mass and prognosis. The function of albumin is to provide oncotic pressure and prevent edema. Increased catabolism of albumin in rats with fibrosarcoma may be a result of protein oxidation. In addition to decreased albumin levels, cysteine/thiol and glutathione disulfide/glutathione ratios are increased in these rats [15].

These studies on the formation of carbonyl groups and thiol oxidation indicate a relationship between protein oxidation and cancer. In our study, the level of carbonyl groups in the blood samples taken immediately after

the diagnosis in breast cancer patients was significantly higher than the level measured after treatment. However, studies investigating the relationship between the chemotherapeutic agents used and oxidative damage are limited. In a study conducted by Li et al., the relationship between MDA level, which is one of the indicators of oxidative damage, and medications used in treatment could not be fully determined [16].

In a study by Zoli et al., 5-FU and adriamycin were used in breast cancer patients, and apoptosis was investigated before and after chemotherapy and no significant difference was found [17]. Another study conducted by Burcombe et al. on breast cancer patients reported that there was no significant difference between the chemotherapeutics and apoptosis in terms of clinical, pathological, and radiological criteria [18].

After radiotherapy, pyknosis and karyorrhexis develop within hours and cell loss occurs [19]. In a study examining radiation-induced enteropathy, it was determined that radiation therapy of actinomycin-D, adriamycin, methotrexate, vinca alkaloids, 5-FU, hydroxyurea, bleomycin, cyclophosphamide, and agents in the nitrous urea group increased the negative effects on the intestinal mucosa [20,21].

Furthermore, most chemotherapeutic agents, including radiation, are known to induce apoptosis. Being able to induce apoptosis is desirable in cancer treatment. Anticancer drugs such as cisplatin, topotecan, gemcitabine have been shown to induce apoptosis in non-small cell lung tumors, and cause caspase-8 activation [22]. In addition, Tanaka et al. investigated apoptosis and P53 gene to demonstrate the efficacy of postoperative and adjuvant 5-FU administration, and demonstrated that apoptosis was induced by these applications [23].

In conclusion, we determined in this study that carbonyl group levels, which is a marker of oxidative damage in breast cancer patients and indicates protein oxidation, increased and there was a significant decrease in these levels after chemotherapy. We think that serum protein carbonyl groups can be used for monitoring these patients for complications and prognosis. For this purpose, future studies may be conducted with a larger sample and longer follow-up.

REFERENCES

[1] S. L. Robbins, V. Kumar,*BasicPathology*.4thed., 1987;247.

[2] R. K. Murray, D. K. Granner, P. A. Mayes, V. W. Rodwell, *Harper's Biochemistry*, 24 th ed, Appleton and Lange 1996;757.

[3] B. Van Der Loo, et al.,"Enhanced peroxynitrite formation is associated with vascular aging,"*J Exp Med,*vol. 192, pp. 1731-1744, 2000.

[4] K. B. Kim andB. M. Lee,"Oxidative stress to DNA, protein and antioxiadant enzymes (superoxide dismutase and catalase) in rats treated with benzoapyrene,"*Cancer Lett,*vol. 113, pp. 205-212, 1997.

[5] L. Selmeci, "Advanced oxidation protein products (AOPP):novel uremic toxins, or components of the non-enzymatic antioxidant system of the plasma proteome?" *Free RadicalResearch*, vol. 45, no. 10, pp. 1115–1123, 2011.

[6] C. Capeillere-Blandin, V. Gausson, B. Descamps-Latscha, andV. Witko-Sarsat, "Biochemical and spectrophotometric significance of advanced oxidized protein products," *Biochimica et Biophysica Acta—Molecular Basis of Disease,* vol. 1689, no. 2, pp.91–102, 2004.

[7] M. Iqbal, S. D. Sharma, A. Rahman, P. Trikha and M. Athar, "Evidence that ferric nitrilotriacetate mediates oxidative stress by down-regulating DT-diaporase activity: implications for carcinogenesis,"*Cancer Lett*, vol. 141, pp.151-157, 1999.

[8] R. L. Levine, et al., "Determination of carbonyl content in oxidatively modified proteins,"*Met In Enzimology,*vol. 186, pp. 464-478, 1990.

[9] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, "Protein mesaurement with the Folin phenol reagent,"*J Biol Biochem*, vol. 193, pp. 145-157, 1951.

[10] I. Dalle-Donne, R. Rossi, D. Giustarini, A. Milzani and R. Colombo,"Protein carbonyl groups as biomarkers of oxidative stress,"*Clinica Chemica Acta*, vol. 329, pp. 23-38, 2003.

[11] E. R. Stadtman and B. S. Berlett,"Reactive oxygen-mediated protein oxidation in aging and disease,"*Chem Res Toxicol*, vol. 10, pp. 485-494, 1997.

[12] S. Popadiuk, M. Korzon, J. Renke and M. Wozniak,"Carbonyl groups content on the basis of protein peroxidation analysis with total antioxidant status in blood of children with cancers,"*Wiad Lek*, vol. 51, pp. 107-112, 1998.

[13] R. J. Keller, N. C. Halmes, J. A. Hinson and N. R. Pumford, "Immunochemical detection of oxidized proteins,"*Chem. Res. Toicol*, vol. 6, pp. 430-433, 1993.

[14] I. Yılmaz, T. Akcay, U. Cakatay, A. Telci, S. Atausand V. Yalcın, "Relation between bladder cancer and protein oxidation," *Inter. Urology and Neph*, vol. 35, pp.345-350, 2003.

[15] A. Ushmorov, V. Hack and W. Dröge, "Differential reconstitution of mitochondrial respiratory chain activity and plasma redox state by cysteine and ornithine in a model of cancer cachexia," *Cancer Research,* vol. 59, pp. 3527-3534, 1999.

[16] B. Li, P. L. Gutierrez, P. Amstad and N. V. Blough,"Hydroxyl radical production by Mouse epidermal cell line in the presence of guinone anti-cancer compounds,"*Chem Res Toxicol*, vol. 12, pp. 1042-1049, 1999.

[17] W. Zoli, et al.,"Addition of 5-fluorouracil to doxorubicin-paclitaxel sequence increases caspase-dependent apoptosos in breast cancer cell lines,"*Breast Cancer Res*, vol. 7, pp. 681-689, 2005.

[18] R. Burcombe, et al., "Evaluation of Ki-67 proliferation and apoptotic index before, during and after neoadjuvant chemothrepy for primary breast cancer," *Breast Cancer Res,* vol. 8, pp. 3-31, 2006.

[19] M. Berthrong and L. F. Fajardo, "Radiation injury in surgical pathology. Part II. Alimantry Tract," *Am J Surg Pathol,* vol. 5, pp. 153-178, 1981.

[20] M. Rotman, L. Rogow, G. Deleon and N. Heskel, "Supporative therapy in radiation oncology," *Cancer*, vol. 39, pp. 744-750, 1977.

[21] J. R. Stewart and F. A. Gibbs, "Prevention of radiation injury: predictability and preventability of complications of radiation therapy,"*Annu Rev Med*, vol. 33, pp. 385-395,1982.

[22] M. Macluskey, R. Baillie, L. M. Chndrachud, N. Pendleton and A. M. Schor, "High levels of apoptosis are associated with improved survival in non-small cell lungh cancer," *Anticancer Res,* vol.20, pp. 2123-2128, *2000*.

[23] F. Tanaka, et al., "Apoptosis and P53 status predict the efficacy of postoperative administration of UFT in non-small lungh cancer,"*Br J Cancer*, vol. 84, pp. 263-269, 2001.