

Comparison of protein-binding malondialdehyde level between breast cancer, fibroadenoma and normal tissues in cases of breast surgery; a pilot study

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Abstract

Introduction: Breast cancer is the most common malignant solid tumor in women. Like other solid tumors, an inflammatory microenvironment with oxidative stress provides a suitable condition for tumor growth.

Objectives: This pilot study was designed to compare the protein-binding level of malondialdehyde (MDA) – as an oxidative stress index – among breast cancer, fibroadenoma and normal tissues in Iranian cases of breast surgery.

Materials and Methods: A total of 17 surgical cases were selected in operation room including 6 cases of invasive ductal carcinoma (breast cancer), 6 cases of fibroadenoma and 5 cases of mastoplasty surgery (control group). All the cancer patients had received neoadjuvant chemotherapy. The total protein was extracted from tissues and then the level of MDA was studied using spectrophotometry.

Results: Concentration of MDA was 46.84 (± 11.90), 53.78 (± 1.69) and 52.96 (± 2.29) μM in invasive ductal carcinoma, fibroadenoma and control groups, respectively. According to analysis of variance (ANOVA) the differences were not statistically significant ($P=0.238$).

Conclusion: MDA level was not different between the groups of study. This lack of association might be due to the wide distribution of data in cancer group which in turn might be resulted from personalized responses of the tumors to neoadjuvant chemotherapy.

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Introduction

In the human body, there are free radicals called as reactive oxygen species (ROS) and antioxidant agents. Imbalance between ROS and antioxidants is known as oxidative stress (1,2). Oxidative stress can be associated with a lot of diseases such as cancers (3), atherosclerosis (4) and neurological disorders (5). The pathogenesis of oxidative stress in cancer is attributed to increase inflammation, angiogenesis and epithelial to mesenchymal transmission (3). Oxidative stress is also associated with silencing tumor suppressor genes like adenomatous polyposis coli (*APC*) and breast cancer susceptibility gene 1 (*BRCA1*) via increasing global hyper-methylation (6). Controversially, the chemotherapeutic agents used for cancer therapy increase oxidative stress. It is not clear whether this oxidative stress has harmful or beneficial

Core tip

Malondialdehyde (MDA) can be measured also in extracted total protein. The previous studies were performed on serum or tissue homogenates. Concentration of MDA in malignant tumors can be individualized.

effect, however, using antioxidants along with chemotherapy has been recommended (7). Lipid peroxidation is a process in which lipids are degenerated by oxidative stress. Aldehydes such as malondialdehyde (MDA) are the end products of lipid peroxidation. Therefore, MDA level is assayed in biological samples to study lipid peroxidation and oxidative stress (8).

Breast cancer is the most common solid tumor in women. In Iran breast cancer has incidence of 24 per 100000 according to the report of 2009 (9). A national registry in Iran showed incidence of 22.1-23.1 per

100000 according to the report of 2015. Breast cancer covers about 24.6% of all cancers in Iranian women (10). Anthropology wise, different ethnicities may different patterns in expression of breast cancer related receptors (11). In addition, breast cancer has global importance which accounts for 25.1% of all cancers (12).

Like many other cancers, breast cancer is associated with increased oxidative stress. As we mentioned, this oxidative stress may be either the reason of cancer due to changing microenvironment (13) or the consequence of antitumor activity (7). Since *BRCA1* is a tumor suppressor gene, *BRCA1*-deficiency is associated with increased oxidative stress (14). The most common malignant tumor of breast is invasive ductal carcinoma (15) and the most benign tumor of breast is fibroadenoma (16).

Although the roles of oxidative stress and MDA level are currently known in breast cancer, it was necessary to compare MDA level between the breast cancer patients received neoadjuvant chemotherapy and the patients with benign fibroadenoma and normal breast tissue. This is a lacuna of research whether benign tumors are associated with oxidative stress or whether chemotherapy has increasing or decreasing effect on MDA level.

Objectives

This study was designed to compare the protein-binding level of MDA between the extracted proteome of breast invasive ductal carcinoma tissue in patients who had received neoadjuvant chemotherapy, extracted proteome of breast fibroadenoma tissue, and extracted proteome of breast normal fibro-glandular tissue in Iranian cases of breast surgery.

Patients and Methods

Study design

This observational study had a cross sectional design with case control sampling approach in order to have a pilot result for creation of further hypotheses. The groups of study were independent variables and MDA level was the outcome variable.

Study population

A total of 17 cases of breast surgery were selected via convenient sampling from operation room of Firoozgar general hospital, Tehran, during the second half of 2018. Six of them had invasive ductal carcinoma (at any stages, and both groups of radical mastectomy and lumpectomy) and had received neoadjuvant chemotherapy. Six of them had fibroadenoma. All of the diagnoses were approved by a pathologist. Five of the participants were cases of mammoplasty surgery and used as the control group. The inclusion criteria were female gender, having detectable and resectable mass (according to physical examination, imaging and conditions during surgery), and lack of ethical issue. The exclusion criteria were regression of mass (after imaging and before surgery due to response

to neoadjuvant chemotherapy) and having change of diagnosis after surgery (Based on the report of pathology).

Laboratory investigation

A particle of tumor nidus about 2 mm was taken from each mass. In the cases of fibroadenoma, the particles were taken in the very operation room. In the cases of cancer, the particles were taken in pathology room in order to save surgical margins of the tumor for frozen section study.

The particles were homogenized in liquid Azote. Total protein was extracted using a columnar kit (Norgen, Canada). The concentration of total protein was assayed with nanodrop spectrophotometry. MDA assay kit (Teb Pazhouhan Razi, Iran) was used to assay concentration of MDA according to manuals of the company. The optical density (OD) was read with Elisa reader and the ODs were converted to concentration (μM).

Statistical analysis

One way analysis of variance (ANOVA) was used to compare the mean of MDA level between the groups. Kolmogorov-Smirnov test was used to assay normal distribution. *P* value <0.05 considered as the significance level. The analyses were done in Stata 14 (StataCorp LLC, US).

Ethical considerations

The research followed the tenets of the Declaration of Helsinki. All the patients signed a hospital form for using their information and biological samples for research and educational purposes. All of them gave us informed consent. The ethical protocol of taking the main samples had been approved by Iran and Lorestan universities of medical sciences for another project (IR.IUMS.REC.1397.983, and IR.LUMS.REC.1398.194).

Results

The concentration of the extracted total protein reached 80-90 mg/mL. An amount of 20 μL was taken from each proteome sample, and then they were diluted up to the volume 100 μL . The MDA levels were obtained 25-58.46 μM for these diluted protein samples.

Concentration of MDA was 46.84 (± 11.90), 53.78 (± 1.69) and 52.96 (± 2.29) μM in invasive ductal carcinoma, fibroadenoma and control groups, respectively. According to one way ANOVA the differences were not statistically significant ($F = 1.59$, $P = 0.238$) (Table 1). No significant post hoc association was found. Bartlett's test for equal variances was reported as chi-square = 17.28 and $P < 0.001$ by the software below the report of ANOVA.

Box plot was used to show the distribution of the data. The control group showed the narrowest distribution whereas the cancer group showed the widest distribution. The minimum and maximum amounts of MDA were for the cancer group (Figure 1).

Table 1. Concentration of MDA in the groups of study

Group	Mean (μM)	Standard deviation	Min-Max
Cancer	46.84	11.90	25-58.46
Fibroadenoma	53.78	1.69	51.27-55.92
Control	52.96	2.29	51.27-56.77

$F=1.59$, $P=0.238$; one way ANOVA.

Discussion

This study was designed to compare protein-binding MDA level between tissue proteome of invasive ductal carcinoma, fibroadenoma and normal breast tissue. No significant difference was observed between the groups of study. Concentration of MDA had wider distribution in the cancer group. According to the Bartlett's test for equal variances, this difference in variances was statistically significant. It showed that breast cancer tissue had personalized response to chemotherapy in oxidative and anti-oxidative systems. In addition, lack of difference between fibroadenoma and normal tissue groups showed that formation of benign fibroadenoma was not associated with impaired anti-oxidative system. In the present study we found that MDA level could also be measured in total proteome like biological liquid samples.

Previously, Abdel-Salam et al in Egypt, compared serum MDA level between metastatic and non-metastatic cases of breast cancer and control group. All the cancer patients had received chemotherapy. MDA level had a significant decrease in cancer patients without significant difference between metastatic and non-metastatic cases (17). Khoshbin et al in Iran, compared plasma MDA level between before and after starting chemotherapy in breast cancer patients. They found no significant difference (18). In our study we studied MDA in tumor proteome and in line with Khoshbin et al results, we found no significant difference. Amin et al in Egypt, compared MDA level of blood samples between 20 breast cancer patients receiving chemotherapy, 20 breast cancer patients without chemotherapy and 20 healthy women. MDA level had increased in breast cancer patients in comparison to healthy women and also in breast cancer patients after chemotherapy in comparison to those who had not received chemotherapy (19). Other than breast cancer, there was a study also in non-small cell lung cancer. Mohan et al in India, compared plasma level of MDA before and after receiving chemotherapy in patients with non-small cell lung cancer. They found no significant difference (20). In our study it was the first time to assay MDA level in extracted proteins of breast tissue.

Conclusion

Protein-binding level of MDA was not significantly different between breast tumor tissue, breast fibroadenoma tissue and breast normal tissue. This lack of association might be due to the wide distribution of data in the cancer group which in turn might be resulted from personalized

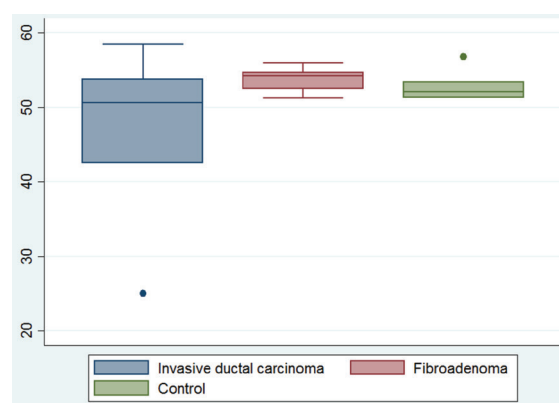


Figure 1. Box plot of concentration of MDA (μM) in the groups of study to show the wide range of distribution in the cancer group.

responses of the tumors to neoadjuvant chemotherapy.

Limitations of the study

Lack of investigation of MDA level in tissue homogenates and blood serum, lack of investigation of other oxidative stress indices, low sample size and lack of subgroups were our limitations.

Acknowledgements

The present study was performed on the extracted proteins of another project. The ethical protocol of taking the main samples had been approved by Iran and Lorestan universities of medical sciences, and no further registration number was needed according to the decision of the ethical committee.

Authors' contribution

SS: sample taking, design and conceptualization. ZM: laboratory study. SAYA: primary draft and analysis. RN: supervision and critical approval.

Conflicts of interest

The MDA assay kit was produced by a knowledge-based company in the same institution.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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