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ORIGINAL ARTICLE

EVALUATION OF MALONDIALDEHYDE, HOMOCYSTEINE AND ANTIOXIDANT INFLUENCE CHEMOTHERAPY IN BREAST CANCER PATIENTS

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Summary

The study was designed to evaluate the medical relevance of total homocysteine (tHcy), total antioxidant capacity (TAC), and malondialdehyde (MDA) before and after chemotherapy for women with breast cancer (BRCA). Blood samples were taken from Oncology Unit in Merjian Teaching hospital in Hilla city (Iraq). Sixty patients suffering from breast cancer (BRCA) were enrolled in this study, and twenty-one apparently healthy subjects were considered as a control group control. We found that significantly increased lipid peroxidation, measured as MDA, was demonstrated in the serum of BRCA patients who were not taken any medication was higher than in the control group ($P<0.01$) were (25±15) nmol/ml of MDA levels in BRCA patient and (14.5±7.9) nmol/ml of MDA levels in healthy controls. We found that treatment by chemotherapy resulted in a significant increase in MDA levels when compared with MDA levels in patients who were not taken any medication. The tHcy level in BRCA patients before and after treatment were changed. In addition, it is found that the mean serum TAC levels in BRCA patients were significantly less than in the control group. Moreover, a positive correlation was observed between the activity of chemotherapy and MDA levels in the patient and the same correlation between tHcy levels and TAC levels while a negative correlation was observed between TAC levels with MDA levels in the patient group.

Key words: Malondialdehyde; Total antioxidant capacity; Total homocysteine; Oxidative stress; Breast cancer

Introduction

Oxygen; a vital molecule to our life, could turn into a deleterious agent affecting our cells and organ (1). Once mismatched electron pairs happen, a new agent with free-floating electrons is produced called free radicals (2). Two types of which exist; reactive oxygen species and reactive nitrogen species. Its produced as a byproduct of a metabolic pathway for energy production or exogenously due to exposure to radiation or smoking (3). Chronic diseases could increase production, such as hyperlipidemia (4,5), diabetes (6,7), metabolic syndrome (8), vascular diseases (9), and multiple sclerosis (10). Antioxidant vitamins (11) could help tackle this abnormal situation through

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scavenging free radicals via oxidizing themselves thereby restoring the antioxidant status, avoiding cell and/or tissue damage and restoring the quasi-equilibrium milieu in the vicinity.

An imbalance between the release of reactive oxygen species (ROS) and antioxidant cell abilities is recognized as oxidative stress (OS). Highly ROS have long been known to be destructive remnants of natural oxygen metabolism and to have an important role in several malignancies. Meanwhile, for so many decades, the controlled manufacturing of radicals was already regarded as an effective process of cellular signalling which promotes the cell's homeostasis (12,13).

Essential fatty acids, such as linoleic acid and arachidonic acid, as these are numerous in plasma membranes, are also the main target of cells from oxidative stress. Lipid peroxidation, or the oxidation of lipids by superoxide anion ($O2^{\bullet-}$), results in the production of a considerable variety of essential (hydroperoxides) and sequel (aldehydes) products with a variety of biochemical capabilities (14-16). The far more recent tHcy has to have a function in copper transport in the body, reducing cupric to cuprous, leading to higher cell damage (17,18).

tHcy, the sum of free and bound with cysteine and protein, accumulates in the cell and is exported to the recirculation in plasma generally in its electron carrier from tethered to protein, cysteine, and is measured as tHcy (19,20). The intracellular demethylation of methionine yields tHcy, as a result, hyperhomocysteinemia is linked to a higher risk of cancer in patients with irritable bowel syndrome illness (21), and that could be linked to hypomethylation (22). Tumour cells generally display significant methylation of DNA (23, 24).

The body constantly resists itself against the manufacture and destruction of these oxidants using only a mixture of enzymatic and non-enzymatic protective mechanisms. Antioxidant species are agents capable of neutralizing programs that encourage oxygen and enable a non-cytotoxic level of public radicals to be stabilized at the cell and organism levels. It lowers or resists the oxidation of other organic compounds, among other things (25,26).

Oral antioxidant supplementation was already reported to reduce the negative effects of chemo and radiotherapy, bringing in a better lifestyle (27,28). However, the effect of antioxidant supplementation in cancer treatment is contentious, because it has been linked to inferior therapeutic efficacy and cancer recurrence (29-31). Our target was to see that there was an association between tHcy levels and TAC and MDA as an oxidative stress markers in breast cancer (BRCA).

Materials and Methods

Study Design: This study's Sample collection was conducted during the period from Sep2021 to Feb 2022, and has been subjected to the present study

- Group 1: Apparently healthy (Control).
- Group 2: Patients who are not taking any medication (n=17).
- Group 3: Patients who are taking chemotherapy for once time (n=17).
- Group 4: Patients who are taking chemotherapy twice the time (n=17).
- Group 5: Patients who are taking chemotherapy for thrice time (n=17).

Sample collection and preparation

Blood Sample Collection: Three millilitres of venous blood were drawn from each of the patients and control group, the first group was the control, the second group was patients before treatment, the third group was receiving one dose of chemotherapy drug, the fourth group was the second dose of chemotherapy the fifth group was receiving the third dose of chemotherapy. The blood samples were placed in gel tubes and then left at room temperature for a period of (20) minutes for coagulation, then centrifuged (at 3000 X g) for 20 minutes for serum separation. The sera were divided into five Eppendorf tubes and stored at (-20C^{o}) until the time of biochemical estimation.

The study of patients in Merjian Teaching hospital in Hilla city excluded patients with other chronic diseases, smokers, systematic immune disease, gestational diabetes and also a pregnant woman.

Assay Principle of studied parameters

This kit is a sandwich kit for the accurate quantitative detection of human TAC, MDA and tHcy in serum, an Enzyme-Linked Immunosorbent Assay (ELISA).

Result

Patients with carcinoma had more MDA levels than the healthy group are shown in Table 1.

Table 1. TAC(U/ml), tHcy (nmol/ml), and MDA (nmol/ml) in studied groups.

	patients			control			P value
Parameters	N	Mean	SD	N	Mean	SD	
TAC	60	5.5	4.1	21	16.5	29.1	0.001
tHcy	60	3.7	3.7	21	21.2	54.6	0.001
MDA	60	24.9	15.0	21	14.4	7.9	0.008

The TAC levels tended to decrease in these patients who were not taken any medication, the mean value of them in patients was (5.3±4.5 U/ml) while the mean value for control was (16.8±29 U/ml), respectively. Inversely, we found mean MDA levels and tHcy levels were increased in these patients who were taking chemotherapy in the group (4). The mean value of them in patients were (24.9±14.9) for MDA and (3.9±3.8) for tHcy these mean values of MDA and tHcy were more than the mean value of MDA and tHcy for patients who were not taken any medication, the mean value of them in patients was (20.1±10.8) nmol/ml of MDA and (3.7±5.1) nmol/ml of tHcy. On the other hand, MDA levels and tHcy levels were returned to decrease in these patients who were taken chemotherapy in the group, the mean value of them in patients was (17.3±8.9) nmol/ml of MDA and (2.9±3.7) nmol/ml of tHcy, less than the mean value (20.1±10.8) nmol/ml of MDA and (3.7±5.1) nmol/ml of tHcy for patients who were not taken any medication.

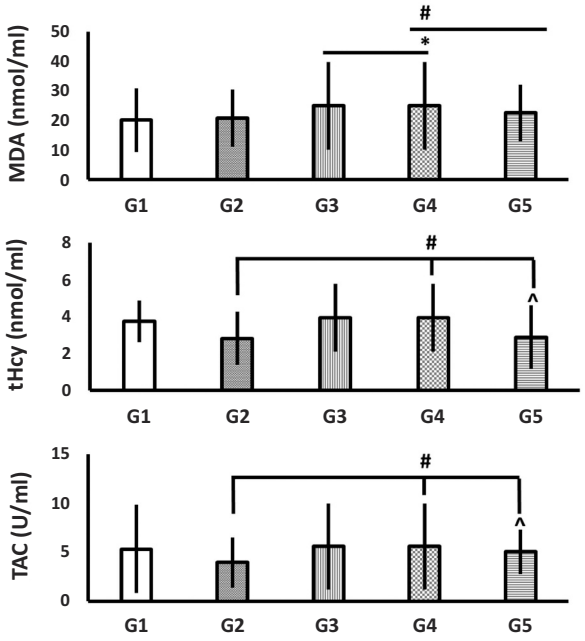

Figure 1. Comparison between measured parameters in the studied groups. Data expressed as mean±SD. *#p<0.05. MDA=malondialdehyde, tHcy= Total homocysteine, TAC=total antioxidant capacity. Group 1= healthy (Control), Group 2= Patients who are not taking any medication, and Group 3= Patients who are taking chemotherapy for once time. Group 4=Patients who are taking chemotherapy twice a time. Group 5= Patients who are taking chemotherapy thrice the time.

Discussion

ROS are present in the cell at reasonable concentrations, their concentration is regulated by the balance between their production rate and their rate of elimination by antioxidant systems. Thus, in the quiescent state, it is said that the balance of antioxidants/pro-oxidants (balance redox) is in balance (32).

However, this redox homeostasis can be broken, either by excessive production of ROS (as in ageing or atherosclerosis) or by a decrease in antioxidant capacities (as in people suffering from obesity and smokers). This is called oxidative stress. Such imbalance can be caused in a controlled manner by the activation of ROS production systems (33, 34). The antioxidant response is then effective to compensate for this production and the imbalance is transient. On the other hand, in certain pathological situations (cancer), the production of ROS is more important and prolonged, and the antioxidant response is insufficient (35, 36).

In our study, MDA levels were increased in carcinoma patients. In so far as carcinoma is concerned, indicated excess oxidative stress in patients. Shukla S. *et al.* (2020) have pretended to increase LPO in cancer patients compared with the healthy group through the alternation in peroxidant-antioxidant status in various cancer patients (37). Khalil T. T. *et al.* (2019) have supposed to increase MDA in ovaries cancer patients compared with healthy groups (38). Recent reports concluded that elevated MDA in cancer patients and related to DNA damage (39). Our findings are in correspond with previous studies proposing that ovary cancer patients might be injured the cell. Therefore, we proposed excess LPO which causes an increase in an MDA product that is transported in the circulation system, then elevated MDA levels in carcinoma patients.

Gatt A. found that elevated tHcy levels may enhance the production of OH, known LPO initiators, by tHcy autoxidation (40). Yoshida *et al.* proposed that found elevated MDA levels in patients point out elevated an LPO of the membrane and prospect further oxidation damage (41). Gupta et al. showed the correlation between tHcy and MDA when elevated tHcy in patients (42). Our studying is in correspond with the previous studies, which found positive relation them because elevated tHcy cause increased production of free radical and then, excess in the production of MDA.

Xiang M. *et al.* 2019 have a postulate OS index, and TAC was significantly different between patients with lung cancer and healthy controls (43). In the current research, both MDA levels and tHcy levels were elevated in carcinoma patients who were taken chemotherapy. Kasapović J. *et al*. (2010), have supposed that patient's cancer who were taking triple chemotherapy (5-fluorouracil, doxorubicin, and cyclophosphamide) affect the activity of the antioxidant enzyme (e.g. catalase, glutathione peroxidase, glutathione) in BRCA due to increased oxidative stress (44). Taherkhani M. et al. (2017) have concluded AC chemotherapy with BRCA increases oxidative stress in the higher stages of the BRCA (45). Our current study corresponds with previous reports, an increase of oxidative stress in cancer patients dealing with chemotherapy. in addition to tHcy may elevate ROS, thereby the MDA levels enhance in patients who have chemotherapy.

From this point of view, our finding suggested that elevated MDA, tHcy levels and decreased TAC depending on cancer progression may be due, at least in part, to elevate oxidative stress depending on chemotherapy effect in the treatment of BRCA.

Conclusion

Our findings show that MDA levels, tHcy levels, and TAC levels markers may be beneficial indeed for patients with BRCA in the appreciation of chemotherapy effects. Our study specified that chemotherapy-induced changes in MDA as LPO marker, tHcy levels, and TAC levels within treatment by chemotherapy in breast cancer patients. The oxidative stress levels are considered an indicator of the impact of chemotherapy.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee in the College of Science for Women, the University of Babylon as a part of Master's student projects.

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