BIOCHEMICAL AND MOLECULAR MARKERS IN BREAST CANCER PATIENTS

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ABSTRACT

The present study was designed to evaluate the DNA damage markers, antioxidants markers and lipid peroxidation in breast cancer patients.

About 5ml of venous blood was collected from 10 healthy persons and 30 breast cancer patients before chemotherapy. Comet assay used to measure DNA damage and antioxidants were assessed biochemically. Lipid peroxidation was estimated by the Thiobarbituric acid assay.

The results of present study showed that the DNA damage markers such as comet length, tail length, DNA percentage in tail and tail moments were significantly increased at p<0.05 in Breast cancer patients as compared with healthy control group. Also biochemical markers such as SOD and CAT in significantly high in patients and MDA was significantly elevated in the patients while GPx and GSH decreased as compared with the healthy control. This indicates high level of ROS, tumor transformation and invasion that play as a good early marker for cancer recurrence. The COMET assays described in this study help in evaluating single and double-strand breaks and monitor the ability of the cells to repair such damage.

Keywords: Breast Cancer, COMET, Antioxidants, ROS

INTRODUCTION

Breast cancer (BC) is the most common cancer and the main cause of mortality among women in the world and there was a sharp rise in BC worldwide (¹).
Reactive oxygen species (ROS) like $\text{H}_2\text{O}_2$, $\text{O}_2^-$, $\text{OH}^-$ are produced by cells in normal conditions but when they be in high level under stress, they lead to macromolecules and plasma or organelle membranes damage\(^{(2,3,4)}\). ROS are increased in cancer cells due to different cellular dysfunctions and genetic mutations\(^{(5)}\). Therefore, as a response, cancer cells have high level of molecules that scavenge ROS as antioxidant that control ROS accumulation enzymatically (superoxide dismutase (SOD) or catalase (CAT)) and nonenzymatically\(^{(6-10)}\).

In cancer progression, GSH has a dual role that it is important in carcinogens detoxification and GSH high levels protect cancer cells by developing resistance against chemotherapeutic drugs\(^{(11,12)}\). Therefore, cancer cells are maintaining antioxidant levels balance in order to survive and metastasize where ROS regulate cancer cells metastasis potency and high level of GSH promotes cancer metastasis\(^{(13-15)}\).

Comet assay is a very important, economic and simple technique to measure DNA damage (single-strand and double strand breaks) in individual cells\(^{(16)}\).

This study was conducted to evaluate and estimate the ROS and tumor metastasis level through the anti-oxidants enzymes in addition to DNA damage.

**MATERIALS AND METHODS**

**DNA damage measurements**

About 5ml of venous blood was collected from 10 healthy persons and 30 breast cancer patients.

DNA damage was measured following\(^{(16)}\) method. Briefly, about 100µl of venous blood placed in a 2ml phosphate buffer containing microfuge tube then 40µl of proteinase K added. After centrifugation, total of 7.5µl mixed with 75µl of low melting agarose and immediately spread on the clear part of a comet slide. Slides were stored in lysis solution at 4°C for 60 minutes, after that, the lysis solution removed and replace by alkaline solution containing 6g NaOH and 500µl of 0.5% Na\(_2\)EDTA for 60 minutes at room temperature in dark. Slides were washed gently by immersing in 1X TBE buffer for 5 minutes then transferred to horizontal electrophoresis apparatus and ran for 60 min. at 70V. Excess water was removed 70% ethanol then stained using ethidium bromide and stored for 24 hours before viewing by fluorescence microscope. Comets were quantitated using the Comet analysis software through examining ≥ 100 cells for each case.

**Antioxidants measurements**

SOD activity was determined by autoxidation of Pyrogallol according to\(^{(17)}\). While CAT activity was measured according to procedure of\(^{(18,19)}\). GSH activity was measured according to\(^{(20)}\), the acid soluble sulfhydryl groups form a yellow colored complex with dithionitrobenzene (DTNB). The activity of GPx was investigated according to procedure of\(^{(21)}\).

**Lipid peroxidation measurement**
Lipid peroxidation estimated using Thiobarbituric acid assay for Malondialdehyde (MDA) concentration according to (22,23).

Statistical analysis

Statistics was done using SPSS (V.20) to find means, standard deviation and least significant differences by ANOVA. Values with p<0.05 is represented significant.

RESULTS

DNA damage Markers

According to figure (1), the DNA damage markers showed significant differences in control and patient (p<0.05). The Comet length recorded highest (Mean±SD) in patients which reached (92.5±3.6µm) compared with control (29.2±2.1µm)(figure 2). The tail length was recorded in patients (20.18±1.9µm) while in control (0.78±0.13µm)(figure3), while the DNA% in tail in control of serum was (0.54±0.02%) while in patients reached to (3.23±0.54%)(figure 4). Thetail moments in patients(0.62±0.14µm) and (0.11±0.01µm) in control(figure 5).

Figure 1: Comet assay in Breast Cancer patients and healthy control A: Healthy control, B: Breast cancer patient.
Figure 2: Comet length in control and cancer patients

Figure 3: Tail length in control and cancer patients
Biochemical Markers

All biochemical markers showed significant differences between control and patients. The superoxide dismutase SOD activity in serum of control was (28±2.4U/mg), in patient it reached (39±3U/mg) while the activity of catalase in control was (20±2.8U/mg) and in breast cancer patients which elevated to reach (38.3±4.4U/mg).

The Glutathione GSH activity was showed significantly differences between control and cancer patient, its activity in control was (52.7±3.5µmol/ml), while activity in the patient of cancer was declined to (30.3±3.3µmol/ml).
Significant differences between control and patients in glutathione peroxidase GPx activity, it’s activity in serum control was (291±53.5µmol/ml) and the activity were declined to (187±34.5µmol/ml) in cancer patients. The concentration of MalondialdehydeMDA was significantly different between control and patients in serum, it’s activity in control of serum was recorded(18±1.07µmol/ml), its activity was increased in patients to reach (283±6.7µmol/ml)(figure 6).

![Figure 6: The biochemical markers in control and patients of Breast cancer patients before chemotherapy (BCh).](image)

**DISCUSSION**

Many factors increase ROS level including infections and pollution that generate oxidative stress, causing DNA and other molecules damage. The Elevation of some concentration of antioxidants, help in reducing the risk of many diseases including cancer, diabetes and heart disease. The antioxidants concentrations may interfere with the cancer patients’ treatment program including radiation and some chemotherapy types since those treatments generate free radicals that attack tumor cells.

Antioxidant components are exhausted where lower concentration for some antioxidants is present in patients under oxidative stress. Enhanced lipid peroxidation has been reported recently in breast cancer tissues, which support the oxidative stress hypothesis in breast carcinogenesis. Low level of SOD and CAT and high level of MDA was reported as an indication of oxidative stress and lipid peroxidation that support tumor transformation and invasion. (Woolston et al., 2011) found no correlation between GSH levels and recurrence or overall survival.
DNA damage is intimately related to breast cancer with repair failures where breast tissue has an increased opportunity for remodeling and DNA damage throughout a woman’s life (30).

Endogenous and exogenous factors, DNA damage type and the genes involved relationships are important in breast cancer treatments guidance (31).

CONCLUSION

The biochemical results indicate high level of ROS, tumor transformation and invasion that play as a good early marker for cancer recurrence.

The COMET assays described in this study help in evaluating single and double-strand breaks and monitor the ability of the cells to repair such damage.

REFERENCES


