Elevated lipid peroxidants in breast cancer patients

A.Nath¹, Aseem Kumar¹, Priyanka¹, J.K Singh², Reena Sinha³, Shailendra kumar¹ and Chandan kumar Singh¹

¹Research Centre, Mahavir Cancer Institute, Patna
²Department of oncology
³Department of Pathology Mahavir Cancer Institute and Research Centre, Patna

Abstract: Malondialdehyde is an important biomarker for lipid peroxidation. It has the ability to react majorly with deoxyguanosine and deoxyadenosine of DNA which causes mutation. It promotes the development of cardiovascular disease, atherosclerosis and several types of cancer including breast cancer. Breast cancer is the leading cause of death among women worldwide. Blood samples of 180 breast cancer patients were collected at Department of Pathology, Mahavir Cancer Institute, India. Lipid peroxidation assay was performed to determine MDA level in serum.

In conclusion, the present study shows the association between MDA level and breast cancer. The decreased level of MDA in serum of grade III breast cancer patients needs to be explored further to understand the exact role of MDA in breast cancer.

The MDA levels in serum of breast cancer patients were found significantly higher than the normal range (p<0.05). The mean value of MDA level in different grades of breast cancer patients was measured and found to be 41.59 (nMol/ml) for grade I, 45.85 (nMol/ml) for Grade II and 36.27 (nMol/ml) for Grade III with a p-value < .048. Histopathological study of tissue from breast cancer patients unveiled some major significant changes in the architecture of the cells.

Therefore, the present study was undertaken to estimate level of MDA in serum of 180 breast cancer patients and to observe histopathological changes in same patients and subsequent correlation with the MDA level.

Keywords: LPO, MDA, Breast cancer, tissue, blood.

I. Introduction

Malondialdehyde is an important indicator of lipid peroxidation which is produced by enzymatic and oxygen radical induced lipid peroxidation [4]. Formation of MDA leads to decrease in fluidity of plasma membrane, inactivation of membrane bound enzymes and receptors and also disrupts nonspecific calcium ion permeability [2and11]. MDA exhibits its property to react with deoxyguanosine and deoxyadenosine of DNA which ultimately lead to mutation. Several studies have confirmed the mutagenic property of MDA in human as well as in animal models. In humans, it promotes the development of cardiovascular diseases, atherosclerosis and several types of cancer including breast cancer. Breast cancer is the most leading type of cancer causing death among women in worldwide [20] including India. Approximately, 0.4 Million women die from breast cancer out of 1 Million patients being diagnosed, annually [16]. Estrogen metabolism in human body may act as a source of oxidative stress [5 and 3]. The generation of MDA occurs in both plasma and tissue of breast cancer patients. Besides, MDA levels have been implicated to be elevated in the blood and in malignant breast tissues of breast cancer patients [8,12,17]. Histopathological classification of breast cancer is based upon observation of biopsy specimen under light microscopy. Three most common histopathological types are invasive ductal carcinoma, ductal carcinoma in situ and invasive lobular carcinoma which account for 55%, 13%and 5% of total breast cancer respectively. The most acceptable grading system of breast cancer is based on Nottingham modification [19] which classifies breast cancer as grade I (well differentiated tumor), grade II (moderately differentiated tumor) and grade III (poorly differentiated tumor). The formation of MDA and its association with cancer progression has drawn the attention of scientists for further research.

Therefore, the present study was undertaken to estimate the MDA level in serum of breast cancer patients and to observe the associated histopathological changes in tissue of breast cancer patients.

II. Material and Methods

Blood and tissue samples were collected from 180 breast cancer patients and normal women who came for their treatment at Mahavir Cancer Institute, Patna, India, from the Department of Pathology, of the same institute, with the prior consent of the patients.
Elevated lipid peroxidants in breast cancer patients

2.1 TBARs assay
Serum of 180 breast cancer patients were assayed for lipid peroxidation by determining their malondialdehyde (MDA) levels. MDA level in each breast cancer patients was estimated by standard procedure with slight modifications [10]. Blood samples were centrifuged at 3000rpm for 10 min to obtain serum. 2.5 ml of 10% of TCA was added to 0.5ml of test serum, incubated at 95°C for 15 min and the solution was centrifuged at 3000rpm for 10 min. The supernatant was collected and 0.675% of TBA was added to the same and incubated again at 95°C for 15 min. The color reaction was obtained to measure optical density using spectrophotometer and the amount of TBARs was calculated with the help of standard procedure.

2.2 Histopathological study
Histopathological parameter was studied by collecting tissues of breast cancer patients. Tissues were fixed in neutral formalin fixative and dehydrated in ascending concentration of alcohol. Tissues were kept in paraffin wax and blocks were prepared. 5 µm thick sections were cut and fixed with the help of Mayer’s solution. Double staining was done after 6hrs and the slides were kept in xylene and hydrated in descending concentration of alcohol. The slides were stained with hematoxylin and dehydrated up to 70% alcohol. Again, the slides were stained with eosin and then dehydrated in 90% alcohol and absolute alcohol finally was mounted with DPX to observe under light microscope.

Nottingham Histological score system was used to grade the cancer slides. Gland formation has been seen in more than 75% of tumor area. Three other cellular component were cosider; nuclei, nucleoli and mitotic count; Nature of nuclei and number of nucleoli with their status and mitotic count were consider for the Nottingham Histopathology score system.

2.3 Statistical analysis
Mean and standard deviations were calculated using MS Excel 2010. Statistical analysis was performed using One way ANOVA and SPSS software package 11.5.

III. Results
Text Fig. 1 shows the MDA level in normal and breast cancer patients. Mean level of MDA in normal women was found 20.85nMol/ml. Highest mean level of MDA was observed as 45.85nMol/ml in grade II breast cancer patients which was more than two fold high than the normal. Surprisingly, grade III breast cancer patients show decreased level of MDA in their serum which was 36.27n Mol/ml. Table 1 shows mean and standard deviation of MDA levels in the serum of different grades of breast cancer patients.

![Text Fig1](image)

(P-value< 0.05)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Grades</th>
<th>Mean±S.D of Lipid peroxidation level (MDA nM/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients CaBr</td>
<td>I</td>
<td>41.59±4.18</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>45.85±5.33</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>36.27±2.83</td>
</tr>
<tr>
<td>Normal</td>
<td>NA</td>
<td>20.85±2.91</td>
</tr>
</tbody>
</table>

Table 1- Mean and standard deviation of MDA levels in breast cancer patients and control.
Elevated lipid peroxidants in breast cancer patients

Plate 1- X400 Photomicrograph

Fig. 01 - (A) Showing normal structure of breast tissue, (B) breast cancer (grade I) (C) Microphotograph of breast cancer, grade II (D) infiltrating duct carcinoma of breast cancer, grade III. Straight arrows barb at vesicular nuclei and black elbow arrow connectors pointed at rapid formation of nuclei. Note the increased number of nucleoli (NL). Inset showing vesicular nuclei and lobular structure visible.

Fig 02-Photomicrograph (100X) of breast cancer tissue grade II, showing cytoplasmic diffusion, its loss and irregular shape of cytoplasm (straight arrows).

Normal tissue section of breast cancer showed normal architecture of the tissue (fig 1A). Section illustrates lobules lined by uniform ductal cells with blan nuclei lined by myoepithelial cells.

Upon a histopathological investigation of breast cancer it has been observed that the tissue is complex and is made up of stromal and neoplastic cells. Its microenvironment comprises of adipocytes, endothelial and immune cells in addition to neoplastic cells. The appearance of the cancerous tissue is quite heterogenous, ranging from tumors with well-developed tubule formation and low grade nuclei to tumors consisting of sheets of anaplastic cells. The tumor margins are usually irregular but are occasionally pushing and circumscribed.
Elevated lipid peroxidants in breast cancer patients

IV. Discussion
The study reflects substantial increase in the peroxidation level of lipids in the serum of breast cancer patients when compared to the normal which is consistent with few studies [18]. Elevated level of MDA in serum or plasma apart from the breast cancer has also been reported in several cancer types such as oral cancer [1], uterine cancer [13] colorectal cancer [7] etc.

The presence of increased level of MDA in cancer has been shown to be associated with the oxidative stress of the body which leads to loss of PUFA in plasma membrane and its integrity is defied. Reports confirm that oxidative stress leads to mitochondrial dysfunction and up regulation of important factors like nuclear respiratory factor 1, NRF1 [17]. Further, a significant property of lipid peroxidation is the formation of DNA adducts. Few studies report the same in breast cancer patients [14]. At present, the correlation between formations of this MDA-DNA adducts and the risk of breast cancer remains unknown.

In the present study it has been observed that the level of MDA in grade II patients was higher than the MDA level among all other grades of breast cancer patients. Cytoplasmic diffusion and its loss was also observed to be the highest in grade II breast cancer patients. Interestingly, MDA levels in other types of cancer (gastrointestinal tract tumors) have also shown lower levels in grade III as compared to early grades[15]. The reason might be attributed to the nuclei that have been found to be enlarged and vesicular in grades I and II, respectively. On the other hand, in grade III, nuclei are spread all over the tumor area and found irregular in shape, which means formation of new cells and nuclei occurred. Since older cells have increased ROS than new cells [6], hence due to appearance of new cells, decreased level of ROS may be observed in grade III, as a result of fresh electron transport chain machinery. Moreover, new cells emanate with larger amount of antioxidant enzymes and molecules than older cells do. These antioxidants might be able to compensate for lost ones, which should depreciate the level of MDA in grade III. However, the same could unlatch the concealed reasons of sudden lowering of MDA level in grade III and its irregularity in different grades. In another report [9], CA-125 gene, like MDA level, shows irregular pattern of its expression in different stages of ovarian cancer. However, study is under progress and reasons behind low peaked TBARs level in grade III as compared to grades I and II might soon be known.

V. Conclusion
On the basis of the above study, it can be concluded that increased level of MDA in breast cancer patients is a significant biomarker for the breast cancer patients. Further, its low level in grade III breast cancer patients needs more study to understand the overall mechanism of action of MDA in breast cancer. Future work will need to analyze the MDA level in numerous breast and other cancer patients also. So that, MDA level in serum can be used as prognostic marker for breast cancer patients.

Acknowledgement
Authors are indebted to the Department of Science and Technology, Ministry of Scienceand Technology, Government of India, for financial support. We are thankful to the Department of Oncology and Pathology, Mahavir Cancer Institute and Research Centre, Patna for their co-operation.

Reference
Elevated lipid peroxidants in breast cancer patients


