EPILOGUE

Great is the art of beginning, but greater is the art of ending.

-Henry Wadsworth Longfellow
Because of its increasing incidence, breast cancer is one of the major health challenges for scientists, clinicians and policy makers. Oxidative stress has emerged as an important etiological factor for breast cancer. The enzymatic and non-enzymatic antioxidant defense system counteract the oxidative stress. The major damage due to oxidative stress occurs to lipids which constitutes a large portion of cell membrane. This damage leads to lipid peroxidation of the cell membrane. Thiol is an important endogenous antioxidant. It also maintains redox state in the cell. The higher oxidation favors the oxidized state in the cell. The cytoplasmic antioxidant hsp70 helps other proteins and macromolecules to fold or refold and reach their final, native conformation. Abrupt changes of cellular redox status lead to hsp induction. It is also observed that oxidative stress may cause imbalance of proteolytic-antiproteolytic system leading to enhanced proteolysis and destruction of ECM proteins and metastasis. Therefore, the current study was undertaken to assess enzymatic and non-enzymatic antioxidants, lipid profile parameters, oxidative stress related markers, hsp70 expression, MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in controls, patients with BBD and breast cancer patients as well as their follow-up samples of breast cancer patients. The observations obtained by analysis of the parameters using highly specific, sensitive and sophisticated methods were as follows:

**Summary:**

**Enzymatic antioxidants:**

- Plasma and RBC GST activity were lower and plasma GR, RBC GR and RBC SOD activities were higher in breast cancer patients as compared to the controls.
- ROC curve analysis suggested that RBC GST and plasma and RBC GR could significantly discriminate between controls and breast cancer patients.
OR analysis suggested that higher OR with increasing activities of plasma and RBC GR as well as RBC SOD were associated with increased risk of breast cancer. Whereas, lower OR with increasing activity of RBC GST suggested reduction in breast cancer risk.

RBC GST activity was lower and RBC SOD activity was higher in patients with BBD as compared to the controls.

Plasma and RBC GR activities were higher in breast cancer patients as compared to the patients with BBD.

Multivariate analysis with clinicopathological parameters showed significant association of plasma and RBC GST, plasma GR, and RBC catalase with nuclear grade of the tumor.

Out of the 25 DNA samples studied for GST-μ genotype, the null genotype was observed in 44% breast cancer patients.

Non-enzymatic antioxidants:

Plasma non-enzymatic antioxidants β-carotene, vitamin-E and vitamin-C levels were significantly lower and plasma vitamin A levels were significantly higher in breast cancer patients as compared to the controls.

ROC curve analysis documented that, plasma levels of β-carotene, vitamin-A and vitamin-E could significantly discriminate between controls and cancer patients.

OR analysis indicated that higher OR with increasing levels of plasma vitamin-A was associated with increased risk of breast cancer. Whereas, lower OR with increasing β-carotene, vitamin-E and vitamin-C were associated with reduced risk.

Comparison of plasma non-enzymatic antioxidants exhibited higher levels of plasma vitamin-A in patients with BBD as compared to the controls.

Plasma levels of β-carotene, vitamin-C were significantly lower and plasma vitamin-A levels were significantly higher in breast cancer patients as compared to the patients with BBD.
Multivariate analysis with clinicopathological parameters in breast cancer patients showed significant association of plasma vitamin-E levels with lymph node involvement and BR score and plasma vitamin-C levels with nuclear grade of the tumor.

**Lipid profile parameters:**

- Comparison of plasma lipid profile parameters between controls and breast cancer patients demonstrated significantly lower levels of plasma cholesterol and HDL in breast cancer patients as compared to the controls. The levels of plasma VLDL and TG were significantly higher in breast cancer patients as compared to the controls.
- ROC curve revealed that plasma HDL, VLDL and TG levels can significantly discriminate between controls and breast cancer patients.
- OR analysis suggested that lower OR with elevated levels of cholesterol and HDL were associated with decrease in breast cancer risk. Whereas, increased OR with higher levels VLDL and TG were significantly associated with increased risk of breast cancer.
- Significantly lower levels of plasma cholesterol, LDL VLDL and TG were observed in patients with BBD as compared to the controls.
- The levels of VLDL and TG in breast cancer patients were significantly higher as compared to the patients with BBD.
- Multivariate analysis with clinicopathological parameters in breast cancer patients revealed significant association of plasma VLDL and TG with nuclear grade of the tumor.

**Oxidative stress related markers:**

- Plasma levels of thiol were significantly lower and plasma LPx levels was higher in breast cancer patients as compared to the controls.
- ROC curve illustrated that plasma thiol levels can significantly discriminate between controls and breast cancer patients.
OR demonstrate that lower OR with higher levels of the plasma thiol were significantly associated with reduced risk of breast cancer.

Plasma levels of thiol and LPx were comparable in controls and patients with BBD.

Plasma thiol levels were significantly lower in breast cancer patients as compared to patients with BBD.

**hsp70:**

- hsp70 expression was significantly higher in malignant breast tissues as compared to adjacent normal breast tissues.
- Inducible form of hsp70 was observed in 50% of malignant tissues.
- Multivariate analysis showed that hsp70 expression was an independent variable.

**MMPs and TIMPs:**

- Active MMP-2, total MMP-2 and activation ratio of MMP-2 were significantly higher in malignant breast tissues as compared to the adjacent normal tissues. ProMMP-2 levels were significantly lower in malignant tissues as compared to adjacent normal tissues.
- Active MMP-9 and activation ratio of MMP-9 levels were significantly higher in malignant tissues as compared to adjacent normal breast tissues.
- ROC curve revealed that proMMP-2, active MMP-2 and active MMP-9 levels could significantly discriminate between malignant and adjacent normal breast tissues.
- Activation ratio of MMP-2 and MMP-9 was significantly higher in tumors showing lymph node involvement.
- Multivariate analysis between different forms of MMP-2 and clinicopathological parameters showed that proMMP-2 levels were significantly associated with lymphnode involvement in adjacent normal...
tissues. Active MMP-2 was significantly associated with lymph node involvement and BR score in malignant breast tissues. Total MMP-2 was significantly associated with lymph node involvement in adjacent normal tissues and early vs. advanced stage in malignant tissues. Activation ratio of MMP-2 was significantly associated with lymph node involvement and nuclear grade in adjacent normal tissues.

☑ Multivariate analysis between different forms of MMP-9 and clinicopathological parameters showed significant association of proMMP-9 levels with lymph node involvement in adjacent normal tissues.

☑ Plasma MMP-2 and TIMP-1 levels were significantly lower, and MMP-9 and TIMP-2 levels were significantly higher in breast cancer patients as compared to the controls.

☑ Plasma TIMP-1 levels were significantly lower in patients with BBD as compared to the controls.

☑ ROC curve documented that plasma MMP-2 and MMP-9 could significantly discriminate between control and breast cancer patients.

☑ Plasma MMP-2 levels were significantly higher and MMP-9 levels were lower in CR as compared to PT. Paired sample analysis of PT and CR suggested that plasma MMP-2 and TIMP-1 levels were significantly higher and MMP-9 levels were significantly lower in CR as compared to PT.

**Conclusion:**

The present study revealed that, higher RBC SOD activity and lower levels of β-carotene, vitamin-E and vitamin-C as well as altered lipid profile parameters suggested higher ROS status in breast cancer patients. Lower RBC and plasma GST and higher RBC and plasma GR activity in association with lower levels of thiol indicated higher oxidized state in the cell resulting in higher oxidative stress in breast cancer patients. Plasma vitamin A levels were higher in patients with BBD and breast cancer patients as compared to the controls. The oxidative stress level was further confirmed by higher expression of hsp70 in malignant breast
tissues. Oxidative stress resulted in altered proteolytic–antiproyteolytic balance evident from high MMP-2 and MMP-9 levels in malignant tissues which was strengthen by lower levels of circulating MMP-2 and TIMP-1 levels and higher MMP-9 and TIMP-2 levels in breast cancer patients. In addition, circulating levels of MMP-2, MMP-9 and TIMP-1 can be used in treatment monitoring of breast cancer patients. Taken together, the trends of current study implicate interesting clue to the cause as well as management of breast cancer and warrants indeapth study in this interesting area.

Concluding Remarks:
Oxygen radical generation is an inevitable consequence of aerobic life. It is the price the body pays for extracting 12 extra ATP molecules from every acetyl CoA that the cells combust. Our bodies constructs star wars like defenses to scavenges oxidants and civil defense team to repair the damage. However, the defenses are not always perfect. Escape of oxidant from these antioxidant defenses leads to the cascade of events. The present study revealed significant clues on various aspoects of this cascade.

Therefore in furtherance, study of Mn SOD, an important antioxidant with potent antitumor activity will add a significant to the knowledge. Because it has been hypothesized that over expression of Mn SOD results in the activation of MMPs which is possibly based on the transcriptional activation of the redox sensitive transcription factors AP-1 and/or NF-kB by elevated $\text{H}_2\text{O}_2$. Study of Mn SOD, GPX as well as AP-1 and NF-kB may enlighten better and specific understanding of the regulation of anti-proliferative pathways and its control of tumor invasion might aid in the design of novel therapies targeting the respective molecular pathways.

The second approach might be the study of CRBP and RAR$\beta$ in vitamin A homeostasis, which may give clue to the role of vitamin A, CRBP and RAR$\beta$ in
breast carcinogenesis. Study regarding cellular retinol binding proteins and retinoic acid receptors may aid to unravel the discrepancy regarding increased plasma retinol level in the current study, as retinol is homeostatically regulated. A preliminary work is carried out in the final phase of the study.

**Targets for inhibiting MMP expression and activity**

The current results of MMPs and TIMPs suggested that MMPs are important in invasion and metastasis. The up-regulation of MMPs during invasion can be caused by a variety of factors including increased production through cytokines and growth factors, increased activation through mechanisms such as uPA and decreased inhibition by reduced levels of TIMP-1. Because of the protein nature and multiplicity of action, it is unlikely that TIMPs will be widely used as anticancer molecules. Developing orally active synthetic inhibitor of MMPs is a possible treatment for controlling the metastatic potential of tumors. As variety of factors are associated in up-regulation of MMPs.