ORIGINAL ARTICLE

Expression of Prophetic Variables of Analytical Importance and their Potential Role in Breast Cancer Patient Underwent Surgical Procedure

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ABSTRACT

Objective: Study the role of prophetic variables and their role in breast cancer patients especially those underwent surgical procedures.

Study Design: Cross-sectional study

Place and Duration: Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore.

Martials and Methods: Twenty diagnosed patients of breast cancer facing surgical procedure were selected from the surgical department, Jinnah hospital, Lahore and twenty normal females were included in the study. Complete blood count (CBC) of the selected individuals was performed. Other biochemical markers Malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-Hydroxynonenol (4-HNE), Isoprostanes F2α (IsoP- F2α), Interleukin-6 (IL-6), Matrix metalloproteinases-9 (MMP-9), Tumor Necrosis factor-alpha (TNF-α), Prostaglindin-E2 (PGE-2) were measured by commercially available kits.

Results: The mean weight, BMI, WBCs, platelet count, lymphocyte and neutrophil value were higher in patients compared to the controls with statistically significant differences ($p < 0.05$). However, the mean MDA, 8-OHdG, 4-HNE, Iso-P2α, IL-6, MMP-9, TNF-α and PGE-2 value significantly higher than the control subjects ($p < 0.05$)**.**

Conclusion: Findings of the study demonstrated the significant role of the said prophetic variables that were having their medicinal importance in the invagination of breast cancer among the patients undergone surgical interventions. It shows the significant increases in the levels of discussed markers that show increased oxidative stress leading to cell damage that can be observed within the studied group. Therefore, early investigation of the said variables and ruling out the true causes could help in addressing the oncological complications that one undergoes after being through the surgical interventions and in providing the best possible treatments

Keywords: Epidermal growth factor receptor, metastatic invasion, breast cancer surgery, matrix metalloproteinase, DNA adduct

INTRODUCTION

Breast cancer is by far the most common cancer among the women of today [1]. There is a higher incidence rate in Western nations but a higher mortality rate in less developed countries like India, Pakistan. In all age groups, Black women are diagnosed at a more advanced stage which depict a higher mortality rate than other racial groups worldwide [2]. This fact can be explained by several biochemical factors including lymph node metastasis, other organ metastasis and the frequency of triple negative tumors in various ethnic groups. Triple negative tumors are described as those tumors in which there is no hormone receptors expression (HRE) or there is an overexpression of Human epidermal growth factor receptor 2 (HER2) on the plasma membrane of the breast cancer cells. Breast cancer management has been involved with breast conserving surgery (BCS) during the recent era to improve the quality of life of breast cancer patients. However, the BCS is not the suitable choice for some patients with multiple tumors or metastatic tumor in situ [3]. In such circumstances, mastectomy is the best option to achieve the optimum control of the disease but it impacts significant biochemical and physiological changes in the female body. Anemia is the most common complication observed in breast cancer females underwent with surgery perhaps due to excessive bleeding, nutritional deficiencies, tumor cells infiltration and bone marrow damage and the metastatic process itself [4-5].

The inflammatory cytokines overexpressed in tumorigenesis such as Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNF-α), matrix metalloproteinase 9 (MMP-9) and 4-Hydroxynonenol (4-HNE) interfere with the proliferation of the erythrocytic progenitors [6-7]. MMP-9 due to its property of the key modulator of epithelial to mesenchymal transition and formation of tumor microenvironment has been an attractive cytokine in breast cancer patients underwent with surgery [8]. MMP-9 highly secreted by breast cancer cells initiate the signaling of MAPK (mitogen activated protein kinase) pathway cross talking with c-Jun N-terminal kinases (JNKs) followed by TNF-α, TGF-β and NF-kB expression. High serum levels of IL-6, MDA and 8-hydroxydeoxyguanosine (8-OHdG) also correlates with the increase in the oxidative stress in patients undergone breast removal surgery stimulating other biochemical cascades creating DNA methylations in tumor associated microenvironments [9-10]. F2-isoprostanes and prostaglandins E-2 (PGE-2) also increase the incidence of free radical generation by triggering p53activation directly mediating apoptosis and cellular senescence [11]. Changes in complete blood count (CBC) especially red blood cells, white blood cells and platelets play a key role in attaining cellular integrity, malignant proliferation of the breast tissue in women with increased expression of oxidative stress transcriptional factors due to breast surgery[12-13]. The aim of this study is to illustrate the significance of oxidative stress biomarkers that significantly impact the biochemistry and physiology of the breast cancer females who had undergone breast removal surgical procedure.

MATERIALS AND METHODS

Sample Collection: Current study is a cross-sectional study comprising twenty diagnosed patients of breast cancer facing surgical procedure were selected from the surgical department, Jinnah Hospital, Allama Iqbal Medical Collage Lahore-Pakistan) during the period (September, 2019-September, 2020). Inclusion and exclusion criteria was as follow females having breast cancer undergone surgical interventions were included in the current study while, females with any other congenital diseases and medication for hypertension, hypocholesteremia were excluded out of the current study. None of the control individuals was on medications such as multivitamins, cigarette and alcohol nor having depression, liver cancer, diabetes mellitus, and multinutrition syndrome. Research Ethical Committee of The Institute of Molecular Biology and Biotechnology (IMBB) approved the research protocol. Five ml of venous blood sample from each participant into the anti-cubital

vein. The sample bottle was centrifuged within one hour of sample collection and serum was separated for storage at 70°C until assayed.

Biochemical Analysis: Complete blood count (CBC) of the selected individuals was performed on automated hematology blood analyzer by Sysmex version XP-2100. The levels of Malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8-OHdG), 4-Hydroxynonenol (4-HNE), Isoprostanes F2α (IsoP- F2α), Interleukin-6 (IL-6), Matrix metalloproteinases-9 (MMP-9), Tumor Necrosis factor-alpha (TNF-α), Prostaglindin-E2 (PGE-2) were measured by commercially available kits (Glory Science Human Eliza Kits).

Statistical Analysis: The data was represented as mean and standard deviation. The significant value of the result was measured by independent sample t-test and p-value was considered to be statistically significant with less than 0.05.

RESULTS

The laboratory blood parameters of breast cancer patients and control group are depicted in table 01. The mean values of BMI, WBC, Platelets, HCT and neutrophils were significantly higher in breast cancer patients compared to the control group (BMI= 31.25 vs 22.25, WBC= 14.59 vs 8.48, PLT= 298.59 vs 291.58, HCT 65.29 vs 42.59, neutrophils 156.35 vs 71.59. however, RBC=3.29 vs 5.66 and Hemoglobin 10.59 vs 14.33 decreased in breast cancer patients as compared to the healthy individuals as depicted in table 01.

Table-1: Demographic and Hematological Variables in CA Breast

Variables	Control (n=20)	Subject (n=20)	P value
Weight	65.29±5.49	66.35±7.59	0.058
Bmi	22.259±4.59	31.25±7.59	0.001
Rbc	5.66±0.55	3.29±0.185	0.042
Wbc	8.48±2.59	14.59±3.29	0.016
Hb	14.33±3.55	10.59±2.88	0.011
Plt	291.58±12.56	298.59±12.58	0.056
Hct	42.59±7.59	65.29±5.65	0.412
Neutrophils (%)	71.59±10.25	156.35±4.259	0.006

These significant differences in control and breast cancer patients realized that the women underwent breast surgical procedures experiences severe hematological disturbances which ultimately affect the whole biochemistry of the patient. In this regard, the levels of some biochemical markers of inflammation in breast cancer surgery were also analyzed (Table: 02)

Table 2: Prophetic Variables of Analytical Importance CA Breast

Variables	Control(n=20)	Subjects (n=20)	(P<0.05)
Malondialdehyde (MDA) nmol/ml	0.965±0.015	9.86±2.45	0.015
8-hydroxy-2-deoxyguanosine (8-OHdG) ng/ml	16.359±3.25	32.29±5.88	0.001
4-Hydroxynonenol (4-HNE) µmol/l	4.29±1.55	18.35±3.29	0.000
Isoprostanes F2α (IsoP- F2α) (pg/ml)	6.359±2.58	66.35±10.258	0.015
Interleukin-6 (IL-6) pg/ml	6.359±1.99	21.59±3.58	0.008
Matrix metalloproteinases-9 (MMP-9) ng/ml	29.68±7.59	256.35±14.59	0.017
Tumor Necrosis factor-alpha (TNF-α) pg/ml	18.59±3.28	56.35±4.59	0.000
Prostaglindin-E2 (PGE-2) pg/ml	1.022±0.114	5.655±1.256	0.018

MDA and 8-OHdG increased significantly in breast cancer subjects as compared to the normal individuals (9.86 vs 0.965mol/ml) which indicates the formation of guanosine adducts with other nitrogenous bases confirming the DNA damage. HNE also depicted upregulated expression showing increased lipid peroxidation which is capable of DNA interaction and triggering mutagenesis. Similarly other biochemical parameters such as IsoP- F2α, IL-6, MMP-9, and TNF-α and PGE-2 were also upregulated showing higher levels in serum (Table 02) These results are in clear agreement that overproduction of ROS in BC patients underwent with surgery leads to the pathogenesis of many other inflammations, overexpression of apoptosis and cellular senescence.

DISCUSSION

Metastasis is one of the major issues in breast cancer patients [14-15]. In order to address this, some surgical procedures are adopted to avoid tumor invasion. Although these surgical procedures impact the biochemistry of the patients very significantly [16]. Oxygen free radicals which are generated through several enzymatic and non-enzymatic reactions due to surgery attack various biomolecules including lipids, carbohydrates and proteins and initiate the signaling of various inflammatory cytokines such as IL-6, MMP-9, PGE-2, Isoprostanes and TNF-α [17-18-19].

Table 3: Pearson S' Correlation Coefficient Matrix of Variables of Analytical Importance CA Breast

Variables	MDA	8-OHdG	4-HNE	IsoP- F2α	IL-6	MMP-9	TNF-α	PGE-2
MDA	-	0.862**	0.654*	0.525*	0.658*	0.659*	0.584*	0.635*
8-OHdG		-	0.215	0.014	0.856*	0.922**	0.759**	0.159
4-HNE			-	0.231	0.045	0.265	0.368	0.159
IsoP- F2α				-	0.147	0.231	0.056	0.849**
IL-6					-	0.568	0.756	0.125
MMP-9						-	0.995***	0.128
TNF-α							-	0.223
PGE-2								-

In our current study, Pearson correlation matrix showed a highly significant relationship of MMP-9 with 8OHdG vs (r=0.922, p=0.001) and with TNF-α (r=0.995, p=0.000) (Table: 3) which means the formation of 8-hydroxyguanosine DNA adduct is significantly dysfunctioning the mitochondria producing MMP-9 specific for breast cancer invasion and releasing mitochondrial oxidase increasing TNF- α. This causes the fibroblast proliferation influencing various physiological factors such as weight gain and a high BMI. Higher levels of neutrophils in the blood count and overexpression of 4-HNE depicts that demargination of neutrophils in the tumor cells occur occupying the vascular spaces resulting into lymph vascular invasion [20]. Similarly, the highly significant relationship of IL-6 with 8-OHdG (r=0.856, p=0.008) (Table: 3). Secreted IL-6 induces overexpression of antiapoptotic proteins like

cyclin D1, Bcl-xL, VEGHF and MMPs controlling the induction of G1 cell cycle progression and proliferation [21-22]. Thus, it is important for a clinician to inform the patients of these possible biochemical outcomes of the breast surgery in an understandable way to achieve the optimum health outcomes.

CONCLUSION

To conclude that, it is clear that breast cancer treatment through surgery is a field that is undergoing continuous improvements. Hematological increased levels of platelets, WBCs, Lymphocytes, neutrophils and increased expression of inflammatory cytokines of MMP-9, 8-OHdG, IL-6, Isoprostanes, PGE-2 and TNF-α are the gold standard in breast cancer screening however, these parameters also encompass serious complications due to the

surgery which leads to oncological results. It is mandatory for the clinicians to manage these afore mentioned upregulated biochemical changes before surgery of breast cancer patients in order to achieve the best possible results.

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