Role of Single Nucleotide Polymorphism in Paraoxonase-1 and Oxidative Stress in Breast Cancer Progression among Pakistani Females

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ABSTRACT

Breast cancer is a chronic disorder that usually arise from milk conducting lobules or ducts. As reported by W.H.O, 2.1 million females are diagnosed with breast cancer each year. Pakistan has highest breast cancer incidence rate among Asian population. It may be due to inaccurate molecular mechanism that defines genetic vulnerability to breast carcinogenesis. Pakistan has highest proportion of breast carcinoma in Asian region. Improved quality of life and disease free survival is not achieved by current available therapies. It may be due to inaccurate molecular mechanism that defines genetic vulnerability to the disease. Among various risk factors oxidative damage pose great stress in development of breast malignancy. Oxidative stress is the accretion of reactive oxygen species (ROS) weakly encountered by antioxidant defense system. Oxidative stress plays a potential role in accelerating irregular cell multiplication. Amount of ROS that are generated in normal metabolic conditions are increased during disease condition. It affects normal function of signaling pathways, which in turns trigger abnormal cell division. Gene polymorphism of Paraoxonase-1 (PON1), an antioxidant enzyme has been focus of study in many life threatening diseases. The purpose of current study is to examine possible association of an antioxidant enzyme with the increased incidence rate of breast malignancy among local population. The correlation of PON1 and lipid peroxidation is measured in patients as compare to normal individuals. PON1 L55M genetic polymorphism was also observed. These findings deliberate the association of oxidative stress in the progression of BC that may contribute to develop better therapeutic strategies.

Keywords: Breast cancer – Gene Polymorphism –Lipid peroxidation–Oxidative stress – Paraoxonase-1– Tetra-ARMS PCR

INTRODUCTION

Globally in 2018, rate of cancer incidence and related deaths elevated up to 18.1 million and 9.6 million respectively.It is estimated that the frequency of the disease will rise to 29.5 million by 2040. Including all types of cancer, breast carcinoma is the most common cause of incidence (11.6%) and mortality (6.6%) among females worldwide. Discovery of precise biomarker is immediately required to identify novel diagnostic and therapeutic targets.Pakistan has 2.5 times highestrate of breast cancer as compare to their neighboring countries.Number of risk factors involved in the progression of disease including age, family history, hormonal and reproductive factors and many more. Including all of them oxidative stress play a key role in disease enhancemnts. Oxidative damage occurs when there is an imbalance between pro-oxidants and antioxidant enzyme system.Under normal physiological constraints reactive oxygen species (ROS) are produced, and their levels are elevated in disease conditions. Paraoxonase-1 (PON1) is a calcium-dependent antioxidant enzyme, secreted in blood stream and binds with high density lipoprotein (HDL) to perform its function. Approximately, 200 single nucleotide polymorphisms were identified in PON1 gene. Out of them eight are located in promoter

region of PON1 gene.Genetic polymorphism of PON1 L55Mhas been focus of study in many life threatening diseases and it is greatly linked with its enzyme activity and its concentration.The purpose of current study is to detect possible interrelation of an antioxidant enzyme with the increasedincidencerate of breast cancer among local population.

OBJECTIVES

- To determine PON1 L55M polymorphism in breast cancer patients and normal individuals.
- To asses activity of PON1 in serum samples of both disease and normal groups.
- To measure extent of lipid peroxidation as oxidative stress marker among Pakistani women.
- To rule out possible association between single nucleotide polymorphism PON1 L55M and oxidative stress.

METHODOLOGY

Blood samples were collected after taking IBC approval and informed consent from study participants. To determine PON1 L55M Single nucleotide polymorphism, Pallet-based extraction method was performed to extract DNA (Kleines et al., 2003, p.5273). These extracted DNA were then subjected to Tetra-ARMS PCR in which combination of four primers are used simultaneously. Two of them are non-allele specific while remaining are allele specific. Amplicon were then analyzed using Agarose gel electrophoresis. PON1 enzyme activities,paraoxonase and arylesterase were measured by providing substrates paraoxon and phenylacetate at 412nm and 270nm respectively, according to the protocol described earlier (Gan& Karen, 1991, p.100). Enzyme activity were measured using molar extinction coefficient after taking absorbance for 3 minutes time interval.To check extent of lipid peroxidation, MDA levels were measured at 532nm according to described protocol (Ohkawa et al., 1979, p.351)

RESULT AND CONCLUSIONS

From current study it is concluded that homozygous mutant M allele of PON1 L55M polymorphism has significant association with the disease as compare to healthy females that might be considered as potential risk factor for breast cancer progression. We also observed significant reduced activity of serum paraoxonase and arylesteraseenzymes in diseased samples. Our study proposed that PON1 may play protective role against breast malignancy.MDA levels are significantly higher inbreast cancer patients as compare to healthy control suggesting increased oxidative stress in cancerous patients.M allele was found significantly higher with concomitant decrease in PON1 activity. Findings of present study proposed that M allele is a risk allele associated with carcinogenesis with increased oxidative stress.

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