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Serum Adenosine Deaminase and Xanthine Oxidase in Breast Cancer Patients

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ABSTRACT

Introduction: *Breast cancer most commonly originates from the inner lining of milk ducts or the lobules. Diagnosing cancer at an early stage may help in offering best treatment and can reduce the morbidity and mortality. Adenosine deaminase (ADA, E.C.3.5.4.4) and xanthine oxidase (XO), are involved in nucleotide breakdown. A change in the activity of these enzymes can occur in malignant conditions. ADA is an enzyme that catalyses the hydrolytic deamination of adenosine. (XO) is an enzyme involved in the degradation of purines. Immunohistochemical localisation has shown its presence in various tissues, such as mammary glands, lung, spleen and liver. As XO produces reactive oxygen species (ROS) during the catabolism of purines, its excess activity can lead to increased oxidative stress, mutagenesis and possibly cancer.*

Objective: *To investigate serum ADA and XO activities in patients with biopsy-proven breast cancer.*

Materials and Methods: *This prospective cross-sectional study included 46 female patients (Mean age = 56±12 years) who were newly diagnosed (preoperative) with breast cancer.*

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Results: *Levels of serum ADA and XO proportionately increased with advancement of breast cancer.*
Conclusion: *We observed a significant increase in the levels of serum ADA and XO in breast cancer patients. Biochemical pathways associated with these markers might be potentially important in cancer initiation and progression.*

Running title– *Adenosine Deaminase , Xanthine Oxidase , Breast Cancer*

Introduction

Breast cancer most commonly originates from the inner lining of milk ducts or the lobules [1]. Most breast cancer are either invasive ductal carcinoma or invasive lobular carcinoma. Diagnosing cancer at an early stage may help in offering best treatment and can reduce the morbidity and mortality. Many serological, molecular or genetic markers have been proposed for breast cancer. Antigen 15-3, cancer antigen 27-29 and carcino embryonic antigen (CEA) is the most common tumour markers used for screening breast cancer but most lack specificity, sensitivity, prognostic value and clinical effectiveness[2]. So there is a need to develop a simple and cost -effective biochemical marker for breast cancer.

Adenosine deaminase (ADA, E.C.3.5.4.4) and xanthine oxidase (XO) are involved in nucleotide breakdown [3]. A change in the activity of these enzymes can occur in malignant conditions. ADA is an enzyme that catalyses the hydrolytic deamination of adenosine [4]. ADA in humans helps in the development and maintenance of the immune system particularly by helping in differentiation and proliferation of T-cells [5]. Its association has also been observed with epithelial cell differentiation, neurotransmission, and maintenance of gestation. [6]. Increased levels of serum ADA have been observed in various cancers such as liver cancer [7],and colorectal cancer [8]. XO is an enzyme involved in the degradation of purines. Immunohistochemical localisation has shown its presence in various tissues, such as mammary glands, lung, spleen and liver[9]. As XO produces reactive oxygen species (ROS) during the catabolism of purines, its excess activity can lead to increased oxidative stress, mutagenesis and possibly cancer[10]. Therefore, the aim of this study was to investigate serum ADA and XO activities in the serum of patients with biopsy proven breast cancer.

Materials and methods

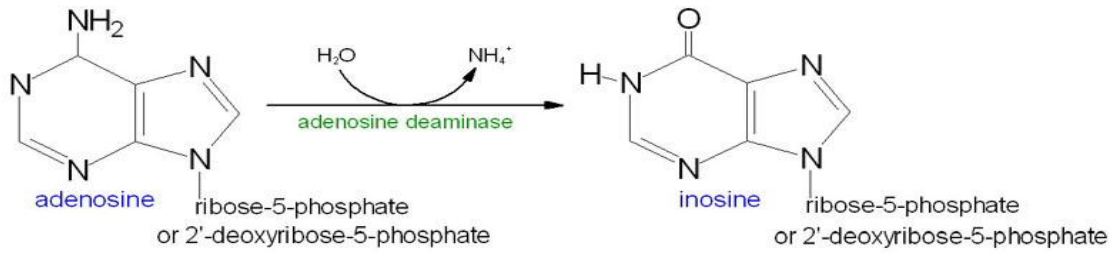
This prospective cross-sectional study included 46 female patients
(Mean Age =56±12 years) who were newly diagnosed (preoperative) with breast cancer between March 2014 and October 2014. All patients were non-smokers non-alcoholic and were not on any long term medications. None of the patients had any other serious medical or surgical Illness. Controls consisted of 46 healthy females randomly selected from a group of healthy non-smoking volunteers with no history of previous disease, drug or alcohol consumption.

Blood collection

Blood samples were collected from all the subjects into empty red capped vacutainer which was immediately kept at 4 °C. The serum was then separated and stored at – 20°C until use. Serum was used for the measurement of ADA and XO levels

Measurement of adenosine deaminase activity

Serum ADA activity was estimated spectrophotometrically by the method of Giusti, which is based on the direct measurement ammonia formation, which occurs when ADA is in the presence of excess adenosine [11]



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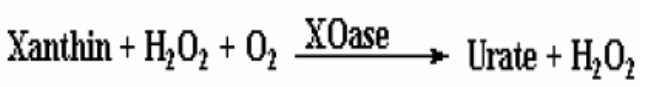
Measurement of xanthine oxidase activity

Xnathine oxidase (XO) activity was determined by the method of Ackermann.

This method depends on the enzymatic oxidation of xanthine which is followed spectrophotometrically by measuring uric acid formation at 293 nm [12]



Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) version 20. Statistical analysis of the obtained parameters in patients with breast cancer and control groups was done using Student's t- test. The mean and standard deviation (S.D) for each of the outcome were computed. The comparison between controls and various stages of breast cancer subjects was done by analysis of variance (ANOVA). A $p < 0.05$ was considered statistically significant.

Results

The mean (± SD) age of cases and controls were 56± 10 and 50 ± 6.0 respectively.

Table 1: Comparison of serum ADA and XO levels in controls and breast cancer patients

Characteristics	Controls (n=46)	Breast Cancer Patients (n=46)	*p - value
ADA (U/L) (Mean ± SD)	23.39 ± 2.21	41.45 ± 6.62	*P= 0.001
XO (U/L) (Mean ± SD)	21.82±4.34	39.26± 6.12	*P= 0.001

Student t-test*

Table 2: Comparision of serum ADA in different stages of breast cancer

Group	Number	Serum ADA(U/L) (Mean ± SD)	*p-value
Stage 2	16	39.75 ±7.1	0.332[a]
Stage 3	21	42.90 ± 6.70	0.776[b]
Stage 4	09	41.11 ± 5.3	0.875[c]

*ANOVA
(A) *P*- value Stage- 2 vs Stage 3- Not significant
(B) *P* -value Stage- 3 vs Stage 4 - Not significant
(C) *P*- value Stage- 4 vs Stage 2–Not significant

Table 3: Comparison of serum XO in different stages of breast cancer

Group	Number	Serum XO(U/L) (Mean ± SD)	*p-value
Stage 2	16	38.00 ±6.97	0.962[a]
Stage 3	21	38.52 ± 5.91	0.127[b]
Stage 4	09	43.22 ± 3.19	0.099[c]

*ANOVA
(A) *p*- value Stage 2 vs Stage 3 - Not significant
(B) *p*- value Stage 3 vs Stage 4 - Not significant
(C) *p*- value Stage 4 vs Stage 2 –Not significant

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Discussion

The present study showed that levels of serum ADA and XO level proportionately increased with advancement of breast cancer . This is in accordance with other studies viz. Chaudhari et al., [13], Pirincci [14], who found an increase in their levels in breast cancer patients. They had shown that because of local hypoxia and accelerated salvage pathway, solid tumours produce increased nucleotide metabolism leading to an increase in purine metabolizing enzyme ADA. This can provide a selective advantage to cancerous cells to grow and develop more rapidly . Further, adenosine inhibits the cell mediated anti- tumour immune response, that can promote tumour cell migration and angiogenesis leading to more proliferation of tumour cells. Thus, serum ADA activity is also increased in cancer cells.[15] . In our study a significant increase in serum ADA was observed in breast cancer patients as compared to controls.

Limitation: Serum ADA and XO are not specific markers for breast cancer and their activity may alter in cancer of other tissues . These markers may be used as an adjuvant tools in management of breast cancer provided other disease conditions which can alter ADA and XO are ruled out. Due to smaller sample size, results of this study cannot be generalised. Also, since XO is involved in purine catabolism, higher levels can occur due to increasing age and body mass index (BMI) of patient.

Conclusion: As we observed a significant increase in the levels of serum ADA and XO in breast cancer patients, pathways leading to these markers might be potentially important in cancer initiation and progression.

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Conflicts of interest

There are no conflicts of interest.

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