



## ORIGINAL RESEARCH PAPER

Biochemistry

**A STUDY OF SERUM ADENOSINE DEAMINASE AND GAMMA GLUTAMYL TRANSFERASE ACTIVITY IN PATIENTS WITH AND WITHOUT CARCINOMA BREAST**
**KEY WORDS:** ADA activity, GGT activity, CA breast

**DR.RASHMI RAJKAKATI**

ASSOCIATE PROFESSOR, DEPARTMENT OF BIOCHEMISTRY, ASSAM MEDICAL COLLEGE, DIBRUGARH, ASSAM

**DR.SARAJU MEDHI**

MD BIOCHEMISTRY, Assam Medical College, Dibrugarh.

**DR.REENA KOULI**

ASSOCIATE PROFESSOR, DEPARTMENT OF PATHOLOGY, ASSAM MEDICAL COLLEGE, DIBRUGARH, ASSAM.

**DR.DIPAK KUMAR SARMA**

ASSOCIATE PROFESSOR, DEPARTMENT OF SURGERY, ASSAM MEDICAL COLLEGE, DIBRUGARH, ASSAM

**DR.PRATIM GUPTA**

DEMONSTRATOR, DEPARTMENT OF BIOCHEMISTRY, ASSAM MEDICAL COLLEGE, DIBRUGARH, ASSAM

**ABSTRACT**

The study "Serum Adenosine Deaminase and Gamma Glutamyl Transferase Activity in Carcinoma Breast" in Assam Medical College and Hospital, Dibrugarh- a case-control study was an attempt to compare the differences between the activities of these enzymes in breast cancer patients to age matched healthy controls and also to find correlation between the activities of these two enzymes in breast carcinoma.

Serum Adenosine Deaminase and Gamma Glutamyl Transferase activities in Breast Carcinoma patients were comparatively higher than in age matched healthy controls. A positive correlation existed between activities of serum Adenosine Deaminase and Gamma Glutamyl Transferase and the p-value was extremely statistically significant ( $p < 0.001$ ).

**INTRODUCTION**

The situation of breast cancer in India is, in a certain sense, fairly typical of the situation in many countries of Asia and other regions where dramatic economic and social change is taking place. Breast cancer is the second most common cancer among women in India and accounts for 7% of global burden of breast cancer and one-fifth of all cancers among women in India. Over 50,000 women are estimated to die of it annually in India<sup>1</sup>. It is the most common cancer among women in urban areas of India. The incidence of breast cancer is approximately three times higher in urban areas compared to rural areas.

In the North-East region, Aizawl recorded maximum number of cases (30.3% in India) and Kamrup Urban district recording 22.8% (National Cancer Registry Programme, 2009-2011).

Total ADA activity has been studied in patients with various tumors. Some suggest that high ADA activities take an important part in the salvage pathway activity of cancerous tissues and cells<sup>2,3</sup>, while some suggest that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates due to accelerated purine and pyrimidine metabolism in the cancerous tissues.<sup>4</sup>

Gamma-glutamyltransferase (GGT) is involved in glutathione metabolism and its activity is increased in malignancies. In the past years, many studies have focused on the possible role of GGT in tumor progression and invasion. Elevated serum levels of GGT are seen in oxidative stress, due to factors including alcohol, heavy metals, cardiovascular disease and diabetes<sup>5</sup> and has shown similar results in carcinomas.

**AIMS AND OBJECTIVES**

To measure and compare serum Adenosine Deaminase and Gamma Glutamyl Transferase activity in clinically established and histopathologically confirmed cases of carcinoma breast, with that of healthy controls.

To find correlation, if any, between activities of serum Adenosine Deaminase and Gamma Glutamyl Transferase in clinically established and histopathologically confirmed cases of carcinoma

breast.

**BACKGROUND**

Adenosine Deaminase is an aminohydrolase which participates in the purine metabolism where it degrades either adenosine or 2'-deoxyadenosine producing inosine or 2'-deoxyinosine, respectively. Adenosine Deaminase is expressed in a variety of tissues, but in humans, lymphocytes have the highest activity of this cytoplasmic enzyme<sup>6</sup>.

The enzyme contains a parallel alpha/beta-barrel motif with eight central beta strands and eight peripheral alpha helices which is a common structure found in 1/10 of known enzymes; it also contains five additional helices<sup>7</sup>. The product of human ADA gene consists of 363 amino acids (41 kDa) and there is a high degree of amino acids sequence conservation amongst species.

ADA modulates ligand binding and signaling through A1R on DDT1MF-2 cells, a smooth muscle cell line<sup>8,9</sup>. ADA seems to be necessary for the high affinity binding of agonists to A1R<sup>10,11</sup>. Some studies have indicated presence of two distinct isoforms of ADA in humans, distinguished electrophoretically<sup>12</sup>.

Some authors suggest that high ADA activities play an important role in the salvage pathway<sup>3,14</sup> whereas others suggest that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates, adenosine<sup>15,16</sup>. There are many possible sources of adenosine in tumor cells including accelerated purine and pyrimidine metabolism, cell death and nucleotide degradation, ischemia and ATP breakdown, AMP release and hydrolysis of S-Adenosyl Homocysteine. Furthermore, high ADA activity is against the high toxicity of deoxyadenosine and its derivatives dAMP, dADP and dATP, which are potent inhibitors of nucleic acid biosynthesis<sup>17</sup>. High ADA activity in malignant tumors may give selective advantage to the cancer cells via production of high amounts of hypoxanthine, substrate of hypoxanthine guanine phosphoribosyl transferase (HGPRT). It is a key enzyme for the salvage pathway<sup>18</sup>.

**Adenosine deaminase and breast cancer.**

With respect to breast cancer there are only a few reports. Canbol

et al investigated the activity of ADA in malignant breast tissues irrespective of oestrogen receptor status and found a higher activity of the enzyme when compared to the non-cancer ones<sup>19</sup>.

Concerning the relationship between ADA expression and oestrogen receptor, there is only one study so far, that has been reported by Xie et al<sup>20</sup>. They demonstrated the induction of ADA mRNA by oestradiol in MCF7 human breast cancer cell lines. In one of the preliminary studies performed on a small number of breast cancer patients, the activity of adenosine deaminase was significantly increased in the serum<sup>21</sup>.

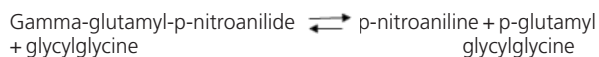
Majoomdar M et al (2004) in his study also found progressive increase in ADA activity when interstage comparison was done<sup>22</sup>. Mohammad Hashemi et al (2005) along with his coworkers found significant increase in activity of ADA in estrogen receptor positive (ER+) cell line (MCF-7) than that of estrogen receptor negative breast cancer cell line (MDA-MB468)<sup>23</sup>. In another study, Mahmoud Aghaei et al (2010) along with his coworkers found that raised activity for total ADA and ADA2 in the serum and tumor of Benign Breast Diseases and breast cancer when compared to healthy controls.<sup>24</sup>

**Gamma Glutamyl Transferase<sup>25</sup>**

Peptidases are enzymes that catalyze the hydrolytic cleavage of peptides to form aminoacids or smaller peptides. They constitute a broad group of enzymes of varied specificity, and some individual enzymes act as amino acid transferases and catalyze the transfer of aminoacids from one peptide to another aminoacid or peptide.

Gamma glutamyl transferase( EC 2.3.2.2) catalyzes the transfer of gamma glutamyl group from peptides and compounds to an acceptor. The gamma glutamyl acceptor is the substrate itself, some amino acid or peptide, or even water, in which case simple hydrolysis takes place. The enzyme acts only on peptides or peptide like compounds containing a terminal glutamate residue joined to the remainder of the compound through the terminal carboxyl. Glycylglycine is five times more effective as an acceptor than is glycine or the tetrapeptide (gly-gly-gly), but little is known about the optimal properties of the acceptor cosubstrate. The peptidase transfer reaction is considerably faster than the simple hydrolysis reaction.

GGT



Substrate (donor) (acceptor) (donor residue) (transfer product)

GGT is present in proximal renal tubule, liver, pancreas and intestine. The enzyme is present in cytoplasm (microsomes), but the larger fraction is located in the cell membrane and may transport aminoacid and peptides into the cell across the cell membrane in the form of gamma glutamyl peptides. GGT is critical for the maintenance of adequate intracellular levels of reduced glutathione, a major antioxidant agent. GGT activity in serum comes primarily from liver. The enzyme in serum is heterogenous with respect to both net molecular charge and size.

**GGT and breast cancer**

.Experimental work has documented that active enzyme is present in atherosclerotic plaques, and this appears related to the ability of GGT to mediate redox/pro-oxidant reactions at a cellular level.

A series of 283 438 first attendants at the Vienna General Hospital gave blood for GGT analysis and were followed up for upto 13 years to determine all-cause mortality (Kazemi-Shirazi et al,2007). For both males and females with elevated serum GGT, there was significantly increased mortality from all causes, cardiovascular disease, hepatobiliary disease and cancer. Mortality risk was particularly increased for those aged <30 years<sup>26</sup>. Recently, Strasak et al (2008) examined cancer incidence in relation to GGT levels

among the 92 983 females participating in the Vorarlberg study<sup>27</sup>. After a median follow-up of 13.5 years, 4884 cancer cases were diagnosed. The normal low level of GGT was taken as 17.99 IU/L and, compared with this, there was a highly significant increase in hazard ratio (HR) for cancer incidence with increasing levels of GGT, so that among those with levels 472 IU/L, HR was 1.43 (95% confidence interval (CI): 1.28–1.61). In terms of site specificity, elevated GGT was associated with cancers of the digestive tract, respiratory tract, breast/female genital organs and haematopoetic system.

**MATERIALS AND METHODS**

The present study was carried out on diagnosed cases of breast cancer who attended and/or was admitted in Department of Surgery, Assam Medical College and Hospital, Dibrugarh.

**SOURCE OF DATA** : The patients were selected from OPD and indoor ward of the department of Surgery, Assam Medical College and Hospital, Dibrugarh from May,2015-April 2016.

**ETHICAL CLEARANCE** : Clearance certificate from the Institutional Human Ethics Committee of AMCH was obtained prior to the commencement of the study.

**CRITERIA FOR SELECTION OF CASES:**

**INCLUSION CRITERIA:**

All the clinically established and histopathologically confirmed cases of carcinoma breast coming to AMCH, Dibrugarh.

**EXCLUSION CRITERIA:**

1. Patients who refused to give consent
2. Known cases of Tuberculosis, Rheumatic fever, Hemolytic anemia, Jaundice, Hepatobiliary disease, Alcoholic liver disease, Bone disease, Pancreatic disease, CCF,MI, Ulcerative colitis, Kidney disease, Other malignancies and
3. Patients of breast carcinoma who had already received chemotherapy/radiotherapy.

**Determination of Serum Adenosine Deaminase Activity<sup>28,29</sup>**

**Method:** Colorimetric Method

**Principle:**

Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.

ADA

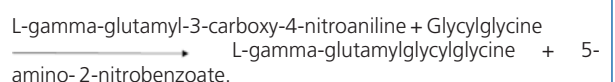


**Determination of Serum Gamma Glutamyl Transferase Activity**

**Method:** Carboxy Substrate Method

**Principle:** The GGT catalyses the transfer of amino group between L-gamma-glutamyl-3-carboxy-4-nitroanilide and glycylglycine to form L-glutamylglycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is measured as an increase in absorbance which is proportional to the GGT activity in the sample.

GGT



**RESULTS AND OBSERVATIONS**

The present study included 50 cases of breast carcinoma patients and 50 age matched healthy controls. The results and observations detected in the participants are recorded and analyzed statistically using "Pearson Correlation" and "Student's t test". The findings of the tests are as follows:

**DISTRIBUTION OF CASES AND CONTROLS ON THE BASIS OF AGE**

**TABLE 1.1**

Age Group	Case		Control	
	No.	%	No.	%
>30	2	4	2	4
30-39	10	20	10	20
40-49	21	42	21	42
50-59	7	14	7	14
≥ 60	10	20	10	20
Total	50	100.0	50	100.0

MEAN, S.D. AND SIGNIFICANCE IN THE MEAN SERUM ADENOSINE DEAMINASE ACTIVITY (U/L) BETWEEN STUDY GROUPS.

**TABLE 1.2**

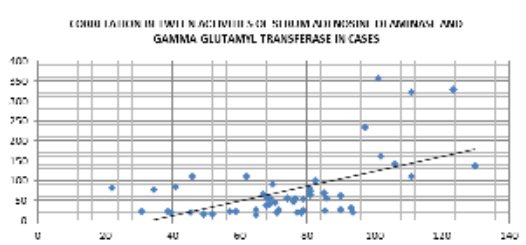
Study Group	Range	Mean ± SD	P- values
Case	22.01 - 130	74.07 ± 23.37	<0.0001
Control	12 - 29.43	20.84 ± 5.83	

MEAN, S.D. AND SIGNIFICANCE IN THE MEAN SERUM GAMMA GLUTAMYL TRANSFERASE ACTIVITY BETWEEN STUDY GROUPS.

**TABLE 1.3**

Study Group	Range	Mean ± SD	P- value
Case	14 - 358	75.46 ± 79.72	<0.0001
Control	9.98 - 41.99	24.67 ± 8.50	

**FIGURE-1.4: CORRELATION BETWEEN ACTIVITIES OFSERUM ADENOSINE DEAMINASE AND GAMMA GLUTAMYL TRANSFERASE IN CASES**



From the above mentioned table and figure, we find that there is positive correlation between the activities of serum Adenosine deaminase and Gamma glutamyl transferase in breast cancer patients with  $r = 0.55$  and the  $p$ -value was less than 0.0001 which was found to be extremely statistically significant.

**DISCUSSION AND CONCLUSION**  
**ADENOSINE DEAMINASE ACTIVITY IN BREAST CANCER**

In our study, the mean of serum Adenosine deaminase activity of the breast cancer patients are higher than the healthy controls, (74.07 ± 23.37 in cases and 20.84 ± 5.83 in controls respectively) with  $p$ -value <0.0001 which was found to be extremely statistically significant. Borzencko BG et al found elevated ADA activities in breast cancer patients on interstage comparison<sup>31</sup> Walia M et al found similar results<sup>21</sup>. Canbolat et al found raised ADA activities in breast cancer patients when compared to normal subjects<sup>19</sup>. Mazoomdar M et al (2004) also found raised ADA activities in breast cancer patients on interstage comparison<sup>22</sup>.

In 2005, Mohammad Hashemi, Fatemeh Karami Tehrani, Saeid Ghavami and Majid Sirati Sabet et al in their study "Adenosine deaminase activity in estrogen receptor positive and negative human breast cancer cell lines" found that the activity of enzyme in estrogen receptor positive (ER+) cell line (MCF-7) was

significantly higher than that of estrogen receptor negative breast cancer cell line (MDA-MB468)<sup>23</sup>.

In 2010, Mahmoud Aghaei, Fatemeh Karami-Tehrani, Siamak Salami, and Morteza Atri et al in their study "Diagnostic Value of Adenosine Deaminase Activity in Benign and Malignant Breast Tumors" found that the mean values for total ADA and ADA2 activities in the serum and tumor of Benign Breast Diseases were significantly higher than those of healthy controls ( $p < 0.01$ ). Furthermore, the mean values for total ADA and ADA2 activities of patients with breast cancer were significantly higher than those of the benign group ( $p < 0.005$ ) and healthy subjects ( $p < 0.0001$ )<sup>24</sup>.

Our finding was in agreement with the findings of the workers stated above in respect to ADA activities.

**GAMMA GLUTAMYL TRANSFERASE ACTIVITY IN BREAST CANCER**

In our study, the mean and standard deviation of serum Gamma glutamyl transferase activity of the breast cancer patients are higher than the healthy controls, (75.46 ± 79.72 in cases and 24.67 ± 8.50 in controls respectively) with  $p$ -value <0.0001 which was found to be extremely statistically significant.

In 2010, I S Fentiman and D S Allen et al in their study "Gamma Glutamyl Transferase and breast cancer risk" also found similar results. After adjustment for age at entry, height, weight, age at menarche and first birth with nulliparity, there was a highly significant relationship between elevated GGT and breast cancer risk. In the highest quartile, the hazard ratio (HR) was 2.17 (95% confidence interval (CI): 1.19, 3.93). When subdivided by menopausal status, there was a reduced non-significant effect in postmenopausal women, whereas for premenopausal women in the highest quartile, HR was 3.81 (95% CI: 1.37, 10.59). Premenopausal women with serum GGT levels above the normal range had a significantly elevated HR of 4.90 (95% CI: 1.86, 12.94)<sup>32</sup>.

Our finding was in agreement with the finding of the authors stated above.

To conclude measurement of these two important enzymes in serum of breast carcinoma patients was helpful. These may help in the early detection of breast cancer and so these can be better biomarkers. Also these tests are easy to be done even in small laboratories, these tests are cheaper so that the general population can afford these tests.

Our findings are in accordance with the findings of the previous studies. However, as we are constrained by the limitation of time and relatively smaller sample size, it would probably be more predictive with larger sample size and longer period of study to explore more deep into this area.

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