



Original Research Article

Evaluation of serum adenosine deaminase and gamma glutamyl transferase in cancer cervix -A low-cost diagnostic tool

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ABSTRACT

Introduction: Cancer cervix is one of the leading causes of death in women worldwide. It is a slow progressive carcinoma and if detected early mortality can be reduced. Most cases occur in low economic women and in developing countries. So, if a low-cost diagnostic tool is utilised it can be detected early.

Aims and Objectives: To estimate serum adenosine deaminase and gamma glutamyl transferase activity in carcinoma cervix patient and in healthy controls so that it can be used as a supportive diagnostic tool.

Materials and Methods: The present cross sectional study was conducted on 30 patients of clinically diagnosed cervical cancer in the age group of 30-70 years and 30 age matched controls. Serum Adenosine Deaminase and Serum Gamma Glutamyl Transferase was determined using Galanti and Giusti Method and Carboxy substrate method respectively. Student's 't' test was used to compare the results.

Results: A significant increase in adenosine deaminase and Serum Gamma Glutamyl Transferase were observed in cervical cancer patients when compared to controls with a p value of less than 0.0001.

Conclusion: By present study we conclude that serum adenosine deaminase and gamma glutamyl transferase may be used for diagnostic purposes as support parameters and may be included further for finding the prognosis. Such biochemical parameters are cheap, rapid, easy to test and can be effectively analysed even in the smaller laboratories which have not been exposed to any advanced technology.

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1. Introduction

Cervical cancer is a neoplasia of malignancy that originates from cells of the cervix.¹ Cervical cancer was the fourth most common cancer among women worldwide, listed following breast cancer, colorectal cancer and lung cancer.² An estimated 570 000 women were diagnosed with cervical cancer worldwide in 2018 and the global mortality figures for the first time rose to 3,00,000 women, and those are going to grow particularly in marginalized and underprivileged communities.² The reported cases of new cancer cervix are 500,000 a year, 79 per cent of which occur in developing countries.³ Together, China and India contributed more than 33% of the global cervical burden, with 97 000 cases and 60 000 deaths in India.² Uterine cervix carcinoma is a major health problem facing Indian

women and around 120,000 women develop this disease every year.⁴ India accounts for 15.2 per cent of the total cervical cancer deaths in the world.⁵ Current data suggests that 90% of all cases occur in low- and middle-income countries, due largely to poor access to screening and early detection and treatment of both pre-cancers and cancer.⁶ Nearly all cases of cervical cancer (99 per cent) are associated with high-risk human papillomavirus (HPV) infection, an extremely infectious virus transmitted via sexual contact. Cervical cancer steadily progresses from intraepithelial cervical neoplasia to invasive cancer.¹ As suggested by the evidences, carcinoma cervix is preventable and highly suitable for primary prevention.⁷

An increase in activity of enzymes in serum is indicative of cell damage or over production in a tissue rich in enzyme concerned. In necrotic conditions, cell destruction leads to appearance of both mitochondrial and cell sac enzymes in the serum. Increased enzymes in systemic circulation is due

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to change in the membrane permeability or tumor necrosis of cancer cells. In these cells enzymes are in much high concentration.⁸

Adenosine deaminase is an ectoenzyme found in human tissue, mainly in lymphoid tissue. Cellular activation to detoxify toxic metabolites for energy demand is a major cause for increased ADA activity. Tissues of solid cancers produce increased levels of purine nucleoside adenosine because of local hypoxia.⁹ Tumor cell migration, proliferation and angiogenesis is promoted by adenosine and cell mediated anti-tumor response is inhibited by adenosine. Increased ADA activity can detoxify high amount of toxic adenosine and deoxyadenosine substrates which are produced in cancerous tissues due to accelerated purine metabolisms. It can also provide substrate to cancer cells to accelerate salvage pathway.¹⁰ Gamma glutamyl transferase is a microsomal enzyme containing glycoprotein. L-amino acid accepts gamma glutamyl group from gamma glutamyl peptide in presence of GGT.¹¹ With alteration of normal epithelial environment, there is occurrence of GGT activity. Tumor formation involves indirect role of GGT such that GGT is involved in the various stages of cervical cancer, specifically in transformation of cervical intraepithelial neoplasia to invasive cancer. GGT and oxidative stress form part of a biological pathway linked to cancer incident development.¹²

In low-resource domains, facilities for screening asymptomatic women are not readily available and diagnostic methods for detection are accessible in well-equipped centres with present-day technology which are expensive. Considering this the present study was undertaken for providing some of the promising enzyme markers namely Adenosine deaminase (ADA), Gamma glutamyl transferase (GTT) which are inexpensive, accurate, identified by easy method of detection and validated that may support the diagnosis and prognosis of the disease.

2. Materials and Methods

This study was conducted after obtaining clearance from Institutional Ethical Committee. The present cross-sectional study was conducted on patients of clinically diagnosed cervical cancer who visited hospital OPD. Study comprised of about 30 healthy females in age group 30-70 years as controls and about 30 clinically and histopathologically confirmed cases of cervical cancer of the same age group. Patients suffering from Hepatocellular damage, Pancreatic disease, Renal failure, Diabetes Mellitus, Tuberculosis and other malignancies were excluded. All cases were evaluated and selected by simple random technique after fulfilling the selection criteria. Clinically and histopathologically confirmed cases of cervical cancer reported to Department of Obstetrics and Gynaecology were screened. After finding

the suitability as per inclusion and exclusion criteria they were requested to participate in the study and briefed about the nature of the study, the interventions used and written informed consent was obtained. The patients who gave consent were enrolled in the present study. Participants data such as name, age, sex, comprehensive history were collected by interviewing participants and reported on pre structured proforma. About 5 ml of venous blood was collected from antecubital vein under aseptic precautionary measures using sterile disposable syringe. The collected blood was allowed to clot and serum was separated by centrifugation and stored at 4°C. The estimation of parameters were carried out immediately. The following methods were used for the assay of enzymes:

1. Serum Adenosine Deaminase (ADA) : Galanti and Giusti Method.¹³
2. Serum Gamma Glutamyl Transferase (γ GT) : Carboxy substrate method.¹⁴

Estimation of adenosine deaminase in serum by Galanti and Giusti method

Principle: Adenosine deaminase hydrolyses adenosine to ammonia and to inosine. Within an alkaline medium the ammonia produced further reacts with phenol and hypochlorite to form a blue indophenol complex with catalyst acting sodium nitroprusside. The intensity of the colored indophenol complex produced in blue is directly proportional to the sample amount of ADA.

Estimation of gamma glutamyl transferase in serum by carboxy substrate methods

Principle: Gamma glutamyl transferase catalyzes the transfer of amino group between L γ -Glutamyl- 3-carboxy-4 nitroanilide and glycylglycine to form L- γ - glutamyl glycyl glycine and 5- amino-2- nitrobenzoate. The rate of 5- amino-2-nitrobenzoate formation is calculated as an increase in absorbance proportional to the GGT level in the sample. Serum Adenosine deaminase and serum Gamma glutamyl transferase will be estimated by Semi-auto analyser.

2.1. Statistical analysis

Statistical analysis of all the obtained parameters in patients with cervical cancer and control groups were done using student's 't' test. The mean and standard deviation (S.D.) for each of the outcome was computed.

3. Results

The present study comprises of 60 participants, 30 healthy controls and 30 cases of cervical cancer, which was confirmed by clinical and histopathological examination. The mean age for cases of cervical cancer was 50.63 ± 5.51 and for controls was 46.83 ± 4.3 with $p=0.0042$. Hence age is matched in two groups.

Table 1: ADA and γ GT of controls and cervical cancer patients

Enzymes	Controls		Cases		'p' value
	Mean	S.D.	Mean	S.D.	
ADA (U/L)	23.2	3.96	53.21	14.5	<0.0001
γ GT (U/L)	18.66	8.89	60.97	15.35	<0.0001

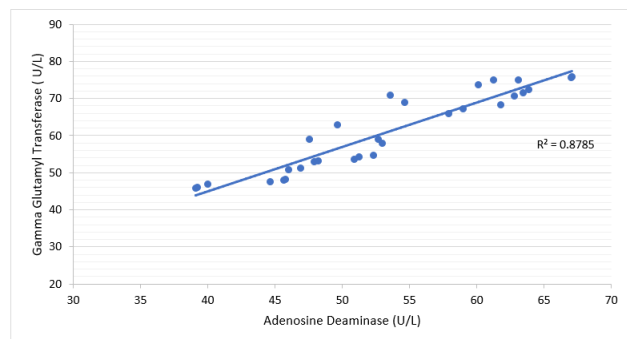
**Fig. 1:** Correlation between adenosine deaminase and Gamma Glutamyl transferase in cervical Carcinoma

Table 1 shows mean adenosine deaminase level in cases clinically diagnosed with cervical cancer and in controls. A highly significant increase in serum ADA was observed in cervical cancer patients as compared to controls with p value less than 0.0001.

Table 1 shows the mean serum Gamma Glutamyl Transferase level in cases clinically diagnosed with cervical cancer and in controls. A significant increase in γ GT was observed in cervical cancer patients when compared to controls with a p value of less than 0.0001. As the level of γ GT increases in cases clinically diagnosed with cervical cancer, there is seen an increase in the levels of ADA too at a constant rate of $y = mx + c$ with r value=0.87.(Figure 1)

4. Discussion

In the present study we estimated serum adenosine deaminase and gamma glutamyl transferase activity in carcinoma cervix patient and in healthy controls and also assessed the reliability of serum adenosine deaminase and gamma glutamyl transferase in carcinoma cervix patients as supportive parameters for diagnostic purpose.

In present study, a significant increase in Adenosine deaminase was observed in cervical cancer patients as compared to controls. Results are in accordance to a study done by Borzenko BG on serum of oncologic patients. They studied activity of adenosine and thymidine metabolism enzymes in serum of oncologic patients. They concluded that, due to local hypoxia, there is production of increased levels of purine nucleoside adenosine deaminase in tissues of solid cancers. Adenosine prevents anti-tumor response mediated by cells, facilitates tumor cell migration, and angiogenesis, and induces tumor cell proliferation. Due

to accelerated purine metabolism in cancerous tissue, there is increased production of toxic adenosine and deoxyadenosine substrates. In order to detoxify adenosine and deoxyadenosine substrates, there is increased serum ADA activity.¹⁵ Speechia G and co investigators reported that lymphocyte ADA activity in patients with gynecologic malignancies shows a broad range of variability.¹⁶

Naidu SK and co-researchers conducted a study that showed upsurge lipid per oxidation in cervical cancer patients which is a consequence of increased free radical generation. Due to this there is profound alterations in function of cell membrane and also structural disorganization of DNA leading to mutations.¹⁷

According to our study, serum gamma glutamyl transferase level was significantly increased in cervical cancer patients when compared to controls. An interstage comparison showed a non-significant increase in serum γ GT level from stage I to stage III. Our study is in accordance with the study conducted by Malkin A and Co-investigators, they studied serum γ GT along with carcino embryonic antigen, pregnancy associated macroglobulin and placental alkaline phosphatase in patients with ovarian and cervical cancer and concluded that, the frequency of γ GT positivity increased with more advanced disease in cervical cancer and γ GT reflected tumor burden in carcinoma of the cervix.¹⁸

Similar results were also documented by de Camargo and co-investigators in their study, the data showed γ GT positive cells in cervical smears of patients with inflammatory or paraneoplastic or neoplastic conditions of the cervix. They concluded that cytochemically detectable transpeptidase activity appears whenever alterations of the normal epithelial microenvironment occur.¹⁹

In developing countries women with higher risk of cervical cancer are women with low socioeconomic status. Poor survival rate of cancer in developing countries, mostly due to late diagnostic stage and limited access to timely and standard treatment.²⁰ To reduce the incidence of cervical cancer in our country, we must spread awareness and educate the population about the importance of cervical screening. Due to lack of awareness, cervical cancer screening is not done in early stages, 70-80% of the patients are diagnosed at advanced stages (stage III and stage IV) with poor long-term survival.²¹

Being the leading cause of cancer associated death in women of our country, successful cervical cancer screening programs have been difficult to implement at low source setting because of many factors. Foremost is

the expense of maintaining the infrastructure and technical expertise required for cytological screening. Hence, as an alternative low-cost rapid screening technology like estimating the levels of ADA and γ GT in cervical cancer should be introduced. Cytology based screening programs for cervical cancer cannot be provided on a large scale in developing countries because they are based on transportation, communication, follow up and training.

5. Conclusions

By present study we conclude that serum adenosine deaminase and gamma glutamyl transferase may be used as supportive parameters for diagnostic purpose and may add further for prognostic information. Such biochemical parameters are cheap, rapid, easy to test and can be effectively analyzed even in the smaller laboratories which have not been exposed to any advanced technology.

However study on lipid peroxidation is required for correlating with release of membrane associated enzyme ADA into the circulation. Further studies on a larger sample with longer follow up are needed to substantiate our findings before firm conclusions can be drawn on the utility of these enzymes for the diagnosis and assessment of progression of cervical cancer.

6. Source of Funding

None.

7. Conflict of Interest

None.

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