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ORIGINAL ARTICLE

# The Diagnostic And Prognostic Value Of Serum Adenosine Deaminase Levels In Head And Neck Cancer

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**Abstract**

Serum adenosine deaminase (ADA) levels were estimated in 63 patients with histologically proven squamous cell carcinoma of the head and neck region of the body in different stages. Serum ADA levels were also estimated in 30 healthy controls. Serum ADA levels in cases (34.89 ± 6.80 IU/L) was significantly increased when compared to the control group (20.47 ± 3.33 I U/L). There was a highly significant correlation between the serum ADA level and the increasing disease stage (severity of the disease). The tumour status and metastasis of the tumour to the neck nodes has shown a correlation with serum ADA levels. After the treatment of head and neck cancers by different modalities, the serum ADA levels were found to be decreased (24.74 ± 3.91) when compared to the serum ADA activity before treatment (34.89 ± 6.80 IU/L).

**Key words:** Adenosine deaminase, Head and neck cancer

**Key messages:** Serum Adenosine deaminase levels are found to be increased in head and neck cancers. ADA levels can be used as an additional tool for diagnosis of head and neck cancer. It can also be used for the follow-up of the treated cases. There is a scope for further study of serum ADA levels and its usefulness in the diagnosis and follow-up of head and neck cancer in a larger population.

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## Introduction

Head and neck cancer is one of the most common cancers in the world, accounting for up to 30 to 40% of malignancies in India [1]. Oral cancer has become the fourth reason for cancer death in males in Taiwan [2]. Tobacco smoking (or chewing), alcohol consumption and betel quid chewing are shown to be independent risk factors for oral, pharyngeal and oesophageal cancers [3,4].

Adenosine deaminase (ADA: 3.5.4.4) is a cytosolic enzyme which catalyzes the hydrolytic deamination of adenosine to form inosine and 2' deoxy adenosine to 2' deoxy inosine, respectively. The physiological function of ADA is critical in controlling the effects of these metabolites on immunological,

neurological and vascular systems. ADA is also involved in the development of B and T lymphocytes, as is evident from the fact that ADA deficient animals suffer from B and T lymphopaenia [5].

The levels of enzymes in T-lymphocytes vary according to cellular differentiation [6]. The activity of the ADA enzyme is subjected to changes, depending upon the degree of activity of the cell [7]. The evidence of high ADA activity during rapid and stimulated growth of normal tissues is of importance in making a fully functional purine salvage pathway possible [8]. An increased serum ADA level is associated with oesophagus tumours [9], liver cancer [10], breast cancer [11] and colorectal cancer [12]. In addition, ADA is the most sensitive marker for tuberculosis [13].

The present study was designed to evaluate the diagnostic and prognostic importance of ADA activity in head and neck cancer, and to evaluate its usefulness as a possible marker of head and neck cancer progression.

## Material and methods

The present study was conducted in the department of otorhinolarygology and Department of Biochemistry, after obtaining clearance from the hospital ethics committee.

**Cases**

The study group consisted of 63 patients with histologically proven squamous cell carcinoma of the head and neck region of the body in different stages, who had not taken any prior treatment. Care was taken to exclude patients with head and neck cancer, who had already taken the treatment, and those patients with tuberculosis. Cancer staging had been done according to the TNM classification of head and neck cancer [14].

**Controls**

The control group consisted of 30 age and sex matched normal healthy individuals who came to the hospital for the health checkup.

**Sample collection**

Blood samples were collected from controls and patients by using aseptic precautions, after obtaining their consent. They were immediately processed to obtain serum for the estimation of serum ADA level. The ADA level in serum was assayed by the colorimetric method of Giusti [15], in which adenosine is used as substrate, and the ammonia liberated by the action of ADA on adenosine is measured as blue indophenol. Serum ADA activity was expressed as IU/L (1 IU/L is defined as one micromole of ammonia formed per minute per liter of serum).

Patients with head and neck cancer were treated with different modalities (Radiotherapy, Chemo-radiation, surgery). One week after the treatment of head and neck cancer, with the consent of the patient, once again, a venous blood sample was collected and the serum ADA level was estimated.

**Statistical analysis**

Serum ADA level between the controls and cases was compared by t-test. Serum ADA activity in different stages of cancer was compared by analysis of variance (ANOVA). Serum ADA activity was compared between different tumour status and nodal status by the Bonferrine test. Serum ADA activity in cases before and one week after the treatment was compared by t-test.

**Results and discussion**

There was statistically significant increase in the ADA level in cases (34.89 ± 6.80 IU/L) when compared to control group (20.47 ± 3.33 I U/L) [Table/Fig 1].

**Table/Fig: 1 Serum ADA levels (U/L) in controls and cases**

| | Mean | Std. Deviation | N | t - test |
|---|---|---|---|---|
| Adenosine deaminase levels in | | | | |
| Controls | 20.47 | 3.33 | 30 | t(91)=10.977 p<0.001 |
| Cases | 34.89 | 6.80 | 63 | |

Lal et al [16] reported that mean value of ADA was significantly higher in cases, compared to controls. Our findings were consistent with that of Lal's study. Walia M, Mahajan M and Singh K [17] have reported that serum adenosine deaminase is a better parameter for the detection of breast cancer, and the assessment of the development of various stages of cancer.

According to the staging of the head and neck cancer done by considering the tumour status and nodal status, 18 of the patients were in stage IV, 16 in stage III, 17 in stage II, and 12 were in stage I. The majority of the patients studied, were in stage IV.

Serum ADA level was compared between the different stages of the disease by analysis of variance (ANOVA) [Table/Fig 2].

**Table/Fig 2: Serum ADA level (IU/L) before treatment at various stages of disease.**

| Stage | Mean | Std. Deviation | N | |
|---|---|---|---|---|
| I | 27.32 | 2.54 | 12 | F(3.59)=45.352 P<0.001 |
| II | 31.18 | 1.39 | 17 | |
| III | 35.86 | 4.61 | 16 | |
| IV | 42.58 | 5.12 | 18 | |

There was a statistically significant increase in the serum ADA level as the disease stage progressed from stage I to stage IV disease. Serum ADA level was more in cases with stage IV disease, when compared to patients with stage III disease. Significant increase was also found between stage II disease and stage III disease. Serum ADA level was more in cases with stage II disease, as compared to stage I. This was statistically analyzed by pair wise comparisons (Bonferrine test) [Table/Fig 3]

**Table/Fig 3: Comparison of serum ADA level (IU/L) between different stages of head and neck cancer**

| Stage (I) | Stage (J) | Mean difference (I – J) | Std Error | p |
|---|---|---|---|---|
| I | II | -3.857 | 1.447 | >0.059 Not significant |
| | III | -8.544 | 1.465 | 0.001 |
| | IV | -15.264 | 1.430 | 0.001 |
| II | III | -4.686 | 1.336 | 0.005 |
| | IV | -11.407 | 1.296 | 0.001 |
| III | IV | -6.720 | 1.318 | 0.001 |

Serum ADA level was compared between different tumour status [Table/Fig 4]. Serum ADA level appeared to be increased as the tumour status progressed from T1 through T3. There is a statistically significant correlation between the serum ADA level and the tumour status when pair wise comparison (Bonferroni test) was done [Table/Fig 5].

**Table/Fig 4: Serum ADA level (IU/L) according to the tumour status, before treatment**

| T Stage | Mean | Std. deviation | N | |
|---|---|---|---|---|
| T1 | 28.55 | 3.89 | 16 | F=(2.60)= 24.28 P<0.001 |
| T2 | 34.24 | 5.50 | 25 | |
| T3 | 40.25 | 5.48 | 22 | |

**Table/Fig 5: Comparison of serum ADA level (IU/L) between different tumour statuses**

| (I) | (J) | Mean difference (I – J) | Std Error | P |
|---|---|---|---|---|
| T1 | T2 | -5.688 | 1.646 | 0.003 |
| | T3 | - 11.694 | 1.690 | 0.001 |
| T2 | T3 | - 6.006 | 1.503 | 0.001 |

Several studies suggest that there is increase in the activity of purine salvage enzymes including ADA, as the adenocarcinoma of the colon becomes more invasive [18, 19]. ADA activity was highest at the mucosa adjacent to the carcinoma of the colon. The ADA synthesis is increased in tissues surrounding cancer, and it has got a role in progression and invasion of colon cancer [20] .

Serum ADA activity was compared between different nodal status [Table/Fig 6].

**Table/Fig: 6 ADA level (IU/L) according to Nodal status, before treatment**

| N Stage | Mean | Std. deviation | N | |
|---|---|---|---|---|
| N0 | 30.37 | 3.48 | 35 | F=(2.60)= 910.761 p=0.001 |
| N1 | 36.89 | 4.59 | 10 | |
| N2 | 42.58 | 5.12 | 18 | |

**Table/Fig 7: Comparison of serum ADA level (IU/L) between different nodal statuses**

| (I) | (J) | Mean difference (I – J) | Std Error | p |
|---|---|---|---|---|
| N0 | N1 | -6.519 | 1.500 | 0.003 |
| | N2 | - 12.217 | 1.213 | 0.001 |
| N1 | N2 | -5.699 | 1.650 | 0.001 |

There was a statistically significant correlation between different nodal status and the serum ADA activity, as determined by the pair wise comparison (Bonferroni test) [Table/Fig 7]. The main physiological activity of ADA is found in T-lymphocytes, and is related to lymphocytic proliferation. Cell mediated immune response particularly mediated by the lymphocytes have been shown to be important in patients with transitional cell carcinoma of the bladder. A fully functioning cell mediated immune response is partly dependant on the purine salvage enzyme, ADA [21].

Serum ADA is sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation [22]. The activity of ADA is increased in very rapidly growing malignancies, while slow growing, well differentiated tumours, do not express pronounced ADA activity [23, 24]. The treatment of colon carcinoma cells with deoxycoformine, an ADA inhibitor, resulted in inhibition of cell growth [25, 26]. This shows that ADA plays a metabolic role in supporting a rapid growth of tissues by reutilization of nucleotides which are required for the RNA and DNA synthesis.

Serum ADA levels showed a significant increase in cases, when compared to the control group. Significant increase in serum ADA levels was related to the nodal status and the tumour status. There was a correlation between different stages of head and neck malignancy and serum ADA levels. The increase in the serum ADA levels may be a result of the lymphoid proliferation in the metastatic lymph nodes or

leakage of the enzyme from the primary tumour cells.

The serum ADA is not a specific marker of head and neck malignancy. Its activity is increased even in cancer of other tissues like colon, bladder, breast, ovary, oesophagus, liver [20, 21, 27, 28, 9, 10], and also in tuberculosis [13] and leprosy [29]. Serum ADA levels can be used as a diagnostic tool in head and neck cancer, in addition to other investigative procedures, provided different disease conditions that show raise in serum ADA activity should be ruled out.

Following treatment by different modalities, 91.7% of those with stage I disease remained in the same stage, and in 3.8% of those who underwent surgery, there was no recurrence of tumour. All the patients in stage II downstaged to stage I. Among the patients with stage III, 50% downstaged to stage II, and 12.5% to stage I. 12.5% who underwent surgery, had no recurrence, and the other 18.7% remained in the same stage. 6.3% of the patients advanced to stage IV. Among the patients with stage IV, 50% have downstaged to stage III, and the other 50% remained in the same stage

Serum ADA levels were estimated in cases, one week after the treatment of head and neck malignancies, and it was compared with the pretreatment serum ADA activity. There was a statistically significant decrease in the serum ADA levels, one week after the treatment, when compared to the pretreatment serum ADA levels [Table/Fig 8].

**Table/Fig 8: ADA level (IU/L) before and after the treatment of head and neck cancer**

| | Mean | Std. Deviation | N | t - test |
|---|---|---|---|---|
| Adenosine deaminase before treatment | 34.90 | 6.80 | 63 | t(62)=14.182 p=0.001 |
| Adenosine deaminase after the treatment | 24.74 | 3.91 | 63 | |

Serum ADA activity was declined following treatment of head and neck malignancies. Nishihara [30] has reported decrease in the serum adenosine deaminase levels in cases with lung cancer, following surgery and radiotherapy, by nearly 85%. The decrease correlated well with the decrease in tumour mass and improvement in the patient's clinical condition. Hence serum ADA levels also can be used to assess the prognosis of head and neck malignancies.

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