Original Research Article

Study of serum adenosine deaminase levels in head and neck malignancies

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ABSTRACT

Background: Head and neck malignancies are the malignancies occurring at various sites like scalp, ear, nose and paranasal sinuses, nasopharynx, oral cavity, oropharynx, hypopharynx, larynx, and salivary glands. There are various biomarkers which aid in early diagnosis and management of head and neck malignancies. In order to study various parameters aiding in early diagnosis, this study has been taken up to estimate serum adenosine deaminase levels among these patients.
Methods: Eighty-eight clinically diagnosed head and neck malignancy patients of both genders were included in this study on simple random basis. After obtaining the written informed consent from the patient, a peripheral venous sample of blood was taken and serum ADA levels were estimated. Data was analyzed using IBM SPSS Version 22.
Results: Serum ADA levels are found to be statistically significant with respect to various stages of head and neck malignancies including malignancy of unknown origin. Mean ADA level in this study was 30.33 IU/l. Mean ADA levels in MUO, primary tumor with neck secondaries were 37 IU/l, 38.7 IU/l respectively. Mean ADA levels in patients with primary tumor without neck secondaries was 27.9 IU/l.
Conclusions: The Serum ADA levels among head and neck malignancy patients including MUO is a simple, inexpensive diagnostic biomarker enzyme which is easy and rapid to estimate. This study emphasizes on using Serum ADA levels as a prognostic indicator among head and neck malignancies who are on various modalities of treatment.

Keywords: Adenosine deaminase, Malignancy, Diagnostic, Biomarker

INTRODUCTION

Head and neck malignancies are the malignancies occurring at various sites like scalp, ear, nose and paranasal sinuses, nasopharynx, oral cavity, oropharynx, hypopharynx, larynx, and salivary glands.[1] Age greater than 55, male gender, low socioeconomic status, smoking and alcohol are some of the predisposing factors which are responsible for its high incidence.[2] The most common tumor markers for head and neck squamous cell carcinoma are serum SCCA (squamous cell carcinoma antigen), CYFRA 21-1 (cytokeratin fragment 21.1) and

CEA (carcinoembryonic antigen) whose diagnostic sensitivity at an early stage still requires improvement.[3]

Adenosine deaminase (ADA) is an enzyme which is involved in purine salvage pathway, catalyzes the hydrolytic deamination of adenosine to form inosine and 20-deoxyadenosine to 20-deoxyionosine respectively.[4,5] It helps in the development and maintenance of the immune system particularly in the process of differentiation and proliferation of T cells.[6] It is associated with epithelial cell differentiation, neurotransmission, and gestation maintenance as well.[7] Neoplastic states cause the release of cytoplasmic enzymes. The degree of membrane

damage is reflected by the concentration of the enzymes released. Increase in the level of serum ADA has been observed in various cancers in other parts of the body also.[8-10]

There are various biomarkers which aid in early diagnosis and management of head and neck malignancies. In order to study various parameters aiding in early diagnosis, this study has been taken up to estimate serum ADA levels among these patients.

METHODS

Current study is a prospective study conducted for a period of two years from June 2018 to May 2020 in the department of otorhinolaryngology head and neck surgery, VIMS, Ballari, Karnataka, India. The patients were selected on simple random basis after taking informed written consent from the patients. Eighty-eight clinically diagnosed patients of head and neck malignancies of all age groups and both gender attending the outpatient and/or as inpatient in the department of otorhinolaryngology and head and neck surgery were included in this study.

Objectives of this study

Objectives of current study were to determine the serum ADA levels in all clinically diagnosed cases of head and neck malignancies among all age groups and both genders population.

Inclusion criteria

Inclusion criterion for current study was all cases that are diagnosed of head and neck malignancies of all age groups and of both genders are included in this study.

Exclusion criteria

Exclusion criteria for current study were patients of head and neck malignancies who were on any modality of treatment for it and head and neck malignancy patients who were associated with chronic granulomatous diseases.

A detailed history taking and clinical examination was done to the patients. These patients were subjected to radiological investigations and cytopathology for the information and were included in the study. After taking written and informed consent a peripheral venous sample was drawn for the estimation of serum ADA levels.

Adenosine deaminase level estimation

Adenosine deaminase levels were estimated by using calorimetric method of Galantine and Guisti, using ADA-MTB Microxpress Tulip diagnostics kit. Adenosine deaminase hydrolyses adenosine to ammonia and inosine. A blue indophenol complex is produced when the formed ammonia in an alkaline medium reacts with phenol and hypochlorite with catalyst being sodium nitroprusside. The intensity of this blue indophenol complex is directly proportional to the ADA levels in the sample. The results were expressed in IU/l.[11]

Statistical analysis

Data was collected by using a structured proforma. Data entered in MS excel sheet was analyzed by using IBM SPSS Version 22. Qualitative data was expressed in terms of frequency and percentage. Mean and SD value was calculated for continuous variables. Mean values between the groups were analysed by using ANOVA with Turkey's Post Hoe multiple comparision. $p < 0.05$ was considered as statistically significant whereas a $p < 0.001$ was considered as highly significant.

RESULTS

Among 88 (n) patients who were included in the study, 70 (79.5%) patients were males and 18 (20.5%) patients were females. Male to female ratio was 3.9:1 (Figure 1). Mean age was 54.74 years. Minimum age was 26 years and maximum was 85 years. Those aging <40 years were 8 (9.1%) patients, in the range of 40-49 years were 21 (23.9%) patients, 50-59 years were 21 (23.9%) patients, 60-69 years were 28 (31.8%) patients, >70years were 10 (11.4%) patients (Figure 2). The number of patients presenting with difficulty in swallowing were 16 (18.2%) patients, swelling in the site of lesion were 16 (18.2%) patients, swelling in the neck were 5 (17%) patients, noisy breathing were 13 (14.8%) patients, pain while swallowing were 6 (6.8%) patients, change in voice were 8 (9.1%) patients, throat pain were 6 (6.8%) patients, swelling over the face were 4 (4.5%) patients, pain in the ear were 3 (3.4%) patients, blocked nose and bleeding from nose was 1 (1.1%) patients (Figure 3).
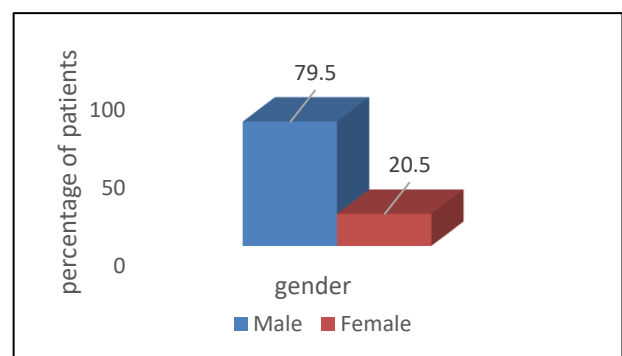

Figure 1: Distribution of patients according to their gender.

Among 88 (n) patients, 32 (36.4%) patients were smokers, 28 (31.8%) patients were both smokers and alcoholic, 13 (14.8%) patients chewed tobacco, 4 (4.5%) patients were smokers and chewed tobacco, 1 (1.1%) patient was both alcoholic and chewed tobacco, 10 (11.4%) patients did not have any habits (Figure 4).

Among 88 (n) patients, 48 (54.5%) patients presented with symptoms whose duration was between 1-6 months, 27 (30.7%) patients with <1 month, 7 (8%) patients with 7-12 months, 6 (6.8%) patients with >12 months (Figure 5). In this study (n=88), 67 (76.1%) patients had primary tumor without neck secondaries, 13 (14.8%) patients had primary tumor with neck secondaries and 8 (9.1%) patients were malignancy of unknown origin (MUO) (Figure 6).
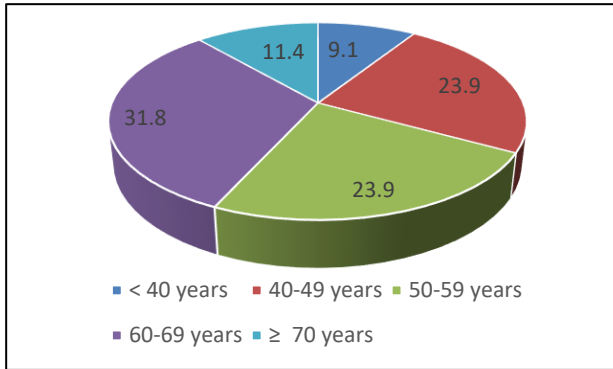

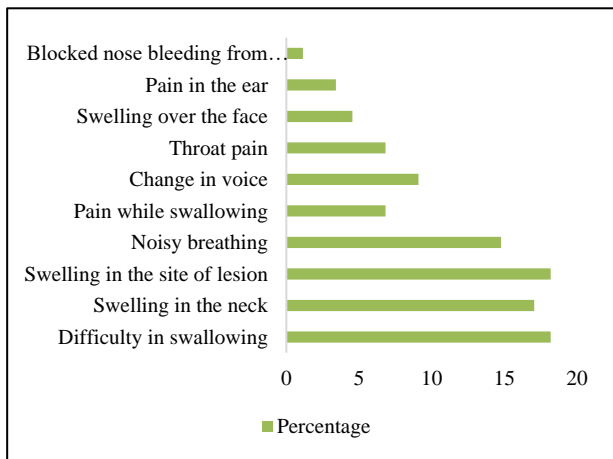**Figure 2: Distribution of patients according to their age.**

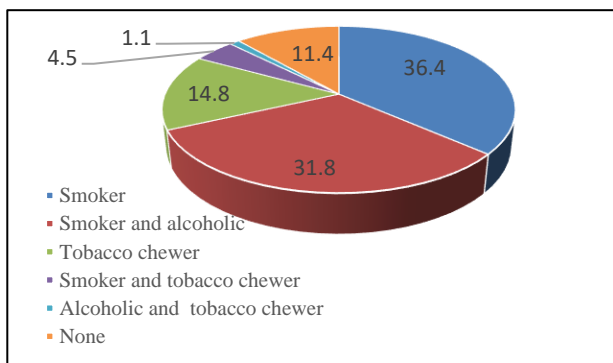**Figure 3: Distribution of patients according to their symptoms.**

Figure 4: Distribution of the patients according to their personal habits.
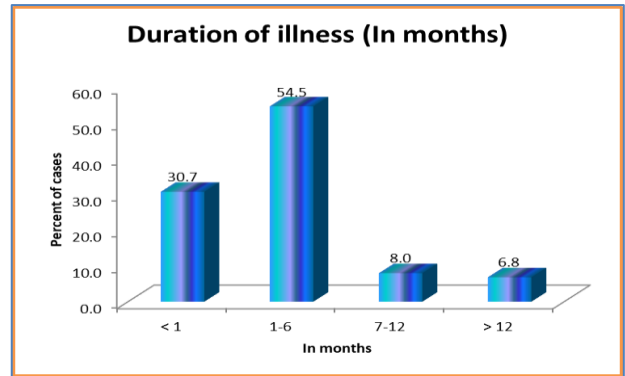

Figure 5: Distribution of the patients according to their symptom duration.
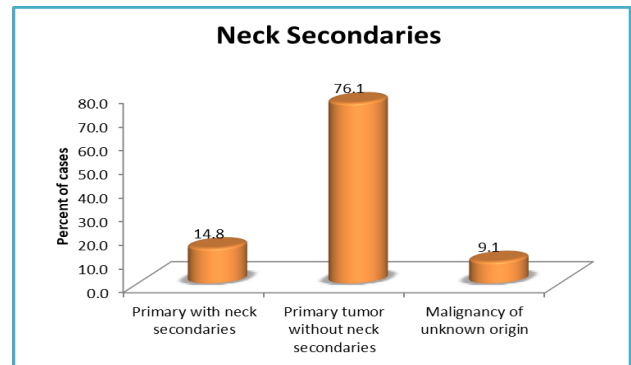

Figure 6: Distribution of the patients according to their neck secondaries.

In this study the mean ADA levels is 30.33 IU/l, minimum value being 3.50 IU/l and maximum value being 98.6 IU/l. The mean ADA levels in primary tumor with neck secondaries, malignancy of unknown origin (MUO) was 38.7 IU/l, 37 IU/l respectively. Mean ADA levels in patients with primary tumor without neck secondaries was 27.9 IU/l. The correlation between mean ADA levels and primary tumor with neck secondaries, malignancy of unknown origin and primary tumor without neck secondaries were highly statistically significant (Figure 7). In this study (n=88) 12 (13.63%) patients were in stage I, 31 (35.22%) patients in stage II, 27 (30.68%) patients in stage III and 9 (10.22%) patients in stage IV of the disease. Mean ADA levels in stage I, II, III and IV were found to be 23.88 IU/l, 27.68 IU/l, 30.50 IU/l and 41.88 IU/l respectively, which are statistically significant for respective stage of the malignancy. Comparatively, stages I to stage IV ADA levels in this study show a trend of increase in the levels and are found to be statistically significant (Figure 8). An interstage comparision of mean ADA levels was done using Turkey Post Hoc Multiple comparision and it is concluded that the interstage comparision of mean ADA levels between stage I and stage II is statistically highly significant. Interstage comparision of mean ADA levels between stage II and stage IV is also statistically significant (Table 1).
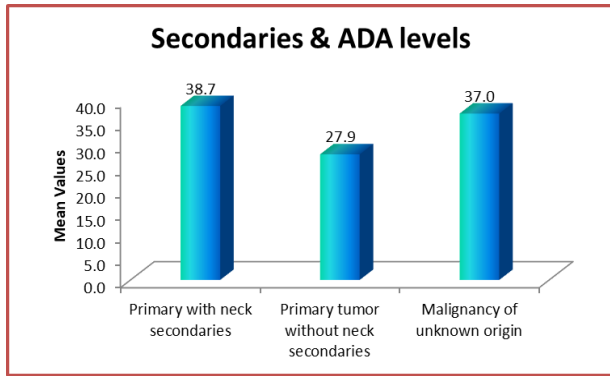
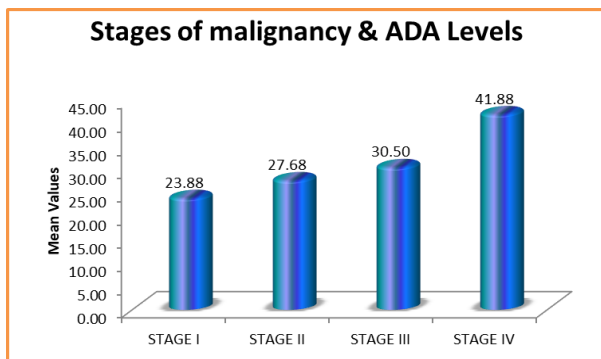**Figure 7: Comparison of mean ADA levels with the neck secondaries; ANOVA p<0.008 highly significant.**

Figure 8: Comparison of mean ADA levels in various stages of head and neck malignancies; ANOVA p<0.01 significant.

Table 1: Interstage comparison of mean ADA levels in various stages (Tukey's Post Hoc Multiple comparison).

Stages	p value	Significance
I vs. IV	p<0.009	Highly Significant
II vs. IV	p<0.01	Significant

DISCUSSION

The genes that regulate the fundamental pathways in cell function are mutated resulting in malignancies and uncontrolled growth of tumor cells.[12] Malignancies causes active proliferation signaling, evades growth suppressing mechanisms, resists apoptosis, permits replicative everlasting status, induces angiogenesis and enables metastasis. These are the 6 hallmarks of malignancy.[13] In association with CD 26 with dipeptidyl peptidase IV activity, ADA exists on the cell surface and intracellularly as well, without own transmembrane domain.[14] Solid tumors release adenosine due to adenine nucleotide degradation which is caused by severe hypoxia and necrosis which in turn is caused by aggressive growth of the tumor.[15] ADA enzyme prevents the accumulation of toxic metabolites in rapidly proliferating cells.[16] It is stated that in the aggressively proliferating malignant tissues, ADA plays a pivot role in the salvage pathway activity and there is an increase in ADA activity resulting in the production of more nucleotides thus helps in maintaining DNA synthesis in these cells.[17] Among the healthy individuals, the levels of serum ADA level is found to be 18-22 IU/l.[18] Serum enzymes can be used as a prognostic indicator as they reflect the tumor burden and are also used for detecting the recurrences and monitoring the therapeutic effects.[18] The aggressive nature of the malignancy is denoted by elevated enzyme levels, unchanged or increasing enzyme levels indicate lack of response and following effective treatment there is a decrease or and to the normal levels.[19]

Hence this study is emphasized on a simple method of estimating a serum biomarker which is feasible, easily available and cost effective.

In our study there was male predominance which correlates with a study conducted by Simard et al who found that there was two to five fold greater risk for head and neck malignancies in males when compared to females.[20] In our study the most common age group involved was of 60-69 years. This result was consistent with a study conducted by Vigneswaran et al.[21] In our study tobacco smoking was found to be most prevalent risk factor followed by combined use of tobacco smoking and alcohol. This was consistent with the study conducted by Alam et al.[22] However, there were 10 (11.4%) patients in our study who had no risk habit yet developed malignancy. Most of the patients had symptom duration of 1-6 months indicating the early onset and rapid progression of the disease. Most of these patients presented with rapid onset of symptoms hence can be explained about the presenting with no neck secondaries. However, there were 8 (9.1%) patients presenting with malignancy of unknown origin (MUO) which can also be a mode of presentation. This result was similar to the study conducted by Waltomen et al who found that MUO consisted of 8.1% of study population.[23]

In our study the mean ADA levels was 30.33 IU/l. The ADA level was found to be higher in malignancy of unknown origin and patients with neck secondaries hence indicating the rise in the levels with the spread of malignancy. A study was conducted by Ashok et al in 2008 found that there was a highly significant correlation between the serum ADA level and the increasing disease stage (severity of the disease), the tumor status and metastasis of the tumor to the neck nodes. They concluded that Serum ADA levels can be used as one of the diagnostic tools in head and neck cancer.[24]

In our study most of the patients presented in stage II and stage III of malignancy and there is statistically significant (p<0.01) increasing trend in the level of serum ADA levels as the disease progressed. Serum ADA level was more in stage IV of the disease when compared to stage III and serum ADA level was more in stage II cases, as compared to stage I cases. A study by Lal et al

proved that there was a rise in serum ADA activity with the increasing stage of the cancer indicating that it was directly proportional to the primary tumor mass and also reported that the rise in serum ADA levels were more in ulcerative growth than the proliferative growth.[25] In our study we found no statistical significance (p>0.716) between the serum ADA levels and the duration of the symptoms presentation, hence we conclude that the rise in serum ADA level was independent of the symptom duration. A similar study conducted by Mishra et al came to the conclusion that the rise in serum ADA level did not depend on the duration of the disease and was directly proportional to the stage of the disease. The study also concluded that the activity of ADA reduced with radiotherapy and after surgery.[26] A study by Sharma et al showed that serum ADA level was significantly high in histologically proven cases of laryngeal cancers and the ADA level changed with respect to the severity of the disease.[27] In a study conducted by Chandrakiran et al the serum ADA levels were quantified in patients diagnosed with oropharyngeal, hypopharyngeal and laryngeal malignancies, immediately after diagnosis and 3 months after completion of treatment was there was a significant fall in ADA levels following treatment.[28]

In our study we found no statistical significance (p>0.667) in the serum ADA levels in both male and female patients and hence we conclude that serum ADA levels were not dependent on the gender of the patient. A study was conducted by Rai et al in 2011 estimated the adenosine deaminase level in saliva in patients of squamous cell carcinoma of tongue concluded that the levels of ADA in saliva was more in male patients than female patients. The concentration of enzyme increased along with the increasing stages of the disease in both genders.[29]

A study was conducted by Canbolt et al suggested that the expression of serum ADA as well as superoxide dismutase is more in patients with laryngeal carcinoma as compared to healthy people and it was noted that the levels of ADA and superoxide dismutase in serum did not differ before and after the surgical removal of tumor, suggesting that the enzyme leaks may occur not only from primary tissues but also from adjacent tissues and/or malignant cells.[30] It can also be reasoned out that the high levels of serum enzymes after the surgical removal might be due to the half life of these previously released enzymes whose high enzyme activity persists for more than a month.[30] A study was conducted by Dhankhar et al in 2011 studied regarding various enzymes which included serum ADA level and found that levels were significantly higher in patients of head and neck cancers as compared to the levels in controls.[31] A study by Sharma et al proved that serum ADA can be used as a diagnostic parameter in cases of head and neck malignancies.[32]

CONCLUSION

The serum ADA levels among head and neck malignancy patients including MUO is a simple, inexpensive diagnostic biomarker enzyme which is easy and rapid to estimate. This study emphasizes on using serum ADA levels as a prognostic indicator among head and neck malignancies who are on various modalities of treatment.

ACKNOWLEDGEMENTS

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