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## ORIGINAL ARTICLE

# Adenosine deaminase and 5′-nucleotidase activities in saliva from patients with oral and laryngeal cancer

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**OBJECTIVE: The aim of this study was to evaluate saliva's activities of adenosine deaminase (ADA) and 5′-nucleotidase (5′-NT) enzymes and their utility as diagnostic and therapeutic markers in oral and laryngeal cancer.**

**MATERIALS AND METHODS: Pre- and post-operative saliva's activities of ADA and 5′-NT enzymes were measured in patients with squamous cell oral (*n* = 10) and laryngeal cancer (*n* = 17) and compared with control saliva samples (*n* = 19).**

**RESULTS: The ADA was found to be lower in saliva of the patients with oral cancer compared with the laryngeal cancer and controls. However, no significant differences were found between pre- and post-operative values for both enzymes in the patient groups. We also could not find statistically significant differences between saliva's activities of 5′-NT in patients and control subjects.**

**CONCLUSIONS: Low activity of ADA observed in saliva of the patients with oral cancer has been suggested as a compensatory mechanism against rapid purine and DNA metabolism in cancer cells. The current study does not support the hypothesis that saliva's activities of these enzymes may be used as additional diagnostic and prognostic cancer markers.**

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**Keywords:** adenosine deaminase; 5′-nucleotidase; oral and laryngeal cancer; saliva

## Introduction

Saliva is an essential fluid required for maintenance of the ecological balance in the oral cavity. Saliva has been widely used to study a variety of molecules and biochemical substances. Biochemical methods have also been used to define enzymatic activities involved in various phases of the cell cycle, such an in DNA synthesis.

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Adenosine deaminase (ADA; EC 3.5.4.4) and 5′-nucleotidase (5′-NT; EC 3.1.3.5) are important enzymes participating in purine and DNA metabolisms. ADA is an enzyme in purine salvage pathway and catalyses the irreversible conversion of either adenosine and/or deoxyadenosine to inosine and deoxyinosine, respectively. Defects in this enzyme often results in an intracellular accumulation of the substrates of ADA, namely adenosine and deoxyadenosine. These substrates are very toxic to the living cells (Lizuka *et al*, 1981). Therefore, detoxication of adenosine and deoxyadenosine is important. Several mechanisms have been suggested for their toxicity that deoxyadenosine causes dATP accumulation, which is a strong inhibitor of ribonucleotide reductase and cause some aberrations in DNA synthesis (Donofrio *et al*, 1978). In fact, deoxyadenosine inactivates S-adenosyl homocystein hydrolase, inhibition of which causes interference with critical methylation-dependent processes such as synthesis, maturation or function of DNA (Hersfield and Kredich, 1980). An increase in adenosine results in an accumulation of 5′-nucleotides (primarily 3,5-cyclic AMP; Meisel *et al*, 1979). In several studies, ADA activities were found increased in cancerous tissues and cells (Sufrin *et al*, 1978; Camici *et al*, 1990; Durak *et al*, 1994; Öztürk *et al*, 1998). However, some found low ADA activities in cancer (Dasmahapatra *et al*, 1986; Durak *et al*, 1993).

The 5′-NT is another enzyme functioning in nucleotide metabolism. This enzyme generates nucleosides from various types of nucleotides and is recognized to be a plasma membrane-bound enzyme of mammalian cells. Similarly, 5′-NT activities were found increased (Öztürk *et al*, 1998), unchanged (Durak *et al*, 1994) or decreased (Camici *et al*, 1990; Durak *et al*, 1993) in some cancerous tissue and cell systems. Decreased activity was evaluated as an attempt of the cancer cell to preserve the mononucleotide pool (Camici *et al*, 1990), and increased activity was mostly evaluated as an attempt to supply salvage pathway activity (Dornand *et al*, 1982).

There is however, no report on saliva's activities of ADA and 5′-NT enzymes in patients with oral and laryngeal cancer. The aim of this study was to investigate saliva's activities of ADA and 5′-NT enzymes before and after surgical removal of oral and laryngeal

cancer and to compare the results with those of the control subjects. The objective was to assess whether saliva's activities of these enzymes might be useful as diagnostic and therapeutic markers.

## Materials and methods

Present study included patients who had oral cancer (mean age of 51.8 years, range: 36–73), laryngeal cancer (mean age of 49.4 years, range: 43–73) and healthy subjects (mean age of 56.1 years, range: 38–84). Saliva samples were obtained from 10 patients with oral cancer and 17 patients with laryngeal cancer before and 1 month after the surgical operation and from 19 healthy volunteer subjects. All patients had histologically confirmed squamous cell oral and laryngeal cancer. None of the patients had been treated with additional radiotherapy or chemotherapy for any residual tumour. All patients were staged by the (T: Extent of Primary Tumour; N: Condition of Regional Lymph Nodes; M: Presence of Distant Metastasis) TNM classification [American Joint Committee on Cancer (AJCC)]. All patients were in stage I. Patients in poor general condition were excluded from this study. All cancer patients were smokers for about 10 years and none of them was alcoholic. They were asked not to eat or drink for 2 h before the specimen collection. Moreover, the patients were required to stop any medications and to refrain from cigarette smoking for at least 12–24 h before the study. Unstimulated whole mixed saliva samples were collected after the mouth had been rinsed thoroughly with distilled water. The subjects were asked to drool into a clean 50-ml container. The samples were pipetted into a sterile 20-ml plastic tubes and preserved for 3 months at −20°C. ADA and 5′-NT activities were measured as previously described (Guisti, 1974; Donald, 1986). Results were expressed in IU $ml^{-1}$ and given as mean ± s.d. Statistical analyses were performed using the SPSS statistical software package. The results were evaluated statistically by using Kruskal–Wallis variance analysis with statistical significance being accepted at 5%.

## Results

Mean saliva ADA and 5′-NT activities of the patients and control groups are given in Table 1. There are statistically significant differences between mean ADA enzyme activities in oral cancer, laryngeal cancer and controls. ADA activities are decreased in saliva of the patients with oral cancer compared with laryngeal cancer and control values ($P < 0.05$). There were no significant differences between pre- and post-operative saliva's ADA activities for the patients ($P > 0.05$). As to the 5′-NT activities, there were however, no statistically significant differences between control and any of the patient groups ($P > 0.05$).

## Discussion

Saliva is a readily available specimen, which can be collected by non-invasive procedures and contains many hormones, drugs and antibodies of interest in screening and diagnosis (Brandtzaeg, 1989). Several methods have been described for the collection of saliva, i.e. whole mouth resting or stimulated saliva, or individual salivary gland's secretions. Methodological differences in both the collection and storage of saliva specimens might affect the contents of saliva. Therefore, in this study we used whole mouth unstimulated saliva and stored at −20°C.

It has long been known that cancer development follows a series of biochemical changes. Although there are several reports on serum and tissue ADA activities in the diagnosis and prognosis of cancer, contradictory results were reported (Sufrin *et al*, 1978; Öztürk *et al*, 1998).

Canbolat *et al* (1994) had reported that serum ADA levels were higher in laryngeal cancer than in control subjects. They found that no significant differences between pre- and post-operative serum enzyme activities for the patients. Lal *et al* (1987) demonstrated that serum ADA activities were higher in patients with head and neck cancer when compared with control subjects. However, they had also reported that serum ADA levels were decreased in patients with head and neck cancer following radiotherapy. Similar results were also obtained by Nishihara *et al* (1970). In another study, Sufrin *et al* (1978) had reported that serum ADA levels were significantly reduced in patients with lung cancer following surgery. They suggested that increased serum ADA levels might be a result of the leakage of the enzyme from the primary tumour or from metastases and, after surgery or radiotherapy, enzymes leakage was eliminated. To the contrary, in a study carried out by Durak *et al* (1993) it was found that ADA and 5′-NT activities were depressed in cancerous laryngeal tissues. Dasmahapatra *et al* (1986) also found low lymphocyte ADA activities in head and neck cancers. These researchers suggested that low lymphocyte ADA activities might be a more sensitive indicator of suppressed cellular immunity.

Similarly, we found that saliva's activities of ADA in patients with oral cancer were lower than those of laryngeal cancer and control group. Low ADA activity observed in this study may be a compensatory mechanism

**Table 1** Mean ± s.d. values of ADA, 5′-NT activities

| *Group* | *ADA (IU $ml^{-1}$)* | *5′-NT (IU $ml^{-1}$)* |
|---|---|---|
| (A) Control group ($n = 19$) | 0.0785 ± 0.07371 | 0.0265 ± 0.0203 |
| (B) Oral cancer, preoperative ($n = 10$) | 0.0249 ± 0.0111 | 0.0309 ± 0.0233 |
| (C) Oral cancer, postoperative ($n = 10$) | 0.0870 ± 0.0942 | 0.0100 ± 0.0124 |
| (D) Laryngeal cancer, preoperative ($n = 17$) | 0.0318 ± 0.0129 | 0.0482 ± 0.0394 |
| (E) Laryngeal cancer, postoperative ($n = 17$) | 0.0612 ± 0.0666 | 0.0248 ± 0.0210 |

(A) *vs* (B): $P < 0.05$; (B) *vs* (D): $P < 0.05$. All others are not significant.
ADA, adenosine deaminase; 5′-NT, 5′-nucleotidase.

against rapid purine and DNA metabolism in cancer cells. It has been suggested that decreased ADA activities cause higher dATP and dAMP concentrations in the cell, thereby lowering dATP/dAMP ratio and leading to decreased energy production (Schmalsteig *et al*, 1977). This may be another limiting factor acting against rapid proliferation of cancer cells.

Lal *et al* (1987) demonstrated that the rise in serum ADA activity was related to the stage of cancer. Sufrin *et al* (1978) also reported a significant association between an increase in lymphocyte ADA and the stage of tumour in patients with transitional cell carcinoma of the bladder. In this study, all patients had stage I squamous cell carcinoma. Therefore, the effects of the stage of tumour on activity of enzymes were not evaluated.

The controversial outcomes in the studies of enzymes might depend on the histological type of tumour, stage and therapy of cancer, the methods employed for material collection and the methods of analysis which are used (Sufrin *et al*, 1978; Durak *et al*, 1994; Nurkka *et al*, 2003). The enzyme metabolism in cancer cells might show greater differences depending on cancerous tissues studied, and the underlying mechanisms might be specific for each cancer. However, these different findings could result from the carcinogenesis process itself, but personal habits such as alcohol use, smoking, etc. might be also involved in the event (Durak *et al*, 1993). Unfortunately, we could not investigate possible effects of smoking due to lack of information of smoking status of control subjects.

While, some studies revealed that 5′-NT activities were higher in some types of cancerous tissues while lower in others (Dornand *et al*, 1982; Camici *et al*, 1990; Durak *et al*, 1993, 1994), we could not find statistically significant differences between saliva's activities of 5′-NT in patients with oral and laryngeal cancer and control subjects. These different results might arise from the fact that analyses were carried out in different organs and tissues. 5′-NT activities in salivas of patients with oral and laryngeal cancer were higher compared with control subjects. However, increases in enzyme activities were generally not statistically significant. This finding showed that 5′-NT activity was not a limiting factor in accelerated purine metabolism of oral and laryngeal cancer. Similar results were also obtained by Durak *et al* (1994).

In conclusion, although ADA activities in saliva of patients with oral cancer were established as lower than that of the controls and laryngeal cancer before surgical removal of tumour tissue, in our opinion it is very difficult to put forward the hypothesis that saliva activities of this enzyme may be used as additional diagnostic and prognostic cancer markers as proposed by several other researchers (Sufrin *et al*, 1978; Dasmahapatra *et al*, 1986; Öztürk *et al*, 1998).

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