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ORIGINAL ARTICLE

Adenosine deaminase in saliva as a diagnostic marker of squamous cell carcinoma of tongue

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Abstract Tongue cancer is amongst the most common and fatal types of cancers in the world. The abnormalities in purine metabolism are characteristic features of many human tumors. Little is known about the correlation between the activities of key enzymes of purine nucleotide pathway and clinical indicators of tongue cancer invasiveness and aggressiveness. Fifty patients (M: F 25:25; mean age: 55.6 years (range 45-60; SD 1.8)) with diagnosed squamous cell carcinoma of the tongue (test group) and 30 normal subjects (M: F 15:15) without any systemic disease (control group) were recruited after obtaining informed consent. All patients were staged by the TNM classification. Salivary adenosine deaminase (ADA) activity was assessed in cancerous patients (test group) and normal healthy subjects (control group). Statistically significant differences between test and control groups were observed in salivary ADA ($P<0.001$). Furthermore, serum ADA levels significantly increased as the disease stage progressed from stage I to stage III of squamous cell carcinoma of the tongue in both genders ($P<0.001$). Salivary ADA might be used as a diagnostic tool for early detection of squamous cell carcinoma of tongue.

Keywords Saliva · Adenosine deaminase · Squamous cell carcinoma · Tongue cancer · Staging of cancer · Diagnostic marker

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Introduction

Adenosine deaminase (ADA) is an important enzyme participating in purine and DNA metabolism [1]. In the purine salvage pathway, it catalyzes the irreversible conversion of either adenosine or deoxyadenosine to inosine and deoxyinosine. Defects in this enzyme often result in an intracellular accumulation of substrates of adenosine deaminase, namely, adenosine and deoxyadenosine. These substrates are very toxic to living cells [2]. It has been suggested that deoxyadenosine toxicity causes dATP(2′-deoxyadenosine 5′-triphosphate) accumulation. The latter is a strong inhibitor of ribonucleotide reductase, causing some aberrations in DNA synthesis [3]. There is some ongoing debate as to whether it has been reported that adenosine deaminase activity is augmented in the cancerous tissues and cells [4], while other studies oppose this concept 5. ADA is also involved in the development of B and T lymphocytes, as it is evident from the fact that ADA deficient animals suffer from B and T lymphopaenia [1–5]. The levels of enzymes in T-lymphocytes vary according to

cellular differentiation [6]. The activity of the ADA enzyme is subject to change 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 possible fully functional purine salvage pathway [8]. An increased serum ADA level is associated with esophagus tumors, liver cancer, breast cancer, and colorectal cancer [8–12]. Hence, the present study was aimed to estimate the salivary adenosine deaminase in different gradings of squamous cell carcinoma of the tongue.

Material and methods

The 50 patients (25:25;M:F) of squamous cell carcinoma of tongue and 30 normal subjects (15:15;M:F), without any systemic disease, aged 45-60 years attending Bhagwan Dental Clinic, Jind (Haryana) were selected after obtaining informed consent. All patients were staged by the TNM classification. 10, 25, and 15 patients were in stage I, II, and III respectively. Patients in poor general conditions as well as smokers and alcoholic subjects were excluded from the study. During the examination, paraffin wax stimulated whole saliva was collected, and samples were stored at −20°C until analyzed. Saliva was centrifuged at 8,000×*g* for 9 min and levels of adenosine deaminase in supernatant were determined using a Guisti method [5]. Ammonia forms under conversion of adenosine to adenosine deaminase causing intensely blue indophenols with sodium hypochlorite and phenol in an alkaline solution as determined by modification of a Berthelot reaction. Sodium nitroprusside was used as the catalyst. The ammonia concentration was directly proportional to the absorbance of the indophenol measured at a wavelength of 620 nm. The reaction catalyzed by ADA was stopped at the end of 1 h incubation at 37°C by the addition of phenol nitroprusside solution. ADA activity was expressed in international units (IU) using the formula as follows: (absorbance of sample/absorbance of standard)×50 IU/l. Adenosine was obtained from SRL Chemicals, India and all the other chemicals were of analytical grade and obtained from MERCK Company, Mumbai (India). Inter-assay variance was 7.2 and the reliability coefficients presented here were estimated using values as provided by the laboratory. Analyses performed after assigning all values below the sensitivity levels to 0.5 nmol/l, midpoint between 0 and the sensitivity level, gave similar results and were, therefore, not presented.

Results were expressed in International Unit per milliliter and given as mean (SD) Statistical analyses were performed using the SPSS statistical software package (version SPSS Inc., version 11.5, Chicago, IL, USA). The results were evaluated statistically by using Kruskal–Wallis variance analysis with statistical significance being accepted at 5%.

Results

Statistically significant differences were noted between mean salivary ADA enzyme activities in squamous cell carcinoma of tongue as compared to the controls (Table 1, $P<0.001$). There was a statistically significant increase in the serum ADA levels as the disease stage progressed from stage I to stage III of cancer in both genders (Table 1, $P<0.001$). There was a tendency towards significance for the gender differences, with males having a more pronounced increase than females.

Discussion

The levels of salivary ADA were significantly higher in patients compared to controls as demonstrated in the previous studies [10–14]. This could not be confirmed in one particular study [15]. Furthermore, there was a statistically significant increase in the serum ADA level as the disease stage progressed from stage I to stage III. Salivary ADA levels were higher in cases with stage III disease, when compared to patients with stage II and I disease. Salivary ADA levels as reported in previous study

Table 1 The mean (SD) adenosine deaminase levels of (squamous cell carcinoma of tongue stage I, II, and III) patients and controls

Subjects	Sex and number of subjects	Adenosine deaminase levels (IU/ml)
Stage III	M (8)	0.0397 (0.0098)
	F (7)	0.0308 (0.0123)
Stage II	M (13)	0.0289 (0.0087)
	F (12)	0.0231 (0.0064)
Stage I	M (5)	0.0201 (0.0101)
	F (5)	0.0198 (0.0105)
Normal healthy	M (15)	0.0067 (0.0056)
	F (15)	0.0056 (0.0034)

[11]. It may imply that purine metabolism and the salvage pathway activity of purine nucleotides are accelerated in the cancerous human oral tissues. 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 [16, 17] as in this study. The ADA synthesis is increased in tissues surrounding cancer, and it has got a role in progression and invasion of cancer [4]. Serum ADA is sensitive to stimulation by growth factors and cytokines during rapid tissue proliferation [18] as salivary ADA in squamous cell carcinoma of tongue. The activity of serum ADA is increased in very rapidly growing malignancies, while, slow growing and well-differentiated tumors do not express pronounced ADA activity [8, 19]. The treatment of colon carcinoma cells with deoxycoformine, an ADA inhibitor, results in inhibition of cell growth [20, 21]. This may show 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. The controversial outcomes in the studies of enzymes might depend on the histological type of tumor, stage and therapy of cancer, the methods employed for material collection, and analysis [4]. The enzyme metabolism in cancer cells might show larger differences depending on cancerous tissues studied and the underlying mechanisms might be specific for each cancer. However, these distinct findings could result from the carcinogenesis process itself, but personal habits such as alcohol use, smoking, etc. might be also involved in the event [21]. Unfortunately, in this study, smoking and alcoholic were not the risk factors of carcinoma because smoking and alcohol abuse were considered contraindications for this study.

In conclusion, salivary ADA seems increased in subjects suffering squamous cell carcinoma of the tongue s, with levels increasing with carcinoma staging. Our findings support the hypothesis that salivary ADA activity may serve as an interesting additional diagnostic and prognostic cancer marker.

Conflicts of interest statement None declared.

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