PLACE OF SALIVARY β GLUCURONIDASE ACTIVITY IN HEAD & NECK CANCERS

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ABSTRACT

Salivary β -glucuronidase activity was studied in 20 healthy subjects without habits and 10 with habits, 38 untreated patients of advanced head and neck cancer and 21 patients after treatment either after surgery or radiation.

Salivary β -glucuronidase activity was elevated in untreated cases of head and neck cancer ($p < 0.001$), with a fall in the activity after treatment. The post operative fall in the activity of salivary β -glucuronidase activity was highly significant ($p < 0.001$) as compared to post radiation. This test can be utilized in the diagnosis and also for monitoring the course of the disease.

INTRODUCTION

The use of enzyme determinations as a diagnostic aid in patients with cancer requires that the assay define the disease of the involved organ, describe the extent of the cancer, and permit the clinician to evaluate disease progression or regression. Traditionally, serum enzymes have been used for these purposes but in recent years, in response to the need for more specific diagnostic tools, attempts have been made to also measure enzyme activity in tissues, body fluids, and cavity washings. The urinary levels of β -glucuronidase has been used in screening and follow up of primary urinary tract malignancy (1, 2). The serum and urine levels of β -glucuronidase could be useful in the differentiation of patients depending on tobacco smoking(3). The enzyme has wide application in determining urinary steroids (4). The salivary β -glucuronidase is found to have application in the detection of head and neck cancer (5).

The purpose of this report is to find out the utility of the enzyme studies in assessing the effect of different modalities of treatment in head and neck cancer, and subsequent changes in enzyme levels, to assess its value as a diagnostic index, to find out the high risk group patients going in for malignancy, who are known addicts of tobacco in one or other form and to study the changes of leukoplakia.

Oral cancer and other head and neck malignancies account for a majority of cancer patients at the Tata Memorial Hospital. A high incidence of this cancer is attributed to several pre-disposing factors, and a critical study of some of them has been in progress at our laboratories for some time past.

MATERIALS AND METHODS

The material for the study comprised of 20 healthy normal between the age group 20-40 years without any habits, 10 healthy individuals between the age group of 20-70 years with the habits of tobacco chewing and bidi smoking to the extent of 50-70 bidis per day and using highly carcinogenic tobacco quid for a considerable length of time (3-4 times in the mouth at least for 10 years or more), 38 untreated cases which include advanced oral cancer, patients who have histological diagnosis of squamous cell carcinoma of head and neck regions with minimal neck nodes. 21 patients were followed up after surgery and radiation of which 12 of these patients were subjected to primary surgical treatment and 9 were subjected to radiation.

The clear saliva was obtained after a mouth wash in the morning without the use of outside stimulating agents. The samples were centrifuged and the supernatant was analyzed on the same day. The activity was determined by the method of Gabor Szasz (6) using p-nitrophenyl glucuronide obtained from Sigma Chemicals Co. U.S.A. The system for the assay of β glucuronidase consisted of 0.2 ml of acetate buffer (0.2 M, pH 5.0), 1.0 ml of glycine (0.6 M, pH 11.7), 0.1 ml serum and 0.1ml of p-nitrophenyl glucuronide (50 mM) as substrate with appropriate serum and reagent blank. After incubation at 37°C for 2 hours, absorbance at 546 nm was read on a spectrophotometer against water blank.

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The activity is expressed in milliunits (mU=0.001 IU) using the equation

$$\text{Mu} = \text{Ax} - (\text{Asb} + \text{Arb}) \times 10^6 / \text{E} \times \text{TV} / \text{SV} \times 1/t$$

In which Ax=absorbance of unknown sample, Asb=serum blank absorbance, t=incubation time, Arb=reagent blank absorbance, TV=total volume, SV=sample volume and E=molar absorptivity. Molar absorptivity of p-nitrophenyl glucuronide is 18,500.

The amount of p-nitrophenol liberated was calculated using the above formula. The protein content of the saliva was estimated by the micro-kjeldahl (7) method and the Nitrogen content was calculated as follows

$$\text{Protein Value} / 6.25 = \text{X gm/dl of nitrogen}$$

The enzyme is then expressed as mU per mg. of nitrogen. The results are statistically evaluated using student's 't' test.

RESULTS AND DISCUSSION

The results of the assay are tabulated in Table I.

A review of the literature on estimation of β glucuronidase activity in the body fluids indicated that

the enzyme activity is determined by the method of Fishman *et al.* (8) using phenolphthalein glucuronide as substrate. Gabor Szasz carried out a comparative study using p-nitrophenyl glucuronide and phenolphthalein glucuronide (6) as substrate for the estimation of β -glucuronidase activity which was later on modified by Khanolkar *et al.* (9).

Table I indicates that there is a rise in the salivary β glucuronidase activity in tobacco chewers as compared to the non chewers. Table I clearly indicates that on comparing the mean levels of the enzyme of the normal (without habits and with habits) with that of the untreated head and neck cancer there is a significant elevation in the activity of the enzyme ($p < 0.001$). This finding is in confirmation with the findings of Harvey *et al.* (10).

The result obtained for salivary β glucuronidase in normal subjects without habits were in the range of 0.03-0.37 mU/unit nitrogen with a mean value of 0.1267 ± 0.0848 and those with habits had a range of 0.190-1.500 mU/unit nitrogen with a mean of 0.7683 ± 0.4470 . The total untreated cases of head and neck cancer had a mean of 5.5125 ± 4.65 and a range of 0.442-15.0 mU/unit nitrogen, which included the

Table 1. Salivary β glucuronidase activity in normals subjects, untreated and treated head and neck cancer, pre and post-operative patients with head and neck cancer, pre and post radiation head and neck cancer and advanced head and neck cancer.

Group	Number of Cases		Salivary β glucuronidase mU/unit nitrogen
Normals without Habits	20	Range	0.03 - 0.37
		Mean + S.D.	0.1267 ± 0.0848
Normals with Habits	10	Range	0.19 - 1.50
		Mean + S.D.	0.7683 ± 0.4470
Untreated head & neck cancer	38	Range	0.442 - 15.0
		Mean + S.D.	$5.5125 \pm 4.65^*$
Treated head & neck cancer	21	Range	0.250 - 9.43
		Mean + S.D.	$2.805 \pm 2.55^{**}$
Advanced head & neck cancer	6	Range	5.08 - 15.0
		Mean + S.D.	$10.805 \pm 3.944^*$
Pre-operative head & neck cancer	12	Range	2.50 - 11.4
		Mean + S.D.	$5.1575 \pm 3.024^*$
Post-operative head & neck cancer	12	Range	0.250 - 2.80
		Mean + S.D.	$1.1794 \pm 0.8903^*$
Pre-radiated head & neck cancer	9	Range	2.225 - 14.2
		Mean + S.D.	$7.437 \pm 4.9474^*$
Post-radiated head & neck cancer	9	Range	1.2 - 9.43
		Mean + S.D.	4.973 ± 2.7318
Cancer due to smoking & chewing tobacco	11	Range	0.442 - 4.3
		Mean + S.D.	$1.438 \pm 1.1426^*$

* $p < 0.001$; ** $p < 0.05$

advanced head and neck cancer with a range of 5.08-15.0 mU/unit nitrogen with a mean of 10.805 ± 3.944 , cancer due to smoking and chewing tobacco which had a mean value of 1.438 ± 1.1426 and a range from 0.442 - 4.300 mU/unit nitrogen, pre operative head and neck cancer and pre radiation oral cancer with a mean of 5.1575 ± 3.024 and 7.437 ± 4.9474 and range of 2.5-11.4 and 2.225-14.2 mU/unit nitrogen respectively. After surgery and radiation the levels of the enzyme were found to have a range of 0.250-9.43 and 1.2-9.43 mU/unit nitrogen with a mean of 1.1794 ± 0.8903 and 4.973 ± 2.7318 respectively (Table 1).

The Table 1 clearly indicates that there is a rise in salivary β glucuronidase activity in healthy tobacco chewers as compared to healthy non chewers. The levels were elevated in untreated cases of oral and head neck cancer as compared to healthy subjects both chewers and non chewers. The selection of our patients who had undergone surgery has been such that they had minimal and mobile neck nodes at the initial examination, the surgery involved total excision of the primary lesion with or without block dissection of the lymph node in the neck and the enzyme had reached a remarkable level in T_3 , T_4 tumor before surgery when there were no nodes palpable and had come to a low level after surgery. It appears that the tumor itself may be contributing to this increase in the activity in these patients.

Patients who received radiation also showed a fall in the activity of the enzyme. This as a pilot study, so far has convinced us in its value in the high risk group and this could be used as a marker utilizable by physicians.

A few cases of advanced cancer were also selected where the activity is very high. These patients died between 6 weeks to 3 months from the date of their diagnosis indicating that the activity of salivary β glucuronidase can be useful guide for the prognosis and course of the disease.

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