RESEARCH ARTICLE

Serum and Salivary Estimation of Dipeptidyl Peptidase IV, as a Prognostic Indicator in Oral Squamous Cell Carcinoma before and during Radiotherapy

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ABSTRACT

Increase in serum and salivary levels of Dipeptidyl peptidase IV (DPP IV) was reported earlier in the patients of oral squamous cell carcinoma. A study was conducted in the department to assess the serum and salivary levels of Dipeptidyl peptidase IV in patients of oral squamous cell carcinoma before and after radiotherapy.

The study was performed on 50 patients with different stages of oral squamous cell carcinoma as a study group and 50 control healthy group. The mean DPP IV activity in study group before radiotherapy in serum and saliva was significantly lower than control group. Amongst the study group, well differentiated carcinoma had the highest mean serum DPP IV activity than moderately and poorly differentiated carcinoma. There was an increase in mean DPP IV levels in study group after radiotherapy.

We hence conclude that DPP IV activity in serum and saliva can be used as a prognostic indicator in oral squamous cell carcinoma patients.

Keywords: Oral squamous cell carcinoma, Serum and salivary dipeptidyl peptidase IV levels.

INTRODUCTION

Oral cancer is sixth to eighth most common cancers in the world with a great variability of incidence among different countries.1,2 In countries like India, oral cancer is the most commonly occurring malignancy accounting to about 40% of all malignancies.3,4 The incidence of cancer is increasing with each year and it is attributed to the changes in lifestyle and increase in life expectancy.3 It is a disease of increasing age. Approximately 95% of oral cancer occurs in people older than 40 years, with average age of diagnosis 60 years.5,6

The majority of oral cancers involve buccal mucosa, lower alveolus (Fig. 1), tongue (Fig. 2), oropharynx and floor of the mouth. The single most important factor to improve the result is the early diagnosis of cancer. A number of cell products especially components of cell surface of malignant cell and enzymes involved in the metabolism of nucleic acid may be shed, which can circulate in blood. Dipeptidyl peptidase IV (DPP IV) is a cell membrane bound enzyme, which is found in serum and saliva. Its activity may be below normal limits reflecting the clinical stage of the tumor.7 This study was carried out to quantitatively estimate and compare the activity of enzyme DPP IV in serum and saliva of oral squamous cell carcinoma patients before and after radiotherapy.

Dipeptidyl Peptidase IV (DPP IV)

DPP IV was discovered in 1966 by Hopsu-Havu and Glenner as a new dipeptidyl naphthylamidase enzyme hydrolyzing glycylprolyl B-naphthylamide as substrate in rat liver and kidney.8 Hydrolysis of amino acid β-naphthylamides by amino peptidase in human parotid saliva and human serum were studied by Nagatsu I et al9 in 1968. They observed the activity of glycyl propyl β-naphthylamidase in human parotid saliva to be very low (1120 ± 692 μmol/min/L) as compared with that in serum (25.1 μmol/min/L). Serum DPP 4 activities in normal sera and in sera of patients with malignant tumor of oral lesions were assayed by Fukasawa K10 in 1982.

MATERIALS AND METHODS

For the present study, 100 patients were selected at random from OPD of Oral Medicine and Radiology Department of GDCH, Nagpur, Radiotherapy Department of GMC, Nagpur and Rashtra Santa Tukdoji (RST) Cancer Hospital, Nagpur. They were divided into 2 groups:

• Group 1 (control group) consisted of 50 healthy individuals, who gave no history nor presented with any signs of systemic diseases or pathological lesions.
• Group 2 consisted of 50 individuals with clinically and histopathologically confirmed oral squamous cell carcinoma.
This group was further divided into 3 groups applying Brynes criteria based upon degree of differentiation:

a. Well-differentiated squamous cell carcinoma
b. Moderately differentiated squamous cell carcinoma
c. Poorly differentiated squamous cell carcinoma.

The age ranged between 40 to 70 years with the mean age of 46.72 years in group 1 and 54.24 years in group 2 (Table 1 and Graph 1). Patients with carcinoma of pancreas, liver, hemopoietic system, stomach hepatobiliary diseases rheumatoid arthritis, SLE were excluded from this study. Standard proforma recorded patients’ details, habits, lesion and histological finding. Blood sample was collected before onset of radiotherapy and one month after radiotherapy (4000 rads). Blood was allowed to clot at room temperature for 30 minutes. Serum was separated by centrifuging it at 3000 rpm for 15 minutes. Unstimulated whole saliva was collected. After collection saliva, was centrifuged at 3000 rpm for 15 minutes and the resultant supernatant was used. Sera and supernatant was stored at −20°C until used. The enzyme activity in serum and saliva for the hydrolysis of glycyl proline-p-nitroanilide was assayed by direct photometric method. DPP IV is an enzyme that splits N terminal X proline from peptides by using substrate glycyl proline-p-nitroanilide. This enzyme hydrolysis of glycyl proline-p-nitroanilide produces p-nitroaniline and glycylyproline, which is further hydrolyzed to glycine and proline by immidodipeptidase in serum.

Glycyl proline p-nitroanilide (Sigma chemical company) was used as substrate. 0.3 μmol glycyl proline p-nitroanilide was obtained by dissolving 9.864 mg of substrate with 100 ml of distilled water containing 0.2 gm of Triton X. Glycyl proline p-nitroanilide is unstable. Hence Triton X 100 (a detergent) was added to increase stability.

In direct photometric method, the experimental tube contained 0.1 ml of 0.3 mol/liter glycine/NaOH buffer [pH 8.7], 0.1 ml of 0.3 mol/liter glycyl proline p-nitroanilide, 180 ml of water and 20 ml of serum/saliva. Instead of serum/saliva, the blank and standard tubes contained 20 ml of 3 mmol/ltr, p-nitroaniline in a mixture of methanol and Triton X solution in water. All the tubes were incubated at 37°C for 30 minutes and reaction was stopped by adding 1M acetate buffer [pH 4.2]. To the control tube, 20 ml of serum was added after stopping the reaction. The photometer [Beckman Model 35], the absorbance of experimental [E], control [C], and standard [S] were read at 385 nm in curette with 1 cm light path. P-nitroaniline liberated by the enzyme reaction.

\[
E - C \times \frac{150 \text{ nmol}}{S} \times 1 \times \frac{1}{30} = 0.000051
\]

\[
\frac{100(E-C)}{S} \mu \text{mol/min liter of serum (37°C)}
\]

The students ‘t’ test was used to test the difference between the mean values of two independent groups. Paired ‘t’ test was used to test the difference between the two mean values of the
same group. The statistical level of significance was determined by table value at 1% (p < 0.01).

DISCUSSION AND CONCLUSION

1. The mean DPP IV activity in study group before radiotherapy in serum [28.89 ± 4.464] and saliva [4.717 ± 1.439] was significantly lower than DPP IV activity in control group in serum [63.622 ± 4.513] and saliva [8.77 ± 1.349] (Table 2 and Graph 2). This observation was comparable to that observed by Fukasawa K et al. 7

2. The study showed that well-differentiated squamous cell carcinoma had the highest mean serum DPP IV activity [32 + 2.437] than moderately differentiated squamous cell carcinoma [26.666 ± 1.336] and poorly differentiated squamous cell carcinoma [23.025 ± 0.382], suggesting the mean DPP IV activity could be used for histological grading of squamous cell carcinoma (Table 3 and Graph no 3). Fukasawa K et al. and Urade et al. 12 also reported lower levels of serum DPP IV levels in the poorly differentiated squamous cell carcinoma patients.

3. Salivary DPP IV activity was significantly decreased in study group than control group (Table 3 and Graph 3). However, variation depending on the degree of differentiation was not statistically significant.

4. There was definite increase in the mean DPP IV serum and salivary activity in patients undergoing radiotherapy, i.e. after 1 month [4000 rads] in all three study groups.

5. No correlation was obtained between degrees of differentiation in malignant lesion in DPP IV activities after radiotherapy.

Table 2: Comparison of mean DPP IV activity in control and study group

<table>
<thead>
<tr>
<th>Mean DPP IV activity</th>
<th>Control group</th>
<th>Study group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before RT</td>
<td>After RT</td>
</tr>
<tr>
<td>Serum</td>
<td>63.622 ± 4.513</td>
<td>28.89 ± 4.464</td>
</tr>
<tr>
<td>Saliva</td>
<td>8.77 ± 1.349</td>
<td>4.717 ± 1.439</td>
</tr>
</tbody>
</table>

Graph 2: Comparison of mean DPP IV activity in control and study group in serum and saliva; before and after radiotherapy

Graph 3: Mean DPP IV activity in serum and saliva samples of oral squamous cell carcinoma patients classified according to their degree of differentiation

SUMMARY

Thus it can be concluded that pre- and post-treatment levels of DPP IV in serum as well as saliva can be used as prognostic indicator. A low DPP IV activity could indicate poor prognosis. Further studies are necessary to elucidate the precise mechanism of reduction of serum and salivary DPP IV activity.

REFERENCES


