Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(1):312-315
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Quantitative analysis of serum dipeptidyl peptidase IV enzyme in oral squamous cell carcinoma

Prashant V. Kandekar[1], Avinash Tamgadge[2] and Suresh Meshram[3]

[1]Ibn Sina National College For Medical Studies, Jeddah, KSA
[2]DY Patil Dental College and Hospital, Navi Mumbai, India
[3]Government Dental College and Hospital, Mumbai, India

ABSTRACT

Dipeptidyl Peptidase IV (DPP IV) is an enzyme which splits N-terminal X-proline from peptides. The objective of this study was to present a reliable test to quantitatively estimate the serum Dipeptidyl Peptidase IV enzyme in oral cancer patients and to ascertain if Dipeptidyl Peptidase IV can be used as a tumor marker in the early diagnosis of cancer. The study was performed on 31 patients with different stages of oral squamous cell carcinoma as a study group and 10 control healthy group. The mean DPP IV activity in study group was significantly lower than control group. Among the study group, well differentiated squamous cell carcinoma had the highest mean serum DPP IV activity than moderately and poorly differentiated squamous cell carcinoma. We hence conclude that DPP IV activity in serum can be used as a biochemical marker in the diagnosis of oral squamous cell carcinoma.

Key words: Oral squamous cell carcinoma, Dipeptidyl Peptidase IV, biochemical marker.

INTRODUCTION

It is now a well established fact that oral cancer is one of the most important cause of mortality and morbidity. The term “oral cancer” is used to describe any malignancy that arises from oral tissues and 90% of all malignancies are oral squamous cell carcinomas. Although in the last few years remarkable progress has taken place in the understanding of its biologic and biochemical nature, diagnosis and management of malignancies has always challenged the oral health care professionals, as the cruel disease almost often devours its victims (1).

It has now been recognized that many substances are produced inappropriately or in high amounts in patients with malignancy. The measurement of these substances in the body fluid can help in the diagnosis of cancer as well as monitoring the extent of the disease.

Dipeptidyl peptidase IV (DPP IV) is one such substance. It is also known as CD 26. It is a highly sialylated glycoprotein. It is a cell membrane bound enzyme (2, 3) and is also present in a soluble form in the serum. The origin of DPP IV in serum remains unknown. Since it is known that antigens existing on the surfaces of normal and cancer cells are released from the cell surface to serum or medium by shedding, it is considered that DPP IV activity in serum is derived from a variety of cells or tissues.

The estimation of serum DPP IV has been used for the early detection of hepatic carcinoma, renal carcinoma and various other malignancies (3). This study was carried out to quantitatively analyse enzyme DPP IV in oral squamous cell carcinoma and to determine a relationship between enzyme and degree of dysplasia.

MATERIALS AND METHODS

For the present study, 41 patients were selected at random from out-patient department of Government Dental College and Hospital, Mumbai. They were divided into 2 groups:

1. Control group included10 healthy individuals (7 males and 3 females) with no history and any sign of pathologic lesions or systemic diseases.
2. Study group included 31 individuals (24 males and 7 females) with clinically and histopathologically confirmed oral squamous cell carcinoma (Table1).

The study group was further divided into 3 groups applying Bryne's criteria (4) based upon degree of differentiation:
a. Well-differentiated squamous cell carcinoma-20 cases
b. Moderately differentiated squamous cell carcinoma-9 cases
c. Poorly differentiated squamous cell carcinoma-2 cases (Table 2).

Standard proforma recorded patient details, habits, lesions and histopathological findings. Blood sample was collected and allowed to clot at room temperature for 30 minutes. Serum was separated by centrifuging blood samples at 3000 rpm for 15 minutes. The serum was stored at -20℃ until used. The enzyme activity in serum for the hydrolysis of glycyl proline p-nitroanilide tosylate (Sigma Chemical Company) was estimated by direct photometric method. DPP IV is an enzyme that splits N-terminal X-proline from peptides by using substrate glycyl proline p-nitroanilide, producing p-nitroaniline (5, 6).

Table 1: Sample size, age and sex distribution

Group No.	Group Name	Age Range	Sample Size	Sex Distribution	
				Male	Female
1	Control	20-54	10	7	3
2	Study	30-75	31	24	7

Table 2: Study group distribution according to Histopathological grading

Sr. No.	Histopathological Grades	Sample Size
1	Well differentiated squamous cell carcinoma	20
2	Moderately differentiated squamous cell carcinoma	9
3	Poorly differentiated squamous cell carcinoma	2

In direct photometric method, the experimental tube contained 0.1 ml of 0.3 mol/litre glycine/NaOH buffer (pH 8.7), 0.1ml of 0.3 mol/litre glycyl proline p-nitroanilide tosylate, 18ml of water and 20 ml of serum. Instead of serum, the blank and standard tubes contained 20ml of 3mmol/litre P-nitroaniline in a mixture of methanol and Triton X solution in water. All the tubes were incubated at 37^0 C for 30 minutes and reaction was stopped by adding 1M acetate buffer (pH 4.2). To the control tube, 20ml of serum was added after stopping the reaction. In the spectrophotometer, the absorbance of experimental (E), control (C), and standard (S) were read at 385nm in a cuvette with 1cm light path.

Calculation of P-nitroaniline liberated by Dipeptidyl peptidase IV enzyme reaction is done by the following formula

$$\text{DPPIV activity} = E - C \times \frac{150\text{nmol}}{S} \times 1 \times \frac{1}{30} - 0.000051$$
$$= \frac{100(E-C)}{S} \text{umol/ min litre of serum (37℃)}$$

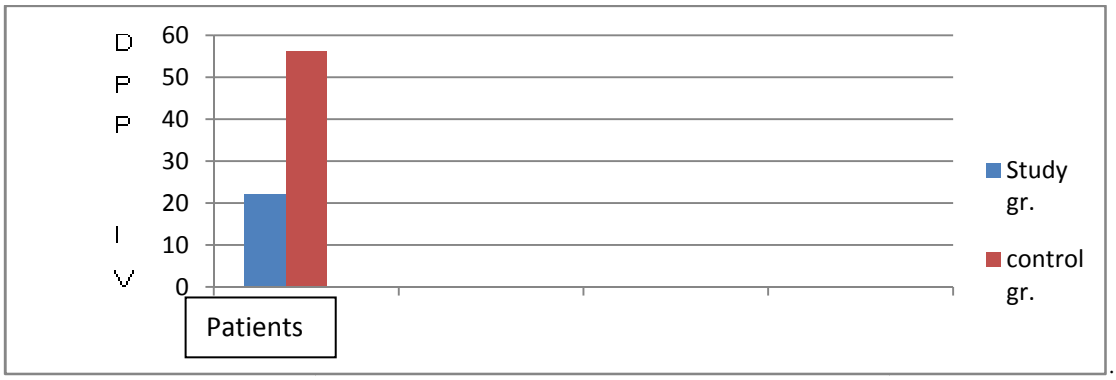
The student t test was used to test the difference between the mean value of two independent groups. Paired t test was performed to test the difference between the two mean values of the same group. The statistical level of significance was determined by table value at 1% ($p < 0.01$).

RESULTS AND DISCUSSION

a. The mean DPP IV activity in malignant cases (22.1± 4.96) was significantly lower than normal control group (56.09 ± 4.70). This observation was comparable to that observed by Fukasawa et al (2) and Masahiro et al (7). (Graph 1)
b. The study showed that well-differentiated squamous cell carcinoma had the highest mean value (27.7 ± 4.091) than moderately differentiated squamous cell carcinoma (18.8 ± 3.555) and poorly differentiated squamous cell

carcinoma (14 ± 1.414), suggesting the estimation of mean DPP IV activity could be used for histological grading of squamous cell carcinoma. Fukasawa et al (2) and Masahiro et al (7) also reported similar findings.
(Graph 2)

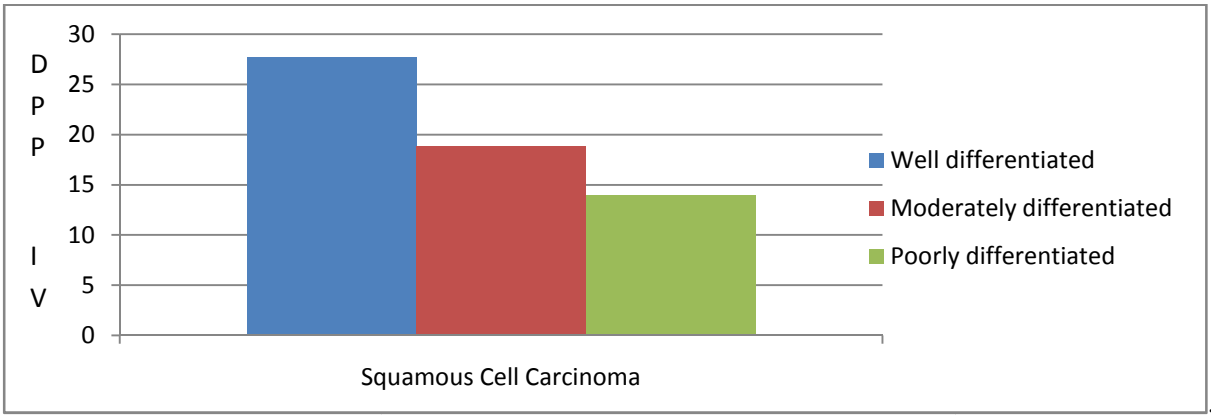
Graph 1: Comparison of the mean DPP IV levels in study group and control group



The cause of the decrease in the serum enzyme activity in patients with malignancy remains to be elucidated. Few hypotheses are conceivable:

- It may be due to small amounts of enzyme protein, arising from decomposition by neuraminidase, followed by proteases in the blood or the tumor cells synthesizing this enzyme protein less than normal cells.
- It may be due to incomplete biosynthesis of surface glycoprotein by transformed cells giving rise to partial asialoglycoconjugates.
- Decreased surface expression and altered protein processing resulting in premature degradation may account for the loss of it.
- It may be because of the influence of the native inhibitor (s) or activator (s) on the serum enzyme activity (3).

Graph 2: Comparison of mean DPP IV levels in different grades of oral squamous cell carcinoma



We have shown that the serum enzyme activity was decreased in patients with squamous cell carcinoma of the oral region, and seemed to reflect the histopathological grading of the tumor. It may be of supplementary value in the assessment of the stage of the tumor, in combination with other indicators.

This procedure of estimation of enzyme by UV spectrophotometer is simple, easy to perform, convenient for measuring multiple samples simultaneously, less time consuming and non-invasive; thus suitable for the routine assay of the enzyme.

CONCLUSION

The obtained results can suggest DPP IV activity in oral squamous cell carcinoma patients is inversely correlated with the grades of the disease. It can be suggested that estimation of serum DPP IV using spectrophotometer can be used as a biochemical marker in the diagnosis of oral squamous cell carcinoma.

Acknowledgement
The author thanks Dr. Suresh Meshram, Dean of Government Dental College and Hospital, Mumbai and Biochemistry department, J. J. Hospital, Mumbai for the necessary support.

REFERENCES

[1] Brad A Neville, CA *Cancer journal clinics*, **2002**, 52, 192-215.
[2] Katasuhiko Fukasawa at al., *International Journal of Oral Surgery*, **1982**, 11, 246-250.
[3] D. A. Pereira et al., *Brazilian Journal of medical and biological research*, **2003**, 36, 567-578.
[4] Bryne et al., *Journal of Oral Pathology and Medicine*, **1991**, 20, 413-420.
[5] Masami Hino et al., *Clinical chemistry*, **1976**, 22, 8, 1256-1261.
[6] Nagatsu T, Hino M, Fuyamada H, Hayakawa T, Sakakibara S, Nakagawa Y, Takemoto, *Anal Biochem*,**1976**, 74, 466-476.
[7] Masahiro Urade et al., *Cancer*, **1989**, 64, 1274-1280.