

## Research Article

# Significance of salivary phosphodiesterase level in oral squamous cell carcinoma patients

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## ABSTRACT

Oral cancer, more specifically oral squamous cell carcinoma (OSCC) consider as common cancer that 300,000 people diagnosed per year worldwide. The only effective treatment for OSCC is surgical intervention. Over the past two decades, overall disease condition has not improved although advancement of treatment has considerably increased. The phosphodiesterase (PDEs) are responsible for the hydrolysis of the second messengers with a fundamental role in the transduction of the intracellular signals. In numerous pathological conditions such as cellular differentiation, apoptosis, and tumor invasivity the different PDF activity has been observed that shown role in pathophysiological mechanism. The role of PDEs as an intervention factor for activation of angiogenesis by influencing a tumor growth has been shown. The objective of this study was to estimate and compare salivary PDEs levels in healthy controls and biopsy-proven oral cancer patients before definitive therapy. Study was done in patients age between 25-65 years biopsy proven oral cancer patients and control group. After obtaining prior consent from biopsy-proven oral cancer patients (n=26) (before onset of any definitive treatment) and age- and sex-matched healthy controls (n=29), salivary sample was collected for estimation of the activity of phosphodiesterases (PDEs).

**Keywords:** Oral cancer, Oral squamous cell carcinoma (OSCC), Phosphodiesterases (PDEs)

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is a one of the major cause of death due to cancer and it represent a major health problem worldwide. According to the world health organization, carcinoma of the oral cavity is the sixth commonest in developing countries.<sup>1-3</sup> There are numerous factor found to be associated in development of OSCC, such as the use of tobacco, alcohol or betel nut chewing, human papillomavirus infection, and the presence of incompatible prosthetic materials. Tobacco is known to be one of the major causes of development of OSCC. Smokers is known to develop OSCC 7-10 times higher than non-smoker.<sup>4-6</sup>

On an account of Indian cancer registry data, it is estimated about 75,000-80,000 new oral cancer cases develop annually in India.<sup>3</sup> Only 50% of advanced stage would survive for 5 years, as this rate has been constant over the last two decades. In order to decrease mortality rate, a new tools are require for early stage diagnosis because in initial stage treatment of cancer would decrease the mortality rate.<sup>7</sup>

Finding a new biomarkers (or molecular biomarker) which could help to detect disease progression is require for better management of this disorder. Saliva is of great importance for diagnosis of several systemic diseases, and its use for diagnosis of OSCC has been used

extensively. Many salivary enzymes along with DNA, RNA and protein obtained from saliva, cancerous cells and inflammatory cells of oral cavity. Extensive studies carried out from genomic and proteomic perspective to identify the potential biomarkers in body fluid as well as saliva and blood for diagnosis and prognosis of OSCC. Variations on these parameters have been correlated to different pathological mechanisms, such as cellular differentiation, apoptosis, and tumor invasivity. Some of these markers are also known to play a role in tumor growth by influencing angiogenesis.

Many therapeutic drug monitoring can be done by analyzing saliva, the composition of saliva comprises the hormonal level and is used in immunology, toxicology and infectious disease markers.<sup>8</sup> Consequently, this fluid provides a source for the monitoring of oral and also systemic health. The non-invasive nature of collection, the direct contact to the oral cavity, and the relationship between oral fluid and blood levels make saliva a useful and promising specimen to detect potential biomarkers for OSCC. As a bio fluid, it provides a perfect medium to observe disease onset, progression, recurrence and treatment outcome through non-invasive means.<sup>9</sup> In this study we measured salivary PDEs level as an enzyme that involve in cellular differentiation, apoptosis, tumor invasion<sup>10,11</sup> angiogenesis<sup>12</sup> and its found a significant increase in its salivary level hence PDEs in oral cancer patients and it may be used as a potential non-invasive salivary biomarker to monitor the improvement of oral cancer patient's condition.

## METHODS

The study was carried out after obtaining approval from the Institutional Ethics Committee. In this study, 55 subjects, aged between 25 and 65 years of either sex, were used. Of them, 26 were biopsy proven oral cancer patients and 29 were healthy controls. The oral cancer patients were admitted under Radiotherapy and Oncology department, Kasturba Medical College, Manipal University between June 2011 and June 2012. Cancer cases with associated serious diseases like liver disease, diabetes, renal disease, and those who were on any long-term medications were excluded. The control group comprised of age- and gender-matched healthy subjects. Among oral cancer patients, 23 were males and 3 were females.

The subjects were instructed not to eat, drink or smoke for 1 h before sampling. Unstimulated whole saliva was collected, after allowing saliva to pool in the floor of the mouth for 5 min by leaning forward and letting saliva drain into a sampling tube. Since the salivary composition, show diurnal variations, a specific time was fixed for sample collection 9.00 am to 12.00 am. Immediately after collection, the samples were analyzed (within 4-6 h, meanwhile it was stored on crushed ice). The sample was taken in eppendorf tube and centrifuged for 10 min at 8000 rpm. The supernatant, free of debris was used for analysis of the parameter.

## Principle PDE Assay

Paranitrophenyl phosphate (4-nitrophenyl phosphate) is hydrolyzed by PDE to 4-nitrophenol (4-hydroxynitrobenzene) and inorganic phosphate. The yellow color formed due to liberation of 4-nitrophenolate at pH 9 was measured spectrophotometrically at 400 nm.<sup>13</sup>

## Procedure

In a clean dry test tube, 1 ml of assay mixture containing 500 µl of Tris HCl, 100 µl of MgCl<sub>2</sub>, 100 µl of paranitrophenyl phosphate, and 300 µl of distilled water was taken. The mixture was incubated at 37°C for 5 minutes. Then, to this mixture, 10 µl of saliva (10 µl of double distilled water in case of blank) was added. The mixture was again incubated at 37°C for 10 minutes. 2 ml of NaOH with EDTA was added to each test tube to stop the reaction. Activity was calculated using molar absorption coefficient of the product of chemical reaction, 4-nitrophenol.<sup>13</sup>

## RESULTS

Pre-treatment salivary PDE levels were significantly elevated in oral cancer patients ( $P < 0.016$ ) as compared with the controls. This may implicate a role for serum PDE in pathophysiology of oral cancer (Table 1).

**Table 1: Comparison of pretreatment serum PDE levels of oral cancer patients with healthy controls.**

Groups	Number	Pretreatment salivary PDE (µmol/l) Median (IQR)
Controls	29	0 (0, 59.85)
Cases	26	85.51 (21.15, 183.84)
*P value		<0.016
*Mann: Whitney Test, IQR: Interquartile range		

## DISCUSSION

Squamous cell carcinoma (OSCC) in 90% of oral cancer patients, is the sixth most common cancer recognized now a days. If OSCC is detected at the early stage (T-1 stage), the 5-year survival rate is close to 80%. If OSCC is detected at the later stages (T-3 or T-4 stage), the 5-year survival rate decreases to 20-40%, indicating early detection methods are necessary for increasing long-term patient survival. A vast number of potential biomarkers have been correlated with OSCC outcome, illustrating the complex events leading to carcinogenesis and cancer progression. Some of the proposed markers are frequently debated and sometimes results seem to contradict each other. Several factors may explain this situation, such as

the small number of individuals included in each study or the heterogeneity of selected patients, which frequently differ in various features, notably tumor location.<sup>14</sup> Local tumor recurrence affects approximately 60% of patients and metastasis develops in 15-25%.<sup>15</sup> The prevention and management of this disease is likely to greatly benefit from the identification of molecular markers and targets.<sup>16,17</sup>

However, little is known about the molecular basis of OSCC compared with other malignancies. Molecular alterations in a number of oncogenes and tumor suppressor genes (TSGs) associated with the development of OSCC may be significant clues with which to address these problems.<sup>18,19</sup>

cAMP is an important “second messenger” transferring information into cells and exists ubiquitously in the tissues and mammalian cell. The cAMP signal pathway is involved in many metabolic pathways in cells, and regulates many physiological processes, such as cell metabolism, proliferation, and cell death. The PDEs are responsible for the hydrolysis of the second messengers, with a fundamental role in the transduction of the intracellular signals. Variations in PDE activity have been found in different pathologies, and they have also been correlated to different pathophysiological mechanisms, such as cellular differentiation, apoptosis, and tumor invasivity.<sup>20,21</sup>

PDEs are also known to play a role in tumor growth by influencing angiogenesis.<sup>22,23</sup> Inhibition of selective PDE isoforms, which raises the levels of intracellular cAMP and cGMP, has been shown to induce apoptosis and cell cycle arrest in a broad spectrum of tumor cells.<sup>24,25</sup> Our results showed a significant increase in pre-treatment salivary PDE levels in cancer patients as compared with controls. This may imply a possible role for inhibitors of specific PDE isoenzymes which may selectively restore normal intracellular signaling, providing antitumor therapy with reduced adverse effects.

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