RESEARCH ARTICLE

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Evaluate the Role of Phosphodiesterase, Myeloperoxidase and Iron in Oral Cancer

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Abstract

Objective: The present study aimed to estimate the levels of phosphodiesterase (PDE), myeloperoxidase (MPO) and iron levels in patients with oral cancer. **Method:** 50 patients reporting to the Department of Oral Medicine and Radiology A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore were recruited for the study after taking informed consent. The study group consists of 50 control groups with no complications and 50 oral cancer patients. A 5 ml venous Blood sample was taken, centrifuged and serum was collected for subsequent analysis. 50 normal control samples were also collected for comparison purposes. PDE, MPO and iron levels were estimated. **Results:** PDE levels were significantly elevated in the case group compared to the control. MPO and iron levels were significantly reduced in the case group compared to the control. **Conclusion:** Immunological and biochemical assessment of oral precancer and cancer patients may help in earlier diagnosis and/or prognosis of these lesions. This may also serve in predicting the malignant potential of the pre-malignant lesions.

Keywords: phosphodiesterase (PDE)- myeloperoxidase (MPO)- iron- oral cancer

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Introduction

Cancer is a significant global public health issue. Every year, almost 11 million people are diagnosed with cancer. The Global Cancer Statistics 2020 research reported 10.0 million cancer-related deaths and 19.3 million newly diagnosed cases. In 2020, cancer killed over 10.0 million people, making it a top cause of death worldwide [1]. More than 90% of oral cancers are squamous cell carcinomas (OSCCs) , with other tumours of the mouth cavity including salivary minor glands, melanomas, and lymphomas [2]. OSCC can have varying amounts of differentiation and frequently result in node metastases. Lymphatic extending into the neck is directly proportional to the T stage, depth of invasion, and tumour thickness [3].

Oral cancer, together with oropharyngeal cancer, is the world's sixth most prevalent malignancy. Every year, over 400,000 new cases of oral cancer are expected to be detected worldwide, with Asian nations such as Sri Lanka, Indonesia, India, Pakistan, and Bangladesh accounting for two-thirds of these [4]. In these high-risk countries, oral cancer is the most frequent malignancy, accounting for more than 25% of all new cancer cases each year. The incidence of oral cancer rises with age, peaking at 60 years old, despite an increase in incidences among those under the age of 40. Oral cancer has a terrible prognosis, with overall 5-year survival rates as low as 40%. However, if discovered in the early stages (I and II), survival rates can surpass 80% Up to 50% of oral cancers are identified in an advanced stage (stage III and IV), because most people are not symptomatic in the early stages and do not seek medical help until they display visible symptoms such as pain, bleeding, or a mass in the mouth or neck [4].

When the diagnostic delay exceeds one month, the risk of developing advanced-stage oral cancer is dramatically increased in most cases, the patient is responsible for a large part of the diagnostic delay; however, delay can also be the result of an incorrect medical approach by

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not suspecting an oral malignancy and not diagnosing and treating it promptly and adequately. In general, the prognosis decreases as the disease progresses and the tumour site becomes less accessible. The clinical and pathological stage of diagnosis remains the most critical factor impacting prognosis [5]. Given the high mortality rate, early detection of oral cancer and anticipation of diagnosis resulted in better prognosis and survival rates, as well as less morbidity from treatment [6].

cAMP is an important "second messenger" that transfers information into cells and is found throughout mammalian tissues and cells. The cAMP signal pathway governs a variety of physiological activities, including cell metabolism, proliferation, and cell death. PDEs hydrolyze second messengers and play an important role in intracellular signal transduction. Variations in PDE activity have been observed in a variety of illnesses and have been linked to many pathophysiological mechanisms, including cellular differentiation, death, and tumour invasivity [7-9]. PDEs are also known to influence angiogenesis, which promotes tumour growth. In humans, rats, and mice, the PDE superfamily consists of 11 different gene families (PDEs 1–11), each of which encodes at least 100 unique PDE isoforms via alternative mRNA splicing, various promoters, and transcription start sites. The 11 PDEs can be classified into three major categories based on sequence homology, substrate specificity, and selectivity. PDE4, PDE7, and PDE8 are selective for cAMP hydrolysis; PDE5, PDE6, and PDE9 are specific for cGMP hydrolysis; while PDE1, PDE2, PDE3, PDE10, and PDE11 have dual specificity, operating on cAMP and cGMP with varying affinities depending on the isoform [10]. Recently, genetic changes or overexpression of PDE genes have been linked to tumour growth. Polymorphisms in the PDE8A and PDE11A genes have been linked to an increased risk of developing specific malignancies, including adrenocortical, testicular, and prostate . More crucially, PDE5 overexpression has been documented in a variety of malignancies [11-12, 16].

Despite increased interest in the role of neutrophils in carcinogenesis, cancer development, and metastasis, many of the fundamental processes of neutrophil activity in cancer are still unknown. In this sense, neutrophil-derived enzymes have emerged as promising targets for understanding some of the neutrophil functions in cancer [13]. MPO is a heme-containing peroxidase found mostly in neutrophil azurophilic granules. MPO is generated during neutrophil activation and degranulation and interacts with H_2O_2 and halides to catalyze the generation of ROS, including HOCl, hypothiocyanous acid (HOSCN), hypobromous acid (HOBr), and hypoiodous acid (HOI), respectively [14, 15]. MPO has the potential to promote (light pink boxes) or restrict (light green boxes) cancer development through a number of mechanisms. After stimulation, neutrophils in the blood and tissue can release MPO. MPO-derived HOCl and other oxidants can damage DNA, induce mutagenesis, and aid in the conversion of pre-carcinogens to carcinogens, hence aiding tumour formation.

MPO, a highly positively charged peroxidase enzyme, is mostly generated by neutrophils [16]. Some subpopulations of macrophages can generate MPO, but at lesser quantities.MPO's most significant impacts are associated with its enzymatic activity and the production of highly reactive compounds. These products can react with and affect proteins, DNA, lipids, and other oxidizable groups, resulting in cellular changes and genetic abnormalities . However, there have been instances of MPO having effects on various cells that are independent of its enzymatic activity . Furthermore, MPO has been found to attach and internalize into several cells, including platelets , endothelial cells , macrophages , and epithelial cells [17-20], thus altering signalling pathways.

MPO has been demonstrated to promote breast cancer cell migration and invasion in vitro while also promoting lung metastasis in vivo. MPO's function in cancer has attracted attention in recent years, despite the fact that it is a relatively young topic of research. Several studies have shown that MPO plays a role in cancer control. MPO has both pro- and anti-tumor activities, although the majority of the evidence suggests that it favours tumour start and progression [21]. Research indicates that neutrophils produce more MPO during the early stages of OSCC development, suggesting a role in both initiation and progression.There are few studies examining blood and salivary MPO levels in oral cancer, despite previous research on the topic [22, 23].

Anaemia is linked to poor response to antineoplastic therapy and lower survival rates in patients with OSCC. Anaemia is commonly associated with resistance to radiotherapy due to low haemoglobin (Hb) levels, which are crucial for tumour oxygenation. Low haemoglobin levels contribute to tumour hypoxia and impaired radiosensitivity. Radiation dosages required to eliminate hypoxic cells are estimated to be 2-3 times higher than those required to destroy well-oxygenated cancer cells. Prolonged hypoxia can cause tumour cells to become resistant to apoptosis by stimulating genetic alterations that lead to increased aggressiveness [24, 25].

With this background, we aimed to estimate the levels of PDEs, MPO and iron level in patients with Oral cancer.

Materials and Methods

Ethical statement

This study was approved by the Ethics Committee of A.B Shetty Memorial Institute of Dental Science Deralakatte, Mangalore (ABSM/EC/118/2009) dated 25th August. 50 patients reporting to the Department of Oral Medicine and Radiology A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore were recruited for the study after taking informed consent. 50 normal control samples were also collected for comparison purposes.

Sample collections

The study group consists of 50 control groups with no complications and 50 oral cancer patients from the Department of Oral Surgery, A.B Shetty Memorial Institute of Dental Science. A 5 ml venous Blood sample was taken, centrifuged and serum was collected for subsequent analysis.

Estimation of Serum Phosphodiesterase by Bis-PNPP method

For standard

Para nitro phenol was pipetted out into a working standard solution in 5 different test tubes in the range of 0.2 to 1 ml. Volume was made up to 1 ml with Tris HCl buffer. 1 ml of Tris HCl buffer was taken in a separate test tube which will serve as blank. Tubes were incubated at 27 o C for 1 hour.0.4 ml of NaOH was added to all the test tubes to arrest the reaction and read the absorbance at 405 nm against the blank.

For sample

1ml of the serum sample was taken in a test tube.1ml of Tris HCl Buffer was added to make the volume to 2 ml. 1ml of Bis PNPP of 50 mM was added to the tubes and kept for incubation at 37 0 C for 1 hour. After incubation 0.4 ml of NaOH was added to all the test tubes to arrest the reaction and read the absorbance at 405nm against the blank [26]

Calculation

$$\frac{\text{O.D of test}}{\text{O.D of standard}} \times \frac{\text{Amount of the standard}}{\text{Volume of sample}} \times 1000 = \text{'y' IU}$$

Estimation of Serum myeloperoxidase:

Procedure

1.5ml of 4-amino antipyrine was pipetted into a clean test tube.0.4ml of phosphate buffer was added to it. 1 ml of hydrogen peroxide was added. Then 0.1 ml of serum was added. Optical density was measured every 30 seconds for 5 minutes at 512nm. Average changes in absorbance were recorded. MPO activity was calculated.

Estimation of Serum iron by Banthophenanthpoline method

Procedure Standardization

Working standard (Ammonium ferrous sulfate) prepared in aliquots of 0.1, 0.2, 0.3, 0.4,

0.5mL respectively in different test tubes. The volume of solution was made up to 1mL by adding deionized water.2 ml of the chromogen solution was added to all the tubes and placed at room temperature for 5 minutes. The optical density of all the reaction mixtures was measured Spectro photometrically at 535 nm against the blank replacing the working standard with deionised water. The obtained optical densities were then plotted on a graph with the concentrations on the X-axis and the respective optical densities along the Y-axis.

Estimation of iron in sample

100 µL of the sample (serum/saliva) was taken in a clean microcentrifuge tube and made to 250µL with deionized water. Added 500 µL of protein precipitating solution. Centrifuged the mixture at 2000 rpm for 10 minutes. 500 µL of the supernatant was taken and added to 500 µL of the chromogen solution. The optical density immediately (within 10 minutes) was measured at 535nm against a blank treated similarly to the test wherein the sample is replaced with deionized water.

Calculations

The concentration of iron in the sample is obtained by plotting the optical densities of the test against the standard graph. The obtained concentration is then multiplied by 2.5 (dilution factor) [27]

Statistical analysis

Values are expressed in mean ± SD. Data was collected and statistically analyzed by the Students T-test. $P < 0.05$ is considered as significant. All statistical analysis were carried out using the statistical package for social science (SPSS 16.).

Table 1. Demographic Summary of the Study Population:

Parameters	Control	Oral cancer
Mean age	35.17±3.14	52.15±5.23
Sex		
Males	25 (50%)	38 (76%)
Females	25 (50%)	12 (24%)

Results

The present study aimed to estimate the levels of PDEs, MPO and iron in patients with oral cancer. The study group consists of 50 control groups with no complications and 50 oral cancer patients. A 5 ml venous blood sample was collected, centrifuged and serum was utilized for subsequent analysis. The mean age of the study population in the control sample was 35.17±3.14, the case was 52.15±5.23. Males (76%) were predominantly affected by oral cancer compared to females (24%) (Table 1).

PDE levels were significantly elevated in the case group compared to the control. MPO and iron levels were significantly reduced in the case group compared to the control (Table 2 and Figure 1).

Discussion

Oral cancer begins with a small, unusual, unexplained growth or sore in the mouthparts, which comprise the lips, cheeks, sinuses, tongue, hard and soft palates, and the base of the mouth extending to the oropharynx. Globally, mouth cancer is ranked sixth among all cancers. India has the highest number of cases of oral cancer, accounting for one-third of the global burden. Oral cancer is a severe health concern in countries experiencing economic transformation [27]. India reports over 77,000

Table 2. Comparison of the Levels of PDE, MPO and Iron in Oral Cancer Patients with Normal Control

Parameters	Normal	Oral Cancer	P Value
Phosphodiesterase (ug/dl)	150.8±45.14	328.1±43.82	<0.00001****
Myeloperoxidase (pM/L)	32.09±8.67	17.78±4.5	=0.002***
Iron (ug/dl)	110±17.00	68.00±34.25	=0.0027***

Level of significance; ****P<0.0001 is extremely significant; *** P=0.0027 is statistically significance

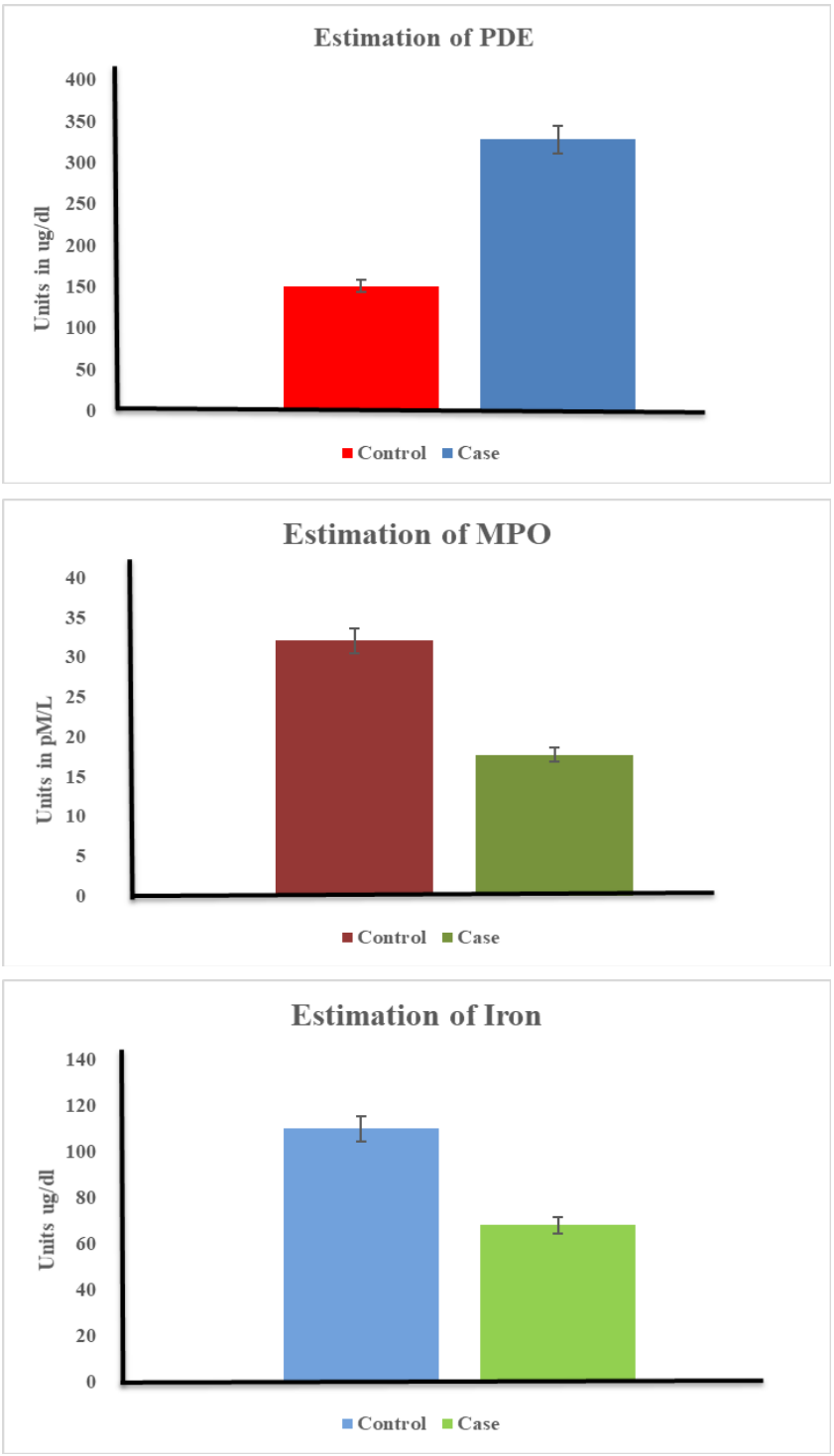

Figure 1. Shows the levels of PDE, MPO and Iron in Control and Oral Cancer Patients

new cases and 52,000 deaths every year, accounting for almost one-fourth of global incidents. Oral squamous cell carcinoma (OSCC) accounts for 84-97% of oral cancers. OSCC is usually caused by possibly malignant lesions or normal epithelial linings. Indicators of the preclinical stage of oral cancer include inflammatory oral submucosa, fibrosis, erythroplakia, leukoplakia, candidal leukoplakia, dyskeratosis congenital, and lichen planus [28, 29].

In the present study, we aimed to investigate the level of Phosphodiesterase, Myeloperoxidase and iron in oral cancer patients. The blood sample was collected from the oral cancer patients along with the control group of normal individuals. From this study, we found that there is a significant increase in the level of Phosphodiesterase in the serum samples of oral cancer when compared to the normal. The cAMP signal pathway governs a variety of physiological activities, including cell metabolism, proliferation, and cell death. PDEs hydrolyze second messengers and play an important role in intracellular signal transduction. Variations in PDE activity have been observed in a variety of illnesses and have been linked to many pathophysiological mechanisms. Our study is in consistent with previous studies [7].We found that there is a significant decrease in the level of

myeloperoxidase in the serum samples of oral cancer patients when compared to the normal and it is extremely significant. Many cancers, including ovarian cancer, have been linked to elevated myeloperoxidase levels. Up to 65% of the invasive ovarian tumours examined had increased myeloperoxidase levels. Further research on myeloperoxidase expression in ovarian cancer cells is necessary and needed [30].

In conclusion, the development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. Leukoplakia is the most common precancer representing 85% of such lesions. The level of Phosphodiesterase in the serum of oral cancer patients was increased when compared with the serum of normal people In this study the result shows that phosphodiesterases, most probably play a role in the progression of oral cancer. The exact mechanism of phosphodiesterase which is how it is responsible for increasing the chances of cancer is not clear. Much more work is desired in this field. Myeloperoxidase level was gradually decreased in the oral cancer patient when compared to the normal. Myeloperoxidase is an iron-containing enzyme and it is also decreased along with serum iron. Serum iron levels are considered as a biochemical indicators for nutritional assessment. Utilization of iron in collagen synthesis by the hydroxylation of proline and lysine leads to decreased serum iron levels in oral cancer patients. Reduction in the serum iron level may be due to malnutrition caused by the tumor burden in cancer patients. Immunological and biochemical alterations in the sera of such patients can help not only in early diagnosis, and appropriate treatment but also as indicators of prognosis, as the disease progresses. It can be suggested that immunological and biochemical assessment of oral precancer and cancer patients may help in earlier diagnosis and/or prognosis of these lesions. This may also serve in predicting the malignant potential of the pre-malignant lesions.

Scope for the future work

The development of oral cancer is a multistep process arising from pre-existing potentially malignant lesions. More study is needed in different stages of oral cancer which can give us a better idea about the levels of these markers in different stages of oral cancer which helps in better diagnosis.

Author Contribution Statement

Madhura M M, Pushparaj Shetty, Shwetha A Neralakatte, Preethi Hegde, Rajeshwari and Nireeksha conceived and designed the work. Pushparaj Shetty, Mehaboob Ali, Kundubai A Mamatha Kshatriya were involved in the laboratory investigation, analysis and drafting the manuscript. Naresh Yajamanam helped in standardisation oif the methods and statistical analysis. All authors discussed the results and contributed to the final manuscript.

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Conflict of Interest

The author declares no conflict of Interest.

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