Original Research Article
Journal of Pierre Fauchard Academy (India Section), *Vol 38(3-4), DOI: 10.18311/jpfa/2024/46643, July-December 2024 p. 40-45*
ISSN (Print) : 0970-2199
ISSN (Online) : 2405-772X

A Quantitative Analysis of Serum Alkaline Phosphatase in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma in Population of East Singhbhum District: A Comparative Study

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Abstract

Background: Serum Alkaline Phosphatase (ALP) is an enzyme and a potential marker for the early detection of oral malignancies. Numerous studies have been conducted to explore its role as a serum biomarker. **Aims and Objectives:** To evaluate the diagnostic potential of serum ALP in identifying Oral Potentially Malignant Disorders (OPMD) and Oral Squamous Cell Carcinoma (OSCC). **Materials and Methods:** Samples were collected from patients visiting the outpatient department of Awadh Dental College & Hospital after obtaining informed consent. Serum ALP levels were measured and statistical analysis was performed. A literature search was conducted using databases PubMed, Google Scholar, EBSCO, and Wiley Online. Keywords used included "serum ALP," "oral potentially malignant disorders," "oral squamous cell carcinoma," and "serum biomarkers." A review of literature of relevant studies concerning the regulation and role of ALP in individuals with these disorders and conditions is also presented. **Results:** The mean Serum Alkaline Phosphatase (ALP) levels in the three study groups were 72.7 mg/dl for the control group, 119.7 mg/dl for the OPMD group, and 175.6 mg/dl for the OSCC group, with corresponding Standard Deviations (SD) of 16.4, 34.1, and 53.8, respectively. **Conclusion:** This study suggests that serum ALP holds promise as a reliable serum biomarker for detecting oral cancer. Several studies over the past decade have also supported this finding.

Keywords: Serum ALP, Oral Potentially Malignant Disorders, Oral Squamous Cell Carcinoma, Serum Biomarkers

1. Introduction

The concept of malignant changes in the oral cavity has been recognized for quite some time. One of the earliest observations was made by Sir James Paget in 1870[1], who noted carcinomatous changes in a lesion located on the tongue. Since then, the understanding of oral malignancies has evolved considerably. Today, the World Health Organization (WHO) classifies a group of disorders under the term "Oral Potentially Malignant Disorders" (OPMD), which includes both lesions and conditions that carry a risk of malignancy, either at the time of diagnosis or in the future[2,3].

OPMD is categorized into two main groups: precancerous lesions and precancerous conditions. A precancerous lesion refers to tissue that has undergone morphological changes, making it more susceptible to developing cancer[3-5]. Leukoplakia, Actinic keratosis,

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Article Received on: 22.10.2024 Revised on: 25.10.2024 Accepted on: 13.11.2024

Actinic cheilitis, Bowen disease etc., are a few examples of pre-cancerous lesions. In contrast, precancerous conditions may not show significant changes in the appearance of the tissue, but they are associated with an increased risk of future malignancy[5]. Lichen planus, Submucous fibrosis, Epidermolysis bullosa, Discoid lupus erythematous etc., are a few examples of pre-cancerous conditions. This distinction is important in clinical settings as it guides treatment approaches and follow-up protocols. Among the lesions included in OPMD, the overall malignant transformation rate has been observed to be 7.9%, with variations across specific subgroups. For example, the malignant transformation rate for Lichen Planus (LP) is 1.4% (as per recent studies the rate of malignant transformation of lichen planus is debatable due to stringent diagnostic criteria), Leukoplakia (LE) is 9.5%, Oral Lichen Lesions (OLL) is 3.8%, Oral Submucous Fibrosis (OSF) is 5.2%, Oral Erythroplakia (OE) is 33.1%, and Proliferative Verrucous Leukoplakia (PVL) has a transformation rate of 49.5%[3,4].

Oral Squamous Cell Carcinoma (OSCC) is the most common form of oral cancer, accounting for up to 90% of all oral malignancies. Its incidence varies significantly across different geographic regions and between genders. Depending on the location and demographic characteristics of the patient population, the oral cavity ranks between the 6th and 9th most common site for cancer. Despite advances in medical science, the mortality rate associated with OSCC remains high, underscoring the importance of early detection in improving patient outcomes. One of the key areas of ongoing research is identifying reliable biomarkers that can aid in the early detection and prognosis of OSCC, which is the focus of this study. According to the FDA-NIH Biomarker Working Group, a biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention."[5] Serum biomarkers are particularly valuable because they are easily accessible through blood tests and can be quantitatively measured. One such biomarker that has garnered attention in recent years is serum Alkaline Phosphatase (ALP).

Alkaline Phosphatase (ALP) is an enzyme belonging to the hydrolase group, functioning optimally in an alkaline environment. It is primarily found in tissues such as the liver, biliary epithelium, and bone. While its normal values fall within a specific range, ALP levels increase in liver and bone diseases and show moderate elevation in conditions like Hodgkin's disease and congestive heart failure.

At high pH, ALP catalyzes the hydrolysis of p-Nitrophenylphosphate into p-Nitrophenol and phosphate. The formation of p-Nitrophenol can be measured by the increase in absorbance, which is directly proportional to the ALP activity in the sample.

Reaction: p-Nitrophenylphosphate → p-Nitrophenol + Phosphate

In clinical practice, ALP levels are often measured as part of liver function tests, but elevated ALP levels have also been associated with several conditions, including bone diseases, periodontitis, dental caries, and oral potentially malignant disorders. The normal range of serum ALP is between 20 and 140 IU/L, but deviations from this range have been linked to malignancies. For example, elevated ALP levels are commonly observed in patients with metastatic cancers, such as liver metastasis from colorectal cancer or bone and liver metastases in breast cancer[7]. Interestingly, high ALP levels are sometimes observed even in the absence of metastasis, raising questions about its potential role in primary tumors. In the case of OSCC, the relationship between elevated ALP levels and cancer progression remains unclear, and further studies are needed to explore this association[6,7].

Given the potential utility of ALP as a biomarker in various diseases, this study aims to investigate whether there are changes in serum ALP levels in patients with OPMD and OSCC[8]. The aim is to determine whether ALP levels are elevated in patients with OPMD, or whether the increased quantity is observed only in cases of established OSCC. By exploring the role of ALP as a biomarker, this study seeks to contribute to the early detection and better management of oral cancers.

2. Materials and Method

2.1 Sample Selection

The study included a total of 60 subjects from East Singhbhum area divided into three groups: 20 healthy subjects (controls), 20 subjects diagnosed with Oral Potentially Malignant Disorders (OPMD), and 20 subjects with Oral Squamous Cell Carcinoma

(OSCC). These individuals, aged between 30 and 70 years, were selected from those visiting the outpatient department of Awadh Dental College and Hospital during the years 2020 and 2021. The study does not consider confounding factors (Sex, comorbidities, or medications that may impact liver function) which might act as a limitation of the study. Patients were asked about medical history prior to consideration and cases were chosen on the basis of clinical and histopathological findings.

2.2 Sample Collection

Before collecting blood samples from the subjects, informed consent was obtained, and a detailed case history was recorded. All procedures were conducted following the necessary sterilization protocols to ensure safety during sample collection.

Each patient was informed about the procedure in advance. 5ml of venous blood was drawn from the antecubital vein while the patient was in an upright or supine position. A small needle was inserted into the vein, and a collection tube was attached to gather the blood sample. Following collection, the blood samples were centrifuged to obtain the serum, which was then transferred into a clean, dry test tube for further analysis.

2.3 Procedure for Measuring Serum Alkaline Phosphatase (ALP)

1. Preparation of Working Reagent: A working reagent tablet was added to 3.2 ml of buffer liquid and dissolved completely to form the working reagent. This prepared reagent could be used for up to 15 days.
2. Sample Preparation: A clean, dry test tube was used, and 1 ml of the working reagent was added. The solution was incubated for 3-4 minutes.
3. Sample Addition and Incubation: A 20-microliter serum sample was added to the prepared working reagent. The mixture was shaken well and then incubated for 5 minutes.
4. Measurement: The serum ALP levels were measured using a chemical biochemistry semi-autoanalyzer.

3. Results

On comparing the serum ALP levels among different groups, malignant cases showed higher values compared to the other groups. Among the malignant lesions, the poorly differentiated showed a higher value compared to early squamous cell carcinoma. The mean serum Alkaline Phosphatase (ALP) levels in the three study groups were 72.7 mg/dl for the control group, 119.7 mg/dl for the OPMD group, and 175.6 mg/dl for the OSCC group, with corresponding standard deviations (SD) of 16.4, 34.1, and 53.8, respectively. This data shows a consistent increase in serum ALP levels across the groups, with values progressively higher from controls to OPMD and OSCC (Control < OPMD < OSCC). The p-value was found to be 0.001, indicating that the results are statistically significant (Table 1, Figure 1).

Table 1. Comparison of mean serum ALP levels between the groups

Groups	Mean ALP concentration	SD	p-value
Control	72.7	16.4	0.001*
Oral potentially malignant disorders	119.7	34.1	
Squamous cell carcinoma	175.6	53.8	

*Statistically significant difference exists between the groups ($p \leq 0.05$)

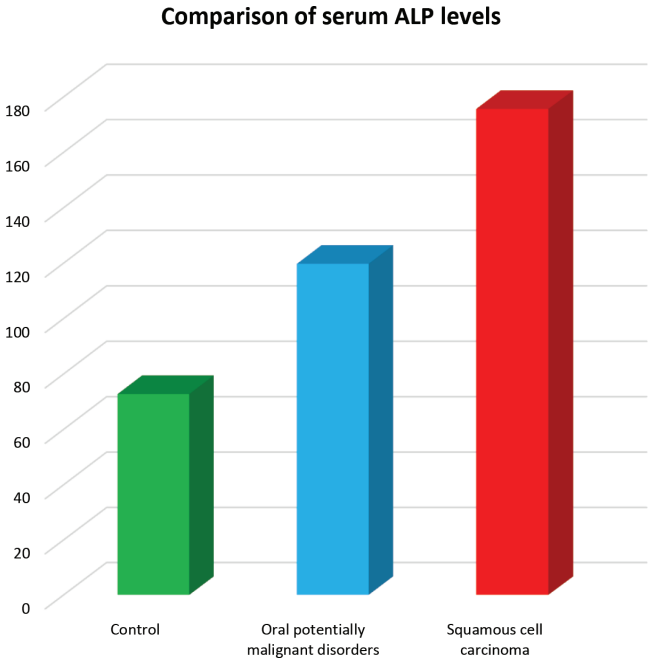


Figure 1. Mean serum alkaline phosphatase levels in three groups; Healthy subjects (controls), subjects with Oral Potentially Malignant Disorders (OPMD), and Oral Squamous Cell Carcinoma (OSCC).

4. Discussion

Alkaline Phosphatase (ALP) has long been studied as a potential biomarker in malignancy. The level of alkaline phosphatase usually increases in cancer patients due to various reasons, such as metastases, active bone formation, tumor growth etc. When cancer spreads to bones, the tissues release ALP into the blood. ALP also indicates osteoblast activity. Early work by Fishman and Stolbach highlighted the elevation of ALP, particularly the Regan isoenzyme, in patients with established cancers[7]. Subsequent research by Saif, Alexander, and Wilcox demonstrated that ALP levels are notably elevated in carcinoma patients, especially in the presence of metastasis[8]. Klompje *et al.,* further corroborated this, observing that patients with elevated ALP were more likely to have metastatic disease. These foundational studies have laid the groundwork for understanding the role of ALP in cancer diagnosis and prognosis[9].

In the current study, serum ALP levels were measured across three distinct groups: a control group of healthy individuals, a group of individuals with Oral Potentially Malignant Disorders (OPMD), and a group of individuals diagnosed with Oral Squamous Cell Carcinoma (OSCC). The findings demonstrated a clear and statistically significant elevation of serum ALP across these groups. Specifically, the control group had a mean ALP level of 72.7 mg/dl, the OPMD group exhibited a mean of 119.7 mg/dl, and the OSCC group had the highest mean at 175.7 mg/dl. With a p-value of 0.001, these differences were found to be statistically significant, indicating that the elevation of ALP is indeed associated with disease progression (Control < OPMD < OSCC).

The findings align with previous studies. For example, in 1992, Van Hoof and colleagues compared serum ALP levels among healthy individuals, cancer patients, and individuals with non-cancerous liver and bone diseases. Their study also found significant variations in ALP levels across these groups, supporting the current study's observation that ALP levels increase in the presence of malignancy[10]. Similarly, Rasam *et al.,* (2009) emphasized the role of heat-stable ALP as a biomarker in head and neck cancers, though they noted that general serum ALP elevation was not always significant in these cancers[11].

Other researchers have confirmed the association between ALP elevation and cancer progression, particularly in cases where metastasis or nodal involvement is present. Aminian *et al.,* reported that patients with esophageal carcinoma experienced a 3.29-fold increase in serum ALP when metastasis or nodal involvement was detected[12], while Marina *et al.,* (2013) observed a two-fold increase in ALP in metastatic cancer patients. These studies emphasize that while ALP levels may be elevated in primary tumors, the increase becomes more pronounced in advanced or metastatic disease. The present study's results, which show a substantial elevation in ALP levels in OSCC patients, are consistent with these findings.

Dhivyalakshmi and Maheswari (2014) also observed an increase in ALP levels in squamous carcinoma, corroborating the findings of the present study. Acharya *et al.,* (2017)[13] and Bhattacharya *et al.,* (2018) similarly reported elevated ALP levels in squamous cell carcinoma patients, with Acharya *et al.,*[14] noting a 24% increase and Bhattacharya *et al.,* observing that 19% of OSCC patients had elevated serum ALP levels[15] .

It is important to note that not all studies have found significant associations between ALP elevation and head and neck cancers. Banseria *et al.,* (2013) collected serum ALP data from patients with head and neck Squamous Cell Carcinoma (SCC) but found that the elevation in ALP levels was not statistically significant. Similarly, while Rasam *et al.,* identified the presence of heat-stable ALP as a potential biomarker, they concluded that overall serum ALP elevation was not significant in the same group. This discrepancy may be due to differences in study design, sample sizes, or the specific cancers under investigation.

Despite these conflicting results, the present study provides compelling evidence that serum ALP levels increase significantly in patients with OPMD and OSCC[16]. The consistent rise in ALP from the control group to the OPMD group and further to the OSCC group suggests that serum ALP could serve as a useful biomarker for early detection and monitoring of disease progression in oral cancers. While further studies are needed to confirm these findings and explore the mechanisms underlying ALP elevation in OSCC, the results of this study are a significant step toward understanding the potential role of ALP as a biomarker in oral malignancies (Table 2).

Table 2. Review of literature of various studies concerning serum alkaline phosphatase levels in patients diagnosed with OPMD and OSCC

S. No.	Year	Author	Article, Journal	Findings
1	1992	Van Hoof et al.[10]	Alkaline phosphatase isoenzyme pattern in malignant disease. Clin chem. 1992; 38(12):2546-51.	Cases with liver metastasis show higher ALP value than without metastasis.
2	2009	Rasam et al.[11]	Heat-Stable Alkaline Phosphatase A putative tumor marker of head and neck squamous cell carcinoma. Acta Oncologica.1995; 34(1):49-52.	SCC patients showing higher levels of Heat Stable Alkaline Phosphatase
3	2005	Saif, Alexandar, Wicox et al.[8]	Serum Alkaline Phosphatase Level as a Prognostic Tool in Colorectal Cancer: A Study of 105 Patients. J Appl Res. 2005; 5(1): 88–95.	Level of serum ALP is 5.5 times in the presence of metastasis
4	2010	Merza K S,Alaaraji S B, Abullah B H[17]	Comparative study on lactate dehydrogenase, Alkaline phosphatase and immunoglobulins in Serum and saliva of acute leukaemia and oral Squamous cell carcinoma patients. Iraqi Journal of Science.2010; 51(2):262-70.	In OSCC patient's serum ALP is 11.06+_3.06 and that of control is 6.67+_2.7.
5	2011	Aminian et al.[12]	Correlation of serum alkaline phosphatase with clinicopathological characteristics of patients with oesophageal cancer. Eastern Med Health J. 2011; 17(11):862-6.	Cancer patients with nodal involvement show a 3.29 times higher ALP value.
6.	2013	Marina et al.[21]	Values of alkaline phosphatase and their isoenzyme profiles in patients with cancer with respect to bone and liver metastasis. Arch Oncol. 2013; 21(1):14-6.	In case of malignancy ALP activity is higher
7.	2014	Dhivyalaxmi, Maheshwari et al. [13]	Expression of Salivary Biomarkers – Alkaline Phosphatase & Lactate dehydrogenase in Oral Leukoplakia.2014; 6(5):3014-8.	Salivary ALP value is higher in OSCC and OPMD than control
8.	2017	Carroll et al.[18]	Elevated Alkaline Phosphatase in a Cancer Patient: Think You Know the Source? J App Lab.2017; 2(3):440-3.	Ewing sarcoma has elevated ALP value
9.	2017	Shetty et al[19]	Salivary alkaline phosphatase and oral health: A review. Ital J Dent Med.2017; 2(2):55-8.	OPMD showed elevated ALP value
10.	2017	Acharya et al[14]	Serum alkaline phosphatase in oral squamous cell carcinoma and its association with clinicopathological characteristics. South Asian J Cancer.2017; 6:125-8.	OSCC had a higher value of ALP
11.	2017	Manimegalai et al[20]	Role of Salivary Alkaline Phosphate in Detecting Oral Diseases- A Review. J Med Sci Clin Res.2017; 5(12):31904-7.	S-ALP value is higher in OSCC
12.	2018	Bhattacharjee et al[15]	Correlation of serum lactate dehydrogenase and alkaline phosphatase in different histological grades of head and neck squamous cell carcinoma and premalignant lesions. J Can Res Ther.2018; 14:934-40	ALP value is higher in cancer patients but not statistically significant

5. Conclusion

In conclusion, this study demonstrates a statistically significant elevation of serum ALP in patients with OPMD and OSCC, supporting the hypothesis that ALP may serve as a useful biomarker for early detection and disease progression in oral cancer. Given the established role of ALP in other malignancies, these findings warrant further investigation into its potential clinical applications in oral squamous cell carcinoma.

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