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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF SALIVARY ALPHA ENOLASE LEVELS IN PATIENTS WITH SUBMUCOUS FIBROSIS AND HEALTHY CONTROLS

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ABSTRACT

Oral submucous fibrosis (OSMF) is presently recognized as a potentially malignant disorder (PMD), with the likelihood of developing to oral squamous cell carcinoma ranging between 7 to 30%. Alpha-enolase, a lysosomal enzyme essential to glucose metabolism, is associated in disrupted energy production during carcinogenesis, guiding uncontrolled cell proliferation in numerous cancers. Identifying the connection between OSMF and salivary alpha-enolase could offer a helpful means for early diagnosis of malignancy, establishing the path for its application as a biomarker in both diagnosis and treatment. This study seeks to investigate and assess the salivary alpha-enolase levels in OSMF patients to healthy controls, proposing to find possible variations that might contribute to an early diagnostic breakthrough.

Material and methods: Saliva samples were taken from 20 participants in each group (OSMF sufferers and healthy controls). Salivary alpha-enolase levels were identified using the sensitive and precise enzyme-linked immunosorbent assay (ELISA). Statistical analysis were carried out using the Independent Sample t-test to establish significance.

Results: The results indicated that salivary alpha-enolase levels in OSMF patients were significantly higher in comparison to those in healthy people.

Conclusion: Salivary alpha-enolase could become a reliable biomarker for early OSF identification, thereby offering an opportunity for timely intervention, more efficient treatment planning, and improved outcomes for patients.

Keywords: Oral submucous fibrosis, Salivary alpha enolase, Oral premalignant disorders, ENO1 gene

1. INTRODUCTION

OSMF is defined by the excessive buildup of collagen and is recognised as a precancerous state, with the tendency to develop to malignant tumours.[1] This disorder provides a high likelihood (7–30%) of developing into oral squamous cell cancer.[2] The main feature of OSMF is its extensive detrimental impact on the oral cavity, which frequently results in major implications such as tissue scarring, dysphagia, and trismus.[3]

The specific origin of OSMF is uncertain; nevertheless, it is thought to develop from a combination of elements, such as areca nut chewing, eating spicy foods, genetics, immunological responses, and malnutrition.[4] These features lead to prevalent symptoms comprising restricted mouth opening, sensation of burning, and stiffness of the oral mucosa.[5] The epithelium often displays significant modifications, such as epithelial atrophy and, in certain instances, hyperplasia with or without dysplasia.[6]

Many molecular pathways play a part in the development of OSMF. Principal factors involve myofibroblasts, cytokines, and noncoding RNAs, all of which promote the progression of the disease.[7]

A biomarker is a discernible and quantifiable feature that indicates normal biological process, pathological process or respond to exposure or treatment.[8] Because molecular markers play a vital role in diagnosing, monitoring as well as treating OSMF, the screening of the biomarkers has become increasingly popular in recent years.[9] Enolase, an important enzyme in glycolysis has a vital part in glucose metabolism, which is closely linked to altered energy production during cancer development. These changes contribute to abnormal cell growth and metastasis.[10] The ENO (Enolase) gene family includes three isoforms ENO1, ENO2, and ENO3 (Enolase1, Enolase2, Enolase 3) each with distinct functions. Alpha-enolase (ENO1) is particularly important as it is found on the cell surface, where it functions as a plasminogen receptor, progressing cell migration and promoting cancer metastasis.[11]ENO1 is involved in numerous pathological and biological processes, mainly in cancer and autoimmune diseases.[12] Its expression is strongly associated with decreased survival rates and poor detection across many cancer types.[13] Overexpression of ENO1 and its post-translational changes have significant diagnostic and predictive implications in several cancers.[14] Notably, ENO1 can be translated into a 37 kDa (kilo Dalton) protein known as (MBP-1) c-myc promoter-binding protein through an alternate start codon.[15]

ENO1 interacts with MBP-1 in the transcriptional regulation of the oncogene c-myc.[16] Additionally, ENO1 has the ability to bind to DNA (Deoxyribonucleic Acid), mRNA (messenger Ribonucleic Acid), lncRNA (Long noncoding RNA), and tRNA Lys (Lysin Transferase Ribonucleic Acid), thereby influencing gene transcription and translation in cancer cells.[17] This emphasizes on the significant role of ENO1 in the molecular mechanisms underlying cancer progression and metastasis.[18]

The current focus is on developing minimally invasive and non-invasive diagnostic procedures for detecting lesions. Recent discoveries in scientific technology has led to the discovery of specific salivary biomarkers for various clinical conditions, including cancer.[19] Saliva is an ideal diagnostic medium as it is easily accessed and can be collected in a non-invasive manner.[20]

Using saliva for diagnosis offers numerous benefits, such as ease of collection, minimal staff training, rapid sampling, convenient storage, simple transportation, lower susceptibility to clotting, and reduced health risks.[21] Salivary biomarkers are rapidly emerging as promising diagnostic tools, offering speed, accuracy, non-invasiveness, and higher patient acceptance.[22,23] These advantages make salivary diagnostics a valuable and patient-friendly alternative in clinical practice. Prior to this, no other previous studies have evaluated salivary alpha-enolase as a biomarker in OSMF patients. Therefore, the objective of this study was to assess the salivary alpha-enolase levels in patients with OSMF and healthy controls and comparing the results between the two groups.

2. MATERIAL AND METHODS

The study was approved by Krishna Vishwa Vidyapeeth Ethics Committee of Karad (ref no.- KVV/IEC/10/2024) India. Written informed consent was taken from all the participants before saliva collection in accordance with ethical guidelines.

2.1 Recruitment of Study Subjects

The study comprised 20 cases of OSMF and 20 healthy controls from the School of Dental sciences Karad Outpatient Department. Medical records and demographic details were collected. Patients with any infectious diseases within a month prior to saliva sampling, active dental abscesses, systemic illness, collagen vascular diseases, or were undergoing any form of medical or dental treatment were excluded. Pregnant and lactating individuals were also excluded. None of the control participants presented with oral lesions.

2.2 Sample collection

Saliva samples were obtained between 9:00 and 12:00 A.M. under non-stimulatory conditions. Participants were instructed to abstain from eating, chewing, or drinking for at least one hour prior to collection. A total of 4 to 5 milliliters of saliva was collected from OSMF patients before any therapeutic interventions. The samples were then centrifuged immediately to remove cellular debris, and the supernatant was stored at −80°C for subsequent analysis.

2.3 Estimation of alpha enolase

Alpha enolase concentrations in saliva were quantified using a commercial ELISA Kit from Bioassay Technology, following the manufacturer's instructions. The assay was performed according to the provided protocol, with absorbance measured at 450 nm using a Robonik ELISA plate reader. The results were expressed in units of nanograms per milliliter (ng/ml) for saliva samples.

2.4 Statistical analysis

The data were analyzed using IBM SPSS statistics 21. Independent sample t-test is used to compare means of two groups to determine if they are statistically different. The descriptive statistics such as mean and standard deviation were calculated for each group.

3. RESULTS

The study included 20 control participants and 20 participants with Oral Submucous Fibrosis (OSMF), matched for age and sex. The age range for the control group was 19 to 30 years, while for the OSMF group, it was 24 to 50 years. Both groups were predominantly male. All participants in the OSMF group had a history of tobacco or paan chewing, smoking, or alcohol use.

Table 1. Demographic Parameters in Healthy and OSMF Patients Descriptive statistics

	N	Range	Minimum	Maximum	Mean	Std Error	Std deviation
Healthy salivary α-enolase concentration	20	.26	.52	.78	.65	.02	.08
OSMF salivary α-enolase concentration	20	.18	.83	1.01	.93	013	.06

Table 1. shows OSMF patients salivary α-enolase concentration values (0.83–1.01) are consistently higher compared to healthy patients (0.52–0.78), indicating a potential biomarker for differentiation. OSMF patients demonstrate significantly high salivary α-enolase concentration compared to healthy individuals, which could serve as diagnostic markers. The higher variability in age among OSMF patients may reflect a broader demographic susceptibility. The minimal variability in measurements across groups suggests reliable data collection methods.

Table 2. Descriptive Statistics and Distribution Analysis of salivary α-enolase Concentrations in Healthy and OSMF Patients

			Statistic	Std. Error
Healthy patients salivary α-enolase concentration	Mean		.66	.01852
	95% Confidence Interval for Mean	Lower Bound	.62	
		Upper Bound	.69	
	5% Trimmed Mean		.66	
	Median		.66	
	Variance		.007	
	Std. Deviation		.083	
	Minimum		.52	
	Maximum		.78	
	Range		.26	
	Interquartile Range		.13	
	Skewness		-.211	.512
	Kurtosis		-1.13	.992
OSMF patients salivary α-enolase concentration	Mean		.93	.01309
	95% Confidence Interval for Mean	Lower Bound	.90	
		Upper Bound	.96	
	5% Trimmed Mean		.93	
	Median		.93	
	Variance		.003	
	Std. Deviation		.06	
	Minimum		.83	
	Maximum		1.01	
	Range		.18	
	Interquartile Range		.10	
	Skewness		-.09	.512
	Kurtosis		-1.22	.992

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Table 2 shows α-enolase Concentration in Healthy Patients. The mean concentration is **0.66**, with minimal variability (std. deviation **0.083**) and a narrow range (**0.26**). The skewness (**-0.211**) and kurtosis (**-1.13**). The mean salivary α-enolase concentration (**0.93**) is significantly higher than in healthy patients (**0.66**), with minimal variability (std. deviation **0.06**) and a narrow range (**0.18**). The distribution metrics (skewness: **-0.09**, kurtosis: **-1.22**). OSMF patients have consistently higher salivary α-enolase concentration values than healthy individuals. These parameters could serve as biomarkers for diagnosing OSMF. Both groups show slight left skewness and flatter distributions, but the differences in means and ranges highlight clear distinctions between healthy and OSMF groups. Narrow confidence intervals and low standard errors across all metrics reflect reliable data and precise measurements.
Table 2 highlights the potential diagnostic significance of salivary α-enolase concentrations in distinguishing between healthy and OSMF patients.

Table 3. Normality Test Results for salivary α-enolase Concentration Measurements in Healthy and OSMF patients

Tests of Normality						
	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
α-enolase concentration in healthy patients	.155	20	.200*	.931	20	.162
α-enolase concentration in OSMF patients	.101	20	.200*	.947	20	.327
*. This is a lower bound of the true significance.						
a. Lilliefors Significance Correction						

Table 3 indicate the tests of normality, including the Kolmogorov-Smirnov test with Lilliefors correction and the Shapiro-Wilk test, were conducted to evaluate the distribution of α-enolase concentration measurements in healthy and OSMF patients. For healthy patients α-enolase Concentration showed non-significant p-values in both tests ($p > 0.05$), indicating no significant deviation from normality. Similarly, for OSMF patients, salivary α-enolase Concentration also demonstrated non-significant results in both tests, confirming normal distribution.

Healthy patients salivary α-enolase concentration

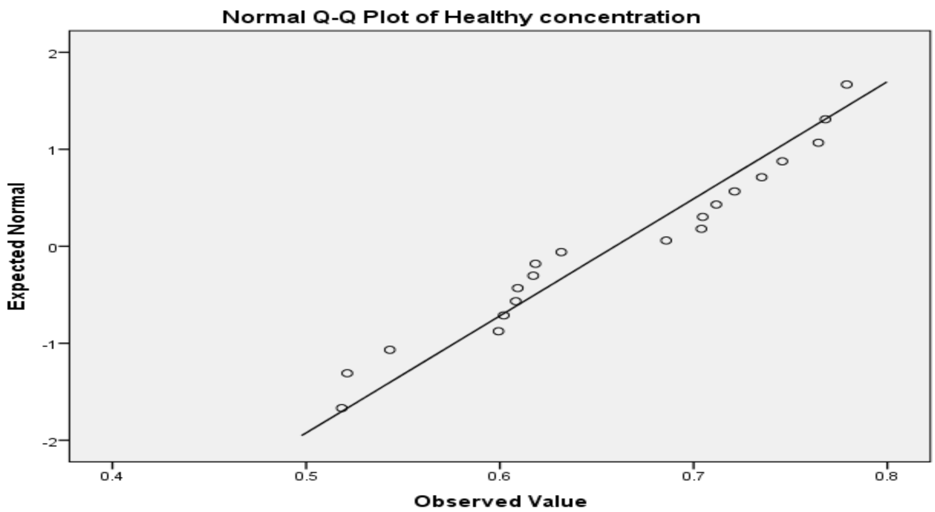

Figure 1. Normal Q-Q Plot for Healthy patients salivary α-enolase concentration

Figure 1 illustrates the Normal Q-Q Plot of salivary α-enolase concentration in healthy patients, comparing the measured values to a theoretical normal distribution. The points nearly align with the diagonal line, suggesting that the data seems to be regularly distributed. Minor deviations are noted at the extremes; nevertheless, they are insignificant, indicating that the assumption of normality applies for this dataset.

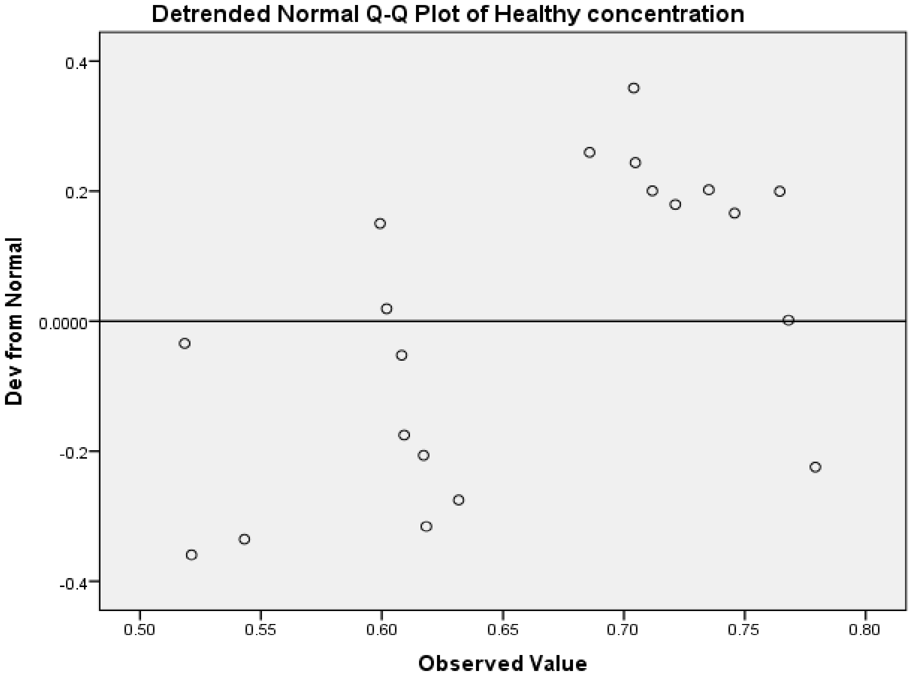

Figure 2. Detrended Q-Q Plot for Normality Assessment of Healthy patients salivary α-enolase concentration

Figure 2 illustrates the Detrended Normal Q-Q Plot of salivary α-enolase concentration deviations from normality in healthy patients by plotting the residuals (the differences between observed and predicted values under normality). The majority of dots are clustered near the zero line, signifying negligible deviations and corroborating the hypothesis of normalcy. Nevertheless, few points diverge marginally, especially near the extremes, indicating tiny deviations from an ideal normal distribution. The data seems to conform to the principles of normality.

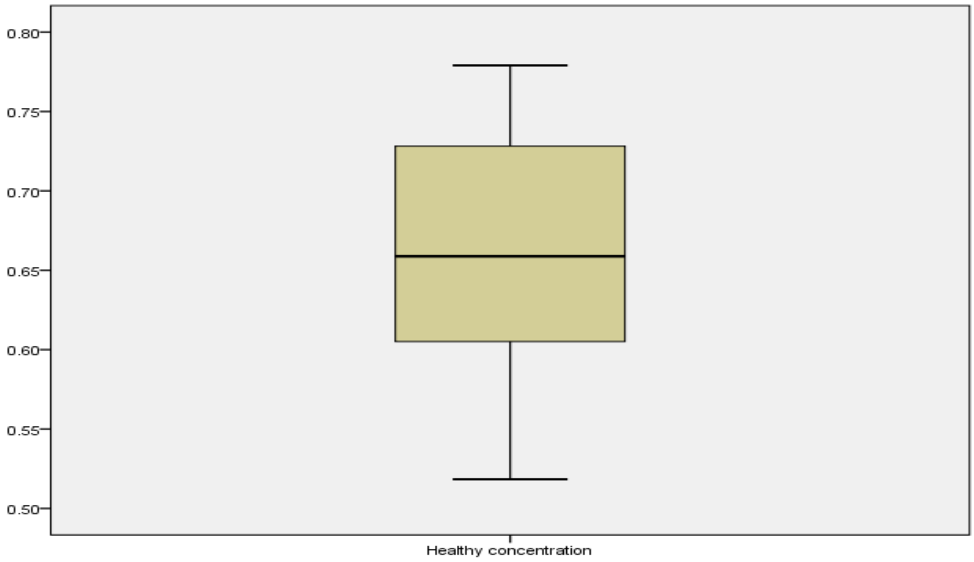

Figure 3. Boxplot of Healthy patients salivary α-enolase concentration

Figure 3 shows the boxplot for healthy patients salivary α-enolase concentration distribution of data through the interquartile range (IQR) and central tendency. The median, represented by the line inside the box, is around 0.66, indicating a central value close to 0.65. The box itself covers the middle 50% of the data, with the lower quartile around 0.05 and the upper quartile around 0.10. The whiskers extend from the box to the minimum (0.52) and maximum (0.77) values, capturing the range of the data. There appear to be no extreme outliers, as the data is relatively evenly distributed within the box and whiskers. This suggests a fairly normal distribution with moderate variability.

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OSMF patients α-enolase concentration

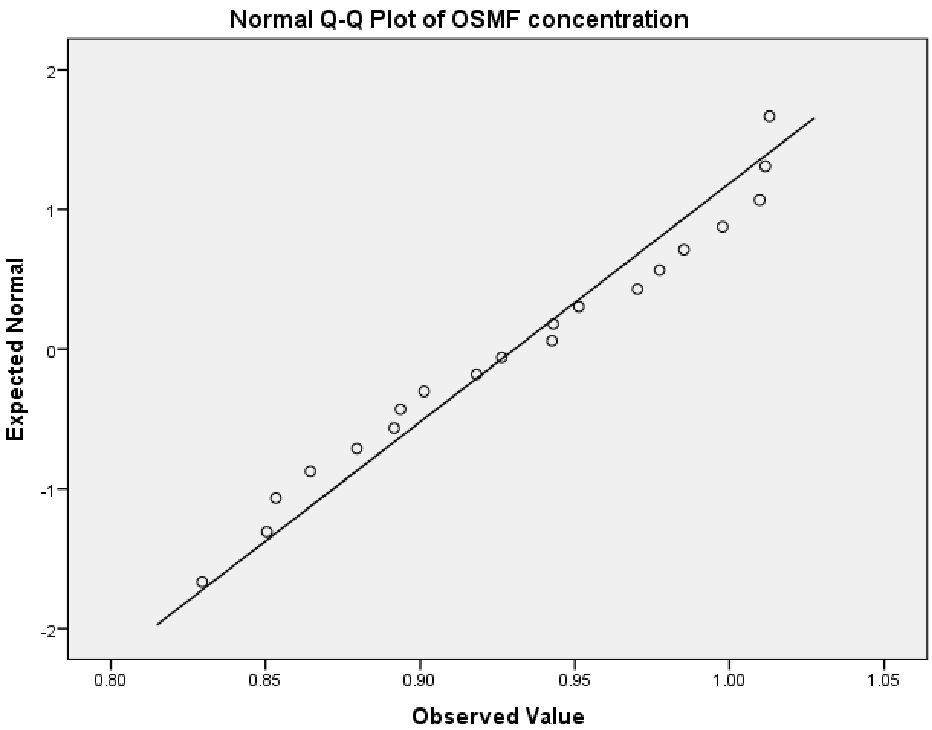

Figure 4. Normal Q-Q Plot for OSMF patients salivary α-enolase Concentration

Figure 4 illustrates the Normal Q-Q Plot of salivary α-enolase concentration in OSMF patients, comparing the observed values to a theoretical normal distribution. The points nearly align with the diagonal line, suggesting that the data is approximately regularly distributed. Minor deviations are noted at the extremes; nonetheless, they are insignificant, indicating that the assumption of normality holds for this dataset.

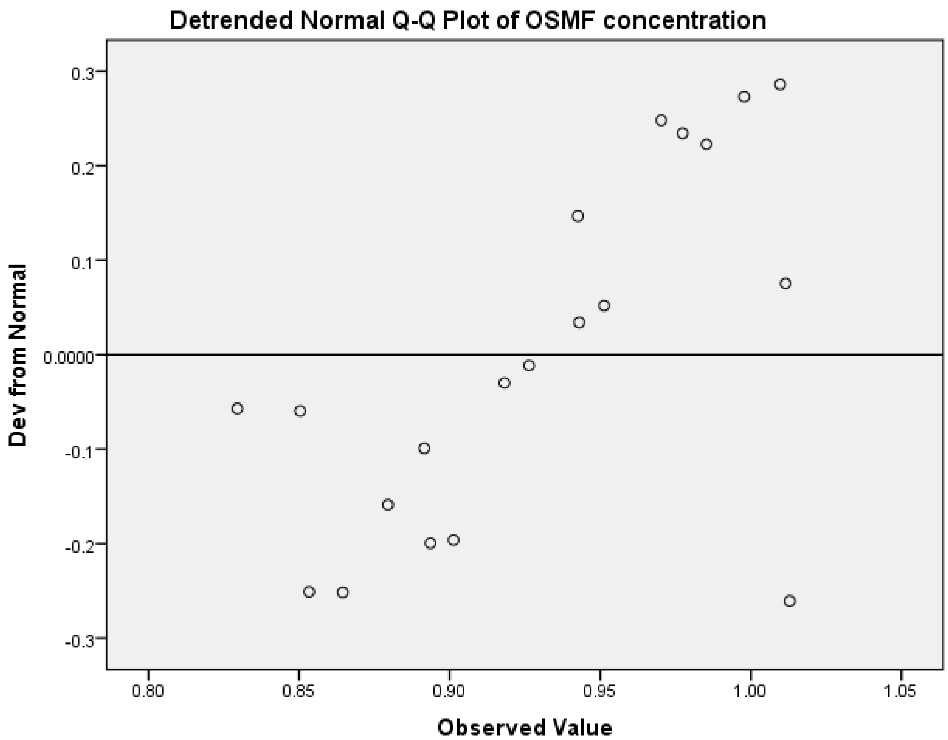

Figure 5. Detrended Q-Q Plot for Normality Assessment of OSMF patients salivary α-enolase Concentration

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Figure 5 shows the Detrended Normal Q-Q Plot of OSMF patients salivary α-enolase concentration deviations from normality by plotting the residuals (differences between observed and expected values under normality). Most points are scattered closely around the zero line, indicating minimal deviations and supporting the assumption of normality. However, a few points deviate slightly, particularly at the extremes, suggesting minor departures from a perfectly normal distribution. Overall, the data appears to align well with normality.

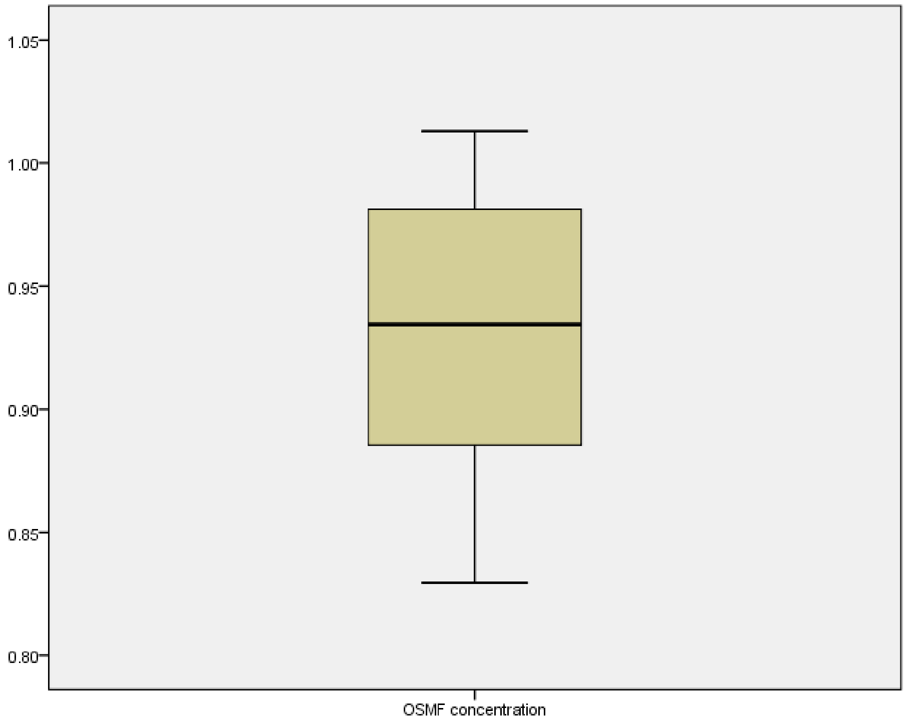

Figure 6 boxplot shows OSMF patients salivary α-enolase concentration average distribution of data through the interquartile range (IQR) and central tendency. The median, represented by the line inside the box, is around 0.93, indicating a central value close to 1. The box itself covers the middle 50% of the data, with the lower quartile around 0.05 and the upper quartile around 0.50. The whiskers extend from the box to the minimum (0.83) and maximum (1.20) values, capturing the range of the data.

Table 5. Comparison of salivary alpha enolase Concentration in ng/mL between Patients with Oral Submucous Fibrosis (OSMF) and Healthy Individuals

Comparison		N	Mean	Std. Deviation	Std. Error Mean	t	Sig. (2-tailed
Comparison of salivary alpha enolase Concentration in ng/mL in patient s with OSMF as compared to Healthy individuals	Healthy individuals	20	.66	.08	.018	-11.951	.000*

Table 5 compares the salivary α-enolase concentration (ng/mL) between patients with Oral Submucous Fibrosis (OSMF) and healthy individuals. Healthy Individuals (Mean - 0.66±0.08) had lower salivary α-enolase concentration with minimal variability. OSMF Patients (Mean - 0.93°±0.06) had higher salivary α-enolase concentration, suggesting an association with disease status. The difference (t = -11.951, Sig. (2-tailed) = 0.000) is statistically significant, indicating a clear distinction in concentration levels between groups.

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The findings highlight significant differences between OSMF patients and healthy individuals in terms of concentration. Concentration levels (ng/mL) are elevated in OSMF patients. The statistical significance ($p < 0.001$) across all comparisons indicates a strong correlation between these measures and the presence of OSMF. These results suggest concentration may serve as reliable markers for identifying or studying OSMF.

Table 6. Independent Samples t-Test for Comparison of alpha enolase Concentration between Patients with OSMF and Healthy Individuals

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Comparison of Concentration in ng/mL in patients with OSMF as compared to Healthy individuals	Equal variances assumed	4.868	.033	-11.951	38	.000*	-.27106	.02268	-.31698	-.22514
	Equal variances not assumed				34.185	.000*	-.27106	.02268	-.31714	-.22498

Table 6 shows the independent samples t-test results significant differences between patients with Oral Submucous Fibrosis (OSMF) and healthy individuals across all comparisons, for the salivary alpha enolase concentration in ng/mL, the t-test also revealed a significant difference ($t = -11.951$, $p < 0.000$), with a mean difference of -0.27106. The confidence intervals for all comparisons did not include zero, indicating robust statistical significance. Overall, these results demonstrate that patients with OSMF have notably higher concentrations than healthy individuals, highlighting potential biochemical or physiological changes associated with the condition.

4. DISCUSSION

Oral potentially malignant disorders (OPMDs), including erythroplakia, leukoplakia, and OSMF, are known to precede the development of oral squamous cell carcinoma (OSCC), with OSMF being the most commonly encountered, particularly in Southeast Asia.[24] OSMF is a chronic, insidious, and progressive condition, often leading to trismus due to submucosal fibrosis in various parts of the oral cavity. Individuals affected by OSMF face a significantly higher risk of developing oral cancer, with the malignant transformation rate to squamous cell carcinoma estimated to range between 7% and 30%.[25]

Enolase, or phosphopyruvate hydratase, was initially discovered in 1934 by Lohman and Mayerhof.[26] It is among the most widely expressed cytosolic proteins in many organisms and is essential for glycolysis, hence playing a critical role in glucose metabolism. Enolase has been associated with modified energy generation during carcinogenesis, as well as with dysregulated cellular proliferation and metastasis.[27] The ENO1 gene, encoding alpha enolase, is thought to be significantly linked to cancer formation in the oral epithelium.[28]

Assessing alpha-enolase (ENO1) levels provides an appealing strategy for monitoring potentially malignant oral illnesses and how they become malignancies. Oral submucous fibrosis (OSMF), a potentially malignant disorder, might greatly benefit from the discovery of salivary alpha-enolase as an essential biomarker for early identification and confirmation of malignant transformation.[29] Regarding the significant correlation between the ENO1 gene and oral epithelial carcinogenesis, it is important that researchers identify biomarkers that are both reliable as well as therapeutically efficient.

This study examined salivary alpha enolase levels in both groups, finding a mean value of 0.66 ng/ml for the

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control group and 0.93 ng/ml for the OSMF group. The concentration of salivary alpha enolase increased in the OSMF group in comparison to the control group.

The results of this study align with previous research[30], which demonstrated an apparent spike in alpha enolase levels in biopsies of OSMF patients. The present research is the first to examine the use of saliva samples, as biopsies are intrusive and generate discomfort. This non-invasive method is a more comfortable option for patients while nevertheless providing important diagnostic data. In addition, salivary alpha-enolase levels were markedly elevated in patients with OSMF relative to the control group.

Future scope: These findings underscore the potential of salivary alpha-enolase as a reliable biomarker for identifying alterations in persons with oral submucous fibrosis and oral cancer. The found protein marker, α-enolase, may be pivotal in the early management of OSMF, potentially alleviating pain, decreasing treatment expenses, and markedly improving patients' overall quality of life.

This investigation revealed that salivary α-enolase levels in the control group exhibited relatively minor fluctuations, which lacked statistical significance. In the OSMF group, salivary α-enolase levels demonstrated a high degree of statistical significance, indicating that they may be a viable biomarker for the early detection of precancer and cancer. Nevertheless, additional research with larger sample sizes is necessary to assess salivary α-enolase levels in a variety of OSMF and OSCC grades.

Limitations: This research has specific limitations. The sample size is really limited and could not fairly reflect the general population; so, a larger sample would be required for additional validation. Furthermore, a refined comprehension of salivary alpha enolase levels in relation to clinical staging and histological grading of OSMF and OSCC may elucidate its role in the progression of oral cancer.

5. CONCLUSION

The level of alpha-enolase was markedly increased in the saliva of OSMF patients relative to the control group. A discernible, incremental rise in alpha-enolase levels was noted from the control group to OSMF patients, highlighting its potential as a dependable, non-invasive, and economical diagnostic biomarker. This finding highlights the promise of salivary alpha-enolase in detecting early changes in oral health, offering a simple yet powerful tool for clinical use**.**

List of abbreviations:

1. OSMF – Oral submucous fibrosis
2. ENO – Enolase
3. ENO1,ENO2,ENO3 – Enolase1, Enolase2, Enolase3
4. 37 kDa -37 kilo Dalton
5. MBP-1 - c-myc promoter-binding protein
6. DNA – Deoxy ribonuclic Acid
7. mRNA - messenger Ribonuclic Acid
8. lncRNA - Long noncoding RNA
9. tRNA Lys -Lysin Transferase Ribonucleic Acid
10. IQR - Interquartile range
11. PMD – Potentially malignant disorders
12. OSCC – Oral squamous cell carcinoma

DECLARATIONS

Ethical approval and consent to participate

The study was approved by the Krishna Vishwa Vidyapeeth Ethics Committee of Karad (ref no. KVV/IEC/10/2024), India. In line with ethical standards, written informed consent was obtained from all participants before saliva collection**.**

Consent for publication

This study does not include individuals any kind of information hence NOT Applicable

Availability of data and material

All data generated or analysed during this study are included in the published article [and its supplementary links are given in references].

Competing interest I declare that there are no competing interest.

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1.Study and collection - 400
2.Analysis - 25000
3.Interpretation of data
4.Manuscript writing

Authors contribution: Divyani Patil - Study of the topic data collection ‘analysis ‘interpretation of the data, manuscript writing**; Madhura Mahajan -** Study of the topic data collection ‘analysis, interpretation of the data, manuscript writing

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