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Diagnostic potential of salivary *IL-1β*, *IL-8*, *SAT*, *S100P*, and *OAZ1* in oral squamous cell carcinoma, oral submucous fibrosis, and oral lichen planus based on findings from a Sri Lankan cohort

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The research examined the salivary concentrations of various biomarkers, such as *OAZ1*, *SAT*, *S100P*, *IL-1β*, and *IL-8* aiming to detect early-stage oral squamous cell carcinoma (OSCC). These biomarkers show potential as indicators for detecting both pre-cancerous and cancerous states within the oral cavity. Analyzing these specific molecules in saliva could help clinicians enhance diagnostic accuracy and refine early detection methods for OSCC. The research encompassed a cohort of nine OSCC patients, ten with oral submucous fibrosis (OSF), eleven individuals with oral lichen planus (OLP), and ten healthy controls. The study focused on assessing the expression levels of key biomarkers—*IL-1β*, *IL-8*, *SAT*, *S100P*, and *OAZ1* mRNA—in extracellular RNA extracted from saliva samples. This evaluation was conducted using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) with sequence-specific primers. Additionally, receiver operating characteristic curve (ROC) curve analysis was employed to gauge the efficacy of these biomarkers in detecting OSCC. Based on the results we observe, when these five biomarkers are used together, they give a 90% predictive probability for patients with OLP, an 80% predictive probability for OSF, and an impressive 100% predictive probability for patients with OSCC (AUC = 1.000, $p = 0.000$). This study demonstrates the efficacy of salivary transcriptome diagnostics in detecting OSCC. This novel clinical technique has the potential to be a powerful, efficient, and reliable tool for early detection of cancer. Salivary transcriptomes can be further analyzed to evaluate their effectiveness in other important illness contexts and for regular health monitoring.

Keywords Salivary biomarkers, Oral cancer, mRNA, Saliva, Precancer

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Abbreviations

AUC	Area under the curve
GLOBOCAN	Global cancer observatory
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IL	Interleukin
OLP	Oral lichen planus
OPMD	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
OSF	Oral submucous fibrosis
OAZ	Ornithine decarboxylase antizyme
PVL	Proliferative verrucous leukoplakia
RT-PCR	Real-time reverse transcription polymerase chain reaction
ROC	Receiver operating characteristic curve
SAT1	Spermidine/spermine N1-acetyltransferase

One of the most pressing issues in public health today is the alarming rise of oral cancer. From 1990 to 2017, the prevalence, death rate, and disability-adjusted life years linked to this specific illness increased by about 1.0-fold, according to the available academic literature[1]. The Global Cancer Observatory (GLOBOCAN) incidence and mortality data indicate that there were 377,713 new cases and 177,757 deaths attributed to lip and oral cavity cancer in 2020[2]. Most oral malignancies are classified as oral squamous cell carcinomas (OSCC), a highly aggressive condition characterized by a pronounced propensity for distant and localized metastasis. The phenomenon above substantially influences the quality of life for those affected and the broader societal fabric. Because OSCC is typically misdiagnosed and treated late, the overall 5-year survival rate is only about 51.7%[3].

OSCC is a type of malignancy that arises in the cells lining the oral cavity. It has multiple causes, including genetic factors, changes in gene activity, and habits, including using tobacco, chewing areca nuts, smoking cigarettes, and drinking alcohol. Microbes also play a role in its development[4,5]. OSCC arises from pre-existing oral potentially malignant disorders (OPMD), which are clinical conditions characterized by observable alterations in the oral mucosa. The presence of OPMD increases the likelihood of developing OSCC[6].

Numerous OPMD have been identified with a notably heightened risk of progressing to malignancy. These include oral submucous fibrosis (OSF), erythroplakia, oral lichen planus (OLP), leukoplakia, proliferative verrucous leukoplakia (PVL), actinic cheilitis, palatal lesions associated with discoid lupus erythematosus, dyskeratosis congenita, oral lichenoid lesions, oral graft versus host disease, and reverse cigar smoking[7]. Figure 1 illustrates the typical examples of clinical cases of OSCC and common OPMD.

Several biomarkers have been suggested for identifying OSCC and their potential utility in screening[8]. However, it should be noted that the biomarkers mentioned in existing literature have not undergone thorough or large-scale studies. This study examines saliva samples' five specific mRNA markers (*IL-1β*, *IL-8*, *OAZ1*, *SAT*, *and S100P* mRNAs). These markers have been previously identified as significantly increasing in individuals

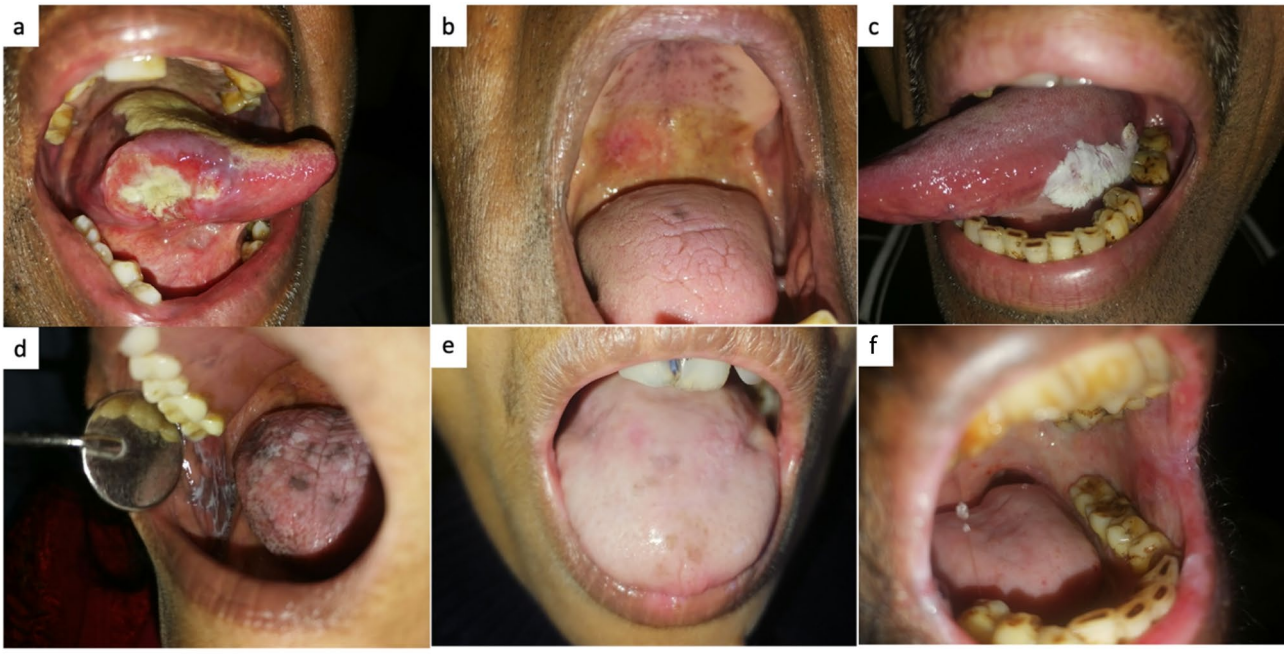

Fig. 1. Clinical pictures of oral cancer and oral potentially malignant disorders: (**a**) oral squamous cell carcinoma; (**b**) oral erythroplakia; (**c**) oral leukoplakia; (**d**) oral lichen planus; and (**e**) and (**f**) oral submucous fibrosis.

diagnosed with OSCC. Multiple studies have observed this association, including a multicenter study[9]. The first identification of mRNA molecules in saliva was made in 2004[10]. However, later studies have cast doubt on their existence in this body fluids[11]. The use of salivary mRNAs for forensic identification of human bodily fluids and tissues has subsequently been the subject of a significant amount of academic literature[12–14]. Additionally, these mRNAs have been investigated concerning the diagnosis of periodontal disease [15; 16], Sjögren's syndrome[17–19], and OSCC detection [9;20].

To our current understanding, there has been limited exploration into the potential use of *IL-1β*, *IL-8*, *OAZ1*, *SAT*, and *S100P* mRNAs as biomarkers for early identification of OSCC, OLP, and OSF. Saliva emerges as a promising diagnostic medium for early OSCC detection, and this study aims to validate that hypothesis. Furthermore, we aim to utilize these identified saliva biomarkers to develop a practical screening tool for distinguishing OSCC, OLP, and OSF. Our investigation (a preliminary study) seeks to establish saliva's viability as a diagnostic tool for early recognition and differentiation of these conditions from individuals without them, by quantifying the levels of the aforementioned biomarkers.

Methods

Patient selection

In this study, being a preliminary exploration into the diagnostic potential of salivary biomarkers, this study utilized a smaller sample size ($n=40$) to identify potential trends and feasibility before committing to larger, more resource-intensive studies. The sample size was determined based on the funding and resources available to conduct this study.

Thirty patients with histologically confirmed OSCC, OSF, and OLP were recruited from the Diagnostic Clinic at the Dental Teaching Hospital in Peradeniya, Sri Lanka, between May and August 2023. The subsequent phases of the study were conducted from September to November 2023 at the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka. Exclusion criteria included individuals under 18 years of age and those physically or mentally incapable of responding to the data collection tools. Participants over 18 years old, without any physical or mental incapacities, who were newly diagnosed and had not undergone any prior interventions such as chemotherapy, radiation, surgery, or alternative therapies were selected. Histopathological assessments were conducted on all primary tumor sections. Ten control participants were meticulously matched with the experimental group by age and sex; they were selected following a detailed oral cavity examination by a specialist in oral medicine, ensuring the absence of any pathological lesions. None of the patients had a medical history of cancer, hepatitis, HIV infection, immunodeficiency, or autoimmune disorders. Each participant willingly participated after signing a consent form. Table 1 summarizes the pathological and clinical characteristics of all individuals examined in the study. Here is a breakdown of the participant groups:

- **Controls**: Ten individuals without any of the studied conditions.
- **Patients with OSCC**: Nine newly diagnosed OSCC patients.
- **Patients with OLP**: Eleven individuals with OLP.
- **Patients with OSF**: Ten individuals with OSF.

Collection of saliva

Saliva samples were collected from patients during the morning, specifically between 9:00 a.m. and 12:00 p.m., to mitigate the impact of daily fluctuations. Participants were advised to refrain from consuming food, brushing their teeth, using mouthwash, smoking, chewing betel quid, and consuming alcohol for at least 2 h before sample collection. Donors were further instructed to rinse their mouths with potable water and eliminate any liquid, followed by a 5-minute period of rest with their heads inclined forward and refraining from swallowing to facilitate saliva collection.

Afterward, participants were instructed to expel the collected oral fluid (2–5 mL) into a sterile plastic falcon tube (15 mL) positioned on ice[21].

Stabilization of saliva

For the stabilization of saliva for use with the Qiagen RNeasy Protect Saliva Kit*, we aliquoted 1 mL of RNA protect Saliva Reagent* into 2 mL Eppendorf tubes. While the manufacturer's instructions recommend placing 200 µL of saliva directly into 1 mL of RNA protect Saliva Reagent*, we made a modification for this study. Specifically, 1 mL of saliva was introduced into a 2 mL tube and promptly centrifuged at 3000 revolutions per minute for 10 min at 4 °C. Afterward, approximately 200 mL of supernatant along with the pellet was combined with the 2 mL tubes containing RNAprotect Saliva Reagent*. The Eppendorf tubes were then vortexed, placed on ice, and preserved at − 80 °C until the complete RNA extraction process.

Extraction of salivary RNA

Total RNA extraction followed the manufacturer's protocol using the Qiagen RNeasy Protect Saliva Mini Kit*. This kit features a silica-membrane RNeasy spin column capable of binding up to 100 µg of total RNA. To ensure RNA amplification accuracy on the RT-qPCR platform, each sample underwent DNase treatment with Qiagen's RNase-free DNase Set according to the manufacturer's instructions. Ethanol solutions of 70% and 80% were freshly prepared and utilized throughout the extraction process. The final elution volume for the extracted total RNA was approximately 12 µL. Subsequently, the samples were stored at -80 °C for further analysis.

Parameters	Study population ($n=40$)			
	OSCC	OSF	OLP	HC
Total	9	10	11	10
Age (years)				
Median range	58 (37–69)	42 (22–74)	58 (19–82)	49.50 (24–76)
Gender				
Male	7 (77.8)	9 (90)	3 (27.3)	7 (70)
Female	2 (22.2)	1 (10)	8 (72.7)	3 (30)
Past medical history				
Yes	4 (44.4)	3 (30)	8 (72.7)	1 (10)
No	5 (55.6)	7 (70)	3 (27.3)	9 (90)
Areca nut chewing				
Yes	7 (77.8)	10 (100)	3 (27.3)	1 (10)
No	2 (22.2)	0 (0)	8 (72.7)	9 (90)
Smoking history				
Yes	3 (33.3)	7 (70)	1 (9.1)	1 (10)
No	6 (66.7)	3 (30)	10 (90.9)	9 (90)
Alcohol consumption				
Yes	7 (77.8)	9 (90)	1 (9.1)	4 (40)
No	2 (22.2)	1 (10)	10 (90.9)	6 (60)
Histological grading				
Well-differentiated	4 (44.4)	–	–	–
Moderately differentiated	2 (22.2)	–	–	–
Poorly differentiated	1 (22.2)	–	–	–
Early invasive	2 (22.2)	–	–	–
Mild to moderate epithelial dysplasia	–	1 (10)	–	–
Mild epithelial dysplasia	–	2 (20)	–	–
Focal mild epithelial dysplasia	–	1 (10)	–	–
No epithelial dysplasia	–	6 (60)	–	–
Oral lichen planus	–	–	11 (100)	–

Table 1. Clinical and pathological characteristics of the patients in the study ($n=40$).

Gene symbol	Primer sequence	Genbank ID	Product size	References
IL-8	F: GAGGGTTGTGGAGAAGTTTTTG R: CTGGCATCTTCACTGATTCTTG	NM_000584	88	[10]
IL-1β	F: GTGCTGAATGTGGACTCAATCC R: ACCCTAAGGCAGGCAGTTG	M15330	120	
S100P	F: GAGTTCATCGTGTTCGTGGCTG R: CTCCAGGGCATCATTTGAGTCC	NM_005980	82	
SAT	F: CCAGTGAAGAGGGTTGGAGAC R: TGGAGGTTGTCATCTACAGCAG	NM_002970	99	
OAZ1	F: AGAGAGAGTCTTCGGGAGAGG R: AGATGAGCGAGTCTACGGTTC	D87914	113	
GAPDH	F: TGAAGGTCGGAGTCAACGGATTTGGT R: CATGTGGGCCATGAGGTCCACCAC	NM_002046.7	983	[22]

Table 2. Gene symbols, accession numbers and references for salivary transcriptomics study.

Qualitative assessment of salivary RNA

The purity and concentration of the isolated RNA were assessed using spectrophotometric analysis with a NanoDrop spectrophotometer, specifically by measuring the A260/A280 ratio.

cDNA synthesis

Reverse transcription was done for the RNA using the High-Capacity cDNA Reverse Transcription Kit obtained from Applied Biosystems (California, USA). The protocol of the manufacturer was meticulously followed during this process. Subsequently, the samples were preserved at − 20 °C until further analysis.

SYBR-based quantitative real-time qPCR

For each sample, qRT-PCR was executed three times on each of the forty individuals who participated in the study for the *IL-1β*, *IL-8*, *OAZ1*, *SAT*, *S100P* and *GAPDH* genes. 10 µL of GoTaq® qPCR Master Mix (Promega Corporation, Madison, USA), 2 µL of salivary cDNA, and 10 µmol of sense and antisense primers were used for transcription quantification using QuantStudioTM 6 Flex Real-Time PCR (Thermo Fisher Scientific).

Sequences of PCR primer sets (in 5'–3' direction) were tabulated in Table 2.

The standard curve that was previously defined was used to extrapolate the initial amount of cDNA/RNA of a given template[23]. All samples were measured in triplicates and relative expression of each specific product was determined using the $2^{-\Delta\Delta CT}$ method. CT refers to the fluorescence threshold value, ΔCT represents the difference between the CT values of the target gene and the reference gene (*GAPDH*), and ΔΔCT is the difference between the ΔCT values of the oral cancer/OPMD sample and the calibrator sample.

Statistical method

The statistical analyses and diagram creation were performed using GraphPad Prism 4.0 software. Specifically, the Wilcoxon signed-rank test was employed for two specific comparisons: OSCC versus controls and OSCC versus OPMD. To assess the biomarker's ability to distinguish between tumor and control samples in individuals' saliva without relying on a subjective threshold, the data were visualized using a ROC curve. This graph illustrates the relationship between sensitivity (true positives) and 1-specificity (false positives), considering each recorded value as a potential cutoff point. The Area Under the Curve (AUC) was calculated to evaluate the marker's overall discriminatory efficacy. A marker lacking discriminatory value would have an ROC curve closely following the diagonal line, resulting in an AUC value around 0.5. Conversely, a reliable discriminatory test would shift the ROC curve toward the upper left corner, yielding an AUC value close to 1.0. This analysis provides valuable insights into the diagnostic performance of the biomarker.

Ethical considerations

This study was conducted in accordance with the Helsinki Declaration of 1964 (as amended in October 2013 by the World Medical Association General Assembly). This study was approved by the Ethical Review Committee, Faculty of Dental Sciences, University of Peradeniya (Ref. No: ERC/FDS/UOP/E/2021/14). All participants were informed about the study objectives. Participation was voluntary and the participants could withdraw from the study at any time without penalty. All participants gave informed consent before participating in the study. Names, emails, or any other personal identifiers were not included in the data collected.

Results

The study cohort comprised 40 individuals, 26 were men, and 14 were women, indicating a slight preference for men, with an average age of 59.5 ± 15.6 years. The study included nine patients diagnosed with OSCC, 10 with OSF displaying diverse degrees of dysplasia (1 with mild to moderate epithelial dysplasia, 2 with mild epithelial dysplasia, 1 with focal mild epithelial dysplasia, and 6 with no epithelial dysplasia), and ten meticulously matched healthy control subjects. The results affirmed a notable increase in saliva transcripts of five candidate mRNAs *IL-8*, *IL-1β*, *S100P*, *SAT*, and *OAZ1*—in patients with OSCC, OSF, and OLP (Wilcoxon signed-rank test $p < 0.05$). These genes were stratified into three tiers based on the magnitude of elevation: high up-regulated mRNA, encompassing OAZ1 (198.956 fold-OSCC; 73.817 fold-OSF; 44.343 fold-OLP); moderate up-regulated mRNAs, including IL-8 (14.083 fold-OSCC; 15.475 fold-OSF; 2.260 fold-OLP), S100P (24.996 fold-OSCC; 24.035 fold-OSF; 3.972 fold-OLP), and IL-1β (14.083 fold-OSCC; 15.475 fold-OSF; 2.260 fold-OLP); and low up-regulated mRNAs, comprising SAT (7.148 fold-OSCC; 8.800 fold-OSF; 2.375 fold-OLP) (Fig. 2). Significantly, OLP demonstrated diminished levels of up-regulated mRNAs for all five candidate biomarkers (Fig. 2). Binary logistic regression analysis of the four biomarkers—*OAZ1*, *SAT*, *S100P*, *IL-8*, and *IL-1β*—were provided in Tables 3, 4 and 5. None of the biomarkers proved significant for samples from patients with OSCC, OSF, and OLP.

Following the binary logistic regression analysis, we gathered predictive probability values and employed them to construct ROC curves (as depicted in Fig. 3). These ROC curves were then leveraged to compute the AUC for each biomarker's prediction probability, as outlined in Table 6. However, the binary regression analysis data for the salivary transcriptomics study was not significant, and this might be due to multiple factors, including small sample size, high biological variability in salivary biomarker levels among participants which could have masked true associations, and uncontrolled confounding factors (such as variations in oral hygiene, diet, medication use, and systemic health conditions) which may have influenced the results. In addition, the biomarkers selected might not have been sufficiently sensitive or specific, and limitations in the study design could have further impacted the findings. Hence, addressing these issues in future research could improve the likelihood of obtaining significant results in salivary transcriptomics studies.

Combining multiple significant biomarkers effectively enhances discriminatory power compared to individual or non-significant markers. The predictive probability for detecting OSCC using the combined five biomarkers is highly statistically significant, with an area under the curve of 1.000 ($p < 0.001$). Similarly, the predictive probabilities for detecting OSF and OLP, utilizing all five biomarkers, were also statistically significant, with areas under the curves of 0.96 ($p = 0.001$) and 0.945 ($p = 0.001$), respectively. Table 6 illustrates the optimal predictive probability coordinates in the ROC curves, depicting sensitivity and 1-specificity for the combined biomarkers associated with OSCC, OLP, and OSF across varying degrees of dysplasia. Notably, the table underscores that the most favorable values for sensitivity and specificity are observed in the ROC curves for OSCC, OSF, and OLP.

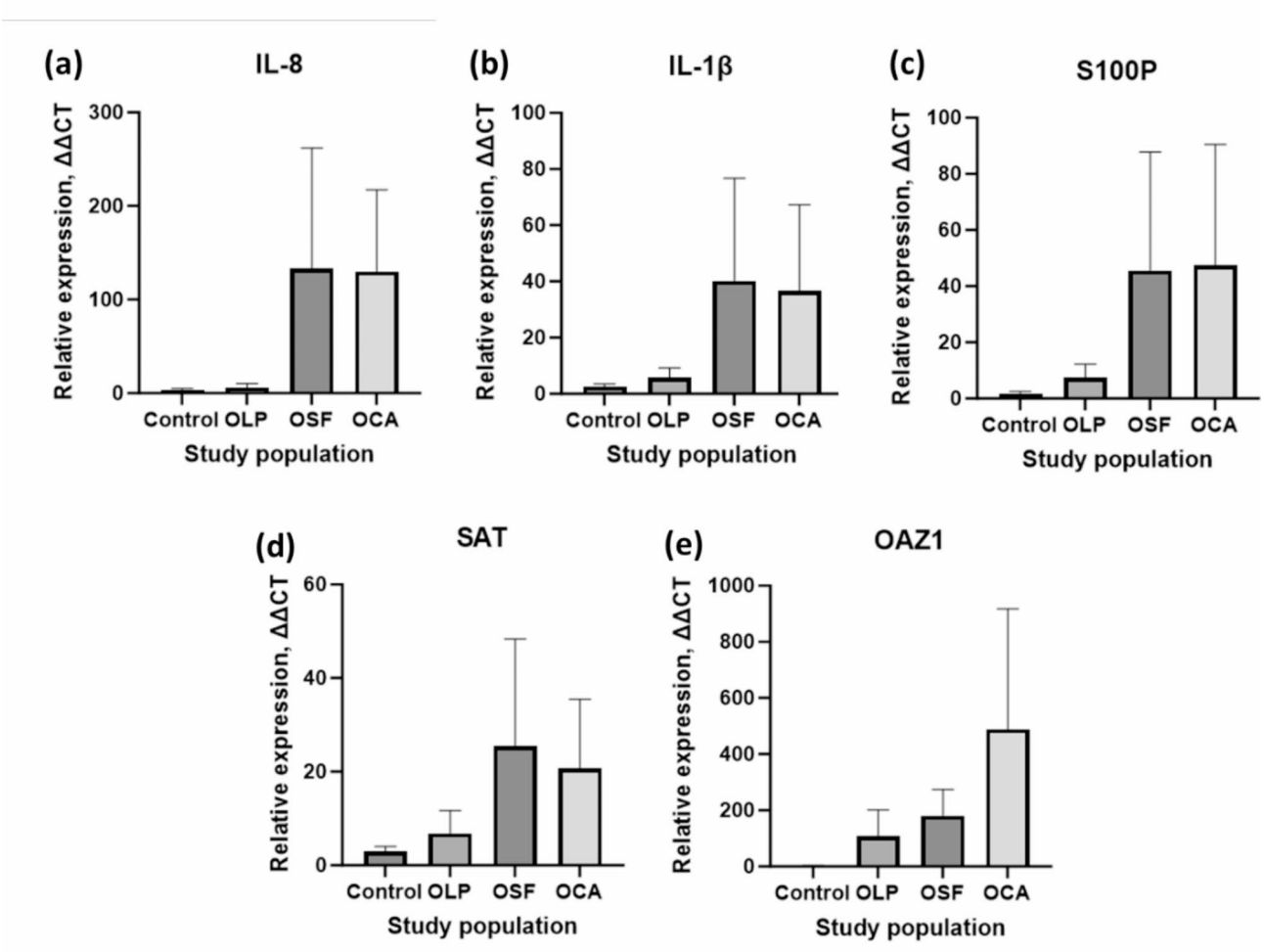

Fig. 2. Relative expression of candidate biomarkers in saliva for OSCC, OLP, OSF and compared to control samples. (**a**) The box-plot diagram shows *IL-8* mRNA expression levels. (**b**) The box-plot graph illustrates the *IL-1β* mRNA expression levels. (**c**) The box-plot graph depicts the expression levels of *S100P* mRNA. (**d**) The box-plot diagram shows *SAT* mRNA expression levels. (**e**) The box-plot diagram shows *OAZ1* mRNA expression levels.

Biomarkers	B	S.E	Wald	df	$p = Sig$	Exp(B)	Asymptotic 95% confidence intervals	
							Lower	Upper
IL-8	6.013	230.089	0.001	1	0.979	408.810	0.000	2.910E+198
IL-1β	− 79.682	3224.512	0.001	1	0.980	0.000	0.000	–
S100P	53.297	1618.366	0.001	1	0.974	1407E+23	0.000	–
SAT	9.087	759.326	0.000	1	0.990	8841.135	0.000	–
OAZ1	3.936	166.145	0.001	1	0.981	51.205	0.000	1.356E+143
Constant	− 54.117	1647.692	0.001	1	0.974	0.000	–	–

Table 3. Binary logistic regression analysis of the biomarkers in saliva samples of patients with OSCC. Hosmer and Lemenshow test, $p = 1.000$, Sample size: N(1) = 19, B: Binary logistic regression coefficients, S.E: Standard Error of B, Wald squared value. df: degrees of freedom, p: level of significance, Exp(B): Odds ratio.

Discussion

In the realm of global health, South and Central Asia stand out with some of the highest rates of both incidence and mortality for OSCC. The primary risk factor driving OSCC development in this region is the consumption of areca nut, whether or not it is combined with tobacco. The number of newly diagnosed OSCC patients rose from 1941 in 2011 to 2199 in 2014, according to the National Cancer Control Programme of Sri Lanka[24,25]. Based

Biomarkers	B	S.E	Wald	df	$p = Sig$	Exp(B)	Asymptotic 95% Confidence Intervals	
							Lower	Upper
IL-8	0.887	1.054	0.707	1	0.400	2.427	0.307	19.158
IL-1β	− 0.417	3.843	0.012	1	0.914	0.659	0.000	1229.885
S100P	5.531	6.834	0.655	1	0.418	252.289	0.000	165,535,534.108
SAT	− 10.937	9.318	1.378	1	0.240	0.000	0.000	1519.221
OAZ1	0.489	0.389	1.583	1	0.208	1.631	0.761	3.494
Constant	− 1.248	1.486	0.705	1	0.401	0.287	–	–

Table 4. Binary logistic regression analysis of the biomarkers in saliva samples of patients with OSF. Hosmer and Lemenshow test, $p = 0.591$, Sample size: N(1) = 20, B: Binary logistic regression coefficients, S.E: Standard Error of B, Wald squared value. df: degrees of freedom, p: level of significance, Exp(B): Odds ratio.

Biomarkers	B	S.E	Wald	df	$p = Sig$	Exp(B)	Asymptotic 95% Confidence Intervals	
							Lower	Upper
IL-8	0.407	0.821	0.246	1	0.620	1.502	0.300	7.516
IL-1β	5.414	5.521	0.962	1	0.327	224.494	0.004	11234289.096
S100P	− 7.835	7.568	1.072	1	0.301	0.000	0.000	1093.675
SAT	4.070	3.536	1.325	1	0.250	58.552	0.057	59904.061
OAZ1	− 0.490	0.378	1.686	1	0.194	0.612	0.292	1.284
Constant	1.239	1.214	1.042	1	0.307	3.452	–	–

Table 5. Binary logistic regression analysis of the biomarkers in saliva samples of patients with OLP. Hosmer and Lemenshow test, $p = 0.861$, sample size: N(1) = 21, B: binary logistic regression coefficients, S.E: Standard Error of B, Wald squared value. Df: degrees of freedom, p: level of significance, exp(B): odds ratio.

on these results, it appears that OSCC cases have been on the rise[26]. OLP is widely recognized within the medical and research communities as a chronic or recurring condition with an uncertain etiology. The pathophysiology of OLP is likely rooted in an autoimmune assault mediated by T-lymphocytes. Furthermore, after a period of debates[27], there is an increasing body of evidence suggesting that OLP displays features that are characteristic of an OPMD. As a result, individuals affected by this condition often face a notably heightened susceptibility of developing OSCC during the course of the illness[28]. OSF is a chronic ailment intricately linked to malignancy[29]. Regarded as a precancerous condition, OSF possesses a concerning propensity to progress into OSCC. Its genesis is primarily attributed to the habitual consumption of betel nuts. OSF in affected individuals undergoes rapid accumulation of abnormal collagen, propelled by specific components present in betel nuts, such as polyphenols, arecoline, adipose elements, and crude fibers [30,31]. OSF is more prevalent among Southeast Asians, but it is rare in Caucasians, implying potential differences in the mechanisms leading to oral cancer development between these populations. Despite ethnic and behavioral distinctions, the biomarkers originally designed for the Western population and employed in this study exhibited outstanding accuracy in discriminating between OSCC, OLP, OSF patients, and control subjects in Sri Lankan individuals. This was evident from the high values of the AUC and sensitivity. Notably, these biomarkers had previously undergone testing in diverse populations from Serbia and the United States, where alcohol and tobacco usage are the primary causative factors for OSCC [9; 20]. The performance of the biomarkers in those studies was comparable to what was noted in this present study.

Biomarkers associated with both malignant and benign oral lesions display distinctive levels in saliva due to their close proximity to the lesion. Moreover, they can provide valuable insights into forecasting, prognosis, and recurrence of carcinomatous lesions[32]. Interleukin-1β belongs to the IL-1 family, comprising 11 closely related cytokines linked with inflammation, including IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33, IL-36α, IL-36β, IL-36γ, and IL-38[33].

The primary focus of the investigation was on specific cytokines serving as salivary indicators in the progression of oral leukoplakia and OLP to OSCC[14,34–37]. Research has shown that certain tumors can independently produce cytokines, which then promote angiogenesis and enhance immune responses supporting the tumor[38]. Interleukin-1β (IL-1β) and interleukin-8 (IL-8) are synthesized by various cell types, including oral keratinocytes[38]. Moreover, significant levels of these substances have been found in the serum and saliva of individuals diagnosed with OSCC, suggesting their potential utility as biomarkers for the disease[35]. IL-1β, a cytokine commonly associated with the cancer-inflammation link, may significantly influence OSCC development. Evidence suggests that OSCC cell development inhibition primarily occurs through IL-1β suppression, a process regulated by genes like TGFβ[39]. Additionally, IL-1β has been identified as a critical gene in the tumor microenvironment during oral cancer progression[39]. Interleukin-8 is released in response to various stimuli, including IL-1[40]. Elevated IL-8 levels have been detected in the saliva of individuals with OSCC, indicating its potential as a biomarker for oral malignancy development[40–42].

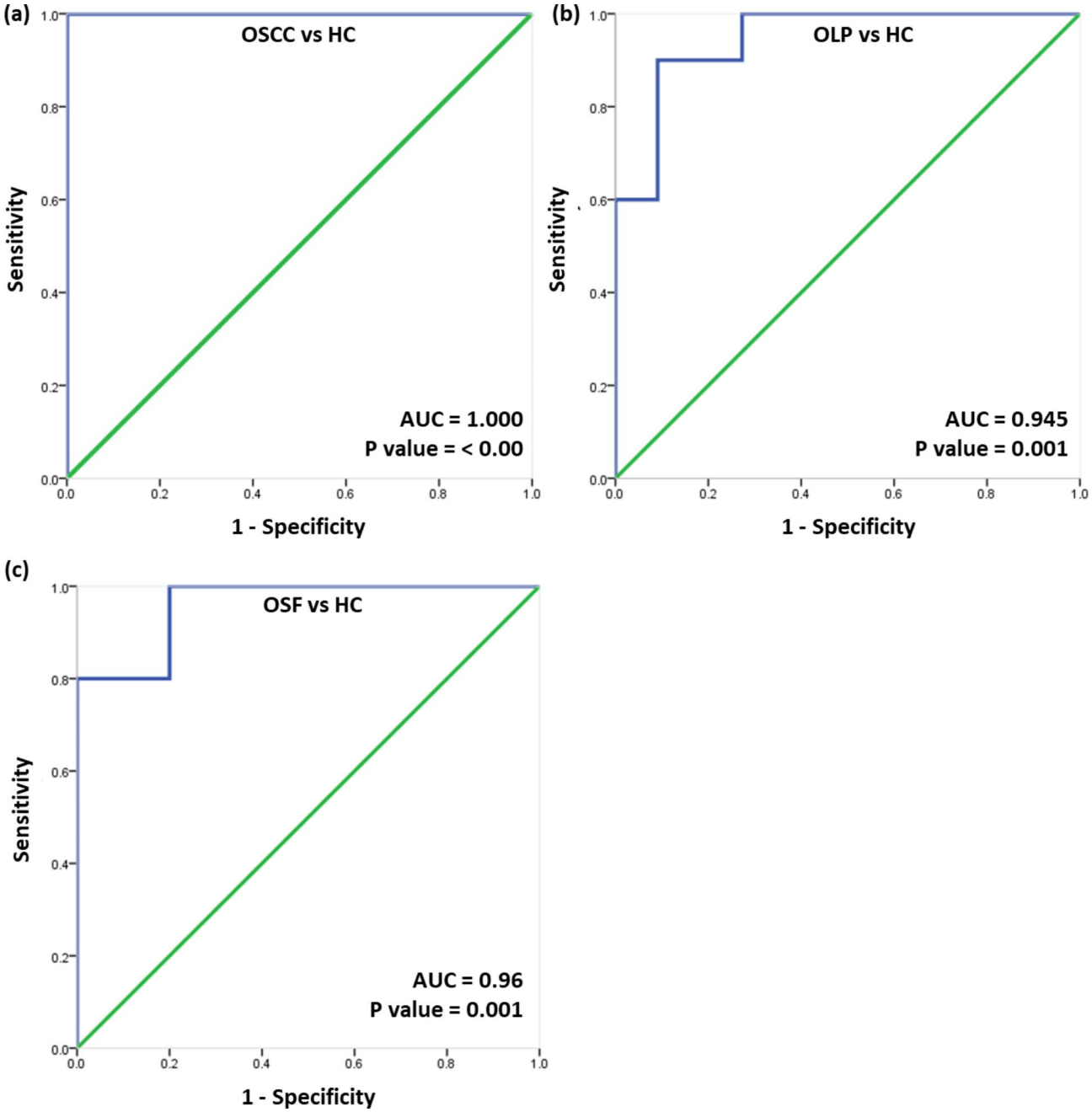

Fig. 3. ROC curve evaluation of coupled salivary mRNA biomarkers for predictive power. Five salivary mRNA biomarkers—*IL-1β*, *OAZ1*, *SAT*, *S100P,* and *IL-8*—were included in the final logistic model.

Predictive probabilities	Area	Std error	Asymptotic sig.	Sensitivity (%)	Specificity (%)	Asymtotic 95% C.I. Intervals	
						Lower bound	Upper bound
OSCC	1.000	0.000	0.000	100	100	1.000	1.000
OLP	0.945	0.039	0.001	90	90.9	0.883	1.000
OSF	0.960	0.039	0.001	80	90	0.883	1.000

Table 6. Areas under the curve (AUC) of the five combined biomarkers null hypothesis: actual area 0.50. Using a cutoff probability of 50%, 49.3%, and 55.7%, we obtained sensitivity and specificity by ROC, respectively, for OSCC, OLP, and OSF.

Ornithine decarboxylase antizyme (OAZ) inhibits the activity of ornithine decarboxylase (ODC), the enzyme responsible for controlling polyamine synthesis rates[43]. Elevated ODC activity is prevalent in most human malignancies[44], suggesting that OAZ may act as a tumor suppressor[45]. OAZ1 plays a crucial role in DNA repair and contributes to the metastatic potential of cancer cells in OSCC cell lines[46]. As OAZ expression is triggered by a mechanism dependent on polyamines[47], the detection of increased levels of salivary OAZ1 mRNAs in OSCC patients indicates heightened polyamine levels in OSCC[8].

S100P belongs to the S100 calcium-binding protein family[48]. Its overexpression has been noted in various malignancies, such as lung, pancreatic, colon, breast, and prostate cancers, and is linked to acquiring malignant traits like increased cell proliferation, survival, mobility, and invasion[49]. Notably, higher *S100P* mRNA expression has been observed in OSCC cells resistant to anoikis (a form of detachment-induced apoptosis) compared to anoikis-sensitive OSCC cells, suggesting a potential role for S100P in OSCC metastasis[50]. However, a study by Sapkota et al., examining mRNA expression patterns of 16 members of the S100 gene family, including S100P, found no significant elevations in S100P mRNA levels in OSCC tissue samples[51]. Currently, it remains uncertain whether salivary mRNAs accurately reflect mRNA alterations in OSCC tissue, necessitating further research to investigate modifications in S100P expression in OSCC[8]. SAT1, also known as spermidine/spermine N1-acetyltransferase 1, is a protein functioning as an acetyltransferase involved in polyamine degradation. In a study by Brinkmann et al., SAT1 mRNA was identified as one of the four proteins in a transcriptome panel showing increased levels in advanced-stage oral squamous cell carcinoma (OSCC)[20].

The outcomes of this research highlight the reliability and effectiveness of salivary biomarkers as discerning indicators for distinguishing individuals with OSCC, OSF, OLP, and those in a control group within a Sri Lankan cohort. This is particularly noteworthy as it suggests that salivary biomarkers for OSCC are likely independent of ethnicity[20]. The markers identified through transcriptome analysis exhibit a robust predictive capability for the overall occurrence of OSCC, as evidenced by an AUC value of 1.000, 0.945 for OLP and 0.96 for OSF. The markers exhibit a sensitivity of 100% and specificity of 100% for OSCC. For OLP, the sensitivity is 90%, and the specificity is 90.9%. Additionally, for OSF, the sensitivity is 80%, and the specificity is 90%. One might question why a singular biomarker lacks the potency to effectively differentiate between OSCC and controls independently. The answer lies in acknowledging the multifaceted and heterogeneous pathogenesis of OSCC. By incorporating a greater number of biomarkers, we consider the diverse factors contributing to OSCC, thereby enhancing the discriminatory capacity of individual biomarkers and elevating them to a level of high performance when used in combination[20].

This study serves as a preliminary exploration into the diagnostic potential of salivary biomarkers *IL-1β*, *IL-8*, *SAT*, *S100P*, and *OAZ1* for OSCC, OSF, and OLP. As this an initial exploratory study we utilized a smaller sample size to identify potential trends and feasibility before committing to larger, more resource-intensive studies. Conducting studies with larger sample sizes requires substantial resources, including time, funding, and personnel. Given these constraints, we opted to conduct a smaller-scale study to gather preliminary data, which can inform and justify the allocation of resources for larger future studies.

Unraveling the origins of salivary biomarkers associated with OSCC continues to pose a significant challenge. Factors such as exfoliating cancer cells, oral mucosa cells, variations in salivary gland secretion patterns (including submandibular, sublingual, parotid, and minor salivary glands), or gingival crevice fluid may contribute to the overall salivary biomarker profile. Despite the ongoing importance of refining biomarker sources, directing attention solely toward an effective screening method that utilizes readily obtainable whole saliva is critical for widespread clinical adoption. While the current findings hold promise and underscore the potential of salivary transcriptome and proteome markers, further investigations with larger patient cohorts are imperative for enabling population-level clinical applications.

Data availability

The authors confirm that the data supporting the findings of this study is available upon request from the corresponding authors.

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Author contributions
Study conception and design: KS, TANM, and RDJ; Data analysis and interpretation: KS, TANM; Writing original manuscript: KS; Writing reviewing and editing manuscript: KS, TANM, NUJ, CUG, and KKK; Supervision: TANM, NUJ, JR, CUG, UP, BS, RDJ and KKK; Publication funding acquisition: KKK; All authors have read and approved the manuscript.

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Declarations

Competing interests
Ruwan Duminda Jayasinghe is an editorial board member of Scientific Reports and a co-author of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication. Other authors declare that they have no conflict of interest involved with their work in this study.

Ethics approval and consent to participate
This study was approved by the Ethical Review Committee, Faculty of Dental Sciences, University of Peradeniya (Ref. No: ERC/FDS/UOP/E/2021/14). All participants were informed about the study objectives. Participation was voluntary and the participant could withdraw from the study at any time without penalty. All participants gave informed consent before participating in the study. Names, emails, or any other personal identifiers were not included in the data collected.

Additional information
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