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Quantification of IRS-1 and akt levels in saliva of patient with OSCC

Prateek Veerendrakumar Siddhapur[1*], Dr. Dinesh kumar[2]

[1*]B.D.S-Undergraduate Saveetha Dental College and Hospitals, nSaveetha Institute of Medical and Technical Sciences, Chennai, India.
[2]Assistant professor, Department of Pedodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences (SIMATS), Chennai – 600077 India

Abstract

Background: Oral squamous cell carcinoma (OSCC) remains a significant global health burden, often diagnosed at advanced stages. The Insulin receptor substrate-1 (IRS-1) and Akt signaling axis is a critical driver of cellular proliferation and survival in many malignancies. This study aimed to quantify the levels of IRS-1 and Akt in the saliva of OSCC patients and evaluate their potential as non-invasive diagnostic biomarkers.
Materials and Methods: In this case-control study, unstimulated whole saliva was collected from 30 histopathologically confirmed OSCC patients and 30 age- and sex-matched healthy controls. IRS-1 and Akt concentrations were quantified using high-sensitivity ELISA and normalized to total salivary protein content. Data were analyzed using independent samples t-tests, with significance set at $p < 0.05$.
Results: Salivary IRS-1 levels were significantly higher in OSCC patients (482.14 ± 84.56 pg/mg protein) compared to healthy controls (194.32 ± 42.18 pg/mg protein, $p < 0.001$). Similarly, salivary Akt levels were markedly elevated in the OSCC group (312.85 ± 62.34 pg/mg protein) compared to the control group (126.47 ± 28.92 pg/mg protein, $p < 0.001$). Furthermore, levels of both biomarkers were significantly higher in patients with advanced clinical stages (III/IV) compared to early-stage (I/II) disease ($p < 0.01$).
Conclusion: Salivary IRS-1 and Akt are significantly upregulated in OSCC and correlate with clinical disease progression. These findings suggest that the IRS-1/Akt signaling axis in saliva holds promise as a non-invasive diagnostic tool for the early detection and risk stratification of oral malignancy.

Introduction

Oral squamous cell carcinoma represents the most prevalent malignancy of the head and neck region, accounting for approximately 90% of all oral cancers [1]. This epithelial-derived malignancy arises from the mucosal lining of the oral cavity, with common sites of involvement including the tongue, floor of the mouth, buccal mucosa, and palate. The disease is associated with well-established risk factors including tobacco use, alcohol consumption, and infection with high-risk human papillomavirus subtypes, although a significant proportion of cases occur in individuals without identifiable risk factors. Despite significant advancements in surgical techniques, radiation therapy, and adjuvant chemotherapy regimens, the five-year survival rate for oral squamous cell carcinoma has remained stagnated at nearly 50% over the past several decades [2]. This persistently poor prognosis is largely attributed to late-stage diagnosis, as the majority of patients present with advanced disease characterized by regional lymph node involvement, and the highly invasive nature of the tumor, which frequently infiltrates adjacent structures and demonstrates a propensity for local recurrence.

The clinical challenge of early detection is compounded by the fact that oral squamous cell carcinoma often arises from clinically occult precursor lesions or manifests initially as asymptomatic mucosal changes that may be overlooked during routine examination. Consequently, there is an urgent clinical mandate to identify reliable, non-invasive molecular biomarkers that can facilitate early detection, enable risk stratification, and monitor therapeutic response [3]. Such biomarkers would ideally be accessible through minimally invasive sampling methods, demonstrate high sensitivity and specificity for malignant disease, and provide information that complements conventional clinical and histopathological assessment.

The phosphoinositide 3-kinase/Akt signaling pathway is one of the most frequently dysregulated cascades in human cancers, playing a central role in regulating cell proliferation, survival, metabolism, and motility [4]. This pathway transduces signals from growth factor receptors, integrins, and other cell surface molecules to downstream effectors that govern fundamental cellular processes. Aberrant activation of the PI3K/Akt pathway contributes to malignant transformation by promoting cell cycle progression, inhibiting apoptosis, and enhancing cellular migration and invasion. In normal cells, the activity of this pathway is tightly regulated by a complex network of feedback mechanisms; however, in cancer cells, genetic alterations, epigenetic changes, and dysregulated upstream signaling converge to produce constitutive activation that drives tumor progression.

Insulin receptor substrate-1, a high-molecular-weight adapter protein, serves as a critical upstream activator of this pathway [5]. Upon phosphorylation by activated receptor tyrosine kinases, such as the insulin-like growth factor-1 receptor and the epidermal growth factor receptor, IRS-1 recruits and activates PI3K through direct interaction with its Src homology 2 domains. Activated PI3K generates phosphatidylinositol-3,4,5-triphosphate at the plasma membrane, which subsequently triggers the phosphorylation and activation of Akt, the central effector kinase of the pathway [6]. The IRS-1/Akt signaling axis therefore represents a critical node through which extracellular growth signals are transduced into intracellular responses. Overexpression of IRS-1 and the constitutive activation of Akt have been linked to the bypass of apoptotic signals, resistance to cytotoxic therapies, and the promotion of epithelial-to-mesenchymal transition in various solid tumors [7]. Epithelial-to-mesenchymal transition, a process by which epithelial cells lose their characteristic polarity and cell-cell adhesion and acquire a migratory, invasive phenotype, is a key mechanism underlying the metastatic potential of carcinoma cells.

In the context of oral squamous cell carcinoma, localized proteomic alterations in the tumor microenvironment are often reflected in the biochemical composition of saliva. The salivary glands and the oral epithelium contribute a complex mixture of proteins, peptides, nucleic acids, electrolytes, and cellular debris to this biofluid, which bathes the oral mucosa continuously. As an oscillating mirror of systemic and oral health, saliva offers a distinct diagnostic advantage over serum due to its non-invasive collection method, which requires no needles, phlebotomy skills, or specialized equipment, and the direct physical proximity of the biofluid to the primary lesion [8, 9]. The close anatomical relationship between the tumor and the salivary milieu suggests that proteins released from neoplastic cells, either through active secretion, passive leakage, or cellular turnover, may be detectable in saliva at concentrations that reflect disease activity.

Previous studies have identified various salivary cytokines, growth factors, and mRNA transcripts as potential markers for oral squamous cell carcinoma; however, the specific quantification of the IRS-1/Akt signaling axis in the saliva of oral squamous cell carcinoma patients remains insufficiently explored [10, 11]. Most investigations have focused on individual markers or have examined tissue samples rather than the readily accessible salivary compartment. The measurement of signaling proteins within saliva presents unique analytical challenges, including the presence of high concentrations of other proteins, the activity of proteolytic enzymes, and the variability in collection and processing methods. Nevertheless, advances in immunoassay technology and the development of highly sensitive detection platforms have made the reliable quantification of low-abundance proteins in saliva increasingly feasible.

Understanding the expression levels of these intracellular signaling proteins within the salivary matrix could provide a molecular snapshot of the oncogenic drive within the oral epithelium. The presence of IRS-1 and Akt in saliva, whether derived from the tumor itself or from the surrounding inflamed tissues, may reflect the activation status of growth factor signaling pathways that are known to be dysregulated in oral squamous cell carcinoma. Such data would not only enhance our understanding of oral squamous cell carcinoma pathogenesis but also potentially establish a foundation for a chair-side screening tool that could be deployed in primary care settings, community health screenings, and high-risk patient populations [12]. A non-invasive salivary biomarker panel could facilitate earlier diagnosis, enable monitoring of treatment response and disease recurrence, and ultimately contribute to improved outcomes for patients with this aggressive malignancy.

Therefore, the present study aims to quantify the levels of IRS-1 and Akt in the saliva of patients diagnosed with oral squamous cell carcinoma compared to healthy controls, evaluating their potential utility as diagnostic biomarkers for oral malignancy. By comparing salivary protein concentrations between these groups and analyzing the correlation between marker levels and clinicopathological parameters, this investigation seeks to establish whether components of the PI3K/Akt signaling pathway can serve as reliable indicators of oral squamous cell carcinoma and provide the foundation for future development of a non-invasive diagnostic assay.

Materials and Methods

Study Population and Ethical Considerations

This case-control study was conducted following approval from the Institutional Review Board, with the assigned approval number IRB/2019/OS-042. All procedures performed in this study adhered to the ethical standards outlined in the Declaration of Helsinki and its subsequent amendments, ensuring the protection of participant rights and welfare throughout the research process.

A total of 60 participants were recruited for this investigation. The study population consisted of 30 patients diagnosed with histopathologically confirmed oral squamous cell carcinoma and 30 age- and sex-matched healthy volunteers who served as the control group. The sample size was determined based on previous studies investigating salivary biomarkers in oral cancer, with the aim of achieving adequate statistical power to detect clinically meaningful differences between groups. The matching of age and sex between groups was implemented to minimize confounding effects that could arise from demographic variations.

Exclusion criteria were applied to both groups to eliminate factors that could confound the measurement of IRS-1 and Akt levels in saliva. Exclusion criteria for both groups included a history of other systemic malignancies, as concurrent neoplasms could contribute to systemic alterations in signaling pathway activity. The presence of active inflammatory conditions, such as periodontitis or oral lichen planus, was grounds for exclusion, as these conditions are associated with elevated levels of inflammatory mediators that could influence salivary protein composition. A recent history of radiotherapy or chemotherapy was excluded, as these treatment modalities induce significant changes in salivary gland function and tissue architecture that could alter protein expression profiles. Systemic metabolic disorders, including diabetes mellitus, were excluded due to the central role of insulin signaling in regulating IRS-1 and Akt expression, and the potential for metabolic disease to independently alter these pathway components. Informed written consent was obtained from all participants prior to sample collection, with the nature of the study and the procedures involved explained in detail.

Saliva Collection and Processing

Unstimulated whole saliva samples were collected from all participants between 9:00 AM and 11:00 AM to minimize diurnal variation, as salivary protein composition is known to fluctuate throughout the day in response to circadian rhythms and changes in physiological status. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least two hours before collection to prevent contamination of samples with food debris, dietary constituents, or oral care products that could interfere with subsequent protein quantification.

The spitting method was employed for saliva collection, a well-established technique for obtaining unstimulated whole saliva. Participants were asked to expectorate into sterile 50 milliliter polypropylene centrifuge tubes over a period of 10 minutes while seated in a relaxed upright position. This duration was selected to collect an adequate volume of saliva for subsequent analyses while minimizing participant fatigue and ensuring sample consistency. Following collection, the samples were immediately placed on ice to preserve protein integrity and transported to the laboratory within 30 minutes of collection.

To remove cellular debris, exfoliated epithelial cells, and mucins that could interfere with the enzyme-linked immunosorbent assay, the saliva was centrifuged at 10,000 × g for 15 minutes at 4°C. This centrifugation speed and duration were selected to effectively pellet particulate matter while maintaining the soluble protein fraction in the supernatant. The resulting supernatant was carefully aspirated, taking care not to disturb the pellet, and aliquoted into microcentrifuge tubes. The aliquoted samples were stored at -80°C until biochemical analysis to prevent protein degradation and preserve sample stability for subsequent assays.

Quantification of IRS-1 and Akt

The concentrations of IRS-1 and total Akt in the salivary supernatants were quantified using commercially available high-sensitivity solid-phase enzyme-linked immunosorbent assay kits specifically validated for human biofluids. These assays utilize the sandwich enzyme-linked immunosorbent assay principle, which provides high specificity and sensitivity for the target proteins.

For the IRS-1 assay, 100 microliters of salivary supernatant was added to pre-coated microtiter plates containing capture antibodies specific to IRS-1. After incubation to allow for antigen capture and subsequent washing to remove unbound material, a biotin-conjugated antibody specific to IRS-1 was applied to form an antibody-antigen-antibody sandwich complex. This was followed by the addition of Avidin-conjugated Horseradish Peroxidase, which binds with high affinity to the biotin-conjugated detection antibody. For the Akt assay, a similar sandwich enzyme-linked immunosorbent assay protocol was followed using monoclonal antibodies specific to the human Akt protein, with the same detection methodology employed.

In both assays, the colorimetric reaction was developed using tetramethylbenzidine substrate, which undergoes oxidation in the presence of horseradish peroxidase to produce a blue product. The reaction was terminated with a sulfuric acid stop solution, which converts the blue product to a yellow color stable for spectrophotometric measurement. The optical density was measured spectrophotometrically at a wavelength of 450 nanometers using a microplate reader equipped with appropriate filters. The concentration of each protein was determined by interpolation from standard curves generated using known concentrations of recombinant IRS-1 and Akt proteins provided with each kit.

Protein Normalization

To account for variations in salivary flow rate, hydration status, and glandular function that can affect the concentration of individual proteins in saliva, the total protein concentration in each salivary sample was determined using the Bradford protein assay. This colorimetric assay is based on the shift in absorbance maximum of Coomassie Brilliant Blue G-250 dye from 465 to 595 nanometers upon binding to protein, with the degree of color change proportional to the protein concentration.

Bovine serum albumin was used as the standard for calibration, with a series of known concentrations prepared to generate a standard curve. The absorbance of each sample was measured at 595 nanometers, and the total protein concentration was calculated from the standard curve. The final concentrations of IRS-1 and Akt were expressed as picograms per milligram of total salivary protein. This normalization approach adjusts for inter-individual variability in salivary protein content and provides a standardized measure that facilitates comparison between groups and across studies.

Statistical Analysis

Data were analyzed using SPSS software, version 25.0. The distribution of the data was assessed using the Shapiro-Wilk test to determine whether parametric or non-parametric statistical methods were appropriate for analysis. Continuous variables were expressed as mean ± standard deviation to summarize the central tendency and variability of the measurements.

Differences in IRS-1 and Akt levels between the oral squamous cell carcinoma group and the control group were analyzed using the independent samples t-test, a parametric test appropriate for comparing means between two independent groups. This test was selected based on the assumption that the data were normally distributed and that the variances between groups were comparable. A p-value of less than 0.05 was considered statistically significant for all comparisons, representing a 5% probability of incorrectly rejecting the null hypothesis of no difference between groups. All statistical analyses were performed with the groups coded to maintain blinding of the investigator to the clinical status of the samples until after the completion of data analysis.

Results

Demographic and Clinical Characteristics

The study population consisted of 60 individuals, with an equal distribution between the oral squamous cell carcinoma group and the healthy control group, each comprising 30 participants. The demographic characteristics of both groups were comparable, ensuring that any observed differences in biomarker levels could be attributed to disease status rather than demographic disparities.

The mean age of the oral squamous cell carcinoma group was 54.2 ± 8.6 years, while the control group had a mean age of 52.8 ± 7.4 years. Statistical analysis confirmed that this difference was not significant, with a p-value greater than 0.05, indicating that the groups were adequately age-matched. Gender distribution was balanced across both cohorts, with 18 males and 12 females in each group, representing a male-to-female ratio of 1.5:1 in both populations.

Among the oral squamous cell carcinoma patients, the distribution of disease severity revealed that 60% presented with Stage III or IV disease at the time of diagnosis, reflecting the advanced stage at which many oral cancer patients are typically identified. The remaining 40% presented with Stage I or II disease. The most common primary site of involvement was the lateral border of the tongue, accounting for 43.3% of cases, followed by the buccal mucosa, which constituted 30% of cases. The remaining cases involved the floor of the mouth, retromolar trigone, and hard palate.

Quantification of Salivary IRS-1

The concentrations of IRS-1 were significantly elevated in the saliva of patients with oral squamous cell carcinoma compared to the healthy control group. The mean salivary IRS-1 level in the oral squamous cell carcinoma group was 482.14 ± 84.56 picograms per milligram of total protein, representing a substantial increase over the control group values. In contrast, the healthy control group exhibited a mean IRS-1 level of 194.32 ± 42.18 picograms per milligram of total protein. This difference was statistically significant, with a p-value of less than 0.001, indicating that the observed elevation in IRS-1 among oral squamous cell carcinoma patients is highly unlikely to be attributable to chance variation.

The magnitude of difference between the two groups was notable, with oral squamous cell carcinoma patients demonstrating approximately 2.5-fold higher levels of salivary IRS-1 compared to healthy controls. The standard deviations within each group indicate moderate variability, which is expected given the biological heterogeneity inherent in clinical populations.

Quantification of Salivary Akt

Similarly, salivary Akt levels demonstrated a marked increase in the malignant cohort compared to healthy controls. The oral squamous cell carcinoma group showed a mean Akt concentration of 312.85 ± 62.34 picograms per milligram of total protein, while the healthy control group exhibited a mean Akt level of 126.47 ± 28.92 picograms per milligram of total protein. This difference was highly significant, with a p-value of less than 0.001, indicating that salivary Akt levels are substantially elevated in patients with oral squamous cell carcinoma.

The ratio of Akt levels between the oral squamous cell carcinoma and control groups was approximately 2.5:1, consistent with the pattern observed for IRS-1. The standard deviations within both groups reflect the range of biomarker expression across individual patients, with the oral squamous cell carcinoma group showing greater variability, which is consistent with the heterogeneity of tumor biology and disease stage.

Table 1: Comparison of Salivary IRS-1 and Akt Levels Between OSCC Patients and Healthy Controls

Biomarker	OSCC Group (n=30)	Control Group (n=30)	p-value
IRS-1 (pg/mg protein)	482.14 ± 84.56	194.32 ± 42.18	< 0.001*
Akt (pg/mg protein)	312.85 ± 62.34	126.47 ± 28.92	< 0.001*

*Statistically significant at $p < 0.05$

Table 1 presents the comparative data for both biomarkers, demonstrating consistent elevation of both IRS-1 and Akt in the saliva of oral squamous cell carcinoma patients. The p-values indicate highly significant differences for both proteins, confirming their potential utility as discriminatory markers between malignant and healthy states.

Salivary Biomarker Levels by Clinical Stage

Analysis of biomarker levels in relation to disease stage revealed that more advanced disease was associated with higher concentrations of both IRS-1 and Akt in saliva. Among the 30 oral squamous cell carcinoma patients, those with Stage I or II disease demonstrated lower biomarker levels compared to those with Stage III or IV disease.

For IRS-1, patients with Stage I or II disease exhibited a mean concentration of 412.65 ± 55.40 picograms per milligram of total protein, while those with Stage III or IV disease demonstrated a mean concentration of 528.47 ± 72.88 picograms per milligram of total protein. This difference was statistically significant, with a p-value of less than 0.01.

For Akt, patients with Stage I or II disease showed a mean concentration of 268.42 ± 41.15 picograms per milligram of total protein, compared to 342.47 ± 58.20 picograms per milligram of total protein in patients with Stage III or IV disease. This difference was also statistically significant, with a p-value of less than 0.01.

Table 2: Salivary Biomarker Levels Categorized by TNM Clinical Staging in OSCC Patients

Clinical Stage	n	IRS-1 (pg/mg protein)	Akt (pg/mg protein)
Stage I/II	12	412.65 ± 55.40	268.42 ± 41.15
Stage III/IV	18	528.47 ± 72.88	342.47 ± 58.20
p-value		< 0.01	< 0.01

Table 2 illustrates the progressive increase in both biomarkers with advancing clinical stage. The consistent pattern of higher biomarker levels in advanced disease suggests that these proteins not only discriminate between healthy and malignant states but also reflect disease burden or biological aggressiveness. The narrower standard deviations in the Stage I/II group compared to the Stage III/IV group indicate greater homogeneity in biomarker expression among patients with early-stage disease, while the wider variability in advanced-stage patients may reflect the heterogeneity of tumor biology, treatment history, and individual patient factors that become more pronounced in advanced disease.

Discussion

The identification of non-invasive biomarkers in saliva represents a transformative shift in the early detection and monitoring of oral squamous cell carcinoma, offering the potential to complement existing diagnostic modalities with a simple, painless, and cost-effective approach. Our findings demonstrate a significant elevation of both IRS-1 and Akt levels in the saliva of oral squamous cell carcinoma patients compared to healthy controls, suggesting that these signaling proteins may serve as viable indicators of the oncogenic state within the oral cavity [13]. The observed correlation between advanced clinical stages, specifically Stage III and IV, and higher protein concentrations further underscores the potential of the IRS-1/Akt axis to reflect tumor burden and disease progression, providing not only diagnostic information but also insights into the biological aggressiveness of the malignancy [14].

The marked increase in salivary IRS-1 in our oral squamous cell carcinoma cohort aligns with previous tissue-based studies that have characterized IRS-1 as a key driver of head and neck carcinogenesis. IRS-1 acts as a molecular bridge, facilitating the transfer of signals from growth factor receptors, including the insulin-like growth factor-1 receptor and epidermal growth factor receptor, to downstream effectors such as PI3K [15]. This adapter protein integrates signals from multiple upstream activators and serves as a critical point of convergence for various growth-promoting pathways. In oral malignancy, the overexpression of IRS-1 often leads to the sustained activation of the Akt pathway, which is a hallmark of cellular transformation and resistance to apoptosis [16, 17]. The constitutive activation of this pathway enables tumor cells to bypass normal growth control mechanisms, resist programmed cell death, and continue proliferating under conditions that would be inhibitory to normal cells. Our data suggest that these intracellular changes are not confined to the tumor cells themselves but are detectable in the salivary biofluid, likely due to the shedding of tumor-derived exosomes, the release of soluble proteins from necrotic or apoptotic cells, or direct secretion by the malignant epithelium into the surrounding oral environment [18]. The presence of these intracellular signaling proteins in saliva implies that the molecular alterations driving tumorigenesis are reflected in the accessible biofluid that bathes the oral mucosa.

The concomitant rise in salivary Akt levels further supports the hypothesis of a hyperactive PI3K/Akt pathway in oral squamous cell carcinoma patients. Akt, also known as protein kinase B, is a central node in cell signaling that regulates a diverse array of cellular processes including cell survival, proliferation, metabolism, and migration. Its activation is known to promote the expression of matrix metalloproteinases, which degrade the extracellular matrix and facilitate local invasion, and vascular endothelial growth factor, which stimulates angiogenesis to supply nutrients to the growing tumor [19]. The coordinated elevation of both upstream adapter and downstream effector in the same signaling pathway provides convergent evidence for pathway activation, strengthening the biological plausibility of these biomarkers. Previous proteomic analyses of saliva have reported alterations in general protein profiles in oral cancer patients, but the specific quantification of Akt provides a more targeted view of the survival signaling pathways currently active in the patient [20, 21]. Unlike broad proteomic approaches that generate large datasets requiring extensive bioinformatic interpretation, targeted quantification of pathway-specific proteins offers a more direct link to the underlying biology of the disease.

The significant difference observed between early-stage, Stage I and II, and late-stage, Stage III and IV, patients, as presented in Table 2, suggests that Akt levels could potentially assist clinicians in risk stratification and treatment planning [22]. Patients with higher levels of these signaling proteins may harbor more biologically aggressive tumors, potentially warranting more intensive therapeutic approaches or closer surveillance. Conversely, patients with lower biomarker levels may have less aggressive disease that could be managed with less extensive treatment protocols. The correlation with clinical stage also raises the possibility that these markers could be used to monitor treatment response, with declining levels expected following successful surgical resection or chemoradiotherapy. The ability to non-invasively monitor molecular changes over time would represent a significant advance in the management of oral cancer patients, enabling early detection of recurrence and timely intervention.

While these results are promising, several limitations must be acknowledged to contextualize the findings and guide the design of future studies. The sample size, while statistically adequate to detect the observed differences between groups, was relatively small and limited to a single institutional center. Larger, multi-center studies would be necessary to validate these findings across diverse populations and to establish normative reference ranges for clinical use. Additionally, while we excluded patients with systemic metabolic disorders such as diabetes mellitus, which could independently alter insulin signaling pathways, the influence of localized oral inflammatory conditions, including periodontitis and oral lichen planus, on the IRS-1/Akt axis requires further investigation in larger, multi-centric trials [23, 24]. These conditions are common in the general population and could potentially confound biomarker interpretation if they independently affect the levels of these signaling proteins. The specificity of these biomarkers for oral squamous cell carcinoma versus other oral pathologies remains to be established. The cross-sectional design of this study, while appropriate for initial biomarker discovery, does not permit assessment of the temporal relationship between biomarker levels and disease progression or treatment response. Longitudinal studies following patients over time, from diagnosis through treatment and into surveillance, would provide more definitive evidence of the utility of these markers for monitoring disease status.

Future studies should also explore the molecular forms of IRS-1 and Akt present in saliva, as phosphorylation status, rather than total protein levels, may provide even more specific information about pathway activation. The analysis of exosomal content, which may better reflect tumor-derived material than whole saliva, represents another promising direction for refining these biomarkers. Additionally, investigations into the potential utility of these markers for early detection in high-risk populations, such as smokers and alcohol users, and for screening of oral potentially malignant disorders would help define the clinical role of this approach. The longitudinal changes in these salivary markers following surgical resection or chemoradiotherapy should be evaluated to determine their efficacy in monitoring for recurrence, as the ability to detect recurrence earlier than clinical examination alone would represent a significant clinical advance.

In conclusion, our study provides evidence that salivary IRS-1 and Akt are significantly upregulated in oral squamous cell carcinoma and correlate with the clinical stage of the disease. These findings highlight the potential of these proteins as a non-invasive diagnostic panel that could complement current clinical and histopathological examinations, which often require tissue biopsy and may not fully capture the molecular heterogeneity of the disease. The ability to obtain molecular information through a simple, non-invasive saliva collection has the potential to enhance early detection, guide treatment planning, and facilitate monitoring of disease status, ultimately contributing to earlier intervention and improved patient outcomes [25]. Further validation in larger, prospective studies and exploration of the mechanistic basis for the elevation of these signaling proteins in saliva will be essential steps toward the clinical translation of these findings.

Conclusion

The present study successfully quantified salivary IRS-1 and Akt levels, revealing a significant upregulation of both proteins in patients with oral squamous cell carcinoma compared to healthy individuals. The marked elevation in these signaling molecules within the salivary matrix mirrors the known dysregulation of the PI3K/Akt pathway observed in malignant oral tissues. Furthermore, the significant correlation between higher concentrations of these biomarkers and advanced TNM clinical stages suggests that salivary IRS-1 and Akt may serve as reliable indicators of disease severity and tumor progression.

These results underscore the clinical potential of saliva as a diagnostic medium, offering a non-invasive, cost-effective, and patient-friendly alternative to traditional serum-based or invasive biopsy procedures. The integration of IRS-1 and Akt quantification into a salivary diagnostic panel could significantly enhance the early detection of OSCC, providing a window for more conservative and effective therapeutic interventions. While further longitudinal research is necessary to validate these markers for post-treatment monitoring and recurrence, this study establishes a strong foundation for the use of the IRS-1/Akt signaling axis as a biological signature of oral malignancy.

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