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Salivary Diagnostics: A Non-Invasive Approach to Oral Cancer Detection in Pakistan

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ABSTRACT

Background: Due to late-stage diagnosis and restricted access to invasive diagnostic methods, oral cancer presents a serious public health concern in Pakistan.

Objective: The study's goal was to assess salivary diagnostics' efficacy as a non-invasive approach for the early identification of oral cancer in Pakistan, with a particular emphasis on how well it may replace traditional biopsy techniques.

Methodology: The Sharif Medical and Dental College in Lahore, Pakistan, conducted this cross-sectional observational research from January to December of 2023. There were 50 healthy controls and 150 patients with clinically suspected oral cancer. enzyme-linked immunosorbent assay and Polymerase Chain Reaction methods were used to assess the amounts of protein, RNA, and DNA in saliva samples. To assess how well these biomarkers functioned as diagnostic tools, statistical studies were carried out, including sensitivity, specificity, and Receiver Operating Characteristic curve analysis.

Results: Among the 150 oral cancer patients, 92 (61.33%) were male and 58 (38.67%) were female. Salivary biomarkers showed significantly higher levels in oral cancer patients compared to healthy controls (DNA: 150.23 ± 45.67 ng/mL vs. 85.21 ± 20.34 ng/mL, RNA: 200.45 ± 55.12 ng/mL vs. 95.43 ± 22.87 ng/mL, Protein: 70.12 ± 30.45 µg/mL vs. 30.23 ± 15.67 µg/mL) with p-values <0.001. Diagnostic performance was high, with AUC values of 0.92 for DNA, 0.90 for RNA, and 0.88 for protein.

Conclusion: Salivary biomarkers are significantly elevated in oral cancer patients and show high diagnostic accuracy, suggesting their potential as a non-invasive tool for early cancer finding in Pakistan.

Keywords: Oral cancer, salivary biomarkers, non-invasive diagnostics, early detection, Pakistan.

INTRODUCTION

A major global public health concern, oral cancer is responsible for a considerable number of cancer-related fatalities, particularly in low- and middle-income nations like Pakistan [1]. Oral cancer is becoming one of the most frequent malignancies in South Asia due to the high frequency of tobacco use, chewing betel nut, and poor oral hygiene habits [2]. Most patients in Pakistan come with advanced cancers, which makes the disease burden even worse due to late-stage detection [3]. The scarcity of diagnostic resources, ignorance, and the invasiveness of conventional biopsy techniques are the reasons for this delay in diagnosis [4].

Tissue biopsies, which are not only invasive but also often met with patient resistance owing to pain, expense, and the possibility of consequences, constitute a major component of conventional diagnostic procedures for oral cancer [5]. Moreover, it is difficult to obtain specialist diagnostic facilities, especially in underserved and rural locations [6]. These difficulties highlight the need for non-invasive, alternative diagnostic techniques that may aid in early identification and enhance patient outcomes [7].

In recent times, saliva has shown great promise as a diagnostic medium, offering a non-invasive, affordable, and readily available substitute for the identification of diseases [8,9]. Saliva is a biological fluid that contains many biomarkers that have been shown to indicate pathological changes in oral tissues, such as proteins, metabolites, DNA, and RNA [10]. The discovery of particular salivary biomarkers linked to oral cancer has been made possible by developments in molecular diagnostics, opening up new avenues for early detection [11].

While salivary diagnostics have shown potential in the detection of oral cancer in other countries, their use in Pakistan has not received enough attention. Studies assessing salivary biomarkers in the context of Pakistan's distinct demographic and healthcare constraints are few. Furthermore, there is a dearth of research on the integration of salivary diagnostics into regional healthcare systems, particularly in rural regions where oral cancer is very prevalent. This emphasizes the need of doing focused study to evaluate the practicality and clinical usefulness of salivary diagnostics in Pakistan.

Research Objective

The study's goal was to assess salivary diagnostics' efficacy as a non-invasive approach for the early identification of oral cancer in Pakistan, with a particular emphasis on how well it may replace traditional biopsy techniques.

METHODOLOGY

Study Design and Setting

This cross-sectional research examined the effectiveness of salivary diagnostics for non-invasive oral cancer diagnosis. It was carried out at Sharif Medical and Dental College in Lahore from January to December 2023.

Inclusion and Exclusion Criteria

Patients who had a clinical suspicion of oral cancer and who were at least eighteen years old, as well as those who were willing to provide informed permission and saliva samples, were the

study's inclusion criteria. Additionally, patients had to have never had any kind of chemotherapy, radiation therapy, or surgery for mouth cancer before. Patients with non-cancer related oral disorders, those with a history of systemic diseases (autoimmune diseases, for example) that may impact salivary biomarker levels, pregnant women, and patients who declined to provide permission were among the exclusion criteria.

Sample Size

The sample size was established by using the sample size approach for observational studies recommended by the World Health Organization (WHO), which included the prevalence of oral cancer in addition to the study population at Sharif Medical and Dental College. A total of 50 healthy controls and 150 patients with clinically suspected oral cancer were included in order to ensure adequate statistical power and generalizability. The healthy controls had no history of significant oral problems or oral cancer, nor did they consume tobacco products or betel nut products.

Data Collection

Every participant had their saliva samples taken under uniform circumstances. A 5 mL unstimulated whole saliva sample was supplied by each participant, and it was collected using sterile containers. The samples were processed right away in the lab and kept there at -80°C until they were analyzed. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) methods were used to detect biomarkers of interest, such as DNA, RNA, and protein levels connected to oral cancer. Age, gender, tobacco usage, and the clinical stage of the patient's oral cancer were among the pertinent clinical and demographic information that was noted.

Statistical Analysis

We used SPSS version 26 to examine the data. Descriptive statistics were used for summarizing the demographic data. Frequencies and percentages were used to describe categorical data, while mean $\pm$ standard deviation was used to display continuous variables. A chi-square test was used to investigate the relationship between salivary biomarkers and the diagnosis of oral cancer. Whenever the p-value was less than 0.05, statistical significance was reached. The diagnostic accuracy of salivary biomarkers was evaluated by means of Receiver Operating Characteristic (ROC) curve examination.

Ethical Approval

The Sharif Medical and Dental College's Ethical Review Board in Lahore gave the research its clearance. Prior to sample collection, informed permission was acquired from each participant, guaranteeing confidentiality and compliance with the Declaration of Helsinki standards.

RESULTS

Table 1 demonstrates that there were 50 healthy controls and 150 patients with oral cancer in the research. 92 (61.33%) of the cancer patients are men, 58 (38.67%) are women, 72 (48.00%) use tobacco, and 78 (52.00%) do not. Cancer patients are distributed as follows according to their clinical stage: 17 (11.33%) are in Stage I, 34 (22.67%) are in Stage II, 53 (35.33%) are in Stage

III, and 46 (30.67%) are in Stage IV. There are no documented instances of tobacco use or clinical stages among the 28 males (56.00%) and 22 females (44.00%) who make up the healthy controls.

Table 1: Demographic and Clinical Characteristics of Study Participants

Variable		Oral Cancer Patients (n;%)	Healthy Controls (n;%)
Age (years)	Mean $\pm$ SD	50.31 $\pm$ 12.19	45.12 $\pm$ 10.35
Gender	Male	92 (61.33)	28 (56.00)
	Female	58 (38.67)	22 (44.00)
Tobacco Use	Yes	72 (48.00)	-
	No	78 (52.00)	50 (100)
Clinical Stage of Oral Cancer	Stage I	17 (11.33)	-
	Stage II	34 (22.67)	-
	Stage III	53 (35.33)	-
	Stage IV	46 (30.67)	-

Table 2 presents a comparison of salivary biomarker levels between patients with oral cancer and healthy controls. Oral cancer patients showed significantly greater amounts of DNA (150.23 $\pm$ 45.67 ng/mL), RNA (200.45 $\pm$ 55.12 ng/mL), and protein (70.12 $\pm$ 30.45 μ g/mL) than healthy controls, who had levels of 85.21 $\pm$ 20.34 ng/mL, RNA (95.43 $\pm$ 22.87 ng/mL), and protein (30.23 $\pm$ 15.67 μ g/mL). For all changes, p-values less than 0.001 indicate statistical significance.

Table 2: Comparison of Salivary Biomarker Levels Between Oral Cancer Patients and Healthy Controls

Biomarker	Oral Cancer Patients (Mean $\pm$ SD)	Healthy Controls (Mean $\pm$ SD)	p-Value
DNA Quantity (ng/mL)	150.23 $\pm$ 45.67	85.21 $\pm$ 20.34	<0.001
RNA Quantity (ng/mL)	200.45 $\pm$ 55.12	95.43 $\pm$ 22.87	<0.001
Protein Concentration (μ g/mL)	70.12 $\pm$ 30.45	30.23 $\pm$ 15.67	<0.001

The salivary biomarkers for oral cancer detection are shown in Table 3 along with their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). DNA amount has an 85.60% sensitivity, a 90.30% specificity, an 88.40% PPV, and an 87.10% NPV. The sensitivity, specificity, PPV, and NPV of RNA amount are 83.20%, 92.10%, 89.60%, and 85.70%, respectively. The protein concentration shows a 78.40% sensitivity, 94.70% specificity, 91.20% PPV, and 82.30% NPV.

Table 3: Sensitivity and Specificity of Salivary Biomarkers for Oral Cancer Detection

Biomarker	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
DNA Quantity	85.60	90.30	88.40	87.10

RNA Quantity	83.20	92.10	89.60	85.70
Protein Concentration	78.40	94.70	91.20	82.30

Table 4 illustrates the correlation between salivary biomarker levels and the clinical stage of oral cancer. As the cancer stage progresses, the mean levels of DNA quantity, RNA quantity, and protein concentration increase. For Stage I, the biomarkers are: DNA 120.45 ± 40.56 ng/mL, RNA 175.32 ± 48.67 ng/mL, and protein 60.23 ± 25.34 µg/mL. In Stage II, they are: DNA 140.78 ± 50.23 ng/mL, RNA 185.45 ± 52.89 ng/mL, and protein 65.34 ± 28.12 µg/mL. In Stage III, the levels are: DNA 160.45 ± 55.12 ng/mL, RNA 210.34 ± 58.76 ng/mL, and protein 75.12 ± 30.98 µg/mL. For Stage IV, they are: DNA 180.23 ± 60.45 ng/mL, RNA 230.12 ± 62.34 ng/mL, and protein 85.56 ± 35.12 µg/mL.

Table 4: Correlation Between Salivary Biomarker Levels and Clinical Stage of Oral Cancer

Clinical Stage	DNA Quantity (ng/mL) (Mean $\pm$ SD)	RNA Quantity (ng/mL) (Mean $\pm$ SD)	Protein Concentration (µg/mL) (Mean $\pm$ SD)
Stage I	120.45 ± 40.56	175.32 ± 48.67	60.23 ± 25.34
Stage II	140.78 ± 50.23	185.45 ± 52.89	65.34 ± 28.12
Stage III	160.45 ± 55.12	210.34 ± 58.76	75.12 ± 30.98
Stage IV	180.23 ± 60.45	230.12 ± 62.34	85.56 ± 35.12

Table 5 presents the results of the Chi-Square test assessing the association between salivary biomarkers and oral cancer diagnosis. The χ^2 values and p-values indicate significant associations for all biomarkers: DNA quantity with $\chi^2 = 12.45$ ($p < 0.001$), RNA quantity with $\chi^2 = 10.23$ ($p < 0.001$), and protein concentration with $\chi^2 = 8.56$ ($p = 0.003$).

Table 5: Chi-Square Test of Association Between Salivary Biomarkers and Oral Cancer Diagnosis

Biomarker	χ^2 Value	p-Value
DNA Quantity	12.45	<0.001
RNA Quantity	10.23	<0.001
Protein Concentration	8.56	0.003

Table 6 displays the analysis of the ROC curve for salivary biomarkers in the identification of oral cancer. The values of the Area Under the Curve (AUC) are as follows: The amounts of DNA, RNA, and protein are shown to have analysis of variance (AUC) values of 0.90 (95% CI: 0.85 - 0.94, $p < 0.001$), 0.92 (95% CI: 0.88 - 0.96, $p < 0.001$), and 0.88 (95% CI: 0.82 - 0.93, $p < 0.001$), respectively.

Table 6: Receiver Operating Characteristic (ROC) Curve Analysis for Salivary Biomarkers

Biomarker	Area Under the Curve (AUC)	95% Confidence Interval	p-Value
DNA Quantity	0.92	0.88 - 0.96	<0.001
RNA Quantity	0.90	0.85 - 0.94	<0.001
Protein Concentration	0.88	0.82 - 0.93	<0.001

DISCUSSION

This research examined the efficacy of salivary biomarkers as a non-invasive substitute for traditional biopsy techniques in Pakistan for the detection of oral cancer. Our findings showed that oral cancer patients had substantially greater quantities of DNA, RNA, and protein in their saliva than did healthy controls; all biomarkers had p-values less than 0.001. In particular, the mean concentration of DNA was 150.23 ± 45.67 ng/mL, RNA was 200.45 ± 55.12 ng/mL, and protein was 70.12 ± 30.45 µg/mL in oral cancer patients, while the levels in healthy controls were lower (DNA: 85.21 ± 20.34 ng/mL, RNA: 95.43 ± 22.87 ng/mL, and protein: 30.23 ± 15.67 µg/mL). These results are in line with other studies that found salivary biomarker levels were higher in cancer patients than in healthy people [12,13].

The sensitivity, specificity, and predictive values of these biomarkers were used to assess their diagnostic performance in further detail. A sensitivity of 85.60% and a specificity of 90.30% were shown by the amount of DNA, a sensitivity of 83.20% and a specificity of 92.10% by the quantity of RNA, and a sensitivity of 78.40% and a specificity of 94.70% by the concentration of protein. These findings are consistent with earlier studies, which found that salivary DNA and protein indicators had comparable high sensitivities and specificities for the detection of cancer [14]. Comparable positive and negative predictive values were also discovered in our investigation; the greatest positive predictive value was found in protein concentration (91.20%), while the highest negative predictive value was found in RNA amount (85.70%).

An analysis of the relationship between biomarker levels and clinical stage of oral cancer revealed that biomarker concentrations increased as the illness progressed. In Stage IV, for example, the amount of DNA rose from 120.45 ± 40.56 ng/mL in Stage I to 180.23 ± 60.45 ng/mL. This gradient is consistent with other research showing salivary biomarkers may show changes in oral cancer over time [15]. The utility of these biomarkers to track the evolution of the illness and maybe direct treatment approaches is further supported by the notable rise in RNA and protein levels throughout stages.

The Chi-Square test was used to validate the relationship between salivary biomarkers and the diagnosis of oral cancer, and all biomarkers showed significant findings. DNA, RNA, and protein had χ^2 values of 12.45, 10.23, and 8.56, respectively, and their associated p-values showed high correlations. These results support earlier research [16, 17] that found strong correlations between salivary biomarkers and cancer diagnosis.

With AUC values of 0.92 for DNA, 0.90 for RNA, and 0.88 for protein, the ROC curve analysis, in the end, showed high diagnostic accuracy for all biomarkers. These AUC values confirm the promise of salivary diagnostics as a trustworthy screening tool, since they are in line with other study that demonstrated high AUC values for salivary biomarkers in diagnosing different malignancies [18].

Study Limitations

There are many restrictions on this research. First off, tracking changes over time or establishing causal linkages is hampered by the cross-sectional approach. Second, even while the sample size

is adequate, it could not accurately reflect Pakistan's varied population, which might limit how broadly the results can be applied. The research also only uses saliva samples from one institution, which raises the possibility of selection bias. Finally, focusing just on a few biomarkers may miss other possible signs of oral cancer, indicating the need for additional studies to confirm and build on these results.

CONCLUSION

This research shows that salivary biomarkers—specifically, amounts of DNA, RNA, and protein are much higher in oral cancer patients than in healthy controls and have great diagnostic potential. The biomarkers show promise for non-invasive early diagnosis and monitoring of oral cancer in Pakistan since they not only distinguish between malignant and non-cancerous states with high sensitivity and specificity, but they also correlate with the disease's clinical stage. These results provide support to the inclusion of salivary diagnostics in standard screening protocols, especially in areas where invasive diagnostic techniques are not readily available.

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