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Original Article

ASSESSMENT OF THE PROPERTIES (SENSITIVITY AND SPECIFICITY) OF CYFRA 21-1 IN THE DIAGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Objectives: *To evaluate the sensitivity and specificity of the salivary CYFRA 21-1, to be applied in a defined clinical context.*

Materials and Methods: *A case-control design was employed, conducted in Sardar Begum Dental College, and the data was collected from Khyber College of Dentistry, with a total of 101 diagnosed but untreated individuals of Oral squamous cell carcinoma (cases) with biopsy reports and 101 controls, non-disease (matched for age and gender) were selected for the research. Saliva samples were taken from both groups and followed by, testing CYFRA 21-1 using a commercially available ELISA kit to determine its concentration and its sensitivity and specificity.*

Results: *The findings demonstrated that CYFRA 21-1 exhibits strong diagnostic performance for Oral squamous cell carcinoma, with a sensitivity of 93.6%, Specificity of 93%, a positive predictive value of 92.5%, and a negative predictive value of 93.75%.*

Conclusion: *The results highlight the validity of CYFRA 21-1, as a promising biomarker of the saliva for the very initial identification of Oral Squamous Cell Carcinoma.*

Key words: *CYFRA 21-1, Oral Squamous Cell Carcinoma, Sensitivity, Specificity*

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INTRODUCTION

Malignancies of the head and neck have been ranked as the 6th most prevalent malignancies globally. Almost 90% of all head and neck cancers are OSCC[1]. Over the last three decades, the survival rate (five-year) for OSCC remained 50%. Because of its diverse and non-specific clinical manifestations, this highly aggressive tumor is mostly diagnosed at advanced stages, leading to a high mortality rate[2].

Moreover, OSCC is higher in developing regions, particularly South-East Asia[3].This is because of their lifestyle which has exposed them to predisposing factors, like tobacco use, alcohol intake, betel nut habit, human papillomavirus (HPV) infection and ultraviolet radiation[3]. Early detection and timely treatment planning can help prevent the advancement

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of aggressive OSCC[4].

In the diagnosis of OSCC, biopsy is the gold standard[2]. However, due to its complicated procedure that cannot be painful for the patient but also affects the accuracy of the procedure due to the heterogeneous nature of the tumor[5]. With the changing era, a shift from older invasive diagnostic procedures to less invasive methods like (saliva samples) are now considered[6]. Recent findings have revealed that saliva is a preferred medium for diagnosis because of its biomolecules that can help detect various diseases at an early stage, leading to a better prognosis[7-9].

Recently, the salivary biomarker Cytokeratin-19 Fragment (CYFRA 21-1), has been established[10]. This protein can detect not only cancer cells but also variations in epithelial cells and is especially expressed during the death of these cancer cells, giving it great diagnostic potential[10-13].

In lung cancer (Squamous type), CYFRA 21-1 demonstrated greater sensitivity than other epithelial tumor markers, showing higher diagnostic performance than carcinoembryonic antigen (CEA) and tissue polypeptide antigen (TPA)[14]. Previous systematic reviews have shown that salivary biomarkers such as, CYFRA 21-1 and Matrix Metalloproteinase-9 (MMP-9) demonstrate variable diagnostic accuracy for assessing OSCC, with CYFRA 21-1 demonstrating high sensitivity and specificity in lung cancer[15].

The goal in our research is to evaluate the properties of CYFRA 21-1 as an indicative marker for OSCC, specifically its sensitivity, specificity, positive predictive value, and negative predictive value, as we must determine the clinical validity of CYFRA 21-1 which will reflect on the proficiency of the marker between the individuals with OSCC and without OSCC.

MATERIALS AND METHODS

A case-control analysis was conducted according to the STROBE checklist, over a period of one year from April 2021 to May 2022. The sample size was calculated through the WHO sample size calculator, which determined 101 participants of histologically confirmed cases of OSCC individuals, aged between 34 to 83 years. Another 101, related to the case group and without OSCC, were included as the control group. Written informed consent was obtained from all participants before enrollment (cases and controls). The sampling technique used was non-probability judgmental to select the desired participants.

Ethical approval for this study was acquired from Khyber College of Dentistry (KCD) Peshawar under ethical approval number 15/ADR/KCD on 28th April 2021 and data was collected with approval.

Saliva samples were collected after the final diagnosis but prior to the initiation of treatment. Individuals taking medications such as antidepressants, anxiolytics, antihistamines, antihypertensives, or antipsychotics, as well as those with allergies or active dental abscesses within the past month, patients with systemic or autoimmune diseases, and pregnant or lactating women, conditions that could alter salivary flow or chemical composition were excluded.

Individuals from both groups of disease and non-disease were instructed to rinse with sterile saline solution prior to sample collection, to wash up all the food particles or debris that can contaminate the saliva sample. Then, they were asked to fill 3ml of saliva without any stimulation into a labeled container. Then 12ml was added in the saliva, stored in the icebox and transported to laboratory at Mercy Hospital, Peshawar. The collected samples of saliva with saline were centrifuged in the laboratory using 15 ml Falcon tubes. Following the standard laboratory protocol, the tubes were centrifuged at 1000 rpm for 20 minutes. The supernatant achieved was then transferred into 1.5 ml clear micro-Eppendorf tubes and stored in an ice box. After the samples were collected, transferred and preserved at –80 °C in the freezer of Peshawar Medical College laboratory. Once all the samples were collected, the supernatants were analyzed using the ELISA kit assay.

Following the manufacturer's instructions. 100 ul sample was dispended into the wells and then incubated for 90 minutes at the temperature of 37˚C. After discarding the liquid, 100 ul of the Biotinylated Detection Antibody working solution was promptly added to every well. Then at 37˚C, the wells were incubated for 60 minutes. The wells were aspirated and followed by three wash cycles, before adding 100ul Horseradish Peroxidase (HRP) Conjugate working solution. Then again at 37˚C, incubated for 30 minutes. Aspirated and plates were washed 5 times. Then 90ul substrate Reagent was dispensed and incubated for 15 minutes at a temperature of

37˚C. In order, to top the process stop solution of 50ul, was dispensed into the well. 450 nm of optical density was measured instantly.

The calibration curve for the CYFRA 21-1 ELISA kit was generated on the PICOSS II AMP, AUSRTIA (ELISA plate reader) in the laboratory, using standard concentrations ranging from low to high levels of the analyte. Each standard produced a corresponding absorbance value, plotted on the Y-axis versus concentration on the X-axis. A smooth, linear increase in absorbance with rising concentrations, indicating that the assay follows a linear response pattern within the tested range. The strong alignment of the plotted calibration points with the regression line shows good assay precision and reliability. This calibration curve was then used by the ELISA software to calculate the CYFRA 21-1 concentrations in the saliva samples based on their absorbance readings. During laboratory analysis, 21 salivary samples were found unsuitable for testing due to insufficient volume and therefore excluded. Correspondingly, 21 samples from healthy controls, matched by the excluded patient samples, were also removed to maintain balance in the study design. As a result, 80 salivary specimens from patients and 80 specimens from healthy individuals were incorporated in the final analysis, in accordance with the study protocol.

Using the latest version of SPSS (version 22) all the data was analyzed. Confusion matrix was implemented to evaluate the sensitivity (with the disease), specificity (disease-free), positive predictive value (PPV) (patients correctly identified as having the disease by a positive test), and negative predictive value (NPV) (patients with the negative test who do not have the disease), of salivary CYFRA 21-1.

RESULT

Based on the result of this study, that is to measure the properties of CYFRA-21-1 (sensitivity and specificity). As determined from the confusion matrix, a total of 160 samples were analyzed, including OSCC cases and controls. As described in the table below (Table 1.0). Among the OSCC group, 74 samples were correctly identified as positive, while 5 were incorrectly classified as negative. In the control group, 75 samples were correctly identified as negative, whereas 6 were falsely classified as positive. Overall, the test yielded 80 positive results and 80 negative results across both groups.

The values were put in the Table 2.0, with the aim of evaluating the Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of salivary CYFRA 21-1.

The ratio of true negatives to total negatives is 93.75 percent. In this scenario, the program properly identifies 93.75 percent of patients without the disease. The ratio of true positives to total positives is 92.5 percent. This shows that the model properly identifies a substantial number of individuals with the illness (in this example, 92.5% of patients with the ailment). There are 97.38562% accurate categories across all classes. This suggests that the model is capable of accurately classifying the majority of cases.

Positive Predictive Value (PPV), or the percentage of genuine positives among all positive patients, is 92.5 percent. This suggests that 92.5% of individuals categorized by the model as having the illness are in fact affected by it. The Negative Predictive Value (NPV) indicates that 93.75 percent of all patients categorized as negative are real negatives. This shows that 93.75 percent of individuals categorized by the algorithm as lacking the disease are, in fact, healthy.

Table 1: Demonstrating Sensitivity, Specificity, PPV, and NPV for OSCC and Control (healthy individuals).

Result	OSCC	Control	Total
Positive	A	B	80
	74	6	
Negative	C	D	80
	5	75	
	79	81	160

Table 2: Computation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of salivary CYFRA 21-1 using the confusion matrix.

Parameter	Formula	Substitution	Calculation	Result (%)
Sensitivity	TP / (TP + FN) × 100	74 / (74 + 5) × 100	74 / 79 × 100	93.6%
Specificity	TN / (TN + FP) × 100	75 / (75 + 5) × 100	75 / 80 × 100	93.0%
Positive Predictive Value (PPV)	TP / (TP + FP) × 100	74 / (74 + 6) × 100	74 / 80 × 100	92.5%
Negative Predictive Value (NPV)	TN / (TN + FN) × 100	75 / (75 + 6) × 100	75 / 81 × 100	93.75%

DISCUSSION

According to R Simon et al in 2015, sensitivity, specificity, positive predictive value (PPV), Negative predictive value (NPV) are key measures to be used for predictive biomarkers and asses there effect on treatment and survival rate of cancer diagnosed patients[16]. According to a study by Khurshid et al. (2018) suggested that due to direct interaction with the oral mucosa, saliva has been widely explored for its clinical potential in detecting various tumor markers, such as P53, P16, cytokines, interleukin-8, and other biomarkers. In fact, over 100 potential salivary biomarkers have been identified in different studies[17].

The current study indicated high sensitivity and specificity (92.5% and 93.75%, respectively) of the salivary CYFRA 21-1 model, supporting its reliability as a diagnostic tool for OSCC. Similar findings were observed by Malhotra et al[18], who reported a sensitivity of 93.8%. However, these results differ from prior research by Wieskopf et al[19] and Zhong et al[20], which showed lower sensitivities of 19% and 57%, respectively[18-20].

Our study also demonstrated an impressive overall specificity of 92.5% for CYFRA 21-1, aligning closely with the findings of Wieskopf et al. (1994) (94.4%), Zhong et al. 2007) [1] (96.4%), and Malhotra et al. (2016) (84.3%)[18-20]. The coherent result determines the diagnostic performance of CYFRA 21-1 as a potential biomarker for OSCC, but in small cell and non-small lung cancer, according to Chen et al (2021) evaluated sensitivity of 50.5 %, specificity of 58.1% segregating the oral cancers from lung cancers (small and non-small)[21].

Uncovering further documentation, a meta-analysis conducted recently by Liang et al (2024) conducted in China, affirmed the reliability of the diagnostic test, of salivary CYFRA 21-1, which is in accordance with our study[22]. Saliva samples confirmed a sensitivity of 88% and specificity of 98% which shows excellent diagnostic potential. Thus, suggesting that CYFAR 21-1, when analyzed via ELISA, serves as a remarkable sensitive and specific biomarker for early diagnosis of OSCC[22].

Our study findings on CYFRA 21-1 are consistent with previous evidence from systematic reviews, by A.M AlAli et al in 2020 which reported that CYFRA 21-1 exhibits high diagnostic accuracy in identification of OSCC, with sensitivity ranging from 0.84 to 0.94 and specificity from 0.84 to 0.96[23]. These values indicate that CYFRA 21-1 can reliably identify patients with OSCC while correctly excluding healthy individuals. In contrast, matrix metalloproteinase-9 (MMP-9) showed more variable results, with sensitivity between 0.76 and 1.00 but specificity ranging widely from 0.27 to 1.00, suggesting that MMP-9 may have limited reliability as a single diagnostic biomarker for OSCC.15 A meta-analysis conducted by Lihui Liu et al. (2019) reported an overall sensitivity of 53% for CYFRA 21-1 when diagnostic studies primarily were compromised on serum samples were combined[23]. This sensitivity is quite lower than the findings of our research, which elicited a much higher sensitivity using salivary samples. However, their findings, specifically 97%, are consistent with our results[22]. These differences highlight the potential use of saliva as a non-invasive and reliable diagnostic medium in evaluating CYFRA 21-1 for OSCC. The outcome of this study indicated that salivary CYFRA 21-1 has reliable properties of sensitivity and specificity. Many other potential serum and salivary biomarkers for OSCC were not included for comparison due to limited resources and financial constraints as this study focused solely on sensitivity and specificity of CYFRA 21-1 as a salivary biomarker for oral squamous cell carcinoma (OSCC).

CONCLUSION

The sensitivity, specificity, PPV and NPV proves that Cyfra 21-1 biomarker of saliva, can be effective non-invasive diagnostic medium for OSCC. However, conventional diagnostic methods such as tissue biopsy and imaging remain essential for definitive diagnosis and staging. To establish the clinical utility and validity of salivary CYFRA 21-1, Additional research is needed to evaluate its sensitivity and specificity alongside other serum and salivary biomarkers associated with epithelial pathologies. Moreover, determining optimal cut-off values through larger, well-designed studies will be crucial to validate its role as an authentic diagnostic marker for OSCC.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	GM, SHD, SAA, IM, A, SS, BF
Acquisition, Analysis or Interpretation of Data:	GM, SHD, SAA, IM, A, SS, BF
Manuscript Writing & Approval:	GM, SHD, SAA, IM, A, SS, BF

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.