DOI: https://doi.org/10.53350/pjmhs020231711179

ORIGINAL ARTICLE

Salivary Biomarkers CYFRA 21-1 and MMP9: Predictive Indicators of Disease Progression in Oral Squamous Cell Carcinoma

WAQAR-UN-NISA[1], MOOMAL ASLAM KHAN[2], FAIZA AGHA[3], SHUMAILA KHAN[4] , SARA FATIMA[5] , PADMA RATHORE[6]
[1]*Associate Professor, Department of Oral Pathology, Bacha Khan College of Dentistry, Mardan, Pakistan.*
[2]*Senior Lecturer, Department of Pathology, United Medical and Dental College, Karachi, Pakistan*
[3]*Assistant Professor, Department of Biochemistry, Liaquat National Hospital & Medical College, Karachi, Pakistan*
[4]*Lecturer, Department of Biochemistry, Baqai Medical University, Karachi, Pakistan*
[5]*Senior Registrar, Department of Oral Medicine, Baqai Dental College, Karachi, Pakistan*
[6]*Assistant Professor, Department of Physiology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan*
Correspondence to: Waqar-Un-Nisa, Email: dr.waqarunnisanoor@gmail.com

ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is a prevalent cancer with high mortality rates. Identifying non-invasive biomarkers for early diagnosis and monitoring the disease's progression is crucial.
Objective: This study aims to assess the salivary levels of CYFRA 21-1 and MMP-9 in OSCC patients and their correlation with clinicopathological characteristics and histological grades.
Methods: A total of 90 participants, including 45 healthy controls and 45 OSCC patients, were enrolled. Demographic and clinicopathological data were collected, including gender, habits (smoking, gutkha, betel nut, pan/naswar), habit duration, and tumor site. Salivary levels of CYFRA 21-1 and MMP-9 were measured using enzyme-linked immunosorbent assay (ELISA). The OSCC patients were categorized into well-differentiated, moderately differentiated, and poorly differentiated histological grades.
Results: Among OSCC patients, males (78%) were predominant. Smoking and gutkha use were the most common habits. The highest percentage of patients reported habit durations >16 years. CYFRA 21-1 and MMP-9 levels were significantly higher in poorly differentiated OSCC compared to moderately and well-differentiated cases ($p = 0.01$ and $p = 0.002$, respectively). MMP-9 levels showed a significant increase with longer habit durations ($p = 0.002$). The tumor site predominantly involved the tongue, especially in poorly differentiated cases (46.6%).
Conclusion: Salivary CYFRA 21-1 and MMP-9 are promising biomarkers for OSCC, with elevated levels correlating with histological grade and habit duration. These biomarkers may aid in early diagnosis and monitoring the progression of OSCC.
Keywords: CYFRA 21-1, MMP-9, Oral Squamous Cell Carcinoma, Saliva, Histological Grade.

INTRODUCTION

Oral carcinoma represents a significant global health distress as a result to its rising occurrence and rise in death rate [1]. It ranks as the 6th primary reason of cancer-related deaths globally and the second most predominant cancer in Pakistan [2]. The yearly prevalence rate of oral cancer is 3 lakh cases, with a death rate of around 1 lakh 45 thousand cases globally [3].

Oral squamous cell carcinoma (OSCC) constitutes more than 90% of all oral carcinoma cases and is among the most prevalent oral malignancies worldwide. Each year, more than 500,000 new OSCC cases are reported, underscoring its substantial impact on global health [4]. The buccal mucosa, tongue, and floor of the mouth are the most typical areas where OSCC occurs [5]. Its development is closely associated with risk factors such as tobacco use, alcohol consumption, and smokeless tobacco chewing. Additional contributing factors comprise infections with HPV16 and HPV18, genetic susceptibility, industrial hazards, ultraviolet radiation exposure, and poor nutrition caused by low fruit and vegetable intake [6].

The ultimate standard for diagnosing oral squamous cell carcinoma (OSCC) involves a histopathological examination of a biopsy, which is invasive, painful, costly, and raises concerns about cancer spread, leading to late diagnosis. These challenges limit its practicality due to the tumor's nature and therapeutic response. The main treatment strategies for OSCC include surgery, radiation therapy, and chemo-therapy [7]. However, regardless of progressions in these therapeutic, the 5-year survival rate for OSCC remains 50% [8]. As a result, non-invasive techniques for identifying oral squamous cell cancer are receiving more attention [9]. Human genetic alterations can be detected in bodily fluids such urine, blood serum, saliva, and cerebrospinal fluid. These fluids are useful biomarkers for the early identification of oral squamous cell carcinoma because they show changes in proteins and nucleic acids [10].

Salivary biomarkers are thought to be an excellent and promising diagnostic and surveillance tool for OSCC [11]. Among the many benefits of using saliva as a diagnostic medium are its affordability, ease of use, safety, and non-invasiveness. It also makes collecting, transportation, and processing simple [12]. There are a number of salivary biomarkers for oral squamous cell carcinoma that can be utilized as prognostic and diagnostic indicators for OSCC.

CYFRA 21-1 (Cytokeratin 19 Fragment) and Matrix Metalloproteinase-9 (MMP9) have emerged as promising biomarkers in various epithelial cancers. CYFRA 21-1 is a fragment of cytokeratin-19, an intermediate filament protein found in epithelial tissues. Elevated levels of CYFRA 21-1 in bodily fluids, including saliva, are indicative of increased cellular turnover, apoptosis, and tumor invasion, which are hallmarks of OSCC progression[13,14]. MMPs are also associated with disease advancement, with increased expression in oral cancer linked to poor prognosis [15]. The aim of the study is to evaluate the diagnosis of salivary CYFRA 21-1 and MMP9 levels in OSCC patients among histological grades

MATERIAL AND METHODS

This study was designed as a comparative, cross-sectional analysis to evaluate salivary biomarkers CYFRA 21-1 and MMP-9 levels in patients with oral squamous cell carcinoma (OSCC) and their association with histological grades of the disease. The study was conducted from January 2023 to June 2023 following approval from the Institutional Review Board (IRB). Recruitment of participants with oral squamous cell carcinoma (OSCC) was done at the dental outpatient department (OPD) of a tertiary care hospital in Karachi.

Participants for the control group were recruited from the attendants, friends, family, and colleagues of patients, ensuring they met the inclusion criteria for healthy controls

In this investigation, 90 participants were recruited and allocated into three categories: well-differentiated OSCC (n = 15), moderately differentiated OSCC (n = 15), and poorly differentiated OSCC (n = 15), alongside healthy controls (n = 45). Patients were identified through clinical assessment and verified via histopathological analysis. Control participants were age- and

Received on 11-07-2023
Accepted on 22-10-2023

gender-matched individuals with no history of malignancy or habits linked to OSCC risk factors.

Participants included OSCC patients aged 18–70 years with histologically confirmed diagnoses and no prior history of treatment, including surgery, radiotherapy, or chemotherapy. Exclusion criteria encompassed patients with systemic diseases, infections, or autoimmune conditions, those with prior malignancies or non-OSCC oral pathologies, and pregnant or lactating women.

Saliva samples were chosen due to their non-invasive nature and ease of collection. Participants were advised to abstain from consuming food, beverages, or smoking for a minimum of 30 minutes before the collection to prevent contamination. They were also asked to rinse their mouths with water to remove any food or debris that might interfere with the sample. Each participant was asked to sit comfortably and allow saliva to naturally accumulate in their mouth. Using the passive drooling method, participants were instructed to spit into a sterile collection container. This process was carried out in a controlled environment, ensuring no external contamination of the sample. The amount of saliva collected was approximately 5-10 mL, which was considered sufficient for analysis. To ensure consistency, saliva was collected in the morning when salivary flow is typically more stable. Following collection, the saliva was promptly placed into properly categorized sterile tubes. After that, saliva samples were immediately taken to laboratory and centrifuged for 15 min, 4 °C at 8000 rpm. The salivary samples obtained were preserved at -80°C until subsequent analysis. The samples were aliquoted into smaller portions to avoid repeated freeze-thaw cycles, which could degrade the biomarkers.

Protein estimation was performed using Bradford's method. Salivary MMP-9 and CYFRA 21-1 levels for all study groups were quantified using the Enzyme-Linked Immunosorbent Assay (ELISA) sandwich technique as per the manufacturer's instructions in the Bioassay Technology KIT. Each sample was analyzed in triplicate to ensure the accuracy and consistency of the results.

This study was carried out in adherence to the ethical guidelines set forth in the Declaration of Helsinki. Ethical approval was granted by the Institutional Review Board (IRB) before the study began. All participants were completely informed about the study's objectives, methods, future risks, and benefits in terms they could easily understand.

Written informed consent was acquired from all participants prior to their inclusion in the research. The consent process emphasized the voluntary nature of participation, ensuring that individuals could withdraw at any stage without any repercussions. The privacy and secrecy of the participants were safeguarded throughout the study.

Statistical Analysis: Data were analyzed using SPSS version 25. Mean and standard deviation values were calculated for salivary CYFRA 21-1 and MMP-9 levels. One-way ANOVA with post hoc Tukey's test was applied to compare biomarker levels among histological grades of OSCC. A p-value of <0.05 was considered statistically significant.

RESULTS

The study analyzed demographic, clinicopathological characteristics, and salivary biomarker levels in healthy controls and OSCC patients. The study included 45 healthy controls and 45 OSCC patients.

Table 1 shows demographic and clinicopathological characteristics. Among OSCC patients, males were predominant (78%) compared to females (22%). Habits such as smoking (31%) and gutkha use (29%) were most frequent, followed by betel nut chewing (18%) and pan/naswar (22%). Notably, 38% of OSCC patients reported a habit duration of >16 years, the highest among duration categories. In contrast, controls reported no significant habits (90% had no habits).

Table 1: Demographic and Clinicopathological Characteristics of Healthy Controls and OSCC

Category	Controls (N = 45)	OSCC (N = 45)
Age in years		
Gender		
Male	27 (60%)	35 (78%)
Female	18 (40%)	10 (22%)
Habits		
No Habits	40 (90%)	0 (0%)
Pan/Naswar	1 (2%)	10 (22%)
Gutkha	1 (2%)	13 (29%)
Betel Nuts	1 (2%)	8 (18%)
Smoking	2 (4%)	14 (31%)
Duration (years)		
No Duration	40 (89%)	0 (0%)
<5	5 (11%)	9 (20%)
5–10	0 (0%)	9 (20%)
11–15	0 (0%)	10 (22%)
16–20	0 (0%)	17 (38%)

The table compares demographic and clinical characteristics between control and OSCC groups.

Duration refers to the years of habits exposure (e.g., Pan/Naswar, Gutkha, Betel Nuts, or Smoking).

Statistical analysis was performed using frequency and percentages

Table 2: Distribution of Demographic and Clinical Characteristics among OSCC Patients Based on Histological Grades

Category	Well Differentiated OSCC Total = 15 N (%)	Moderately Differentiated OSCC Total = 15 N (%)	Poorly Differentiated OSCC Total = 15 N (%)	P- value
Gender				
Male	12 (80%)	11 (73.3%)	12 (80%)	
Female	3 (20%)	4 (26.7%)	3 (20%)	0.02
Habits				
Pan/Naswar (%)	2 (13.3%)	5 (33.3%)	3 (20%)	
Gutkha (%)	4 (26.7%)	3 (20%)	6 (40%)	
Betel Nuts (%)	3 (20%)	2 (13.3%)	3 (20%)	
Smoking (%)	6 (40%)	5 (33.3%)	3 (20%)	0.08
Duration (years)				
<5 (%)	2 (13.3%)	5 (33.3%)	2 (13.3%)	
5–10 (%)	5 (33.3%)	4 (26.7%)	3 (20%)	
11–15 (%)	3 (20%)	4 (26.7%)	4 (26.7%)	
16–20 (%)	5 (33.3%)	2 (13.3%)	6 (40%)	0.55
Site				
Buccal mucosa	4 (26.7%)	4 (26.7%)	7(46.6%)	
Tongue	8 (53.3%)	7 (46.6%)	6 (40%)	
Lower buccal sulcus	1 (6.7%)	1 (20%)	1 (20%)	
Lower lip	1(6.7%)	1(6.7%)	1 (6.7%)	
Palate (%)	1 (6.7%)	2 (13.3%)	0 (0%)	0.04

Table 2 details the distribution of clinical characteristics among OSCC patients by histological grade. Poorly differentiated OSCC exhibited the highest percentages for smoking (40%) and gutkha use (40%), while well-differentiated OSCC showed higher

rates for betel nut chewing (20%) and pan/naswar use (13.3%). Habit duration >16 years was most common in poorly differentiated OSCC (40%) compared to well-differentiated cases (33.3%). Tumors in the tongue were predominant across all grades, with poorly differentiated OSCC showing the highest occurrence (46.6%). Significant differences were noted in gender (p = 0.02), habits (p = 0.08), and tumor site (p = 0.04).

Statistical analysis was performed using the chi-square test for categorical variables.

CYFRA 21-1: cytokeratin 19 fragments, and MMP-9: matrix metalloproteinase-9.

A p-value <0.05 was considered statistically significant.

Gender and site comparisons showed significant differences, while habits and duration did not show statistical significance across OSCC grades.

Table 3 presents the comparison of salivary CYFRA 21-1 and MMP-9 levels among histological grades of OSCC. Poorly differentiated OSCC exhibited the highest mean levels for both biomarkers. CYFRA 21-1 levels were significantly elevated in poorly differentiated OSCC (35.23 ± 10.29 ng/mL), followed by moderately differentiated OSCC (25.34 ± 7.82 ng/mL) and well-differentiated OSCC (18.30 ± 9.32 ng/mL), with a statistically significant difference (p = 0.01). Similarly, MMP-9 levels were highest in poorly differentiated OSCC (59.23 ± 18.76 ng/mL), compared to moderately differentiated OSCC (52.12 ± 15.21 ng/mL) and well-differentiated OSCC (40.67 ± 12.34 ng/mL), also showing a significant difference (p = 0.002).

Table 3: Intra-group Comparison of Salivary CYFRA 21-1 and MMP-9 Levels among Histological Grades of OSCC

Histological Grade	Salivary CYFRA 21-1 (ng/mL)	P-Value (CYFRA 21-1)	Salivary MMP-9 (ng/mL)	P-Value (MMP-9)
Well Differentiated	18.30 ± 9.32	0.01	40.67 ± 12.34	0.002
Moderately Differentiated	25.34 ± 7.82		52.12 ± 15.21	
Poorly Differentiated	35.23 ± 10.29		59.23 ± 18.76	

CYFRA 21-1: cytokeratin 19 fragments, and MMP-9: matrix metalloproteinase-9. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's test. A p-value <0.05 was considered statistically significant.

Table 4 showed Salivary CYFRA 21-1 and MMP-9 levels were highest in tongue lesions but showed no significant association with lesion site. However, both markers significantly increased with the duration of OSCC, with CYFRA 21-1 rising from 18.30 ng/mL to 35.23 ng/mL and MMP-9 from 40.67 ng/mL to 55.23 ng/mL over 16–20 years. These findings highlight their potential as biomarkers for disease progression. Statistical significance was noted for duration (P = 0.01 and P = 0.002, respectively).

Table 4: Association of Salivary CYFRA 21-1 and MMP-9 with Sites and Duration in OSCC Patients

Category	Details	Salivary CYFRA 21-1 (ng/mL)	P-Value	Salivary MMP-9 (ng/mL)	P-Value
Site	Buccal Mucosa	19.45 ± 6.23	0.09	48.12 ± 10.34	0.78
	Tongue	23.78 ± 7.45		52.45 ± 12.21	
	Lower Buccal Sulcus	17.56 ± 5.67		44.78 ± 9.89	
	Lower Lip	16.23 ± 6.34		42.67 ± 8.56	
	Palate	18.45 ± 7.12		39.23 ± 9.12	
Duration (years)	<5	18.30 ± 9.32	0.01	40.67 ± 12.34	0.002
	5–10	25.34 ± 7.82		48.12 ± 15.21	
	11–15	29.45 ± 8.12		53.34 ± 14.45	
	16–20	35.23 ± 10.29		55.23 ± 18.76	

CYFRA 21-1: cytokeratin 19 fragments, and MMP-9: matrix metalloproteinase-9. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's test. A p-value <0.05 was considered statistically significant.

DISCUSSION

Oral cancer, particularly oral squamous cell carcinoma (OSCC), remains a noteworthy health challenge worldwide and its early detection is crucial for improving survival rates [16]. The 5-year survival rate for OSCC remains disappointing at beyond 40%, with most cases diagnosed at later stages, which significantly impacts treatment outcomes [17].

The elevated expression of both CYFRA 21-1 and MMP-9 in various metastatic and invasive cancers underscores their potential as biomarkers for early detection, prognosis, and monitoring therapeutic responses in cancers like OSCC, gastric cancer, lung cancer, and breast cancer [14].

The current study highlighted the investigative potential of salivary biomarkers CYFRA 21-1 in OSCC patients, emphasizing its association with histological grades. The findings revealed a important association between elevated salivary levels of CYFRA 21-1 and disease severity, with the highest levels observed in poorly differentiated OSCC. Our results are constant with preceding research that reported elevated levels of CYFRA 21-1 in OSCC patients related to healthy controls, predominantly in advanced disease stages. Additionally, CYFRA 21-1 showed a significant association with the histologic grading and staging of OSCC, demonstrating a positive correlation between its serum and salivary levels [18]. The study demonstrated that elevated salivary CYFRA 21-1 levels in OSCC and potentially malignant lesions compared to healthy controls, highlighting its potential for early oral cancer diagnosis [19]. A strong association was also observed between oral lesions and habits like alcohol consumption, smoking, and tobacco chewing, reinforcing the diagnostic significance of salivary biomarkers in malignancy detection [20]. A meta-analysis validates CYFRA 21-1 as a dependable diagnostic biomarker for OSCC, with saliva demonstrating enhanced sensitivity and specificity over serum[19]. These results advocate for the use of salivary CYFRA 21-1 in the early and precise detection of OSCC. This enhanced expression may result from alterations in the differentiation process during malignant transformation, causing supra-basal cells to retain and overexpress CK19 in large amounts [19]. It has been proposed that the elevated CK19 undergoes caspase 3-mediated cleavage during apoptosis, releasing fragments into extracellular spaces. This process results in the presence of CYFRA 21-1 in various body fluids, including blood, saliva, and CSF [18].

Similarly, MMP-9, a protease involved in extracellular matrix degradation and tumor invasion, has been widely implicated in various cancers, including OSCC [21]. Our study witnessed a noteworthy increase in MMP-9 levels in poorly differentiated OSCC (p = 0.002), which is consistent with the notion that higher MMP-9 expression correlates with more aggressive disease. The findings align with those by Smriti et al., who demonstrated higher levels of salivary MMP-9 concentration in OSCC subjects compared to those with tobacco habits and control groups [22]. A study stated a significant increase in serum MMP-9 levels in OSCC patients,

showing a +28% mean difference (393.21 pg/ml) with a p-value of 0.001 compared to healthy controls. The elevated MMP-9 levels were also linked to tumor stage and nodal involvement, highlighting its potential as a prognostic marker for OSCC[23]. The research demonstrated that MMP-9 immunoexpression elevated with the progression of both the stage and histological grade of OSCC, with statistically significant findings. This implies that MMP-9 is integral to invasion and metastasis, highlighting its potential as an independent prognostic marker for OSCC [24].

MMP-9 has been linked to the progression of dysplasia to cancer, with its polymorphism correlating strongly with a higher risk for OSCC development. It shows a significant role in OSCC cell migration and lymph node metastasis, contributing to a worse prognosis. Increased MMP-9 expression in metastatic OSCC, particularly in gingival cancers, further supports its involvement in metastasis [15].

These findings highlight the potential of salivary biomarkers CYFRA 21-1 and MMP-9 for the early detection and prognosis of OSCC, aiming to improve management and outcomes.

CONCLUSION

In conclusion, salivary biomarkers CYFRA 21-1 and MMP-9 exhibit considerable promise as non-invasive diagnostic markers for oral squamous cell carcinoma (OSCC). Elevated levels of these biomarkers correlate with higher histological grades and longer duration of harmful habits, particularly smoking and gutkha use. The study highlights the role of these biomarkers in distinguishing between different OSCC grades, suggesting their relevance in early detection and monitoring disease progression. Further studies are necessary to validate these findings and assess their clinical applicability for routine OSCC screening and management.

Author Contributions

1. Dr. Waqar-Un-Nisa (WUN): Conceptualization, methodology, supervision, project administration, and manuscript review.
2. Dr. Moomal Aslam Khan (MAK): Data analysis, interpretation and original draft preparation.
3. Dr. Faiza Agha (FA): Investigation and Data Collection.
4. Dr. Shumaila Khan (SK): Data compilation, Statistical Analysis and manuscript editing.
5. Dr. Sara Fatima (SF): Writing—review and editing, and funding acquisition.
6. Dr. Padma Rathore (PR): Supervision, validation, and final approval of the manuscript.

Conflict of interest: None to declare.

Grant support and financial disclosure: None to disclose

REFERENCES

1. Chaves FN, Bezerra TMM, Moraes DC, dos Santos Costa SF, Silva PGB, Alves APNN, et al. Loss of heterozygosity and immunoexpression of PTEN in oral epithelial dysplasia and squamous cell carcinoma. Experimental and Molecular Pathology. 2020;112:104341.
2. Sahu S, Routray S. Assessing the analytical efficacy of TEX in diagnosing oral cancer using a systematic review approach. Journal of Oral Pathology & Medicine. 2021;50(2):123-8.
3. Pérot P, Falguieres M, Arowas L, Laude H, Foy J-P, Goudot P, et al. Investigation of viral etiology in potentially malignant disorders and oral squamous cell carcinomas in non-smoking, non-drinking patients. PLoS One. 2020;15(4):e0232138.
4. Givony S. Oral squamous cell carcinoma (OSCC) an overview. J Med Sci. 2020;8:67-74.
5. Zahid E, Bhatti O, Zahid MA, Stubbs M. Overview of common oral lesions. Malaysian Family Physician: the Official Journal of the Academy of Family Physicians of Malaysia. 2022;17(3):9.
6. Dikova V, Jantus-Lewintre E, Bagan J. Potential non-invasive biomarkers for early diagnosis of oral squamous cell carcinoma. Journal of Clinical Medicine. 2021;10(8):1658.
7. de Barros-Silva PG, Fontes-Borges MM, Costa-Dias C, Mota-Lemos JV, do Perpétuo Socorro-Saldanha-Cunha M, Fernandes-Souza E, et al. Clinical-pathological and sociodemographic factors associated with the distant metastasis and overall survival of oral cavity and oropharynx squamous cell carcinoma. Medicina oral, patologia oral y cirugia bucal. 2020;25(3):e375.
8. Moya-Garcia CR, Okuyama H, Sadeghi N, Li J, Tabrizian M, Li-Jessen NY. In vitro models for head and neck cancer: Current status and future perspective. Frontiers in Oncology. 2022;12:960340.
9. Liu D, Xin Z, Guo S, Li S, Cheng J, Jiang H. Blood and salivary microRNAs for diagnosis of oral squamous cell carcinoma: a systematic review and meta-analysis. Journal of Oral and Maxillofacial Surgery. 2021;79(5):1082. e1-. e13.
10. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. Journal of oral biology and craniofacial research. 2016;6(1):67-76.
11. Naz S, Salah K, Khurshid A, Hashmi AA, Faridi N. Head and neck squamous cell carcinoma-comparative evaluation of pathological parameters in young and old patients. Asian Pacific Journal of Cancer Prevention. 2015;16(9):4061-3.
12. Diesch T, Filippi C, Fritschi N, Filippi A, Ritz N. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: A systematic review. Cytokine. 2021;143:155506.
13. Yoon S, Lim YK, Kim HR, Lee M-K, Kweon OJ. Establishment of Reference Intervals of Cytokeratin 19 Fragment Antigen 21-1 in Korean Adults. Annals of laboratory medicine. 2023;43(1):82-5.
14. AlAli A, Walsh T, Maranzano M. CYFRA 21-1 and MMP-9 as salivary biomarkers for the detection of oral squamous cell carcinoma: a systematic review of diagnostic test accuracy. International journal of oral and maxillofacial surgery. 2020;49(8):973-83.
15. Monea M, Pop AM. The use of salivary levels of matrix metalloproteinases as an adjuvant method in the early diagnosis of oral squamous cell carcinoma: a narrative literature review. Current Issues in Molecular Biology. 2022;44(12):6306-22.
16. Anwar N, Pervez S, Chundriger Q, Awan S, Moatter T, Ali TS. Oral cancer: Clinicopathological features and associated risk factors in a high risk population presenting to a major tertiary care center in Pakistan. Plos one. 2020;15(8):e0236359.
17. Abati S, Bramati C, Bondi S, Lissoni A, Trimarchi M. Oral cancer and precancer: a narrative review on the relevance of early diagnosis. International journal of environmental research and public health. 2020;17(24):9160.
18. Adusumilli P, Babburi S, Venigalla A, Benarji KA, Sai SK, Soujanya P. Estimation of salivary and serum CYFRA 21-1 levels in patients with oral squamous cell carcinoma. Journal of Oral and Maxillofacial Pathology. 2023;27(1):98-102.
19. Rajkumar K, Ramya R, Nandhini G, Rajashree P, Ramesh Kumar A, Nirmala Anandan S. Salivary and serum level of CYFRA 21-1 in oral precancer and oral squamous cell carcinoma. Oral diseases. 2015;21(1):90-6.
20. Goldoni R, Scolaro A, Boccalari E, Dolci C, Scarano A, Inchingolo F, et al. Malignancies and biosensors: A focus on oral cancer detection through salivary biomarkers. Biosensors. 2021;11(10):396.
21. Mondal S, Adhikari N, Banerjee S, Amin SA, Jha T. Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview. European journal of medicinal chemistry. 2020;194:112260.
22. Smriti K, Ray M, Chatterjee T, Shenoy R-P, Gadicherla S, Pentapati K-C, et al. Salivary MMP-9 as a biomarker for the diagnosis of oral potentially malignant disorders and oral squamous cell carcinoma. Asian Pacific journal of cancer prevention: APJCP. 2020;21(1):233.
23. Benitha G, Ramani P, Jayaraman S, Abilasha R, Ramalingam K, Krishnan M. Evaluation of Serum Levels of Matrix MetalloProteinase-9 (MMP-9) in Oral Squamous Cell Carcinoma and Its Clinicopathological Correlation. Cureus. 2023;15(2).
24. Patil R, Mahajan A, Pradeep G, Prakash N, Patil S, Khan SM. Expression of matrix metalloproteinase-9 in histological grades of oral squamous cell carcinoma: An immunohistochemical study. Journal of Oral and Maxillofacial Pathology. 2021;25(2):239-46.

This article may be cited as: Nisa WU, Khan MA, Agha F, Khan S, Fatima S, Rathore P: Salivary Biomarkers CYFRA 21-1 and MMP9: Predictive Indicators of Disease Progression in Oral Squamous Cell Carcinoma. Pak J Med Health Sci, 2023;18(11):179-182.