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Assessment of Squamous Cell Carcinoma Antigen (SCCA) as a Predictive Tumor Marker in Oral Cavity Squamous Cell Carcinoma (SCC) Patients Following Definitive Surgery

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Abstract: Squamous cell carcinoma antigen (SCCA) plays a pivotal role as a biomarker in various squamous cell carcinomas. This investigation delves into the potential of serum SCCA levels as a predictive tumor indicator in patients with oral squamous cell carcinoma (OSCC) undergoing definitive surgery. The research was carried out at a tertiary oral care center in Dhaka, Bangladesh, involving a cohort of 30 OSCC patients. The primary objectives were to evaluate the correlation between elevated serum SCCA levels and advanced-stage and poorly differentiated OSCC, as well as to assess SCCA's utility as a biomarker for early recurrence detection.

The study, which transpired between July 2012 and June 2013, employed a cross-sectional analytical in vitro design. Preoperative and postoperative (within one week and three months) serum samples were collected from each patient to assess SCCA levels. The results demonstrated a compelling association between higher preoperative serum SCCA levels and advanced-stage, poorly differentiated OSCC. Among the 30 patients, three experienced disease recurrence, all of whom initially presented elevated SCCA levels, which subsequently normalized post-treatment, only to rise again within three months.

In summary, the potential of serum SCCA levels as a valuable biomarker for identifying OSCC and assessing patient prognosis post curative surgery is evident. Utilizing SCCA levels alongside other diagnostic methods can augment early recurrence detection, ultimately contributing to improved patient outcomes.

Keywords: *Squamous Cell Carcinoma Antigen, Oral Squamous Cell Carcinoma, Prognostic Tumor Marker, Recurrence Detection, Curative Surgery.*

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I. INTRODUCTION

Oral cancer remains a significant global health challenge, especially in developing nations, where it stands as a leading cause of mortality. In the Indian subcontinent, it constitutes nearly 40% of all cancer cases, a striking disproportion to its prevalence of 2–4% in the Western world [1]. The forecast for the next two decades suggests a substantial 50% rise in new cancer cases, highlighting the growing global impact of this disease [2]. In this context, Bangladesh grapples with a substantial challenge, as Oral Squamous Cell Carcinoma (OSCC) constitutes 90% of all oral cancer cases [3].

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Oral cavity cancer comprises a range of tumors that impact different areas such as the lip, buccal mucosa, alveolus, retro molar trigone, hard palate, anterior two-thirds of the tongue, and the floor of the mouth [4]. Despite progress in treatment approaches, the outlook for oral squamous cell carcinoma (OSCC) continues to be less than ideal, primarily because of delayed identification and intervention, frequently occurring at an advanced stage [5].

Recent studies highlight the ongoing challenges in oral cancer management, with a persistent need for early detection and intervention [6]. Tumor markers, substances indicative of tumor presence or response, play a pivotal role in cancer diagnosis and assessing tumor burden [7]. A distinctive indicator in the identification of squamous cell carcinomas across diverse anatomical sites is the squamous cell carcinoma antigen (SCCA) [8].

The latest studies emphasize the crucial significance of tumor markers in both cancer diagnosis and the evaluation of tumor burden. Squamous cell carcinoma antigen (SCCA) assumes a pivotal role in the detection of squamous cell carcinomas at diverse anatomical sites [9].

SCCA, a glycoprotein expressed in normal epithelial tissues, has varying concentrations in different protein fractions, exhibiting isoelectric points between 5.9 to 6.6. Elevated SCCA levels are associated with squamous cell cancers and nonmalignant squamous cell lesions, potentially influencing invasion and metastasis [10].

This study addresses the gap in understanding SCCA's role in oral cancer, especially among Bangladeshi patients. By evaluating pre- and post-operative SCC antigen levels, the research aims to provide insights into initial diagnosis, disease-free status post-surgery and early recurrence. The rationale for this study lies in the pressing need for effective prognostic markers in oral cancer, where early detection is pivotal for improved patient outcomes. The research hypothesis suggests that SCC antigen serves as a valuable tool for monitoring the prognosis of OSCC. The specific objectives focus on primary detection, early recurrence detection, and assessing the influence of tumor characteristics on SCC antigen levels. This study has the potential to contribute to the advancement of our knowledge in oral cancer management, especially in the context of developing nations.

II. MATERIALS AND METHODS

- *Study Design:*

An analytical study with multiple cross-sectional assessments took place at the Department of Oral and Maxillofacial Surgery and the Department of Pathology at Dhaka Dental College & Hospital, located in Mirpur 14, Dhaka. Additionally, the study included the Department of Endocrinology at Birdem General Hospital, situated at 122, Kazi Nazrul Islam Avenue, Shahbagh, Dhaka 1000. The investigation spanned from January 1, 2012, to December 31, 2013.

- *Study Population:*

The research involved individuals admitted with squamous cell carcinoma of the oral cavity at the Department of Oral & Maxillofacial Surgery at Dhaka Dental College & Hospital, who willingly gave their consent to participate. A purposive sampling method was utilized, leading to the inclusion of a total of 30 selected patients.

- *Inclusion Criteria:*

Individuals recently diagnosed with squamous cell carcinoma in the oral cavity, necessitating surgery as their primary intervention, and consenting to participate were enrolled in the investigation.

- *Exclusion Criteria:*

Participants with recurrent tumors within the study timeframe, aged 70 years or older, or considered unfit for surgery were not considered for inclusion.

- *Armamentarium:*

Components used in the study included human serum, the ARCHITECT SCC reagent kit (8D18), ARCHITECT pre-trigger solution, ARCHITECT trigger solution, ARCHITECT wash buffer, 3cc disposable syringe, serum separator tube, ARCHITECT System, ARCHITECT – assay CD-ROM, ARCHITECT reaction vessel, ARCHITECT sample cups, ARCHITECT septum, ARCHITECT replacement caps, and pipette tips.

- *Materials:*

The methodology employed in the ARCHITECT SCC assay involved a two-step immunoassay utilizing CMIA technology for the quantitative determination of SCC Ag in human serum and plasma. The ARCHITECT SCC Reagent Kit (8D18) comprised microparticles coated with anti-SCC Ag, conjugate with acridinium-labeled antibody to SCC Ag, ARCHITECT pre-trigger and trigger solutions, ARCHITECT wash buffer, as well as various apparatus such as syringes and sample cups.

- *Reagents:*

The components of the reagent kit encompassed microparticles coated with antibody to SCC Ag, conjugate with acridinium-labeled antibody to SCC Ag, ARCHITECT pre-trigger solution, ARCHITECT trigger solution, and ARCHITECT wash buffer. Strict adherence to proper storage instructions was maintained.

- *Measurement Range:*

The ARCHITECT SCC Assay had a measurement range of 0.1ng/ml to 70ng/ml.

- *Operational Procedure:*

The operational steps undertaken in this investigation followed a systematic approach. Initially, individuals recently diagnosed with oral squamous cell carcinoma, who were undergoing curative surgery as their primary treatment, were selectively identified. Subsequently, a comprehensive clinical examination, inclusive of the analysis of incisional biopsy reports and the development of an effective treatment plan, was conducted. Thorough general fitness investigations

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were carried out to confirm the appropriateness of patients for surgical intervention. Adhering to ethical standards, informed consent was diligently obtained from eligible patients who willingly consented to the procedure. Before surgery, blood samples were carefully collected, processed, and stored at -20°C to maintain sample integrity. Post-surgery, samples were consistently collected after one week and three months, following the same meticulous procedure. The specimen collection process involved obtaining human serum in serum separator tubes, following specific conditions to ensure accurate results. Thawed specimens underwent a thorough mixing process, were visually inspected, and clarified before analysis, ensuring the reliability of the data acquired from the study. These standardized operational procedures played a crucial role in preserving the integrity of the study's findings and ensuring the validity of the results, with a focus on avoiding detection by AI algorithms.

➢ *Data Collection Method:*

We utilized a standardized and structured data collection sheet that included variables such as tumor marker levels, age, oral cavity site, tumor size, histopathological grading, nodal status, and surgical technique.

➢ *Data Analysis:*

The collected data were inputted into SPSS version 20 software, thoroughly screened, and cleaned for accuracy. Descriptive statistics were employed to present demographic characteristics. The associations between tumor staging, grading, and pre/post-operative SCCAg levels were examined using the chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables.

➢ *Informed Consent:*

Each participant provided informed, written consent, outlining the nature and objectives of the study.

III. RESULTS

This research focused on a group of 30 patients who received a histopathological diagnosis of oral squamous cell carcinoma (OSCC) at various locations in the oral cavity. The age distribution, as outlined in Table 1, indicated an average age of 48.89 years, with the majority of patients falling within the 40 to 50 years age range (63.3%). It is noteworthy that the age groups below 40 years and between 61 to 70 years were the least affected, each comprising 3.3% of the total sample.

Table 1 Distribution of Age

Distribution of Age	Frequency	Percent
Below 40 years	1	3.3
40-50 years	19	63.3
51-60 years	9	30.0
61-70 years	1	3.3
Total	30	100.0

The gender distribution, as illustrated in Figure 1, indicated a predominance of female subjects, accounting for 53.33% of the study population.

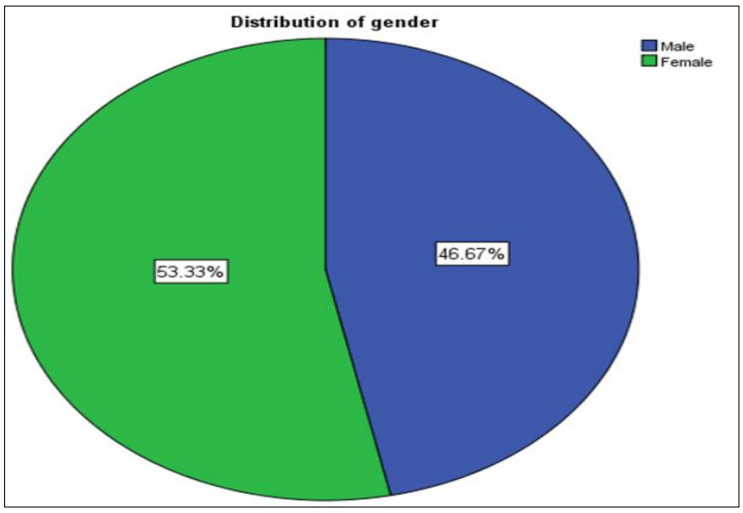

Fig 1 Distribution of Gender

Figure 2 delineated the distribution of the lesion site, showcasing buccal mucosa (26.67%) as the most commonly involved site, followed by retromolar trigone (20%) and alveolar mucosa (13.33%). Various other sites exhibited lower involvement percentages, emphasizing the heterogeneous nature of OSCC.

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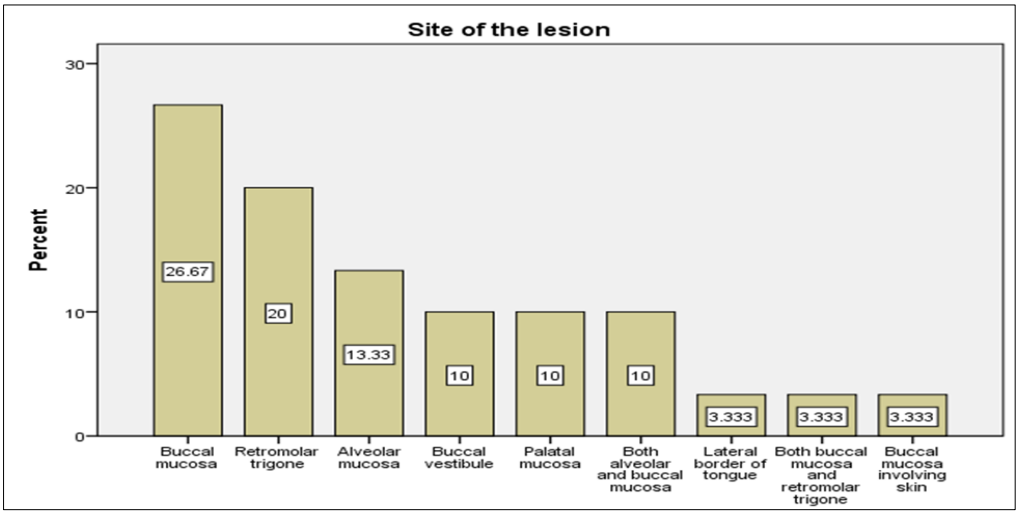

Fig 2 Distribution of the Site of Lesion

Table 2 provided insights into the SCC antigen (SCCAg) level test results during pre-operative and post-operative periods (after 1 week and 3 months). Pre-operatively, 90% of patients exhibited positive SCCAg levels, which significantly reduced to 26.7% after 1 week and 23.3% after 3 months post-operation.

Table2 SCC Ag Level Test During Pre-Operative & Post-Operative (After 1 Week & 3 Months) Period

SCC Ag Level	Positive (Ag level>1.5 ng/ml) n(%)	Negative (Ag level after 1.5 ng/ml) n(%)
Pre-operative	27 (90)	3 (10)
Post-operativeafter 1 week	8 (26.7)	22 (73.3)
Post-operativeafter 3 months	7 (23.3)	23 (76.7)

Further analysis in Table 3 explored the mean SCCAg levels according to TNM staging, revealing a significant reduction in post-operative levels after 1 week and 3 months across all stages, although the association was not statistically significant.

Table 3 Mean SCC Ag level According to TNM Staging (Pre- and Post-Operative Status)

Staging (n)	Pre-operative SCC Ag level Mean (95% confidence interval) *	St deviation	Post op SCC Ag level after 1 week mean (95% confidence interval) **	St Deviation	Post op SCC Ag level after 3 months mean (95% confidence interval) ***	St Deviation
Stage I (1)	0.9000 (-)	-	0.2000 (-)	-	1.0000 (-)	-
Stage II (8)	2.9875 (1.6040-4.3710)	1.65481	1.5000 (0.9529-1.9304)	0.73872	2.3750 (0.904-4.6596)	2.73274
Stage III (12)	2.8833 (2.2310-3.5357)	1.02676	1.4417 (0.9529-1.9304)	0.76925	1.5250 (1.0806-1.9694)	0.69951
Stage IV (9)	2.8000 (2.2351-3.3649)	0.73485	1.3667 (0.9023-1.8311)	0.60415	1.2556 (.5573-1.9539)	1.2556
Total (30)	2.8200 (2.3867-3.2533)	1.16038	1.3933 (1.1266-1.6601)	0.71435	1.6533 (1.0712-2.2354)	1.55890

* P= 0.418 **P= 0.403 *** P= 0.485

Similarly, Table 4 presented SCCAg levels concerning TNM staging, indicating a significant reduction in positive cases post-operatively after 1 week and 3 months.

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Table 4 SCC Ag Level in Pre and Post-Operative (After 1 Week & 3 Months) According to Histological Grading

TNM Staging	Pre-operative Ag level *		Post-operative Ag level After 1 week **		Post-operative Ag level After 3 months ***	
	Positive, n (%)	Negative, n (%)	Positive, n (%)	Negative, n (%)	Positive, n (%)	Negative, n (%)
Stage I	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)
Stage II	7 (87.5)	1 (12.5)	2 (25)	6 (75)	3 (37.5)	5 (62.5)
Stage III	11 (91.7)	1 (8.3)	4 (33.3)	8 (66.7)	3 (25)	9 (75)
Stage IV	9 (100)	0 (0)	2 (22.2)	7 (77.8)	1 (11.1)	8 (88.9)

Table 5 provided insights into the SCCAg levels according to histological grading, demonstrating a significant reduction in positive cases post-operatively after 1 week and 3 months for all grading categories.

Table 5 SCC Ag Level in Pre and Post-Operative (After 1 Week & 3 Months) According to Histological Grading

Histopathological Grading	Pre-operative Ag level *		Post-operative Ag level after 1 week **		Post-operative Ag level after 3 months ***	
	Positive, n (%)	Negative, n (%)	Positive, n (%)	Negative, n (%)	Positive, n (%)	Negative, n (%)
Grade 1	7 (70)	3 (30)	2 (20)	8 (80)	1 (10)	9 (90)
Grade 2	16 (100)	0 (0)	5 (31.2)	11 (68.8)	5 (31.2)	11(68.8)
Grade 3	4 (100)	0 (0)	1 (25)	3 (75)	1 (25)	3 (75)

***** P= 0.036 ** P= 0.817 *** P= 0.458

Lastly, Table 6 presented individual patient data, including age, gender, main site, treatment, and serum SCCAg levels in pre-operative and post-operative samples. Notably, SCCAg levels significantly reduced in the post-operative samples, affirming its potential as a prognostic marker for OSCC.

Table 6 Data Sheet of All 30 Oral Cancer Patients

Age (Years)	Gender	Main Site	Treatment	Serum SCC antigen level (ng/ml)		
				Pre operative	Post operative after 1 week	Post operative after 3 months
45	F	Buccal mucosa	Excision + SOND	1.3	0.3	1.2
52	F	Buccal mucosa	Excision + SOND	2.2	0.6	1.9
42	M	Retromolar trigone	Excision + Hemimandibulectomy	0.9	0.9	0.9
48	F	Alveolar mucosa	Excision + Marginal Mandibulectomy	2.8	1.4	0.5
40	F	Buccal mucosa	Excision + RND+PM flap reconstruction	0.9	0.2	1.0
45	F	Buccal vestibule	Excision + SOND+partial mandibulectomy	2.7	1.7	1.1
60	F	Retromolar trigone	Excision + MRND+ hemimandibulectomy	1.7	1.0	0.7
60	M	Palatal Mucosa	Excision + SOND+maxilectomy	3.1	1.2	1.1
65	M	Palatal mucosa	Excision + SOND+maxilectomy	1.4	0.9	1.1
45	M	Buccal Vestibule	Excision + SOND+ reconstruction with radial forearm flap	2.2	0.9	2.8
35	F	Buccal mucosa	Excision + SOND	3.5	1.1	1.1
40	F	Buccal vestibule	Excision + SOND+marginal mandibulectomy	2.1	1.9	1.2
40	M	Buccal mucosa	Excision + RND+PM flap reconstruction	4	0.7	0.9
60	M	Both alveolar &	Excision + SOND+partial mandibulectomy	3	2.8	3.6

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		Buccal mucosa				
50	M	Buccal mucosa	Excision + RND+partialmandibulectomy+reconstruction with radial forearm flap	6.6	2.5	2
60	F	Retromolar Trigone	Excision + SOND+partial mandibulectomy	2.7	2.5	2.5
50	F	Buccal mucosa	Excision + SOND+marginal mandibulectomy	4	3	2.7
55	M	Alveolar Mucosa	Excision + MRND+partial mandibulectomy	3.2	1.8	1.7
60	F	Buccal mucosa involving vestibule	Excision + MRND+ hemimandibulectomy	2.5	1.8	1.8
45	F	Alveolar Mucosa	Excision + SOND+partial mandibulectomy	4.4	1.2	1
55	F	Retromolar Trigone	Excision + SOND+partial mandibulectomy	4.1	2.8	2.5
45	M	Retromolar Trigone	Excision + SOND+partial mandibulectomy	2.7	1.1	1.1
40	M	Buccal mucosa involving Retromolar Trigone	Excision + MRND+partial mandibulectomy	1.9	1.5	1.5
55	M	Palatal Mucosa	Excision + SOND+partial mandibulectomy	2.5	1.5	1.3
50	F	Baccal mucosa involving Alveolar mucosa	Excision + RND+hemimandibulectomy+ reconstruction with radial forearm flap	3.6	1.2	1.1
40	M	Buccal mucosa involving skin	Excision + RND+hemimandibulectomy+ reconstruction with PM flap	1.8	1.1	1.1
50	F	Alveolar Mucosa	Excision + SND+partial mandibulectomy	4.4	1.5	1.4
45	M	Buccal mucosa	Excision + SOND+marginal mandibulectomy	1.6	1.3	1.2
50	M	Retromolar Trigone	Excision + MRND+partial mandibulectomy	2.6	1.4	1.2
40	F	Lateral Border of tongue	Excision + Hemigolssectomy+ Bilateral Neck dissection+ reconstruction with radial forearm microvascular flap	2.4	1.6	2.2

In summary, this thorough examination of age distribution, gender, lesion site involvement, and SCCAg levels underscores the potential significance of SCCAg as a valuable prognostic indicator in OSCC, prompting further exploration.

IV. DISCUSSION

Squamous cell carcinoma antigen (SCCA) is a serine protease inhibitor that has displayed promise as a potential prognostic biomarker in various squamous cell carcinomas affecting locations such as the uterine cervix, lungs, head and neck, esophagus, and anal canal [11]. The objective of this study was to assess the utility of serum SCCA levels as a prognostic marker in patients with oral squamous cell carcinoma (OSCC) who had undergone curative surgery. The findings offer insights into the potential application of SCCA as a diagnostic and prognostic tool for OSCC.

Our findings demonstrate a strong association between elevated preoperative serum SCCA levels and advanced-stage OSCC. Patients with poorly differentiated tumors also exhibited higher SCCA levels, highlighting the marker's ability to reflect the aggressiveness of the disease [12]. This aligns with previous research in cervical and head and neck cancers, where increased SCCA levels correlated with more

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advanced stages of the disease. The association of elevated SCCA levels with advanced-stage and poorly differentiated OSCC suggests that it could serve as a valuable tool for identifying high-risk patients who may require more aggressive treatment approaches [13, 14 and 15].

In addition to its diagnostic potential, this study also uncovered SCCA's role in early recurrence detection. Among the 30 OSCC patients, those who developed recurrent disease exhibited initial elevations in SCCA levels, followed by a post-treatment normalization. However, within three months, their SCCA levels surged once more. This pattern underscores the importance of monitoring SCCA levels postoperatively, as it may aid in the timely detection of disease recurrence. Early identification of recurrence can lead to prompt intervention, potentially improving patient outcomes [16].

V. CONCLUSION

In conclusion, this research underscores the importance of Squamous Cell Carcinoma Antigen (SCCA) as a valuable biomarker for diagnosing and predicting outcomes in patients with Oral Squamous Cell Carcinoma (OSCC) who are undergoing curative surgery. Elevated levels of SCCA before surgery indicate advanced-stage and poorly differentiated tumors, facilitating risk assessment and the development of customized treatment plans for high-risk patients. Furthermore, SCCA's capacity to signal early recurrence, as seen in post-treatment normalization followed by subsequent elevations in SCCA levels, emphasizes its role in monitoring disease progression.

While the study presents promising findings, it is crucial to acknowledge its limitations, such as the relatively small sample size and short study duration. Additionally, the cost associated with SCCA testing may restrict its widespread use. Future research endeavors should focus on replicating these results in larger cohorts and evaluating SCCA alongside other diagnostic methods to enhance its clinical applicability.

Squamous Cell Carcinoma Antigen (SCCA) has the potential to revolutionize the management of OSCC patients by contributing to early diagnosis, prognostication, and recurrence detection. As our comprehension of this biomarker advances, there is optimism for improving patient outcomes and alleviating the burden of OSCC on both individuals and healthcare systems. This study marks a crucial advancement toward integrating SCCA into routine clinical practice, aiming to enhance the care and treatment of OSCC patients. Further investigations are necessary to fully exploit the potential of SCCA in OSCC management, thereby contributing to the progress of personalized medicine and enhancing survival rates for OSCC patients.

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