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ORIGINAL ARTICLE

Role of pre and post incisional biopsy levels of Serum Cytokeratin-19 for detection of the dissemination of oral squamous cell carcinoma.

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ABSTRACT… Objective: To evaluate the diagnostic efficacy of cytokeratin 19 for the presence and spread of oral squamous cell carcinoma following incisional biopsy. **Study Design:** Descriptive Cross-sectional. **Setting:** Isra Dental College Hospital, Isra University Hyderabad. **Period:** November 2020 to October 2021. **Material & Methods:** Consenting patients of either gender, age 20-70 years, with clinical suspicion of OSCC were included in the study. Patients with a previous history of chemotherapy and radiotherapy, those having history of previous biopsy, recurrence of carcinoma were excluded. After detailed examination, blood samples of all patients were collected before and after incisional biopsy and sent to the laboratory for further evaluation for CK-19 level. **Results:** Of total 60 patients, majority (86.6%) were male. Over half (53.3%) of the participants belongs to age-group 40-59 years. Before incisional biopsy, all participating OSCC patients were negative for the Cytokeratin RT-PCR assay. While RT-PCR (CK-19) was detected positive for 12 (20%) of patients within 15 minutes of biopsy whereas, remaining 48 (80%) showed negativity for CK-19 assay. Statistically significant difference ($p<0.05$) was observed for the post incisional biopsy CK-19 +ve and –ve patients. Male patients and patients well differentiated OSCC tumors had positive post incisional biopsy PCR compared with their counterparts ($p<0.05$). **Conclusion:** Incisional biopsy promotes the propagation of cancerous cells and increases the risk of developing metastatic lesions. Additionally, RT-PCR is a reliable tool for the detection of CK-19 transcripts in the blood.

Key words: Biopsy, Histo-pathological Grading, Squamous Cell Carcinoma.

INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC), the most common malignancies that arise in the head and neck, are a major public health burden.[1] The global burden of HNSCC varies considerably across different geographical regions and is vastly associated with numerous predisposing factors such as exposure to tobacco and/or its derivatives, superfluous consumption of alcohol.[2] In many cases, tobacco and alcohol use potentiate the carcinogenic properties of one another. Additionally, prior infection with certain oncogenic strains of Human papillomavirus (HPV), such as HPV-16 and HPV-18, has also been attributed to the causation of HNSCC.[2,3]

Although the availability of modern therapeutic tools, including radiotherapy, chemotherapy, and various newer surgical interventions, the overall rate of survival for HNSCC still falls short of 60%.[2,4]

Arising from the mucosal surface of the oral cavity, the oral squamous cell carcinomas (OSCC) constitute some of the most common HNSCCs, which have a notorious tendency to metastasize to the neighboring lymph nodes.[2,5] In most cases, the mortality is attributed to the recurrence of OSCC in local areas while almost 30% of cases have shown to develop a metastatic cancerous lesion in distant areas during the post-diagnosis 5-year follow-up period.[6]

With more than 850,000 yearly new cases and more than 400,000 yearly deaths, HNSCC ranks

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as the sixth most common cancer globally.[2,7] This incidence of HNSCC is expected to rise by 30% by the year 2030, as projected by the Global Cancer Observatory (GLOBOCAN).[7,8] According to a study conducted at the Pakistan Medical and research council, OSCC account for 10% of all malignancies in the country.[9] While a study conducted at the Jinnah Postgraduate Medical Center showed that one fifth of all cancer patients were suffering from OSCC.[10]

For the prompt treatment and optimal prognosis, a proper diagnosis of the cancerous lesion is crucial. One of the most frequently used diagnostic methods for such lesions is incisional biopsy. However, many clinicians believe that the risk of tumor metastasis is increased by incisional biopsy because when the tumorous mass is cut from the depths of the lesion, cancerous cells gain access to the normal surrounding tissue via the exposed local channels and blood vessels.[11,12] This allows to cancer cells to migrate from the primary site, travel via the local channels or blood stream to a secondary site where they lodge into the surrounding vessels and tissues and proliferate.

The cytoskeleton of the both the normal as well as tumor cells of epithelial origin tumors is made up of Cytokeratins (CK). Among the different members of the CK family, cytokeratin 19 (CK-19) has gained a lot of attention in recent studies of OSCC.[13] Since the CK-19 is an intermediate filament, which belongs to the keratin family of epithelial proteins, the presence of CK-19 in peripheral blood samples is strongly suggestive of OSCC in suspected patients.[14]

However, there presence of CK-19 in incisional biopsy of OSCCs is still not extensively explored which itself poses as a significant knowledge gap. Therefore, the objective of the current study was to assess the diagnostic efficacy of CK-19 for the presence of OSCC and to determine whether CK-19 acts as an indicator for the spread of OSCC following incisional biopsy.

MATERIAL & METHODS

Descriptive cross sectional study was conducted at the Outpatient Department, Isra Dental College Hospital, Isra University Hyderabad from November 2020 to October 2021. All patients belongs to either gender, between the age of 20 and 70 years, with clinical suspicion of OSCC and given consent of participation were included in the study. While patients with a previous history of chemotherapy and radiotherapy, those having history of previous biopsy, recurrence of OSCC and those not fulfilling the inclusion criteria were excluded. Patients were selected by using Non-probability purposive sampling technique. The sample size of 60 for the study was calculated by using rao soft online calculator using 95%confidence interval and margin of error at 10%.

Study was ethically approved from the research ethics committee of Isra University, Hyderabad (IU/RR-10-IRC-20/N/2020-192). Informed consent from all patients was taken after explaining the procedure as well purpose of the study. A detailed history was recorded in a pre-designed written pre-tested proforma. While detailed clinical and oral examination of all patients was performed for any relevant clinical features by an experienced dental surgeon. All clinical findings were then recorded on same proforma. After that, blood sample was collected and sent to the laboratory for further evaluation.

Procedure

Under all aspect measures, the incisional biopsy of the oral lesion was performed by experienced oral maxillofacial surgeon under local anesthesia. The tissue specimen were collected after excision of tissue portion and processed in different concentrations of alcohol. Later the specimen were fixed in 10% buffered formalin then routinely processed overnight and were embedded in paraffin. Tissue sections were prepared and dyed with Hematoxylin and eosin sections were prepared for light microscopic evaluation. The microscopic diagnosis was rendered conjointly by the oral pathologists. Relevant histological findings were recorded on the profroma.

Prior to performing the incisional biopsy, two peripheral blood samples of 5 ml were collected.

For the collection of samples, the median forearm vein was selected using 18 gauge disposable syringes under apposite sterile measures. First sample was collected immediately before performing the incisional biopsy (sample 1) while other sample collected after 15 minutes of incisional biopsy (sample 2). The collected samples were then stored in citrate. RT-PCR was performed for the detection of CK-19 mRNA expression. The gene specific primers for CK-19 were taken according to Dattaetal[9] CK19 (Forward CK1): -5'-AAG CTA ACC ATG CAG AAC CTC AAC GAC CGC - 3' CK19 (Reverse CK2): - 5' -TTA TTG GCA GGT CAG GAG AAG AGC C - 3'. The extraction of RNA was performed by using QIAGEN RNA blood Mini-Kit. All the findings were then recorded on profroma.

Collected data was entered and analyzed using SPSS ver. 23. The qualitative variables were presented as frequency and percentages and was analyzed by Chi-square test or Fisher's exact test whichever applied. Level of significance of set at $p<0.05$.

RESULTS

Total 60 patients of OSCC fulfilled the selection criteria. Of which, majority were male compared with their counterparts. While over half of the participants belongs to age-group 40-59 years with the mean age participants was 39.8±16.4 (range 21-64 years). Over 60% participants were addicted to smokeless tobacco followed by smoking and alcohol. (Table-I)

Prior to incisional biopsy, all participating OSCC patients were negative for the Cytokeratin RT-PCR (CK-19) assay. While RT-PCR (CK-19) was detected positive for 12 (20%) of patients within 15 minutes of incisional biopsy whereas, remaining 48 (80%) showed negativity for CK-19 assay. There was a statistically significant difference ($p<0.05$) was observed for the post incisional biopsy CK-19 +ve and –ve patients. (Table-II)

Table-III is demonstrating the Age, gender and histo-pathological grading wise distribution of post incisional biopsy on Cytokeratin-19 test. The findings revealed that more male patients had positive post incisional biopsy PCR compared with their counterparts ($p<0.05$). While patients belongs to 40 and above have more positive CK-19 after incisional biopsy. Based on histo-pathological grading, patients with well differentiated OSCC tumors had more positive CK-19 then those with moderately differentiated tumor ($p<0.05$). (Table-III)

	N (%)
Age group	
• 21-39	25 (41.6%)
• 40-59	32 (53.3%)
• 60 and over	3 (5.1%)
Gender	
• Male	52 (86.6%)
• Female	08 (13.4%)
Substance of addiction	
• Smokeless tobacco	44 (73.3%)
• Smoking	07 (11.6%)
• Alcohol consumption	01 (1.6%)
• No addiction	08 (13.3%)
Tumor Location	
• Lip	05 (8.3%)
• Buccal Mucosa	26 (43.3%)
• Tongue	05 (8.3%)
• Palate	04 (6.6%)
• Alveolus	15 (25.0%)
• Others	05 (8.2%)
Histo-pathological findings	
• Well differentiated	52 (86.6%)
• Moderately differentiated	08 (13.4%)

Table-I. Socio-demographic and personal details of study participants (n=60)

	CK-19	No. (%)	P-Value
Cytokeratin-19 (Post incisional biopsy)	Positive	12 (20%)	0.000*
	Negative	48 (80%)	

Table-II. Post incisional biopsy Cytokeratin-19 test findings in study population (n=60)
***Chi-square test ($p<0.05$)**

	Cytokeratin-19		P-Value
	Positive	Negative	
Gender			
Male	08	44	0.04*
Female	04	04	
Age groups (years)			
≤ 39	05	20	1.00
40 and above	07	28	
Histo-pathological grading			
Well differentiated	08	44	0.04*
• Moderately differentiated	04	04	

Table-III. Age, gender and histo-pathological grading wise distribution of post incisional biopsy on Cytokeratin-19 test findings (n=60)

DISCUSSION

For the best possible treatment, a firm diagnosis is critical. One of the most important tools for the diagnosis of oral lesions is an incisional biopsy.[15] Lately, however, the procedure of incisional biopsy has been a matter of great concern among several clinicians, who believe that incisional biopsy may lead to the spread of cancerous cells into the neighboring normal tissues.[11,12] Since the intact basement membrane of the oral epithelium acts as a barricade against metastasis, surgical intervention may compromise the structural integrity of this basement membrane and hence lead to the local spread of the primary cancerous lesion as well as distant metastatic lesions.

In light of this belief, doctors are facing a great moral dilemma of carrying out a potentially fatal procedure. There have been several case reports where the incidence of neck metastasis has increased in patients after they went through the process of incisional biopsy.[16] Among the various markers used to detect the spread of HNSCC including OSCC, CK-19 ranks at the topmost.[17] Using the highly sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) technique for the detection of CK-19 is in turn an innovatory method for detecting the presence of any notorious metastatic lesions. Several previous studies have reported the reliability of CK-19 as a potentially efficacious marker of metastasis.[18,19]

Thakare et al. studied the expression of CK-19 in patients suffering from non-malignant tumors such as ameloblastoma and reported no detection of CK-19 from the lymph nodes of the patients as well as from healthy controls.[20] This finding signifies the sensitivity and specificity of CK-19 as a reliable marker for OSCC spread.

The current study sought to determine the pre and post incisional biopsy CK-19 levels by the usage of RT-PCR in patients suffering from OSCCs. In our study, CK-19 was found to be a good marker (20%) for the assessment of cancer advancement which was evident from the significant p-value (<0.05) among the CK-19 positive and CK-19 negative cases.

Similar findings were observed by Dyavanagoudar et al., who reported the presence of CK-19 in 16% of post-incisional biopsy samples in their study.[21] The frequency of CK-19 is comparatively lower in this study by Dyavanagoudar et al. but this could be attributed to the comparatively lower sample size.

The findings of the current study are also in accordance with the study conducted by Kusukawa et al. who reported a 20% positivity rate for CK-19 transcripts in post-incisional samples of OSCC patients.[22]

In another study, Kusukawa et al. found that the overall incidence of neck metastasis in stage I and II OSCCs treated with excisional biopsy was significantly lower than those OSCCs which underwent excision after incisional biopsy.[23]

The current study was, however, not devoid of certain limitations including monetary restraints, availability of a larger sample size and limitation of resources. Therefore, further research studies are recommended for the correlation of local nodal as well as distant metastatic lesions with CK-19 levels.

CONCLUSION

The current study hence concludes that incisional biopsy promotes the propagation of cancerous cells and thereby increases the risk of developing metastatic lesions. Additionally, the findings of the current study also suggest that RT-PCR reliable tool for the detection of CK-19 transcripts in the blood.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Uzma Tariq	Data analysis and interpratation, Study design.	
3	Arsalan Ahmed	Drafting of manuscript and proof reading, Literature review.	
4	Khushbu Lohana	Assistance in experimental work, Data collection, Drafting, References.	
5	Sobia Masood	Data collection, Literature review.	
6	Ameet Kumar Maheshwari	Assistance in experimental work.	