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Estimation of salivary biomarkers in patients with squamous cell carcinoma

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Abstract
Introduction: Squamous cell carcinoma (SCC) is an epithelial malignancy that occurs in organs that are normally covered with squamous epithelium which includes several different anatomic sites. The present study was conducted to determine the role of salivary biomarkers in detection of SCC.
Methods: The present study was conducted on 62 cases of oral squamous cell carcinoma of both genders. Subjects were divided into 2 groups. Group I had SCC patients whereas group II had control. Unstimulated whole saliva was collected and CYFRA 21-1 and CA19-9 were estimated by ELISA method while LDH level was assessed based on standard kit method.
Results: Out of 62 patients, males were 42 and females were 20. The mean salivary biomarkers levels of LDH in group I was 424.8 and 112.5 in group II, CA19-9 was 22.4 in group I and 20.5 in group II and CYFRA 21-1 was 18.5 in group I and 3.6 in group II. The difference was significant ($P< 0.05$).
Conclusions: Authors found raised level of LDH and CYFRA 21-1 level in patients with squamous cell carcinoma as compared to controls.

Keywords: CYFRA 21-1, LDH, salivary

Introduction
Oral cancer is the sixth most prevalent cancer worldwide. In the Indian subcontinent, it ranks among the three most common types of cancer [1] Squamous cell carcinoma (SCC) accounts for nearly 90% cases of malignancy of oral cavity. The history of oral cancer shows that it is preceded in more than 70% of the patients by a recognized premalignant lesion (PML) and intervention at this stage may result in regression of the lesion [2].
Squamous cell carcinoma (SCC) is an epithelial malignancy that occurs in organs that are normally covered with squamous epithelium which includes several different anatomic sites, including the skin, lips, mouth, esophagus, urinary tract, prostate, lungs, vagina, and cervix. Of these anatomic sites, there are four which make up the majority of SCC cases: non-melanoma skin cancer, head and neck cancer, esophageal cancer, and non-small cell lung cancer. Given the range of tissues in which it arises, SCC represents the most common cancer capable of metastatic spread in the US and worldwide [3]
Saliva contains multiple salivary markers. CYFRA 21-1, which is the soluble fragment of cytokeratin 19 has emerged as a novel marker of SCC. CA19-9, total protein, amylase, and lactate dehydrogenase (LDH) have also been shown to be significantly altered in patients with OSCC as compared to premalignant states and healthy controls [4] The present study was conducted to determine the role of salivary biomarkers in detection of SCC.

Materials & Methods
The present study was conducted in the department of general pathology. It comprised of 62 cases of oral squamous cell carcinoma of both genders. Age and gender matched controls were also selected. The study protocol was approved from institutional ethical committee. All were informed regarding the study and written consent was obtained.
General information such as name, age, gender etc. was recorded. Subjects were divided into 2 groups. Group I had SCC patients whereas group II had control. Unstimulated whole saliva was collected and the saliva sample was centrifuged at 3000 rpm for 15 min to remove squamous cells and debris. The resulting supernatant was used for further biochemical analysis. Saliva samples were stored in deep freezer at −80°C until analyzed. CYFRA 21-1 and CA19-9 were estimated by ELISA method while LDH level was assessed based on standard kit method.
Results thus obtained were subjected to statistical analysis. P value less than 0.05 was considered significant.

Results

Table 1: Distribution of patients

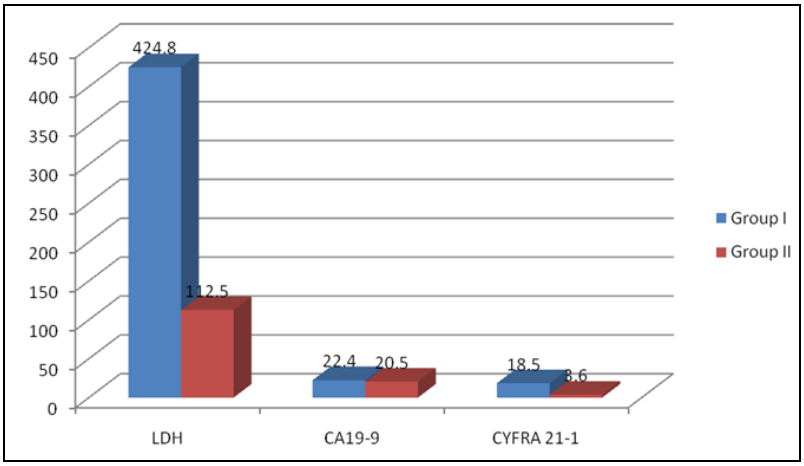
Total- 62		
Gender	**Males**	**Females**
Number	42	20

Table 1 shows that out of 62 patients, males were 42 and females were 20.

Table 2: Salivary biomarkers in both groups

Salivary biomarkers	Group I	Group II	P value
LDH	424.8	112.5	0.01
CA19-9	22.4	20.5	0.54
CYFRA 21-1	18.5	3.6	0.05

Table 2 shows that mean salivary biomarkers levels of LDH in group I was 424.8 and 112.5 in group II, CA19-9 was 22.4 in group I and 20.5 in group II and CYFRA 21-1 was 18.5 in group I and 3.6 in group II. The difference was significant (P<0.05).



Graph 1: Salivary biomarkers in both groups

Discussion

Numerous genetic alterations have been described in SCC sub-types, although the molecular mechanisms contributing to tumor initiation and progression are still poorly understood. SCCs share many phenotypic and molecular characteristics with each other, thus molecular insights, new markers, or drug targets discovered in individual SCCs may shed light on this type of cancer as a whole. Head and neck squamous cell carcinomas (HNSCC) make up the vast majority (more than 90%) of head and neck cancers and rank as the sixth most common cancer worldwide, with 45,660 new cases of HNSCC diagnosed in 2007 and 35,720 new cases reported in the US during 2009. [5] The present study was conducted to determine the role of salivary biomarkers in detection of SCC.

In present study, out of 62 patients, males were 42 and females were 20. We found that mean salivary biomarkers levels of LDH in group I was 424.8 and 112.5 in group II, CA19-9 was 22.4 in group I and 20.5 in group II and CYFRA 21-1 was 18.5 in group I and 3.6 in group II.

Increased serum lactate dehydrogenase (LDH) activity is considered as a marker of cellular necrosis and serum LDH levels have been used as a biochemical marker in diagnosis in various cancers such as oral, laryngeal and breast cancer. Studies have also suggested that increased levels of LDH in serum are seen in patients with oral leukoplakia (OL), OSMF and oral squamous cell carcinoma (OSCC). Lactate dehydrogenase activity is mainly due to genomic changes during malignant transformation. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein. Value of LDH elevates in OSCC and potentially malignant disorders; this finding can be used for benefit of the patient in predicting prognosis [6, 7]

Rathore *et al.* [8] found that serum LDH activity was significantly (P<0.05) increased in patients with OSCC, OL and OSMF. Serum LDH estimation can prove to be a valuable tool as a biochemical marker as it is a simple, non-invasive procedure and is easily accepted by the patient.

Singh *et al.* [9] found that there was a statistically significant difference (P<0.001) of mean serum Cyfra 21-1 levels (ng/mL) between OSCC cases (1.76 ± 0.81) and healthy controls (0.43 ± 0.14). The difference between controls and Stage III and Stage IV was also statistically significant. The mean serum Cyfra 21-1 level was lower in histopathological Grade I cases (1.72 ± 0.78) than Grade II, but the difference was not statistically significant. Yazigi *et al.* [10] showed increased level according to clinical stage: 33% in Stage I, 36% in Stage II, and 67% in Stage III.

Conclusion

Authors found raised level of LDH and CYFRA 21-1 level in patients with squamous cell carcinoma as compared to controls.

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