Original Research

Assessment of salivary biomarkers in patients with squamous cell carcinoma

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ABSTRACT
Background: Oral cancer is the sixth most frequent type of cancer worldwide. In the Indian subcontinent, it is among the top three types of cancer.[1] About 90% of cancer cases in the oral cavity are caused by squamous cell carcinoma (SCC). The present study was conducted to assess salivary biomarkers in patients with squamous cell carcinoma.
Materials & Methods: 54 patients with SCC patients of both genders were put in group I and healthy control in group II. 5 ml of unstimulated saliva was collected and the saliva sample was centrifuged at 3000 rpm for 15 min to remove squamous cells and debris. Assessment of IL1b, IL8, SAT1, OAZ1 was done with PCR and LDH with standard kit method.
Results: Out of 82 SCC patients, 50 were males and 32 were females. OAZ1 was 24.7 in group I and 14.2 in group II, LDH level was 412.5 in group I and 121.8 in group II, IL 1 b level was 104.2 in group I and 27.5 in group II, IL 8 was 224. 8 in group I and 29.6 in group II, SAT 1 was 26.6 in group I and 23.4 in group II. The difference was significant (P< 0.05).
Conclusion: Higher concentrations of all salivary biomarkers in SCC patients' saliva than in those of healthy individuals. Saliva can therefore be used as a diagnostic tool.
Key words: PCR, Salivary biomarkers, squamous cells carcinoma

Introduction

Oral cancer is the sixth most frequent type of cancer worldwide. In the Indian subcontinent, it is among the top three types of cancer.[1] About 90% of cancer cases in the oral cavity are caused by squamous cell carcinoma (SCC). More than 70% of patients with oral cancer had a recognized premalignant lesion (PML) before the disease developed, and treatment at this stage may cause the lesion to disappear.[2] Saliva has long been thought to be a useful source of biologic information for diagnosing human diseases. In addition to the obvious connection with the oral mucosa surface, research has demonstrated a synergistic interaction between the expression of molecular markers in saliva and systemic or remote site problems.[3]

A biomarker is defined as 'a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease' by the National Cancer Institute.[4] The biomarker, also called a molecular marker, has a wide range of applications in diagnosis, monitoring of treatment, and the prognosis of a disease or condition. It has been discovered that metabolites, proteins, coding and noncoding RNAs, and DNA are all present in sick people's saliva and can be used as useful diagnostic tools. Saliva contains a number of signs that help identify SCC.[5] The present study was conducted to assess salivary biomarkers in patients with squamous cell carcinoma.

Materials & Methods

The present study was conducted on 54 patients with squamous cell carcinoma of both genders. All gave their written consent to participate in the study.
Data such as age, gender etc. was recorded. Group I comprised of 54 SCC patients and group II had 54 healthy controls. To get rid of squamous cells and debris, 5 ml of unstimulated saliva was taken, and the saliva sample was centrifuged for 15 minutes at 3000 rpm. Additional biochemical analysis was conducted using the resultant supernatant. Using the conventional kit approach, PCR and LDH were used to assess IL1b,

569

IL8, SAT1, and OAZ1. Results thus obtained were subjected to statistical analysis. P value less than 0.05 was considered significant.

Results

Table: I Distribution of patients

| Total- 54		
Gender	Males	Females
Number	38	16

Table I shows that out of 54 SCC patients, 38 were males and 16 were females.

Table: II Assessment of salivary biomarkers

Salivary biomarkers	Group I	Group II	P value
LDH	384.6	128.4	0.01
IL1b	116.2	34.6	0.01
IL8	216.4	31.5	0.01
SAT1	23.2	22.8	0.53
OAZ1	25.6	16.4	0.05

Table II, graph I shows that LDH level was 384.6 in group I and 128.4 in group II, IL 1b level was 116.2 in group I and 34.6 in group II, IL 8 was 216. 4 in group I and 31.5 in group II, SAT 1 was 23.2 in group I and 22.8 in group II. OAZ1 was 25.6 in group I and 16.4 in group II. The difference was significant (P< 0.05).

Graph: I Assessment of salivary biomarkers
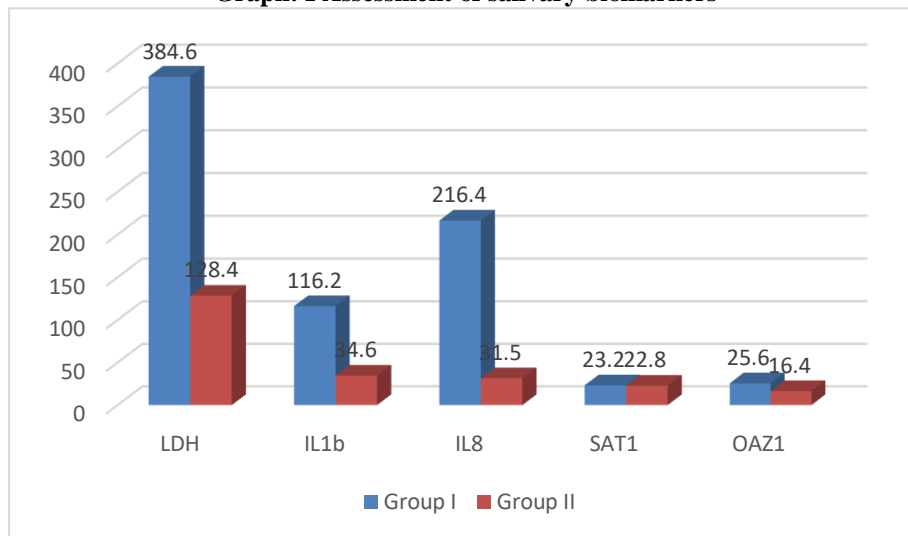
Discussion

Whole saliva also contains a variety of non-organic and organic substances from the serum, gingival crevicular fluid, as well as oral microorganisms and their products. In addition to a diversity of biomarkers for many diseases, saliva's collection is noninvasive and convenient, and the transportation and storage are easy, therefore saliva sampling is cost effective and efficient.[6] These advantages demonstrate that saliva is a potential body fluid for laboratory tests compared to serum and tissue samples. Biomarkers are detected and determined by various molecular techniques.[7,8] For the genomic biomarkers (including DNA, mitochondrial DNA [mt.DNA], RNA, messenger RNA [mRNA], microRNA [miRNA]), the utilized techniques can be DNA microarrays, polymerase chain reaction (PCR), Southern blot analysis, restriction fragment length polymorphism (RFLP), and cross-linking immunoprecipitation (CLIP).[9,10] The present study was conducted to assess salivary biomarkers in patients with squamous cell carcinoma.

We found that out of 54 SCC patients, 38 were males and 16 were females. A total of 180 samples were used in the investigation, comprising 60 OSCC patients, 60 controls, and 60 PMOD patients, according to Li et al.[11] Seven transcriptome markers (IL8, IL1b, SAT1, OAZ1, DUSP1, S100P, and H3F3A) were examined by qPCR, while two proteome indications (IL8 and IL1b) were evaluated by ELISA. DUSP1, one of seven transcriptome indicators, had a significantly lower

570

transcript level in OSCC patients than in PMOD and control groups. OSCC patients exhibited significantly higher protein concentrations of IL8 and IL1b among the proteomic indicators compared to controls and dysplasia patients. According to univariate fractional polynomial (FP) models, salivary IL8 protein (IL8p) has the highest AUC value between OSCC patients and PMOD patients (0.72) and between OSCC patients and controls (0.74). Salivary IL8p combined with IL1b and H3F3AmRNA yielded the highest AUC value for distinguishing between patients with OSCC and controls, as well as between patients with PMOD and OSCC, using a two-marker FP model. The optimal combinatory variables were created by multivariate models analysis combining salivary analytes and risk factor exposure associated to oral carcinogenesis in order to distinguish between OSCC and PMOL (AUC ¼ 0.80), OSCC and controls (AUC ¼ 0.87), and PMOD and controls (AUC ¼ 0.78).

We found that LDH level was 384.6 in group I and 128.4 in group II, IL 1b level was 116.2 in group I and 34.6 in group II, IL 8 was 216. 4 in group I and 31.5 in group II, SAT 1 was 23.2 in group I and 22.8 in group II. OAZ1 was 25.6 in group I and 16.4 in group II. Franzmann et al[12] reported elevated levels of CD 44 in saliva (oral rinse) of oral squamous cell carcinoma patients (n = 102) compared to controls (n = 69). St John et al[12] detected higher concentrations of IL-8 in saliva and higher concentrations of IL-6 in serum of patients with OSCC and concluded that IL-8 in saliva and IL-6 in serum are the informative biomarkers for OSCC.

Conclusion

Authors found greater levels of every salivary biomarker in SCC patients' saliva compared to healthy people's. Therefore, saliva can be employed as a diagnostic tool.

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