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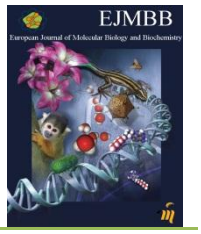


# SALIVARY FLUID: A MOLECULAR TOOL

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**ABSTRACT**
The 90% of oral cancers are oral squamous cell carcinoma. This cancer, when found early, has an 80 to 90% survival rate. At the moment, a lack in national screening programs together with a lack of definitive and satisfactory biological markers for early oral cancer detection has resulted in late stage diagnosis of oral cancer. Analysis of salivary parameters such as salivary flow rate, pH, buffer capacity, lactobacillus, and yeast content, presence of IgG, IgM and anti-La autoantibodies and raised protein levels such as that of lactoferrin and cystatin C as has been proposed for the diagnosis of Sjogren's syndrome. Concerning cancer diagnostics and follow up altered levels of certain mRNA molecules have been detected in saliva in oral cancer patients and of certain proteins in several cancers.

## INTRODUCTION

Oral cancer refers to all malignancies arising from the lips, the oral cavity, and pharynx [1] and it affects more than 481,000 new patients worldwide. The 90% of oral cancers are oral squamous cell carcinoma. This cancer, when found early, has an 80 to 90% survival rate. Despite this fact and the great treatment advances, the World Health Organization has reported oral cancer as having one of the highest mortality ratios amongst other malignancies with a death rate at five years from diagnosis at 45% [2].

## METHODOLOGY

At the moment, a lack in national screening programs together with a lack of definitive and satisfactory biological markers for early oral cancer detection has resulted in late stage diagnosis of oral cancer [3]. An increasing number of systemic diseases and conditions, amongst them oral cancer, have been shown to be reflected diagnostically in saliva. Moreover, using saliva as a diagnostic fluid meets the demands for inexpensive, noninvasive, and accessible diagnostic methodology. epithelial cells and food debris. Saliva has been long proposed and used as a diagnostic medium because it is easily accessible and its collection is non-invasive, not time-consuming, inexpensive, requires minimal training and can be used for the mass screening of large population samples [4,5].

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## DISCUSSION

Saliva as a Perfect Diagnostic Medium Whole saliva is the product of the secretions of the 3 major salivary glands (parotid, submandibular, sublingual) and the numerous minor salivary glands mixed with crevicular fluid, bronchial and nasal secretions, blood constituents from wounds or bleeding gum, bacteria, viruses, fungi, exfoliated. Analysis of salivary parameters such as salivary flow rate, pH, buffer capacity, lactobacillus, and yeast content, presence of IgG, IgM and anti-La autoantibodies and raised protein levels such as that of lactoferrin and cystatin C as has been proposed for the diagnosis of Sjogren's syndrome [6,7].

Concerning cancer diagnostics and follow up altered levels of certain mRNA molecules [8] have been detected in saliva in oral cancer patients and of certain proteins in several cancers. Cell-free nucleic acids and proteins in saliva may derive from serum or can be locally produced [9]. Serum derived nucleic acids and proteins in the saliva may be part of the normal salivary secretion (by the acinar cells) [10] or come there either via intracellular

routes such as active transport or passive diffusion from the serum to saliva across cell membranes or extracellular routes such as ultrafiltration through tight junctions or as constituents of the outflowing crevicular fluid. Cell free nucleic acids and proteins in saliva however can be locally produced by cell necrosis, lysis or apoptosis and trauma and may even be actively released by normal epithelial or cancerous cells. Cell necrosis is a possible mechanism leading to the release of cell free nucleic acids and proteins in the saliva and this idea is also supported by the large amount of DNA in the plasma of patients with cancers in an advanced stage. Moreover, mounting evidence exists concerning the presence of cell-free nucleic acids and proteins in apoptotic bodies which also protect these molecules from degradation.

The active release of these molecules in exosomes or microvesicles is another strong possibility. Exosomes or microvesicles are released by living cells. They are membrane vesicles, 40–100-nm in diameter, originating from the endoplasmic reticulum and are released when fused with the cell membrane. They contain mRNA, miRNA and proteins and are thought to play a role in the cell-free intercellular communication [11,12,13], Table 1.

Salivary Markers for Oral Cancer Detection Molecular markers for the diagnosis of OSCC can be quested in 3 levels; (I) changes in the cellular DNA, which result in (II) altered mRNA transcripts, leading to (III) altered protein levels (intracellularly, on the cell surface or extracellularly). All these markers are summarized in Table 2.

**Table 1. Genotypic and Phenotypic Markers in the Saliva**

| | | |
|---|---|---|
| Cell-free nucleic acids & proteins in saliva | Serum derived | Normal salivary secretion Passive diffusion Active transport Ultrafiltration through tight junctions Outflow of crevicular fluid |
| Cell-free nucleic acids & proteins in saliva | Locally produced | Cell necrosis, lysis Apoptosis Trauma Active release |

**Table 2. Diagnosis of Oral Squamous Cell Carcinoma by Molecular Markers**

| Changes in the cellular DNA | Altered mRNA transcripts | Altered protein markers |
|---|---|---|
| Allelic loss on chromosomes 9p | Presence of IL8 | Elevated levels of defensin-1 |
| Mitochondrial DNA mutations | Presence of IL1B | Elevated CD44 |
| p53 gene mutations | DUSP1 (dual specificity phosphatase 1) | Elevated IL-6 andIL-8 |
| Promoter hypermethylation of genes (p16, MGMT, or DAP-K) | H3F3A (H3 histone, family 3A) | Inhibitors of apoptosis (IAP) |
| Cyclin D1 gene amplification | OAZ1 (ornithine decarboxylase antizyme 1) | Squamous cell carcinoma associated antigen (SCC-Ag) |
| Increase of Ki67 markers | S100P (S100 calcium binding protein P) | Carcino- embryonic antigen (CEA) |
| Microsatellite alterations of DNA | SAT (spermidine/spermine N1-acetyltransferase) | Carcino-antigen (CA19-9) |
| Presence of HPV | | CA128 |
| | | Serum tumor marker (CA125) Intermediate filament protein (Cyfra 21-1) Tissue polypeptide specific antigen (TPS) |
| | | Reactive nitrogen species (RNS) |
| | | 8-OHdG DNA damage marker |
| | | Lactate dehydrogenase (LDH) |
| | | Immunoglobulin (IgG) s-IgA |
| | | Insulin growth factor (IGF) |
| | | Metalloproteinases MMP-2 and MMP-11 |

## CONCLUSION

The completion of Human Genome project and the development of technology such as microarray and proteomics provide new avenues for developing informative biomarkers. The successful identification of all proteins in human saliva by the joint effort Human Salivary Proteome project is representative of the promise for these technologies in discovering salivary analytes for normal health maintenance and disease detection [14]. Combination of mRNA and protein markers may further push the power of predictability toward real-world biomarker application. Recent advances in bioinformatics tools will no doubt incorporate these multiple analyte categories to produce highly discriminatory "fingerprints" for the early detection and assessment of disease

progression. The multiple avenues to salivary biomarker discovery can provide optimism for the future of saliva diagnostics for oral cancer.

Despite the scepticism in the scientific community and the conservatism of the patients, saliva seems to emerge as a valuable tool in cancer diagnostics and mass population screening. In our opinion much attention must be given to the saliva collecting method. An attempt to integrate the simultaneous testing of different salivary molecular markers in order to raise the possibility of an accurate diagnosis by simply using micro- and nano-electrical-mechanical systems biosensors is on the way raising much hope in its future applications [15].

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**CONFLICT OF INTEREST**:
The authors declare that they have no conflict of interest.

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