



Saliva as a Non-Invasive Diagnostic Medium in Proteomics for Oral Squamous Cell Carcinoma Detection

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Abstract

Non-invasive diagnostic techniques are essential for the screening of oral cancer to reduce high mortality associated with it. The acknowledgement of various research advancements namely omic research and technological advances in the field of genomes has led to an increased use of saliva collection. Obtaining saliva for diagnosis is simple and non-invasive and is safe for both the health worker and the patient. It also permits easy and cost-effective storage methods. Around 3000 differently expressed proteins and peptides have been identified in various proteomic studies involving saliva, and hence, salivary proteomics is being progressively used for the early diagnosis of numerous diseases such as oral cancer, breast cancer, and several autoimmune disorders, among others. The salivary analysis riding on the recent advancements in technology, can prove to be of much more importance in near future.

Keywords: Biomarkers, Diagnosis, Oral Squamous Cell Carcinoma, Oral Potentially Malignant Disorders, Saliva

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Introduction

Oral cancer indicates all malignancies that arise from the lips, the oral cavity, and pharynx, with over 481 000 new cases occurring worldwide annually and 90% of them being oral squamous cell carcinoma (OSCC) type.¹ The most common type of head and neck malignant tumor, OSCC occupies the eighth spot in terms of frequencies among all cancer types worldwide.² The standardized incidence rate of oral cancer in India stands at 12.6 per 100 000 people.³ Major risk factors for OSCC include consumption of tobacco, alcohol intake and infections with human papilloma virus.⁴ Any form of tobacco use and excess intake of alcohol constitute the major risk factors of oral cancer. The symptoms of OSCC are presented in late stages and encountering the disease at late stages results in recurrences after treatment. The patients previously treated for OSCC, constitute another group that is at high risk for developing OSCC, because they are prone to recurring OSCC or a secondary lesion, as well as those who have history of some other malignancies other than OSCC.⁵ Oral cancer can occasionally be preceded by lesions of oral precancer which predominantly includes oral submucous fibrosis and leukoplakia.⁶ This distinction proposes a two-step development of oral cancer, i.e., the occurrence of a precursor initially followed by its transformation into cancer and is well substantiated. Currently identified oral potentially malignant disorders (OPMDs) include oral submucous fibrosis, erythroplakia, leukoplakia, oral lichen planus, palatal lesions

associated with reverse smoking, actinic cheilitis and discoid lupus erythematosus.⁷ The prevention and timely recognition of such OPMDs not only favours a decreased rate of oral cancer but also improves the chances of survival in subjects developing oral cancer.⁸ The 5-year survival rate of oral cancer has remained persistently at a dismal rate of around 50% despite major advances in its treatment.⁹ There has been a continuous effort towards developing novel diagnostic techniques, including new panels of OSSC biomarkers. As saliva is easy to obtain and can be collected non-invasively, it makes a great choice for this purpose.¹⁰ Research exploring biomarkers in saliva for diagnosis of various cancers is being published routinely.¹¹⁻¹⁴ The use of saliva for identifying oral cancer biomarkers has gained attention due to many reasons, one among them being its direct contact with the lesion.¹⁵ The use of saliva as liquid biopsy medium offers an edge over blood, as collection of saliva is safe, non-invasive, easy and cost-effective.¹⁶ Saliva constitutes a composite collection of cytokines, enzymes, hormones, antibodies and antimicrobial agents.¹⁷ The low molecular weight proteins constitute about 40% to 50% of proteins in the salivary glands.¹⁸ The chromosomes 4, 12 and 20 contain the genes that encode for these proteins.¹⁹ The low molecular weight proteins in saliva were grouped into six main classes, namely, basic proline-rich proteins, glycosylated proline-rich proteins histatins, acidic proline-rich proteins, cystatins and statherins.^{20,21} Around 3000 differentially expressed peptides and proteins,

a number of them being microbiological in origin have been characterized recently after analysing salivary proteome of humans.²² Salivaomics, omics procedures utilizing saliva,²³ are at present confined to proteomics, transcriptomics, metabolomics, microbiomics and micro-RNA-omics.²⁴ Proteomics is the new science of estimating the protein characteristics, their composition in the various organs and tissues of the body, at a previously inapproachable level.²⁵ This makes it possible to make a significant advancement in the investigation of saliva, which owing to its accessibility and relation to blood has long gained the attention of researchers.

Discussion

OSCC with over 300 000 fresh cases and about 145 000 deaths annually throughout the world constitutes a high prevalence and morbidity.²⁶ The high mortality and morbidity associated with OSCC, can still be attributed to its late diagnosis. A large number of OSCC cases, around two-thirds of all the cases are first encountered at an advanced stage.²⁷ The early stages of OSCC have a fair prognosis with a cure rate of 80% in Stage I and 65% in Stage II.²⁸ As most of the cases are diagnosed at late Stages of either III or IV, they have a poor survival 5 year rate of less than 50%.²⁹ It is therefore of critical importance to detect OSCC early to reduce its morbidity and mortality. The incisional biopsy together with histopathological investigation constitutes the current gold standard in the diagnosis of OSCC.³⁰ The scalpel biopsy although a gold standard for diagnosis, but being invasive, patients may show reluctance to accept the procedure. The expert clinical examination and histopathological investigation fails to detect the presence of early OSCC in most of the suspicious looking cases, thereby raising the need for the early detection of OSCC through improvement of diagnostic techniques.³¹

The effectiveness of saliva is still limited in clinical settings and requires additional research for its routine use as a diagnostic medium. The gold standard technique, biopsy, can confirm or even change the diagnosis of cancer with clear histopathological picture and shows grading of the disease ; it also reveals the type of cancer and any local invasion but is invasive in nature. The non-invasive and economical nature of saliva makes it an appealing diagnostic medium for various diseases.³² Although oral cavity is full of complexity owing to a large number of factors involved in maintaining its homeostasis, the use of saliva as a reliable diagnostic medium has been attempted and encouraging steps have been taken to prove its efficiency to detect protein biomarkers.²² Saliva is a very dilute body fluid which contains proteins, electrolytes and nitrogenous products. Among its protein content, the cystatins, α -amylase, serum albumin, proline rich peptides and mucins are the most plentiful in Saliva.³³ In addition to the most abundantly proteins found in saliva, the lesser ones have also been seen to have a definite diagnostic advantage in various diseases.^{34,35} The profiling of proteomes may be of significant importance to understand the pathogenesis of diseases and resultant identification of biomarkers, as proteins constitute the end products of gene information and act as final effectors for many cellular functions.³⁶ The main methods to

study salivary proteome include mass spectrometry (MS) and Two-dimensional electrophoresis (2D electrophoresis). These techniques have worked wonders in evaluation of salivary protein components, in combination with other techniques like drop electrophoresis, chromatography, and gel filtration. Several publications have summarized methodology bases of saliva associated proteomic research.³⁷⁻³⁹ The accumulation of data and the development of various tools like bioinformatics has resulted in the foundation of salivary proteome databases, Salvaomic database,⁴⁰ and SDXMART-BIOMART portal; data can be found here ranging from saliva proteome, peptidome, metabolome, and transcriptome.⁴¹ The first systemization of salivary proteins was published in 2008 after analysing saliva proteome for the first time.⁴²

The serum proteomic studies in head and neck cancer (HNC) reveal that, proteins expressed differently can differentiate HNC patients from the control groups with a high degree of sensitivity (68% to 83.3%),^{43,44} and specificity (76%-90%).^{44,45} The studies employing salivary proteomics in a similar fashion have shown comparable reliability in detecting OSCC with a sensitivity of 90% and specificity of 83%.⁴⁶

Gleber Netto et al, concluded that salivary proteomics is valuable in the diagnosis of OSCC and OPMDs and such salivary analytes can diagnose OPMDs with high discriminatory powers enabling early detection of patients with high risk for OSCC development.⁴⁷ Gallo et al, confirmed numerous significant modifications shown by salivary proteomes in OSCC patients; furthermore, few of these modifications are associated with nodal status, therefore, offering better prospects of understanding the process of carcinogenesis, and that these proteomes also help to discover useful potential biomarkers with dependable clinical utilities.³⁶ Salivary proteomics is really worth for continuous evaluation until more discoveries are made in the struggle against OSCC. Chen et al, improved upon the workflow for salivary protein digestion and assessed quantification performance, strength and technical impediments in analysing clinical samples based on targeted proteomics using liquid chromatography (LC), multiple reaction monitoring (MRM) with MS detection (LCMRM/MS).⁴⁸ They identified 56 proteins, previously seen associated with OSCC patients and found most of these significantly elevated in saliva samples from OSCC patients when compared with healthy controls. The developed platform assayed for the most highly multiplexed panel of salivary protein biomarkers, establishing the advantage of MRM in OSCC biomarker research.

Carnielli et al, combined the discovery and targeted proteomics methods to detect predictive proteomic signatures for OSCC patients.² The discovery (proteomic analysis) phase of this study was performed on formalin-fixed paraffin-embedded OSCC tissues and combined with clinical status of the participants, establishing several proteins (CSTB, NDRG1, LTA4H, PGK1, COL6A1, ITGAV, and MB), expressed in a distinct pattern between inner tumor and invasive tumor front, and thereby proposing a likely prognostic value of clinical and pathological analysis. This was followed by a targeted phase, with two separate approaches, through clinical significance

and histochemical staining and the second one being selected reaction monitoring-mass spectrometry (SRM-MS) to evaluate the abundance of seven proteins in salivary samples of independent group of OSCC patients. The combination of COLA1-, LTA4H-, and CSTB- specific salivary peptides, in their study demonstrated the ability to discriminate between the nodal status of the patients with and without metastasis with a decent and better than the prediction value of single or grouped proteins.

Cosz et al, evaluated a non-invasive salivary diagnostic method for detection of useful biomarkers for early detection of OSCC by a Luminex-based multiplex kit for (VEGF, IL-6, IL-8, IL-1 α , IL-1 β , TNF- α) and SRM-based targeted proteomic method for (CD59, catalase, galectin-3-binding protein, profilin-1, S100A9, CD44, thioredoxin and keratin-19).⁴⁹ ELISA was used for validation of selected potential OSCC biomarkers, and revealed SA1009 and IL-6 as useful OSCC biomarkers for detection and thus improving the diagnostic precision for OSCC. Shan et al, selected three proteomics salivary biomarkers for validation, S100A2, SLC3A2, and IL1RN from the 21 significantly altered proteins when OSCC was compared with both OPMDs and Controls.⁵⁰ The findings of this study establishes the combination of SLC3A2, S100A2, and IL1RN as encouraging salivary biomarkers of OSCC. After additional validation the purely non-invasive salivary screening assay, may reveal better performance for early diagnosis of OSCC.

The medical diagnostic field is progressing by proteomic analyses because of its ability to reliably identify proteins, that were previously inaccessible. It is imperative to identify non-invasive sensitive and specific biomarkers for early diagnosis of OSCC. The ability to detect molecules in saliva, such as proteins, metabolites, DNA, coding and noncoding RNAs indicates that, saliva has enormous value in the early diagnosis of OSCC.⁵¹

Conclusions

Saliva has been in use since a long time for routine clinical laboratory practices notable among them being evaluation of hormones, cortisol and secretory antibodies. As a diagnostic medium it enjoys several advantages from, undemanding collection technique, safer to handle, non-invasive and being able to be shipped and stored in an easy and cost-effective approach. Salivary proteome has recently been explored for understanding the efficiency of saliva as a diagnostic medium. The various proteomics studies mentioned in this review favour diagnostic role of saliva in discriminating OSCC from oral precancer and healthy controls, thereby providing effective means of monitoring and arresting the development of oral cancer. Various attempts have been made to develop rapid salivary methods of OSCC detection with optimistic outcome. Today, proteomic technologies are extremely complex, expensive and of limited accessibility. The time is not far when further continuous research in -omics related studies will produce simple, reasonably priced and ergonomic instruments, that can be effectively applied to saliva for diagnosing OSCC at a rapid, cost-effective and non-invasion fashion.

Authors' Contributions

SAB performed literature survey and compiled the manuscript. LC supervised the manuscript, generation and performed critical analysis.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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