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Review

Saliva: A potential media for disease diagnostics and monitoring

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SUMMARY

Within the past 10 years, the use of saliva as a diagnostic tool has gained considerable attention and become a well-accepted method. As a diagnostic fluid, saliva offers superiority over serum due to both a noninvasive collection method by specially trained persons and a cost-effective approach for screening of large populations. Collection of saliva offers a reduced risk of infection compared to the collection of serum. Moreover, obtaining saliva samples from infant, disabled or anxious patients, is much easier than obtaining other samples. There is a lot of useful components-changing information in saliva when a person is in sick. Therefore, we define these changing components as "biomarkers". The utilization of biomarkers as early predictors for clinical disease not only contributes to the effective prevention and treatment of diseases, but also enhances the assessment of potential health risks. In this article, we have reviewed the properties of saliva, the salivary analysis method for biomarker discovery, and the diagnostic potentials of salivary biomarkers in monitoring and detecting periodontal disease, Oral and Breast cancers, and Sjögren's syndrome. We also discussed some barriers of applications of saliva as a diagnostic media as well as recent improvements. We also prospected the future processing directions of using biomarkers in disease diagnosis and draw a conclusion that saliva is indeed an effective media in various disease monitoring and diagnosis.

Introduction

Early detection of disease plays a significant role in successful clinical treatment. In most cases of various diseases, early detection and diagnosis lead to a greater survival rate with a reduced chance of the disease re-emerging. Successful monitoring of a disease, especially in its early stage, may also reduce any severe impacts on a patient's health or help to prevent and/or delay succeeding complications. The ability to evaluate physiological conditions, trace disease progression, and monitor post-treatment therapeutic resulting through a noninvasive method is one of the primary objectives in the field of healthcare research. Saliva, a multi-constituent oral fluid that can be collected through noninvasive means, has considerable potential for the surveillance of general health and disease. Human saliva contains many kinds of proteins and peptides, each of them carries several significant biological functions. With the advancement of novel technological means (such as bioinformatics, metabolomics, genomics and proteomics), saliva, as a clinical tool, has become a more and more attractive option because of its ability to mirror both oral and systemic health conditions.[1] But in order for saliva-based diagnostics to be useful, two prerequisites must be fulfilled: (1) discovering biomarkers for various diseases among the complicated composition of saliva, and (2) evaluating the sensitivity and specificity of biomarkers through a series of continuous developments.[2]

Saliva profile

Water is the most abundant component in saliva, representing 99% of saliva's total composition. The solid components soluble in the aqueous phase differ from person to person, and can even vary in the same individual at distinct times during a day. The inorganic species are mainly composed of weak and strong ions including Na^+, K^+, Cl^-, Ca^{2+}, HPO_3^{2-}, HCO_3^-, Mg^{2+}, and NH_3. The organic species (see Table 1) consist of body secretion products (urea, uric acid and creatinine); putrefaction products (putrescine and cadaverine); lipids (cholesterol and fatty acids), and more than 400 types of protein. Among those proteins, the most relevant ones are glandular in origin (alphaamylase, histatins, cystatins, lactoferrins, lysozymes, mucins, and proline-rich proteins (PRPs)) or are plasma-derivatives (albumin, secretory immunoglobulin A (sIgA), and transferrin).[3]

Human saliva proteome (HSP) analysis is inherently challenging because human saliva contains an inherently large variety of proteins with an equally wide range of concentrations. For example, α-amylase, the most abundant protein in human saliva, is at mg/ml level, whereas cytokines are typically within the range of pg/ml.[4]

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Table 1
Salivary proteins.[3]

Salivary protein	Origin	Functions	Concentrations
Total proteins			0.47 ± 0.19 mg/ml, 0.9 ± 0.2 mg/ml, 4.3–710.0 mg/dl, 2.67 ± 0.54 mg/ml
α-Amylase		Starch digestion	3257 ± 1682 U/ml, 1080.0 ± 135.6 IU/l, 476 ± 191 μg/ml
Albumin	Plasma	Mainly from plasma leakage	0.2 ± 0.1 mg/ml, 0.8–192 mg/dl
Cystatins group	SM > SL	Antimicrobial(cistein-proteinase inhibitor)	14.3 kDa form 58 ± 25 μg/ml; 14.2 kDa form 91 ± 46 μg/ml
Hystatin	P	Antifungal	1190 ± 313 μg/ml
Secretory IgA	B lymphocytes	Antimicrobial	124.3–335.3 μg/ml
Lactoferrin	Mucous > serous	Antimicrobial	3.7 ± 2.5 μg/ml
Lysozyme	SL > SM,P	Antimicrobial	3.5–92.0 μg/ml, 21.8 ± 2.5 mg/dl, 59.7–1062.3 μg/ml
Mucins group	Mucous glands	Lubrication	MUC5B: 2.4 ± 1.7 U/ml
PRPs	P	Binding to bacteria and with dietary tannins	Acidic PRP: 456 ± 139 μg/ml, Basic PRP:165 ± 69 μg/ml
Statherin		Ca^{++} binding	4.93 ± 0.61 μmol/l, 36 ± 18 μg/ml
Transferrin	Plasma		0.58 ± 0.2 mg/dl

SM = submandibular; SL = sublingual; P = parotid.

The reason why saliva can potentially be used as a specimen for diagnosis is because of its exchange with substances existing in human serum. A thin layer of epithelial cells separating the salivary ducts from the systemic circulation enables the transfer of substances to the saliva by means of active carriage, diffusion through the cell membrane, or passive diffusion via a concentration gradient.

One of the principal advantages of using saliva as a diagnostic media is that its sampling is easy and noninvasive, thus eliminating any discomfort and pain associated with blood collection while also avoiding privacy issues associated with urine collection. Additionally, compared with blood, saliva contains a smaller quantity of proteins, therefore decreasing any potential risk of non-specific interference and hydrostatic interactions. Within blood, the protein concentration can vary over several orders of magnitude, with protein half-lives ranging from a few seconds to several months or longer. The composition of saliva, however, is not as complex or varying as serum, and should more accurately reflect the current condition of the body at any given time.

Ultimately, saliva may contain locally expressed proteins and other substances that can be used as indicators of diseases. These components, called biomarkers, can be closely related to an individual's health condition and can change greatly when diseases afflict the body.

Biomarker

According to the National Institutes of Health, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmaceutical responses to a therapeutic intervention.[5] Generally speaking, a biomarker can be any biomolecule or specific characteristic, feature, or indicator of an alteration in any biological constitution and function that can objectively reflect the state of a living organism.[6]

Criterion for biomarker

- A major product of oxidative modification that may be implicated directly in the development of a disease;
- A stable product, not susceptible to artefactual induction, not easy to lose, or not changeable during storage;
- Representative of the balance between oxidative damage generation and clearance;
- Determined by an analytical assay that is specific, sensitive, reproducible and robust;
- Free of confounding and interference factors from dietary intake;
- Accessible in a target tissue or a valid surrogate tissue such as a leukocyte;
- Detectable and measurable within the limits of detection of a reliable analytical procedure.[7]

The discovery–validation–implementation paradigm

A biomarker must be verified and validated before it can have any impact or application on health risk assessment. The verificating process might be considered as a process that is conceptually similar to therapeutic drug evaluation. There are six prerequisites before a biomarker can be used in a clinical assay: (1) preclinical testing: developing in vitro or in animal models; (2) preliminary testing: developing preliminary assays on patient samples; (3) feasibility analysis: testing on a small group of patients to determine its ability to discriminate between healthy or diseased subjects; (4) validation of the accuracy of assays; (5) statistical analysis: determining in large patient populations; (6) post-approval reporting and testing. A general recommendation is that the validation effort should concentrate on those biomarkers directly involved in the causal pathway of disease, since the closer to the causal pathway the biomarker is, the more precisely it will predict disease.[8]

Saliva analysis

In the last few years, remarkable efforts have been devoted to the identification of proteins in human and parotid saliva by using diverse proteomic approaches. High-resolution liquid separation is a critical component in both shotgun and random proteome analysis. Pre-fractionation of proteins using liquid-based separation techniques is often required for a comprehensive analysis. Separations can be performed based on the physiochemical properties of the interested protein using capillary isoelectric focusing (IEF),[9] gel filtration liquid chromatography (LC), reversed-phase (RP) LC, strong cation exchange LC or ZOOM IEF. The fractions are collected and digested using proteolytic enzymes and the resulting peptides are analyzed with 1D-LC/MS/MS or 2D-LC/MS/MS, either online or offline. The online 2D-LC separation uses a single capillary column packed with two types of LC separation media[10] or an automatic column-switching technique. Free-flow electrophoresis can be coupled with RP-LC to greatly enhance the separation of peptides prior to MS/MS analysis.[11]

In other cases, investigators have used two-dimensional (2D) gel electrophoresis (GE) to separate protein components, followed by mass spectrometry (MS) to subsequently identify the peptides produced from in-gel digestion of the proteins of interest. This approach revealed that more than 300 proteins exist within saliva. When separations were performed using liquid chromatography