| | |
|---|---|
| **原文題目(出處)：** | Potential uses of human salivary protein and peptide analysis in the diagnosis of disease. Arch Oral Biol 2012;7:1-9 |
| **原文作者姓名：** | Kawas SAl, Rahim ZHA, Ferguson DB |
| **通訊作者學校：** | Department of Oral & Craniofacial Health Sciences, College of Dentistry, University of Sharjah, Sharjah, United Arab Emirates |
| **報告者姓名(組別)：** | 李懿修 Intern K 組 |
| **報告日期：** | 2012/07/09 |

內文：

Introduction

- Mainly a mixture of the secretions from the three pairs of major salivary glands each secreting a characteristic type of saliva
- Contains gingival crevicular fluid, many microbial contaminants and from the desquamated cells of the oral epithelium
- Obtained by spitting into a test tube
- More sensitive and more specific markers for oral disease like squamous cell carcinoma
- Diagnostic tools to monitor both oral and systemic disease
- Advantage: Collection non-invasive, reducing patient discomfort, low cost, easy to collect
- Disadvantage: Requires centrifugation or filtration to remove precipitated mucins and cellular contaminants
- Complete analysis of salivary proteins and peptides with proteomic techniques and using salivary proteins and peptides as a biomarkers for systemic disease

Analysis of the proteome of saliva

- Abundance of proteins with a wide range of functional properties included immune response and antimicrobial activity (lysozyme, lactoferrin)
- Salivary proteome-map out the total protein and peptide composition of the saliva
- Huq et al stated that over 1380 proteins have been detected in saliva but only around 100 of these are present in relatively high abundance
- Proline-rich, peptides, alpha-amylase, cystatins, histatins, mucins, secretory IgA and carbonic anhydrase well-known by protein analysis technique
- Techniques
    - Separation of the salivary proteins by two-dimensional electrophoresis and detecting them with a suitable stain.
    - Shotgun proteome analysis (enhanced resolutions)
    - Combining surface chromatography with matrix-assisted laser desorption/ionisation time of flight mass spectrometry--rapid
    - high performance liquid chromatography combined with mass spectrometry in the evaluation of the smallest salivary proteins and peptides
    - Ionization methods such as electrospray ionization(ESI) and matri-assisted laser desorption ionization(MALDI)–can deplete proteins in order to improve identification of lower abundance proteins
    - All these techniques together have extended proteome of whole saliva to comprise of more than 1050 proteins
    - Well known--proline-rich peptides, alpha-amylase, cystatins, histatins,

mucins, secretory IgA and carbonic anhydrase.

### Proteome of normal human saliva

- Identified 914 proteins in parotid saliva and 917 proteins in submandibular/sublingual saliva from a total of 23 subjects (12 females and 11 males)
- 174 of the 657 proteins found in plasma and 236 of the 467 proteins found in tears are also expressed in saliva.

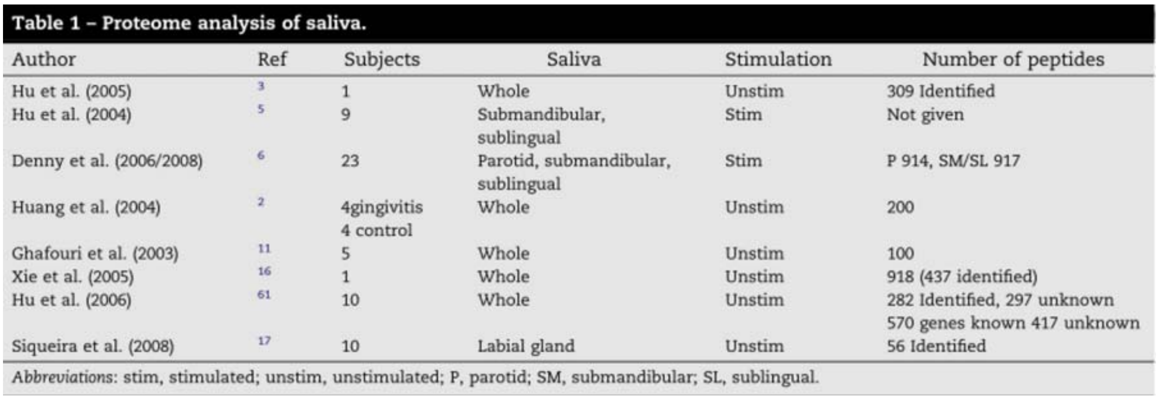

**Table 1 – Proteome analysis of saliva.**

| Author | Ref | Subjects | Saliva | Stimulation | Number of peptides |
|---|---|---|---|---|---|
| Hu et al. (2005) | 3 | 1 | Whole | Unstim | 309 Identified |
| Hu et al. (2004) | 5 | 9 | Submandibular, sublingual | Stim | Not given |
| Denny et al. (2006/2008) | 6 | 23 | Parotid, submandibular, sublingual | Stim | P 914, SM/SL 917 |
| Huang et al. (2004) | 2 | 4gingivitis<br>4 control | Whole | Unstim | 200 |
| Ghafouri et al. (2003) | 11 | 5 | Whole | Unstim | 100 |
| Xie et al. (2005) | 16 | 1 | Whole | Unstim | 918 (437 identified) |
| Hu et al. (2006) | 61 | 10 | Whole | Unstim | 282 Identified, 297 unknown<br>570 genes known 417 unknown |
| Siqueira et al. (2008) | 17 | 10 | Labial gland | Unstim | 56 Identified |

*Abbreviations*: stim, stimulated; unstim, unstimulated; P, parotid; SM, submandibular; SL, sublingual.

### Proteome of normal human saliva

- Major proteins reported: alpha-amylase, proline-rich proteins, cystatin, histatins and mucin
- Most involved in metabolic processes, complementand clotting cascades, cell adhesion and communication, cell cycle progression and regulation of the actin cytoskeleton

### Problems of standardization

- Simply allowing saliva to accumulate in the mouth and then allowing it to dribble out, is subject to considerable variation as the patient tries
- Using wax stimulated saliva, or giving the patient a sterilised piece of soft plastic surgical cannula to chew
- Cellular and fluid components will be more in stimulated saliva
- A whole saliva free of bacterial and epithelial cell contamination is required.
- It is recommended that saliva samples should always be collected at the same time of day to reduce circadian variation

### Concept of biomarkers

- A single molecular species which is present in samples from a subject with a particular disease or status, and is not present in other subjects.
- The range of variation which can be regarded as normal must be ascertained for a number of substances in a large population
- Degradation of proteins in whole saliva also contributes to variety in the proteins and peptides observed.
- cErb2 is found in the saliva of patients with mammary carcinoma but at very much lower concentrations in the saliva

### Salivary proteomes in relation to oral diseases

- Dental caries susceptibility

- Evaluation of periodontal disease
- Diagnosis of oral squamous cell carcinoma
- Salivary variation in other oral inflammatory diseases

### Dental caries susceptibility

- The two most common oral diseases are dental caries and periodontal disease
- Evaluate caries risk by measuring the amounts of selected oligosaccharides whose concentrations have shown a correlation with caries experience in young adults
- Changes in the saliva output during ageing correlated with greater risk of dental caries and may be indicators of caries risk
- Lower median values of alpha-defensins
- Statherin and cystatin S are the best predictors of occlusal caries in saliva

### Evaluation of periodontal disease

Enzyme immunosorbent assays test for 3 potential biomarkers:

1. Interleukin-1 beta as a marker of inflammation
2. Matrix metalloproteinase-8 as a marker of collagen breakdown
3. Osteoprotegerin as a marker for bone turnover compared salivary proteomes from patients with periodontitis

1. Blood proteins and immunoglobulins ↑
2. Cystatins ↓
3. Alpha-amylase ↑

Biomarkers in saliva for three distinct phases of periodontal breakdown were identified

1. Interleukins 6 and 7
2. Matrix metalloproteinases 8 and 9
3. Osteoprotegerin and ICTP

### Diagnosis of oral squamous cell carcinoma

- Heavy cigarette smokers:interleukin-8 and Thioredoxin(predisposing factor)
- CD44 ↑ distinguish cancer from benign tumours
- Three tumour markers:
    1. Cytokeratin 19 fragment
    2. Tissue polypeptide antigen
    3. Cancer antigen 125

### Salivary variation in other oral inflammatory diseases

- Oral lichen planus:
    1. Urinary prokallikrein ↑
    2. Expression of the palate, lung and nasal epithelium carcinoma associated protein ↓

### Salivary proteomes in relation to systemic diseases

- Saliva has been used in the diagnosis of auto-immune deficiency syndrome (AIDS) and other viral infections such as hepatitis B and C.
- Detection of cErb2 in the saliva:
    - higher levels of cErb2 in saliva with breast cancer

  - Theoretically other exocrine gland tumors might also raise salivary cErb2 levels in saliva

Practical issues

- Simple tests using whole saliva, unstimulated or stimulated, and they can be performed easily in standard clinical laboratories.
- It must be simple to use and preferably adapted for use in surgery or clinic

Conclusions

- It is essential that a comprehensive database be established for the proteome of whole saliva collected and processed under standard conditions.
- It must show advantages over other tests, be sufficiently easy in use, and, preferably, able to give a rapid and accurate result in the clinical consultation. It should also be patient friendly.
- More research is required to validate the various discovered potential biomarkers for early disease detection that will lead to more effective treatment.

| 題號 | 題目 |
|---|---|
| 1 | What is the most common site for salivary gland tumor?<br>(A) Submandibular gland<br>(B) Sublingual gland<br>(C) Parotid gland<br>(D) Minor salivary gland |
| 答案( C) | 出處：Oral and Maxillo Facial Pathology p. 473 |
| 題號 | 題目 |
| 2 | What is the most common benign tumor?<br>(A) Pleomorphic adenoma<br>(B) Myoepithelioma<br>(C) Basal cell adenoma<br>(D) Warthin tumor |
| 答案( A) | 出處：Oral and Maxillofacial Pathology p. 473 |