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Salivary Acute Phase Proteins as Biomarker in Oral and Systemic Disease

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1. Introduction

The purpose of this review is to present the latest research data regarding the physiological and diagnostic significance of acute phase proteins concentrations in saliva during oral and systemic diseases. We also show some interesting advantages in using saliva sample as a diagnostic fluid.

2. Saliva as diagnostic tool

Saliva is a unique biological fluid, with an important role in the oral physiology. It is a major player in the process of oral and general health maintenance (Humphrey, 2001). According to recent data it mirrors general health condition thus reflecting various systemic changes in the body (Chiappelli, 2006; Nagler, 2002, 2008).

Saliva is a colorless viscous liquid mixture of oral fluids which includes secretions from both the major and minor salivary glands. Additionally, it contains several constituents of non-salivary origin: gingival crevicular fluid, expectorated bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, leukocytes, electrolytes, immunoglobulins, proteins and enzymes, food debris and a small portion is gastro-esophageal reflux, etc. (Edgar, 1990; Nagler, 2002).

These facts motivate more extended use of saliva samples for diagnosis of different oral and systemic diseases.

2.1 Advantages of saliva as research material

Recently, saliva has been proven to be among credible diagnostic tools for detecting different biomarkers. Its promising future relies on two main reasons. Firstly, characteristic biomarkers for different diseases were found in significant concentrations among the components of the saliva. Second, the improvement of existing technologies and development of new, highly sensitive methods has succeeded to reveal the acceptable sensitivity and specificity of salivary biomarkers in term of different local and systemic conditions.

Additionally, the raised interest in saliva as a useful diagnostic fluid during last few decades is motivated by the proven ability to monitor the general health, to discover the disease onset and to follow up the progression to be used in large scale screening and epidemiologic studies (Irwin, 1990; Jentsch, 2004; Yu-Hsiang, 2009).

Compared to the other diagnostic media (such as tissue samples, serum, cerebrospinal fluid, etc.) saliva sampling is easy collectable, cost-effective, non-invasive diagnostic tool for research and often is preferred as an alternative diagnostic approach.

Advantages of saliva sampling are mostly seen in some patient's categories which are historically problematic to manage as children, IVU or any other case when venepuncture is difficult to achieve. The saliva collection does not cause anxiety or discomfort and studies are reproducible (Hu, 2008). It could be performed by the individuals themselves after minimal training, without the help of medical staff. Furthermore it is relatively safe to obtain numerous samples during the course of the disease with minimal risk of contamination both for the patient and the healthcare staff.

2.2 Whole or gland-specific saliva?

Using the whole saliva and gland-specific saliva as objects of diagnostic investigations has some specific concerns. Most frequently whole saliva is examined mainly because of its simple and facile mode of collection. There are some difficulties to collect and separate the gland-specific saliva from individual salivary glands: parotid, submandibular, sublingual, and minor salivary glands. When gland-specific samples are compulsory needed, use of specific devices is advised (Navazesh, 1993). In case of inflammatory and obstructive diseases of the particular salivary gland, examination of gland-specific saliva is more accurate and thus is preferable.

Analysis of the saliva may be useful in increasing number of clinical situations, such as infectious diseases, hereditary disorders, autoimmune diseases, malignant and endocrine disorders. It is also beneficial in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use (Yu-Hsiang, 2009). The list of the implicated clinical and research use of the saliva samples is kept growing.

Furthermore, the whole, unstimulated saliva contains serum and local constituents, which reliably reflect changes in the local immunity. These components are derived from the local vasculature of the salivary glands and also reach the oral cavity via the flow of gingival fluid.

2.3 The collection of saliva

The preanalytic stage is essential for obtaining reliable and reproducible study results. This is the reason many authors to focus particular attention to the prerequisites which patients and devices need to meet before collection of the saliva sample. Usually several conditions have to be fulfilled. Avoiding of any oral-hygienic procedures on the study day, restrain of food, liquids and smoking before sample collection are among most popular, but might vary according to the objectives of the study (Dawes, 1990, Jentsch, 2004). It is found that best approach is to obtain samples at the same time of the day, most often between 8 and 9 a.m. (Dawes, 1990).

Importantly, in healthy subjects, gingival crevicular fluid from the tooth/gum margin may constitute up to 0.5% of the volume of mixed saliva (Cimasoni, 1974). During the oral-hygiene procedures minor abrasions appear and plasma exudate from in the mouth increased. Therefore, it is usually recommended that subjects should not brush their teeth or practice any other methods of oral hygiene for several hours before collecting a saliva sample (Hold, 1999).

2.3.1 Collection time of saliva

From practical point of view, time for collection of the saliva is usually from 5 to 15 minutes. The necessary quantity is 2-10 ml according of the number of tested components (Dawes, 1990, Jentsch, 2004; Marton, 2004). The samples are gathered in special sterile containers, which might be plastic, glass, polypropylene, etc. However, small molecules tend to stick to the polyethylene container for collection. Therefore, the most suitable plastic material is polypropylene, but not if recycled (Hold, 1999).

2.3.2 Methods of saliva collection

Techniques for the collection of saliva

Several methods have been described for the collection of mixed saliva:

- Draining method - the patients are instructed to swallow before collecting saliva. The saliva is secreted in the collecting bottle using a funnel, held near the mouth (Navazesh, 1993).
- Spitting method - saliva is collected in the mouth while patient keeps lips closed. At the end of each minute the patient emerged oral samples in sterile bottles (Navazesh, 1993).
- Suction method - the procedure starts with swallowing, to release the mouth of available saliva. Special devices are placed under the tongue and attached to the suction pump that drains saliva. At the end of the procedure the suction tube is placed in the vestibulum and in the floor of mouth and, likewise, is collecting residual saliva (Navazesh, 1993).
- Absorbent method - absorbent tampons or sponges (0.2 x 0.6 cm) are placed under the tongue near the exit channels of the sublingual and submandibular glands. Two other sponges are placed between the gingiva and cheek at the exit channel of the parotid gland (Navazesh, 1993).

2.4 Stimulated and unstimulated saliva

Although most patients prefer donating saliva rather than blood, a substantial social barrier exists to "spitting". For this and other reasons, subjects often experience decreased salivary secretion (dry mouth) if asked to provide a sample. Many researchers have found it advantageous to further stimulate salivation and a number of stimuli have been used: mechanical with inert material - paraffin wax, parafilm, rubber bands, pieces of teflon, polytetrafluoroethylene (PTFE), chewing gum or chemical stimulation with 2% citric acid, acid lemon drops or a few drops of 0.5 mol/l citric acid are among the most potent of taste stimuli and will generally induce a maximal secretion of 5 to 10 ml/min (Dawes, 1992; Hold, 1999; Ruhl, 2004).

Some authors recommend that, when these types of stimuli are used, the subject should allow saliva to accumulate in the mouth until the desire to swallow occurs, at which time the fluid can be expelled smoothly into a vessel. Repeated expectorations should be avoided since this introduces bubbles, which may result in changes in pH leading to errors in interpretation of the saliva/plasma concentration ratio (S/P ratio) (Hold, 1999).

There are several advantages of stimulating salivary flow. Firstly, large volumes of saliva can be obtained within a short time. Secondly, the pH of stimulated saliva mostly lies within a narrow range around the value of 7.4, whereas the pH of unstimulated saliva shows a larger variability. It may be of importance for the salivary secretion of weak acidic and basic compounds (Feller, 1977; Ritschel, 1983). The last, but not at least the intersubject variability

in the S/P ratio may be diminished when stimulated saliva is used, as has been reported for digoxin (Hold, 1999).

2.5 Storage of saliva

Some authors believe that saliva rest stable at room temperature for several days (George, 1997; Schramm, 1991, Wade, 1991). Other recommend elimination of bacteria and enzymes prior to storage or otherwise stabilizers might need to be added stabilizers (Frerichs, 1992). Most authors suggest freezing the material immediately after collecting, especially when evaluating levels of hormones, immunoglobulins and interleukins (Zapryanova, 1989). Further processing of collecting saliva depends on the methods of study that will be used, but most usually saliva is centrifuged at 15 000G 4 ° C for 10 minutes (Jentisch, 2004; Rohan, 2000; Ruhl, 2004; Zapryanova, 1989).

2.6 Diagnostic features of saliva

Currently, three major restrictions limit the development of clinical diagnostics:

- The lack of definitive molecular biomarkers for specific diseases;
- The lack of easy and inexpensive method of sampling with minimal discomfort;
- The lack of accurate, easy to use and portable platform to facilitate early detection disease (Yu-Hsiang, 2009).

Since 2002, the National Institute of Dental and Craniofacial Research (NIDCR) has created opportunities to overcome these limitations by exploring oral fluids as a diagnostic tool for assessment of health and disease status. Through the visionary investment by the NIDCR, the discovery of salivary biomarkers and ongoing development of salivary diagnostics technologies now provide promising solutions for the rest (Yu-Hsiang, 2009).

2.6.1 Tested substances in saliva

Like blood, saliva is a complex fluid containing a variety of numerous organic and inorganic constituents, of which the most frequent subject of investigations is: proteins, enzymes, hormones, carbohydrates, calcium, sodium, magnesium, copper and others.

Also it is important that saliva is a biologic medium for the study of genomic DNA (Ng, 2004). Therefore it is a unique fluid for the development of molecular diagnostics. Unfortunately its potential seems to be frequently underestimated due to technological barriers. Further studies are awaited to prove if it meets the requirements necessary to screen saliva containing complex constituents with low concentration (Yu-Hsiang, 2009).

Some of the components exert similar concentrations in blood and saliva and it is possible to easily move from their determination in serum to saliva. It is supposed that the saliva is functionally equivalent to serum in reflecting the physiological state of the body, including emotional, hormonal, nutritional, and metabolic variations (Ivanova, 2009). Others substances (like cholesterol, creatinine, uric acid etc) have very low concentration making their determination with the available techniques in saliva unreliable and clinically unfeasible (Ivanova, 2009).

2.6.2 Techniques for investigating saliva

The concentration of many substances in saliva was significantly lower than in blood plasma and this requires the use of highly sensitive tests and methods designed for saliva, and the proper collection of material.

At this level of knowledge the researchers aim to improve the sensitivity of the different methods that is believe to help further implementation of saliva sampling in diagnostic process in various disease processes. The low concentration of different substances compared with levels in the blood may prevent salivary diagnostics from being clinically practical. However this limitation was recently overcome as new and highly sensitive techniques (e.g., molecular diagnostics, nanotechnology) were developed. Unfortunately, they are still available only in a limited number of specifically equipped labs (Yu-Hsiang, 2009).

Most commonly radial immunodiffusion, immunoelectrophoresis, turbidimetry, nephelometry are used for determining salivary protein levels. For detecting cytokines levels ELISA, flowcytometry, ELISPOT, RT-PCR are preferred. The most common ELISA method is characterized with quite low sensitivity. The ELISPOT assays surpass ELISA techniques in terms of higher sensitivity achieved, but the proceeding times are usually longer. Compared to precipitation methods, ELISA essays are more sensitive thanks to the use of monoclonal antibodies (Altankova, 2001).

Based on currently emerging biotechnology in salivary diagnostics became possible to determine the number of biomarkers for various diseases. The list includes malignancies (Kaufman, 2000), hereditary, autoimmune, viral and bacterial infections, cardiovascular diseases, HIV related conditions. We cannot miss the convenient use of saliva diagnostics in therapeutic drug monitoring, measurement of hormone levels, and of course diagnosis of local oral disease. These developments have extended the range of saliva-based diagnostics from the simple oral cavity to the whole physiological system. Thus, saliva-based diagnostics is on the cutting edge of diagnostic technology, and may offer a robust alternative for clinicians to use in the near future to make clinical decisions and predict post-treatment outcomes (Yu-Hsiang, 2009).

For the past two decades, salivary diagnostic approaches have been focused on the oral diseases. Interest represents a concentration of proven proteins in saliva, on the other hand their attitude to medical conditions and diseases affecting not only the oral cavity and the body. Therefore we would like to direct the focus of this review on determination the positive and negative acute phase protein in saliva in oral and systemic diseases.

3. Acute phase proteins in saliva

3.1 Origin of acute phase proteins in saliva

The serum concentration of the most acute phase proteins increases in response to tissue injury, inflammation or infection. The changes in serum/plasma concentrations correlate with increased hepatic synthesis (Fournier, 2000). The majority of acute phase proteins are synthesized in the liver and some of them diffuse or are actively transported into saliva from the blood. Others are locally produced, including into the salivary glands.

Although haptoglobin is mainly produced in the liver, some other organ may contribute: skin, lungs, kidneys, adipose tissue (Li, 2005). A local synthesis in oral cavity of transferrin, alpha 1-acid glycoprotein, alpha 1-antitrypsin and haptoglobin was observed in infants (Szabo, 1988). Increased CRP mRNA expression in the submandibular glands of rats with experimentally induced inflammation was recently reported (Dillon, 2010). It is now widely accepted that acute phase protein synthesis may take place in extra-hepatic cell types. Presumably, the same inflammatory mediators as observed in hepatocytes regulate the process (Fournier, 2000). Depending on the quantity of measured level, acute phase

response might be positive or negative. Some protein levels are found elevated during the acute phase (positive acute phase proteins), while the production of other proteins is decreased (negative acute phase proteins).

When the concentration of specific component in saliva strongly correlates the serum one, a serum source is accepted (Nagler, 2008). However, the lack of a high correlation between concentrations of a component in saliva and in serum does not necessarily reject the serum origin. It may simply reflect variability in the diffusion process or local synthesis for the component (Nagler, 2008).

The determination of the salivary acute phase proteins' concentration is an object of many studies. Basically it is not a routine practice in the daily dentistry, but we hope it would be soon.

3.2 Positive acute-phase proteins in saliva (positive app)

Abnormal serum levels of positive acute phase proteins have been reported in various studies on myocardial infarction, Inflammatory bowel disease psoriasis, and malignancy. Some oral diseases as parodontitis, oral lichen planus, oral leukoplakia, oral squamous cell carcinoma etc. are also characterized with increased positive APP.

3.2.1 Haptoglobin

The diagnostic and prognostic value of haptoglobin and CRP in different diseases motivates further interest of their salivary concentrations and dynamics (Rocha-Pereira, 2004; Chen, 1989; Clayman, 1992).

There are a limited number of studies regarding the salivary haptoglobin (Backhausz, 1975; Grimoud, 1998; Katnik, 1990). This might be at least partially explained by the difficulties in determination of the acute phase protein in the oral cavity due to their low concentrations (Backhausz, 1975; Grimoud, 1998; Katnik, 1990; Keur, 1994).

Some authors reported immunoenzyme method to determine haptoglobin in different biofluids, including saliva, which ranged 5 to 150 µg/l (Katnik, 1990). The salivary haptoglobin measurement is commonly used in the veterinary practice (Hiss, 2003; Parra, 2005).

Human studies show that saliva haptoglobin increases in patients with parodontitis (Backhausz, 1975). Bell's palsy (Keur, 1994) and in oral manifestation of AIDS (Grimoud, 1998).

Backhausz et al. investigated salivary samples from 60 individuals by immuno-electrophoresis. Transferrin and haptoglobin levels in subjects with periodontal inflammation were found twice as frequent as in control individuals, which is indicative of a correlation with the inflammatory process (Backhausz, 1975).

Keur et al. evaluated the flow rate of extra-parotid and parotid saliva in patients with Bell's palsy compared to healthy volunteers. Concentration of total protein and seven acute-phase proteins were measured. Either extra-parotid or parotid saliva APP levels do not differ by means of patient's age. Interestingly, an elevated concentration of haptoglobin, alfa2-macroglobulin and ceruloplasmin were found in extra-parotid saliva, while only haptoglobin and ceruloplasmin were increased into saliva from parotid gland (Keur, 1994). This study demonstrates that haptoglobin presence in parotid and extraparotid saliva might serve as a reliable marker and it is worth to determine it in whole as well as in gland-specific saliva.

In HIV positive patients presented with typical oral mucosal manifestations a correlation between salivary and serum concentrations of IgA, haptoglobin and alpha1-protease inhibitor were observed (Grimoud, 1998). This is additional confirmation of reliability of saliva sampling for studying the diseases natural history and may successfully replace or at least add value to serum samples.

There is a number of data confirming that haptoglobin levels can predict the clinical outcome. In 2003 S. Hiss et al. created an immune-enzyme technique to determine haptoglobin in different swine fluids with sensitivity threshold of 0.02 mg/l. haptoglobin concentrations in swine’s saliva were found between 0.3 and 3.0 microg/ml and correlated poor with serum levels (n=93, r=0.35, p<0.001) (Hiss, 2003). Parra et al. reported highly sensitive immunofluorimetric method for evaluation of dog serum and saliva haptoglobin. The detection level was 0.002 microg/ml. Saliva haptoglobin levels in diseased dogs were found significantly higher than in healthy ones (Parra, 2005).

3.2.2 CRP (C-reactive protein)

CRP serum concentration usually increases in case of systemic inflammation. Explicably CRP is the most commonly used acute phase protein in clinical practice. Few studies evaluated the CRP levels in oral cavity. It was detected in whole saliva in study which included 45 adults. The concentrations (range from 0 to 472 pg/ml) were found higher in patients with gingivitis, moderate and severe periodontitis (Pederson, 1995). Additionally, edentulous volunteers showed lower levels than healthy subjects (Pederson, 1995, Sibraa, 1991). Some authors used monoclonal antibodies and a radioisotope assay to measure CRP levels in gingival crevicular fluid (GCF) from 24 periodontitis patients. CRP did not differ significantly between diseased and healthy sites in same patients (Sibraa, 1991).

Salivary haptoglobin and CRP in oral neoplasm

The serum levels of C-reactive protein and haptoglobin have prognostic and diagnostic value in patients with oral cancer, inflammatory bowel disease and psoriasis (Vucelić, 1990, Florin, 2006; Solem, 2005; Landowski, 2007; Koelewijn, 2008).

It is known that serum levels of both proteins reflect disease activity and treatment outcome and might challenge the prognosis. Lead by the question if these changes are also observed in saliva, we conducted prospective study to evaluate if the saliva concentrations followed the same trend. We prospectively studied the haptoglobin and CRP levels in saliva samples from 16 patients with parotid tumors and 35 treatment-naïve oral squamous cell carcinoma. Thirty-one healthy subjects served as matched controls. The results clearly showed elevated CRP and haptoglobin levels in both study subgroups as compared to the controls (Table 1).

Groups	CRP (mg/1)	Haptoglobin (mg/l)
Healthy subjects (n=31)	0,105 ± 0,115	15 ± 12,9
Patients with parotid tumors (n=16)	0,185 ± 0,89*	38 ± 58*
Patients with oral squamous cell carcinoma (n=35)	0,346 ± 0,602*	45 ± 61*

*p<0.05 versus healthy subjects

Table 1. Saliva CRP and haptoglobin levels in patients with parotid tumors and oral squamous cell carcinoma

Three months even insignificant decrease of CRP and haptoglobin was found (Krasteva, 2009).

As discussed above, the haptoglobin is found in the cytoplasm of epidermal Langerhans cells (Li, 2005). Basically, we believe that that increased levels found in squamous cell carcinomas patients are related to local production and in oral mucosa. Possibility of systemic/serum origin however could not be overlooked. The levels of CRP in untreated oncology patients were almost three times higher than in control subjects almost 25% of the untreated cancer patients had levels above the established highest value in controls. Following appropriate treatment (chemotherapy, radiotherapy, surgery) 15 patients had salivary haptoglobin and CRP which were not different from the control subjects (mean 17 ± 36 mg/l and $0,136 \pm 0,72$ mg/l respectively). Salivary haptoglobin and CRP levels decreased after treatment as expected due to the removal of tumor and subsiding of the inflammatory process (Krasteva, 2009). We found moderate correlation between CRP and haptoglobin ($r = 0,502$, $p = 0,008$) which provides additional evidence to confirm the same trend of elevation of both APP the parallel movement (increase) of two acute phase proteins (Krasteva, 2009).

Salivary haptoglobin and CRP in systemic disease

In 32 currently untreated psoriatic patients with PASI >10 we observed increased salivary CRP and haptoglobin levels (Table 2).

Groups	CRP (mg/l)	Haptoglobin (mg/l)
Healthy subjects (n=31)	$0,10 \pm 0,115$	$15 \pm 12,9$
Patients with psoriasis (n=32)	$0,166 \pm 0,55$	$26,9 \pm 24,6$

*p<0.05 versus healthy subjects

Table 2. Saliva level of CRP and haptoglobin in psoriatic patients

We suggest that elevated salivary haptoglobin levels may be due to transudation or local production in Langerhans cells and keratinocytes of oral mucosa. This could reflect a kind of local defense mechanisms involving systemic inflammatory process, which underline the basis of psoriasis. Furthermore the salivary levels of haptoglobin and CRP in 31 patients with inflammatory bowel disease (IBD) under immunosuppressive therapy were also examined. No significant difference in the salivary concentrations as compared with healthy individuals was found (Table 3).

Groups	CRP (mg/l)	Haptoglobin (mg/l)
Healthy subjects (n=31)	$0,105 \pm 0,115$	$15 \pm 12,9$
IBD (n=31)	$0,175 \pm 0,246$	$20,6 \pm 28,8$

Table 3. Saliva level of CRP and haptoglobin in psoriatic patients

This could be partially explained by the lack of oral inflammation or might reflect the treatment-induced control of the disease. In patients with more active disease we observed slight increase of CRP values (slight elevation of Mayo index and CDAI index) (Krasteva, 2009). Published data and our results has driven the conclusion that routine determination of CRP and haptoglobin in saliva in oral diseases as well as in systemic disorders. They could be

used as biomarkers in addition to diagnostic and prognostic value in patients. It would be most useful if they could be determined at the baseline and during the treatment with a regular follow up at first, third, sixth and twelfth month.

There are just a few data for the presence of the other positive acute phase proteins in saliva.

3.2.3 Alpha 2-macroglobulin

Alpha-2-macroglobulin is a large plasma protein produced by the liver. It is able to inactivate an enormous variety of proteinases (including serine-, cysteine-, aspartic- and metalloproteinases).

Alpha 2-macroglobulin was studied in patients with oral diseases (Sengupta, 1988; Pederson, 2005, Rao, 1995). Alpha 2-macroglobulin levels in whole saliva were found between 0 and 4941 ng/ml using ELISA (Pederson, 2005). It has been often studied in patients with gingivitis and periodontitis (Sengupta, 1988; Pederson, 2005, Rao, 1995).and the salivary levels was found even higher in severe disease (Pederson, 2005).

The concentration of alpha 2-macroglobulin in human gingival sulci has been investigated in gingival washings of experimental gingivitis and in crevicular fluid before and after initial periodontal therapy (Condacci, 1982). The alpha 2-M was found elevated in the washings. The absolute amount of alpha 2-M in the crevicular fluid showed a significant decrease and ever reached undetectable levels after treatment (Condacci, 1982).

In opposite of the above studies, Sibraa et al. found in gingival crevicular fluid lower levels of alpha-2-macroglobulin in periodontally diseased than healthy sites ($p < 0.001$) (Sibraa, 1991). Gautam et al. found lower concentrations both in the serum and saliva in phenytoin treated patients with varying grades of gingival enlargement compared to phenytoin-naïve subjects with normal gingiva. The mean serum and salivary concentrations increased after the fading the inflammatory process (Gautam, 2009).

Recently, Chen et al. both alpha-2-macroglobulin and alpha-2-macroglobulin-like protein are essential components of the salivary innate immunity acting like a natural inhibitor against swine origin influenza A virus (Chen, 2010). Additionally, alpha 2-macroglobulin was reported between salivary biomarkers of type-2 diabetes (Rao, 1995).

3.2.4 Alpha 1-antitrypsin (A1AT)

Alpha 1-antitrypsin inhibits a wide variety of proteases. It protects tissues from enzymes of inflammatory cells, especially neutrophil elastase.

There is very few data for alpha 1-antitrypsin in saliva. Alpha 1-antitrypsin was measured in whole saliva between 2-2271 ng/ml by ELISA (Pederson, 1995). In contrast to alpha-2-macroglobulin, A1AT was not found to increase in case with gingivitis (Pederson, 1995). However, similarly to alpha-2-macroglobulin it was lower in phenytoin gingival enlargement compared to normal gingival (Gautam, 2009). Smokers have also low levels of A1AT in crevicular fluid (Persson, 2001).

3.2.5 Alpha-1-acid glycoprotein (A1AG)

Alpha-1-acid glycoprotein or orosomucoid is a 41-43-kDa glycoprotein, one of the major acute phase proteins in humans, rats, mice and other species (Fournier, 2000). A1AG is synthesized primarily in hepatocytes, but mild production was also found in the salivary glands and spleen. Minor expression is detected in all other tissues, including lung, lymph nodes, uterus, ovary, kidney and tongue (Lecchi, 2009). In the setting of established

immunomodulatory and direct antibacterial activity of A1AG, its expression in the salivary glands might be explained with local immunity, even in healthy condition (Lecchi, 2009).

In pre-matured infants the salivary A1AG and albumin concentration were found higher than in controls, due to the increase of the protein transudation. The last is a late consequence of intrauterine malnutrition (Szabó S, 1988).

3.2.6 Fibrinogen

Fibrinogen is a soluble plasma glycoprotein, synthesised by the liver, that is converted by thrombin into fibrin during blood coagulation. It may be elevated in any form of inflammation, it is especially apparent in human gingival tissue during the initial phase of periodontal disease (Page, 1976)

One of the first, but unfortunately unsuccessful attempts for fibrinogen determination in saliva was made in 1965 (Brandtzaeg, 1965). Almost three decades later, Sukharev et al. succeeded to determine salivary lactoferrin and fibrinogen levels in 1205 salivary samples using an agar immunodiffusion test (Sukharev, 1991). Salivary fibrinogen degradation products and lactoferrin detection rates do not depend on the subject's gender but increases with age and intensity of the aggressive admixture effects in inhaled air. These are also related to the intensity of the inflammatory and proliferative processes in the oral cavity and bronchopulmonary system (Sukharev, 1991).

3.2.7 Ferritin

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. In humans, it acts as a buffer against iron deficiency and iron overload.

Inflammation induces the hepatic synthesis of the apo-ferritin, which is released into the circulation, and behaves as an acute phase protein.

Very recent study revealed corresponding salivary and serum ferritin levels in 33 children suffering from protein-energy malnutrition – PEM. The salivary ferritin was measured very low both in serum and saliva in children with PEM even in grade I, as compared to normal children. In PEM grade III the mean ferritin value was reported $3.28 \pm 0.75 \mu\text{g/l}$ versus $169.3 \pm 21.9 \mu\text{g/l}$ in normally nourished children. The salivary ferritin levels in PEM may be used to distinguish the early stage of the disease and to estimate the disease severity (Agarwal, 1984).

3.3 Negative acute phase proteins

3.3.1 Albumin

Albumin is the main protein of plasma; it binds water, cations, fatty acids, hormones, bilirubin and drugs - its main function is to regulate the colloidal osmotic pressure of blood.

Common saliva albumin concentration usually is not found decreased in inflammation and is often used as a marker for the degree of mucositis or inflammation in the oral cavity (Izutsu, 1981). Whole saliva albumin as an indicator of stomatitis in cancer therapy patients. On the contrary, albumin is substantially increased, like lactoferrine, in case of acute inflammation of the salivary glands (Dodds, 2000).

in saliva arises from contamination by either traces of blood or gingival fluids. Normal range of albumin in saliva is still issue of debate. Different concentrations of salivary albumin in healthy adults were reported (Ruhl, 2004 - $S 38.5 \mu\text{g/ml}$ and Vaziri, 2009 - $64.50 \pm 38.40 \mu\text{g/ml}$).

Almost all samples obtained after stimulation of the major salivary glands contain albumin in concentration about or below the detection limit of 18.9 µg/ml (Ruhl, 2004).

Salivary albumin was evaluated in early childhood. High levels of albumin are found in saliva of 63 newborn infants, as early as within the first 10 days of life (Bastian Manfred Seidel) (Selner, 1968).

The median concentrations of serum albumin in whole saliva increased from 1 to 12 µg/ml during the first year of infancy, within an intra-individual range from 10.4 to 105 µg/ml. A shift in median concentrations was observed prior to the eruption of the first teeth. In individual infants, the albumin concentration started to rise about a month before eruption of the first tooth (Ruhl, 2005).

The concentration and the within-child variability of albumin increased with age (from 6 months to 5 years). Between-child variances were greater than the within-child variances by a factor of 1.3 for log (albumin). There were no changes in albumin levels between infection and non-infection periods, suggesting a local immune response rather than serum leakage. (Gleeson, 1991).

There was not any difference in the concentration of salivary albumin depending on pregnancy, as the same levels were measured in pregnant and non-pregnant women (Selby, 1988).

Salivary albumin concentration in subjects with gingivitis or periodontitis is caused by leakage of plasma proteins (Henskens, 1993). The increase in the concentration of albumin in whole saliva was always detected prior to the clinical appearance of stomatitis, suggesting that albumin in whole saliva may be a marker and predictor of this complication. Therefore, the monitoring of salivary albumin can assist in the identification of stomatitis at a pre-clinical stage and enable the chemotherapy dosage to be adjusted or treatment for the stomatitis to be initiated at an early stage (Izutsu, 1981).

Differences between glandular and gingival contributions to the composition of saliva were explored in patients medicated with cyclosporin who exhibited gingival overgrowth (responders), those without gingival overgrowth (non-responders). In responders the albumin level was 88 µg/ml and in non-responders 52 µg/ml (Ruhl, 2004).

Unfortunately, measurement of the salivary albumin failed to predict microvascular impairment in diabetic patients (Fisher, 1991). Twenty years later Vaziri et al. found adults with type 2 diabetes mellitus have higher concentration of salivary albumin (73.47 ± 31.35 µg/ml versus 64.50 ± 38.40 µg/ml in controls). It is discussed it may play a diagnostic role in oral health and disease in diabetic patients (Vaziri, 2009).

Importantly, the saliva albumin and prealbumin concentrations in elderly are correlated with serum ones (albumin: $R^2 = 0.308$, $p = 0.0010$, prealbumin: $R^2 = 0.433$, $p < 0.0001$). Thus salivary determination of these markers might facilitate the evaluation of protein nutritional status in this specific group (Murayama, 1999).

3.3.2 Transcortin

Transcortin, also corticosteroid-binding globulin officially called serpin peptidase inhibitor is an alpha-globulin and binds several steroid hormones: Cortisol, Progesterone, Aldosterone, 11-Deoxycorticosterone.

Early reports failed to detect transcortin in saliva (Riad-Fahmy, 1982), but low transcortin's concentration (about 25 pg/l), was detected some years later in saliva (Hammond, 1986; Chu, 1988). About 15% of salivary cortisol is bound to transcortin, which is a normal

component of uncontaminated parotid saliva. The cortisol in saliva being mainly free, implying that the elevation of the salivary cortisol concentration is not due to higher concentrations of transcortin (Umeda, 1981; Hammond, 1986; Chu, 1988).

One of the first reports regarding detection of salivary transferrin was published by Gugler et al. (Gugler, 1968). The study demonstrated the presence of pre-albumin, albumin, alpha-1-antitrypsin and transferrin in submaxillary saliva. Retinol (pre-albumin) could not be detected in parotid saliva (Gugler, 1968). The salivary transferrin is supposed to be a marker of blood contamination (Schwartz, 2004). This underlies its suggestive use as a biomarker for early detection of oral cancer (Jou, 2010).

It is quite important to know if the blood components are present in the oral mucosa as the quantitative estimates of different salivary tests may be compromised. To provide a quantitative measure of blood contamination in saliva, Schwartz et al. developed an enzyme immunoassay for transferrin, which is usually present in very small amounts (<5 mg/l) in saliva and in high concentration in whole blood (Schwartz, 2004). Transferrin saliva concentration was found below 4.0 mg/l in healthy volunteers. These results suggest that salivary transferrin can serve as a marker for quantification of blood contamination in saliva in the absence of visual evidence of blood contamination (Schwartz, 2004), especially in the absence of oral abnormalities. Further more than three hundreds and sixty children's saliva samples were studied to reveal if blood contamination could influence the measurement of salivary cortisol, testosterone, and dehydroepiandrosterone. Transferrin levels averaged 0.37 mg/dl (3.7 mg/l) that in fact supported previously mentioned results and clearly demonstrated that blood contamination in children saliva samples is quite rare.

Studies, which involved oncology patients, are important as most of them aim to provide new serum or other body fluids biomarkers for early detection of cancer. Very recently Jou et al. studied saliva samples from patients diagnosed with oral cancer patients aiming to identify salivary markers for early cancer detection. In neck squamous cell carcinoma patients the transferrin levels in the saliva were found increased more than 3 times than normal values. Additionally, the increased salivary transferrin levels strongly correlated with the size and stage of the tumor (Jou, 2010). As the saliva biologically is within an immediate surrounding of the developing tumor the detection of Transferrin might be useful. Many experts thus agreed that it is worth to include Transferrin in a panel of biomarkers for therapeutic monitoring and for early detection of HNSCC (Dowling, 2008).

Similarly the iron binding proteins, lactoferrin and transferrin were measured higher in patients with periodontitis, partial dentures and edentulous patients as well as those wearing complete dentures. A probable explanation is their bacteriostatic effect on salivary gram-positive and negative bacteria (Emiko, 2000).

The future

Human saliva proteomics is a novel approach in the search of protein biomarkers for detection of different local and systemic diseases. Currently more than 1400 salivary proteins have been identified (Scarano, 2010). Proteomics is the large-scale study of proteins, particularly their structures and functions. After genomics, proteomics is considered the next step in the study of biological systems. It is much more complicated than genomics mostly because while an organism's genome is more or less constant, the proteome differs from cell to cell and from time to time (Wilkins, 2009; Wasinger, 1995). Understanding the proteome, the structure and function of each protein and the complexities of protein-protein interactions will be critical for developing the most effective diagnostic techniques and

disease treatments in the future. CH Chen using a proteomic approach describes the immune properties of alpha-2-macroglobulin in saliva (Chen, 2010). The emerging proteomic technologies aim to identify biomarkers for head and neck squamous cell carcinoma diagnostics (Drake, 2005).

4. Conclusion

Assess the health in the oral cavity requires specific tests and non-invasive approach to detect and monitor the diseases are preferred. Saliva is a unique clear fluid, composed of electrolytes, immunoglobulins, proteins and enzymes. The basic role of saliva is to assure the integrity of the upper part of the alimentary tract, through: lubrication; buffering action and clearance; maintaining tooth and mucosal integrity; antibacterial and antiviral activity as well as taste and digestion. In the last 10 years, saliva has become the object of various studies. Curiously, there are most of the papers on saliva samples are used for analysing drug abuse individuals as well as detection of various oral and systemic diseases. The most important advantage in collecting saliva samples is that an easy access and non-invasive nature of this approach is permitted. Furthermore, the possibility to measure a wide range of molecules both in saliva and serum allows evaluating microbiological, immunological, hormonal, pharmacological and oncological biomarkers. The FDA defines a biomarker as, "A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". Historically the blood levels of the acute phase proteins are used as biomarkers in physiology and disease. Haptoglobin might serve as a simple example. Its serum levels increases during acute conditions such as infection, injury, tissue destruction, some cancers, burns, surgery, or trauma. Levels are low in some diseases and hemolysis is a classic example of a dramatic decrease. Haptoglobin binds the free hemoglobin released from erythrocytes. Haptoglobin also contributes in the regulation of the host immunity (Huntoon, 2008). There are enough data to support the acute phase proteins determination in saliva. In contrast to the serum levels the negative acute phase proteins are found in higher concentration in saliva in oral diseases. Elevation of albumin in gingivitis and paradontitis as well as those of transferrin in blood contamination has been already discussed above.

However, we still need larger and longitudinal studies to define the normal limits of the salivary concentration of different proteins. Table 4 summarizes the reported levels in healthy subjects. Comparability of the different studies however is still controversial depending on different non standardized methods used.

Determination of the concentrations of the APP in paired serum (plasma) and saliva samples allow easy to calculate "serum-salivary gradient". Table 5 summarizes a proposed relative gradient, calculated from the published serum and saliva upper limits of normal in healthy controls for every of the evaluated proteins. The salivary concentration of the acute phase proteins usually is about 1 - 2% from the plasma (serum) levels. The lower values are difficult to be explained.

Most of the discussed data were obtained from small number of observational studies. We do believe that large, prospective, controlled and longitudinal studies are needed to confirm the range of saliva APP and its correlation with serum levels. This seems to be mandatory before using the saliva APP evaluation routinely in the setting of sepsis, malignancy, inflammation, autoimmune diseases and transplantation.

Protein	Salivary level	Author
Albumin	38.5 µg/ml	Ruhl, 2004
Albumin	64.50 ± 38.40 µg/ml	Vaziri, 2009
Alpha 1-antitrypsin	2-2271 ng/ml	Pederson, 1995
Alpha 2-macroglobulin	0 - 4941 ng/ml	Pederson, 1995
CRP	0 - 472 pg/ml	Pederson, 1995
CRP	0,105 ± 0,115 mg/l	Krasteva, 2009
Ferritin	169.3 ± 21.9 µg/l	Agarwal, 1984
Haptoglobin (in animals)	0.3 - 3.0 µg/ml	Hiss, 2003
Haptoglobin	15 ± 12,9 mg/l	Krasteva, 2009
Transcortin	25 pg/l	Hammond, 1986; Chu, 1988
Transferrin	< 5 mg/l	Schwartz, 2004

Table 4. Salivary level of acute phase proteins in controls

Parameter	Saliva level (%)	Author
Alpha-1 antitrypsin	0,2	Gautam, 2009
Alpha-2 macroglobulin	1	Gautam, 2009
Haptoglobin	1,7	Krasteva, 2009
Transcortin	0,1	Hammond, 1986; Chu, 1988
Transferrin	0,5	Schwartz, 2004
CRP	1,2	Krasteva, 2009

Table 5. Saliva/serum concentration ratio of acute phase protein in controls

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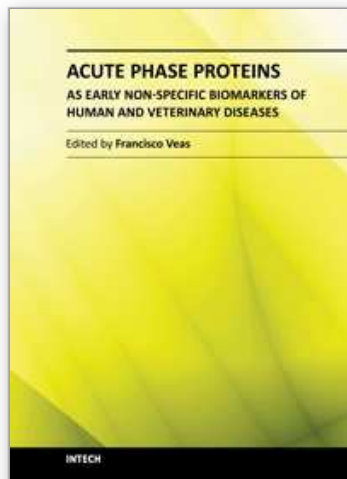
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Edited by Prof. Francisco Veas

ISBN 978-953-307-873-1

Hard cover, 408 pages

Publisher InTech

Published online 10, October, 2011

Published in print edition October, 2011

The two volumes of Acute Phase Proteins book consist of chapters that give a large panel of fundamental and applied knowledge on one of the major elements of the inflammatory process during the acute phase response, i.e., the acute phase proteins expression and functions that regulate homeostasis. We have organized this book in two volumes - the first volume, mainly containing chapters on structure, biology and functions of APP, the second volume discussing different uses of APP as diagnostic tools in human and veterinary medicine.

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Assya Krasteva and Angelina Kisselova (2011). Salivary Acute Phase Proteins as Biomarker in Oral and Systemic Disease, Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases, Prof. Francisco Veas (Ed.), ISBN: 978-953-307-873-1, InTech, Available from:
<http://www.intechopen.com/books/acute-phase-proteins-as-early-non-specific-biomarkers-of-human-and-veterinary-diseases/salivary-acute-phase-proteins-as-biomarker-in-oral-and-systemic-disease>

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