Saliva: functions, composition and chemistry

1-3 Saliva

1-3-1 Secretion and function;

The components of saliva essentialy are produced by acinar cells. Saliva is the main product of the salivary glands. It is an acidic, clear, slightly mucoserous exocrine fluid forming a complex mixture of secretions from major and minor salivary glands and gingival crevicular fluid(Humphrey and Williamson, 2001; Kaufman and Lamster, 2002). This mixture of fluids derived from different salivary glands is termed ‘‘ whole saliva’’, while the fluid which is secreted by single glands is called ‘‘ duct saliva’’(Edgar, 1992).

The constant flow of saliva from the mouth into the gut has a protective action. This flushing effect push, for example, food debris and exogenous and possibly noxious substances into the gut(Tenovuo, 1998).

Saliva is an organic fluid that can indicate local and systemic alterations, such that the components of saliva can be related to the immunologic, hormonal, neurologic, metabolic and nutritional state of the individual(Carlson, 2000).

Saliva is derived from many types of salivary glands. Each type of salivary gland secretes saliva with composition , characteristics and properties. The secretions from these different glands have been shown to be different considerably, to be affected by different forms of stimulation and to be complex in composition, time of day, age, diet , gender, several pharmacological agents and a variety of disease states(Forde *et al.,* 2006; Wong, 2007).

1-3-2 Salivary Composition

Saliva is a clear, slightly acidic (pH 6-7) liquid; it consist of:

Inorganic components

The highest and abundant component in saliva is water (approximately 99%), followed by ions $H_2PO_4^-$, F^-, Na^+, Cl^- Ca^{2+}, K^+, HCO_3^-, I^-, Mg^{2+}, thiocyanate. The ionic composition of saliva is different from the plasma that derived from it.(Humphrey and Williamson, 2001). The hypotonicity hydrates various organic compounds that form a protective coating on the oral mucosa and facilitates taste sensitivity. Resultant bicarbonate act as a buffering agent, also calcium and phosphate neutralize acids that would otherwise inhibit tooth mineral integrity(Humphrey and Williamson, 2001; Van Nieuw Amerongen *et al.,* 2004).

Organic components

Saliva includes a huge number of organic compounds such as: glucose, cholesterol, urea, uric acid, , fatty acids, mono–, di–, and triglycerides, phosphor and neutral lipids, steroid hormones, glycolipids, amino acids, ammonia and proteins that aid in the protection of oral cavity tissues, including mucins, amylases, agglutinins, glycoproteins, lysozymes, peroxidases, lactoferrin and secretory IgA. It also includes of non-immune factors include cystatins, mucin G1 and G2, lactoferrin, lysozyme, defensins, myeloperoxidase and histatins,(Hicks *et al.,* 2004; Kavanagh and Dowd, 2004; De Smet and Contreras, 2005; Dodds *et al.,* 2005).

In addition, these macromolecules form tooth enamel pellicle and a viscoelastic mucosal coat and and cleanse and aggregate debris and bacteria from the oral cavity(Heramia, 2002). Saliva contains growth factors and a variety of antimicrobial constituents(Shugars and Wahl, 1998). There is a strong relationship between functions-constituents of saliva and a number of salivary proteins participate in more than one function. Function of saliva are: mastication, digestion, deglutition, defense (spiting and oxidative stress), protective (antifungal, antibacterial, antiviral activity, lubricant and buffering agent), drug testing, water balance, excretion, chemical communication (kissing or infant salivating) , speaking, denture retention, tasting,(Greabu, 2001; Battino *et al.,* 2002; Pesce and Spitalnik, 2007; Wong, 2007; Zimmermenn *et al.,* 2007). There is a fact that saliva has been used as diagnostic fluid for more than two thousand years. Ancient doctors of traditional Chinese medicine have suggested that blood and saliva are ‘ brothers’ in the body and they come from the same origin. It is believed that salivary changes are indicative of the wellness of the patient.

Saliva offers some distinctive advantages(Tabak, 2001; Kaufman and Lamster, 2002; Forde *et al.,* 2006; Koka *et al.,* 2006; Pesce and Spitalnik, 2007). Smaller sample aliquots, the possibility of a dynamic study, stress free, greater sensitivity, easy collection procedure and non- invasive, a good cooperation with patients, the possibility to collection somewhere and anywhere, not a trained technician and no special equipment are needed for collection, correlation with levels in blood, potentially valuable for older adults and children , could eliminate the potential risk of contracting infectious disease for both a technician and the patient, more accurate than

blood for detection of many oral and systemic diseases, may provide a cost-effective approach for the screening of large populations.

Advances in the use of saliva as a diagnostic fluid have been affected by current technological developments: enzyme-linked fluorescence technique, Western blot assays, polymerase chain reaction (PCR).

Saliva is one of the most important host factors that play a role in prevention of the caries process through its inorganic and organic constituents, besides the physiological functions. The continuous flow of saliva through the mouth removes cariogenic challenges and bathes the dentition with remineralizing ions(Edgar *et al.,* 2005; Fejerskov and Kidd, 2008; Guy, 2012). For that reason saliva plays an important role in the equilibrium between the demineralization and the remineralization of enamel(Choi, 2010).

1-3-3 Diagnostic uses of saliva

Saliva is critical for maintaining and preserving the health of oral tissues and has been used to eliminate of many drugs and as a source of non-invasive investigation of metabolism . However, it receives little attention until its quantity diminishes or its quality becomes altered(Axelsson, 2000; Humphrey and Williamson, 2001; Tabak *,* 2001; Malamud, 2006). At present, saliva represents an increasingly useful auxiliary means of diagnosis(Malamud, 2006). Many researchers have made use of sialochemistry and sialometry to monitoring general health, diagnose systemic illnesses and as an indicator of risk for diseases creating a close relation between systemic and oral health(González and Sánches, 2003).

Recently , saliva has gained attention as an important diagnostic fluid just as for blood and urine. It is now obviuos that saliva contains the same of many biomolecules that are commonly measured in other body fluids. As an example, approximately, 30% of the proteins which found in saliva are also found in blood improving the diagnostic potential of saliva. Saliva tests give us an advantages and preferable in comparison to blood tests due to simple collection being inexpensiv, noninvasive, simple, and with minimal risk of contracting infectious organisms such as HCV, HPV and HIV by the healthcare professional. In addition saliva is an ideal biofluid for children because of no compliances(Pfaffe *et al.,* 2011).

Saliva is a plasma ultra filtrate and contains proteins either derived from blood or synthesized in situ in the salivary glands. It contains biomarkers derived from gingival crevicular fluid, mucosal transudate and serum,. Saliva is produced in the acinar cells and acinar cells are connected to the vasculature which enables molecular transportation from blood into saliva. Salivary components may originate entirely from the salivary glands or may be derived from the blood by active transport or passive diffusion(Aps and Martens, 2005). To date, researchers have identified 2, 340 proteins in saliva(Bandhakavi *et al.,* 2009; Schulz and Cooper, 2012). Saliva resembles the plasma proteome in amanner that proteome has a large dynamic range and it is essential to suppress this dynamic range in order to enable low abundant proteins of diagnostic potential.

1-4 Smoking and changes in saliva that can be used diagnostically:

It has been known for long time that thiocyanate level are increased in the saliva of smokers and still used as a biomarker of smoking activity(tsuge *et al.,* 2000). Because of thiocyanate is a product present in tobacco smoke *,* it is also measures the exposure to passive smoking(Ferguson, 1998).

Determining the concentration of nicotine and cotinine in biological fluids is widely used in both clinical and epidemiological smoking studies(Hatsukami *et al.,* 2003). Both nicotine and cotinine concentrations are used to measure tobacco consumption, to validate abstinence in smoking cessation programmes and to determine exposure to environmental smoke(Hatsukami *et al.,* 2003). Nicotine, when smoked in cigarettes is absorbed across nasal and buccal membranes. The drug has a rapid onset of action with a half-life of 2 h and can be detected in saliva, urine and blood(Hatsukami *et al.,* 2003).

As nicotine is present mainly in the non-ionised form in alkaline pH and it is a weak base (pKa of 8. 0), and hence more easily absorbed with increased levels of pH(Ciolino *et al.,* 2001). Thus, salivary pH changes will affect the amount of nicotine that is absorbed across the buccal mucosa(Zevin *et al.,* 1998). Cotinine, the main metabolite of nicotine, is used widely for estimating exposure to nicotine. This pharmacologically inactive compound has a half-life of 20 h (15 – 40 h), is slowly cleared from the body and it is important to know that Cotinine is specific to tobacco(Hatsukami *et al.,* 2003; Patterson *et al.,* 2003). Urinary levels of cotinine have been shown to be quite variable, because of the differences in nicotine metabolism among individuals(Yang *et al.,* 2001).

Generally, cotinine levels depend of degree of exposure to ETS. increased cotinine levels were predictors of an Increased number of cigarettes smoked at home(Mannino *et al.,* 2001). The study in adolescents in Tenerife established correlation between the degree of ETS exposure and cotinine levels in saliva . The highest values noticed in active smokers who smoked at least more than ten cigarettes daily, was 341. 1 ng/mL. The smokers who smoked fewer number cigarettes, average value of cotinine was 142. 7 ng/mL, while in passive smokers it was 4. 2 ng/mL. In the same study, it is established that persistent cough , bronhospasm as well as infections of lower respiratory tract, are more frequent in adolescents with higher concentrations of cotinine(Suarez, 2001).

1-4-1 Amylases:

Salivary α-amylase is the first enzyme in the gastrointestinal tract for extracting caloric value from food. However, beyond the primary role of α-amylase to begin digestion of sugars, carbohydrates and complex starches, *.* salivary α-amylase is known be a important marker of stress *.* It has also been found that salivary α-amylase may be influenced by psychological and behavioral factors and processes(Kivlighan, 2006).

Human salivary amylase hydrolyses ct-I- 4 glycoside bonds in starch *,* yelding maltotriose, maltose, glucose and dextrins as final products. In spite of and having similar composition and immunological activity and playing the same role as pancreatic amylase(Liang *et al.,* 1999), these enzymes have different molecular weights, catalytic properties and isoelectric points,(Liang *et al.,* 1999). Salivary amylase exists in two families: family A is glycosylated

while family B is nonglycosylated . At least six izoenzymes have been recognized(Liang *et al.,* 1999).

Although playing an important role in the initial digestion of starch(Tseng et at., 1999), the importance of salivary amylase in digestion has been shown to be minor compared to pancreatic, as people who lack it jul to show any digestive perturbations. However, salivary amylase has many important intra-oral functions such as participation in ACDP, modulation of intra-oral microflora and affimity for hydroxyapatite,(Scannapieco *et al.,* 1995; Gong *et al.,* 2000). The catalytic activity of salivary amylase also participate in degradation of sticky starch rich foods which are retained in dental surfaces and theft transformation in slow glucose releasing devices which may play quite a role in dental caries pathogenesis(Tseng *et al.,* 1999).

It has been suggested that amylase represent between 40 to 50% of the total protein produced by salivary gland, most of the enzyme being synthesized in the parotid gland(Noble, 2000). Human submandibular saliva and parotid saliva contain about 45 mg and 30 mg of amylase, respectively, per 100 mg of protein However, it has also been suggested that amylase makes up about 1/3 of the total protein content in parotid saliva, and the content would be lower in whole saliva(Pedersen *et al.,* 2002). The concentration of amylase increases with the increase of salivary flow rate, and it is generally considered to be a reliable marker of serous cell function(Almståhl *et al.,* 2001).

Amylase is also present in human acquired pellicle in vivo(Yao *et al.,* 2001). Fasting has been found to decrease whole saliva amylase levels and

activity(Mäkinen, 1989). The amylase concentrations has been found to be reduced in radiation-induced hyposalivation(Almståhl *et al.,* 2001).

During chewing, some starch is hydrolyzed into dextrins and glucose by salivary α-amylase but the degree of hydrolysis ranges considerably (1 to 27%) depending on the type of food(Woolnough *et al.,* 2010). variation in human salivary α-amylase activity has been reported, with values ranging between 50 and 400 U. mL-1 60(Kivela *et al.,* 1997; Mandel *et al.,* 2010). An indirect measure of α-amylase activity, which is particularly relevant to food application(Gonzalez *et al.,* 2002), can be obtained by measuring the decrease in viscosity of starch pastes with the addition of α-amylase(Collado & Corke, 1999). This assay has been used to study the relationship between sensory analysis of starch thickness perception, α-amylase activity, starch paste and mechanical properties(Evans *et al.,* 1986; de Wijk *et al.,* 2004; Mandel *et al.,* 2010).

Furthermore, the effect of decreased starch viscosity (due to α-amylase activity) affects saltiness perception(Ferry *et al.,* 2006)and aroma release(Ferry *et al.,* 2004; Tietz *et al.,* 2008).

Amylomaltase-treated starches were found to be particularly good fat substitutes in yoghurts and a loss of instrumentally-measured firmness thats because α-amylase was reported in those systems(Alting *et al.,* 2009). It is therefore accepted that α-amylase has a significant 70 impact on a number of critical starch attributes during eating(Engelen & Van Der Bilt, 2008), thickness perception being the main one. In literature reviews, there appeared to be a great variation in sensory analysis of thickness perception

for the same starch-thickened food system which could be due to the natural variation of α-amylase activity between donors. Recently, α-amylase concentration variations in saliva has been linked to genetic differences(Mandel *et al.,* 2010)and this was suggested as an explanation for the natural variation observed in thickness perception of starch-thickene systems.

Moreover, sAA levels are influenced by numerous factors which may lead to variability among individual, thus again undermining the accuracy of sAA as a biomarker for fatigue. For instance, studies have shown that cigarette smoking decreases basal α-amylase activity in saliva and that people who chronically drink alcohol have decreased levels of amylase(Rohleder and Nater *,* 2009).

Activity of amylase was decreased in passive smokers compared to healthy group(Rezaei and Sariri 2011). Similar results have been reported by Granger et al who found lower salivary amylase activity for mothers, not for infants as a result of tobacco smoking exposure(Granger *et al.,* 2007). The results showed also a decrease in salivary amylase smokers as compared to non-smokers were recorded by(Sariri *et al.,* 2008). It was explained that inhibition of salivary amylase by cigarette smoke may be caused by the interaction between SH groups of the enzyme moleculesand smoke aldehydes. Moreover, the percentage of the enzymatic inhibition showed a negative correlation with the basal level of salivary reduced gluthation (GSH). Regular exposure of passive smokers to cigarette smoke may accumulate in their saliva a smoke aldehydes leading to their interaction with –SH group of amylase.

Another study by Greabu et al. Concluded that exposure to cigarette smoke caused a significant decrease in salivary uric acid and amylase.(Greabu *et al.,* 2007).

1-4-2 Proteins:

Human whole saliva has a protein content of about 0. 5 to 3 mg/mL, and parotid saliva has a protein content of about 0. 4 to 4 mg/mL, while sublingual and submandiblar saliva of about 0. 6 to 1. 5 mg/mL. The protein concentration is independent from the flow rateand is rather stable, Besides maintaining buffer capacity and osmolarity, salivary proteins are also involved in several specific functions. The number of distinct salivary proteins is roughly between 100 and 140(Wilmarth *et al.,* 2004; Yao *et al.,* 2003), from which 30. 40 % are produced by the salivary glands, whereas other proteins are originated from serum, from mucosal and/or immune cells, or from microorganisms(Wilmarth *et al.,* 2004). The most important proteins of glandular origin are alpha-amylase, glycoproteins with blood-group substances, cystatins, epidermal growth factor (EGF), gustin, histatins (HRPs), lactoferrine, lysozyme, mucins *,* salivary peroxi dase, proline-rich proteins (PRPs) and statherin. The most important serum derived proteins are albumin, alpha1-antitrypsin, blood-clotting factors (VIII; IXa; XI) and members of the fibri- nolytic system (proactivators, traces of plasminogen activator). Most important proteins that originate from immune cells are myeloperoxidase, calprotectin (Ca2+ binding L1 leukocyte pro- tein), cathepsin G, defensins, elastase, immunoglobulins (90% to 98% sIgA, 1% to 10% IgG, a few IgM, IgD, IgE). Finally, the most important protein constituents of microbial (unknown) or mixed origin are fibronectin, alpha2-

macroglobulin, , DNases, RNases, kallikrein, streptococcal inhibitor, secretory leukocyte protease inhibitor (SLPI), , molecular chaperone (Hsp70), and cystein peptidases. (Data are summarized in Table 1-2).

The most important proteins involved in oral ecosystem maintenance are, lysozyme, agglutinins and histidine , lactoferrin, peroxidases, proline-rich proteins, as well as secretory immunoglobulin A and immunoglobulins G and M(Liébana *et al.,* 2002), Moreover, saliva contains a many types of proteins and some of them might have protective properties. Additionaly, proteins can protect the tooth structure by the formation of a salivary pellicle when tooth are exposed to saliva(Siqueira *et al.,* 2007). This pellicle may act as a barrier for acids(Dawes, 2008). In hyposalivation, caries process and erosive wear are phenomena that occur simultaneously(Lajer *et al.,* 2009).

With respect to the development of caries it was proposed that the salivary pellicle derived from whole saliva has a preventive role(Featherstone *et al.,* 1993). Concentration of salivary total protein did not show considerable variation in passive smokers compared to control(Rezaei and Sariri2011). A similar result was obtained for salivary protein concentration in school children with smoker parents(Granger, 2007).