SALIVA

Saliva is not one of the popular body fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears. Despite the absence of charisma, however a growing number of pediatricians, pharmacologists, clinical and forensic pathologists, psychologists and dentists are finding that saliva provides an easily available, noninvasive diagnostic medium for a rapidly widening range of diseases and clinical situations (1,2).

Saliva is used to describe the combined fluids present in the mouth. However in its strict sense this word refers only to the hypotonic, watery fluid secreted by the major and minor salivary glands. Expressions like “whole saliva”, “mixed saliva” and oral fluids are used for scientific purposes to represent the combined fluids of the mouth. Indeed, whole saliva is a mixture of pure glandular saliva, gingival crevicular fluid, oral epithelial cells, microorganisms and food remnants.

Saliva is not only a pleasant lubricant which makes oral functions such as speech, mastication and swallowing easier, but also a fluid with many important functions in the maintenance of oral and general health. Although saliva is not considered as essential fluid for life in man, it protects the human body and in particular oral tissues, against numerous noxious and harmful agents. Some systemic diseases and hormonal changes can alter the flow and composition of saliva, so that in many cases saliva analysis have diagnostic value.

Compared to blood and urine, saliva is easy to collect, which makes frequent monitoring of excreted substances possible. Furthermore, the amount detectable in saliva has been interpreted to represent the biologically active fraction of a particular compound.

Whole saliva is a complex mixture of parotid, submandibular, sublingual and minor salivary gland secretions mixed with bacteria, leukocytes, sloughed
epithelial cells and crevicular fluid. The use of different stimulants of saliva secretion produces samples, where the secretion from the major salivary glands occur in different proportions. The concentrations of most salivary constituents depend on the flow rate of saliva. Therefore to obtain meaningful results, the collection of saliva needs to be standardized.

As a result of bacterial action, the composition of saliva will change on standing. The saliva should be collected on ice to arrest the bacterial metabolism. The bacterial action can also be stopped by centrifugation of saliva. Centrifugation removes both the cells and the turbidity, which can interfere with many analytical techniques.

Human whole saliva contains a large number of enzymes derived from the salivary glands, oral microorganisms, crevicular fluid, epithelial cells and other sources. Ingestion of food affects the activity of these enzymes and certain foods themselves contain enzymes which temporarily contribute to the overall enzymatic activity of whole saliva. The activity of whole saliva enzymes is also affected by drugs, smoking, state of oral health and oral hygiene, the chemical nature of dental therapeutics used, the general health of the subjects and other factors. The activity and concentrations of salivary enzymes are further affected by the type of stimulus used in salivary collection.

Both whole saliva and pure glandular secretions have their advantages and disadvantages in sialoenzymological studies. Whole saliva is easy to collect and may be the only type of saliva which can be obtained from certain patients. However, whole saliva samples have to be adequately centrifuged before most enzyme analysis and a standardized procedure should be established for centrifugation as well. The whole saliva samples of some subjects remain turbid after centrifugation and this fact may also affect enzyme determinations.

Sialochemical enzyme analysis for diagnostic purposes is still in its infancy. It is indisputably a growing area which will benefit both dentistry and
The enzymes of human saliva have been studied for decades with respect to origin, purification, characterization, distribution among individuals and relationship to pathological conditions of the oral cavity (3). It is known that human saliva contains a large number of glycosidases, esterases, proteases and various other enzymes (4). It is generally agreed that enzymes in saliva originate from oral microorganisms, salivary glands, serum-like gingival crevicular fluid, polymorphonuclear leukocytes, epithelial cells and dietary constituents (5). However, the extent to which each source contribute to the total amount of salivary enzymes is not clear. The relationship between most salivary enzymes and periodontal disease is also unclear.

A number of authors have demonstrated the presence of proteolytic enzymes in saliva and dental plaque (6-10).

Nakamura and Slots in 1983 (11) made the observation that a large portion of salivary enzymes originate from oral microorganisms. In contrast to parotid saliva, serum and polymorphonuclear leukocytes, mixed whole saliva revealed trypsin and hydroxyproline arylamidase activities. These enzymes have been demonstrated in Bacteroides, Capnocytophaga and other dental plaque microorganisms. Furthermore, the higher salivary enzymatic activity of periodontitis patients compared to that of healthy individuals may partly be the result of marked accumulation of bacterial dental plaque in periodontitis patients. Traces of aminopeptidase activity was detected in mixed whole saliva and difference in hydrolytic activity was also found between serum and whole saliva. These findings suggest that whole saliva contains only small quantities of serum enzymes which may imply that the serum like gingival crevicular fluid becomes markedly diluted by saliva when entering the oral cavity.

A large number of salivary enzymes exhibited significantly higher activity in periodontitis patients than in healthy individuals. This suggests that the quantitative activities of certain salivary enzymes may distinguish varying
periodontal conditions. Also an appreciable trypsin-like activity was found only in whole saliva from periodontally diseased patients. This enzyme is produced by Bacteroides gingivalis, Spirochetes and Capnocytophaga (12, 13) organisms which occur in high numbers in periodontal lesions (14), but not by most organisms with suspected low periodontopathic potential. Additional studies are necessary to further delineate the specific salivary enzyme activity, which may be useful in establishing the degree of disease of the periodontal tissues.

Watanabe and his co-workers (15) made the observation that there is a significant correlation between protease activities and the number of epithelial cells, but not leukocytes. It was also found that oral prophylaxis reduced the number of epithelial cells, leukocytes and the protease activities in the whole saliva. However, experimental evidence does not deny that the activity could be related to the amount of leukocyte debris present in whole saliva. Roeste (16) found that the migratory rate of leukocytes is higher in the mouth with inflammation than in healthy mouth.

According to Tzamourani and others (17) the oral prophylaxis, tooth brushing instruction and ultrasonic scaling, reduced the number of leukocytes, epithelial cells and protease activity in saliva. The reduction of leukocyte count can be explained by assuming that the oral prophylaxis reduced gingival fluid.

GINGIVAL CREVICULAR FLUID (GCF)

After some initial studies on proteases in saliva sample, most studies have utilized sulcular fluid as the indicator material for periodontal health.

GCF is an inflammatory exudate (not a continuous transudate). In a strictly normal gingiva, little or no fluid can be collected (18, 19). The components of sulcular fluid can be characterized according to individual proteins (20), specific antibodies, antigens (21) and enzymes of several specificities (23). So far more than 40 compounds found in GCF have been analyzed, but their origin is not
known with certainty (23). These compounds can be host-derived or produced by the bacteria in the gingival crevice. The majority of GCF elements detected so far have been enzymes.

Cellular elements found in the gingival fluid include bacteria, desquamated epithelial cells and leukocytes (PMNs, lymphocytes and monocytes) which migrate through the sulcular epithelium (24).

The total protein content of gingival fluid is much less than that of serum. No significant correlation has been found between the concentration of proteins in the gingival fluid and the severity of gingivitis, pocket depth or extent of bone loss (25).

Monitoring periodontal disease is a complicated task, because there are very few noninvasive procedures that can follow the initiation and progress of the disease. Analysis of GCF constituents in health and disease may be extremely useful because of GCF’s simplicity. But it is clear that site-specific periodontal disease tests involving sulcular fluid samples will be restricted mainly for use by specialist periodontists. Volume of sulcular fluid (sample volume) is also very less and most of the time it will be contaminated with blood or saliva. It is very difficult to collect GCF from normals.

SALIVARY PROTEINS

Proteins present in whole saliva are derived mainly from the parotid, submandibular, sublingual and minor salivary gland secretions. Small amounts of proteins originate from oral microorganisms, crevicular fluid, epithelial cells, polymorphonuclear leukocytes and dietary constituents.

The total protein of human saliva average around 200 mg/100 ml, only about 3% of the protein concentration in plasma. The protein concentration in parotid saliva is higher than in submandibular saliva and results depended on the
method of analysis (26). The flow rate of saliva influenced both the total protein concentration and as well as the proportions of the protein secreted (27).

The effects of stimulation and prolonged stimulation on the relative proportions of various salivary gland proteins were dependent on the origin of the proteins. The proportional contribution of acinar proteins showed an increase when the flow rate is increased above the unstimulated level, but was thereafter relatively independent of the flow rate. Proteins secreted by the plasma cells or duct cells (lysozyme) behaved differently (28).

Intensity of stimulation also affected the levels of salivary proteins (29). The effect of circadian rhythm on the salivary protein concentration had to be taken into consideration if sequential samples were to be collected (30).

The proteins include enzymes, immunoglobulins and other antibacterial factors, mucous glycoproteins (mucins), traces of albumin and certain polypeptides and oligopeptides of importance in oral health (27).

Methods of estimation of salivary protein

Standard methods for serum proteins based on the presence of aromatic amino acids, like the method employing Folin phenol reagent by Lowry et al were widely used for saliva.

More than half of the salivary proteins however lacked aromatic amino acids and the acidic and basic proline-rich proteins as well as the proline rich glycoproteins contained very little aromatic amino acids (31). Thus the method of Lowry as well as the determination of absorbance at 280 nm were not very suitable techniques for the measurement of the protein content of the saliva.

The standard biuret method also used for protein determination was based on the reaction of the peptide bond and could be recommended for saliva. However, it has certain drawbacks like its low sensitivity and the relatively large
sample consumption and interfering factors (32). Protein dye binding methods developed for serum had also been used for saliva (11), like the most common method introduced by Bradford (33) employing the coomassie brilliant blue. This reagent reacts poorly with proline-rich proteins and thus underestimates the concentration of total salivary proteins.

Smith et al (34), devised the BCA method for measurement of proteins. The reagent bicinchoninic acid sodium salt is a stable water soluble compound capable of forming an intense purple complex with cuprous ion in an alkaline environment. This reagent formed the basis of an analytical method capable of monitoring cuprous ion produced in the reaction of the protein with alkaline cupric ion (Cu²⁺). The colour produced was stable and increased in a proportional fashion over a broad range of increasing protein concentrations.

The bicinchoninate reagent has got a greater tolerance towards commonly encountered interferences such as nonionic detergents and simple buffer salts. Thus, the BCA method maintains high sensitivity and is a superior alternative to that of Lowry's and Biuret methods.

Though detergents does not interfere with the BCA method, common metabolites such as glucose, ammonium ion, acetate and phosphate does interfere with the BCA assay but only in high concentrations.

SALIVARY PROTEASES

Connective tissue degradation is a feature of inflammatory periodontal disease (35). Longitudinal studies of untreated chronic periodontitis indicate that it is a site-specific condition which may process episodically (36).

Collagen degradation by host enzymes is a multistage process involving mammalian collagenase and other proteinases. Collagenase is released as a proenzyme which has to be activated by the removal of a portion of the molecule by the other enzymes (37). This function can be carried out by a number of serine
proteinases (38), including neutrophil elastase (39, 40), mast cell tryptase and chymase (41, 42). Mammalian collagenase is unable to cleave the triple helix of collagen until the terminal peptide region, which contains the intramolecular cross-links, is first cleaved by other proteinases (43) including the serine proteinases, leukocyte elastase and cathepsin G (44).

Leukocyte elastase and cathepsin G can also degrade the protein moiety of proteoglycans releasing the glycosaminoglycan side-chain (44). Elastase can also degrade immunoglobulins (45). Tryptase can cleave the third component yielding C3a anaphylatoxin and may thus increase inflammation (46). Mast cell chymase can attack the basal lamina at the epidermal dermal junction (47). This occurs at the lamina lucida and could increase the permeability of the periodontal pocket epithelium. Thus, serine proteinases have the potential to play a major role in progression of chronic periodontitis.

Collagenase

The property of an enzyme which permits it to be defined as a collagenase has been its ability to degrade collagen in the undenatured state at physiological pH and temperature. Vertebrate type collagenase was found to be present in relatively high quantities in whole saliva but not at all in submandibular, sublingual or parotid fluids. In the human body two types of interstitial collagenases are produced. The fibroblast type, which is also synthesized by other connective tissue cells, macrophages and epithelial cells, differs in some respect from the collagenase that is produced by polymorphonuclear leukocytes (PMN) (48). The properties of the salivary collagenase closely resembles that of the PMN type. Its molecular weight is about 70000. The enzyme can be effectively activated by gold thioglucose and it degrades type I and type II collagen faster than type III collagen. These results point to the conclusion that PMNs are probably the main source of the salivary collagenase. Each ml of whole saliva contains 100 000 to 500 000 PMNs, that are in different stages of degeneration.
Most of the salivary collagenase existed in the soluble fraction released from the cells. The rest of the enzyme was either bound to cells or some other insoluble salivary constituents. Most of the leukocytes enter the oral cavity through the gingival sulcus (50). Consequently, the saliva of edentulous subjects contained very little collagenase, markedly less than saliva of subjects with a full dentition and with healthy periodontium. The salivary collagenase appears to resemble closely the sulcular fluid collagenase. In health, most of the enzyme exists in a latent form. In periodontal diseases both fluids contain increased amount of collagenase and it is mostly active (51). Collagenases of both saliva and sulcular fluid undergo rapid changes following collection. During a short period of time, activation of latent collagenase takes place. This is probably due to the presence of several neutral proteases in saliva (52). Subsequently the active collagenase undergo relatively rapid inhibition. This inactivation may be due to denaturation of the enzyme protein or to the presence in saliva of enzyme inhibitors such as tissue inhibitor of metalloproteinases (TIMP). In periodontal disease, however, the amount of TIMP in saliva may be too low to inhibit all the salivary collagenase (53). The substances like methylmercaptan and hydrogen sulphide present in saliva may activate latent collagenase. The levels of these sulfur compounds are increased in the mouth of periodontitis patients (54). In addition, oxygen derived free radicals, especially hydroxyl radicals, have been found to activate PMN collagenase, possibly through reaction with sulfhydryl groups of the enzyme protein (55). Further, myeloperoxidase dependent generation of hypochlorite by PMN has been found to both potentiate the autoactivation of PMN collagenase and to inhibit the active enzyme (56).

Collagenase activity was significantly decreased in saliva following treatment of periodontal disease. The mean activity of the treated subjects was still higher than that of controls. Activity of some peptidases and glycosidases has also been reported to be decreased following periodontal therapy (57). It is speculated that the decrease was due to the reduction in the number of bacteria associated
with periodontal disease. Iijima et al also found higher salivary collagenase levels in patients with periodontal disease than in subjects with healthy periodontium (58). In addition, they also found a significant correlation between gingival sulcular fluid volume and salivary collagenase. Villela et al studied the collagenolytic activity in crevicular fluid from patients with chronic adult periodontitis, localized juvenile periodontitis and gingivitis and from healthy control subjects (59). They found that among patients, collagenase activity increased with the severity of the disease.

Sorsa et al in 1994 characterized the eventual presence and molecular forms of gelatinase/type IV collagenase activity in gingival crevicular fluid and saliva in different forms of periodontitis (60).

To assess the temporal relationship between periodontal tissue destruction and the activity of collagenase, Altken et al (61) collected exudate from inflamed periodontal tissues and latent and active collagenase activity were measured. They found that active collagenase was 5-6 fold higher in the group with progressive loss of connective tissue compared to the groups with either inflamed tissues alone or with inflammation and previous bone loss.

Teng, Sodek, Mc Culloch (62) estimated the gelatinase activity in GCF in different periodontal stages like, maintained periodontitis, rapidly progressive periodontitis and gingivitis. They detected active gelatinase in maintained periodontitis samples about 97.8%, 86.4% in rapidly progressive periodontitis samples, but only 11.4% in gingivitis samples. In addition, the mean active gelatinase activity was found to be significantly higher in rapidly progressive periodontitis than the maintained periodontitis, both of which were higher than the gingivitis group.

Collagenase is an important factor in the pathophysiology of periodontal diseases, since its amount in gingival sulcular fluid and saliva appears to reflect the disease status measurement to distinguish between periodontal health and
disease. Unfortunately, however at present no methods exist that would be sufficiently specific and sensitive and yet at the same time practical in clinical situations. The development of such an assay would probably be an important addition to the diagnosis of periodontal diseases.

**Leukocyte Elastase**

It was known that human neutrophil leukocytes are rich in neutral proteinase activity, this is now known to be largely due to elastase and cathepsin G.

Much of the current interest in the enzyme systems came from the work of Janoff and co-workers (63). They first recognised the enzyme as an elastase and then provided evidence suggesting that it might be involved in a number of important disease states, including pulmonary emphysema associated with a hereditary deficiency of $\alpha_1$-proteinase inhibitor. Leukocyte elastase is one of the major proteins of the azurophil granules of human neutrophils (64).

The molecular weight of leukocyte elastase is close to 30,000. Leukocyte elastase is a serine proteinase that shows maximal activity against most of its substrates in the region of pH 8.5. The enzyme differs from porcine pancreatic elastase in showing a preference for accommodating a valyl rather than an alanyl residue in specificity subsite $S_1$ when acting on synthetic substrates (65). Among the proteins degraded by the enzyme are such important structural proteins as elastin (66), cartilage proteoglycan (67), collagens of type I, II (67), III (68), IV (69) and fibronectin (70).

Reversible inhibitors include some long-chain fatty acids (71), polysaccharide sulfates (72), elastatinal (73), and elasnin (74). Phenylmethyl sulfonyl fluoride inactivates leukocyte elastase eight times more efficiently than it does pancreatic elastase, but a far better inhibitor is Meo - Suc Ala - Ala - Pro - Val-CH$_2$Cl.
Protein inhibitors of leukocyte elastase include kunitz soya bean inhibitor, turkey ovomucoid, inhibitors from leeches, bronchial mucus and the plasma proteins, α₁-proteinase inhibitor and α₂-macroglobulin.

Numerous biochemical constituents of gingival fluid and saliva (e.g. proteases or enzymes released during cell lysis) have been evaluated as markers of periodontal destruction. One logical candidate is elastase, a serine protease that plays a significant role in connective tissue destruction associated with inflammatory process (75). Elastase is released by activated polymorphonuclear leukocytes and can degrade collagen, laminin, fibronectin, proteoglycan and elastin. Recent studies have shown that elastase activity is by far the highest of any protease found in gingival fluid of gingivitis and periodontitis patients (76). Cimasoni and Kowashi demonstrated that a neutral protease thought to be elastase, increased significantly in gingival fluid during experimental gingivitis (77). Thus, gingival fluid elastase may reflect PMN infiltration and could potentially indicate the degree of inflammation at individual periodontal sites.

David, Darany and others (78) studied the relationship of gingival fluid leukocyte elastase activity to gingival fluid flow rate. According to them, gingival fluid flow rate was significantly higher at periodontitis sites with intermediate or deep probing depths than at healthy sites, and higher at deep periodontitis sites than at mild gingivitis sites. Gingival fluid elastase appears to be a less suitable index of periodontal status. Elastase was significantly higher at periodontitis sites with deep probing depths than at healthy sites or mild gingivitis sites. However there was no significant difference in elastase between healthy sites and periodontitis sites with intermediate probing depths (78).

GCF volume, elastase activity per site, elastase activity per μl and the relative activity showed a correlation to probing depth and to attachment loss. The comparison between the gingivitis patients and periodontitis patients showed that
the mean values of GCF volume, elastase activity per site and elastase activity per μl was significantly higher in periodontitis patients (79). The diagnosis of periodontal disease should ideally include not only a description of the present state of the disease, but also information, which would indicate whether the disease is actively progressing or is about to progress. Several studies have examined the potential of measuring levels of various substances found in gingival crevicular fluid as indicators of periodontal disease progression. GCF elastase levels are significantly higher in sites demonstrating progressive periodontal attachment and bone loss assessed 6 months later and may serve as a predictor of future bone and attachment loss (80). Sites with high levels of elastase are significantly greater risk for progressive bone loss as assessed by digital subtraction radiography (81, 82). It is clear, that site-specific periodontal disease tests involving either sulcular fluid or plaque as samples, will be restricted mainly for use by specialist periodontist. Uitto and others used water rinse samples of oral cavity for elastase activity. According to them there was a good correlation between the elastase activity and the number of deep periodontal pockets and the average community periodontal index of the subjects. Elastase activity is not a good indicator of gingivitis. About 45% of gingivitis cases were positive with the elastase test and the enzyme values are not significantly increased in experimental gingivitis. In a longitudinal study of advanced periodontitis cases, elastase levels dropped dramatically as a result of clinically successful therapy, close to the values of the healthy subjects (83).

**Trypsin - like activity**

In addition to metallo enzymes, collagenase, gelatinase and elastase, one more serine protease, trypsin-like activity is also found in gingiva. These proteinases require high salt concentrations for their optimal extraction from tissues. This may be attributed to the dissociation of the enzyme from endogenous inhibitors or their natural substrates (84). The proteinase may also be associated with ionic bonds to other substances such as heparin, that binds mast cell
proteinases in the intracellular granules (85). These trypsin like enzymes in gingiva have several functions, in degradation of structural proteins and also of plasma derived proteins that participate in local defence reactions. These include immunoglobulins (86) and components of complement system (87). In addition to leukocytes, mast cells have been shown to be a major source of neutral proteinases in skin (88). Recently fibroblasts and epithelial cells have also been found to produce neutral proteinases. Inflamed tissue contains a lot of enzymes from inflammatory cells. In addition, bacteria is also a source of gingival enzymes.

The trypsin-like enzyme was inhibited by serine protease inhibitors, phenylmethylsulfonylfluoride and benzamidine and by metalloproteinase inhibitor, EDTA as well as ascorbic acid.

The ability of subgingival plaque to hydrolyze benzoylalanine β-naphthylamide (BANA) is a reliable marker for the presence of high proportions of spirochetes in the plaque sample and possibly could be used clinically to identify those sites or individuals who might require treatment to reduce this spirochetal overgrowth (89).

Much effort has been directed in recent years towards the identification of host and bacterial enzymes in crevicular fluid. However, there has been no evidence for the presence of a mast cell enzyme. The substantial increase in serine protease activity produced by heparin is suggestive of mast cell tryptase. They were also consistent with the properties of tryptase like enzyme identified in extracts of human gingiva. Partial inhibition of crevicular fluid activity by soyabean trypsin inhibitor suggests the additional presence of a second trypsin-like enzyme which might be of host or bacterial origin (90).

**Cysteine Proteinases**

Lysosomal proteinases which are active at acidic pH, are believed to play an important role in connective tissue alterations, resulting from plaque initiated
inflammatory reactions in gingivitis. This consists of ‘acid proteinases’ like cysteine proteinases and to a limited extent cathepsin D (91).

Tamara and others (92) in their study on cathepsin ‘L’, found that the activities of cysteine proteinases mainly cathepsin ‘L’ in gingival fluid contribute remarkably to the acid proteolytic activity, which was previously ascribed solely to cathepsin D (93, 17).

It has been shown that among several cysteine proteinases only cathepsins B, L and N possess collagenolytic activity (94). Cathepsin ‘L’ however, exhibits the highest specific activity with respect to collagen and other proteins as substrates. Cathepsin-L like activity in the GCF increases sharply and more significantly than that of cathepsin-D in patients with deeper periodontal pockets at the site of fluid collection (92).

Cathepsin D activity, as measured on hemoglobin as substrate, was 10 times higher in gingival fluid than in serum and it is increased during experimental gingivitis (95). Cimasoni questioned the role of acid proteolysis in the course of periodontal disease, as the pH of the fluid ranges from 7.5 to 8.5. It is however probable that the pH at specific sites, such as along the junction of a nonphagocytosable surface or at the attachment site of an activated macrophage or a supporting structure, is much lower. Besides, the pH optimum is also dependent on the kind and conformation of the protein substrate, which can be modified by enzymatic processing and ligand binding. The collagenolytic activity of cathepsin L is, for instance much higher at pH 3.5 than at pH 6.0, while cathepsin D degrades proteoglycans at an optimum pH of 5 to 6.0 (96).

Eley and Cox in 1991 studied the cathepsin B-and L-like activities at local gingival sites of chronic periodontitis patients and they observed that, enzyme activity in healthy control tissue was significantly lower than in diseased tissue (97). Enzyme activity in gingival tissue and total tissue from periodontitis patients decreased with increasing pocket depth, clinical attachment level, gingival index
and bleeding index. While cathepsin B activity in granuloma increased with increasing pocket depth and clinical attachment level but not with increasing gingival index or gingival bleeding index. Mean enzyme activity in gingival tissue was 1.6-2.8 times greater than in granuloma. Mean patients did not correlate positively with their mean pocket depth, clinical attachment level, gingival index or gingival bleeding index. These results are best explained by the probable cellular origins of the enzymes and the likely influence of their serum and tissue inhibitors during the disease process. Eley and Cox studied the activities of these proteases before and after periodontal surgery. There were reductions in all clinical parameters and all protease activities after scaling and hygiene treatment and further reductions after periodontal surgery (98).

A novel arginine-specific cysteine proteinase termed 'argingipain' was purified from culture supernatants of Porphyromonas gingivalis, an anaerobe commonly associated with progressive periodontal disease. The purified enzyme was found to be composed of a single polypeptide of Mr 44,000. The proteolytic activity of 'argingipain' is absolutely thiol dependent, but the enzyme also has in part the characteristics of both metallo and serine endopeptidases. The activity is inhibited by metal chelators, chymostatin and the chloromethyl ketones of tosyl-L-lysine and tosyl-L-phenylalanine. However, internal protease inhibitors such as cystatins, tissue inhibitor of metalloproteinases and α₁-antichymotrypsin have no effect on the activity. Despite its narrow specificity for synthetic substrates containing arginine in the P₁ site and hydrophobic aminoacids in the P₂ or P₃ sites, the enzyme extensively degrades collagen (type I and IV) and immunoglobulin-G. Most important, the enzyme has the ability to disrupt the functions of polymorphonuclear leukocytes, as shown by its inhibitory effect on the generation of active oxygen species from the activated cells (99).

The major cysteine proteinases released by Porphyromonas gingivalis hydrolyze peptide bonds only after arginyl (gingipain-R) or lysyl residues (gingipain – K). Both 50 and 95 KDa gingipain-R were effectively inhibited by
alpha-2-macroglobulin, whereas the catalytic activity of gingipain-K could not be eliminated. All the three enzymes were however, inhibited by homologous macroglobulin from rat plasma, α1-inhibitor-3 (100).

**Kallikrein**

Plasma kallikrein is a serine protease which consists of one heavy chain (Mr. 43000 Da) and one light chain (Mr. 35000 Da), which are linked by disulfide bonds (101). The light chain contains the catalytic portion which is homologous to trypsin family of serine proteases. Both prekallikrein and kallikrein form complexes with high molecular weight kininogen (102).

The binding site for high molecular weight (HMW) kininogen is located on the heavy chain region of kallikrein. Further, the heavy chain is required for the potent surface dependent procoagulant activity of kallikrein (103).

Plasma kallikrein converts factor XII to XIIa and XIIf, plasminogen to plasmin and liberates bradykinin from HMW kininogen (104). This was enhanced in the presence of plasmin (105). The platelet bound HMW kininogen is protected from kallikrein proteolysis (106).

The heavy chain of kallikrein might bind to neutrophil membrane receptors and such binding is required prior to the expression of proteolytic activity, that leads to the neutrophil aggregation and elastase release (107).

Burger and co-workers (108) reported that neutrophils incubated with human plasma kallikrein remained unactivated and suggested that kallikrein does not directly activate blood neutrophils. The sequential exposure of polymorphonuclear leukocytes to plasma kallikrein and a formylpeptide increased the superoxide production from neutrophils (109).

Kallikrein as a soluble stimulus, predominantly induces degranulation of specific granules containing collagenase, capable of degrading connective
Secretion of lysozyme and collagenase requires the presence of active kallikrein (110).

Plasma kallikrein circulates in blood as prekallikrein. Prekallikrein is a glycoprotein (Mr. 82 KDa) which occurs at concentration of 40-55 μg/ml of plasma (111). Activation of human prekallikrein seems to involve limited proteolysis. Factor XIIa activates prekallikrein by cleavage of an Arg-Ile bond in presence of HMW kininogen (112).

Five plasma protease inhibitors are known to inactivate kallikrein including cl' inhibitor, α2-macroglobulin, antithrombin-III, α1-proteinase inhibitor and α2-plasmin inhibitor. Human plasma kallikrein forms 1:1 complex with plasma protein ‘C’ inhibitor (PCI) (113). In the reaction between PCI and kallikrein, a complex with Mr. 132,000 was formed. The kinetic inactivation of kallikrein and its light chain are similar, indicating a complex formation between light chain of kallikrein and PCI.

The aminoacid composition, molecular weight, inhibition and kinin generating ability of the parotid salivary proteinase indicate that the enzyme is in fact kallikrein. There is a close similarity in the composition of human salivary kallikrein and the mucous submaxillary-gland proteinase. Moreover both enzymes will cleave only the arginine-glycine bond in salivary protein C. The enzymes may be involved in post-synthetic proteolytic modification of precursor proteins (114).

Human urinary kallikrein has been shown to activate inactive renin (115), and kallikrein may play a role in the conversion of proinsulin to insulin (116). The function of kallikrein in human salivary glands is not clear. The enzyme may play a role in vasodilatation (117) and in electrolyte secretion (118), but this does not explain why it is secreted in saliva. Because salivary kallikrein cleaves a single bond in salivary protein C, it is tempting to suggest that it acts as a processing enzyme. This is not in agreement with glandular location of the enzyme. Acidic proline-rich proteins have been demonstrated in acinar cells of the human parotid
gland and in serous demilunness of the submandibular gland, but at least in the submandibular gland of human and other species, kallikrein is present only in the cells lining the ducts (119), thereby excluding a role of kallikrein as a processing enzyme for proteins synthesized in acinar cells.

PROTEINASE INHIBITORS

The plasma proteinase inhibitors, after albumin and the immunoglobulins, constitute by weight the third largest group of functional proteins in human plasma. Representing nearly 10% of the total protein in plasma, they control a variety of critical events associated with connective tissue turnover, coagulation, fibrinolysis, complement activation and inflammatory reactions. In addition, at least one of the inhibitors, alpha-2- macroglobulin (α2M), plays an important secondary role in backing up the primary function of other inhibitors.

The existence of proteinase inhibitor activity in human plasma was apparently first noted by Fermi and Pernossi in 1894 (120). Since that time a host of investigations have been made to determine the various inhibitory activities in tissue derivative, primarily by adding a larger number of proteinases of varying specificities and catalytic mechanisms to plasma and plasma fractions. These experiments were performed to determine the number, type, concentration and mechanism of action of the various inhibitors and with the hope of understanding their role in the control of proteolytic events within the body.

Alpha,-Proteinase inhibitor (α,-PI)

Human α,-PI has been the subject of intense investigations, primarily because of its role in controlling proteolytic events in tissues. Originally named α,-antitrypsin (121) because of its ability to inactivate pancreatic trypsin, this protein has since been found to be far more effective in controlling the activity of a number of other serine proteolytic enzymes (122).
Human \( \alpha_1 \)PI is a glycoprotein of Mr near 53,000 (123). The inhibitor exists as a single polypeptide chain with no internal disulfide bonds and only a single cysteinyl residue normally intermolecularly disulfide-linked to either cysteine or glutathione (124). The reason for this unusual structure is not clear. However, in its native form \( \alpha_1 \)PI may exist in a reduced state with its free thiol group serving in a protective role as a scavenger for oxidants that could inactivate the inhibitor.

During phagocytosis neutrophils release some oxidants outside the cell, since \( \alpha_1 \)PI exposed to dialyzable components produced by these cells reduced markedly its elastase inhibitory capacity (125). When these studies were extended to blood monocytes and alveolar macrophages, reactive oxygen species were also released, which reduced elastase inhibition (126). Therefore, oxidants produced by phagocytizing cells may play a significant role in the inactivation of \( \alpha_1 \)PI outside the cell.

Tissue destruction during periodontal and other inflammatory diseases can occur when the endogenous inhibitors of these hydrolytic enzymes are reduced in concentration or inactivated such that the ratio of proteinase activity relative to the activity of the endogenous inhibitors is excessive. Elastase, the major serine proteinase released by PMN, is capable of degrading many of the components of extra cellular matrix. Its activity is efficiently regulated by the serine proteinase inhibitor, \( \alpha_1 \)PI, which rapidly forms an inactive 1:1 complex with the enzyme. Several groups in recent years have reported that elevated levels of elastase, and its complex with \( \alpha_1 \)PI in GCF, are both positively correlated with clinical parameters of disease such as pocket depth, gingival attachment loss and alveolar bone loss (127, 128). Free elastase already combined with the elevated levels of \( \alpha_2 \)-MG would be unavailable to react with \( \alpha_1 \)-PI, but could still hydrolyze chromogenic peptide substrates (129).

During inflammation, \( \alpha_1 \)-PI can be inactivated oxidatively or proteolytically. The oxidatively inactivated \( \alpha_1 \)-PI has decreased ability to bind to
its substrate, e.g. PMN elastase. Human neutrophils have been shown to contain two matrix metallo proteinases, collagenase (MMP-8) and gelatinase (MMP-9), which can also inactivate $\alpha_{1}$-PI (130-133) leading to “down regulation”.

**Alpha$_1$-Antichymotrypsin**

$\alpha_{1}$-Antichymotrypsin is a plasma glycoprotein first isolated and characterised without knowledge of its function (134). Subsequently, it was shown to have inhibitory activity towards chymotrypsin (135), together with $\alpha_{1}$-PI and $\alpha_{2}$-M. The inhibitor is a major acute phase protein, whose concentration increases rapidly and dramatically after a variety of events, including surgery (136), burn injuries (137), Crohn’s disease and ulcerative colitis (138) and some types of cancer (139).

$\alpha_{1}$-Antichymotrypsin shows the most immediate response as an acute phase protein, doubling in concentration within eight hours of insult. Thus, this inhibitor might play an important role in controlling specific systems associated with inflammatory episodes. It has relatively low concentration in plasma (25 mg/100 ml).

**Alpha-2-Macroglobulin ($\alpha_{2}$MG)**

Human $\alpha_{2}$MG is a glycoprotein of molecular weight 725,000, containing 8-11% carbohydrate (140-141), and present in plasma at a concentration of 250 mg/100 ml. It is relatively easy to isolate because of its large molecular weight. The purified protein is composed of a tetramer of identical subunits of Mr 185,000, linked in pairs by disulfide bonds. Two pairs associate by non-covalent binding to form the native, tetrameric molecule (142).

The characteristics of the reaction of proteinases with $\alpha_{2}$M suggest a unique reaction mechanism. At least three types of binding reactions may occur during inhibitor-enzyme interactions, including (a) a steric trapping reaction, specific for proteinases, (b) a covalent linking of proteinases and other molecules
containing nucleophilic groups and (c) noncovalent, non steric adherence reaction with a number of other proteins and other molecules, unrelated to proteolytic activity (143).

Alpha$_2$ macroglobulin inhibits a range of proteases from all four classes and which accounts for more than 90% of total trypsin inhibiting capacity in serum (144). The broad specificity of $\alpha_2$MG suggests that this inhibitor may have an important role in defence via the control of exogenous proteases of viral and bacterial origin.

There are a few reports in the literature of the serum levels of $\alpha_2$MG in periodontitis (145). Saito et al (1969) reported elevated serum levels of $\alpha_2$MG in three different types of periodontal disease; periodontitis, periodontosis and gingival recession with inflammation.

PERIODONTAL DISEASES

Some degree of inflammatory periodontal disease is thought to be present in almost all persons with natural teeth. On a worldwide basis, it is the leading cause of tooth loss.

Traditionally, periodontal diseases have been divided into two major categories; gingival diseases and periodontal diseases. The former includes diseases that attack only the gingiva, whereas the latter includes diseases that involve the supporting structures of the tooth.

Gingivitis

Gingivitis is an inflammatory process affecting the soft tissues surrounding the teeth. The inflammatory process does not extend into the alveolar bone, periodontal ligament or cementum. The primary etiologic agent of gingivitis is bacterial plaque.
Plaque-associated gingivitis is the most common form of gingivitis and probably, the most common form of all periodontal diseases. Plaque associated gingivitis has clinical features including some or all of the following: inflammation, edema, bleeding upon probing or spontaneous gingival sensitivity and itching. However, by definition, no loss of attachment or radiographic loss of bone is associated with gingivitis.

Other factors may modify the course and clinical presentation of gingivitis. These factors permit a classification of gingivitis based upon secondary etiologic factors (146).

- Forms of gingivitis are -
  Plaque associated gingivitis
  Acute necrotizing ulcerative gingivitis (ANUG)
  Steroid hormone influenced gingivitis.
  Medication - influenced gingival overgrowth
  Other forms of gingivitis.

ANUG (Vincent’s infection, trench mouth) has several possible secondary etiologic factors. Stress and anxiety are probably significant contributing factors (147, 148). However, other contributing factors may include fatigue, lowered resistance, nutritional impairment, smoking, mouth breathing, calculus, pre-existing gingivitis and gross neglect.

The clinical features of ANUG includes necrosis, which may or may not be covered by a greyish-white pseudomembrane. In addition, acute areas of inflammation contribute to the pain, bleeding, soreness and sensitivity of these lesions. The extent of ANUG lesions may include isolated interproximal areas or may be generalized throughout the entire dentition. Boggy, edematous, keratinized gingiva frequently is present. Lymphadenopathy, malaise and pyrexia are frequent accompanying clinical signs (176).
Plaque associated gingivitis also called chronic marginal gingivitis, may remain stationary for indefinite periods of time or may proceed to destroy the supporting structures.

Steroid hormone - influenced gingivitis results from the presence of steroid hormones, which may amplify clinical inflammatory changes of gingivitis. Increased levels of estrogens and progesterones during pregnancy (149), during adolescence, or in patients who are taking birth control medication may enhance marginal gingival inflammation (150). The clinical signs and symptoms include acute gingival inflammation around one or more teeth and are frequently associated with plaque retentive areas. Spontaneous gingival bleeding or bleeding with gentle instrumentation is frequently present. More severe cases of steroid hormone-influenced gingivitis may progress to pyrogenic granuloma (pregnancy tumor).

Medication - influenced gingival overgrowth frequently results in pseudo pockets. Medications having this potential include phenytoin used for the control of epilepsy (151) and cyclosporin (152) used for immunosuppressive therapy of renal transplant patients. The clinical signs and symptoms include gingival over-growth in the form of a diffuse swelling of the interdental papillae or multiple, tiny nodules on the labial of the interdental papillae of anterior teeth, moderate to acute inflammation, soreness, tenderness and moderate pocket depths.

Other forms of gingivitis may be influenced by nutritional deprivation states (153-155). The clinical signs and symptoms of these nutritionally influenced gingivitis patients include acute inflammation of the marginal gingiva, sensitivity or pain, spontaneous gingival bleeding or bleeding upon probing.
Periodontitis

Periodontitis is defined as inflammation involving the gingival unit (gingiva and alveolar mucosa) and extends to the periodontal ligament, alveolar bone, and cementum.

Periodontitis involves loss of clinical attachment and radiographic loss of bone. The conversion, clinically from gingivitis to periodontitis reflects the progression, histopathologically from the established stage to advanced stage of periodontal lesion. The reasons for this progression remain unclear but may reflect aberrations of host cell responsiveness to plaque infection or may represent colonization and infection by highly pathogenic plaque bacteria.

Clinical observations coupled with basic science research have permitted descriptive information of the various forms of periodontitis. Page and Schroeder presented periodontitis as four major forms: adult periodontitis, rapidly progressive, juvenile and prepubertal (156).

Chronic adult periodontitis

This condition usually occurs in adults over 35 years of age and there appears to be no predilection for either sex. This form of periodontitis is directly related to accumulation of tooth-associated materials (plaque and calculus). The rate of pathogenesis of chronic adult periodontitis commonly takes years and even decades to progress. The extent and distribution of bone loss and loss of attachment is variable and often is related to plaque retentive areas.

Rapidly progressive periodontitis, type – A

As the name implies, is a form of periodontitis displaying relatively rapid loss of clinical attachment and alveolar bone.

In early stages of rapidly progressive periodontitis, selected teeth may be affected by moderate to deep pockets, loss of attachment and early signs of
alveolar bone loss. These clinical signs may be observed in a young adult or teenager who has a non-contributory medical history, has had regular dental care, and has minimal deposits of tooth associated materials.

However, in the later stages of rapidly progressive periodontitis, which may take a few years or even a few months to develop, generalized moderate to deep pockets and more severe loss of attachment and alveolar bone may be observed. The disease progresses relatively rapidly and may have periods of acute, often painful inflammation of the periodontal tissues with hemorrhage, proliferation of the marginal gingiva and exudation (157). Increasing mobility and loss of teeth may be associated with more advanced stages of this disease. Usually, this type (A) of rapidly progressive periodontitis, regardless of stage, affects younger adults and teenagers.

**Rapidly progressive periodontitis, type -B**

Patients having this type (B) of rapidly progressive periodontitis are older than those with type A. This type may not affect all the teeth, and there may or may not be conditions enhancing plaque retention and accumulation. Caries rate is variable. It is agreed that the diagnosis of this type of rapidly progressive periodontitis may overlap or, in fact, may be similar to adult periodontitis occurring in a younger individual.

**Juvenile periodontitis (Periodontosis)**

By definition, the disease affects first molars and incisors and may affect up to two additional teeth. The age group of affected patients is generally 12 to 26 years. Females are affected more than males (3:1) (158). Minimal plaque and calculus deposits are associated with the classic form of juvenile periodontitis (156). Caries rate is low. Although these patients may have gingiva with mild to moderate inflammation, there is frequent evidence of rapid loss of attachment. It has been estimated that the rate of pocket formation is 4 to 5 µm/day (159).
Post-juvenile periodontitis

Post-juvenile periodontitis patients display similar clinical and radiographic features (first molar/incisor) as juvenile periodontitis patients. The sex distribution is approximately 3:1, female to male. Patients with post-juvenile periodontitis are generally older (26 to 35 years) and have a variable caries rate. Tooth-associated materials are frequently present. Post-juvenile periodontitis patients may have a markedly slower progression of disease and may be considered arrested or burnt out. The underlying mechanism for the retarded disease activity and attachment loss may relate to circulating antibody levels (160). Clinically, post-juvenile periodontitis patients may be missing one or more first molars and incisors or osseous defects are evident.

Prepubertal periodontitis

Prepubertal periodontitis patients are under 12 years of age, and usually between ages 5 and 8. The sex ratio is nearly equal. Periodontal lesions may be localized or generalized (161), and caries rate is usually low. Tooth associated materials are low, radiographs depict bone loss and frequently furcation involvement in advanced caries.

Gram negative, facultative, capnophilic or anaerobic microorganisms are the principal bacteria associated with periodontal diseases.

Porphyromonas gingivalis, Prevotella intermedia, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Campylobacter rectus, Treponema denticola and Eikenella species are some of the most common bacteria associated with the disease.
Diabetes mellitus

Diabetes mellitus represents a condition with disordered metabolism of glucose resulting in hyperglycemia due to either an absolute deficiency of insulin secretion or a reduction in its biologic effectiveness or both.

Diabetes has a strong disposition to develop both acute and chronic complications as a result of abnormal fat, protein and lipid metabolism. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar syndrome. Among the chronic complications are atherosclerosis, coronary heart disease, microangiopathy, retinopathy and neuropathy. Additionally diabetics are highly susceptible to bacterial infections and exhibit delayed healing of wounds.

Clinically there are at least three sub types -

a) Juvenile onset insulin dependent diabetes mellitus (type -1)

b) Maturity onset diabetes mellitus.

c) Non-insulin dependent diabetes mellitus (NIDDM-2)
Diabetes may be associated with a wide spectrum of oral manifestations like advanced periodontal disease, high incidence of dental caries, sialosis due to neuropathy of the para sympathetic nerve fibers of the salivary glands, xerostomia due to fatty infiltration of acini of the salivary glands, altered taste sensation, prolonged or recurrent fungal and bacterial infections and burning mouth syndrome. Diabetes when poorly controlled is known to be associated with an increased susceptibility to infection and delayed wound healing.

The possible mechanism for increased periodontal disease in diabetes mellitus may be summarised as follows. "Presence of increased number of pathogens, neutrophil dysfunction and other host protection defects, increased production of inflammatory mediators and connective tissue ability all leading to more severe expression of periodontal disease". Higher glucose levels in tissues and tissue fluids promoting growth and proliferation of pathogens lead to increased periodontal disease (162,163).

Anatomical or histological changes associated with periodontal disease in diabetes include thickening of the vascular basement membrane and vascular degeneration of gingiva (164). Angiopathies of diabetic gingiva may contribute to compromised delivery of nutrients to the surrounding tissues and poor elimination of metabolic waste.

Increased sensitivity of periodontal disease to diabetes mellitus may be due to abnormalities in polymorphonuclear (neutrophil) function, in the form of changes in neutrophil activation and adherence as well as defect in neutrophil chemotaxis. This defect allows invading organisms to continue their ingress into host tissue unimpeded. Therefore, diabetic patients are more susceptible to infections and frequently present with severe periodontal diseases (164).

Several pathogens are equipped with unique mechanisms of virulence and consequently thrive in a hyperglycemic environment. The composition of the periodontal microflora found in periodontally diseased sites of NIDDM patients
appears to be similar to that found in chronic adult periodontitis. Due to salivary dysfunction and biochemical alteration in the saliva of diabetic individuals, the resistance of oral tissue to infection might be altered due to altered immunology of the oral cavity, which might have a bearing on periodontal status in diabetic patients. Davidson et al (1969) reported that fatty infiltration noted in histologic sections of enlarged parotid glands in patients with diabetes mellitus, was identical to that observed in association with chronic liver disease and believed that it could be attributed to a disturbance in lipid metabolism. Histologically, the glandular enlargement was observed to be a non-inflammatory, non-neoplastic fatty infiltration of the parenchyma with a decrease in the number of acinar structures. Other changes included acinar hypertrophy, glycogenic degeneration manifesting as epithelial cells with a foamy appearance, stratification of epithelium in smaller ductules, microangiopathy and varying types of ductal luminal debris which could predispose a patient to salivary calculus formation and duct obstruction (165).

Murrah, et al (1985) assessed the parotid gland basement membrane changes in diabetes mellitus. Results of these investigations revealed parotid gland basement membrane abnormalities in all diabetic subjects as indicated by binding of IgG, albumin, and polyvalent immunoglobulins to ductal and acinar basement membranes (166).

Hand and Weiss (1984) noticed changes in salivary flow and composition in diabetic rats. They stated that leakage of macromolecules through damaged basement membranes of parotid glands in diabetic rats may lead to altered salivary composition. They also noticed changes in the epithelial, vascular and neural components of the parotid glands in diabetic rats (167).

Harrison and Bowen conducted a study to examine the association between control of diabetes and composition of whole saliva in insulin dependent diabetic children (168). Poorly controlled diabetics showed significantly increased concentration of lactoferrin in whole saliva. In their study, they had noticed that
IgA concentration was significantly increased in saliva of poorly controlled diabetics. This increase was probably a result of unchanged production of IgA by plasma cells, but significantly reduced output in saliva.

There are conflicting reports in the literature about relationship of diabetes with periodontal disease status. Though some of the reports mention about positive relationship between diabetes and periodontal disease, others indicate that diabetes per se does not initiate or cause periodontal disease. But if present can modify its course and spread. The current view is that periodontopathy should be considered along with neuropathy, nephropathy and retinopathy as one of the vascular complications of diabetes mellitus (163).

**SALIVARY GLYCOPROTEINS**

The majority of the salivary proteins contain a large proportion of carbohydrates in their molecules. In parotid saliva about 35 percent of the total protein contains appreciable amount of carbohydrate. These glycoproteins contain around 75 percent protein with proline, glycine and glutamic acid as the major aminoacids, and about 25 percent carbohydrate with galactose and mannose making up about a half of this total. There are approximately equal amount of hexosamine (85% glucosamine, 15% galactosamine) and fucose and between 1% and 2% sialic acid.

In submandibular saliva, the mucroproteins represent a higher proportion of the total protein. Two major mucoprotein fractions contain 80% of the hexosamine and fucose in submandibular saliva. These fractions differ from the mucoproteins of parotid saliva in having different proportions of the main sugars, galactose, glucose, mannose and fucose in ratio of 6:1:1:4.

The total amount of bound carbohydrate in parotid saliva is much more than that in submandibular saliva (45g/litre against 0.30g/litre). However, the carbohydrate : protein ratio is higher in submandibular saliva.
Human salivary glycoproteins are classified according to cell origin and then subclassified based upon biochemical properties associated with serous or mucous products. In general, one can group human salivary glycoproteins synthesised by acinar cells into families whose members share common structural features. For example, the salivary mucin glycoproteins constitute a family of at least two members. A multisubunit higher molecular weight species, designated MG, and a lower molecular weight subunit species, MG2. Salivary proline-rich proteins and amylases also occur in multiple forms as glycosylated or non glycosylated species (169-171).

Classification of salivary glycoproteins based upon cellular origin and molecular families

1. **Salivary glycoproteins occurring as members of families (acinar cell origin).**
   a) **Mucous glycoproteins**

   Mostly o-linked oligosaccharides. (Ser/Thr-Gal NAC). Higher molecular weight, greater than 40% carbohydrate, small amounts of mannose may be present.

   Examples: Mucin glycoprotein 1 (MG1)  
               Mucin glycoprotein 2 (MG2)

   b) **Serous glycoproteins**

   N-linked oligosaccharides (Asn-GlcNAC) lower molecular weight, less than 50% carbohydrate. Significant amounts of mannose present.

   Examples: Proline-rich glycoproteins (PGR), α-amylases, salivary peroxidases, carbonic anhydrase.

2. **Salivary glycoproteins occurring as single species (ductal or stromal cell origin).**

   Examples: Secretory IgA  
               Lactoferrin  
               Kallikrein  
               Fibronectin.
**Fucose-rich glycoprotein (FRG)**

It is a multisubunit glycoprotein occurring in a complex of at least 5,000-10,000 kDa, with subunits of approximately 440 kDa. This molecule contains 45% carbohydrate. Analysis of the carbohydrate contents of FRG reveals that 12.5% of the total molecular weight is contributed by fucose, with hexosamines (10.5%) neutral hexoses (16.2%), and sialic acid (5.7%) accounting for the remaining residues. Aspartic acid, serine, threonine and glycine compose 41.5% of the total number of amino acid residues. The contribution of proline is relatively small, representing just 4.3% of the total amino acids.

**Functions of salivary glycoproteins**

1. Tissue coating and formation of intraoral pellicles; salivary glycoproteins are thought to play important roles in the formation of the acquired enamel pellicle and thereby protect hard and soft oral structures.

2. Lubrication

3. Clearance/adherence of microflora

4. Anti microbial activity

5. Utilization as a microbial substrate

6. Posttranslational processing

7. Digestion

8. Buffering capacity

**AIM AND OBJECTIVES**

Useful diagnostic parameters should indicate the presence or absence of periodontal disease, the response to treatment, and the need for additional treatment. Current clinical techniques to detect initial and established pathological changes in the periodontium emphasize clinical and radiographic signs. A dilemma exists as to which signs should be used to assess periodontal status, and thereby indicate the need for treatment. The need for accurate and sensitive objective signs in the assessment of periodontal status has frequently been emphasized and they are essential if the diagnosis and management of periodontal disease is to be placed on a rational, and less empirical basis. The composition of gingival fluid seems promising as a potential medium for the detection of early changes which could indicate the onset of the disease. The origin, the composition and the clinical significance of gingival fluid are now known with more precision and have significantly helped our understanding of the pathogenesis of periodontal disease. On the other hand, some of the legitimate hopes that were inspired when gingival fluid was first discovered have been dashed. Upto now for instance, none of the multiple components analysed in the fluid has improved clinical judgement of the rate of the progress of gingivitis and periodontitis or of the rate of repair of these conditions.

The accurate and reproducible sampling of gingival crevicular fluid is not trivial. A number of studies have demonstrated that the choice of collection device (172), the length of collection time (173), the number of sample repetitions (174) and the calculation of data as absolute measures or as flow rates (175) contribute in a large measure to overall variability. Due to these variables and to expected fluctuations based on the operator sensitive use of collection devices (eg. depth of insertion of filter paper strips or capillary tube), potential contamination by serum components and loss of sample from the collection device. There is no consensus
as to which protocols exhibit the lowest bias, the highest reproducibility and the strongest validity.

These statements summarise the requirement in periodontology for a reliable biochemical marker for disease activity and susceptibility. The limited utilization of saliva for diagnostic purposes in the past may be reflection of the ambivalence that has characterized both the public and professional view of the nature of saliva over the ages.

Saliva provides an easily available, noninvasive diagnostic medium for a rapidly widening range of diseases and clinical situations.

The enzymes of human saliva have been studied for decades with respect to origin, purification, characterization, distribution among individuals and relationship to pathological conditions of the oral cavity (3). It is known that enzymes like glycosidases, esterases and proteases are present in the human saliva. A number of authors have demonstrated the presence of proteolytic enzymes in saliva (6-10).

This study deals with three different groups of biochemical parameters in saliva that can be considered as useful in diagnosis of oral diseases. One group of factors are the salivary proteolytic activity. The second group comprises factors that exhibit antiprotease activities. The third group involves total protein and protein bound neutral hexoses, fucose and sialic acid.

In Chapter III, salivary hydrolytic activity against the chromogenic substrate S-2302 (substrate for kallikrein and trypsin) were examined with respect to their pH profile and effector responses. Attempts were made to correlate the S-2302 hydrolyzing activity with different clinical conditions and also in normal male and female samples. From the results, it was observed that measurement of salivary S-2302 hydrolytic activity when normalized to protein level can be used as a marker in differentiating different stages of periodontitis.
Salivary proteolytic activities against different chromogenic substrates were estimated and this was compared with the serum activity. The changes in the levels of protease activities against different chromogenic substrates as a factor of time and the effect of synthetic and natural inhibitors were studied. Further the effect of temperature on protease inactivation and pH profile was studied. Red sepharose chromatographic pattern of distribution, along with the resolution of proteolytic activities were analysed. Salivary proteolytic activity was estimated in normals, gingivitis, periodontitis and diabetes (without periodontitis) samples. The results are presented in Chapter IV.

Previous studies showed that impaired regulation of proteolysis is an important biochemical aspect in the aetiology of periodontal disease, i.e., increased levels of proteolytic enzymes and varied levels of different protease inhibitors. Salivary proteolytic activity was studied and reported in Chapter III and Chapter IV. Chapter V reports a study on the estimation of salivary antitryptic activity, antielastase and antichymotryptic activity to see if there is any correlation between the levels of protease inhibitory activity and different periodontal status.

Most of the carbohydrate content of saliva is bound carbohydrate. Saliva contains a mixture of glycoproteins and are characterized by containing carbohydrate side-chains. A number of investigations have been conducted on salivary glucose level in diabetes, GCF total protein, fucose and glycosaminoglycans level in periodontal disease. No systematic study on the changes in the levels of carbohydrates in saliva in oral diseases has been undertaken. The present study deals with the estimation of total carbohydrate, protein bound carbohydrate, bound fucose and sialic acid along with total protein in different conditions like gingivitis, periodontitis and caries, the results of which are presented in Chapter VI.