

Variations of some salivary antimicrobial factors in different disease states: A review

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لقد تم التعرف على مجموعة من المواد التي تتواجد في الافرازات اللعابية والتي اثبت علميا انها مضادة لمختلف الجراثيم من بكتيريا وفيروسات وفطريات ، وقد يحدث تغيرات في مستوى افراز هذه المواد بين مختلف الامراض التي تؤثر على الجسم بشكل عام وعلى الفم بشكل خاص.

وللمعرفة نوع العلاقة المتبادلة بين الامراض المختلفة والتغير الحاصل في نوعية الافرازات اللعابية وعلى الاخص العوامل المضادة للجراثيم ، تم عمل مراجعة شاملة للدراسات التي بحثت هذا الموضوع.

لقد لوحظ ان التغيرات التي تحدث في نوعية اللعاب تتأثر بشدة المرض وما اذا كان الجهاز المناعي للجسم قد تآثر بهذا المرض ، كما لوحظ ان الافرازات اللعابية تتأثر بشكل خاص عندما تكون الغدد اللعابية قد تآثرت بهذا المرض.

ما زالت الابحاث غير كافية لتحديد ماهية العلاقة بين الامراض المختلفة والافرازات اللعابية ، ولعل من اهم المتغيرات التي يجب ان تكون ثابتة في جميع الابحاث المتعلقة بهذا الموضوع هو توحيد طرق جمع العينات اللعابية وكذلك طرق قياس العوامل المختلفة المكونة لللعاب ، خاصة وان تركيز هذه العوامل يختلف بين اللعاب المحتفظ واللعاب الناتج من الغدد اللعابية منفردة.

The antimicrobial properties of saliva had been recognized for a long time owing to their importance in the natural defense mechanism of the body, particularly in diseases that have local effects on the tissues of the oral cavity. The immune and non-immune antimicrobial salivary factors have been measured in different disease states to observe the variations in their levels in an attempt to understand the pathogenesis of some systemic diseases and to formulate better lines of treatment particularly for diseases that are either difficult to cure or that are not treatable yet. The aim of this paper was to review the literature on some antimicrobial factors and their variations in some disease states and to understand the inter-relationship between the salivary antimicrobial factors and systemic diseases affecting the oral cavity. In order to have a better understanding of the factors affecting salivary secretions, there has to be a more standardized approach for saliva collection and analysis, more so because of some differences between the composition of mixed saliva and that from the individual salivary glands.

Introduction

Saliva can be described as an essential biological oral fluid known for its importance in combatting the different microbial diseases that might affect the oral cavity such as dental caries and inflammatory mucosal diseases. Many constituents of saliva have proven and potentially protective roles in local immune and non-immune defense mechanisms. The antimicrobial ability has been the focus of many studies that investigated the different antimicrobial factors in health and disease. Studies are also continuously carried out to investigate the natural defense factors of the body so as to utilize them either in prevention or treatment.

Salivary factors which have been proven to play a significant protective role against caries include the bicarbonate and carbon dioxide buffer system, and calcium and phosphate ions.¹ Together these help to resist dental dissolution and encourage remineralisation. Other salivary components have antibacterial properties which include the ability to aggregate bacteria and so prevent the

colonization of mucosal and tooth surfaces.¹ In the vast majority of individuals wounds of the oral mucosa caused by direct mechanical trauma heal rapidly. This is in part due to the excellent blood supply to the mucosa, the antibacterial properties of saliva and the presence in saliva of factors which appear to promote wound healing, possibly by speeding coagulation and possibly also by stimulating neural and epithelial cell growth.²

This paper will review some antimicrobial proteins of saliva which were considered significant in the antimicrobial process and were investigated by many scientists either to know their mechanism of action or to find out the effect of disease on such factors. It will also discuss the disturbances that might affect these factors in conjunction with relevant systemic diseases.

Review

Immune factors (IgA)

There are few diseases that were noted not to be associated with changes in the level of salivary IgA.^{3,5}

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Received 11 April 2001; Revised 24 September 2001; Accepted 18 November 2001

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In a number of diseases, the level of salivary IgA was reduced. Sudh *et al.*⁴ investigated a group of 25 Crohn's patients. IgA of unstimulated saliva was determined by ELISA technique. Although the difference in the level of salivary IgA between patient's group and control group was not statistically significant, 3 patients had undetectable sIgA.⁴

It was also found that children prone to recurrent respiratory infections have lower levels of salivary IgA.⁶ Saliva from lymphoma patients receiving chemotherapy showed decreased concentrations of IgA, however other salivary defensive factors showed no significant decreases.⁷ Lymphoma patients who are on cytostatic drugs had total salivary IgA decreased during cancer therapy, which returned to the baseline level after termination of treatment.⁸ Significantly lower levels of IgA were found in resting saliva of children with chronic protein-energy malnutrition.⁹ Also in thalassaemia major analysis of the parotid saliva showed that the concentration of IgA was significantly lower.¹⁰ The parotid IgA was found to be significantly reduced in HIV-infected patients contributing to the recurrent oral infections that these patients frequently acquire.¹¹ Challacombe and Sweet¹² have also found that whole and parotid saliva contained lower IgA concentration in HIV and AIDS patients.¹² However an earlier study done by Atkinson *et al.*¹³ found that the saliva of HIV-positive patients contained increased levels of IgA in stimulated submandibular saliva.

A study conducted by Schiodt *et al.*¹⁴ revealed that HIV patients with salivary gland disease had significant decrease in the level of salivary protein, and increase in salivary IgA.¹⁴ Insulin-dependent diabetes was shown by Tenovuo *et al.*¹⁵ to be associated with higher levels of salivary IgA than healthy patients. Samples consisted of stimulated whole saliva which were collected from 35 insulin-dependent diabetic patients. Non-insulin dependent diabetics were also shown to have increased levels of salivary IgA.¹⁶ Chronic leukemic patients also had increased level of salivary IgA.¹⁷ Patients with primary Sjogren's syndrome showed higher concentrations of IgA, in whole and parotid saliva reflecting the local inflammatory activity of the salivary glands.¹⁸ Ford *et al.*¹⁹ observed a positive trend in the concentration of total protein and salivary IgA following the administration of radioactive iodine used for the treatment of hyperthyroidism. Patients undergoing open heart surgery also showed increased salivary secretion of IgA.²⁰ In

one variant of amyloidosis, the concentrations of salivary IgA were observed to be higher in patients than in normal subjects.

Non-immune factors

1. Lysozyme

This salivary enzyme was found to be unaffected by some systemic diseases.^{3-4,24} Lysozyme was shown to be reduced in a number of diseases. Patients undergoing open heart surgery showed a decrease in the salivary secretion of non-immune host defense factors including lysozyme.²⁰ Pinducciu *et al.*²² found that insulin-dependent diabetes mellitus (type one) was associated with significantly decreased salivary lysozyme concentration. Other diseases associated with a significant decrease in lysozyme concentration include HIV infection associated with salivary gland disease.¹⁴ In thalassaemia major analysis of the parotid saliva showed that although the concentration of lysozyme was lower, the difference was statistically not significant.¹⁰ Meurman *et al.*⁷ investigated a group of 22 patients treated for Hodgkin's disease and non-Hodgkin's lymphoma by chemotherapy. Lysozyme of stimulated saliva was assessed with a modification of the lysoplate method. They observed that saliva had decreased concentrations of lysozyme. An earlier study done by the same author found that lymphoma patients who are on cytostatic drugs had significant increases in albumin secretion and lysozyme concentration of saliva.⁸

Mandel *et al.*²³ found that salivary lysozyme was increased in HIV patients and that it increased in concentration with time. The saliva of HIV-positive patients was also found to contain increased levels of lysozyme in stimulated submandibular saliva.¹³ Patients with chronic leukemia exhibited normal lysozyme content of saliva which was elevated in those patients with severe periodontal disease.¹⁷ In one variant of amyloidosis, the concentrations of salivary lysozyme, were higher in patients than in normal subjects.

2. Lactoferrin

Normal values of salivary lactoferrin were noticed in a number of diseases.^{3-4,24,26} Other diseases were associated with reduced levels of salivary lactoferrin. Lactoferrin output was shown to be significantly reduced in HIV-positive individuals.¹¹ However, when examining the stimulated submandibular saliva, Atkinson *et al.*¹³

found that saliva of HIV positive patients contained increased levels of lactoferrin. A study conducted by Schiodt *et al.*¹⁴ revealed that HIV patients with salivary gland disease had significantly reduced salivary lactoferrin levels contributing to the recurrent oral infections that these patients frequently acquire. Significantly lower levels of lactoferrin, were found in resting saliva of children with chronic protein-energy malnutrition.⁹ Non-insulin dependent diabetics were shown to have increased levels of lactoferrin.¹⁶ Lactoferrin content was also increased in parotid saliva of irradiated patients.²⁵

3. Amylase

This enzyme was found to be reduced in concentration in certain diseases like the case of lymphoma patients receiving chemotherapy.⁷ However, Laine *et al.*²⁰ found that lymphoma patients who are on cytostatic drugs had no change in total protein and amylase concentrations by chemotherapy.⁸ Patients undergoing open heart surgery have also showed a decrease in the salivary secretion of non-immune host defense factors including amylase. In one variant of amyloidosis, the concentrations of salivary amylase were higher in patients than in normal subjects.²¹

4. Histatins

Lal *et al.*²⁹ found that Histatins were decreasing in AIDS patients. However, Atkinson *et al.* (1990) found that the saliva of HIV-positive patients contained increased levels of histatins in stimulated submandibular saliva.

5. Peroxidase

Normal values of salivary peroxidase were found in a few diseases.^{4,24} Lundgren *et al.*³ found that total protein is high while peroxidase lower in patients with Papillon-lefèvre syndrome. The patients' group consisted of 16 patients with the syndrome. Salivary peroxidase in stimulated saliva was determined using the method of Gothefors and Marlund.²⁰ Patients undergoing open heart surgery also showed a decrease in the salivary secretion of non-immune host defense factors including peroxidase.

In insulin-dependent diabetics the only non-immune factor of saliva that is altered (increased in concentration) is peroxidase.¹⁵ Non-insulin dependent diabetics also had increased levels of salivary peroxidase.¹⁶ Mandel *et al.*²¹ found that salivary peroxidase was increased in

HIV patients and that it increased in concentration with time.²³ In one variant of amyloidosis, the concentrations of peroxidase were higher in patients than in normal subjects.

Discussion

This review of literature aims at realizing the association of important systemic diseases with salivary antimicrobial proteins. In order to be able to study the association of a certain disease with salivary composition, a relatively large number of patients has to be included in the study. This is essential to overcome the individual variations in salivary composition. It is also of prime importance to standardize all the other variables that are known to affect the composition of saliva. However, this is extremely difficult to achieve. The composition of saliva varies according to the salivary flow rate. For example, as the flow rate increases, the concentration of proteins rises. Resting saliva is the ideal state to study saliva as it is the predominant state for most of the day. However, it does not yield the required amount of salivary samples.

Another important issue that faces the investigator is whether the salivary sample mixed (whole) or collected from a particular salivary gland. This is important since the source of saliva may well affect its composition. Intake of food and the circadian rhythmic variations in the concentration of many salivary constituents are two more factors governing the composition of saliva. Considering that salivary collection problems are overcome, there is still one essential factor to be considered. Salivary samples have to be treated and analyzed with caution as many salivary constituents are liable to decomposition if not stored and treated in the appropriate way.

The salivary antimicrobial property may be potentiated by the synergistic action exhibited by some of them. It was shown that salivary antimicrobial proteins may interact in a common system to influence the oral ecology.³¹ This natural defense system may be influenced by the various systemic diseases. It was shown that systemic diseases can induce changes in the salivary composition.³²

It can be observed that the changes that affect saliva composition in the different diseases depend partially on the severity of the disease process and whether it affects the immune system of the body. These changes may also be influenced by whether the salivary glands are affected or not. In certain diseases they are

affected as a part of a generalized disease process e.g. amyloidosis, HIV-associated salivary gland disease, and Sjogren's syndrome.

Salivary immune factors are represented mainly by immunoglobulins, of which IgA is the key immunoglobulin in mucosal immunity.

IgA is present in serum and body secretions like milk, saliva, jejunal fluid, hepatic bile and tears. It mainly acts to inhibit adherence of bacteria to tooth and oral mucosal surfaces and may assist in phagocytosis of bacteria by neutrophils.¹ It also acts by neutralisation of enzymes, toxins, and viruses. It interacts with nonspecific antibacterial factors like lactoferrin, lysozyme and peroxidases. Salivary IgA originates purely from salivary glands. Local inflammatory conditions of the salivary glands will subsequently lead to increased secretion of sIgA. This is evident in Sjogren's syndrome, amyloidosis, diabetes mellitus and HIV infection associated with salivary gland disease. The HIV-associated salivary gland disease has been defined as the presence of enlargement of one or more major salivary glands and/or diminished salivary function in an HIV-infected individual.¹⁴ Some HIV-positive individuals develop a Sjogren's-like syndrome. In most cases of amyloidosis minor salivary gland biopsies are positive for amyloid deposits,³³ and hence they can be used for the diagnosis of familial amyloid neuropathy.³⁴ In conditions requiring more protection for the oral mucosa, there will be more secretion of the sIgA. This is obvious in open heart surgery. Where there is depression of immunity like in HIV infection, thalassemia and malnutrition, IgA levels will fall. It is also hypothesized that the secretion of IgA is impaired in Crohn's disease.⁴

Other essential components of salivary antimicrobial system include amylase and histatins. Amylase is a measure of the secretory function of the salivary glands especially the parotid gland. Histatins are known for being antifungal and bactericidal.²⁷⁻²⁸ Histatins also exhibit affinity to mineral surfaces, inhibit calcium phosphate precipitation and play a role in maintaining teeth integrity.²⁸

Other important components include lysozyme, lactoferrin and peroxidase which exhibit antibacterial effects against cariogenic bacteria.³⁰ Lactoferrin combines with iron and deprives bacteria of this essential element for their growth.¹⁻² There is a synergistic relationship between lactoferrin and lysozyme. It is suggested that lysozyme attaches to sites on the bacterial cell wall rendering the cells more sensitive to lactoferrin. On the other hand, lactoferrin by

removing iron which inhibits lysozyme activity, enhances the antibacterial action of lysozyme.¹ This combined synergistic activity against bacteria, however, does not apply to the fungicidal activity of those two factors where there is no synergism detected.³⁵ Lysozyme originates from both saliva and gingival fluid, and it is found in high concentrations in the secretion of labial mucous glands. Its prime method of action is by destabilising the bacterial cell wall and causing autolysis of the cell. Gram-positive bacteria including the cariogenic *Streptococcus mutans* appear to be most sensitive to its action.²

Local diseases of the salivary glands are noted to increase the levels of lysozyme, amylase and peroxidase as found in amyloidosis. HIV infection itself is associated with increased levels of peroxidase and lysozyme perhaps as an effect of the disease itself on the salivary glands. While IDDM is associated with increased peroxidase levels, it decreases lysozyme levels. It is also noted that the decrease in salivary lysozyme level takes place where there is bone marrow depression. This can be a consequence of chemotherapy. Cytostatic treatment by causing long term immunosuppression affects saliva composition.⁷

While IgA secretion increases with salivary gland disease, it falls in disorders causing depression of the immunity. However, this observation does not apply completely in the case of non-immune defense factors like lysozyme and peroxidase. The fact that some of these factors do not originate purely from salivary glands, might add to the complexity of studying these factors.

Preferably, there should be further research to support or add to our knowledge about this subject. More importantly, the methods for saliva collection and analysis should be more standardized to overcome the differences obtained in measuring the various salivary components. This should then make possible our optimum goal of utilizing the natural components of the body to prevent and eliminate the different diseases particularly those with difficult cure or no cure.

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