THE ROLE AND SIGNIFICANCE OF SOME SALIVARY AMINO ACIDS IN PERIODONTAL DISEASES, INCLUDING PLAQUE-INDUCED GINGIVITIS—A LITERATURE REVIEW

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ABSTRACT

Nowadays the problem of plaque-induced gingivitis, including in the different periods of childhood, is marked by a considerable long-term sociomedical impact on individual, group, population and community levels globally. The purpose of this investigation is to study, analyze and summarize various scientific sources on the role and significance of some salivary amino acids in the presence of periodontal diseases, including plaque-induced gingivitis. This manuscript is based on data obtained from research articles, randomized trials, and systemic reviews published in PubMed and Google Scholar. Different salivary markers are characterized by potential for earlier diagnosis of the progression of oral diseases. We can conclude that some salivary amino acids play a considerable role in the periodontal status, including in plaque-induced gingivitis.

Keywords: *saliva, amino acids, plaque-induced gingivitis, periodontal status*

INTRODUCTION

Nowadays the problem of plaque-induced gingivitis, including in the different periods of childhood, is marked by a considerable long-term sociomedical impact on individual, group, population and community levels globally. The rate of distribution of plaque-induced gingivitis corresponds to the prevalence of tooth decay, affecting hard teeth tissues. Based on scientific literature sources, the short- and long-term effect of gingival inflammation on the general health is neglected to some extent. In addition, the adequate control and efficient management of the carious process is related to the maintenance of proper gingival health (1,2).

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AIM

The purpose of that investigation is to study, analyze and summarize various scientific sources on the role and significance of some salivary amino acids in periodontal diseases, including plaque-induced gingivitis.

MATERIALS AND METHODS

This manuscript is prepared on the base of data obtained from research articles, randomized tri-

als, and systemic reviews published in PubMed and Google Scholar.

LITERATURE REVIEW

Plaque-Induced Gingivitis

In children with primary dentition, the physiological norm of the appearance of gingival tissue has to be taken into consideration, red in color because of abundant blood supply, shiny and smooth, without roughness of the relief. The same clinical sings of gingival hyperemia, shiny and smooth gingival surface, correspond to the identification of a mild degree of gingivitis in children with mixed dentition and these with permanent dentition. The precise diagnosis of gingival inflammation in children definitely corresponds to the age-related morphological and physiological characteristics of gingival tissue (1, 2).

In primary dentition, the clinical manifestation of advanced gingivitis is associated with the activated inflammatory cells, which invade the gingival tissue. The good dental practice requires a profound assessment of the caries risk level and evaluation of the risk of gingivitis in children. Dental medicine doctors, especially pediatric dentists, must not neglect the significance of thorough examination of the state of gingival tissues. Even under conditions of diagnosed mild degree of gingivitis, measures have to be taken for its adequate treatment, thus avoiding deterioration of the overall health of the periodontal apparatus in later periods of childhood and in the early stages of adulthood (1,2).

Plaque-induced gingivitis in children and adolescents is characterized as a non-specific inflammation of gingiva. The accumulation of bacterial dental plaque provokes inflammatory reaction in gingival tissues, with impact upon the immune system reactivity of the macro-organism. Dentists have to examine and analyze not only the clinical indicators and X-ray images, but also to pay attention to family anamnesis of elevated risk of periodontal diseases (3–5).

Based on epidemiological investigations, plaque-induced gingivitis is considered to be a prevailing periodontal noxa at all stages in dentate populations (6–10) and is characterized as the most common form of periodontal disorder (11). The early alterations of gingival tissues in plaque-induced gingivitis may not be identified clinically (12). The emphasis is on clinical determinants for distinguishing physiologic state vs. pathologic condition. Plaque-induced gingivitis affects initially the gingival margin and may afflict the rest of the gingival tissues in later stages of progression. Moderate degree of gingivitis is associated with bleeding provoked by tooth brushing, edematization and hyperemia of gingiva, blood in saliva (3–10). These clinical signs are of considerable importance for the proper planning and implementation of preventive programs and measures (11–16).

Some researchers accentuate on the alterations and fluctuations of the immune reactivity towards periodontal pathogens in different periods of childhood, especially in adolescence. The increase of the risk of gingivitis and its progression is related to the growth and overload of dental plaque-associated pathological microflora, inducing non-specific and specific immune defense responses. Pediatric dentists have to concentrate on the proper diagnosis and treatment, as well as upon the primary, secondary and tertiary prophylactic measures based on the conditions and requirements of the clinical situation itself. Professional dental care provides long-term prevention from increased susceptibility to periodontal disorders, which definitely afflict the common health status of the macro-organism, especially in growing children and adolescents who are influenced by the intensive dynamics of hormones influencing gingival tissues (17).

Clinical Indicators for Plaque-Induced Gingivitis

The conventional diagnostic methods for recording the clinical manifestation and evaluation of the degree of plaque-induced gingivitis include the application of both the Silness-Löe plaque index (PLI) and the gingival index (Löe and Silness) (GI)

Plaque index Silness-Löe is applied for evaluation of dental plaque accumulation based on its registration on Ramfjörd teeth: 16, 22, 24, 36, 42, 44. In primary and mixed dentition, the missing representative teeth are replaced by the mesially situated teeth. The index is recorded by scratching with an atraumatic periodontal probe on the vestibular, oral, mesial and distal surfaces along the contact with the marginal gingiva. The degree of dental plaque accumulation is assessed by the figures 0, 1, 2, 3. The value 0 corresponds to lack of dental plaque on the examined

tooth surface. The figure 1 accounts to a very small amount of dental plaque on the peak of the probe. The figure 2 is related to a moderate amount of dental plaque scratched by the probe. The figure 3 is equivalent to a considerable, naked eye-visible accumulation of dental plaque on the respective tooth surface. The sum of all the figures from all the examined teeth surfaces is divided by the total number of examined surfaces, respectively 24. The obtained results can be interpreted in the context of definite scale intervals. The values of PLI which belong in the interval from 0.1 to 1.1 correspond to very good and good oral hygiene. The values of PLI in the range between 1.2 and 2.0 describe good to satisfactory oral hygiene. The values of PLI from 2.1 to 3.0 indicate satisfactory to poor oral hygiene, parallel to the increase of the level of PLI. In plaque-induced gingivitis the established average value of PLI corresponds to the recorded average value of GI.

The gingival index (Löe and Silness, 1963) is applied for the evaluation of the gingival state and indicates alterations in the gingival tissue. This index is implemented for the assessment of the distribution and severity of gingivitis on individual, group and population level. It records the condition of the marginal and interproximal sites separately in the range between 0 and 3. The figures are: 0 = normal gingiva; 1 = mild inflammation—hyperemia and mild edema, without bleeding; 2 = moderate inflammation—hyperemia, edema and provoked bleeding; 3 = severe inflammation—hyperemia, edema, spontaneous bleeding. The bleeding is assessed by atraumatic probing along the site of soft tissue of the gingival sulcus. The figures of the four surfaces of the gingival tissues encircling the tooth (buccal, mesial, lingual, and distal) can be summarized and divided by four to give the value of GI for the representative teeth. The sum of these values is divided by the total number of examined surfaces, respectively 24, along the representative teeth: 16, 22, 24, 36, 42, 44. In primary and mixed dentition, the missing representative teeth are replaced by the mesially situated teeth. Based on the application of the GI index are determined these diagnostic rates of values: GI = 0 designates normal gingiva; GI values from 0.1 to 1.0 correspond to mild inflammation; GI levels from 1.1 to 2.0 account to moderate inflammation; GI rate from 2.1 to 3.0 signifies severe inflammation (18,19).

These widely applied clinical indicators for identification of the state of gingival tissues serve as important tools, but they record any current deviation of the physiological norm without any prognostic accent upon the tendency of control and management of oral health disorders on individual level. Simultaneously, the contemporary dental medicine is focused on individual specifics in the course of the disease, taking into consideration the macro-organism's reactivity not only on systemic, but also on local level in the oral cavity.

Saliva as a Diagnostic Medium

Personalized dental medicine is conceptually associated with the terms of genomics, transcriptomics, proteomics, salivaomics. The term salivaomics was implemented in 2008. It is related to the advanced researches about the various organic and non-organic ingredients composing saliva. Salivary proteomics explores the salivary proteins and their capacity for evaluation of oral-dental health and the pathogenetic mechanisms of oral cavity diseases (20–22). Saliva is composed of approximately 1166 proteins. Approximately 70% of the proteins in saliva are not present in the circulating blood. These proteins are related to the innate mechanisms of maintaining the dynamic equilibrium of homeostasis in the oral cavity. Some of them have the potential of being diagnostic and prognostic tools in oral-dental disorders. The majority (98%) of the total amount of salivary proteins are these with diagnostic role: proline-rich proteins, Ig A, cystatin, hystatin, statherin, mucin, lactoferrin, lysozyme, albumin, α-amylase (23–26). In periodontitis a decrease in the levels of salivary proteins, which is related to periodontal inflammation, is established. In addition to proteins, some free amino acids in the saliva also have diagnostic potentials. This is related to widening of the diagnostic spectrum of salivary markers in correspondence with prognosis, control and management of oral disorders. Some of these free amino acids are characterized by protective properties essential for the maintenance of proper periodontal and gingival health status (27,28).

Salivary Amino Acids and Periodontal Health Status

Table 1 presents the importance of some salivary amino acids associated with the periodontal status.

Table 1. Some salivary amino acids associated with the periodontal status.

Salivary Amino Acids	Significance for Periodontal Status	*References*
Proline	Increased levels in saliva are indicative for: higher rate of collagen degradation; proline-rich protein's degradation; increased release of proline from the inflamed gingiva	(29) Syrjanen, et al., 1984 (31) Syrjanen, et al., 1990
Hydroxyproline	High levels of hydroxyproline in gingiva and gingival crevicular fluid are found in the group of participants with progressive periodontal disease, compared to periodontally healthy controls, related to collagen degradation	(49) Akalin et al., 1993
Glycine	Increased levels in saliva are indicative for intensive collagen degradation; glycine in saliva could exert anti-inflammatory and immune-modulatory properties under conditions of gingival inflammation	(59) Hino et al., 2016 (60) Schaumann et al., 2013
Lysine	Lysine is important for the maintenance of the continuity and constant recovery of clinical attachment level; inhibition of the process of penetration of microbial products into the gingival sulcus; the depletion of the salivary lysine contribute to periodontal diseases	(67) Tábi et al., 2008 (70) Levine et al., 2017
Hydroxylysine	Increased levels are indicative for intensive collagen degradation; increased hydroxylysine levels are found in whole saliva of periodontal patients	(31) Syrjanen, et al., 1990
Arginine	The role of arginine in the composition of dental biofilm is associated to the dynamics of co-aggregation between various microorganisms; suppresses the co-aggregation of Prevotella oris and Porphyromonas gingivalis, which are related to periodontitis; inhibits the co-aggregation between Porphyromonas gingivalis and Prevotella intermedia/Streptococcus oralis/Actinomyces viscosus/Treponema denticola, respectively; arginine has the potential to influence the qualitative and quantitative traits of dental plaque biofilm	(76) Bernier et al., 2011 (77) Borriello et al., 2006 (78) Sato et al., 2014 (79) Zhu et al., 2007 (80) Huang et al., 2017 (81) Ledder et al., 2017
Glutamine	An increase of gingival glutamic acid is established among patients suffering from periodontal disorders; glutamine has the effect to arrest the progression of periodontal disease; oral glutamine supplementation inhibits the progression of periodontitis	(31) Syrjanen, et al., 1990 (87) Ahmadi-Motamayel et al., 2017 (89) Junior et al., 2018

Proline

Some researchers reported an increased level of some salivary free amino acids among patients with diagnosed periodontal disorders (29). In the context of multi-aspect investigations, the diagnostic potentials and capacity of prognosis of various compounds of saliva have been examined for the purpose of efficient long-term management of periodontal diseases (29–33). The main source of free amino acids in whole mouth saliva is the proteolytic degradation of salivary proteins (31). It is proved that sterile saliva collected from the gland is almost absent of free

amino acids (34–36). Some of the major proteins in human saliva are proline-rich proteins (37). It is assumed that some of the amino acids found in saliva are products of disintegration of proline-rich proteins by bacterial enzymes or from degradation of collagen in case of gingival inflammation (31). Proline is one of the most abundant amino acids in saliva. This amino acid is one of those which are not utilized from oral microbiota. For example, lysine, glycine, glutamate, and arginine are further utilized by microbial activity (34–39).

Plague-induced gingivitis, especially its moderate and severe degree of clinical manifestation, is characterized by inflammation of gingiva, accompanied by reversible impingement on its connective tissue (40–42).

A total of 60% of the amount of proteins in normal healthy gingiva is represented by collagens. These are also a predominant ingredient of periodontal ligaments and alveolar bone. The increased synthesis of enzymes related to the degradation of collagen provokes the initiation and advancing of periodontal disease accompanied by a 70% loss of the collagen. It is established that disintegration of collagen can be associated with the release of hydroxyproline (Hyp), which represents approximately 10% of this protein (43–48). About 60–70 % of the collagen is destroyed in the region affected by gingival inflammation. The afflicted tissues are characterized by a several-fold reduction of the quantity of collagen rather than its abundant accumulation and, as a result, an intensified destruction is established. Other researchers also confirm that Hyp levels in gingiva are a product of the disintegration of collagen (43–46). The quantity of collagen-derived Hyp in gingival crevicular fluid (GCF) corresponds to the intensity of collagen loss and the activity of the pathological process in periodontal diseases. Researchers focus on the potentials of Hyp in GCF as an indicative parameter for loss of collagen in progressive periodontal disease (43–57).

Glycine

One of the most abundant amino acids in non-stimulated mixed saliva is glycine. Based on a study, a considerable increase of the salivary concentration of glycine in elderly people was established. Its levels maintain the ratio of glycine to proline at approximately the same level with that of collagen. It was assumed that the elevation of salivary concentrations of glycine and proline parallel to the aging process (from children to elderly people), maintaining the ratio of both of these amino acids and the levels established for collagen the same, may be because of intensive collagen degradation. Collagen is a product of the activity of mesenchymal cells—fibroblasts, odontoblasts, osteoblasts, intercellular matrix. The oral cavity is characterized by various populations of fibroblasts whose role is to maintain the homeostasis at the level of connective tissue. Proline is hydroxylated first by reducing agents of ascorbic acid, as a result protocollagen is formed in the endoplasmatic reticulum. The protocollagen is released and goes through a process of maturation into the extracellular matrix. Researchers have hypothesized that, as a result of the reduction of intracellular antioxidant levels with age, the levels of hydroxylated proline are decreasing. As a consequence, an increase of the secretion of immature procollagen has been established. The latter is easily afflicted by cellular proteases or bacterial collagenolytic proteases, which considerably increases the quantities of glycine and proline in the saliva (58).

In patients with periodontitis and gingivitis, the inflammatory process is activated by the participation of different immune cells and cytokines. It was reported that the free amino acid glycine in saliva could exert anti-inflammatory and immune-modulatory properties in gingival inflammation (59). Immune cells such as alveolar macrophages, neutrophils, and Kupffer cells have been proven to contain glycine-gated chloride channels (60–62). It was also determined that there is a glycine receptor on epidermal keratinocytes in the oral cavity (59). By an immunohistochemistry method, a high glycine receptor expression capacity within the epithelial layers of the gingival tissue has been established. The existence of a glycine receptor on gingival keratinocytes and the anti-inflammatory properties of glycine were demonstrated by an *in vitro* experiment using gingival epithelial cells after stimulation by IL-1β. In an already present inflammatory process, salivary glycine, in combination with IL-1β, has led to a significant inhibition of the expression of IL-6 (59). The glycine anti-inflammatory potential in oral tissues was demonstrated also by Breivik et al. In their animal experiment, they demonstrated reduction of artifi-

cially induced periodontal infection by supplementation with glycine (60-64).

Lysine

The amino acid lysine is important for the maintenance of the continuity and constant recovery of clinical attachment level, inhibiting the process of penetration of microbial products into the gingival sulcus. Under conditions of improper oral hygiene, various bacterial products invade the lysine-deprived clinical attachment level, which provokes the onset of an inflammatory process (65).

The activity of the enzyme lysine decarboxylase produced by *Eikenella corrodens* is associated with gingivitis. This enzyme catalyzes the conversion of lysine to cadaverine and carbon dioxide, and depletes its concentration in the oral cavity (65, 66). The depletion of lysine concentration indicates the presence of higher amount of bacterial lysine decarboxylase, which may contribute to periodontal diseases. The activity of that enzyme and the produced cadaverine have a protective effect for the microbiome in dental biofilm, stabilizing the development of a dysbiotic microbiome related to periodontitis (67–70). Cadaverine protects the oral biofilm microorganisms from bactericidal and bacteriostatic effects of the generated reactive nitrogen (RNS) and reactive oxygen species (ROS) and increases the development of a butyrate-producing microbiome, which slowly destroys the periodontium (71).

Some researchers find hydroxylysine in whole saliva but not in the gingival crevicular fluid of periodontal patients and healthy controls, and conclude that the amino acids released during collagen degradation are characterized as non-stable metabolic products (31). Hydroxylysine is one of the amino acids constituent of collagen together with proline, hydroxyproline and glycine, and represents approximately two-thirds of the total amino acid residues of collagen. Collagen degradation is increased in gingival inflammation (31).

Arginine

Salivary glands secrete a free form of arginine at the average rate of 50 μmol/L. Arginine is found also in salivary secretions in the forms of peptides and proteins from which this amino acid can be released by the activity of proteases and peptidases (72). In the oral cavity, arginine is catabolized initially with the participation of the arginine deiminase system. The products of this reaction are ammonia, CO2 and ornithine, accompanied by the synthesis of ATP (73). The microbial enzyme of arginine deiminase is characterized by essential physiological activities: maintenance of pH homeostasis in dental biofilm, avoiding lethal acidification of cells, supplementation of ATP ensuring the process of growth (72). The role of arginine in the composition of dental biofilm is associated with the dynamics of co-aggregation between various microorganisms (74–77). It was established that arginine suppresses the co-aggregation of *Prevotella oris* and *Porphyromonas gingivalis,* which are related to periodontitis. Arginine also inhibits the co-aggregation between *Porphyromonas gingivalis* and *Prevotella intermedia/Streptococcus oralis/Actinomyces viscosus/Treponema denticola*, respectively (76).

The amino acid arginine is also characterized by the capacity to exercise an impact upon the growth of *Staphylococcus aureus* into dental plaque biofilm (77). Some studies investigated arginine metabolic activity in polymicrobial dental biofilms. It was established that arginine has the potential to influence the qualitative and quantitative traits of dental plaque biofilm (78, 79).

Glutamine

A ratio of about 20% of the total amount of amino acids in the human body is represented by glutamine (80). A statistically significant increase of gingival glutamic acid is established among patients suffering from periodontal disorders in comparison to a control group of participants (31). A considerable elevation of the rate of glutamic acid and glutamine is recorded in gingival inflammation provoked by either IL-1β or bacterial lipopolysaccharides (59).

Glutamine is characterized by its protective function for the organism's immune reactivity. The reduction in this amino acid results in decreased intensity of proliferation of lymphocytes and antibodies caused by antigens. Supplementation with glutamine leads to a suppression of the infection rate and decreased duration of the hospitalization period (81,82). Periodontal disorders are usually infectious processes related to the initiation and progression of inflammation with impact upon the supporting apparatus of the tooth, affecting the junctional

epithelium level in the gingival sulcus (83). A definite symptom of the progressing periodontal disorder is the establishment of gingival bleeding, which can be accompanied by pain symptoms and serous or purulent secretion (84).

Glutamine arrests the progression of periodontal disease, which is related with its property to suppress the effect of oxidative stress. The latter is related to provoking an inflammatory response, which causes disintegration of periodontal tissues (85). Besides the definite anti-microbial activity of glutamine, there is a focus on its immune-stimulating effect. The periodontopathogenic bacteria can provoke apoptosis, which leads to a series of pathophysiological reactions with the participation of IL-6. On the other hand, in enforced oxidative stress, in cases of periodontitis, glutamine is shown to inhibit the apoptosis in periodontal tissues (86). It was established that oral glutamine supplementation among a group of examined participants resulted in inhibited progression of periodontitis (87).

DISCUSSION

Contemporary diagnostic methods are related to investigations of the diagnostic potentials of saliva based on its qualitative and quantitative characteristics, organic and non-organic compounds, especially in the context of the wide distribution of tooth decay and gingivitis globally (88-90). The procedure of collection of non-stimulated saliva is relatively easily implemented and is non-invasive and atraumatic for the individual which is of great importance regarding children (91,92). Researches are predominantly devoted to the significance of gingival crevicular fluid for diagnostic purposes, with considerable informative and prognostic value of its components. Simultaneously, non-stimulated mixed saliva is characterized by markers, which originate from serum and blood cells from oral wounds, gingival crevicular fluid, desquamated epithelial cells, bacteria and bacterial products, expectorated bronchial secretions. Saliva includes systemically derived and local variables of periodontal diseases (93,94). Assays based on salivary samples have the potential of monitoring patients' periodontal health status. This is related to the capacity of saliva for performance of a target-oriented, efficient diagnostic, prognostic, therapeutic and prophylactic approach (93).

Some salivary markers can potentially help with the earlier diagnosis of the progression of oral diseases including gingivitis. Gingival inflammation caused by pathological accumulation of dental plaque (plaque-induced gingivitis) is characterized as a mild form of periodontal disease (92,93). The prevailing scientific sources devoted to the role and dynamics of salivary amino acids concern diagnosed periodontal disorders characterized by bone loss. A relatively smaller number of investigations are related to their fluctuations in gingival inflammation and inflammatory response on a local level (29,30,31,39,93,94).

CONCLUSION

We can conclude that some salivary amino acids have a definite significance and play a considerable role in the periodontal status, including in plaque-induced gingivitis.

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