

Adaptive-Compensatory Changes in the Oral Fluid at Various Degrees of Adentia

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Abstract

The purpose of this study was to identify some aspects of the mechanism of adaptive-compensatory changes in mixed saliva at various degrees of adentia in humans. Thirty patients (mean age 40.6 ± 3.5 years) with varying degrees of partial adentia were examined. The patients were divided into 3 groups. The first group ($n = 12$) consisted of practically healthy people in whom the integrity of the dentition was preserved. The second group consisted of patients with partial adentia, who had no more than 3 teeth missing ($n = 16$). The third group included patients with partial adentia, who had 4-6 teeth missing ($n = 14$). It was revealed that the intensification of LPO processes, which induces a change and imbalance in the AOD system of saliva in patients with adentia, is confirmed by the results obtained in the study of the activities of GR, HT and the content of reduced and oxidized glutathione. Adentia also leads to a decrease in the nonspecific resistance of the oral fluid, which can lead to a deterioration in reparative processes, complicate adaptation in various types of prosthetics.

Keywords: dentoalveolar region, adentia, depression, dysfunction, defect, immunity, saliva, blood, adaptation.

Introduction

Keeping healthy teeth is one of the most important signs of a high quality of life - they provide nutritional value, aesthetic appearance, and an active lifestyle. As you know, the quality of human life directly depends on the condition of the dentition. The complete or partial loss of teeth affects the choice of food, due to the violation of the physiological processes of chewing and digestion, the conditions for its intake, as well as the appearance and socio-psychological behavior of people. Adentia negatively affects the role of a person in society, significantly disrupting communication between people, causing less satisfaction with life, a sense of their own inferiority, and depression. It should be noted that with defects in the dentition, the anatomical and topographic proportions of the facial skeleton change, osteoporosis, atrophy of the bone, masticatory and facial muscles progresses, dysfunction of the temporomandibular joint occurs, which requires immediate orthopedic treatment.

In recent years, attention has been significantly increased to the study of the unique properties of saliva and the associated diagnostic capabilities. A lot of new data have been obtained on the functions and composition of the oral fluid in healthy people and in various dental diseases. Saliva is the most important factor in maintaining the homeostasis of the oral cavity, and on the other hand, changes in the composition of saliva reflect the shifts that occur in the dentition. It should be noted that the number of studies devoted to the study of physicochemical parameters and indicators of oral fluid metabolism in adentia is limited. Interest in these issues arises in connection with the anatomical unity of the oral cavity, dentition and salivary glands, which independently produce and secrete from the blood the main components that form mixed saliva. The body affects the tissues of the tooth through saliva, which makes it an important factor in maintaining dental health. The study of mixed saliva reveals ample opportunities for identifying individual links in the pathogenesis of various diseases of the oral cavity, including edentia, and allows us to identify the nature of adaptive-compensatory changes at the molecular level and substantiate the possibility of metabolic correction in edentia of various origins. Based on the foregoing, the purpose of this study was to identify some aspects of the mechanism of adaptive-compensatory changes in mixed saliva with various degrees of adentia in humans.

Materials and research methods

We examined 30 patients (mean age 40.6 ± 3.5 years) with varying degrees of partial adentia. The survey carried out included the methods of clinical examination (interview, examination). When examining the dental status, attention was paid to the state of the oral mucosa, gums, and teeth. The

patients were divided into 3 groups. The first group (n = 12) consisted of practically healthy people in whom the integrity of the dentition was preserved. The second group consisted of patients with partial adentia, who had no more than 3 teeth missing (n = 16). The duration of partial adentia in patients of this group did not exceed 0.5 years. The third group included patients with partial adentia, who had 4-6 teeth missing (n = 14). The duration of the existence of adentia in patients of group 3 did not exceed 1 year. In both groups, no additional methods of bone tissue regeneration were used. In patients of the 2nd and 3rd groups, at the initial examination, an unstimulated oral fluid was collected before any therapeutic manipulations were performed. The mixed saliva was collected from the mouth in the morning before meals, centrifuged at 600 g for 10 minutes. For research, a super sedimentary liquid was used. The content of total, reduced (GSH), oxidized (GSSG) glutathione and the ratio of the reduced form of the tripeptide to the oxidized form were determined by the method proposed by M.E. Anderson (1985) modified by I. Rahman et al. (2006). The results are presented in nanomoles per mg of protein. The glutathione reductase activity was assessed by the NADPH-dependent reduction of GSSG with its further interaction with 5,5-dithio-bis (2-nitrobenzoic) acid, leading to the formation of thio-2-nitrobenzoic acid, the aqueous solution of which has an absorption maximum at a wavelength of 412 nm. Glutathione peroxidase activity was determined by the ability to catalyze the reaction of GSH interaction with t-butyl hydroperoxide. The results of the activity of the studied enzymes were expressed in micromoles per minute per 1 mg of protein. Statistical processing of the results was carried out according to the Student's method using the Microstat software package: Microsoft Excel 97. Differences were considered significant at $p < 0.05$. Correlation coefficients were calculated using Spearman's method.

Research results and their discussion.

As you know, the formation of free radicals and reactive oxygen species under certain conditions is of a protective and compensatory nature, however, in concentrations exceeding physiological, they can damage the structures of the cells of the organs of the oral cavity, triggering the development of the pathological process. In this situation, the glutathione-dependent system of enzymatic AO, including GPO, GR, and reduced glutathione, acts as the second link in the body's defense against oxidative damage. Based on the literature data on the work of the glutathione-dependent link, we can talk about a significant imbalance in the glutathione system in various pathological conditions of the oral cavity, as a result of which conditions are created in the saliva of patients for further accumulation of ROS in the oral cavity, which, in turn, can cause oxidative modification and conformational changes in macromolecules in the oral cavity, as well as disrupt their synthesis and degradation, contribute to the progression of pathological processes in the oral cavity. It should be noted that glutathione is involved in the synthesis of leukotrienes and is a cofactor for the enzyme glutathione peroxidase. Glutathione also contains an unusual peptide bond between the amino group of cysteine and the carboxyl group of the side chain of glutamate. The value of glutathione in the cell is determined by its antioxidant properties. In fact, glutathione not only protects the cell from toxic free radicals, but also generally determines the redox characteristics of the intracellular environment. In the cell, thiol groups are in the reduced state (SH) at a concentration of about 5 mM. In fact, such a high concentration of glutathione in the cell leads to the fact that it reduces any disulfide bond (S-S) formed between the cysteine residues of intracellular proteins. In this case, the reduced form of glutathione GSH is converted into oxidized GSSG. As can be seen from the presented research results (table 1), the content of total glutathione decreased as adentia increased, and the lowest rates were noted in group 2 of the examined individuals, where the concentration of the latter in the oral fluid decreased by 46% relative to the indicators of the comparison group. The fraction of oxidized glutathione in the examined patients with adentia increased with tooth loss. So in the 1st group of the surveyed persons, it increased by 19%, while in the second group its values exceeded the initial indicator by 42%. This fact proves its active use in the process of glutathionylation of proteins due to the high reactivity of oxidized glutathione in relation to the SH-groups of proteins. Oxidized glutathione is reduced by the action of the enzyme glutathione reductase, which is constantly in the cell in an active state and is induced during oxidative stress. The ratio of the reduced and oxidized forms of glutathione in the cell is one of the most important parameters that indicates the level of oxidative stress. As indicated by the obtained research results, there was a statistically significant decrease in the value of the GSH / GSSG ratio (1.5 times in the first group of patients and 1.9 times in the second group of examined individuals, $p < 0.05$) compared to healthy individuals. Thus, in addition to glutathione, SH groups of intracellular proteins are actively involved in maintaining redox homeostasis. The obtained research results indicate that high rates of interaction of sensor molecules with ROS are provided by the presence of binding sites that contain a functionally active residue of the molecule capable of directly reacting with an oxidizing agent. In proteins, this role in redox signaling is played by cysteine residues (Cys-SH). When interacting with activated

oxygen metabolites, they can undergo oxidation into cysteine sulfenic (Cys-SOH), cysteine sulfinic (Cys-SO₂H) and cysteine sulfonic (Cys-SO₃H) acids, thereby playing an important role in cellular signaling by changing the conformation and activity of proteins. The formation of oxidative stress under hypoxic conditions was also indicated by an imbalance of enzymes in the glutathione system.

According to the data obtained (Table 1), significant deviations in the activity of antiradical defense enzymes were observed in the oral fluid of patients with partial edentulousness. It is known that glutathione peroxidase protects cells from oxidative damage and catalyzes the reduction of lipid hydroperoxides to the corresponding alcohols and the reduction of hydrogen peroxide to water. The analysis of the obtained research results showed a decrease in its activity in patients of the first group by 34%, in the second group - by 56% relative to the indicators of the comparison group.

Table 1
Dynamics of the reduced glutathione content and the activity of glutathione peroxidase and glutathione reductase in patients with secondary adentia

Indicators	Healthy persons n = 12	I- group n = 16	II-group n = 14
Total glutathione, nmol / mg protein	5,92±0,31	4,74±0,27*	3,21±0,21*
Oxidized glutathione nmol / mg protein	0,31±0,01	0,37±0,02*	0,44±0,03*
Reduced glutathione nmol / mg protein	5,74±0,31	4,61±0,25*	4,24±0,21*
GSH / GSSG, conv. units	18,25	12,46	9,64
Glutathione peroxidase μmol / min / g. squirrel	52,13±4,61	34,42±2,51*	22,89±1,74*
Glutathione reductase μmol / sec / g. squirrel	29,43±1,68	22,03±1,14*	18,31±1,43*

Note: *- reliability of differences P <0.05 relative to the indicators of the comparison group

Oxidized glutathione is reduced by the action of the enzyme glutathione reductase, which is constantly active in the cell and is induced during oxidative stress. It is known that reduced glutathione is required for the normal functioning of GPO, because is a coenzyme of this enzyme. It is interesting that the activity of the glutathione reductase enzyme involved in the conversion of the oxidized form of glutathione into the reduced form, probably under conditions of oxidative stress, inhibits one of the enzymes of the hexose-monophosphate shunt glucose-6-phosphate dehydrogenase, which supplies reduced coenzymes NADPH₂ under the action of glutathione regeneration or, possibly, there is a direct inhibition of GH by active oxygen metabolites. As can be seen from the presented research results in the studied groups of patients in the oral fluid, there was a decrease in the GH activity in all the studied groups. Consequently, changes in the activity of glutathione-dependent enzymes in the oral fluid in patients with edentulousness of varying degrees may reflect the severity of compensatory reactions and serve as a biochemical marker of the efficiency of AOD functioning in the oral cavity. The nature of changes in the activity of antiradical defense enzymes under conditions of secondary adentia of varying severity indicates a serious imbalance in the work of the enzymatic link of the antioxidant system. Our data are consistent with the literature data. Thus, in the oral fluid of patients with secondary adentia, significant disturbances are observed in the exchange of one of the main cellular antioxidants - reduced glutathione, which is capable of both independently reducing reactive oxygen species and, together with HPO, catalyzing the reduction of hydrogen peroxide and organic peroxides. One of the most probable reasons for the detected metabolic shift is a sharp decrease in the activity of GR, which regenerates GSH.

The most informative methods for assessing the state of local immunity in the oral cavity is the determination of the components of the body's immune system. An important role in the protection of the oral mucosa from damaging factors is secretory immunoglobulin A (sIgA), which is responsible for local protection. SlgA can bind toxins and, together with lysozyme, exhibits bactericidal and antiviral activity. The decrease in the concentration of SlgA observed in our studies (table 2) indicates a lack of local immunity function.

IgG is the main serum immunoglobulin and is practically undetectable in the saliva of healthy middle-aged people. Its entry into the gastric fluid is observed only with an increase in the permeability of the hematoparenchymal barrier of the gingival mucosa, which is observed as a result of involutive changes. In the study of the content of secretory IgA, as well as IgG and IgM with adentia of varying severity, significant deviations were revealed. The observed changes indicate a lack of local immune protection in the oral cavity, which directly correlates with the severity of adentia. Along with this, a significant increase in the concentration of class G immunoglobulins in the oral fluid was noted, which reflects the progressive

tension in local immunity with partial and complete loss of teeth and indicates possible violations of trophic and microcirculatory processes in these patients, which, undoubtedly, have a negative effect on reparative processes. A similar nature of the changes was found in relation to the concentration of IgM in saliva, an increase in the content of which correlated with an increase in the severity of adentia. A possible mechanism of the observed increase in the concentration of both IgM and IgG in gastric cancer in adentia may be their increased selective transport across the epithelial barrier caused by a deficiency of secretory IgA.

Table 2
Dynamics of indices of local immunity in the oral fluid in patients with secondary adentia

Indicators	Healthy persons n = 12	I- group n = 16	II-group n = 14
Secretory immunoglobulin A (sIgA) level g / l	0,112±0,009	0,077±0,003*	0,063±0,002*
Immunoglobulin G (IgG) level g / l	0,041±0,001	0,053±0,001*	0,062±0,001*
Immunoglobulin M (IgM) level g / l	0,011±0,001	0,019±0,001*	0,031±0,002*
Lysozyme activity (µg / ml)	21,47±0,67	14,72± 0,43*	10,31± 0,18*

Note: *- reliability of differences $P < 0.05$ relative to indicators comparison groups

The bactericidal properties of the oral fluid are due not only to immunoglobulins, but also to minor glycoproteins and, above all, lysozyme. When studying the activity of lysozyme in the oral fluid of patients with secondary adentia, we found that adentia leads to a decrease in the enzyme activity, therefore, a decrease in the nonspecific resistance of the oral fluid, which can lead to a deterioration of reparative processes, complicate adaptation in various types of prosthetics.

Conclusion

1. In the oral fluid of patients with varying degrees of edentulousness leads to a significant imbalance in the metabolites of the patients' saliva. An increase in LPO processes induces a change and imbalance in the AOD system of saliva in patients with adentia, which is confirmed by the results obtained in the study of the activities of GR, HT and the content of reduced and oxidized glutathione.

2. Adentia leads to a decrease in the nonspecific resistance of the oral fluid, which can lead to a deterioration of reparative processes, complicate adaptation in various types of prosthetics

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