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CORRECTIVE MEASURES TO RESTORE ACID-BASE BALANCE IN THE ORAL CAVITY

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Abstract

Changes of the acid-base balance in the oral cavity are oppositely directed: towards acidosis and alkalosis under the influence of destabilizing factors, such as congenital malformations, food, water, air composition, meteorological and professional factors, smoking and other pernicious habits, oral hygiene means and care, medicines and treatment effects and, finally, tooth fillings and dental prosthesis. Our previous studies have shown that pH-metry methods are rather sensitive to reveal and measure changes in the local homeostasis of the oral cavity. The study was aimed at the comparative evaluation of acid-base balance performance correction in the oral cavity of patients with a number of chronic diseases.

The comparison of indices obtained prior to and after the acid-base balance corrective procedures in mentioned patients allowed to reveal new mechanisms in regulation of this balance at different biotopes of the oral cavity. It was established that the change in the acid- and ammonia-producing activity of the microflora of some biotopes affected the studied indices in other biotopes. A significant influence of the nutrition character and dental hygienic care, as well as medical management options, which promote the enhancement of oral liquid properties towards the acid-base balance in the oral cavity were revealed. This study is of particular importance, since it was carried out directly under the conditions of the oral biofilm functioning.

Keywords: *acid-base balance in the oral cavity, buffer capacity of oral liquid, dental plaque, lingual fur.*

Introduction

Acid-base balance (ABB) is an essential element of local homeostasis in the oral cavity [*Takuma C., 1990; Davidov B. et al., 2000; Rumyantsev V. et al., 2007 a; b*]. All ABB changes in the oral cavity are oppositely directed: towards acidosis and towards alkalosis. A number of local factors destabilize this balance. These factors include congenital malformations [*Gavrilova O. et al., 1998*], food, water, air composition, meteorological and professional factors, smoking and other pernicious habits, oral hygiene means and care, drugs and medical influences, and – finally, fillings and dental prostheses [*Patel V. et al., 2011*]. In ABB regulation the liquids, tissues and organs of the oral cavity are involved [*de Almeida P. et al., 2008; Veerman E. et al., 2011*].

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Our previous studies have shown that pH-metry methods are rather sensitive to reveal and evaluate changes in the local homeostasis of the oral cavity.

Hence, the purpose of this study was to describe and present the comparative analysis of results obtained under the influence of corrective measures directed to the oral cavity ABB restoration in different subgroups of patients.

Material and Methods

The trial involved patients of 3 groups with subsequent sub-group distribution.

The first group involved patients with different health problems; patients with diseases of the oral mucosa made the second group, and the third group embraced actually healthy volunteers with different dietary intake.

Since every subgroup of observed patients had its own peculiarities associated with the health condition (Group 1), diseases of the oral mucosa (Group 2), nature of nutrition (Group 3), their corrective measures differed in composition, scope and duration.

Part of the initially examined patients did not agree to undergo procedures for ABB correction of the oral cavity. Another part of the patients were excluded from further analysis, because they did not fulfill all the requirements of the study (personal hygiene measures, refusal to smoke, changing diet, treatment and preventive measures). Unfortunately, after corrective measures a part of patients was not examined for the reasons independent of researchers.

In this regard, in the first group we analyzed only results of 167 (64.5%) patients from the total number of patients (289), among which were patients with diseases of the gastrointestinal tract (GIT), including ulcers of stomach and duodenum (n=82); subjects with chronic renal failure (CRF) (n=31), and diabetes mellitus (DM) (n=54).

In the second group of all initially examined 132 patients only 74 (56.1%) patients could completely finish the trial: those with impaired salivation function (ISF) (n=11), fungal lesions of oral cavity (FL) (n=44) and leukoplakia of mucosa (LM) (n=19).

In the third group of actually healthy volunteers among the 162 patients 97 (59.9%) subjects were repeatedly surveyed: those, who constantly use regular mixed food (MF) (n = 65) with no clear preference; volunteer athletes, in the diet of which protein foods of animal origin (PFAO; n = 18) made a significant proportion during the past two years and volunteers, who used vegetarian food (VF; n = 14) () for the past two years.

The ABB in the oral cavity was estimated by means of the most informative hydrogen indicator (**pH**). The research was conducted in the morning before meals in identical conditions. Thus, the pH of mixed saliva, gingival fluid, surface of the tongue was determined, and sucrose and carbamide pH-tests in the oral cavity carried out. The speed of non-stimulated salivation was established.

Using pH-meter “Orion 710A+” (“Thermo Electron Corp.”, USA) and standard glass electrodes we measured pH of mixed saliva, pH on the surface of the tongue (with the help of intraoral mini-electrode “Beetrode” (“Word Precision Instruments Inc.”, USA) with a diameter of 0.1 *mm* working part and the standard comparison electrode in 7 points: 2 – in the middle of the back of the tongue, 4 – on lateral surfaces and 1 – on the tongue tip. Values of pH were determined every 5 minutes for half an hour after stimulation.

Upon carrying out the planned research, we constructed typical test curves of mixed saliva pH, which were developed by us earlier. Sucrose test curve (Stephen curve) of mixed saliva and the surface of the tongue was obtained after rinsing the mouth with 15 *ml* 47% sucrose solution for 30 *sec* (Figure 1). The curve amplitude characterizes total activity of acid-producing microflora in the oral cavity [*Petrikas A., Rumyantsev V., 1998*]. Carbamide pH curve was obtained for the examined subjects after similar rinsing of mouth with 15 *ml* of 8% carbamide solution. The amplitude of this curve is proportional to the activity of urease-positive (mainly proteolytic) microflora in the oral cavity, processing urea to the final product – ammonia [*Rumyantsev V., 1998*]. Sucrose and carbamide pH tests allow complex indirect evaluation of both metabolic activity of microflora and the possibility of ABB regulation system directly in the oral cavity [*Volozhin A., Rumyantsev V., 2007*]. In order to minimize the analytical errors, these tests were performed on different days of the investigation: sucrose test was carried out on the first day, while carbamide test – on the next day. The amplitudes of pH test curves were calculated:

A_s=pHi - pHmin, A_c=pHmax - pHi,

where A_s is the amplitude of sucrose test pH-curve;
A_c is the amplitude of carbamide test pH-curve;
pHi is the initial value of pH (prior to stimulation);
pHmin is the minimum pH value on the test curve;
pHmax is the maximum pH value on the test curve.

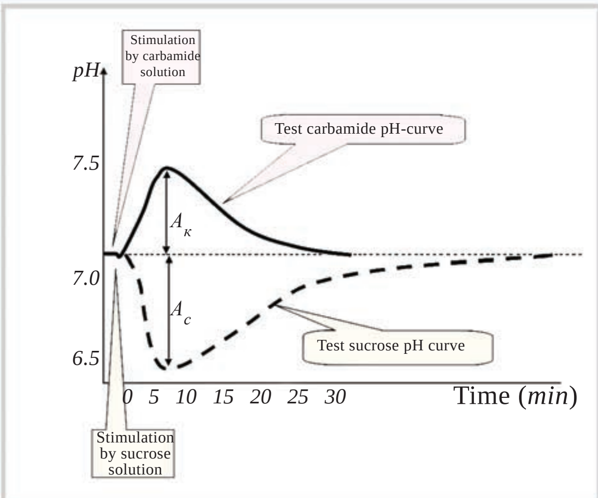

***FIGURE* 1**. *Typical test curves of mixed saliva pH.*

The pH value of gingival fluid pH was determined on strips of laboratory filter paper introduced into the gingival groove or parodontal pockets for 10-15 *sec* to impregnate with the tested fluid. The salivation rate without the prior stimulation was evaluated after patients' spitting (mixed saliva) into the graduated flasks during 5 *min*. Immediately before the study oral cavity professional sanitation of all patients was done. The statistical processing of obtained data was done with the help of parametric and non-parametric criteria.

Analysis on the influence of correction procedures to ABB in the oral cavity was performed by the following identified most important and informative parameters.

1. *Amplitudes of the test sucrose and carbamide pH-curves in the oral liquid, dental plaque and lingual fur.* These parameters indirectly characterize the metabolic activity of the oral microflora directly in its habitat, i.e. in the oral biofilm content. The amplitudes of test pH-curves are sensitive to the quantitative and qualitative changes in the local microbiocenosis (microbiota), as well as the buffer properties of the oral liquid [*Rumyantsev V., 1998; 1999; Rumyantsev V. et al., 2007 a; b; 2009*].

2. *The overall rate of dental plaque – an integrated hygienic index (IHI).* This parameter simultaneously characterizes the amount of dental plaque present on all visible surfaces of the teeth, as well as the hygienic health of the tooth rows, since directly with this index mostly correlate other IHI-indicators and the indices of microbial dissemination of the oral cavity mucous membrane surfaces [*Rumyantsev V., 1999*].

3. *Papillary-Marginal-Alveolar index evaluating the degree of gingival inflammation reaction severity.* The latter essentially depends on the activity and aggressiveness of the dental plaque microflora, especially parodontopathogenic one. Qualitative and quantitative changes in the microbial composition of dental plaque affect the gingival inflammation reaction [*Rakova T., Lazarev A., 2009*].

4. *The index of oral liquid total acidity.* One of ABB indicators of oral cavity that highly correlates with the pH-value, because the microbial production of organic acids increases the total acidity of the oral liquid [*Namestnikova I. et al., 2005*].

5. *Index of sucrose content in the oral liquid.* Since the sucrose is the most "preferred" substrate for acidogenic microflora of the oral cavity, this factor might indirectly indicate the degree of ABB disturbance in the oral cavity [*Leus P., 2008*].

6. *Index of non-stimulated salivation rate*. Taking into account that the oral liquid consisting mainly from the secretion of salivary glands is ABB major regulator in the oral cavity, the evaluation of the amount of secreted saliva allows indirectly consider the state of this balance [*Kleinberg I. et al., 2002; Alamoudi N. et al., 2004; de Almeida P. et al., 2008*].

7. *Indicators of the oral liquid buffer capacity related to acid and base without using the stimulation of salivation.* These indicators along with the pH-value are major ones in the assessment of ABB changes that occur in the oral cavity under the influence of systemic diseases, changed character of nutrition, as well as diseases of organs and tissues of the oral cavity [*Leont'ev V., Suntsov V., 1974; Rumyantsev V. et al., 2009; Zhigulina V., Rumyantsev V., 2011*].

The part of initially observed patients did not agree on having measures related to ABB correction in the oral cavity. Another part of patients was excluded from further analysis because they did not comply with all the requirements of the study (personal hygienic measures, smoking refusal, changing the nutrition character, treatment and preventive measures). The third part of patients, unfortunately, was not been examined after they had corrective measures for reasons independent of the investigators.

To reveal the role of correction measures in different groups of patients we determined the changes of average values of test pH-curves. Data received during the comparative analysis of indices were interpreted graphically (graphs and histograms were constructed) for a more clear visual representation of the dynamics of changes.

RESULTS AND DISCUSSION

The changes in the average values of the amplitudes of test pHcurves of oral liquid in patients from subgroups of Group 1 (subjects with common somatic diseases) before and after corrective measures are presented in Figure 2. The analysis of changes shows that under the influence of corrective measures, the most important of which were the individual correction of oral cavity hygiene, the nature of the usual nutrition and treatment of the main disease, the amplitudes of both sucrose (Figure 2 a) and carbamide (Figure 2 b) pH-curves of oral liquid, in all three subgroups approached close to some common value, which for the sucrose curve was 0.62±0.33 pH units. For carbamide curve, this average value was 0.60±0.21 pH units. It is interesting that the amplitude of sucrose curve in the subgroup of GIT diseases increased, while in the other two subgroups it decreased. In carbamide pH-curve the increase of amplitude was observed only in the subgroup of DM, and decrease in the mean values of the amplitude was marked only in the other two subgroups (Figure 2 b).

The differences between mean values of the amplitudes of both curves in all subgroups before and after the correction measures were significant, despite the small number of studies ($p<0.05$). The revealed regularities indicate that under the influence of ABB corrective procedures in the oral cavity its stabilization occurs with decreasing of value variability amplitudes of test sucrose and carbamide pH-curves in the oral liquid. Thus, in general, the balance of metabolic activity of both the acid- and ammonia-producing microflora is observed in the oral cavity.

It should be noted that the dynamics of changes in values of the mean amplitudes of the test sucrose pH-curves of oral liquid in patient subgroups with CRF and DM was approximately 2 times higher than those in the GIT subgroup (Figure 2). In case of carbamide curve, such difference was not observed (Figure 2 b). The increase of average amplitude of test sucrose pH-curves in GIT subgroup indicates the total increase in microbial acid-production in the oral cavity under the influence of corrective measures. This, though paradoxal at first glance, might be apparently explained by the fact that in patients with peptic ulcer disease, who follow a special diet, the proportion of acid-producing microflora in the oral cavity increases, which is important for the preservation and maintenance of both ABB and microbial balance. The increase in average value of test carbamide pH-curves of the oral liquid revealed in the subgroup of DM testifies an increase of ammonia-producing microflora proportion in the content of oral biofilm, which is rather the result of the approximation to the normal ABB and microbial balance.

Dynamics of changes in the average values of the amplitudes of test pH-curves of oral liquid in patients of Group 2 under the influence of ABB correction procedures in the oral cavity was studied (Figure 2). First of all, the attention should be drawn to the pronounced changes of indices in the subgroup of ISF. It should be noted that in case of stimulation by the test sucrose solutions under the influence of corrective measures in all three subgroups of patients there was mentioned different degree of expressed reduction of the average values of the amplitudes of test pH-curves (Figure 2 a). In FL and LM subgroups the increase was practically the same, but in a subgroup of ISF it was 1.5 times more pronounced ($p<0.05$). Regarding the test carbamide pH-curves it might be said that the difference between the subgroup with ISF and the two other subgroups was even higher, 3-fold ($p<0.05$ (Figure 2 b). Besides, there was found a slight increase in the average values of the amplitudes of carbamide pH-curves in FL and LM subgroups that, likewise the previous cases, were regarded as positive changes indicating normalization of microbiocenosis and ABB in the oral cavity. However, it must be emphasized that, under the influence of corrective measures, to a greater extent, the changes of amplitudes of test sucrose curves were recorded as compared to carbamide ones (Figure 2 a; b).

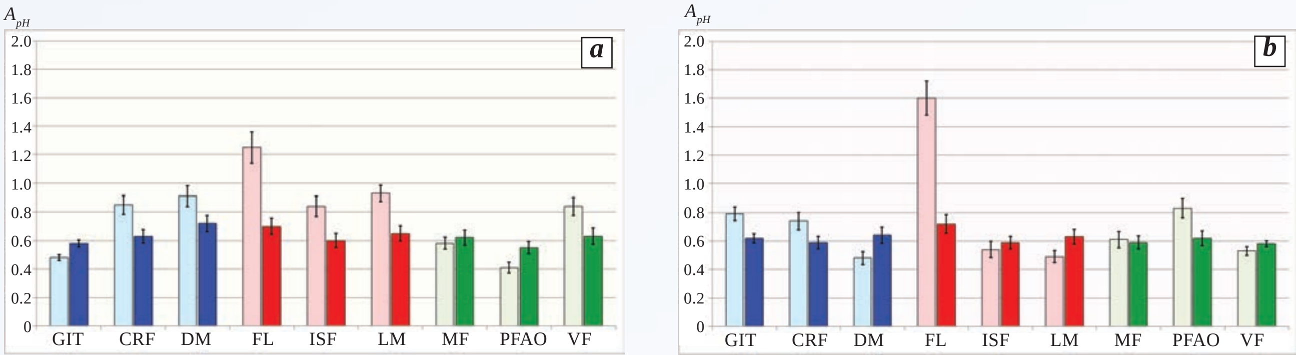

Figure 2. Average values of the amplitudes of test pH-curves of oral liquid before (, ,) and after (, ,) corrective procedures: a – sucrose test; b – carbamide test of acid-base balance in the oral cavity.

This testified that the acid-producing microflora was more sensitive to mentioned measures than the ammonia-producing one. The average values of amplitudes tended to averaged values: for sucrose curve such value made 0.63±0.16 pH units, and for carbamide – 0.64±0.19 pH units.

The dynamics of changes in the average values of the amplitudes of test pH-curves of oral liquid in practically healthy volunteers of Group 3 is presented in Figure 2. The analysis of data revealed that the values of studied indices were at the averaged points. For sucrose test pH-curve such a point was the value equal to 0.61±0.12 pH units, and for carbamide curve it was in the region of 0.58±0.09 pH units. It is interesting to note that in a subgroup of VF the amplitude of sucrose pH-curve decreased under the influence of correction measures, including the individual correction of oral cavity hygiene that was quite logical, since in this case the microbial dissemination of the oral cavity should decrease. However, in two other subgroups, on the contrary, there was noted an increase of amplitudes; this latter was difficult to explain from the point of view of the expected quantitative reduction of acidogenic microflora in the oral cavity. Regarding the test carbamide pHcurves the pattern of regularity was somewhat different. Thus, in MF and VF subgroups a decrease in amplitude was marked, and in the subgroup of patients using PFAO – a slight increase was recorded. Changes in indices during the period of patients observation were statistically significant ($p<0.05$) in the PFAO and VF subgroups (the sucrose pH-curves), as well as in the PFAO subgroup (carbamide pH-curves). In other cases, statistically reliable differences were not found.

Thus, it turned out that the corrective actions carried out in practically healthy patients, which included individual correction of oral cavity hygiene, led to ABB normalization in the oral liquid, but not due to hygiene improving; apparently, it was, to a great extent, due to correction of nutrition. The revealed phenomenon confirms an essential role of nutritional factor in ABB regulation in the oral cavity.

Changes in the average test pH-curve values in dental plaque under the influence of correction measures in patients of Group 1 was graphically presented in Figure 3. In this biotope the pH-changes stimulated by test dose of sucrose almost did not differ in the subgroup of patients with GIT diseases before and after ABB correction ($p>0.05$). However, the changes in other subgroups were significant ($p<0.05$). Likewise the case with oral liquid, in the dental plaque there was observed an approach of the average pH-values of the test sucrose and carbamide curves to the same values. For sucrose curves it made in average 0.94±0.28 pH units, and for carbamide curves – 0.89±0.10 pH units. If being applied to the test sucrose pH-curves the minimal changes were recorded in the subgroup of patients with GIT diseases, in case of carbamide pH-curves it was observed in the subgroup of DM, as indicated by differences in absolute values of amplitude changes (Figure 3 a; b). Thus, in subgroups of GIT and CRF, the dynamics in amplitudes changing twice exceeded that in the subgroup of DM. In our view, this testifies that in patients with DM after changing the nutrition character and individual correction of oral cavity hygiene, the proportion of ammonia-producing microflora in the dental plaque gradually increased, indicating the normalization of microbiocenosis and ABB in this biotope.

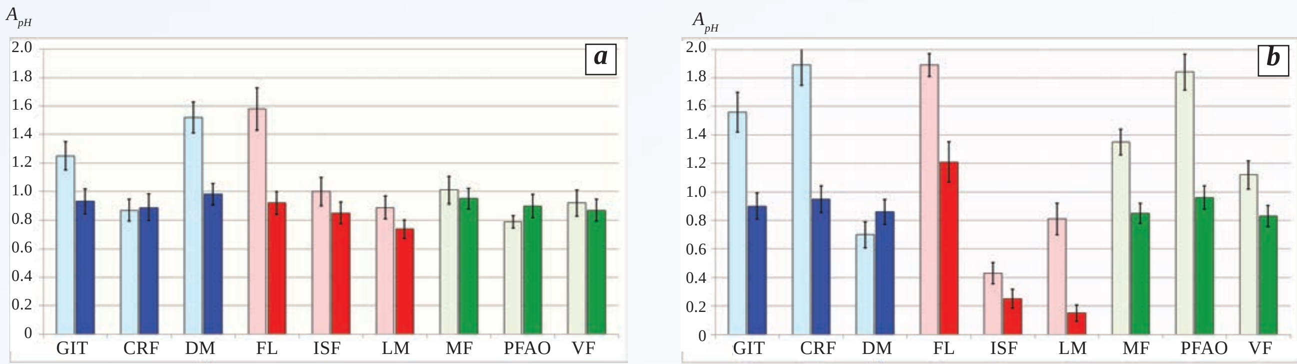

FIGURE 3. Average values of the amplitudes of test pH-curves of dental plaque before (, ,) and after (, ,) corrective measures: a – sucrose test; b – carbamide test.

Figure 3 shows the dynamics of changes in the average amplitudes of pH test-curves of dental plaque in patients of Group 2. In this biotope there was observed a reduction of mentioned indices, both in sucrose and carbamide pH-curves. The average final pH-value in the sucrose curve made 0.64±0.11 pH units, in carbamide curve – 0.94±0.49 pH units. The amplitude of sucrose curve to a large extent changed in the subgroup of patients with ISF in comparison with the two other subgroups, and in carbamide pH-curve in the subgroups of ISF and LM the dynamics of changes was the same. In a subgroup of FL the changes in the amplitudes of both test pH-curves were approximately identical. Thus, the activity of acid- and ammonia-producing microflora in the dental plaque was mostly suppressed under the influence of corrective procedures in the subgroup of patients with ISF.

The dynamics of changes in the average values of amplitudes of the test pH-curves of dental plaque in patients of Group 3 is presented in Figure 3. Likewise the case with oral liquid, there was also marked a noticeable change in indices under the influence of corrective measures. However, in case of sucrose pH-curves, these changes were not statistically reliable (p>0.05), and in case of carbamide curves they were significant (p<0.01) (Figure 3 a; b). It should be noted that in case of stimulation with test sucrose solutions under the influence of corrective measures in the subgroups of MF and VF the amplitude of pH-curve decreased, while in PFAO subgroup it slightly increased. Amplitudes of test carbamide curves in all subgroups of patients decreased. The average value of the point, toward which the indices of amplitude of test sucrose pH-curves tended, made 0.91±0.13 pH units, and those of carbamide curves – 0.89±0.17 pH units. Dynamics of the amplitudes of pH-curves of dental plaque, which is presented at the diagrams, indicates the normalization of the balance in this biotope between acid- and ammonia-producing microflora, as well as the ABB. It is not excluded that the increase in the average value of the amplitude of sucrose pH-curve test revealed in the oral liquid of PFAO group to some extent was explained by a similar increase in the amplitude of pH-curve of dental plaque. At the same time, we cannot explain detected changes in the oral liquid test amplitudes of carbamide pH-curves in PFAO group via the changes in the metabolic activity of the microflora of dental plaque, since we have not detected the unidirectional changes of amplitudes of carbamide pH-curves for dental plaque.

Changes in the average values of amplitudes of test pH-curves of lingual fur under the influence of correction procedures in patients of Group 1 are shown in Figure 4. As in the above mentioned biotopes of the oral cavity, a reduction of variability in average values of indices was observed in the lingual fur, with their tendency towards the same point. For the test sucrose curves this point was 0.74±0.30 pH units; for carbamide curves it made 0.82±0.31 pH units (Figure 4 a; b). Furthermore, the tendency previously identified in other biotopes and characteristic for the sucrose curves in the GIT subgroup (increase in the average carbamide value of the amplitude) was preserved here as well. However, in contrast to the previous cases, the average value of carbamide pH-curve of lingual fur did not increase, but decreased in the DM subgroup. Apparently, due to the small number of studies, these changes were statistically insignificant (p>0.05), but this fact was regarded to be positive under the influence of corrective measures in patients with DM. It justified

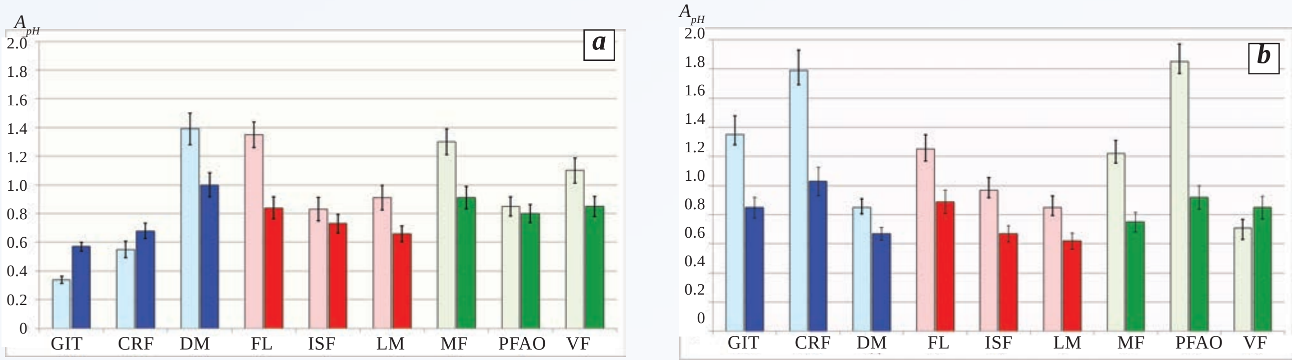

FIGURE 4. Average values of the amplitudes of test pH-curves of lingual fur before (, ,) and after (, ,) corrective procedures: a – sucrose test; b – carbamide test.

that the proportion of ammonia-producing microorganisms decreased in the lingual fur content, which might be a marker of ABB normalization in the oral cavity of this category patients.

Dynamics of changes in the average values of the amplitudes of test pH-curves of lingual fur in patients of Group 2 was also studied (Figure 4). In all subgroups under the influence of corrective procedures of ABB in the oral cavity a decrease in average values of indices was recorded. The analysis of differences in amplitudes demonstrated that the most pronounced changes occurred in the subgroup of patients with ISF, both in sucrose and in the carbamide pH-curves (Figure 4 a; b). The average final value of the amplitude of the test sucrose pH-curve made 0.74±0.35 pH units, and the appropriate value for carbamide was 0.72±0.42 pH units. The revealed regularities indicated a decrease in the activity of acid- and ammonia-producing microflora of lingual fur in all patients, which might be an indicator of ABB normalization.

The research outcomes on dynamics of average values for the amplitudes of test pH-curves of lingual fur in Group 3 patients are presented in Figure 4. It should be emphasized that the mentioned indices decreased in all subgroups under the influence of corrective measures, if as a stimulant the test solutions of sucrose were used. The most pronounced and statistically significant changes were observed in the MF subgroup of patients ($p<0.05$). In the other two subgroups the changes were less pronounced and not confirmed by statistical analysis ($p>0.05$). In case of using test solutions of carbamide as a stimulator, under the influence of ABB corrective procedures in the oral cavity it was possible to identify a decrease in the average values of pH-amplitudes in the subgroups with MF and PFAO. In the latter subgroup we recorded a slight increase in amplitudes. Apparently, this increase can explain the revealed increase in the amplitudes of test carbamide pH-curves of oral liquid in the subgroup with PFAO. The average value of the endpoint, towards which sought the amplitudes of the test sucrose pH-curves of lingual fur made 0.82±0.19 pH units, while with respect to carbamide curves the values were 0.84±0.24 pH units.

CONCLUSION

Summarizing data obtained, we can conclude that the results of carried out comparative study before and after application of ABB corrective procedures, new relationship patterns in the regulation of this balance in different biotopes of the oral cavity of patients were revealed. It was established that the changes in the acid- and ammonia-producing activity of the microflora of some biotopes affected those in other biotopes. A significant influence of the nutrition character and hygienic care on the ABB in the oral cavity was revealed, as well as the impact of activities, which promoted the increase of oral liquid buffer properties. This study is of particular importance, since it was carried out directly under conditions of the oral biofilm functioning.

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