Oral ecology in the case of the most common stomatitises in children – recurrent aphthae and sharp herpetic gingivostomatitis

M. Rashkova[1], Peneva M.[2], Chorbadzhiyska L.[3]

Summary

The different conditions of the oral mucous membrane are the result of disturbed oral ecology. Mucous lesions in particular cause disbalanced eubiosis. This can be an additional reason for the sustenance of a secondary bacterial infection and for the aggrevation of the mucous pathology.

The present study aims at studying oral ecology in the case of the most common stomatitises in children, namely in the case of recurrent aphthae (RA) and sharp herpetic gingivostomatitis (SHGS). The following goals were formulated: (1) the carrying out of a microbiological study of the oral microflora in children with SHGS and RA; (2) the carrying out of a study of the lysosime in the saliva of chidren with RA and SHGS.

The research was done on 92 children aged 1 - 15, with oral mucous conditions: (1) 42 children with sharp herpetic gingivostomatitis (SHGS) and (2) 50 children with recurrent aphthae (RA).

The results show that in the case of the two illnesses studied pretty diverse microflora was found in the mouth cavity – mainly not pathogenic cocci and bacilli as well as Candida in not pathogenic quantities. In the case of SHGS a bigger variety of cocci and rod-like microorganisms was found, the most typical microorganisms being S. salivarius ? Neisseria. The relative high quantities of S.faecalis are indicative of bad oral higiene and for polution of the mouth cavity. The isolation of Peptostreptococcus in 1/5 of the cases with SHGS is indicative of a change in the oral ecosystem towards $?_2$ deficiency. In chidren with SHGS the quantity of anaerobes is much bigger than usual.

In children with RA the quantity of aerobes is bigger than the quantity of anaerobes. The chronic process, having an endogenous immune pathogenesis, brings about slight changes in the oral ecosystem. Microbic association with a much lesser number of MO are found, compared to the microbic associations found in the case of SHGS.

The results show that in both illnesses the lysosime does not exceed the normal limit, the reason being its consumption during the course of the condition.

Key words: oral biological system, oral ecosystem, mucous lesions, stomatitis, sharp herpetic gingivostomatitis, vesiculo-erosive oral mucous lesions, recurrent aphtha, lysosime, oral microflora, aerobes, anaerobes

The mucous membrane as part of the oral biological system is a field for oral mucous pathology – the mirror of the disbalanced eubiosis of the mouth cavity [3, 19,20].

The tremendous diversity and complexity of the microbic flora, the dynamic processes that keep the balance of the oral ecosystem are a precondition for oral health [20].

The various illnesses of the mucous membrane of the mouth cavity are the result of disturbed oral ecology. The mucous lesions can be the reason for disturbed eubiosis. They can be an additional factor for the sustenance of a secondary bacterial infection and for the aggrevation of the mucous pathology [1,2,4,5,6].

[1] Associate professor, Doctor of medicine, Department of Children's dental medicine, Faculty of Dental medicine-Sofia
[2] Associate professor, Doctor of medicine, Department of Children's dental medicine, Faculty of Dental medicine-Sofia
[3] Associate professor, Doctor of medicine, Laboratoty of microbiology, Faculty of Dental medicine-Sofia

It is the prevailing opinion in literature that the plaque of biofilm on the hard parts of the teeth is the main reason for basic conditions of the mouth cavity such as the caries and the periodontal diseases. The role of the oral microflora in the case of the stomatitises has not been studied enough, though. Regardless of the fact that the free bacterial cells in the mouth are less resitent than the bacterial cells in the biofilm, the changes in the parameters of the oral environment (PH, buffer capacity, content of 0_2, etc.), as well as the invasion of forein for the oral environment microorganisms or viruses, can bring in a disbalance in the complex and dynamic equilibrium of the oral environment. The oral environment can acquire a pathogenic potential and initiate hurting of the mucous membrane or deepen oral mucous lesions caused by other endogenous factors (immunopathogens, allergic, deficient conditions) [7,8,9,10,11].

On the other hand, researches are done today proving that biofilm can be formed on the soft mucous surface too. This biofilm is different compared to the plaque. It is made mainly of fungi of the genus Candida. In the case of disrupted eubiosis, the cells of Candida undergo abnormal growth and their patjhogenic potential increased. They acquire specific adhesive qualities with regard to the cells of the epithelium and it is there that their pathogenic potential shows [17,18,19,20].

With the development of contemporary microbiological techniques, of molecular biology and genetics, complex ecological interactions and bacterial phenotypic metamorphoses and mimicries are disclosed, as well as their role in the oral pathology. This development is the basis for an up to date approach in medical treatment involving controlling and influencing the oral environment, including controlling and influencing the oral microflora [12,13, 14,15,16].

So that the influence of the oral ecology on the mucous oral pathology could be studied, two of the most common illnesses of the oral mucous membrane were picked up – the recurrent aphthae (RA) and the sharp herpetic gingivostomatitis (SHGS). These two conditions were chosen because of the following reasons:

According to our own researches, these are the most common stomatitises in children and adolescents.

Regardless of the fact that the causes for aphthae are not completely clear, this kind of stomatitis is a good example of mucous lesions with endogenous reasons – autoimmune, alergic, deficient conditions, etc. The risk factors for a recurrence of the conditioon in children have to do with oral immunity, food allergy, stress and heredity.All these factors indirectly influence the oral ecology [21,22].

Another illness the oral ecology of which was studied was the sharp herpetic gingivostomatitis. It is the first acute viral infection (VHS-1) with a clinical manifestation – with vesiculo-erosive oral mucous lesions that develop very fact and in which the general condition of the patient is affected. The SHGS is an example of the influence of an exogenous local etiological factor – the herpetic virus, which for a couple of days changes the oral ecological environment to an extent that it becomes the cause for sustaining the infection in the mouth [23,24].

The comparison between the oral microflora in the two different types of illnesses of the mucous membrane will show if there is a link between the oral mucous pathology and the oral pathology.

Lysosime, as a nonspecific defensive factor of the saliva, is an indicator of the role of the liquid environment in the mouth on the oral ecology. Its study in the case of the two conditions will cast additional light on the complex processes of interaction in the oral biological system when the oral mucous membrane is ill.

It is the **aim** of the present study:

To study the oral ecology in the case of the most common stomatitises in children – the recurrent aphthae (RA) and the sharp herpetic gingivostomatistises (SHGS).

To achive this goal, the following **tasks** lie ahead**:**

- The making of a microbiological study of the oral microflora in children with RA and SHGS;
- The making of a research of the lysosime in the saliva of children with RA and SHGS.

Material and methods

The research was done with 92 children with oral mucous conditions aged 1 - 15.

- 42 children with sharp herpetic gingivostomatistis /SHGS/;
- 50 children with recurrent aphthae /RA/.

Clinical unit of observation:

Children with primary oral herpetic infection were diagnosed with **„sharp herpetic gingivostomatitis"** if the following criteria – we defined as typical of SHGS - were met:

- general symptoms – general indisposition, irritability, disturbed sleep, high temperature, malnutrition, pains in the mouth cavity, sore throat;

- lesions all over the oral mucous membrane – numerous erosive confluent lesions with a hyper base, uneven outlines, gray whitish coating. Singular preserved vesicles on the tongue and the mucous membrane of the cheeks can be observed, as well as on the skin round the lips;

- gingivitis – from catarrhal to erosive;

- coating of the tongue - frequently differently evinced;

- excessive salivation and bad breath;

Juvenile patients with primary herpetic infections must also fulfil the following additional clinical criteria:

- must be children with the typical characteristics of SHGS, as described by the literature and conforming to our own observations;

- must be children in an early age;

- must be children who have not had the same illness before;

- must be children who are not immunocompromised.

Children with recurrent oral lesions, clinically characterised as "aphtha", were diagnosed with **„recurrent aphthae",** when having:

- alternative lesions with a round form;

- with changeable, smooth edges;

- a bottom covered with grayish-whitish coating;

- a red halo is there around a hyper base;

- a disposition on the movable mucous membrane;

- when different lesions are there in the same place, no confluence occurs.

In the children studied there were no changes in the general condition and the blood count, which made us assume that the patients were healthy during the study. The oral lesions were classified as „idiopathic recurrent aphthae".

Way of collecting the material:

With the help of a sterile wad a smear was taken from an aphtha or herpetic lesion. A sample of not stimulated mixed saliva was taken too. The material for the test was taken on the fourth and the sixth day of the illness, so that the clinical situation in the mouth cavity was identical, as far as the dynamic of the condition was considered.

Indicators tracked:

- isolation of microorganisms, identified as species;

- ascertaining the quantity of lysosime in the saliva.

Methods used for microbiological testing:

For isolation of the microbic cultures. Dextran bouillon and blood agar were used for the purpose. The identification of the species was carried out by means of classical routine methods. Anaerobes were identified with the help of the schemes of Sulter, Citrin, Finegold.

For ascertaining the quantity of Candida. Morphological, cultural and biochemical tests were used (fermentation of saccharides, tests for the assimilation of carbohydrates) so that the different species were identified. Quantitative methods for ascertaining the quantity of Candida in a millilitre of saliva were used too.

For ascertaining the quantity of lysosime in the saliva. The measurement was carried out with the help of the method of Osserman and Lawlor, as modified by Zucker and collaborators, by means of using the highly sensitive strain Lysodeyticum. The results obtained were compared with the lytic activity of a standard lysosim.

Statistical processing

Bearing in mind the basic aim and tasks of the study, as well as the volume and character of the data obtained, the following statistical methods were employed:

Alternative analysis – aimed at assessing the relative share and the standard error of the assessment (S_p) in the case of qualitative criteria;

T-criterion for testing of hypotheses concerning the presence of statistically important difference between the indicators studied. The level of importance of the zero hypothesis used is $P<0.05$.

Results and discussion:

Microbiological study of the oral microflora in children with SHGS and RA

1. Comparative characterisation of the isolated microbic cultures

The microorganisms isolated from the mouth cavity of children with RA and SHGS are shown in Table nr. 1.

Table nr. 1. Microorganisms isolated in children with RA and SHGS

Groups of Ì Î	**Ì Î -genus**	**Ì Î -species**	**quantity**	**+Î $_2$/-Î $_2$**
G+ **cocci**	Streptococci (Ü- haemoliticus) Streptococci (ß- haemoliticus) Staphylococci Peptostreptococci	S.salivarius S. sanguis S.faecalis S.pneumoniae Str.pyogenes S.epidermidis -	10^2-10^5 10^2 10^2 10^3 10^2 10^2-10^4 -	F.anaerobes F.anaerobes F.anaerobes F.anaerobes F.anaerobes F.anaerobes Anaerobes
G- cocci	Neisseria	N.sicca N.subvlava	10^1-10^3 10^1-10^2	Aerobes Aerobes
G+bacilli	Corinebacterium	Cor. xerosa		**Aerobes**
G-bacilli	Bactroides Fuzobacterium Eschirihia	- - Å.coli	- -	Anaerobes Anaerobes F.anaerobes
Fungi	Candida	C.albicans C.tropicalis C.pseudotro-picalis	10^3 - 10^5	F.anaerobes F.anaerobes F.anaerobes

F.anaerobes

F.anaerobes It impressed us that the oral mucous membrane of the children with SHGS and RA contained a variegated microbic flora of cocci and bacilli as well as of fungi from the species Candida. The microbial flora is not pathogenic, the species being opportunistic and evoking no symptoms. In case of decreased resistence these species can cause and sustain a pathological process.

Both aerobic and anaerobic flora were isolated from the oral mucous membrane and the saliva. Among the isolated microorganisms S. salivarius was prevalent, followed by other streptococci. Candida was found in quantities that were not indicative of candidiasis, but sufficient for the sustenance of a mixed secondary bacterial infection.

2. The correlation between pathogenic, conditionally pathogenic and resident microorganisms

In accordance with their pathogenic qualities, microorganisms in the mouth cavity of children with RA and SHGS correlate as follows:

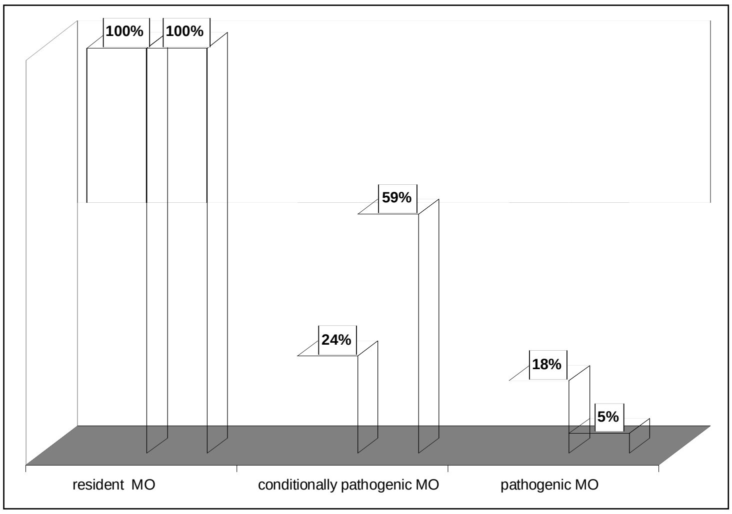

Figure nr. 2. Distribution of isolated microorganism in terms of their pathogenic potential

Microorganisms of the resident oral microflora – Streptococci, S. epidermidis and Neisseria were found in all children with RA and SHGS. The conditionally pathogenic MO – Peptostreptococci, E.coli, Fusobacterium ? Bacteroides were isolated in 24% of the children with RA and 59,52% of the children with SHGS. The difference is statistically important /T= 3,66 P<0,001/, which is indicative of the prevalence of the conditionally pathogenic flora in the case of acute herpetic infection. Pathogenic MO were isolated in a small number of children - in 18% of the children with RA and 5 % of the children with SHGS. These are the microorganisms S.pyogenes and S. pneumoniae. Regardless of the statistically reliable difference /T=2,08 P<0,5/, indicative of a bigger occurrence of MO in children with RA, this fact cannot be seen as a regularity and is most probably coincidental with a concurrent general illness, such as bronchopneumonia and chronic tonsillitis. According to our own pervious researches, these are conditions children with RA suffer from frequently (Fig.2).

3. Types of microorganisms in children with RA and SHGS

The correlation of coccus-like MO or of cocci and bacilli in children with RA and SHGS is shown by Figure nr. 3.

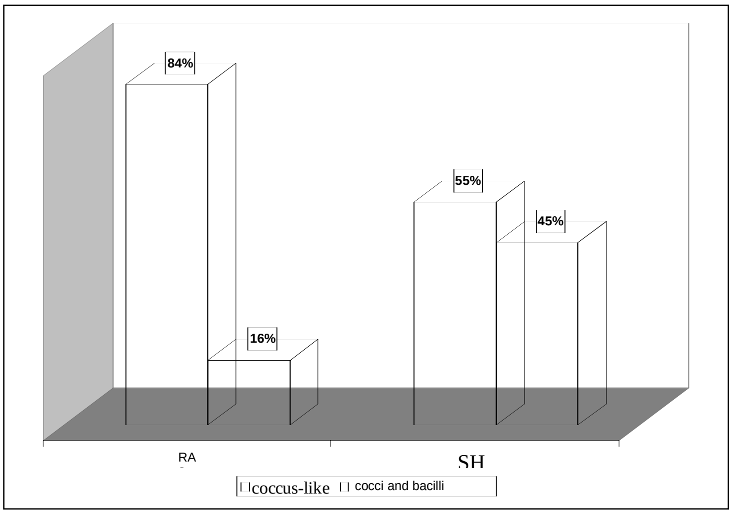

Figure nr. 3. Correlation between cocci and cocci and rods in children with RA and SHGS

Comparing the occurrence of coccus-like microorganisms in children with RA and SHGS, we were impresseed to find out that in the case of RA the occurrence of coccus-like MO is statistically more important /?.1,84 ?< 0,05/. Even bigger is the statistical importance /?= 2.73 ?<0,01/ of the combination cocci+bacilli in the case of children with SHGS.

The distribution of ?? in terms of species is shown by Figure nr. 4.

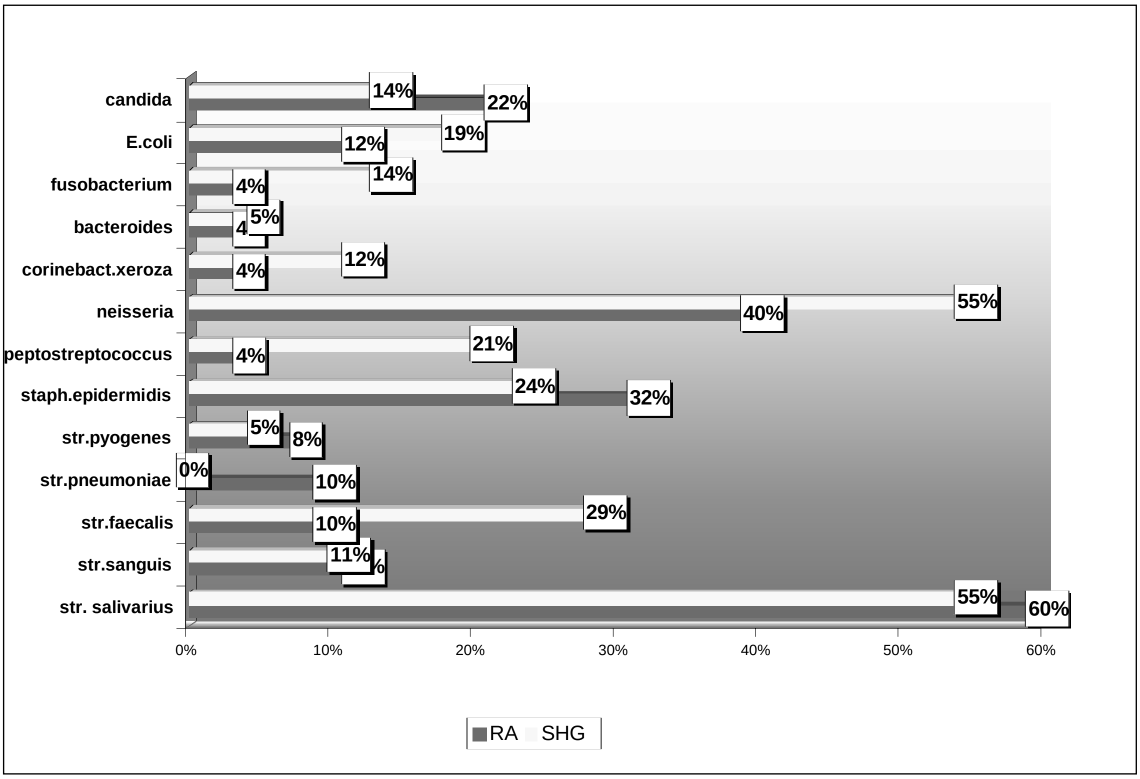

Figure nr. 4. Microorganisms isolated in children with RA and SHGS

Both S.salivarius and Neisseria are usually common to RA and SHGS. We were impressed to find out that that were no statistically important differences between the occurrence of the different species of MO, excluding S.faecalis, which in children with RA is found in 10% of the cases, and in children with SHGS in 28,6% of the cases / T = 2,13 P < 0,01/. The presence of this microorganism is indicative of bad hygiene and pollution of the mouth cavity. Because of the strong painfulness, in SHGS children stop taking food and cleaning their teeth.

Other microorganisms that statistically prevail in SHGS /T= 2,38 P < 0,01/ are the Peptostreptococci (21%). It is an anaerobe and is indicative of the development of an oxygen deficiency in the oral environment of microorganisms. Such a conclusion can be supported by the isolation of other anaerobic microorganisms too, such as Bacteroids and Fusobacterium.

The statistically important occurrence of S. pneumoniae in children with recurrent aphthae / T =3,22 P < 0,001/ is pretty specific too. It is an opportunistic pathogenic microorganism. Its occurrence is accidental and is due to a concomittant general illness, though.

C. albicans is found in children with SHGS in quantities bigger than 10^3 in 14% of the case, and in children with RA - in 22% of the case. C. albicans is not a leading factor for SHGS and RA and its weak quantitative increase is due to the disturbed ecological equilibrium in the oral microflora in both conditions.

4. Distribution of microorganisms according to their oxygen dependancy

Facultative anaerobes were found in all children studied. They are the most adaptable to the diverse and changeable environment in the mouth cavity. In children with RA aerobes are – in statistically reliable terms – more numerous than tha anaerobes /T= 1,86 P<0,05/. Such a correlation is closer to the normal oral ecoenvironment. In children with SHGS there is no statistically reliable difference between aerobes and anaerobes / T = 0,63 P>0,05/. The higher proportion of anaerobes is indicative of a change in the oral environment towards oxygen deficiency. This fact is explained by the relatively less significant changes in the oral ecosystem as well as by the conditions for growth of microorganisms in recurrent aphthae /RA/, compared to the rapid changes in the oral environment in acute viral infections, as is the case with herpetic gingivostomatitis.

The results are shown by Figure nr. 5.

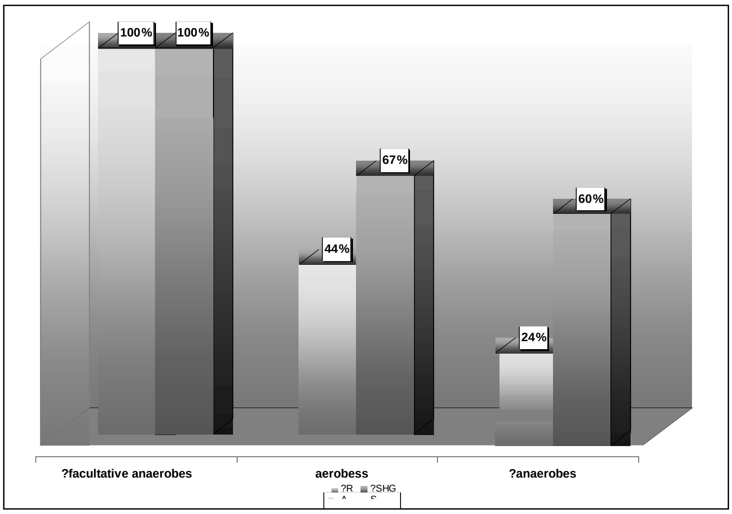

Figure nr. 5 The distribution of microorganisms according to their oxygen dependence

5. The occurrence of microbic associations in children with RA and SHRS

The microorganisms isolated in the mouth cavity participate in microbic associations within which the separate microorganisms evince themselves. The occurrence of microbic associations in children with RA and SHGS is shown by Figure 6.

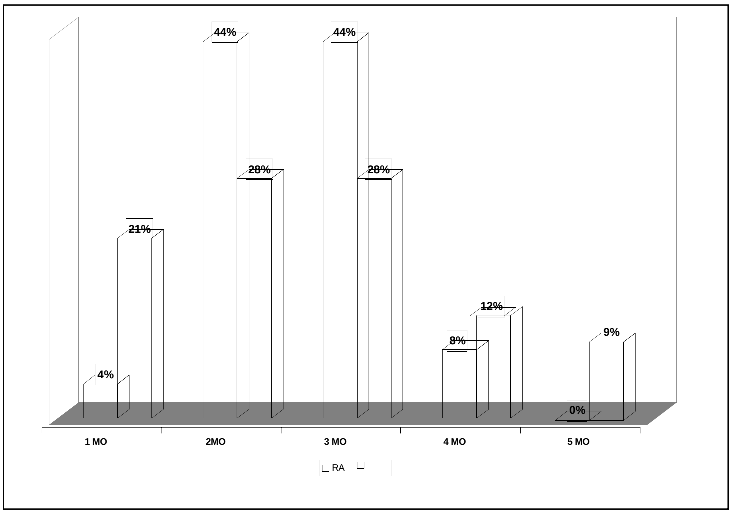

Figure nr. 6 Microbic associations in children with RA and SHGS

It is statistically clear from the diagram that in children with RA associations of 2 or 3 MO prevail /T= 5,53 P <0,001/, while in children with SHGS the diversity of associations is bigger (1 - 5 MO), the cases with 1,2 or 3 MO being considerably more numerous. The later is statistically unverifiable, though.

Streptococci are found in all kinds of associations. Statistical verifiability is only evinced (T= 2,53 P<0,01) in the associations with 4 MO in the children with RA and not in the children with SHGS.

In 22% of the children with RA, Candida occurs independently. When in associations, Candida is found mainly combined with Streptococci, in associations of 1 up to 3 MO. In statistically reliable terms, associations of Candida with 1 MO prevail (T = 3,46, P<0,001). The participation of Candida in more uniform associations can be explained by its supressive effect on the other MO.

Neisseria is another microorganism, which, quite like the streptococci, occurs in associations of 3 up to 6 MO. It is statistically reliable that it prevails in children with RA, in a combimation of 3 MO, the same associations in children with SHGS having a different quotient (T= 2,25 P < 0,05). More rarely, aerobic microorganisms in the case of both illnesses combine in associations of 4, 5 or 6 MO. E.coli prevail in bigger associations in children with SHGS (T= 2,25 P <0,05).

In conclusion, it can said that in the case of recurrent aphthae associations with a smaller number of MO were found, while in the case of herpetic gingivostomatitis the diversity is much bigger, the associations comprising 5 or 6 MO with the participation of anaerobic microorganisms too.

Study of the lysosime in the saliva of children with RA and SHGS

The quantity of lysosime in the saliva of children with RA or SHGS has been studied.

The quantities of lysosime in the saliva of children with RA and SHGS are shown by Figure nr. 7.

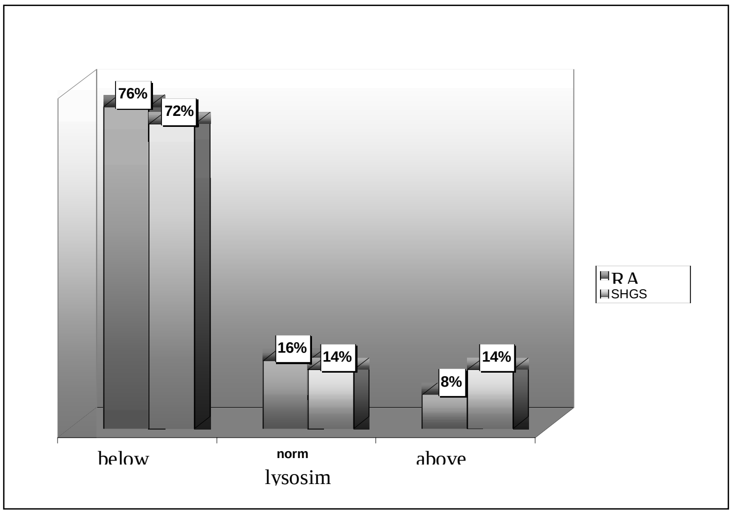

Figure nr. 7 Quantity of lysosime in children with RA and SHGS

It is clear from the diagram that in 72%-76% of the children with RA and SHGS the quantity of lysosime has decreased below the lower limit of the norm -80 ??. The differences are statistically reliable (T= 7,54; P <0,001). Bearing in mind that the study has been conducted during a period when the illness has clinically manifested itself (day 4-6 of the illness), it can be assumed that the lower quantities registered are the result of the consumption of the lysosime as a result of its bacteriacidal effect on the beta-1-4-glucous bridges of the peptidoglycans of the cell membrane.

There are case, though, when the lysosime is within norm - in 16% of the children with RA and 14% of the children with SHGS, as well as above norm – in 14% of the children with SHGS and 8% of the children with RA. The differences are not statistically reliable, though (T=1,24 P> 0,05) because of the small number of such cases. Hence our inability to comment on the possible reasons.

Discussion:

When the oral environment changes as a result of the different illnesses of the oral mucous membrane, microorganisms change their quantitative and qualitative characteristics, join associations and thus participate in the pathogenesis of the different oral lesions. Microorganisms can initiate pathogenic processes in the mucous membrane of the mouth and aggrevate with secondary bacterial infections the current illness.

In the case of the conditions studied – recurrent aphthae and sharp herpetic gingivostomatitis – the bacterial oral flora is not a part of the etiology of the illness. Still, the changes in the oral ecosystem are an important precondition for an additional secondary bacterial infection.

The changes in the oral microflora of children with RA and SHGS is a dynamic process.

In children with RA development of opportunistic microorganisms and microbic associations of three microorganisms, including anaerobes, is observed. Because of the chronic recurrent and endogenous character of the condition, the changes in the oral microbic flora observed are with low intensity.

In the case of herpetic gingivostomatitis the changes in the oral microflora are the consequence of bad oral hygiene, disturbed eubiosis after a viral attack, bigger and diverse microbic associations prevailing in the process. This is due maybe to the stronger dynamic in the change of the oral ecology, to the acute nature of the disease and to the local effect the herpetic virus has on the mucous epithelium. In both illnesses C. albicans is not a leading factor, but the changes in the oral environment do influence its quantity.

Lysosime is a bacteriocidal enzyme, which is found in the secretion of the parotid gland and in the secretion of the salivary gland. It is created and concentrates in the basal cells of the gland ducts. Lysosime has a mucopolysaccharide structure and as a local not specific defence oral factor has the quality to dissolve the beta-1-4-glucous bridges of the peptidoglycans in the cell membrane of some organisms. A sourse of lysosime in the mouth cavity are not only the salivary glands but also the gingival liquid and separate leucocytes that can be found in mixed saliva. The effect of lysosime is varied. It can inhibit certain microorganisms and have no effect on others.

As an element of the oral defence, the lysosime has different effects on both the microbic flora and the oral mucous membrane. Contemprary studies have found out that a large portion of the resident microflora in the mouth cavity is resitent to the lysosime in the saliva. Experiments show that the microorganisms causing caries and the G-organisms are sensitive to lysosime, though.

Other studies have it that the effect of lysosime is not only bacillocidal. Lysosime, they say, has also to do with the process of infammation, i.e. it harms the epithelium of the oral mucous membrane and has a lithic effect on the connective tissue underneath.

When the lysosime is within norm or below norm, it participates in the not specific defense of the organisms, necessary for coping with the pathogenic process in the mouth.

Unfortunately, in children with RA and SHGS lysosime is often below norm. This fact deserves additional study. Here we can assume that lysosime is not only a bacillocidal factor but is also an immunopathogenic fator in the case of illnesses of the oral mucous membrane.

The data obtained from our study do not allow us to conclude whether the presence of Candida in the mouth cavity and the specifics of the pathogenic processes there influence the quantity of lysosime in the saliva. It is a fact, though, that the quantity of lysolime changes differently in oral illnesses with different etiopathogenesis, as well as that the quantity of lysolime depends on the changes in the oral microbic flora in children with oral mucous conditions.

Conclusions:

In the case of both illnesses studied diverse microbic flora is found in the mouth cavity – mainly not pathogenic cocci and rods.

Candida is found in not pathogenic quantities.

Resident microorganisms Streptococci, S. epidermidis, Neisseria are found in all children with any of the two illnesses.

Conditionally pathogenic microorganisms Peptostreptococcus, E.coli, Fusobacterium and Bacteroides prevail in the case of sharp herpetic infection.

In SHGS bigger combinations of cocci and rod-like organisms are found, the most common microorganisms being S. salivarius ? Neisseria. The relatively high quantity of S.faecalis is indicative of bad oral hygiene and polution of the mouth cavity.

The isolation of Peptostreptococcus in 1/5 of the cases with SHGS is indicative of a change in the oral ecosystem towards O_2 deficiency.

In children with RA aerobic microorganisms exceed in number the anaerobic ones. The chronic process, having an endogenous immune pathogenesis, brings about slight changes in the oral ecosystem.

In chidren with SHGS the quantity of anaerobes is much bigger than usual. The acute process and the local effect the herpetic virus has on the mucous epithelium brings about deeper changes in the oral ecosystem.

Microbic association with a much lesser number of MO are found in children with RA, compared to the microbic associations found in the case of SHGS.

The results from our study show that in both illnesses the lysosime does not exceed the normal limit.

References

1. Amir J et al. The Natural History of Primary Herpes Simplex Type 1 Gingivostomatitis in Children. *Pediat Dermatol* , 1999, **22**(4),259 -263;
2. Antoon Y.W., R.L. Miller. Aphthous ulcers - a review of the literature on the etiology pathogenesis diagnosis and treatmen t. *JADA* 1980, **101**, (11), 803 - 808;
3. Auriol M-M. G. Ler Naour.La muqueuse buccale - structure, function et examen. *Act. Odonto Stomatol* 2004, **225**,(3) 7-21;
4. Birek C. Herpesvirus -induced diseases: oral manifestations and current treatment options. *J Calif Dent A ssoc*, 2000, **28**,(12),911-921;
5. Brice SL.Microorganisms in recurrent aphthous ulcerations. *Scand J Dent Res* ,1977, **85**, (6), 426-433 ;
6. Cameron A., R. Widmer - Hendbook of Pediatric Dentristry, *Ed. Mosby-Wolfe- Sydney Australia*, 1997, 146 -160;
7. Essential Microbiolo gy for dentistry . Lakshman Samaranayake . *ed.Elsevier* , 2002, 255 -284;
8. Huber MA. Herpes simplex type -1 virus infection. *J Periodontol*, 2002, **73**,(1),110-114;
9. Ludlow JB, Kutcher MJ, Samuelson A. Recurrent aphthous stomatitis: clinical characteristics and assoc iated systemic disorders . *Semin Cutan Med Surg* , 1997, **16**,(4),278-283;
10. Michael F. Cole et al. Humoral Immunity to Commensal Oral Bacteria in Human Infants: Salivary Secretory Immunoglobulin A Antibodies Reactive with Streptococcus mitis biovar 1, Streptococ cus oralis, Streptococcus mutans, and Enterococcus faecalis during the First Two Years of Life . *Infection and Immunity* , 1999, **67**,(4),1878-1886;
11. Pascal F. et al. Lésions bullose de la cavetébuccale - orientation diagnostique . *Act. Odonto Stomatol* 2004, **225**,(3),73-85;
12. Peter JR; Haney HM . Infections of the oral cavity. *Pediatr Ann* , 1996 **25**, (10), 572-576;
13. Porter SR, Scully C. Aphthous stomatitis - an overview of aetiopathogenesis and management, *ClinExp Dermatol*, 1991, **16**, (1)235-243;
14. Riggio MP, Lennon A, Gho dratnama F, Wray D. Lack of association between Streptococcus oralis and recurrent aphthous stomatitis, *J Oral Pathol Med*, 2000, **29**, (2), 26-32;
15. Sciubba J. - Herpes simplex and aphthous ulcerations: presentation, diagnosis and management--an update, *Gen Dent*, 2003 ,**51**,(6),510-16;
16. Szpirglas H. Ben Slama l. Pathologie de la muqueuse buccale - Paris, *Editions scientifiques et médicales Elsevier*, 1999, 178 -206 ;
17. Zlatev, St. – Microbic infection and immune reactions in the oral region. Publisher - Vassil Petrov, Plovdiv, 2002, 141 -205; Çëàòåâ Ñò. – Ì èêðî áí à àãðåñèÿ è èì óí í è ðåàêöèè â î ðàëí àòà î áëàñò, *èçä.ÅÒ Âàñèë Ï åò ðî â – ÂÀÏ , Ï ëî âäèâ*,2002 ð 141 -205;
18. M.Rashkova – Clinical characteristic of recurrent aphthae in children – «Dentists' Review» 2005, 87, 2, 93-98; Ì .Ðàø êî âà - Êëèí è÷í à õàðàêòåðèñòèêà í à ðåöèäèâèðÿù è àô òè â äåòñêà âúçðàñò- *Çúáî ëåêàðñêè ï ðåãëåä* 2005, **87**,(2),93-98;
19. Oral empbriology, histology and biology, *«East-West» Publishing House*, ed. Milena Peneva, 2007, 191-217; Î ðàëí à åì áðèî ëî ãèÿ õèñòî ëî ãèÿ è áèî ëî ãèÿ, *èçä.»Èçò î ê -Çàï àä»* ï î ä ðåä. Ì . Ï åí åâà, 2007, 191 -217;
20. Rashkova, M. Oral herpetic infections in children and adolescents, 2006, ed. 112ð; Ðàø êî âà Ì . - Î ðàëí è õåðï åñí è èí ô åêöèè â äåòñêî - þ í î ø åñêà âúçðàñò - 2006, 112ð.;
21. Rashkova, M, M.Peneva – Study of the general health statu s in children with recurrent aphthae - *«Dentists' Review»* 2006, **88**, (1), 7-12; Ðàø êî âà Ì ., Ì . Ï åí åâà - Ï ðî ó÷âàí å í à î áù èÿ çäðàâî ñëî âåí ñòàòóñ ï ðè äåöà ñ ðåöèäèâèðàù è àô òè - *Çúáî ëåêàðñêè ï ðåãëåä* 2006, **88**, (1), 7-12;
22. Rashkova, M, M.Peneva –Clinical character istic of oral lesions in children with sharp herpetic gingivostomatitis – «Problems of stomatology», 2005, **31** (1), 85-88; Ðàø êî âà Ì ., Ì . Ï åí åâà - Êëèí è÷í à õàðàêòåðèñòèêà í à î ðàëí èòå ëåçèè ï ðè äåöà ñ î ñòúð õåðï åñåí ãèí ãèâî ñòî ì àòèò - Ï ðî áëåì è í à ñòî ì àòî ëî ãèÿ òà 2005, **31** (1), 85-88;
23. Rashkova, M, M.Peneva – Symptomatology of sharp herpetic gingivostomatitis in children – «Dentists' Review», 2005, **87**, (2), 87-98; Ðàø êî âà Ì ., Ì . Ï åí åâà - Ñèì ï òî ì àòèêà í à î ñòúð õåðï åñåí ãèí ãèâî ñòî ì àòèò â äåòñêà âúçðàñò- *Çúáî ëåêàðñê è ï ðåãëåä* 2005, **87**,(2),87-98;
24. Oral herpetic infections and recurrent aphthae in children and adolescents, M.Rashkova, a dissertation, January 2006; Î ðàëí è õåðï åñí è èí ô åêöèè è ðåöèäèâèðàù è àô òè â äåòñêî - þ í î ø åñêà âúçðàñò, Ì . Ðàø êî âà, Äèñåðòàöèî í åí òðóä, ßí óàðè 2006ã.;

Corresponding Author: Maya Rashkova, Associate professor, Doctor of medicine, Department of Children’s dental medicine, Faculty of Dental medicine-Sofia
Address: 1,G.Sofiyski Str. Sofia 1000, mayarashkova@mail.bg