Dental Diseases and Intestinal Dysbiosis Among Children

T Shishniashvili*/T Suladze**/ M Makhviladze ***/M Kalandaze ****/ V Margvelashvili*****

Objectives: *The goal of the present study correlates dental hard tissue mineralization, mucosal pathologies in the oral cavity and different degrees of intestinal dysbiosis.* ***Study Design:*** *the study examined two groups: the study group (Group I) included 229 children and adolescents aged 1-17 (mean age 5±1years) with oral pathologies (caries, acute or chronic candidiasis) and confirmed dysbiosis of varying severity and stages as well. Group II (the Control Group) was composed of 50 patients aged 1 – 16 (mean age 5±1years) with oral pathologies but with no detected changes in gastrointestinal (GI) flora. Dental caries were examined by DMFT-index; the extent of dental hard tissue mineralization by vital staining (2% methylene blue) and cases of oral candidiasis was investigated by taking cultures from mucosal plaques.* ***Results****: on the basis of the research outcomes the correlation between the different degrees of GI dysbiosis and dental hard tissue mineralization with pathologic expressions in the oral cavity was found. Group I was divided into two subgroups: in the first subgroup that suffered from mild dysbiosis (I and II degree) moderate dental caries was revealed, whereas in the second subgroup with III and IV degree of dysbiosis–high levels of dental caries was detected. In Group II (no GI flora disturbances), the dental hard tissue demineralization indicator was minimal; in children aged 1-3 years the incidence and prevalence of caries were low and increased with age, reaching higher values during puberty (11-16 years).* ***Conclusion****: It may be concluded that dysbiosis of GI microflora influences on a degree of dental hard tissue demineralization, which in turn may predispose to the formation of dental caries.*

Key words: *intestinal dysbiosis in children, dental hard tissues, dental caries*

INTRODUCTION

All organs and consecutive systems of human body are tightly interconnected. Disturbances in metabolism or pathology of any system or organ inevitably lead to functional disorders and cause pathological changes in other systems and organs.[1]

The body's symbiotic microflora is biologically important for the entire life cycle of our organism as it determines numerous significant physiological, biochemical and immunological parameters and serves as a sensitive indicator of a person's health. Microflora in the human gastrointestinal (GI) system plays a major role in homeostasis, disease origin and progression – especially during the period of a child's growth and development. It has been established that normal intestinal microflora promotes the production of secretary immunoglobulin and preserves high levels of mucin.[2]

Normal flora counteracts the colonization and replication of pathogenic organisms. Bifid and *coli* bacteria in the GI tract produce lactic and succinic acid being inhibited by the proliferation of the decaying microbes. Bifidobacteria participates in the synthesis of iron, zinc, calcium and vitamin D as well as in the absorption of amino acids; they also synthesize B vitamins, folic acid, nicotinic and pantothenic acids. Accordingly, qualitative and quantitative changes of GI microflora negatively influence on the organism's ability to fight against infection, and increase the allergic and the mutagenic potential of cells.[3]

Intestinal dysbiosis is defined as a qualitative and quantitative imbalance of GI microflora — i.e. a bacteriological imbalance. This process is mainly caused by a deficiency or complete absence of necessary microorganisms and the growth of others.[4]

There are several factors contributing to the development of intestinal dysbiosis, such as: the degree of pathogenicity of microorganisms, the patient's age, somatic diseases, the influence of harmful ecological and environmental factors, the consumption of antibacterial drugs, or an inappropriate diet.[5]

From the Faculty of Medicine, Tbilisi State Medical University (Georgia)
*T Shishniashvili, MD, PhD, Head of the Department of Child and Adolescent Therapeutic Dentistry.
**T Suladze, PhD student.
***M. Makhviladze, MD, PhD, Assistant professor.
****M. Kalandaze, MD, PhD, Associate professor.
*****V Margvelashvili, MD, PhD,DM Sci, Full professor, Head of Stomatological and Maxillo-Facial Surgery Department.

Send all correspondence to:
T Suladze –Faculty of Medicine, Tbilisi State University (Georgia), Dentist, Dental Clinic № 1, TSSU, Tbilisi, Georgia.
Zemo Vedzisi str. 77. Tbilisi, Georgia
Phone: +995 99 44 74 88
E-mail: tamar_suladze@hotmail.com

It should be noted that the aforementioned factors are especially important and relevant during the continuous process of a child's growth and development. GI dysbiosis in children is often expressed as oral changes caused by the continuance of the GI tract and the oral cavity. The microflora of the oral cavity and GI tract is normally mostly composed of staphylococci, streptococci, lactobacilli, fungi, corynebacteria and various anaerobes. The dysbiosis hypothesis states that the modern diet and lifestyle, as well as the use of antibiotics, have led to the disruption of the normal intestinal microflora. These factors result in alterations in bacterial metabolism, as well as the overgrowth of potentially pathogenic microorganisms. It is believed that the growth of these bacteria in the intestines results in the release of potentially toxic products that play a role in many chronic and degenerative diseases. Many factors can harm the beneficial members of the GI flora, including antibiotic use, psychological and physical stress, radiation, altered GI peristalsis and dietary changes.[6–10]

As it is widely known, there are several processes that depend on calcium absorption intensity, e.g. the primary mineralization process and dental hard tissue formation, which prevents caries in early ages. Calcium itself is delivered to the teeth by pulp vasculature through mineral components in stimulated saliva. Calcium, phosphorus and fluoride preparations are widely used to increase tissue resistance and ensure caries prevention in early stages. Unfortunately, their clinical effectiveness is very low in cases of pathological GI tract changes.[11]

A bacteriophage is a bacterial virus characterized by a specific action against host bacteria. It infects bacterial cells and causes their lysis, yet does not affect human cells (and is therefore safe for them). Bacteriophages are actively used in selected cases of infectious diseases, GI, skin, genitourinary, oral cavity and respiratory tract pathologies.[12-13]

The benefits of phagotherapy:

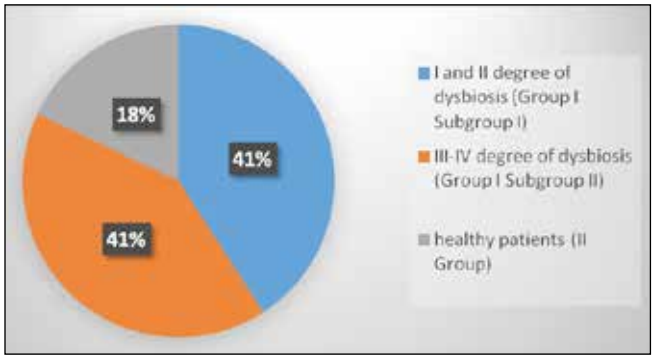
- They are safe for the human body and there are no known complications;
- Phagotherapy can be combined with other therapies; and
- Compared to antibiotic therapy, phagotherapy is cost-effective.

The aim of our study was to find an association between the dental hard tissue mineralization, pathologic oral mucous expressions and different degrees of severity of intestinal dysbiosis.

MATERIALS AND METHOD

A total of 279 patients aged 1-17 years from Tbilisi (Georgia) were included in the survey (as children participating in the study were minors. Informed consent of their parents was obtained according to Georgian legislation). Examined children were divided into two groups: The study group (Group I) included 229 children and adolescents aged 1-17 years (mean age 5±1years), with oral pathologies (caries, acute or chronic candidiasis) as well as confirmed dysbiosis of varying severity and stages. Group II (the Control Group) was composed of 50 patients aged 1 – 16 years (mean age 5±1years) with oral pathologies but with no detected changes in gastrointestinal (GI) flora. Group I was divided into two subgroups: the first subgroup with mild (I and II degree) dysbiosis and the second subgroup with severe (III and IV degree) dysbiosis (Figure 1). The 131 of the 229 patients were recruited in a dental clinic, where examinations were carried out with dental mirror and probes.

Figure 1. The number of examined children according to the degree of dysbiosis



The examination was carried out after obtaining the informed consent from the parents. The prevalence and intensity of dental caries and level of oral hygiene were defined, and special forms were filled consisting of demographic data (age, sex, address, contact info) DMFT and OHI-S indices. The DMFT index was used (DMFT describes the amount of dental caries in an individual and is obtained by calculating the number of Decayed (D), Missing (M) and Filled (F) teeth) [14] to estimate the status of dental caries and the Simplified Oral Hygiene Index(OHI-S index) to study levels of oral hygiene. The Simplified Oral Hygiene Index has two components: the Debris Index and the Calculus Index. The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth. Debris Index was studied as there was no need to study Calculus Index. Debris Index = (The buccal-scores) + (The lingual-scores) / (Total number of examined buccal and lingual surfaces). The higher the index, the worse is the oral hygiene status. Normal Debris Index values may vary from 0 to 3.[15] The 98 of the 229 patients were also examined by a dentist in the Analytical-Diagnostic Centre of the G. Eliava Bacteriophage, Microbiology and Virology Institute Laboratory, where all examined patients' intestinal flora was studied, and the aforementioned indices were also defined. In the laboratory the patient history was filled for every examined child.

Patients (50 children aged 1-16 years) who had no GI dysbiosis, according to laboratory study were included in control group (Group II). Their oral condition was also examined by dentist and appropriate forms were filled. In order to exclude the influence of oral hygiene on dental caries, individuals with satisfactory levels of oral hygiene were selected (OHI-S from 0.7 to 1.6).

Development of dysbiosis is mainly based on quantitative disruption of coli, bifido and lactobacteria's colonies. According to quantitative disruption conditions the degree of dysbiosis is divided into mild – I, moderate – II, severe – III and serious – IV. Therefore, dysbiosis is considered as qualitative and quantitative changes of intestinal flora. Qualitative and quantitative indicators of GI microflora were investigated at the Analytical-Diagnostic Centre of the G. Eliava Bacteriophage, Microbiology and Virology Institute Laboratory in Tbilisi, Georgia. The following components were investigated in 1g of faecal mass: enterococci, lactose deficient and hemolytic strains, as well as bifidobacteria and the total amount of lactobacteria. In cases where the pathogenic microorganisms were identified, the material for investigation had been analysed before antibacterial treatment started.

The qualitative and quantitative values of intestinal microflora were assessed (according to the "Intestinal dysbiosis—SST 91500.11.0004-2003" patient management protocol)[16] in the following manner: if only 10-15% of microbes identified were uncharacteristic of intestinal normobiosis, then the microflora was described as normal. In case the growth of atypical bacteria was higher than 30%, and if a potentially pathogenic flora was apparent (lactose-negative enterobacteria, fungi, cocci and various anaerobes) together with a decreased number of bifidobacteria and/or of lactose-positive enterocoli, then the microflora was considered as dysbiotic.

The results were compiled in appropriate forms, and the data obtained was statistically processed using the Statistical Package for Social Sciences (SPSS; version 21.0 IBM Corp. Released 2012. SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

RESULTS

Our results show a correlation between dental hard tissue mineralization, oral pathologies and different degrees of GI dysbiosis. As Table 1 shows, the degree of dental hard tissue demineralization within the control group (no GI flora disturbances) was minimal. The prevalence and intensity of caries was low in children aged 1-3 years, and they increased with age. They reached the higher values during puberty (11-16 years). In this group, the study showed that besides the normal function of the GI tract, dental caries was mainly associated with inadequate oral hygiene.

Group I was divided into two subgroups: in the first subgroup that suffered from mild dysbiosis (I and II degree) moderate dental caries were revealed (Table 2), and in the second subgroup with III and IV degree of dysbiosis–high levels of dental caries were detected (Table 3).

Both subgroups of the I Group were under the outpatient supervision for 1 year by an infectious disease specialist and, if necessary, the appropriate treatment was prescribed according to the severity of their dysbiosis. This was a complex treatment composed of eubiotics and phages based upon the degree of dysbiosis. In cases of mild to moderate dysbiosis (I and II degree), the treatment was comprised of bacteriophages according to the individual sensitivity.

For cases of severe dysbiosis (2nd subgroup), the sole use of phagotherapy was ineffective and prebiotics and probiotics were prescribed alongside. It is important to point out that in order to increase the stability of dental hard tissue in cases of treated intestinal dysbiosis, dentists were prescribing an appropriate mineralizing product. A year later, the patients who underwent the treatment for dysbiosis did not generate new caries lesions.. According to our research, dental hard tissue mineralization was seen in 92% of cases after GI flora was normalized and consequently an appropriate mineralizing therapy was carried out. In the 8% of patients the process of remineralization was not observed, due to the fact that some patients were not compliant with the prescriptions and recommendations of the dentist and/or infectious disease specialist.

Table 1. Prevalence and intensity of dental caries in the control group (Group II)

Age (Number)	Caries prevalence (%)	DMFT index
1-3 n=29	18.2	0.290.71
4-6 n=11	72.5	2.21.4
7-16 n=10	82	3.750.0

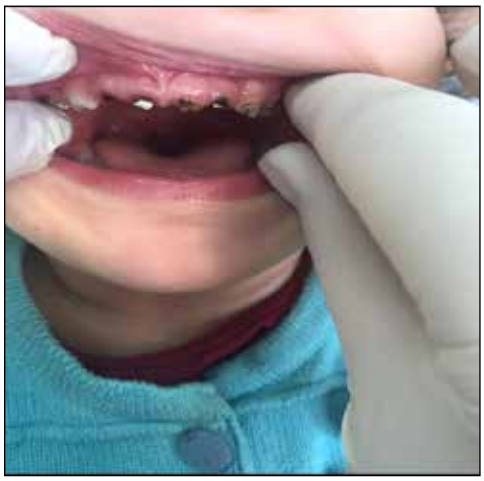
Table 2. Prevalence and intensity of dental caries in patients with I-II degree of dysbiosis

Age (Number)	Caries prevalence (%)	DMFT index	P
1-3 n=50	21.3	0.420.835	<0.05
4-5 n=23	57	2.571.121	<0.05
6-7 n=18	62.7	4.332.196	<0.05
8-9 n=14	64.2	3.51.743	<0.05
10-11 n=3	59.7	3.01.0	>0.05
12-16 n=6	84	2.830.0	>0.05

Table 3. Prevalence and intensity of dental caries in patients with III-IV degree of dysbiosis

Age (Number)	Caries prevalence (%)	DMFT index	P
1-3 n=49	23.2	1.761.601	<0.05
4-5 n=32	72	4.661.911	<0.05
6-7 n=12	79.5	6.171.528	<0.05
8-9 n=9	89.1	5.891.764	<0.05
10-11 n=5	75.2	4.01.000	>0.05
12-16 n=8	91.5	2.501.069	>0.05

Figure 2. The oral condition of a three- year-old girl with IV degree of intestinal dysbiosis



DISCUSSION

The review of Intestinal Dysbiosis by Hawrelak and Myers, state that alterations in the bowel flora and its activities are now believed to be contributing factors to many chronic diseases. Irritable bowel syndrome and inflammatory bowel disease have both been linked to alterations in the intestinal microflora. The intestinal dysbiosis hypothesis suggests a number of factors associated with modern Western living have a detrimental impact on the microflora of the gastrointestinal tract. Factors such as antibiotics, psychological and physical stress, and certain dietary components have been found to contribute to intestinal dysbiosis. If these causes can be eliminated or at least attenuated then treatments aimed at manipulating the microflora may be more successful.[17]

Over the past few decades, antibiotic resistance strains have dramatically increased, mostly as a result of the inappropriate use of antimicrobial therapy. This process has led to increased levels of interest in alternative forms of antibacterial treatment, including bacteriophages. [18–22]It is important to notice the link between the degree of dysbiosis and the high intensity of caries with age.

The present study proved that GI dysbiosis has a great influence on the mineralization of dental hard tissues, in particular, more severe the form of dysbiosis, the higher the intensity of dental caries. The highest correlation between dental caries and severe dysbiosis can be seen in children aged 6-8 (statistical significance p=0,005). This could be related to tooth change, to their active growth process and often to the inappropriate use of antibiotics. In addition, patients' oral condition had improved after treatment with gastroenterologist and improvement of intestinal flora.

CONCLUSION

According to our study, we may conclude that qualitative and quantitative changes of GI microflora can influence the degree of dental hard tissue demineralization, which in turn can predispose to the caries development.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Prof. Inga Giorgadze, Head of the Analytic-Diagnostic Centre of the G. Eliava Bacteriophage, Microbiology and Virology Institute Laboratory.

REFERENCES

1. Shishniashvili T. Dental Disease Prophylaxis. Ed. Tbilisi, Tbilisi; 3 – 4. 2012
2. Makhviladze M, Zubadalashvili M. Comparative Effectiveness of Linex and Lacto-G during Dysbiosis in Adults. Georgian Medical News; 35:45-49. 2009
3. Tamboli G, Neut C, Desreumaux P, Colombel J.F. Dysbiosis in Inflammatory Bowel Disease. Gut; 53:1-4. 2004
4. Hawrelak J.A, Myers S.P. The causes of intestinal dysbiosis. Altern. Med. Rev; 9(2): 180-197. 2004
5. Torrazza R.M, Ukhanova M, Wang X, Sharma R, Hudak M.L, Neu J, Mai V. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. PLoS ONE; 8(12):e83304. 2013
6. De Vrese M, Marteau PH.K. Probiotics and Prebiotics Effects on Diarrhea. J. Nutr; 137:803-811. 2007
7. Koning C.J, Jonkers D.M, Stobberingh E.E, Mukder L, et al. The Effect of a Multispecies Probiotic on the Intestinal Microbiota and Bowel Movements in Healthy Volunteers Taking the Antibiotic Amoxycillin. American Journal of Gastroenterology; 103(1):178-189. 2008
8. Milward M.R. Important Oral Bacteria: The Oral Microflora; School of Dentistry: 19-21. 2012
9. Gill H, Prasad J. Probiotics, Immunomodulation and Health Benefits. Advances in Experimental Medicine and Biology; 606:423-54. 2008
10. Vangay P, Ward T, Gerber J.S., Knights D. Antibiotics, pediatric dysbiosis, and disease. Cell Host & Microbe; 17(5):553-564. 2015
11. SC Kim, GD Ferry. Inflammatory Bowel Diseases In Pediatric and Adolescent Patients: Clinical, Therapeutic, and Psychosocial Considerations. Gastroenterology, Elsevier; 2004
12. Bruttin A. & Brüssow H. Human Volunteers Receiving Escherichia coli Phage T4 Orally: A Safety Test of Phage Therapy. Antimicrobial Agents Chemotherapy; 49(7):2874-2878. 2005
13. Chanishvili N. et al. Phages and their application against drug resistant bacteria. J Chem Technol Biotechnol; 76:1
14. Malmö University. Methods and Indices for Caries prevalence [https://www.mah.se/CAPP/Methods-and-Indices/for-Caries-prevalence/], accessed 12 May 2014.
15. Malmö University. Simplified Oral Hygiene Index (OHI-S) [https://www.mah.se/CAPP/Methods-and-Indices/Oral-Hygiene-Indices/Simplified-Oral-Hygiene-Index—OHI-S/], accessed 5 February 2016.
16. Statement of Ministry of Health of Russian Federation no. 231. Moscow; 112, 2003
17. Jason A. Hawrelak and Stephen P. Myers. The Causes of Intestinal Dysbiosis: A Review. Alternative Medicine Review; 9 (2):180-197. 2004
18. Fischett V.A., Domiel N. & Raymond S. Reinventing phage therapy: Are the parts greater than the sum?. Nature Biotechnology; 24:1508-1511. 2006
19. Skurnik M. & Staunch E. Phage therapy: Facts and fiction. International Journal of Microbiology; 296(1):5-14. 2006
20. Nancy K, Henry, Jay L, Hoecker K, Hable Rhodes. Antimicrobial Therapy for Infants and Children: Guidelines for the Inpatients and Outpatients Practice of Pediatric Infectious Diseases. Mayo Clinic Proceedings; 75(1):86-97. January 2000
21. Wong, D.M., Blumberg, D.A. & Lowe L.G. Guidelines for the Use of Antibiotics in Acute Upper Respiratory Tract Infections. American Family Physician; 74(6):956-966. 2006
22. Joerner, R.D. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poultry Science; 82:640-647. 2003