

Biological approach of dental caries management

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SUMMARY

Dental caries is a disease induced by dental plaque, which can be described as a community of microorganisms (biofilm). Because of genetic and environmental factors a number of changes in the oral microbiome takes place; in case of commensalism and mutualism between biofilm microorganisms and the host, homeostasis in oral microbiome is maintained. However, when a risk factor occurs parasitic relationship starts prevailing and activity of the pathogenic cariogenic microorganisms increases leading to a dental caries. According to the newest technologies of molecular microbiology new cariogenic microorganisms species have been determined. Each individual's oral microbiome is as unique as his/her immune system; therefore, commonly taken caries prevention measures cannot be of the same effectiveness for all individuals. Each person has his own caries risk which is determined by the oral microbiome and immune system influenced by the environmental and genetic factors. Early caries diagnostic, risk assessment and individualized caries prevention plan will allow us to control the disease and achieve a desirable effect. For the dentist the most important thing is not to treat the consequences of the disease – cavities - but be aware of the dental caries as a biological phenomenon.

Key words: dental caries; oral microbiome; prevention; diagnosis.

INTRODUCTION

Dental caries is the most common disease in the world (1). This disease can be perceived in the narrow and general senses. In the narrow sense caries is a cavity, but, in reality, a cavity happens to be a consequence of a certain disease. Dental caries treatment in the clinical practice usually is understood as a restoration of the hard tissues of the teeth in compliance with the existing functional and aesthetical requirements. However, it is not the treatment of a disease as such, it is the treatment of its consequences. Clinical practice of the contemporary dentistry is fully focusing on restorative treatment. In clinical practice a widely applied method of caries treatment covers the removal of the infected tissues, in order to prevent the further progression of a disease. The defect that is left needs to be restored by different restorative materials. It is a well-known fact that fillings are not permanent, in due time they

worn out leaving space for micro cracks to occur, and, consequently, small fillings need to be replaced with bigger ones, more and more tissues of the teeth have to be removed. All the restorations are followed by endodontic treatment, which leads further to prosthodontics dentistry, however, the latter one, happens to be not the final stage in this pathological life cycle of the tooth. In case a tooth needs to be extracted surgically due to some complications the final stage is reached when a dental implant needs to be embedded to recover the function of the tooth (2). It has to be admitted that narrow understanding of a dental caries as a biological phenomenon brings us to such a pitfall. Due to scientific progress in genetics and molecular biology in particular, new discoveries let us change old paradigms with the new ones, create new strategies for caries prevention and treatment. Dental caries in a general sense is understood as a disease, which occurs when ecological balance in the oral microflora gets disrupted. These changes can be influenced by biological and environmental factors. Just by maintaining the mentioned changes the process of dental caries becomes manageable.

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ETIOLOGY OF DENTAL CARIES

In 1924 *Streptococcus mutans* was identified by Clarke (3) and for a long time these microorgan-

isms were one of the main pathological bacteria in the oral cavity (4), firstly, because of their ability to survive in low pH (5), to produce acids (6) and extracellular polysaccharides (7). Although *Streptococcus mutans* is very important at the beginning of the caries process (4), the application of new molecular technologies such as 16S rRNA gene sequencing and polymerase chain reaction (PCR) indicated that in some samples collected from the cavities instead of *Streptococcus mutans* some other acid-producing microorganisms had been identified (8). On the contrary, *Streptococcus mutans* was detected in the patients who had been caries free (9).

With the help of the newest molecular biology approaches there have been identified the following communities of pathogenic microorganisms related to caries: *Bifidobacterium dentium*, *Streptococcus mutans*, *Scardovia wiggsiae*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Prevotella spp.*, *Seleonomonas spp.*, *Lactobacillus spp.* (10). In the future this list is expected to be expanded with the names of more species. Next-generation high-throughput sequencing methods have the potential to reveal the composition and functioning of the biofilm by means of metagenomic and metatranscriptomic analyses (11). Dental caries is a polymicrobial disease, therefore, not only qualitative but quantitative parameters of the biofilm are of great importance to its etiology, but also different microorganisms inter-relationships and their synergistic effect, which has to be evaluated while designing diagnostic, treatment and preventive strategies, should be born in mind (12).

It has been scientifically proved and grounded that the main cause of dental caries is microorganisms in the dental plaque. They form a biofilm where carbohydrates get fermented and acids are produced. Acids damage enamel and dentin that consequently leads to the destruction of proteolytic collagen fibers and exposure of soft infected dentin (13). However, this single factor does not determine the occurrence of caries; it is a multifactorial disease, where the presence of the plaque is not enough. Due to its complicated nature there cannot be just a simple causal connection. The factors determining caries can be divided in two groups: genetic and environmental.

According to the “ecological plaque hypothesis”, when homeostasis in the plaque is disrupted pathological microorganisms start prevailing. Potentially cariogenic microorganisms naturally inhabit the plaque; however, under the condition when pH is neutral they cannot prevail and, thus, occupy just a small part of the whole biofilm. If there are no additional factors cariogenic microorganisms become insignificant, demineralization-rem mineralization cycle

is in balance, and homeostasis is maintained. In case this balance is disrupted acidogenic microorganisms start prevailing. The main factor that contributes to the disruption of microbial homeostasis is low pH in the plaque, which appears because of the frequent intake of the fermentable carbohydrates. Consequently, demineralization develops. Another factor which also affects the balance is low saliva flow rate. The main statements of the “ecological plaque hypothesis” are these: a) pathological microorganisms prevalence is related to changes in the environment and b) it is not always necessary for a disease to have a specific etiology: any species with appropriate features can induce the process of a disease, for example: *Streptococcus mutans* is best adapted to live in cariogenic environment, however, it is not the only one that possesses such abilities, other species like *Streptococcus mitis* also has similar features and can contribute to enamel demineralization (14). Dental plaque being in the same role as a biofilm and a community of microorganisms gain the abilities which help them survive: plaque becomes less sensitive to antimicrobial agents (14, 15) and less virulent microorganisms are enhanced (14).

The newest epidemiology studies suggest that there are populations in which very high caries risk is identified (16), this can be explained by the factor of genetic caries risk (17). In the human body live millions of different microorganisms, they all are related to the person’s health condition and by interacting with each other they may either induce a disease or not. These microorganisms create communities and ecosystems they have their own genotypes and phenotypes, and all of this is called a microbiome (18). According to localization of the colonized surfaces an oral microbiome can be divided into: teeth covering biofilm, tongue, cheek, hard and soft palatals, tonsils. Each microbiome varies by its microbial composition, which is dependent on different niches, which are adapted for groups of certain microorganisms to function (19).

DENTAL CARIES – DISEASE DEPENDED ON CHANGES IN AN ORAL MICROBIOME (BIOFILM)

The individual’s genotype determines the properties of its immune system which affect the composition of a microbiome. If the biodiversity of an oral microbiome is poor there is a higher risk for caries to occur. Biodiversity of the microorganisms in the plaque influences their community lifestyle, due to it they become more resistant to the environmental stress and help each other to adapt and survive (20).

The individual's genotype can also determine favorable environment just for pathogenic bacteria to survive for example, an individual will have such genes that will contribute to the production of a smaller amount of antibodies and proteins in the saliva, and because of this fact accumulation of the plaque will become easier and a risk to the caries increases (19). When among the microorganisms and the host prevails commensalism as well as mutualism, the microbiome is in balance and it is ensured by maintenance of good oral health (21). When the immune system becomes impaired these relationships get destroyed, parasitic relationship starts prevailing, caries progresses, some pathogens help other microorganisms to establish themselves, for example, *Streptococcus mutans* creates acidic environment in which *Veillonella* species thrives, consequently, it enhances further growth of *Streptococcus mutans* (22). Also the impaired immune system inhibits the rate of saliva flow and decreases the substance quantity in them which lets the plaque accumulate in a much easier way (20).

Teeth are part of the oral cavity and saliva is the main fluid in it. Cariogenic bacteria live in the oral cavity; therefore, saliva has a direct impact on their growth and survival (23). It can be stated that saliva has a dual effect: it washes out the products of microorganisms' metabolism and neutralizes acidic pH in dental plaque. Acids are considered to be the main product secreted during metabolism. Acids change biofilm pH; when the latter decreases to 5.5-5.0, demineralization starts and caries develops. The main function of saliva is to neutralize acidic pH of the plaque; it can be done because of the saliva buffer capacity. When a saliva flow rate is stimulated saliva composition changes: the concentrations of protein, sodium, chlorine and bicarbonates rise while the concentrations of magnesium and phosphorus fall. Bicarbonates diffuse into the plaque, neutralize acids in it, increase pH of the plaque and stimulate remineralization of the demineralized enamel and dentin (24). Calcium and phosphate ions in saliva help to maintain the integrity of tooth structure too. Saliva in normal conditions are saturated with these ions.

Studies have showed that both the saliva flow rate and the composition of the oral microbiome in every individual are unique (10). This makes us believe that composition and formation of the plaque in each individual is specific. The different plaque biomass, pH and individuals microbial response, are the reasons why some individuals are more prone to the disease than others despite their oral hygiene habits (20). The composition of a microbiome can be compared to a biomarker, which shows the level of activity of the disease. A healthy microbiome can be maintained by

good oral hygiene and the healthy immune system which functions normally (25).

It is important for a dentist to get aware of dental caries as a disease depended on an oral microbiome and be able to apply this knowledge to clinical practice when a patient's caries treatment and prevention plan is designed individually by appointing different strategies.

DENTAL CARIES PREVENTION

Diet and oral hygiene

Though the significance of the genetic factors in caries etiology has been increasing, yet, alongside with the research development, it cannot be taken for granted. Epigenetic factors (methylated DNA sequences, histones, i.e., protein complexes) can have an impact either on one or the other gene expression (26). These factors are influenced by the environmental factors. There have been identified a few key environmental factors such as diet, smoking, bacteria and inflammation; they may have an impact on oral health through epigenetics changes occurring in the genes which are involved in the immune response in oral mucosa (27).

Thus, it becomes evident that a rational diet and oral hygiene still are the milestones in preventive dentistry. Teeth demineralization is a result of the side effect of the nutrition due to acid production from carbohydrates in the dental plaque. Carbohydrates as such do not do any harm to the teeth directly. When sugar or other carbohydrates get an access to the oral cavity, they are metabolized into acids by microorganisms, pH of the plaque decreases and, consequently, solubility of the hydroxyapatite increases – demineralization starts (28). With pH value decreasing, increase all microorganisms acidurance, and numbers of changes develop in microbiome such as microorganisms phenotypic adaptation and genotypic selection (29). Dental caries is a dynamic process, which depends on environmental acidification, so molecular microbiological studies should also embrace not just identification and quantity parameters of microbiome composition but metabolic activity as well (30). Metabolome analysis is a new tool that might enable us to assess such activity (11).

Many studies have proved that sugar in a diet is one of the main caries etiology factors, which interacts with the oral microflora. In isolated communities where a traditional diet with a low sugar level prevails, there has been identified very low caries prevalence (even when a diet is rich in starch) (31).

It is the food form that determines the time how long carbohydrates stay on the tooth surface. On time

how long aciduric stage prevails, depends acid-induced selection and growth competition and this is the major reasons for the shift in the composition of microflora (29). Liquid foods, drinks, in particular, pass through the mouth cavity very fast and have little contact with the tooth surface. Although, if a soft drink is kept in the mouth for a longer time or sipped constantly there is a higher caries risk. When hard candies, mints or lollipops stay in the mouth too long, sugar is also released gradually and demineralization time gets prolonged (32). Sticky food like caramel or adhesive food such as potato chips should also be avoided.

Irrational diet, avitaminosis, in particular, may have an impact on oral health. Theoretically, vitamin D is associated with metabolism of calcium and mineralization of hard teeth tissues. It has been determined that due to the deficiency of vitamin D, enamel hypoplasia occurs (33). In 1973 in *the Lancet* journal there was published a comprehensive randomized survey, which confirmed the existing correlation between expecting mothers' vitamin D avitaminosis during pregnancy and their offspring's enamel hypoplasia (34). Dental enamel hypoplasia is related to the increased caries risk (35). These correlations encouraged further clinical trials which had been searching for links between vitamin D and caries occurrence. The intake of vitamin D supplements was related to 47% caries risk reduction and there was no difference whether there was used ultraviolet irradiation or the forms of vitamin D2 or D3 (36). It has to be mentioned that vitamin D is also significant to the immune system; its effect is less related to oral health and has not received any proper evaluation by the scientists yet. Vitamin D stimulates the specific gene which regulates production of antimicrobial peptides like cathelicidin that combat bacteria, viruses and fungi. Further to the mentioned features, antimicrobial peptides also hold immune regulatory properties: participate in chemotaxis, cell proliferation, wound healing, cytokine and chemokine production, increase vascular permeability (37), and the condition of the immune system influences changes in the oral microbiome homeostasis (22).

At present a rational diet such as a prevention method becomes of secondary importance, even more, when fluoride compensates a harmful effect of carbohydrates (37). Although from the biological perspective, an irrational, rich in refined carbohydrates diet is one of the main factors, which disrupts microbiome homeostasis; its impact on the patients with high caries risk may be decisive.

Considering the significance of oral hygiene it is important to understand that full elimination of the

biofilm is impossible, even when teeth are brushed perfectly biofilm starts developing immediately because it is impossible to remove all the microorganisms, oral cavity is never sterile, but it takes time for the well-organized community that is responsible for demineralization to get recovered. Biofilm starts developing immediately after tooth brushing and within 24 hours it has individual microcolonies (38). If every time we destroy the biofilm structure when we brush our teeth properly, we will not let microorganism communities, responsible for pathological changes in the teeth hard tissues, recover: we will succeed in maintaining healthy teeth. Daily oral hygiene disrupts the community of biofilm microorganisms, they lose their activity, pathogenic potential gets decreased. Therefore, separate individuals' who have genetic factors responsible for caries development oral hygiene has to be perfect, it will help them if not to avoid the disease then to control caries activity. Oral hygiene is one of the exterior environmental factors which influence changes in the microbiome homeostasis.

Fluoride

Fluoride is considered to be one of the main caries preventive measures. Fluoride can be used either systemically (by drinking fluoridated water, milk, consuming fluoridated salt, pills) or locally. 50 years ago systemic water fluoridation was the basis for the caries prevention programs and it was the most important measure in all 20th century. But today it has been scientifically proven that cariostatic effect of fluoride is experienced as soon as the tooth gets erupted and when it is applied locally. Furthermore, there was an indication of the increased fluorosis prevalence among individuals who had lived in fluoridated areas. Some studies conducted in fluoridated and nonfluoridated areas suggest the conclusions that systemic water fluoridation is not necessary for caries prevention, especially in industrialized countries, since caries is in reduction there due to improved oral hygiene and increased cases of toothpaste with fluoride usage (39). The recent report of the World Health Organization recommends that "every effort must be made to develop affordable fluoridated toothpastes in developing countries" and "to exempt them from the duties of taxation applied to cosmetics" (40). Effect of the locally used fluoride depends on concentration, frequency of the use, duration of the application time and specificity of fluoride compound. The higher fluoride concentration in the toothpaste and the more frequent applications will be done, the lower caries risk become (41).

How does the local fluoride manage to avoid or arrest caries? There are three key mechanisms: a) when plaque is acidified, hydrogen fluoride (HF) molecule diffuses inside the bacterium and inhibits its metabolism b) in the acidic environment the existing fluoride on the crystal surfaces inhibits demineralization, c) it enhances remineralization and produces a protective layer of low solubility similar to fluorapatite on the remineralized crystals (42). Fluoride makes a significant influence to the acid-producing microorganisms in a biofilm: when HF is inside the cell, it dissociates into H and F, because of the higher internal pH of cells; this leads to the accumulation of fluoride and acidification of the cytoplasm, the result is a reduction in both the proton gradient and the glycolytic enzyme (enolase) activity (43). This is known as antimicrobial action of the fluoride.

Therefore, it is recommended to brush teeth twice a day for 2 min with the toothpaste containing fluoride. Many people think that if they brush their teeth they will be protected from caries but it is not enough to brush, especially for children who tend to have a higher caries risk – toothpaste with fluoride is necessary for them. Its concentration should not be lower than 1000 ppm in order fluoride to be effective (44). According to the results of 70 studies, the effect of fluoride toothpaste on the permanent dentition is associated with 24% reduction in decayed, missing and filled tooth surfaces (DMFS) (45). Flossing also should not be forgotten because it is the main interdental hygiene measure. While reserving 2-8 min for oral hygiene per day we gain benefits, i.e., good oral health and fresh breath (40).

When a plan for caries prevention measures is designed for every patient, the approaches have to be chosen individually according to the nature of a patient's caries risk, which depends on the unique oral microbiome and is influenced by genetic and environmental caries risk factors. The deeper the survey is conducted the more evident it becomes that preventive treatment should be individualized; dentistry is not an exception. Then, all the measures applied to the patient individually, according to his/her genetics, immune system, risk factors will be much more effective, state funds for caries prevention programs will be distributed in a more rational way.

DENTAL CARIES PROGRESSION CONTROL

The major challenge in the management of the caries process is to control caries progression (46). If we want to control it the most important thing is to detect early noncavitated caries lesions and assess their

activity. According Nyvad caries diagnostic system it is simple to assess enamel and dentine caries lesions as well as activity/inactivity of these lesions (47). On caries lesions detection and activity assessment depend treatment strategies. Traditional view is that the diagnosis of the caries process is equivalent to that of cavitated lesions and that its treatment should be based on fillings and extractions (48). But the main goal is to reduce noncavitated lesions activity by nonoperative caries treatment methods, trying to avoid caries progression and operative treatment. In clinical practice the patient with active caries lesions shows that there is a dynamic stability changes in his oral microbiome, and he is like clinical disease marker. Without environmental control, which influence microbiome biofilm quantification and qualification, we will fail to stop caries progression. Environmental control may include mechanical plaque control, low sugars diet, application of pH neutralizing techniques such as saliva stimulation (29).

Diagnostics of dental caries lesions and their activity assessment, the patient's personal caries risk assessment, design of a plan for individual preventive measures, caries activity monitoring consume quite much of a dentist's time and is poorly appreciated; therefore, many clinicians treat just the consequences of the disease but not the disease itself. If we want to get away from this restorative dentistry approach we have to overcome many technical, cultural and economic obstacles.

CONCLUSION

In conclusion we could say that dentists should be therapists first of all, whose basic principal since the times of Hippocrates has been not to cause any harm. It is important to get aware of the disease, its causes, and start treating it. During past decades the awareness of a dental caries as a biological phenomenon has been in progress. It is understood that caries occurs because of the impact of environment factors, which affect individual microbiome homeostasis. Technologies of molecular microbiology provide us an opportunity to see the composition of the microbiome in healthy individuals and those who suffer from caries, to identify new species of pathogenic microorganisms, research their interaction, synergistic potential and the impact of these factors on caries occurrence process and its progression. Since each individual has a unique oral microbiome, which depends on the interaction of the immune system and genetic factors, therefore, in order to achieve the most effective result, a caries prevention plan as well as a treatment plan should be individualized. Personalized dentistry is coming.

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