

BACTERIAL FACTORS OF CARIOGENICITY (LITERATURE REVIEW)

BAKTERYJNE CZYNNIKI ROZWOJU PRÓCHNICY (PRZEGLĄD LITERATURY)

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ABSTRACT

Introduction: According to the 2003 WHO report, dental caries affects from 60% to 90% of school-age children and adults that makes it one of the most common diseases worldwide.

The aim was to systematize data about the modern conception of plaque formation and role of microorganisms in its development.

Materials and methods: Biblosemantic, 50 medical literature sources were systematically reviewed as the material for the research.

Review: According to Miller's theory, oral microorganisms can decompose dietary carbohydrates into acids, which in turn dissolve the calcium phosphates found in the enamel, causing its demineralisation. Along with *Streptococcus mutans*, nowadays some other bacteria as *Streptococcus sorbinus*, *Lactobacillus spp.* and *Actinomyces spp.* have been well studied as caries contributors. However, the disease is related to plaque-mediated, because a much larger number of normal oral microflora representatives are involved in creating favourable preconditions for its development. There are a lot of original research papers about a role of bacteria in caries decay but compositions and characters of oral microflora are changing nowadays. Therefore, authors show the main cariogenic bacteria and their factors of pathogenicity which create special conditions for caries lesions. Modern concepts of dental plaque formation and pathogenesis of plaque-associated diseases are presented according to the new actual dental research. A lot of attention is paid to the biochemical properties of cariogenic bacteria and chemical process in biofilm. Role of acid and alkali production by oral bacteria in caries decay are shown in this article. Moreover, mechanisms of bacterial acid-fast and acid-tolerance are presented.

Conclusions: Analysis of literature demonstrates a lot of bacterial pathogenicity factors which play key role in caries development.

KEY WORDS: caries, cariogenicity, *S. mutans*, plaque, biofilm

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INTRODUCTION

Microbial flora of the mouth is a complex and quite resistant to external factors system of microorganisms that are in symbiotic relationship between each other as well as practice commensalism and mutualism with its host, a human body as a biological species [1]. Oral cavity harbours a variety of different and structurally complicated cenoses that result from bacteria colonization on oral surfaces. Most of them are found *throughout* the entire *human body, but there are some only detected* in the oral cavity, e.g. *S. mutans* [2]. There are about 700 species of microorganisms identified on the oral mucosa and dental surfaces in health and some pathological conditions, and a half of them can not be cultivated and have been detected by using bio-molecular techniques. Therefore, the composition of oral microbiota of *Homo sapiens* is still far from being closed issues [3,4,5].

Most of bacteria inhabiting the human oral cavity build up its permanent, or resident, micro biota that emerged during the evolution and has become resistant to factors influencing the oral environment. However, despite the diversity of resident micro flora, there are much more transient microorganisms entering oral cavity [6]. In order to colonize the oral cavity bacteria should possess special properties (ability to stick to hard surfaces, to withstand constant salivation, mechanical stress during movement of the cheeks and tongue, to tolerate changes of the physical parameters of the environment). Therefore, bacteria that are not able to adapt and, thus, to survive in these conditions can be only transient guests in the mouth and the time of their staying is limited [3,7,8].

Nowadays dental caries and periodontitis have been known the commonest oral infectious diseases [7, 9]. They are prevalent worldwide including both developed and developing countries. These conditions can affect the population despite the income level, turning into international public health challenge. Thus, according to the 2003 WHO report, dental caries affects from 60% to 90% of school-age children and adults that makes it one of the most common diseases worldwide [8,9].

THE AIM

The aim was to systematize data about the modern conception of plaque formation and role of microorganisms in its development.

MATERIALS AND METHODS

Biblosemantic, 50 medical literature sources were systematically reviewed as the material for the research.

REVIEW AND DISCUSSION

THE COMPOSITION OF CARIOGENIC MICROFLORA

In 1890, Willoughby D. Miller advanced his well-known chemico-parasitic theory on the development of dental

caries, which is still relevant and the most plausible even today, over 100 years on. According to his theory, oral microorganisms can decompose dietary carbohydrates into acids, which in turn dissolve the calcium phosphates found in the enamel, causing it demineralisation [10]. In 1924 J. Kilian Clarke detected in the patient's carious cavity and described a microbe, a facultatively anaerobic gram-positive coccus, and named it as *Streptococcus mutans*. He suggested it to be a significant contributor to dental caries. However, the conception of bacteria as a causal factor in the development of dental caries was finally recognized in the 50-60-ies ensuing from numerous experiments with sterile animals [3, 11].

Along with *Streptococcus mutans*, nowadays some other acid-resistant bacteria as *Streptococcus sorbinus*, *Lactobacillus spp* and *Actinomyces spp* have been well studied as caries contributors [4, 12]. However, the disease is related to plaque-mediated, because a much larger number of normal oral microflora representatives are involved in creating favourable preconditions for its development [3, 13].

Oral microorganisms build up two types of biofilms on the surface of the teeth, supra-gingival and sub-gingival, which differ significantly in their qualitative and quantitative composition [14]. Supra-gingival biofilm typically contains gram-positive bacteria, among which *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Lactobacillus spp*. are the most prevalent. In turn, sub-gingival biofilm is made of gram-negative anaerobic bacteria, among which *Actinobacillus*, *Campylobacter spp.*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* predominate. Supra-gingival biofilm initiates tooth decay of hard dental tissues, while sub-gingival biofilm is mostly known as etiological factor of periodontal diseases [6, 15].

STAGES OF DENTAL PLAQUE FORMATION

Formation of dental plaque biofilm is a complex process of interaction between bacteria within the entire microbial community of the human body and between each others that develops in three stages [3, 6, 16]. Initiation of the process begins in some minutes following tooth-brushing with the adherence of so called pioneering microorganisms, which are predominantly oral streptococci to hard dental tissues [8, 16]. Interestingly, 2 hours later, bacteria *Streptococcus oralis*, *Streptococcus anguinus*, *Actinomyces spp*. are found in greater numbers, and *Haemophilus spp*. and *Neisseria spp* are found in lesser numbers. These microbes reach their maximum in number in 48 hours. These bacteria produce IgA1-protease, which helps resist to the main element of local protection of the oral cavity, thus creating favourable conditions for the formation of dental plaque [3, 16, 17]. However, these microorganisms do not possess factors that allow them to adhere to the surface of a cleaned tooth. Therefore, pellicle, a thin film of proteins and salivary glycoproteins that grows on the tooth surface within minutes of a professional prophylaxis, mediates between the enamel and bacteria [3, 6, 18]. Non-specific interaction between bacteria and tooth covered with pellicle is determined by ionic and hydrogen

bonds, hydrophobic repulsion and van der Waals forces. The mechanisms of specific interaction provide selectivity in colonization of host tissues as they are mediated by adhesins, specialized superficial proteins of bacterial cells that bind to pellicle receptors [19]. Following the initial colonization of tooth surfaces by microorganisms, co-aggregation, adhering of the varieties of bacteria to the early colonizers, and subsequent bacteria multiplication result in the thickening of biofilm. Some microorganisms which can not directly adhere to pellicle on the dental surfaces interact with bacteria, which are already present in biofilm, staying on the tooth surface (co-adhesion) [20,21,22,23]. Moreover, life activity of bacteria on the tooth surface creates favourable conditions for further colonization with some species demanding proper environment. Thus, *Neisseria spp*. reduces redox potential by using oxygen and releasing carbon dioxide, thus creating conditions for colonizing with anaerobic and facultative anaerobic species [24].

Over time, plaque becomes morphologically heterogeneous and is colonized not only with round cocci, but flexuous and lengthened bacteria as well that are surrounded by amorphous substance of microbial origin [3,4]. In addition, extracellular polymers such as soluble and insoluble glucans, fructans and heteropolymers contribute much in the formation of biofilm [3, 25]. For example, *S. mutans* secretes four extracellular enzymes, three glycosyl transferases and fructosyl transferase, that by breaking up sucrose into glucose and fructose that contribute to the formation of their polymers, glucans and fructans. The latter, in turn, perform receptor function by helping pathogens colonize throughout the surface of the dental pellicle, acting as substrates. Glucans and fructans also protect from the effects of non-specific protective oral factors [3, 6, 26]. Besides *S. mutans*, extracellular polysaccharides are produced by some other bacteria of the oral cavity, such as *S. sanguis*, *S. gordonii*, *A. viscosus* [27,28].

After the bacterial adherence to the tooth surface, there is observed multiplication and differentiation of microcolonies, suppression or activation of gene expression depending on the needs within the biofilm. This results in the formation of a complex multicomponent system of numerous microbial species that uniquely interact with each other through the microchannels for metabolism. Externally, the biofilm is covered with a layer of exopolysaccharides that make it less permeable to environmental fluids, including protective components of saliva or antibiotics [6, 27].

MECHANISMS OF ACID-PRODUCTION AND ACID-TOLERANCE OF ORAL BACTERIA

Among greater contributors to the development of dental caries there are acid-producing microorganisms forming the dental plaque biofilm, as *S. mutans*, *S. sorbinus*, *S. oralis*, *S. sanguinis*, *Lactobacillus spp.*, and *Actinomyces spp* to a lesser extent [29]. In high amount of dietary sugars, they are able to synthesize organic acids, mainly lactic acid, whose ionization constant ($pK_a = 3.5$) is much lower

than that one of the dental enamel that facilitates the dissolution of minerals in the structure of the enamel [30]. For instance, *S.mutans* synthesizes four extracellular enzymes, three glycosyl transferases and fructosyl transferase, which catalyze decomposition of sucrose into glucose and fructose that results in formation of polymers facilitating the adhesiveness. A mixture of both fructan and glucan serve as substrate converted into lactic acid by intracellular glycolytic pathway. Lowering of dental plaque pH to critical level (to 5.5.) evokes imbalance between enamel remineralisation and demineralization, thus, initiating carious process [31].

According to Ann Griswold (University of Florida), for two minutes after the formation of organic acids in the dental plaque, pH is rapidly dropping from neutral values to 5-4,5 and in three minutes reaches 4. It is at this moment that lasts no more than two minutes, at which the tooth enamel begins to demineralise. Starting from the fourth minute, pH can arise by favouring enamel mineralization. This process is completed within an average of thirteen minutes when dental plaque pH returns to its original neutral values. However, in people prone to tooth decay, pH drops much lower and renews much more slowly, therefore, a time interval of critically low pH values and their impact on hard dental surfaces increases [32,33].

It seems that alkali formation in supra-gingival dental plaque reduces its cariogenic potential and in this way counteracts the demineralization of hard dental tissues, but on the other hand, this can lead to unwanted adverse consequences [3,4,34]. Thus, an increase in dental plaque pH promotes the survival of microorganisms unstable to acids as well as results in the precipitation of calcium salts with further formation of dental tartar; cytotoxic ammonia is involved in the development of gingivitis and periodontitis [4,35]. The main ways of alkali formation in dental plaque is urease hydrolysis of urea and arginine metabolism with arginine desaminases, resulting in ammonia formation [35,36]. Among urease positive bacteria in supra-gingival plaque, *Actinomyces spp.*, *Haemophilus parainfluenzae* are prevailing as well as *S.salivariusta* and *S.vestibularis*, which rarely enter into the composition of the dental plaques and influence saliva alkalescence. Activity of arginine desaminases manifests itself by increasing number of oral microorganisms, *S.gordonii*, *S.sanguinis*, *S.rattus*, *S.anginosus*, *Lactobacillus fermentum*, *P.gingivalis*, *T.denticola* [3,37, 38].

The largest share of oral microflora is represented by neutrophils, who function optimally in neutral environment, but species tolerant to acids are found as well [4, 39]. Particularly noteworthy are representatives of genus *Lactobacillus*, which are not only able to withstand a temporary decrease in pH, but even to carry out glycolysis at pH of about 3 [40]. Acidic environment is also favourable for *Candida spp.* due to the action of proton pump - F-ATPase and vegetation at the edge of the plaque [41]. Such environment does not favour the growth of *S.mutans*, known as the main contributor in dental caries aetiology, although this microorganism belongs to acid-tolerant due

to its capacity for glycolysis in acidic environment. At pH 4 *S.mutans* catabolises sugars but can not utilize derived ATP for the growth (it collapses), i.e. in the acidic environment catabolism is separated from anabolism. During the ATP hydrolysis, F-ATPase transfers protons across the cell membrane that directly prevents marked cytoplasm acidulation and, consequently, inactivation of major enzyme systems of the cell. Thus, *S.mutans* is able to “go through” the period of acidification in the dental plaque, whereby the production of acids by this microorganism does not stop at this time. This acid tolerance causes the selection of microorganisms resistant to low pH in the biofilm, while sensitive species disappear [4,42,43].

A wide range of pathogenicity factors caused by cariogenic microorganisms, among which the availability and the synthesis of proteins-adhesins, enzymes that promote the break up of carbohydrates and the formation of organic acids, as well as factors that enhance their stability in acidic environments and help resist local protection factor, creates conditions for the long persistence in the mouth. Normally, there is dynamic balance between indigenous (permanent) microflora and antimicrobial factors in the mouth, while imbalance between them results in the development of common oral diseases, dental caries and periodontitis [44,45].

CONCLUSIONS

The literature review shows the relationship between the development of caries and a large number of representatives of normal oral microflora is an indisputable fact, despite the significant number of other potential etiological factors. However, clinical and experimental studies show that caries-causing bacteria play the most critical role in the development of this pathological process as they can colonize the hard dental tissues, can survive in acidic environment and produce organic acids, which, in turn, lead to enamel demineralization.

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