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Investigations

Meaning of the Oral Hygiene in Children, Due to Atherosclerosis Generation

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Abstract: Research focused on association between periodontal diseases and atherosclerosis has been substantial in recent years. It is now well known that chronic inflammation plays an important role in initiation and evolution of atherosclerosis. The bacterial plaque is a key pathology leading to periodontal inflammation. As reported by other authors, bacteria causing periodontal disease were found within the carotid arteries in adults, thus association between periodontal disease in childhood and peripheral vascular disease seem justifiable. The purpose of this study is to investigate the potential link between certain bacteria found in children with periodontal disease and the process of atherosclerosis in adults. Research was carried out in 97 children of which 58 aged between 6-14 comprised an experimental group, whereas 39 children aged of 6-13 comprised the control group. Based on physical examination, the oral hygiene in children of the experimental group was average or bad, while in children of the control group the oral hygiene was good. Blood samples and subgingival plaques were obtained to proceed with microbiological testing by PCR technique. The results of tests showed *Aggregatibacter actinomycetemcomitans* in 55% and *Prevotella intermedia* in 79% of children in the experimental group. Only 2 cases of *Prevotella intermedia* were detected in the control group, however, no cases of *Porphyromnas gingivalis* were found in either groups. Full blood count, serum homocysteine, CRP, fibrinogen and HDL cholesterol values were normal in both groups. Total cholesterol value was increased in 24,14% in the experimental group vs. 5,15% in the control group, thus increase of total serum cholesterol concentration in children with bad oral hygiene may by a risk factor of atherosclerosis.

Keywords: Atherosclerosis, Childhood, Oral Hygiene, PCR, Periodontal Disease

Introduction

According to 2003 World Health Report, the annual global Cardiovascular Disease (CVD) mortality is 16.7 million (29.2%). CVD typically involves a narrowing or even complete occlusion of the vascular lumen by atherosclerotic deposits, which may lead to death. The pathogenesis of atherosclerosis is multifactorial. There are well-established atherosclerotic risk factors, such as age, male sex, positive family history, diabetes, hypertension, obesity, smoking, low physical activity, elevated Low-Density Lipoprotein (LDL) and total cholesterol levels and decreased High-Density Lipoprotein (HDL) cholesterol (Urban *et al*., 2007; Southerland *et al*., 2006). Recent years have seen an increased interest in the so-called new risk factors for atherosclerosis. This is mainly due to the fact that approximately 25% of patients with premature coronary heart disease show none of the well-established risk factors (Ridker *et al*., 1999). The new risk factors for atherosclerosis include lipoprotein (a), homocysteine, fibrinogen, C-Reactive Protein (CRP), oxidized LDL, small dense LDL particles, Asymmetric Dimethylarginine (ADMA), with a recent addition of an independent risk factor for CVD, i.e. periodontal disease, which causes chronic inflammation (Rech *et al*., 2007; Dave and Van Dyke, 2008). Inflammation, in turn, inherently affects all stages in the development of

atherosclerosis, from adhesion molecule expression on endothelial through fatty-streak and stable atherosclerotic plaque formation, all the way to atherosclerotic plaque destabilization and rupture (Szyguła-Jurkiewicz *et al*., 2004). In 2009, a panel of Polish experts issued a document on the role of periodontal disease in the pathogenesis of cardiovascular disease as well as recommendations as to its prevention (Górska, 2009). In 2010, the European Society of Cardiology published a consensus on the same topic (Bouchard *et al*., 2010). The periodontium is a morphological and functional entity comprising the gingiva, periodontal ligament, cementum and alveolar bone proper. The role of periodontium is to firmly hold teeth within dental alveoli of the maxilla and mandible. One factor that plays an unquestionable role in etiopathogenesis of periodontitis is dental plaque. It is an essential stage in initiating the inflammation typical for most conditions involving periodontal tissues. Dental plaque, together with various general factors, affects the body's immune processes and determines the extent and rate of inflammation. Dental plaque is a soft bacterial film adhering firmly to the surface of teeth and other hard surfaces within the oral cavity, including restorations (Haake *et al*., 1996). Bacteria comprising the plaque exhibit a wide variety, with approximately 300 bacterial species found in the oral cavity. However, the species considered pathogenic with respect to the periodontium are gram-negative anaerobes, including *Aggregatibacter actinomycetemcomitans* (Aa; previously *Actinobacillus actinomycetemcomitans*) (Norskov-Lauritsen and Kilian, 2006), *Porphyromonas gingivalis* (Pg), *Bacteroides forsythus* (Bf), *Treponema denticola* (Td), *Eikenella corrodens* (Ec), *Campylobacter rectus* (Cr), *Prevotella intermedia* (Pi) and *Fusobacterium nucleatum* (Fn). Dental plaque can be divided into supragingival plague colonized mainly by aerobic bacteria and subgingival plaque colonized by anaerobes. Polymerase Chain Reaction (PCR)-based assessments of atherosclerotic lesions in the carotid arteries showed the presence of Pg, Pi and Aa DNA (Górska, 2002). Some studies indicate that the presence of antibodies against bacteria causing periodontal disease may increase the risk of stroke, myocardial infarction and acute coronary syndrome (Pussinen *et al*., 2004; 2007; Renvert *et al*., 2006). Löe *et al*. (1965) demonstrated that the presence or lack of toothbrushing is the key factor determining the development of gingivitis. Those results proved that plaque is the reason of gingivitis. These findings pose a serious concern, especially in children, as epidemiological studies show that dental caries resulting from nonexistent or inadequate oral hygiene is a common problem in children and adolescents, affecting 87% of children aged 3-6 years, nearly 100% of 6-8-year-olds, 79% of 8-12-year-olds and over 90% of 12-19-year-olds (Olczak-Kowalczyk and Bedra, 2003).

Materials and Methods

A total of 97 children aged from 6 to 14 years were included in the study and allocated into two groups. The general health status in all study subjects was good. The study group comprised 58 subjects with fair to poor oral hygiene, including 29 girls and 29 boys. The mean age was 10.42 years. The control group consisted of 39 subjects with good to optimal oral hygiene, including 22 girls and 17 boys. The mean age was 9.31 years. All subjects underwent blood tests and had a sample of dental plaque collected for a microbiological PCR assessment. A questionnaire-based history was taken in all subjects and included the general health status as well as environmental and genetic factors. Oral hygiene status was determined with the Approximal Plaque Index (API) (Lange *et al*. 1978). This index demonstrates the presence or lack of dental plaque in the interproximal surfaces from the oral aspect (quadrants 1 and 3) and the facial aspect (quadrants 2 and 4). The assessment was conducted with a periodontal probe and mirror, without the use of staining. The proportion of plaque-covered surfaces was expressed as a percentage and calculated according to the following formula:

$$\text{API} = \left(\frac{\text{number of plaque} - \text{covered sites}}{\text{number of all sites examined}} \right) \times 100$$

The results were evaluated according to the following scale: API<25% optimum oral hygiene; API 25-39% good oral hygiene; API 40-70% fair oral hygiene requiring improvement; API 70-100% poor oral hygiene. Only otherwise healthy children with API 70-100% or API 40-70% were included in the study group.

Dental plaque samples for a PCR assessment were collected from gingival pockets of all 58 study group subjects and 39 control group subjects. Firstly, the selected sampling site was dried with blotting paper. Then, a swab of supragingival dental plaque was collected, followed by subgingival plaque collection with a sterile periodontal probe. The samples were added into Eppendorf test tubes containing 0.1 mL of physiological saline each and frozen at -25°C until molecular PCR testing. Genomic DNA was isolated from subgingival samples with a Genomie DNA from Tissue kit (Machery-Nagle) according to the manufacturer's instructions.

Multiplex 16S rRNA amplification protocol (Clonit) was used for detection of DNA of the following periodontal pathogens: Aa, Pg and Pi. The PCR mixture for positive and negative controls as well as for the evaluated samples was prepared according to the manufacturer's instructions (Fig. 1-3).

Table 1. Amplification of the isolated DNA, positive control and negative control (included in the kit) was conducted with a Biometria Thermocycler under the following conditions

No. of cycles	Denaturation	Annealing	Extension
1	95°C for 3 min		
30	95°C for 1 min	70°C for 1 min	72°C for 1 min

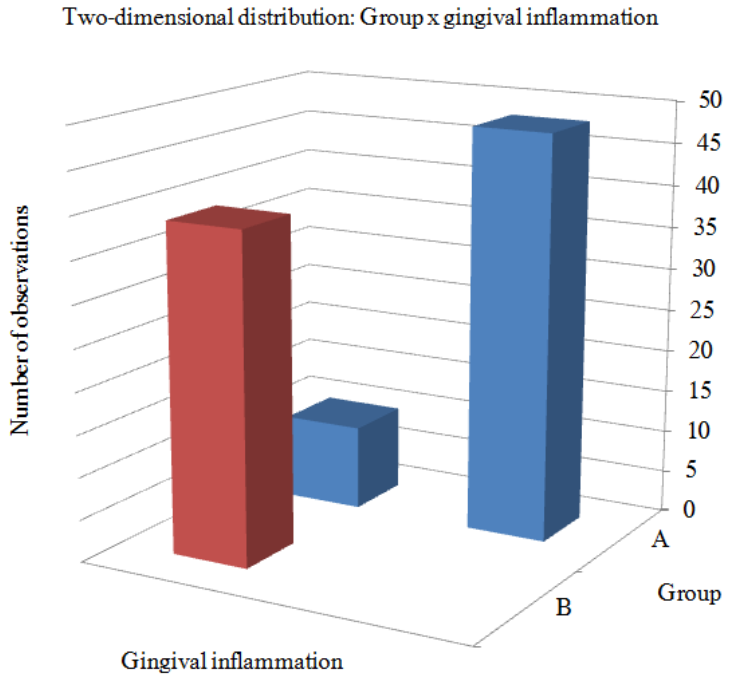

Fig. 1. A graphic representation of the control and study groups in terms of gingivitis (red - group B, blue - group A)

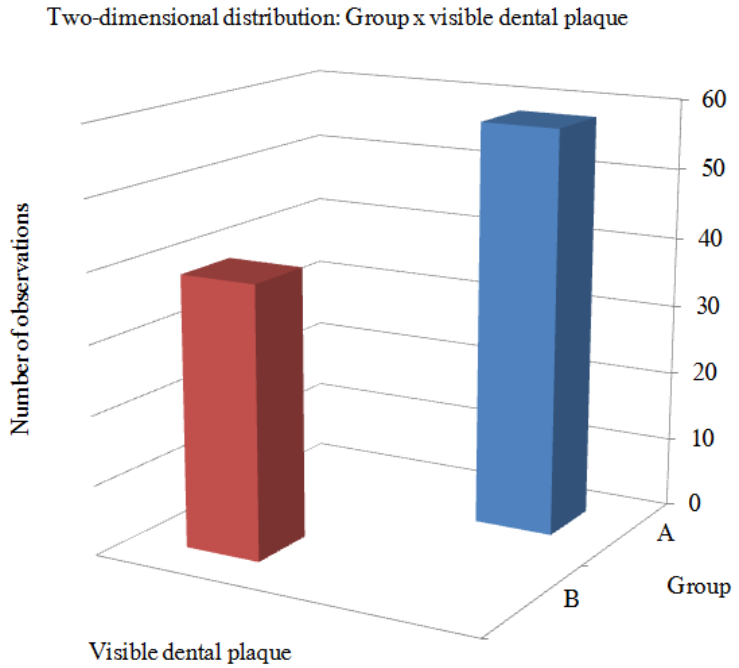

Fig. 2. A graphic representation of the control and study groups in terms of visible dental plaque (red – group B, blue -group A)

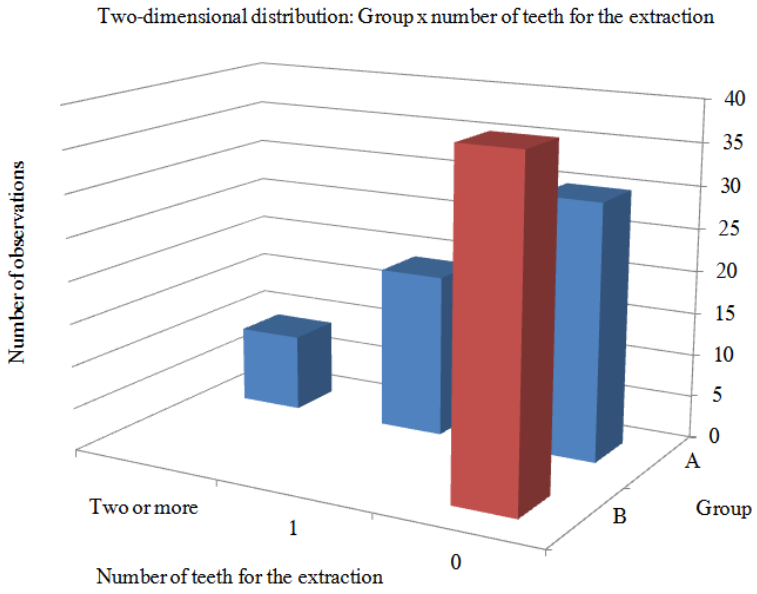

Fig. 3. A graphic representation of the control and study groups in terms of the number of teeth for extraction (red-group B, blue-group A)

Amplification products were detected by an electrophoresis in 2% agarose gel and stained with ethidium bromide (Table 1). A positive result was characterized by the presence of a band characteristic for the given species: 162 bp corresponded to *P. intermedia*, 255 bp corresponded to *A. actinomycetemcomitans* and 530 bp to *P. gingivalis*. Amplification product (band) position was determined with a DNA size marker (included in the kit) and a positive control.

Results

No subjects in the control group (B) showed evidence of gingivitis. In the study group (A), gingivitis was detected in 82.76% of subjects, with the remaining 17.24% of subjects shown to be gingivitis-free. The difference between the groups was statistically significant.

No subject in the control group (B) showed visible dental plaque, whereas dental plaque was detected in 100% of subjects in the study group (A). The difference between these groups was statistically significant.

No tooth extraction was required in 100% of subjects from the control group (B) and in 51.72% of subjects from the study group (A). Extraction of 1 tooth was required in 32.76%, 2 teeth in 10.35% and 3 or more teeth in 5.17% of group A subjects. The difference between the groups was statistically significant.

Fifty-three subjects from the study group had API of 70-100%, which corresponds to poor oral hygiene and 5 had API of 40-70%, which corresponds to fair oral hygiene. Out of the 39 control group subjects, 30 (77%) had optimum oral hygiene (API 70-100%) and 9 (23%) good oral hygiene (API 25-39%). The Mann-Whitney U test showed that the median API% in the study group (poor oral hygiene) was highly significantly greater than that in the control group (good oral hygiene).

The results of PCR-based bacterial DNA detection from the collected dental plaque samples have been presented below.

Figure 4-6, the figures below refer to the study group and show PCR amplification products separated with agarose gel electrophoresis and stained with ethidium bromide; K+ positive control, K- negative control, M size markers; bands: 530 bp *Porphyromonas gingivalis*, 255 bp *Aggregatibacter actinomycetemcomitans*, 162 bp *Prevotella intermedia*; 1-60 analyzed samples.

The DNA of *Porphyromonas gingivalis* was found in none of the subgingival dental plaques. None of the sought-after bacteria were detected in 12 subjects, whereas the DNA of at least one periodontal disease pathogen was found in the remaining 46 subjects. *Prevotella intermedia* was the most common bacterium, found in 46 subjects, including 32 cases where it co-occurred with *Aggregatibacter actinomycetemcomitans*. None of the subgingival dental plaque samples showed evidence of *Porphyromonas gingivalis* DNA. None of the sought-after bacteria were detected in 2 out of 5 subjects with API of 40-70%, while the DNA of both *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* was found in the remaining 3 subjects. The plaque samples collected as part of the study yielded a number of bacteria. Most children from the study group had 2 bacterial species responsible for periodontal disease: *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*. This may suggest an increased risk of periodontal diseases in these patients if their oral hygiene habits do not improve. These bacteria may also have a negative impact on the cardiovascular system.

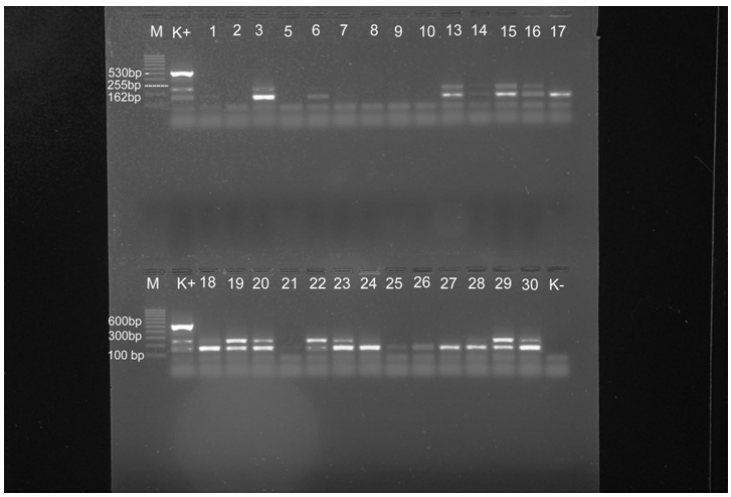

Fig. 4. PCR amplification

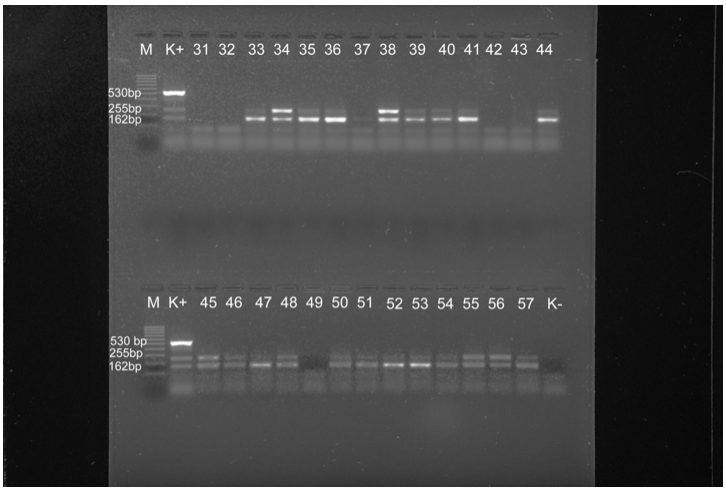

Fig. 5. PCR amplification

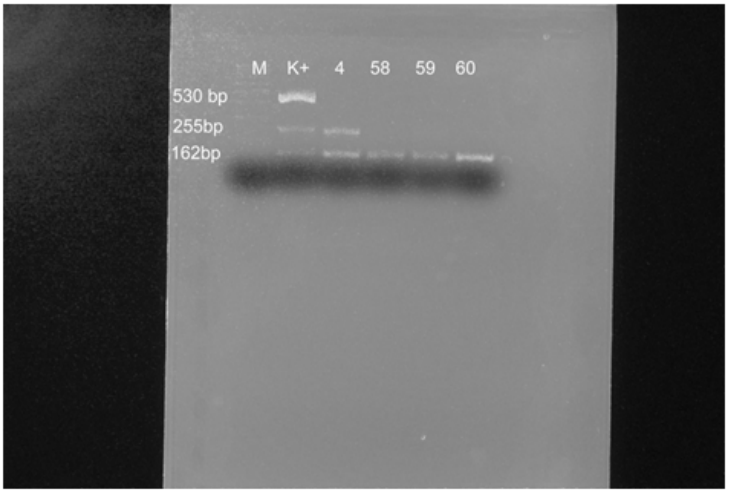

Fig. 6. PCR amplification

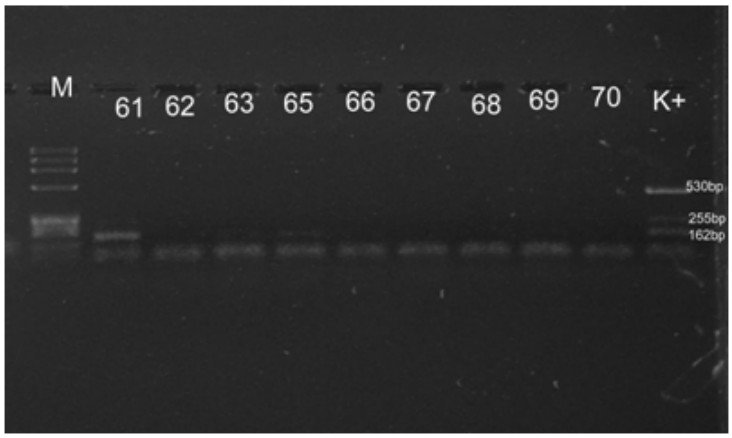

Fig. 7. PCR amplification

Figure 7 the figure below refers to the control group and shows PCR amplification products separated with agarose gel electrophoresis and stained with ethidium bromide; K+ positive control, K-negative control, M size markers; bands: 530 bp *Porphyromonas gingivalis*, 255 bp *Aggregatibacter actinomycetemcomitans*, 162 bp *Prevotella intermedia*; 61-70 analyzed samples.

The Figure above shows the results obtained in the control group (9 samples collected from subjects without apparent dental plaque). A vast majority of samples (37) yielded no bacterial growth. Two samples yielded *Prevotella intermedia*. These findings suggest that maintaining good oral hygiene leads to a dramatic reduction in bacterial flora responsible for periodontal disease.

An analysis of risk factors for atherosclerosis demonstrated that the mean homocysteine levels were significantly higher in the study group than in the control group. The rates of high total cholesterol were significantly higher in the study group. The levels of CRP in the children from the study group tended to indicate a higher risk of atherosclerosis.

Discussion

Nowadays, more and more emphasis is placed both in Europe and worldwide on the relationship between oral and systemic health. According to the prevailing belief, dental medicine teaching programs should include more aspects of general medicine. Dental care has been recognized as an important element of general healthcare. Various studies indicate that mild, chronic inflammation is a significant factor in cardiovascular disease. Such mild, chronic, initially localized inflammation, lasting anywhere from several weeks to several decades, may be caused by periodontal disease. Periodontal disease is believed to be a common inflammatory process and may be an independent risk factor contributing to cardiovascular disease (Rech *et al*., 2007; Dave and Van Dyke, 2008). One of the key risk factors for periodontal disease is poor oral hygiene, i.e. unsystematic and infrequent toothbrushing (while brushing teeth at least twice a day leads to dental plaque removal) (Franek and Górska, 2009). Nonetheless, the relationship between inadequate oral hygiene in children and the risk of developing atherosclerosis remains unknown. There have been a number of studies on this topic, however, these were typically conducted in adult subjects with advanced periodontal disease (Pussinen *et al*., 2004; 2007; Renvert *et al*., 2006). As we did not find any similar studies in children, we compared some of our results to the results of studies conducted in adult study populations.

Based on API assessments, we showed that approximately 91% of children in our study population had a very poor oral hygiene and 9% of children-fair oral hygiene. In contrast, the control group showed 77% of children to have optimum oral hygiene and 23% good oral hygiene. Mean API% was highly significantly greater in the study group than in the control group (children with good oral hygiene). The entire study group requires immediate improvement in oral hygiene. Moreover, a number of studies indicate that oral hygiene status in children is highly unsatisfactory (Olczak-Kowalczyk and Bedra, 2003; Giermakowska *et al*., 1994; Grzesiak and Kaczmarek, 2005; Pires Dos Santos, 2002). The control group was to include children with good oral hygiene and all subjects in this group indeed exhibited sufficient oral hygiene. However, we would like to emphasize that finding subjects for the control group had not been easy because of generally poor oral hygiene in children. This observation demonstrates a very poor condition of oral hygiene in children. Poor oral hygiene in the study group was evidenced by visible dental plaque in all subjects and gingivitis in 82.76% of children from this group. No patients from the control group had either visible dental plaque or gingivitis. The rates of visible dental plaque and gingivitis were highly statistically significant. Already in 1965, Löe *et al*. (1965) discovered that

inadequate oral hygiene may lead to gingivitis (Löe *et al*., 1965). According to international literature, over 82% of American adolescents have evident gingivitis gingivitis (Albandar *et al*., 1998; 2000). The relevant rates in children and adolescents in other parts of the world are comparable or even higher (Albandar *et al*., 2000). Therefore, considering the likely spread of inflammation, it is crucial to detect the inflammation at the stage of gingivitis, when it is still reversible and before it causes deterioration of the periodontium and the general health (Ziętek, 2000). Otherwise, if left untreated, gingivitis may lead to periodontitis, which involves all tissues of the area and is chronic in nature. Long-standing periodontitis may increase the risk of dental plaque bacteria of gingival and, subsequently, periodontal pockets to negatively affect the general condition of the body including the cardiovascular system. This is emphasized by some authors (Beck and Offenbacher, 2005; Arbes *et al*., 1999; Geerts *et al*., 2002; Grzegorczyk-Jaźwińska *et al*., 2001) such as who evaluated the degree of carotid artery stenosis in a group of 411 adults (Schillinger *et al*., 2006). The 6-to-9-month follow-up of the group was to help evaluate the correlation of atherosclerotic lesion progression and oral health parameters: Decayed, Missing, Filled (DMF) index, number of teeth, Plaque Index (API) and the Community Periodontal Index of Treatment Needs (CPITN). The authors demonstrated a positive correlation in the case of the first three parameters. A study by (Briggs *et al*., 2006) included 92 adult males with angiographic evidence of Coronary Artery Disease (CAD). The control group included 79 adult males without CAD, in the same age range as the study group. A comparison of periodontal condition showed a significantly greater number of dental plaque sites, as well as bleeding after probe exploration in the CAD group, which suggested serious neglect of oral hygiene in this group. Moreover, there was a significant difference in the number of periodontal pockets of ≥ 4 mm and ≥ 6 mm in depth. A multivariate logistic regression analysis including socio-economic and systemic risk factors showed that poor periodontal condition combined with $\geq$ 6-mm pockets is associated with CAD.

Another oral hygiene-related problem is caries. A total of 82.05% of children from the control group were cavity-free versus only 12.07% in the study group. Study group subjects with 1 cavity constituted 17.95%, while subjects with 3 and more cavities constituted 29.31% of patients each. A staggering 87.93% of children from the study group were affected by caries, with the mean number of teeth requiring conservative treatment significantly higher than that in the control group. These findings are consistent with those reported by Olczak-Kowalczyk, who showed that nearly 100% of children aged 6-8 years and 79% of children aged 8-12 years were afflicted with caries (Olczak-Kowalczyk and Bedra, 2003). No subject from the control group required tooth extraction due to caries, whereas 51.72% of subjects from the study group did, with 31.76% of children from the study group requiring extraction of one tooth and the remaining children-more than one. The difference between these groups was highly statistically significant. This indicates advanced long-standing caries and poor oral hygiene in study group subjects. According to Olczak-Kowalczyk, approximately 50% of 7-year-olds required one deciduous or permanent tooth extraction due to caries (Olczak-Kowalczyk, 2001). Elter *et al*. (2004), who evaluated adults, reported a higher risk of CAD in patients with a significant number of missing teeth and serious periodontal ligament damage. Holmlund *et al*. (2006), demonstrated a significant correlation between the diagnosis of myocardial infarction and the mean number of missing teeth. Desvarieux *et al*. (2003) reported a positive correlation between the number of missing teeth and the number of atherosclerotic lesions in carotid arteries in an ultrasound examination.

The authors mentioned above (Elter, Holmlund, Desvarieux) claimed that significant number of missing teeth may be an indirect sign of advanced, long-lasting periodontal disease (typically resulting from inadequate oral hygiene). The chronic nature of the disease could have had a negative impact on the general condition, as well as the cardiovascular system. This leads us to believe that poor oral hygiene in children, which indicates inadequate oral care habits and multiple teeth qualifying for extraction due to caries may affect the future condition of the oral cavity as well as the entire body.

One important element in the assessment of future cardiovascular risk in children and adolescents is an assessment for CVD due to atherosclerosis in the closest and more remote relatives of the child. CAD in first-degree relatives (parents, siblings) may be a risk factor. Therefore, any positive family history should be documented from a very young age (Williams *et al*., 2002).

We used the PCR technique for a DNA assessment of bacteria indicative of periodontal disease. Out of the entire oral flora, the bacteria most closely related to the etiology of periodontitis include *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans and Prevotella intermedia* (Buhlin *et al*., 2003; Marat, 2008; Oliveira *et al*., 2010; Accarini and De Codoy, 2006; Fardi and Papadimitriou, 2007; Restaino *et al*., 2007). Over 55% of patients from the study group (poor oral hygiene) exhibited *Aggregatibacter actinomycetemcomitans*. These findings were consistent with those by Leys *et al*. (1994) who used the PCR technique to evaluate dental plaque collected from 52 adults and detected Aa in 60% of them (Leys *et al*., 1994). The rate of Aa isolation from diseased sites demonstrates that Aa is a dominant etiologic factor of early periodontal disease (Armitage, 1999; Affek and Jagusztyn-Krynicka, 2007; Jass *et al*., 2003). This bacterium was not detected in the control group, which suggests that children from this group maintained good oral hygiene. This bacterium, along with *Porphyromonas*

gingivalis and *Prevotella intermedia*, was also detected in the tunica intima (Marques de Silva *et al*., 2005) and atherosclerotic plaques (Kozaeov *et al*., 2005; Oliveira *et al*., 2010).

Aggregatibacter actinomycetemcomitans may spread beyond the oral cavity and cause or contribute to endocarditis (Paturel *et al*., 2004).

The second most common bacterium detected in the subgingival dental plaque in the study group was *Prevotella intermedia*. It was detected in 79% of subjects (46), including 32 subjects in whom it was co-occurring with Aa. These findings indicate a varied colonization of the oral cavity and an increased risk of periodontal disease, including the risk of atherosclerosis in the near or further future. Górska and Marat evaluated atherosclerotic plaques in the carotid artery with the PCR technique and showed DNA of bacteria, including *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Prevotella intermedia* (Górska, 2002; Marat, 2008). Conversely, none of the sought-after bacteria were detected in 37 of 39 subjects in the control group (good oral hygiene). Two samples yielded *Prevotella intermedia*. This may suggest that maintaining good oral hygiene may contribute to a reduction or elimination of bacteria causing periodontal disease. However, the small sample size in the control group did not allow for drawing any conclusions.

Subgingival dental plaque samples of neither group yielded *Porphyromonas gingivalis*. This may be due to the fact that this pathogen is most commonly found in advanced stages of periodontal disease in adults, involving periodontal degeneration and alveolar process resorption, which are conditions very rarely found in children (Lau *et al*., 2004).

A statistical analysis of our study showed a significant difference between the study and control groups in terms of Pi prevalence. This bacterium was detected more often in the study group (poor oral hygiene). A statistical analysis did not reveal any effect of Pi on total cholesterol, HDL cholesterol, homocysteine, CRP, fibrinogen or API% in the study group. These findings may suggest a localized inflammation that has not become generalized yet.

Most subjects from both groups had normal complete blood counts. Additional evaluated parameters included homocysteine, fibrinogen and C-reactive Protein (CRP), which are markers of a general inflammatory response and atherosclerosis. There is a strong correlation between serum CRP levels and the stage of periodontal disease with CRP levels rising in the case of periodontitis (Moutsopoulos and Madianos, 2006; Preshaw *et al*., 2007; Seymour *et al*., 2007). The risk of cardiovascular disease exists in both groups, as CRP levels were elevated in only one subject from the study group. Patients with CRP levels below 1.0 mg/L are believed to have a low risk of developing CVD. Patients with CRP levels ranging from 1.0 to 3.0 mg/L are at a moderate risk of developing CVD and those with CRP levels over 3.0 mg/L are considered to be at high risk. A total of 51 subjects from the study group were at moderate risk (CRP 1.0-3.0 mg/L) and 6 subjects were at high risk (CRP>3.0 mg/L) of developing CVD. Conversely, 38 subjects from the control group showed moderate risk of developing CVD and 1 subject-high risk. This demonstrates a slightly higher risk in the study group. A study by Dye *et al*. (2005) demonstrated that one factor affecting elevated CRP levels is a high level of anti-Pg antibodies. These results show that the presence of this bacterium means an existing severe inflammation in the oral cavity that, in turn, causes elevated CRP levels. We did not detect *Pg* in any subject, which indicates a small, still localized inflammation that corresponds to low CRP levels. Moreover, a statistical analysis did not show significant differences between the two groups in terms of CRP. Homocysteine levels in both groups were within normal limits, as well as fibrinogen levels in most children. However, a statistical analysis showed that the mean homocysteine levels are significantly higher in the study group than those in the control group. Elevated blood homocysteine levels are an independent risk factor for the development of cardiovascular disease. Fibrinogen test results may indicate a lack of current atherosclerotic lesions in the vascular endothelium. We would like to emphasize, nonetheless, that fibrinogen levels were not statistically significant.

A lack of changes in the above markers may indicate a mild, still localized inflammation. If such localized inflammation (as well as poor oral hygiene) persists in children for many years, it may become generalized. In adulthood, those affected are at a higher risk of developing acute coronary syndrome and show elevated plasma CRP and fibrinogen levels (Zaremba *et al*., 2005).

We also assessed total cholesterol and HDL cholesterol levels, with the latter found within normal limits in most subjects. The differences between groups were not statistically significant. However, total cholesterol levels were elevated in 14 (24.14%) children from the study group and in 2 (5.13%) children from the control group. A statistical analysis showed that by informing her that oral care is important for her own health in general and the child's health in particular. Young children, their guardians (parents, nursery and pre-school personnel), as well as pediatricians need health-promoting more stable, less labile total cholesterol levels can be observed in the control group. The Student *t* or Mann-Whitney U tests did not show any statistically significant differences between the groups, however, the test for differences between two structure indicators showed that the groups differed significantly in terms of cholesterol levels, with the study group (poor oral hygiene) showing significantly higher total

cholesterol levels. We found no reports in the available literature on the level of total cholesterol in children with respect to oral hygiene. This may suggest a need for thorough future studies on total cholesterol levels in children with poor and good oral hygiene. Thus, in order to determine whether or not elevated total cholesterol levels may be a risk factor for atherosclerosis in children with poor dental hygiene, further studies are needed because a single study does not allow for adequate conclusions to be drawn.

Both groups of subjects also underwent complete blood count assessment. No relevant abnormalities were detected.

In summary, there are no current studies on whether or not adequate oral hygiene in children affects atherosclerosis formation. Studies on other inflammation markers are currently being planned. This study proved that poor oral hygiene is related to the presence of indicator bacteria of the periodontium in children. This is a disturbing phenomenon that requires more attention and thorough investigation in the nearest future.

This phenomenon is important not only for maintaining oral health from a very young age, but also for maintaining general health. It is important to help develop positive routines of constant oral care in adult life. As the first teachers, parents should ensure systematic toothbrushing in their children, introduction of suitable dietary habits and observing adequate frequency of routine dental visits. It is necessary to make adults (parents) realize that there is a relationship between periodontal disease with the resulting inflammation and other systemic conditions. The greatest medical authority for the parents of a child is a pediatrician who should offer advice on the child's proper oral hygiene and suitable diet. It is a pediatrician who should make parents aware of the necessity to have their child see a dentist who will recommend adequate hygiene of the gums and, later, erupting teeth. The gynecologist who takes care of the mother during her pregnancy and postpartum period also plays an important role educational initiatives. It is very important that neither healthcare professionals nor patients perceive gingivitis only as a pre-cursor of periodontitis, but also realize that gingivitis and periodontitis may negatively affect general health. Such actions may have a positive impact on health-oriented prophylactic habits in the society as well as contribute to reducing cardiac mortality.

Conclusion

- The level of oral hygiene in children is highly unsatisfactory. Thus, good oral care habits need to be cultivated in children from a very young age
- Children with poor and fair oral hygiene (according to API) exhibited periodontal disease-causing bacteria that may induce chronic inflammation, which is a risk factor for atherosclerosis
- Children with good and adequate oral hygiene (according to API) showed a reduction in periodontal disease-causing bacteria in subgingival dental plaque, with no bacteria detected in most cases
- Based on CPR levels, both groups are at risk of cardiovascular disease. That risk tended to increase in the study group (poor oral hygiene)
- Parents should take more measures to help their children maintain oral hygiene by monitoring toothbrushing duration and technique, as well as reviewing thoroughness of brushing in their children
- Dentists should pay more attention to teaching oral hygiene to adults and especially to children. Parents need to be made aware that there is a correlation between good oral hygiene and general health
- Children with positive genetic history, the presence of periodontal disease-causing bacteria and inflammatory reaction constitute a higher risk group for the development of atherosclerosis in the future

Author's Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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