Presence of *Aggregatibacter actinomycetemcomitans* in saliva and cardiac tissue samples of children with congenital heart disease

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**ABSTRACT**

**Aim:** The purpose of this study was to analyze the presence of *Aggregatibacter actinomycetemcomitans* in saliva and cardiac tissue samples of children requiring cardiac surgery in Istanbul, Turkey.

**Subjects and Methods:** Twenty-five patients (mean age: 6.24 ± 2.93) undergoing surgery for congenital heart defects (CHDs) and an age/gender-matched control group of 25 healthy children were enrolled in the study. Saliva samples were collected from all children; plaque index (PI) and gingival index (GI) were also determined. In CHD group, cardiac tissue samples were received during surgery. All samples were evaluated for the presence of *A. actinomycetemcomitans* and its highly leukotoxic JP2 clonal strains using polymerase chain reaction. The findings were analyzed by Mann–Whitney U, Chi-square, and Fisher’s exact tests.

**Results:** No significant differences were found in PI and GI values between the groups. *A. actinomycetemcomitans* was not detected in cardiac tissue samples. *A. actinomycetemcomitans* in saliva was detected in 2 (8%) of the CHD and 5 (20%) of the control children (*p* > 0.05). *A. actinomycetemcomitans* JP2 clonal strains were determined from 1 (4%) of the control group while it was not determined from the samples of the CHD group.

**Conclusions:** Early colonization of *A. actinomycetemcomitans* in oral cavities could be assessed as a risk marker for periodontal disease. Periodontal pathogens may enter bloodstream through bacteremia; thus, the presence of periodontal pathogens in the oral cavity of children should be assessed as a risk marker for cardiac diseases in older ages.

**Key words:** *Aggregatibacter actinomycetemcomitans*, cardiac disease, polymerase chain reaction

Oral health care is of vital importance in children with cardiac disabilities. Cardiac diseases complicate dental care in children and make them more susceptible to bad oral conditions.[1,2] Parents’ negligence of oral hygiene as a result of a greater concern with cardiac disease, chronic intake of sweetened liquid medications could affect the oral and periodontal health in children with cardiac disabilities.[3,4]

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Disease. Especially in children with mixed dentition, it is possible that periodontal pathogens remain in the area surrounding exfoliating primary teeth and continue to survive in the gingival sulcus around permanent teeth; thus, periodontal pathogens survive in the mouth without demonstrating any kind of periodontitis.[5,6]

Various dental procedures and routine oral hygiene habits may lead to the entry of periodontal pathogens into the bloodstream, which may invade coronary endothelium. Dental treatment procedures, which generally cause bleeding are known to be the major causative agent for bacteremia.[7,8]

Aggregatibacter actinomycetemcomitans, a Gram-negative facultative anaerobic coccobacillus, is an important periodontal pathogen which occurs in the oral cavity quite early in childhood.[9,10] A. actinomycetemcomitans is mainly related to aggressive periodontitis; however, it has also been associated with chronic periodontitis.[11,12] Studies have reported the capability of A. actinomycetemcomitans to invade the coronary endothelium and the presence of viable A. actinomycetemcomitans in human atherosclerotic plaque.[13,14]

The JP2 clone of A. actinomycetemcomitans characterized by a 530-base pair (bp) deletion in the promoter region of the leukotoxin gene operon resulting in increased leukotoxin production has most frequently been detected in individuals of African descent. Studies have demonstrated a positive association between the presence of JP2 clone and occurrence of early-onset periodontitis.[15]

The aim of this study is to analyze the presence of A. actinomycetemcomitans and its highly leukotoxic JP2 clonal strain in saliva samples and cardiac tissue specimens of children requiring cardiac surgery.

MATERIALS AND METHODS

Patient selection and sample collection

Twenty-five patients attending the Istanbul University, Institute of Cardiology, Department of Cardiovascular Surgery, at 3–12 years of age (mean age: 6.24 ± 2.93) undergoing elective surgery for congenital heart defects (CHDs) with cardiopulmonary bypass under general anesthesia were enrolled in the study. An age- and gender-matched group of healthy children who attended the Pediatric Dentistry Clinic of Istanbul University Faculty of Dentistry, Istanbul, Turkey, was analyzed as controls. For control group, exclusion criteria were any systemic health problem that may affect oral health and dental plaque retention, and for CHD group, any systemic health problem not associated with the cardiac disease. All clinical procedures were approved by the Local Ethics Committee of Istanbul University Faculty of Medicine (2011/118–457) and informed consent was obtained from each parent before initiating the study.

Oral examinations of CHD and control group were performed by one examiner (Elif Bozdogan) at Pediatric Dentistry Clinic of Istanbul University, Faculty of Dentistry, following biosecurity principles. In CHD group, oral examinations were performed before hospitalization and cardiac surgery. The patients were examined on a medical examination table under good incident light.

Oral hygiene of groups was assessed using Silness–Löe plaque index (PI) which is based on recording both soft debris and mineralized deposits on the teeth.[16] Each of the four surfaces of the teeth (buccal, lingual, mesial, and distal) were given a score from 0 to 3 (score 0 = no dental plaque in the gingiva area, score 1 = a film of dental plaque adhering to the free gingival margin (only upon probing), score 2 = moderate accumulation of dental plaque at the gingival margin (seen by naked eye), score 3 = abundant dental plaque in the gingival margin) and calculated scores were recorded for the PI.

Gingival condition and qualitative changes in the gingiva of CHD and control group were assessed using Löe and Silness gingival index (GI).[17] The bleeding was assessed by probing gently along the wall of soft tissue of the gingival sulcus; the marginal and interproximal tissues scored 0–3 (0 = normal gingiva, 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation) and calculated scores were recorded for the GI.

After oral examinations, paraffin-stimulated saliva samples were collected for 5 min from both CHD and control group. Children were asked to chew a sugar-free gum expectorate into a sterile container. Swab samples from dental surfaces were taken using sterile swab sticks in smaller children, who were unable to chew. The swab samples were then placed in 2-ml sterile saline and homogenized by vortexing. The samples were pooled in a sterile empty tube and stored at −20°C until use. In CHD group, saliva samples were obtained before hospitalization and cardiac tissue samples were received during surgery.

Cardiac tissue specimens of CHD were collected under aseptic conditions during cardiac surgery and placed in 30 ml of prereduced transport medium immediately. The tissue was transported to the laboratory facility where a fraction of it was removed in a laminar-flow hood at aseptic conditions and used for DNA isolation. During the transfer of the cardiac tissues, it was presumed that the remnants of the microcapillary blood from the tissue, estimated at 50 mg/g tissue in vivo, leaked into the medium.

All samples and specimens in CHD and control groups were evaluated for the presence of A. actinomycetemcomitans and its highly leukotoxic JP2 clonal strains.
Microbiological analysis
Polymerase chain reaction (PCR) techniques were used for bacterial detection. For each sample, bacterial DNA was extracted and purified with the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions.

The species-specific primers used in this study are listed in Table 1.[18–20] Primers specific to the IktA gene sequences were used for A. actinomycetemcomitans detection in saliva samples.

A. actinomycetemcomitans positive samples were then analyzed to identify the members of the JP2 clone using the primers span, the Δ530 deletion present in the clone. Escherichia coli 16S ribosomal RNA universal primers common to all bacterial species were also used to detect the presence of nonspecific bacteria in the cardiac specimens.

The final volume of the reaction mixture for each PCR assay was 25 µl consisting of 2.5 µl of DNA sample, 0.25 mM of each deoxynucleotide triphosphate (MBI-Fermentas), 2.5 µl of 10X Standard Taq Reaction Buffer (670 mM Tris-HCL, pH 8.8, 166 mM (NH4)2SO4, 0.1% Tween 20), 1.5 mM (MBI-Fermentas), 0.5 µM of each primer, 1.25U Taq DNA polymerase (MBI-Fermentas), and distilled water. PCRs were performed in a thermal cycler (Eppendorf AG, Master Cycler Personel 22331, Hamburg, Germany) under optimized conditions for each microorganism with an initial denaturation step of 2 min at 95°C [Table 1]. The amplification products were analyzed by 2% agarose gel electrophoresis. The gels were stained with ethidium bromide (0.5 µg/ml) and PCR products were visualized using a computer-aided imaging system (DNR Bio-imaging Systems Ltd, Jerusalem, Israel). The size of the PCR products was estimated using Φ X174 DNA–HAE III Digest (MBI-Fermentas) as a molecular weight marker. The sensitivity of all the PCR methods was evaluated using Δ: 2.3, standard deviation: 0.5, α: 0.05 determined for the minimum number of samples for each group, n = 20, respectively.

RESULTS
Demographic data and clinical measurements of GI and PI of CHD and control groups are presented in Table 2. No significant differences were found in PI and GI values between the groups. Two (8%) of the 25 children with CHD carried A. actinomycetemcomitans in their saliva samples compared to 5/25 (20%) of the healthy children. The difference was not shown to be statistically significant (p > 0.05). No significant differences were found between the age groups and the prevalence of A. actinomycetemcomitans in saliva samples of the groups [Table 3]. A. actinomycetemcomitans was detected in 8.3% and 16.7% of cardiac and healthy children under the age of 6. The minimum age of a subject positive for A. actinomycetemcomitans was 3 years old and in CHD group. Our results showed no significance in the prevalence of A. actinomycetemcomitans between the males and females in groups [Table 4]. In control group, saliva sample of one child was positive for A. actinomycetemcomitans JP2 clonal strain. Table 5 shows the demographic data and the presence of JP2 clonal strain of positive saliva samples in CHD and control groups, respectively. No cardiac tissue samples yielded positive results for bacterial DNA in CHD group.

DISCUSSION
This study reports the presence of A. actinomycetemcomitans in saliva and cardiac tissue samples of children with congenital heart disease.

Table 1: Primer list used in polymerase chain reaction

| Bacteria                        | Primer sequences (5'-3') | Amplification cycles | Amplification size (bp) | References          |
|---------------------------------|--------------------------|----------------------|-------------------------|---------------------|
| Universal bacteria              | AAGGAGGTGATCCAGCGGCA     | 36 cycles            | 1400                    | Choo, 1995[19]      |
|                                 | GAGGTTGATCATGGCTCAG      | 94°C, 45 s           | 94°C, 45 s              |                     |
|                                 |                          | 55°C, 30 s           | 55°C, 30 s              |                     |
|                                 |                          | 72°C, 45 s           | 72°C, 45 s              |                     |
| A. actinomycetemcomitans Ikt    | CTA GGT ATT GCG AAA CAATTT| 30 cycles            | 262                     | Goncharoff et al. 1993[19] |
|                                 | CCGTGAATTTAAG CTG GTAATC | 94°C, 60 s           | 94°C, 60 s              |                     |
|                                 |                          | 55°C, 60 s           | 55°C, 60 s              |                     |
|                                 |                          | 72°C, 60 s           | 72°C, 60 s              |                     |
| A. actinomycetemcomitans JP2 clone | GCCGC ACC AAAGAC AAAGTCT | 30 cycles            | 686                     | Poulsen et al., 2003[20] |
|                                 | GCCCAT AAC CAAGCC ACATA  | 94°C, 60 s           | 94°C, 60 s              |                     |
|                                 |                          | 60°C, 60 s           | 60°C, 60 s              |                     |
|                                 |                          | 72°C, 120 s          | 72°C, 120 s             |                     |

A. actinomycetemcomitans=Aggregatibacter actinomycetemcomitans
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Yuan et al.\[10\] and Ashimoto et al.\[12\] detected the prevalence of A. actinomycetemcomitans in plaque samples of healthy children as 7.7%, 5.5%, and 14%, respectively. Another study reported that 20% of saliva samples of healthy children were positive for A. actinomycetemcomitans.\[8\] In addition, Kulekci et al.\[23\] detected A. actinomycetemcomitans in 24% of saliva samples of healthy children.

In our study, saliva is used for the microbiological analysis of A. actinomycetemcomitans in the oral cavity. Current studies report that proportions of bacterial species differ significantly on different intraoral surfaces.\[24\] Although the microbiota of saliva is most similar to that of the dorsal and lateral surfaces of the tongue, it is worth mentioning that several of the known periodontal pathogens have similar percentages of DNA count in saliva compared to supra and subgingival tooth surfaces.\[24\] Microorganisms in dental plaque can survive in saliva and can utilize salivary components as a substrate. Furthermore, saliva contains a variety of bacteria from different oral sites; and saliva sampling offers a rapid, noninvasive, and easy source for bacterial examination.\[25,26\]

Steelman et al.\[27\] reported 25% of subgingival plaques of cardiac children were positive for A. actinomycetemcomitans compared to the healthy children that had no bacteria in their subgingival plaque samples. In another study,\[28\] author stated 8/12 of subgingival samples of cardiac patients had A. actinomycetemcomitans as compared with 2/12 controls (p < 0.05). In the present study, the detection prevalence of A. actinomycetemcomitans in saliva samples of cardiac and healthy children were 8% and 20%, respectively. Unlike Steelman et al.\[27,28\] findings, these results show that children with cardiac disease had a lower frequency of A. actinomycetemcomitans in their saliva samples compared to healthy children.

Early colonization of periodontal pathogens is suggested as a risk factor for future periodontal diseases for the susceptible host. Tanner et al.\[29\] detected A. actinomycetemcomitans as 30% in tongue samples of children aged 6–18 months. Rotimi et al.\[30\] reported that A. actinomycetemcomitans was detected in 7.5% of the children under the age of 6. On the other hand, Frisken et al.\[31\] reported that A. actinomycetemcomitans was not detected in the children under the age of 2.5. Kónönen et al.\[32\] reported that A. actinomycetemcomitans was not found in saliva and plaque samples of children under the age of 4. In this study, A. actinomycetemcomitans was detected in 8.3% and 16.7% of cardiac and healthy children under the age of 6, respectively. These results supported the studies that reporting A. actinomycetemcomitans was detected in the primary dentition. The youngest child in this study who was found to be positive for A. actinomycetemcomitans was in cardiac group and he was 3 years old.

Some of the studies suggest that prevalence of A. actinomycetemcomitans increases with age.\[33,34\] However, our results have shown no significant differences between the age groups. Although a relationship between the colonization of periodontopathic bacteria and sex hormones has been reported,\[35,36\] the findings in this study showed no significant differences between sex and prevalence of A. actinomycetemcomitans.

Studies have shown that A. actinomycetemcomitans isolates, which demonstrate highly leukotoxic activity, all belong to the JP2 clone. Patients who harbor the JP2 clone have more advanced stages of early-onset periodontitis than patients without the clone. There is a strong association between the occurrence of the JP2 clone and localized aggressive periodontitis.\[37\] Haubeck et al.\[38\] indicated that infection with the JP2 clone of A. actinomycetemcomitans might happen early in life. Åberg et al.\[39\] reported that 8.8% of the subgingival samples of adolescents were positive for JP2 clone. Fine et al.\[40\]

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**Table 2: Demographic data of subjects and index values determined in groups**

| Demographic data | CHD | Control | p   |
|------------------|-----|---------|-----|
| Number of subjects | 25  | 25      | -   |
| Gender           |     |         |     |
| Boys             | 11  | 11      | -   |
| Girls            | 14  | 14      | -   |
| Mean age         | 6.24±2.93 | 6.24±2.93 | -   |
| Gingival index   | 0.10±0.10 | 0.15±0.16 | 0.466 |
| Plaque index     | 0.71±0.30 | 0.78±0.35 | 0.443 |

*Mann-Whitney U-test (P>0.05). CHD=Congenital heart defect*

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**Table 3: Prevalence of Aggregatibacter actinomycetemcomitans in saliva samples of groups according to age**

| Groups       | 0-5 (n=12) | 6-12 (n=13) | p   |
|--------------|------------|-------------|-----|
| CHD (%)      | 1 (8.3)    | 1 (7.7)     | 1.000 |
| Control (%)  | 2 (16.7)   | 3 (23.1)    | 1.000 |

CHD=Congenital heart defect

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**Table 4: Prevalence of Aggregatibacter actinomycetemcomitans in groups according to gender**

| Groups       | Girls (n=14) | Boys (n=11) | p   |
|--------------|--------------|-------------|-----|
| CHD (%)      | 2 (14.3)     | 0 (0)       | 0.487 |
| Control (%)  | 4 (28.6)     | 1 (9.1)     | 0.341 |

CHD=Congenital heart defect

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**Table 5: Demographic data for the presence of Aggregatibacter actinomycetemcomitans and JP2 clone in congenital heart defect and control groups**

| Groups | Subject | Gender | Age | A. actinomycetemcomitans | JP2 |
|--------|---------|--------|-----|-------------------------|-----|
| CHD    | 1       | Female | 9   | +                       | -   |
|        | 2       | Female | 3   | +                       | -   |
| Control| 1       | Female | 12  | +                       | -   |
|        | 2       | Female | 9   | +                       | -   |
|        | 3       | Female | 4   | +                       | -   |
|        | 4       | Male   | 6   | +                       | -   |
|        | 5       | Female | 4   | +                       | -   |

CHD=Congenital heart defect, A. actinomycetemcomitans=Aggregatibacter actinomycetemcomitans

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A. actinomycetemcomitans has been identified in dental and saliva specimens of children using PCR method. Okada et al.\[21\] Yuan et al.\[10\] and Ashimoto et al.\[12\] detected the prevalence of A. actinomycetemcomitans in plaque samples of healthy children as 7.7%, 5.5%, and 14%, respectively. Another study reported that 20% of saliva samples of healthy children were positive for A. actinomycetemcomitans.\[8\] In addition, Kulekci et al.\[23\] detected A. actinomycetemcomitans in 24% of saliva samples of healthy children.

In our study, saliva is used for the microbiological analysis of A. actinomycetemcomitans in the oral cavity. Current studies report that proportions of bacterial species differ significantly on different intraoral surfaces.\[24\] Although the microbiota of saliva is most similar to that of the dorsal and lateral surfaces of the tongue, it is worth mentioning that several of the known periodontal pathogens have similar percentages of DNA count in saliva compared to supra and subgingival tooth surfaces.\[24\] Microorganisms in dental plaque can survive in saliva and can utilize salivary components as a substrate. Furthermore, saliva contains a variety of bacteria from different oral sites; and saliva sampling offers a rapid, noninvasive, and easy source for bacterial examination.\[25,26\]

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detected JP2 clone in 4.8% of school-children who were positive for *A. actinomycetemcomitans*. In our study, JP2 clone was not detected in cardiac group; and 4% of control group (one child) was positive for JP2 clone.

Studies suggest that periodontal pathogens are associated with infective endocarditis (IE) and cardiovascular diseases.[7][39] *A. actinomycetemcomitans* is grouped in the HACEK group of bacteria (*Haemophilus* species, *A. actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*) and it has been reported that members of this group of bacteria cause 3% of all cases and endocarditis due to *A. actinomycetemcomitans* is rare.[40][41] Nakatani et al.[42] indicated 49.5% and 31.7% of the causative microorganism of patients with IE were streptococci and staphylococci, respectively. Niwa et al.[43] reported streptococci and staphylococci are the major pathogens of IE.

Nakano et al.[14] used PCR and found *A. actinomycetemcomitans* in 35% of heart valves and 30.2% of aneurysms. Haraszyth et al.[44] Ishihara et al.[45] and Padilla et al.[46] detected *A. actinomycetemcomitans* in atherosclerotic plaques using PCR as 18%, 23.3%, and 16.6%, respectively. However, detection of *A. actinomycetemcomitans* in cardiovascular tissues has not been confirmed by other studies. Aquino et al.[47] used PCR and found periodontal bacteria in subgingival biofilms of patients but not in carotid atheromas. Furthermore, other study reported that *A. actinomycetemcomitans* was not found in aortic tissue and atherosclerotic plaques using PCR method.[48]

In our study, no cardiac tissue samples yielded positive results for bacterial DNA. It has been estimated that young ages of children for developing atherosclerotic plaques can be a result for negative bacterial DNA isolations; and the findings of this study have shown no invasion of bacteria to cardiovascular tissues.

**CONCLUSIONS**

Data from the present study have shown that children yielded positive results for *A. actinomycetemcomitans* in their saliva samples; thus, early colonization of this periodontal pathogen is a risk marker for periodontal diseases in older ages. Cardiac specimens collected from children undergoing cardiac surgery showed negative results for *A. actinomycetemcomitans*; however, periodontal pathogens may enter bloodstream through bacteremia; thus, positive saliva samples of children should be assessed as a risk marker for cardiac diseases in older ages.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. al-Sarheed M, Angeletou A, Ashley PF, Lucas VS, Whitehead R, Roberts GJ. An investigation of the oral status and reported oral care of children with heart and heart-lung transplants. Int J Paediatr Dent 2000;10:298-305.
2. Balmer R, Bu’Lock FA. The experiences with oral health and dental prevention of children with congenital heart disease. Cardiol Young 2003;13:439-43.
3. Balmer R, Booras G, Parsons J. The oral health of children considered very high risk for infective endocarditis. Int J Paediatr Dent 2010;20:173-8.
4. Saunders CP, Roberts GJ. Dental attitudes, knowledge, and health practices of parents of children with congenital heart disease. Arch Dis Child 1997;76:539-40.
5. Buhlin K, Gustafsson A, Pockley AG, Frostegård J, Klinge B. Risk factors for cardiovascular disease in patients with periodontitis. Eur Heart J 2003;24:2099-107.
6. Casamassimo PS. Relationships between oral and systemic health. Pediatr Clin North Am 2000;47:1149-57.
7. Forner L, Larsen T, Kilian M, Holmstrup P. Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. J Clin Periodontol 2006;33:401-7.
8. Wilson W, Taubert KA, Gewitz M, Lockhart PB, Baddour LM, Levison M, et al. Prevention of infective endocarditis: Guidelines from the American Heart Association: A guideline from the American Heart Association Rheumatic Fever, Endocarditis and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. J Am Dent Assoc 2008;139:3-24.
9. Morinushi T, Lopatin DE, Van Poperin N, Ueda Y. The relationship between gingivitis and colonization by *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* in children. J Periodontol 2000;71:403-9.
10. Yuan K, Hsu PC, Tseng CC, Kiang D, Wang JR. Detection rate of *Actinobacillus actinomycetemcomitans* on the permanent 1st molars of primary school children in Taiwan by polymerase chain reaction. J Clin Periodontol 2001;28:348-52.
11. Yang HW, Asikainen S, Dogan B, Suda R, Lai CH. Relationship of *Actinobacillus actinomycetemcomitans* serotype b to aggressive periodontitis: Frequency in pure cultured isolates. J Periodontol 2004;75:592-9.
12. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol 1996;11:266-73.
13. Nakano K, Inaba H, Nomura R, Nenomo H, Tamura K, Miyamoto E, et al. Detection and serotype distribution of *Actinobacillus actinomycetemcomitans* in cardiovascular specimens from Japanese patients. Oral Microbiol Immunol 2007;22:136-9.
14. Nakano K, Nenomo H, Nomura R, Inaba H, Yoshioka H, Taniguchi K, et al. Detection of oral bacteria in cardiovascular specimens. Oral Microbiol Immunol 2009;24:64-8.
15. Sakellari D, Katsikari A, Slini T, Ioannidou I, Konstantinidou A, Arsenakis M. Prevalence and distribution of *Aggregatibacter actinomycetemcomitans* serotypes and the JP2 clone in a Greek population. J Clin Periodontol 2011;38:108-14.
16. Sílness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
17. Loe H, Sílness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
18. Choo CL. The detection of *Actinobacillus actinomycetemcomitans* in root canals and an investigation of its virulence factors. Master Thesis in
Endodontics. Eastman Dental Institute. University of London; 1995.

19. Goncharoff P, Figurski DH, Stevens RH, Fine DH. Identification of Actinobacillus actinomycetemcomitans: Polymerase chain reaction amplification of lktA-specific sequences. Oral Microbiol Immunol 1993;8:105-10.

20. Poulsen K, Ennibi OK, Haubek D. Improved PCR for detection of the highly leukotoxic JP2 clone of Actinobacillus actinomycetemcomitans in subgingival plaque samples. J Clin Microbiol 2003;41:4829-32.

21. Okada M, Hayashi F, Nagasaka N. Detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in dental plaque samples from children 2 to 12 years of age. J Clin Periodontol 2000;27:763-8.

22. Rotimi VO, Salako NO, Divia M, Asfour L, Kononen E. Prevalence of periodontal bacteria in saliva of Kuwaiti children at different age groups. J Infect Public Health 2013;6:76-82.

23. Kulecki G, Leblebicioglu B, Keskin F, Ciftci S, Badur S. Salivary detection of periodontopathic bacteria in periodontally healthy children. Anaerobe 2008;14:49-54.

24. Mager DL, Ximenez-Fyvie LA, Haffajee AD, Socransky SS. Distribution of selected bacterial species on intraoral surfaces. J Clin Periodontol 2003;30:644-54.

25. Tamura K, Nakano K, Hayashibara T, Nomura R, Fujita K, Shintani S, et al. Detection of 10 periodontal bacteria in saliva samples from Japanese children and their mothers. Arch Oral Biol 2006;51:371-7.

26. Sakai VT, Campos MR, Machado MA, Louris JR, Greene AS, Santos CF. Prevalence of four putative periodontopathic bacteria in saliva of a group of Brazilian children with mixed dentition: 1-year longitudinal study. Int J Paediatr Dent 2007;17:192-9.

27. Steelman R, Rosen DA, Nelson ER, Kenamond LA. Gingival colonization with selective HACEK microbes in children with congenital heart disease. Clin Oral Investig 2003;7:38-40.

28. Steelman R, Einzig S, Balian A, Thomas J, Rosen D, Gustafson R, et al. Increased susceptibility to gingival colonization by specific HACEK microbes in children with congenital heart disease. J Clin Pediatr Dent 2000;25:91-4.

29. Tanner AC, Milgrom PM, Kent R Jr, Mokeem SA, Page RC, Riedy CA, et al. The microbiota of young children from tooth and tongue samples. J Dent Res 2002;81:53-7.

30. Frisken KW, Higgins T, Palmer JM. The incidence of periodontopathic microorganisms in young children. Oral Microbiol Immunol 1990;5:43-5.

31. Könönen E, Askainen S, Saarela M, Karjalainen J, Jousimies-Somer H. The oral gram-negative anaerobic microflora in young children: Longitudinal changes from edentulous to dentate mouth. Oral Microbiol Immunol 1994;9:136-41.

32. Wojcicki CJ, Harper DS, Robinson PJ. Differences in periodontal disease-associated microorganisms of subgingival plaque in prepubertal, pubertal and postpubertal children. J Periodontol 1987;58:219-23.

33. Nakagawa S, Fujii H, Machida Y, Okuda K. A longitudinal study from prepuberty to puberty of gingivitis. Correlation between the occurrence of Prevotella intermedia and sex hormones. J Clin Periodontol 1994;21:658-65.

34. Umeda M, Miwa Z, Takeuchi Y, Ishizuuka M, Huang Y, Noguchi K, et al. The distribution of periodontopathic bacteria among Japanese children and their parents. J Periodontal Res 2004;39:398-404.

35. Brogan JM, Lally ET, Poulsen K, Kilian M, Demuth DR. Regulation of Actinobacillus actinomycetemcomitans leukotoxin expression: Analysis of the promoter regions of leukotoxic and minimally leukotoxic strains. Infect Immun 1994;62:501-8.

36. Haubek D, Westergaard J. Detection of a highly toxic clone of Actinobacillus actinomycetemcomitans (JP2) in a Moroccan immigrant family with multiple cases of localized aggressive periodontitis. Int J Paediatr Dent 2004;14:41-8.

37. Åberg CH, Kvamin F, Claesson R, Johansson A, Haubek D. Presence of JP2 and Non-JP2 Genotypes of Aggregatibacter actinomycetemcomitans and attachment loss in adolescents in Ghana. J Periodontol 2012;83:1520-8.

38. Fine DH, Markowitz K, Furgang D, Fairlie K, Ferrandiz J, Nasri C, et al. Aggregatibacter actinomycetemcomitans and its relationship to initiation of localized aggressive periodontitis: Longitudinal cohort study of initially healthy adolescents. J Clin Microbiol 2007;45:3859-69.

39. Roberts GJ. Dentists are innocent! “Everyday” bacteremia is the real culprit: A review and assessment of the evidence that dental surgical procedures are a principal cause of bacterial endocarditis in children. Pediatr Cardiol 1999;20:317-25.

40. Paturol P, Casalta JP, Habib G, Nezri M, Raoul D. Actinobacillus actinomycetemcomitans endocarditis. Clin Microbiol Infect 2004;10:98-118.

41. Das M, Badley AD, Cockerill FR, Steelberg JM, Wilson WR. Infective endocarditis caused by HACEK microorganisms. Annu Rev Med 1997;48:25-33.

42. Nakatani S, Mitsutake K, Hozumi T, Yoshikawa J, Akiyama M, Yoshida K, et al. Current characteristics of infective endocarditis in Japan: An analysis of 848 cases in 2000 and 2001. Circ J 2003;67:901-5.

43. Niwa K, Nakazawa M, Tateno S, Yoshinaga M, Terai M. Infective endocarditis in congenital heart disease: Japanese national collaboration study. Heart 2005;91:795-800.

44. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71:1554-60.

45. Ishihara K, Nabuchi A, Ito R, Miyachi K, Kuramitsu HK, Okuda K. Correlation between detection rates of periodontopathic bacterial DNA in coronary stenotic artery plaque [corrected] and in dental plaque samples. J Clin Microbiol 2004;42:1313-5.

46. Padilla C, Lobos O, Hubert E, González C, Matus S, Pereira M, et al. Periodontal pathogens in atheromatous plaques isolated from patients with chronic periodontitis. J Periodontal Res 2006;41:350-3.

47. Aquino AR, Lima KC, Paiva MS, Rôças IN, Siqueira JF Jr. Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi. J Periodontal Res 2011;46:303-9.

48. Stielzel M, Conrads G, Pankuweit S, Maisch B, Vogt S, Moosdorf R, et al. Detection of Porphyromonas gingivalis DNA in aortic tissue by PCR. J Periodontol 2002;73:868-70.