Salivary *Streptococcus mutans* and *lactobacillus* sp levels in cardiac children

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This study assesses salivary conditions of 20 children with cardiac disease comparing with a control group of 15 healthy children. The results showed that there was no difference between the groups on salivary flow, buffer capacity and the level of Streptococcus mutans *(Sm). The test group i.e., children with cardiac disease, showed a lower level of* Lactobacillus *sp. The association between the usage of antibiotics and the risk of developing caries, measuring the level of* Streptococcus mutans *and* Lactobacillus *sp., showed that children taking antibiotics frequently had a significant lower level of* Lactobacillus *sp (p<0.05) than healthy children. This association was not found on relation to the levels of* Streptococcus mutans.

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INTRODUCTION

Infective endocarditis is one of the few potentially lethal infections following dental procedures.[1,11,23,29] For the majority of individuals bacteriemia does not cause any major problem, however, for cardiac patients these episodes are life threatening.

It has been recommended, by the American Heart Association, that antibiotic prophylaxis should be used to avoid infective endocarditis in cardiac patients.[2] The maintenance of oral health in cardiac patients seems to be very important to reduce the intensity of the bacteriemia episode.[25] Apart from the rational behind this, the oral health of cardiac individuals has been neglected[12,17,27,30] and dental professionals do not know enough about this subject.[6]

The purpose of our study was to assess salivary conditions in cardiac children, comparing them with healthy children. Salivary conditions were assessed measuring: the salivary flow, the buffer capacity, the level of *Streptococcus mutans* (*Sm*) and the level of *Lactobacillus sp*. Medical history of the patients was collected including antibiotics regimen.

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LITERATURE REVIEW

Patients with cardiovascular diseases are frequently found in the general population. LaCerda *et al.*[21] developed an epidemiological study in the city of Ribeirão Preto, São Paulo, Brazil, and found that 30.3% of this population had some form of cardiovascular disease. Another study, developed by Ramos *et al.*[26] in Niterói, Rio de Janeiro, found a prevalence of 22.48% of cardiac in this population. The prevalence of infective endocarditis in the EUA is around 11 to 50 cases per millions of inhabitants per year, 1.7 to 4.9 episodes per 100,000 habitants/year.[15,33]

Patients at a particular risk of developing endocarditis are well recognised as including individuals with rheumatic fever history, congenital cardiac diseases, prosthetic heart valves and others forms of cardiovascular diseases.[11,23] This disease could be an acute, subacute or chronic infection of the endocardium. It is an infection, which presents a life risk. Many dental procedures result in a bacteriemia, which may cause endocarditis.[1,11,29]

Dental procedures in contaminated tissues may cause a transitory bacteriemia, which rarely persists for more than 15 minutes. Bacteria present in blood vessels could be established in defects or damaged valves inducing infective endocarditis.[11]

The mortality due to infective endocarditis has been found in the literature to be around 14% Bayliss *et al.*[4] and 19 to 46% Bayliss *et al.*[5] However, the morbidity involving the disease is considerable. The data including mortality is directly influenced by the age of the patient, exposure to previous surgery, existence of prosthetic valve(s), among others factors.

A subacute endocarditis is usually caused by low virulent bacteria producing vegetate lesions, bacteria with low invasion capacity causing a slow lesion development. In general, the microorganisms involved are from the indigenous flora. The most frequent organisms causing around 50% of the cases being *Streptococci viridans*, particularly, *Streptococcus sanguis (Ss)*, *Streptococcus mutans (Sm)* and *Streptococcus mitior* (*S. mitior)*. Some of the organisms that could cause infective endocarditis are also etiological agents of common oral diseases as caries and periodontal diseases. Therefore, it has been stated that there is importance for a major oral hygiene control program to patients with a risk of developing infective endocarditis.[4,5,23]

A study covering 544 episodes of infective endocarditis in adult patients showed that 63% were due to *Streptococci*, 19% to *Staphylococci* and 14 % to bowel organisms.[4] Another study, by Tunkel and Mandell,[31] showed that in 2345 episodes of infective endocarditis that occurred between 1933 to 1987, the micro-organisms involved were: *Streptococci* (56.4%), *Staphylococci* (24.9%), Gram negative bacteria (5.7%), others (2.7%), fungi (1%) and negative cultures (9.3%).

There is a controversy around the actual percentage of infective endocarditis caused by dental procedures. Bayliss *et al.*[3] acknowledged that only 4% of the cases have been due to dental procedures. In spite of the difficulty to prove the origin of the *Streptococci*, some authors suggest that around 19% of the cases being from dental origin.[4] Nevertheless, there is enough evidence to agree that oral infections and dental procedures play an important role in the development of infective endocarditis episodes.

There are some studies assessing the oral health of cardiac children,[12,14,17,25,29] however, the results are not all the same. A number of these studies claim that oral health has been neglected in cardiac patients and other studies showed no difference between control and cardiac children.[12,17,27,30] The medical condition seems to take all of the attention of the physicians and parents leaving oral health to second place, which certainly have no sense.

A study by Pollard and Curzon[25] compared caries experience between 100 cardiac children and a control group of 100 healthy children. They found that dental caries experience (dmft) was significantly higher only in the primary teeth of 5 to 9 years old children in the study group, and there was no significant difference in gingivitis, plaque or calculus. The *S. mutans* count was found to be positively correlated to the number of decayed teeth in the study group.

The assessment of oral health of adult and older cardiac patients showed a poor dental health indicating that there is a need for improvement in the provision of dental care for cardiac patients in general.[17,30]

The risk of cardiac patients to develop infective endocarditis when taking antibiotic prophylaxis as recommended by the American Heart Association[2] is lower than not using it. However, this is not always enough for effective prevention of infective endocarditis. A case-control study by Imperiale and Horowitz[18] showed a efficacy of about 90%, while another study by van der Meer *et al*,[32] found a 49% protective efficacy. The American Heart Association recommendation does not always prevent infective endocarditis, probably because not all bacteria that inhabit the periodontal pocket and periapical abscess are susceptible to the systemic concentrations of antibiotic recommended.[24]

The antibiotic prophylaxis has been recommended for all dental procedures that can result in bleeding or produce bacteriemia.[2] The orthodontic treatment is among these procedures. Hobson and Clark,[16] studied the risk of developing infective endocarditis after orthodontic treatment in cardiac patients. Questionnaires were sent to all 1038 members of the British Society for the Study of Orthodontic and the British Association of Orthodontics. A total of 480 were completed in full and analysed. Eight cases of infective endocarditis associated with orthodontic treatment over a 44-year period were reported. A number of replies noted that the medical profession appeared dismissive or unaware of possible risk to patients who are susceptible to infective endocarditis. These authors advise that a high standard of oral hygiene should be established prior to orthodontic treatment for at risk patients.

Antibiotic prophylaxis in orthodontic treatment should be used for procedures, which cause gingival trauma such as band replacement and removal. The daily use of a chlorhexidine mouthwash during treatment and prior to appliance adjustment is recommended.

Roberts *et al.*,[28] investigated 735 children assessing the frequency of bacteriemia after dental treatment. The results were significant for four procedures: 24.5% after dental prophylaxis; 29.4% rubber dam placement; 32.1 % for matrix band with wedge placement; 96.6% for intraligamental injection. In comparison, tooth brushing alone caused a bacteriemia on 38.5% of occasions.

Guntheroth[13] reviewed the experience with infective endocarditis at the University of Washington Hospital. Eighteen patients presenting with congenital heart disease were followed by the pediatric cardiology staff over a 25-year period. None of these patients had an episode of infective endocarditis after dental procedures. From this analysis, the author concluded that the risk of infective endocarditis is mainly from day by day procedures as toothbrushing and mastication than dental procedures as extraction. Although antibiotic prophylaxis for major procedures appears prudent, scrupulous oral and dental hygiene is undoubtedly superior in preventing infective endocarditis.

Bacteriemia may occur spontaneously in patients with poor oral hygiene, patients with periodontal disease, or periapical infection, without any dental procedures done. The incidence and magnitude of the bacteriemia from oral origin are related to the level of inflammation and infection. Individuals at risk of developing infective endocarditis should keep a high standard of oral hygiene.

Several studies showed that dentists should be aware of their role in the prevention of infective endocarditis.[6,8,22] The recommendations should be followed prescribing antibiotic prophylaxis and chlorhexidine mouth rinses (15 ml) immediately before a dental procedure. Systemic antibiotic prophylaxis is recommended for high risk patients: having prosthetic heart valves, a previous history of endocarditis (even in the absence of other heart disease), complex cyanotic congenital heart disease, or surgically constructed systemic pulmonary shunts. Individuals with certain cardiac defects are at a moderate risk: congenital cardiac conditions including; patent ductus arteriosus, ventricular septal defects, bicuspid aortic valve and acquired valvar dysfunction (e.g. due to rheumatic heart disease or collagen vascular disease). The need of prophylaxis for mitral valve prolapse (MVP) is controversial.

Antibiotic prophylaxis for at-risk patients is recommended for some dental procedures including: extractions; periodontal surgery, scaling and root planing and recall maintenance; dental implant placement; replantation of avulsed tooth; endodontic treatment and surgery; subgingival placement of antibiotic fibers, placement of orthodontic bands; intraligamentary local anesthetic injections and cleaning of teeth or implants where bleeding is anticipated.[8,9]

MATERIAL AND METHODS

Thirty five children, 20 cardiac children and 15 healthy children formed both study and control groups, respectively. These children attended the Pedro Ernesto University Hospital, UERJ in Rio de Janeiro, Brazil.

A supervised questionnaire was answered by the parents and a consent letter signed. Demographic data included: age, gender, race, level of education of both child and parents. The study children were examined on the cardiac ward when admitted for medical treatment.

The saliva samples were obtained from each child using a standard method allowing reproducibility. Samples from the controls and patients were collected after paraffin chewing stimulation for 10 minutes and spitted into a universal tube. Salivary assessment included: salivary flow, buffer capacity, and *S. mutans (Sm)* and *Lactobacillus* counting using the *Caritest*® system by Herpo.

The *Caritest*® system is a version of the Cariescreen SM® kit manufactured by APO Diagnostics, Inc., Toronto, Canada, Jordan.[19] These kits use a selective medium for *S. mutans* with sucrose, and bacitracin at critical concentrations, which are tolerated by *S. mutans*, but not by other oral *viridans streptococci*. The basic kit consists of two screw-capped vials. One vial contains modified MSB agar, without bacitracin, dispensed onto both sides of a plastic dip slide. The second vial, which is for sample collection and dilution, contains 24ml of phosphate-buffered saline (PBS). Immediately before use, a tablet containing 440 units of bacitracin is added to the collection vial.

Salivary flow measurement using paraffin was always performed 1 to 2 hours after lunch. The volume of saliva was calculated per minute. This allowed for the classification of the child as having: low salivary flow (less than 0.7 ml/min) or normal when more than 0.7 ml/min.

The saliva buffer capacity was measured using the *Caritest*® system classifying the capacity as low, dubious or normal.

The *Caritest*® system for *S. mutans* was performed using the saliva sample collected. The dip slide containing modified MSB agar is removed from the vial and totally immersed in the diluted saliva sample for a few seconds. Excess liquid is allowed to drain from the dip slide, which is then returned to the original container.

A CO_2 generating tablet is added to the vial, two drops of water are applied to wet the tablet, and the cap holding the dip slide is screwed on tightly. Counting was performed after 2 days of incubation at 37˚C. Cultures from the saliva samples were visually compared with colony formation from the controls.

The *Caritest*® system presents a *S. mutans* colony density chart with values expressed in CFU/ml saliva (low risk being < 100,000 CFU/ml saliva while high risk being (100,000).

The *Caritest*® system for *Lactobacillus* was performed using the same technique with a medium for *Lactobacillus*. The *Caritest*® system presents a *Lactobacillus* colony density chart with values expressed in CFU/ml saliva (low risk being < 10,000 CFU/ml saliva, while high risk being (10,000).

The statistical analyses was performed using Epiinfo statistical software and the difference between the two groups was analysed using Chi-Squared test.

RESULTS

The age of the subjects in the study and control groups ranged from 6 to 14 years of age (Table 1).

Children in the study group were diagnosed and divided in two groups as having congenital cardiac disease or acquired cardiac disease. Fifteen children (75%) were diagnosed as having congenital cardiac disease including: atrial and ventricular defects and Tetral-

Table 1. Characteristics of the study and control groups.

GROUPS	GENDER		MEAN AGE	RACE		
	Female	Male		white	black	mulato
STUDY	9 (45%)	11 (55%)	9.05 ± 2.25	7 (35%)	5 (25%)	8 (40%)
CONTROL	6 (40%)	9 (60%)	9.70 ± 1.90	7 (46.7%)	8 (53.3%)	0 (0%)
TOTAL	15	20	14	13	8	

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Table 2. Salivary flow results: Comparison between study and control groups.

SALIVARY FLOW	CONTROL (n=15)	STUDY (n=20)	TOTAL (n=35)
Low (less than 0.7 ml/min.)	3	3	6
Normal (1 à 2 ml/min.) minimum value =0.7	12	17	29

There was no significant difference (p > 0.05).

ogy of Fallot. Five children (25%) were diagnosed as having acquired cardiac disease due to rheumatic fever.

Considering the medication used by these children, 13 (65%) of the cardiac children used regularly antibiotics (penicillin V or Amoxicillin). Four children (20%) used those antibiotics and some other medication including (ß-blockers, Frusemide, Digoxin and Captopril). The control group, i.e. healthy children, were not regularly taking any medication.

Salivary flow results showed no statistical difference between study and control groups (Table 2).

The saliva buffer capacity results showed no significant difference between study and control groups (Table 3).

Streptococcus mutans (Sm) counting results showed no significant difference between study and control groups (Table 4).

There was a significant difference in *Lactobacillus sp.* between the study group and control group ($p < 0.05$) (Table 5). The cardiac children showed a lower *Lactobacillus sp.* counting when compared with healthy children (controls), $p < 0.05$. This difference was related to the use of antibiotics. Children who used antibiotics regularly presented a significantly lower *Lactobacillus sp.* counting than those who were not taking antibiotics frequently. The same results were not found in relation to *S. mutans* counting.

In this study, qualitative and quantitative results are shown in graphics 1 and 2.

The association between regular use of antibiotics and the risk of developing caries, measured by *Streptococcus mutans (Sm)* counting and *Lactobacillus sp.* counting, showed that children using antibiotics regularly presented a significant lower *Lactobacillus sp.* counting (p<0.05). On the other hand, there was no significant difference in *Streptococcus mutans (Sm)* counting in children using antibiotics.

DISCUSSION

In this study the aim was to assess salivary conditions comparing cardiac children with normal children, in order to, evaluate the susceptibility of these two groups in developing caries.

Children taking not only antibiotics, but also medication that could reduce salivary flow were evaluated.

Table 3. Buffer capacity results: Comparison between study and control groups.

BUFFER CAPACITY	CONTROL (n=15)	STUDY (n=20)	TOTAL (n=35)
Low	0	3	3
Doubtful	6	6	12
Normal	9	11	20

There was no significant difference ($p > 0.05$).

Table 4. Sm counting results: Comparison between study and control groups.

Streptococcus mutans	CONTROL (n=15)	STUDY (n=20)	TOTAL (n=35)
Low (< 100,000)	3	9	12
High (≥100,000)	12	11	23

There was no significant difference ($p > 0.05$).

Table 5. Lactobacillus sp. counting results: Comparison between study and control.

***Lactobacillus sp.* counting**	CONTROL (n=15)	STUDY (n=20)	TOTAL (n=35)
Low (< 10,000)	4	15	19
High (≥10,000)	11	5	16

There was a significant difference between study and control ($p < 0.05$).

Reduction of saliva flow and changes in buffer capacity could interfere with oral health. However, in this study, in the cardiac group we found only four children taking some other medicine than antibiotic. There was no significant difference between cardiac children and healthy children in this aspect. These results were similar to Franco *et al.*[12] findings.

Franco *et al.*[12] investigated the dental and gingival conditions of children with severe congenital cardiac defects. They assessed caries experience, levels of plaque, gingival inflammation, knowledge of dental health practices and loading of *S. mutans, Lactobacillus sp. and Candida sp.* in a total of 120 children (60 with cardiac problems and 60 controls). The results showed similar levels of caries in cardiac patients (dmft 3.9 and DMFT 2.7) and the control (dmft 3.7 and DMFT 2.0). A significant difference was the proportion of untreated carious lesions in the cardiac group (52%) compared to the control group (32%), $p < 0.001$. Although there was no difference between control and cardiac children in the study, both groups had evidence of significant amounts of dental disease that were higher than in the population at large. The explanation for this may be that parents of cardiac children delay or avoid seeking dental treatment because of their preoccupation with the underlying medical problem.

This study also showed no difference in plaque levels and gingival inflammation between control and cardiac groups. The distribution in saliva and plaque of *S. mutans (Sm), Lactobacillus sp.* and *Candida sp.* showed no significant differences between the control and the cardiac group.

The purpose of assessing risk of developing caries through salivary conditions has been studied for decades.[7,19] The majority of those studies emphasised the multifactorial aspects of caries, and the limitation of salivary conditions assessment as predictors of caries development. Nevertheless, there is a role for these salivary tests to understand and monitoring caries development. In this sense, it seems interesting to assess salivary conditions in cardiac patients, especially because it is a non-invasive test that can be easily done.

A neglected oral health in children with cardiac disorders indicates potential morbidity associated to a

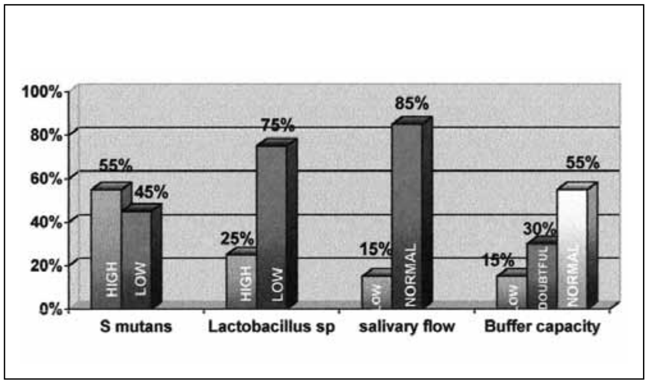

Figure 1. Salivary conditions of cardiac children n=20.

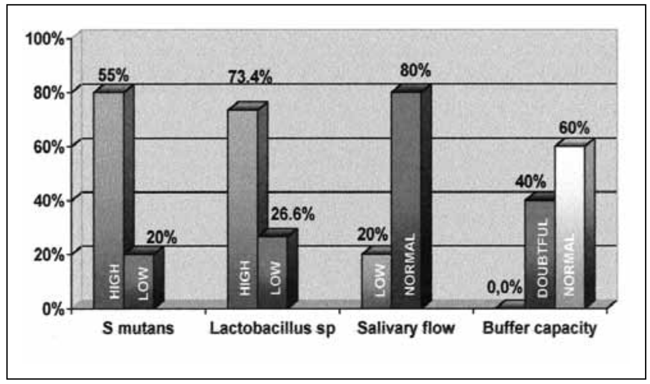

Figure 2. Salivary conditions of controls (n=15).

development of infective endocarditis, and studies focusing the oral health could lead to a better prevention. The improvement of oral health in cardiac patients should be emphasised, as there is a life risk when developing infective endocarditis. The literature on the subject still indicates a lack of knowledge among dentists.

The results showed a difference between the levels of *Streptococcus mutans* and *Lactobacillus sp*. in cardiac children. There was no significant difference in *Streptococcus mutans* counting in cardiac children compared to healthy children (Table 4). On the other hand, there was a significant difference in *Lactobacillus sp.* counting, between cardiac children and healthy children, cardiac children presenting lower levels of *Lactobacillus sp.* than in healthy children. This difference could be related to the regular use of antibiotics as 65% of cardiac children used Amoxicillin and penicillin regularly. The children using regularly antibiotics showed lower levels of *Lactobacillus sp*. ($p<0.05$) than those not using antibiotics. However, the same was not found in relation to *Streptococcus mutans*.

Bearing in mind the small sample, the results of this study showed that regular use of antibiotics had a major influence in the levels of *Lactobacillus sp*. in cardiac children.

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