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Oral microbial colonization in children with sickle cell anaemia under long-term prophylaxis with penicillin

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ABSTRACT

Background and objective: Sickle cell anaemia (SCA) is the most frequent haematological hereditary disease. Children with SCA are submitted to long-term prophylactic therapy with penicillin, but little is known about its impact on oral microflora. The aim of this study was to evaluate the oral microbial colonization of paediatric patients with SCA.
Design: Forty children (4–11 yrs old) with SCA (genotype SS) under long-term prophylactic treatment with penicillin were included in the study. Age/gender-matched control group of healthy children was also included. Scores of dmft/DMFT (number of decayed (D), missing (M), or filled (F) teeth; dmft, for primary dentition; DMFT, for permanent dentition) were obtained and stimulated saliva was sampled. Salivary flow rate and buffering capacity were evaluated. Counts of microorganisms (mutans streptococci, lactobacilli and yeasts) were determined by plating method. Yeasts were identified by API 20C AUX and PCR.
Results: Mean dmft/DMFT values were similar in the studied groups (SCA 2.13/1.60 and control 2.38/1.3). Although no significant differences between cariogenic microorganism counts were observed, significantly higher yeasts oral levels were observed in SCA group. Controls showed lower salivary buffering capacity. *Candida albicans* was the most frequently isolated species in both groups. *Candida famata*, *Candida parapsilosis* and *Candida tropicalis* were also isolated from controls. *Candida dubliniensis*, *Candida rugosa* and *Candida sphaerica* were found only in SCA group.
Conclusions: Based on the results, it could be concluded that paediatric patients with SCA showed significantly higher oral level of yeasts. Uncommon fungal species were found in SCA group. Similar caries prevalence and counts of lactobacilli and streptococci in relation to controls were observed.

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1. Introduction

Sickle cell anaemia (SCA) is the most common haematological genetic disease and it is frequently observed in Africa, Mediterranean, India, Caribbean, Central and South America.[1] The disease can manifest itself in many sites of the body, and unpredictable acute complications can become into life-threatening conditions.[1,2]

Children with sickle cell anaemia have high risk to bacteremia and sepsis, in particular caused by encapsulated microorganisms such as *Streptococcus pneumoniae*.[3] Bitarães et al.[4] estimated that the risk of pneumococci infection in children with sickle cell anaemia is approximately 30–100 times higher than in healthy children. Additionally, invasive bacterial infections in these patients are frequently caused by non-typhi *Salmonella* species and *Haemophilus influenzae* type b (Hib), and are the most common causes of death in children with sickle cell disease below 5 yrs of age.[5] Infections may start and/or be intensified by vaso-occlusive crisis.[6]

Mucosal pallor and late dental eruption are the most commonly observed oral manifestations.[7] Smooth tongue, bone rarefaction and trabecular bone coarsening have been also reported.[8] Also, orofacial pain, paresthesia of the mental nerve, pulpal necrosis and enamel hypomineralization were observed among these patients.[9] No increasing in the severity of periodontal disease was observed in adolescents with sickle cell anaemia.[10] On the other hand, systemic complications induced by periodontal infection in SCA patients were reported.[11] Osteomyelitis of the jaws may occur due to disease-associated tissue hypoxia.[12] Data on dental caries prevalence on SCA patients are not conclusive.[13] Studies on SCA young adults and adults reported controversial results regarding dental caries prevalence. Okafor et al.[14] reported that 35.13% of the studied SCA young adults had caries lesions and this percentage was 54% in the age/gender matched-control group. On the other hand, O'Rourke and Hawley[15] reported similar prevalence of dental caries among SCA patients (also young adults) and controls (mean DMFT = 6.9 and 7.3, respectively).

Due to the increased predisposition to infections, one of the most important interventions in the clinical treatment of the sickle cell anaemia is the long-term prophylaxis with penicillin. The prophylaxis is usually started at 2 months of age and the duration of this preventive measure is still considered a controversial decision.[1,16] It is well established in the literature that the route of administration does not affect prophylactic effect.[17,18]

Data on the effect of the long-term antibiotic prophylaxis in SCA patients on the oral microflora are scarce. Fukuda et al.,[19] using the kit Dentocult SM Strip *mutans*, reported that all SCA children under prophylaxis with oral penicillin were negative to mutans streptococci in saliva, whilst these microorganisms were isolated from 70% of the control group. Considering the lack of information regarding the oral microflora in SCA patients under long-term therapy with antibiotics, the aim of this study was to investigate oral colonization and salivary features in these patients.

2. Patients and methods

This cross-sectional study was previously approved by the ethical committees of the Institutions involved in the study, under the following protocols 082/2007-PH/CEP and CEP 1735/07. The study was conducted from 2008 to 2011. The study group was under treatment at Paulista School of Medicine (EPM–UNIFESP, São Paulo, Brazil), a reference centre in the follow-up of SCA children. Control group was attending dental treatment at São José dos Campos Dental School (UNESP), Brazil. Patients' parents were informed about the aims of the study and procedures, and gave their informed written consent.

Seventy-three SCA children were evaluated and considering the inclusion/exclusion criteria, a sample of 40 individuals (4–11 yrs old, mean 7.83 ± 2.5; 21 male and 19 female) was studied. Inclusion criteria for SCA group were: diagnosis of sickle cell anaemia and long-term prophylaxis with penicillin since 2 months of age. The diagnosis was done by the responsible physician and was based on haemoglobin electrophoresis, associated with hemogram interpretation and clinical manifestations.

Twenty SCA patients under treatment with benzathine penicillin 50,000 UI/kg I.M., every 21 days, and 20 children under treatment with penicillin V 50,000 UI/kg/day, twice a day (oral administration) were included in the study. The therapy was based on the protocol adopted by the Brazilian Ministry of Health. As both drugs are effective for preventing pneumococcal infection in sickle cell disease, the treatment option was based on the preference of parents or guardians. Patients submitted to therapy with other antibiotics in addition to penicillin for the last 3 months were not included in the sample.

In the control group, 40 healthy children without sickle cell anaemia or any other systemic disease, gender/age-matched with SCA group were included. All the patients between 4 and 11 yrs of age under treatment were invited to participate of the study. The patients were randomly selected considering the exclusion and the gender/age-matching criteria.

Exclusion criteria for both groups were: diagnosis of diabetes *mellitus* or other systemic diseases use of orthodontic devices, mouth rinses or pacifiers, and treatment with antidepressant medications or antibiotics/antifungals (except for penicillin treatment in SCA group) in the last 3 months.

Data on the medical history (diagnosis, duration of disease and therapy) were reported by the responsible physician. Anamnesis and clinical examinations were performed to collect clinical data and the dmft/DMFT scores (number of decayed (D), missing (M), or filled (F) teeth; dmft, for primary dentition; DMFT, for permanent dentition) were recorded. All anamnesis and clinical examinations were performed by the same dentist. Also, information about parental education, sugar (sweeties) intake and dental brushing was obtained by a questionnaire.

Stimulated saliva was collected for 5 min in a sterile container. Immediately after the sampling, salivary flow and buffering capacity were evaluated. The salivary flow rate was determined in ml per minutes.[20] For the salivary buffering capacity evaluation, 1 ml of saliva was added to 3 ml of HCl 0.005 N, stirred and kept in an opened container for 10 min, according to Ericsson.[21] After, the pH was measured using a portable pH meter.