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Serum cytokine profile among Brazilian children of African descent with periodontal inflammation and sickle cell anaemia

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ABSTRACT

Objective: The aim of this study was to evaluate possible immunologic relationships between sickle cell anaemia (SCA) and periodontal inflammation and its impact on serum cytokines.

Design: Twenty-five Brazilian children of African descent were involved in this study and divided in two groups: SCA ($n = 10$): confirmed diagnosis of homozygous anaemia; and CTR-control ($n = 15$): no sickle anaemia. Clinical examination included comprehensive medical (routine physical evaluation) and periodontal exams: plaque index (PI), bleeding on probing (BoP), and haematological analysis. Serum samples were collected for cytokine evaluation by microarray. Clinical and laboratorial parameters were compared statistically (alpha = 5%).

Results: The higher values of PI and BoP were similar for both groups ($p > 0.05$) confirming a diagnosis of generalized gingivitis for all individuals. Intergroup analysis showed higher levels of interferon gamma (IFNγ), tumour necrosis alpha (TNFα), interleukin (IL)-4, -5, -8, -10 and 13 only in the SCA group ($p < 0.05$). In addition, PI was negatively correlated with IL-2, IL-4, IL-5, IL-6, IL-8 and IL-13, while BoP was positively correlated with IL-10.

Conclusion: Within the limits of the present study, it was concluded that SCA increase the levels of serum cytokines regardless of the presence of periodontal inflammation. Therefore, a direct immunological relationship between SCA and periodontal inflammation was not established.

1. Introduction

Periodontal diseases are infectious chronic inflammatory states characterized by inflammation of the supporting structures of teeth. They are very prevalent among humans and reported as modifying factors of systemic diseases.[1,2] Persistent release of inflammatory mediators such as cytokines results in the destruction of soft and mineralized periodontal tissues.[3] The tissue destruction at the clinical level begins as gingivitis and progresses to periodontitis through the generation of proteases that degrade the

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extracellular matrix and activate the mechanisms for alveolar bone resorption.[4–6]

Sickle cell anaemia (SCA) is characterized by an abnormal haemoglobin molecule as a result of the substitution of glutamic acid by valine at position 6 of the β-globin chain.[7] It is estimated that SCA is present in 7% of the world's population specially in those of African descent that are very prevalent in Brazil.[8] It is responsible for approximately 3.4% of the deaths in children under 5 years of age in these populations.[8–10] SCA subjects develop childhood chronic anaemia, fragility of erythrocytes as well as characteristics of vaso-occlusive crisis (i.e. thrombosis, fever, splenomegaly, joint pain, infections, lethargy, weakness, and events of stroke and heart failure).[11]

Clinical studies conducted in Africa[12–15] have evaluated possible associations between sickle anaemia and periodontal diseases. These studies indicated that the poor oral hygiene status of these populations could be biasing or masking a true causal effect. As a matter of fact, few studies have reported the presence of an intense immunologic response in both periodontal diseases and SCA.[11,16–21]

It has been reported that the prevention of vaso-occlusive crisis in SCA patients depends on concomitant prevention of infections that target the quality of life of those individuals.[22–24] Infectious complications can reach at least 1% of the SCA population.[15] Therefore periodontal diseases could potentially contribute to the severity of SCA complications by promoting an overexpression of inflammatory cytokines. However the immunologic cumulative effects of both diseases have not yet been investigated.[9–13] Thus, this study aimed to evaluate the serum cytokine profile among Brazilian children of African descent with periodontal inflammation and sickle cell anaemia.

2. Materials and methods

2.1. Study sites and sample population

The study sites were the Health Support and Reference Center (C.U.I.D.A.R.) and the Paediatric Clinic at the Bahian School of Medicine and Public Health (EBMSP) located in Bahia State, Brazil. Twenty-five children of African descent were selected for this study and were matched for age, gender and ethnic background. The SCA group included 10 children in steady state of sickle disease, presenting haemoglobin status conforming to the SS homozygous profile. The control group comprised 15 matched systemically healthy children. Patients that presented other systemic conditions except anaemia, such as autoimmune disease or acquired or induced immunosuppression, recent history of infection, antibiotic use in the last 6 months and those whose parents or legal guardians did not sign the consent form were excluded from the study. The Research Ethics Board (REB/EBMSP, Brazil) approved the study and informed consent was obtained from the parents during children's routine medical consultation.

2.2. Study design

A cross sectional study was conducted in 25 matched children divided in two groups: 10 children in the SCA group with a confirmed diagnosis of SCA; and 15 in the CTR-control group without SCA. A single examiner evaluated the periodontal health status in all subjects. Blood samples were collected for haematological analysis and for cytokine microarray detection. Clinical and laboratory data were statistically compared.

2.3. Medical and periodontal assessments

A comprehensive medical assessment was performed by the C.U.I.D.A.R. haematologists with regard to clinical, infection and pain information using standard medical charting, entailing clinical infection diagnosis, history taking for possible exposure to infectious disease, recording clinical signs and symptoms of fever, rashes, jaundice or swelling as well as evidence of organ specific signs and symptoms such as cough, diarrhoea and dysuria. For the periodontal assessment, a calibrated examiner (kappa = 0.92) performed all the exams in all subjects, using a periodontal probe (CP12 Hu Friedy®, USA). Periodontal parameters that were evaluated included the percentage of visible plaque (PI) and the percentage of bleeding on probing (BoP) in six sites per tooth.[25]

2.4. Blood sampling and hematologic/haemoglobin analysis

Peripheral blood samples were collected during the ambulatory outpatient consultations. Thirteen millilitres of blood were obtained from each individual by venipuncture into vacutainer tubes with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant and also in plain tubes. A fraction of 5 mL was collected in EDTA tubes at the concentration of 1.5 mg/mL[26] for hematologic exams and electrophoresis. Complete blood counts (hematologic values and hematimetric indices) were obtained with an electronic cell counter (Coulter Count T – 890, Beckman Coulter, USA Abbott CELL-DYN® 4000). A fresh hemolysate was prepared from each sample and subjected to cation exchange high-performance liquid chromatography (Bio-Rad VARIANT, Bio-Rad Laboratories, Hercules, CA) to reconfirm the haemoglobin phenotype. The morphologic analysis of erythrocytes was performed through microscopic observation of stained blood smears using the Wright method.[26] Another fraction of 8 mL was also collected in plain tubes (without additives). Serum was separated from the clotted tubes at 1000 × *g* at 4 °C for 10 min, and stored at −80 °C for cytokine evaluation.

2.5. Microarray cytokine analysis

All serum samples that were frozen at −80 °C were thawed at the time of the assay. The concentrations of 10 selected cytokines (interferon gamma (IFNγ), tumour necrosis alpha (TNFα), interleukin 2 (IL-2), IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and granulocyte macrophage colony-stimulating factor GM-CSF) were determined in the serum samples from all subjects, in accordance with the manufacturer's instructions for the use of the arrangement of human T helper (Th)1/Th2 cytokine array (Quantibody Th1/Th2 Protein Human Array®, RayBiotech, USA).[27,28] Briefly, 16 identical antibody arrangements were pre-exposed on a part of a standard glass slide from the kit. In the capture of the 10 specific cytokines, each antibody was