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Proinflammatory cytokines in early childhood caries: Salivary analysis in the mother/children pair

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ABSTRACT

Hypothesis: Proinflammatory cytokines are increased in saliva of mother/children pairs with caries. Design: Case-control study involving caries-free children (n = 20) and children with early childhood caries (ECC) (n = 20), and their mothers (n = 40). The maternal variables analyzed were waist circumference (WC), decayed, missing and filled teeth (DMFT) and sugar intake; and in the children were body mass index (BMI), def-t and sugar intake. Salivary levels of VEGF, IL-6 and TNF-α were analyzed of mother/children pairs. Results: In the mothers, salivary VEGF levels were correlated with DMFT (r = 0.35; p = .03), WC (r = 0.35; p = .02), and sugar intake (r = 0.32; p = .04). Higher salivary IL-6 levels were also correlated with maternal DMFT (r = 0.45; p = .004) and WC (r = 0.32; p = .04). In the children, higher salivary VEGF levels were correlated with higher def-t scores (r = 0.42; p = .008). Children with caries had a 63% higher median salivary VEGF and twofold higher mean IL-6 levels compared to caries-free children. Mothers of children with ECC showed higher mean of salivary IL-6 levels compared to those of children without ECC (p = .03). Conclusion: Salivary proinflammatory cytokines are correlated with the severity of caries in the mother-children pair. Obesity and excessive sugar consumption seem to underlie the associations between proinflammatory cytokines and caries in the family environment.

1. Introduction

Early childhood caries (ECC) is defined as the presence of one or more carious lesions cavitated or not in children up to 71 months of age [1]. Mothers play a decisive role in the occurrence of ECC, with a larger number of carious teeth in the mother being associated with caries in her children [2,3]. Furthermore, a higher maternal body mass index (BMI) [3], greater maternal waist circumference (WC) [4] and children obesity have also been associated with ECC [5,6].

The maternal dietary pattern influences the eating habits of the child, especially the preference for sweets and sugary drinks [7], suggesting that obesogenic behaviors are perpetuated in the family environment.

Systematic reviews of the literature have showed an association of excess intake of sugars with weight gain in children and adults [8], with an epidemy of obesity, metabolic syndrome [9,10] and also insulin resistance [9]. The World Health Organization recommends a reduced intake of free sugars not only for the prevention of caries and obesity, but also to reduce the risk of other chronic noncommunicable diseases [11]. The American Heart Association published recommendations that emphasize the limits of sugar intake by children to reduce the cardiovascular risk [12].

The adipose tissue is an organ secreting numerous cytokines that play a role in the regulation of the systemic inflammatory state [13]. These regulatory cytokines are directly involved in sugar metabolism and obesity. Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are adipokines which have been associated to obesity and hyperinsulinemia [14,15]. These adipokines may regulate the endothelial function [14]. Therefore, cytokines such as vascular endothelial growth factor (VEGF) are also overexpressed in obesity and diabetes [16,17].

High serum levels of IL-6 and TNF-α were also associated to an excessive consumption of added sugars [18]. An increase of salivary

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levels of VEGF are observed in sugar metabolism disorders [19,20]. Elevated salivar levels of IL-6 and TNF-α have been evidenced in presence of high caries severity [21–23].

Based on the premise that the consumption of added sugars in the family environment is associated with both caries and obesity, the hypothesis of this study is that proinflammatory cytokines linked to high consumption of sugar would be increased in saliva of mother/children pairs with severe caries disease.

Therefore, the objective of the present study was to evaluate salivary levels of proinflammatory cytokines (IL-6, TNF-α, and VEGF) and to correlate them with the presence of caries, obesity and sugar intake in mothers and their children.

2. Material and methods

2.1. Ethical aspects

The study was approved by the Research Ethics Committee of the Federal University of Maranhão (UFMA) (Approval No. 23115012534/2008-41). Prior to the study, the mothers signed a free informed consent form containing clear and objective verbal explanations about their participation in the study. Children who required dental treatment were referred to the Pediatric Dental Clinic of UFMA for appropriate therapy.

2.2. Selection of the participant

This was a cross-sectional study nested within a retrospective cohort (n = 400) that involved children aged 24–71 months enrolled in municipal preschools of São Luís, Maranhão, Brazil, and their mothers participating in the research project “Early childhood caries from the mother/child pair perspective”.

The sample size was based on two previous studies evaluating caries disease and inflammatory markers in saliva [21,22]. Children with ECC and caries-free children were selected from original cohort using the “Filter” tool of the Excel® 2010 software; this step was to insure randomization of the sample, increasing sample representativeness (internal validity of the study). Each pair received a numerical identification using the RANDBETWEEN function. Twenty children with ECC and twenty caries-free children and their respective mothers were selected for the analysis of salivary inflammatory markers. Three of the 40 mother/children pairs were lost, including one caries-free child and two children with caries, since they did not have sufficient amounts of saliva for the tests, totaling 37 pairs.

2.3. Data collection

The data were collected at the preschools themselves from August 2013 to March 2015. The mothers answered a structured questionnaire applied by interview that contained socioeconomic and demographic data, as well as a food frequency questionnaire. The maternal variables studied were WC, caries experience and sugar intake. For the children, BMI, ECC and sugar intake were evaluated.

For maternal anthropometric assessment, WC was measured with a seamless Sanny® tape measure at the midpoint between the last costal margin and iliac crest, to the nearest 1 mm. Maternal WC was categorized as follows: < 80 cm (absence of metabolic risk); ≥80–88 cm (high metabolic risk), and > 88 cm (very high metabolic risk) [24].

The height (cm) of the children was measured with an Alturexata® portable stadiometer (Belo Horizonte, MG, Brazil) and body weight (kg) was measured with a Filizola® digital scale (São Paulo, SP, Brazil), with a maximum variation of 100 g. These measurements were obtained in duplicate by two evaluators in a blind manner and the mean value of the two evaluations was recorded. The kappa value was 0.89 (± 0.01) for intraexaminer agreement and 0.91 for interexaminer agreement. The body mass index (BMI) are expressed as Z-score, which calculates the BMI for age in percentiles for children aged 0–5 years according to sex [25]. The WHO Anthro 3.2.2 software was used for children up to 60 months and the WHO Anthro Plus 1.4.0 for children older than 60 months (WHO, Geneva, Switzerland).

Sugar intake by the mothers and children was analyzed using a food frequency questionnaire [26]. The frequency of added sugar intake was obtained as the sum of daily intake of the following foods: soft drinks, processed juices, chocolate drinks, chocolate, cakes, candies, sweets, and cookies. The sum of these frequencies was used as a continuous variable.

Clinical oral examination for analysis of caries experience was performed after tooth brushing using the decayed, missing and filled teeth (DMFT) index for the mothers and the decayed, tooth indicated for extraction and filled teeth (def-t) index for the children [27]. The def-t was modified to include non-cavitated active caries lesions, including rough and opaque white spot lesions.

A flat mouth mirror and exploratory probe with a blunt end were used for clinical examination. The mother and children were examined under natural light sitting on common chairs in the outside patio of the school. Clinical examination of the mother and children was performed at different times to ensure blinding of the evaluator for this variable of the pair. The data were collected by a single, previously calibrated examiner (kappa = 0.86).

Stimulated saliva samples were collected from the mother/children pairs after mastication of Parafilm® for 5 min. The saliva samples were transferred to a 1.5-mL Falcon tube, stored on ice, and sent to the Laboratory of Oral Biochemistry of UFMA for processing.

2.4. Salivary analysis of inflammatory markers

The salivar levels of IL-6 pg/mL, TNF-α pg/mL, and VEGF pg/mL were analyzed saliva using the MILLIPLEX® MAP Human Adipokine Magnetic Bead Panel 2 Kit – HADKMAG-61K (Merck Millipore, BA, USA). The standards, controls and samples were processed according to manufacturer recommendations and the fluorescent beads were read on a Luminex 200 platform (Gen-Probe, CA, USA). The data obtained were analyzed with the Milliplex Analyst program (Merck Millipore, BA, USA).

2.5. Statistical analysis

Continuous variables that showed a normal distribution (maternal DMFT, maternal sugar intake, def-t and children sugar intake) were correlated with the salivary inflammatory markers using Pearson’s correlation test. Categorical variables or non-normally distributed variables (maternal WC and children BMI Z-score) were correlated with the salivary inflammatory markers using Spearman’s correlation test. The correlations were defined as follows: very weak ($0 \leq r < 0.19$), weak ($0.20 \leq r < 0.39$), moderate ($0.40 \leq r < 0.69$), strong ($0.70 \leq r < 0.89$), and very strong ($0.90 \leq r < 1.0$) [28].

In addition, the mean salivary levels of the inflammatory markers were compared between mothers of children without and with caries. In this step, the Student *t*-test was used for normally distributed data (IL-6 and TNF-α) and the Mann-Whitney test for non-normally distributed data (VEGF). The salivary levels of the inflammatory markers were also compared between caries-free children and children with ECC using the Student *t*-test for mean IL-6 and TNF-α levels. VEGF levels are expressed as the median and were compared by the Mann-Whitney test.

All analyses were carried out with the Stata® 14.0 software, adopting a level of significance of 5%.

3. Results

The characteristics of the sample according to the distribution of study variables are described in Table 1.

Analysis of the correlation between maternal variables and maternal proinflammatory cytokines in saliva showed a weak correlation of