Use of low-level laser therapy on children aged 1 to 5 years with energy-protein malnutrition

A clinical trial

Karlla Almeida Vieira, DDS, MSc, PhD, A, Clarissa Moraes BastosB, Marilya Gabriella Correia VitorB, Alessandro Melo Deana, Phys, PhD, PostDocA, Kristianne Porta Santos Fernandes, DDS, MSc, PhD, PostDocA, Maria Fernanda Setúbal Destro Rodrigues, DDS, MSc, PhD, PostDocB, Vanessa Christina Santos Pavesi, DDS, MSc, PhD, Sandra Kalil Bussadori, DDS, MSc, PhD, PostDocA.

Abstract

Background: Episodes of malnutrition in early childhood can produce alterations in the salivary glands. The investigation of mechanisms that can reduce the impact of malnutrition on the defenses of the organism is of the utmost importance and interest to public health. The aim of this study is to evaluate the effect of low-level laser on the saliva of children aged 1 to 5 years with energy-protein malnutrition.

Methods: Mandatory inclusion criteria are diagnosis of malnutrition. The sample will consist of 50 men and women malnourished children aged 12 to 71 months. Saliva will be collected and the volume of saliva will be measured and the salivary flow rate will be determined (mL/min). Concentrations of salivary IgA in all samples will be measured using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit. Low-level laser (laser diode) will be administered in the region of the parotid glands bilaterally as well as in the regions of the submandibular and sublingual glands.

Discussion: This study will be the first that investigate the effects of local laser therapy on the salivary glands of malnourished children.

Trial registration: Clinical.trials.gov as NCT03355313, first received in 21 November 2017.
1. Background

Malnutrition is a clinical–social disease caused by multiple prenatal, intrauterine, and postnatal factors as well as social, political, and cultural determinants (distal causes). Despite the global and national reductions in the number of cases, malnutrition continues to be a public health problem, with greater prevalence in pockets of poverty found in the northern and northeastern regions of Brazil.3,4

Energy-protein malnutrition occurs when there is an imbalance between food intake and the physiological expenditure of energy and nutrients in all cells of the body, including those responsible for the formation of dental tissue, saliva, and the oral epithelium.5,6 Thus, malnutrition means that cells do not receive the nutrients necessary to perform their functions of energy production, tissue formation/repair, and the regulation of their own functioning.5,6,9

Malnutrition in the early years of life leads to growth deficits in childhood, as reflected by anthropometric indicators. This condition is also associated with a higher child mortality rate, greater probability of acquiring infectious diseases, impaired psychomotor development, and poorer academic performance as well as lower productivity in adulthood.10,11

Episodes of malnutrition in early childhood, with consequent calcium, phosphate, and vitamin A, C and D deficiencies, can increase one’s susceptibility to dental caries through 3 probable mechanisms: defects in tooth formation (odontogenesis), delayed tooth eruption, and alterations in the salivary glands.12,13

It is likely that the significant increase in susceptibility to caries in malnourished individuals stems from alterations in the salivary secretion rate, since a reduction in salivary flow (salivary gland atrophy) increases the susceptibility to both dental caries and dental erosion.14–16 As saliva is the main defense factor of the oral cavity, a reduction/change in its physical properties (secretion rate and buffering capacity) can cause immunological disorders that affect an individual’s defense capacity.17,18

Studies have demonstrated that salivary Immunoglobulin A (IgA) also plays an important role in the immunity of the oral mucosa.118–20 Indeed, patients with IgA deficiency can experience recurring upper airway (tonsillitis, ear infection, and sinusitis), lower airway (pneumonia), and gastrointestinal (diarrhea and parasitosis) infections.21,22

IgA was first identified in 1959 by Heremans23 and is found in serum and organic fluids, such as saliva. There are 2 types of this antibody. IgA1 is produced by B cells of the bone marrow and IgA2 is produced by B cells of the mucosa, accounting for approximately 80% of the total production. Once produced by plasmocytes (differentiated from activated B cells) in the lamina propria of the epithelium of the salivary glands, IgA binds to a polymeric Ig receptor in the base of the epithelial cells, where it is transported and released through proteolytic cleavage to join other substances that compose the salivary mucus.21,23

Stimulated and non-stimulated salivary flow rates can be reduced significantly in individuals who have suffered severe malnutrition in early childhood as well as those with continued nutritional stress. Thus, malnutrition can exert a continual effect on the reduction in salivary gland function in adolescence as result of malnutrition in early childhood, which suggests that exocrine systems can be compromised for long periods after exposure to malnutrition.24

The investigation of mechanisms that can reduce the impact of malnutrition on the defenses of the organism is of the utmost important and interest to public health. Among such mechanisms, low-level laser therapy has demonstrated effectiveness in the treatment of diverse conditions and disease through the promotion of the biomodulation of the cell metabolism and due to its analgesic and anti-inflammatory properties with no mutagenic or photothermal effects.25 The conversion of laser light into useful energy for cells through photochemical and photophysical reactions can simulate the production of mitochondrial adenosine triphosphate (ATP), cell proliferation, and protein synthesis.26,27

Laser stimulation of the major salivary glands to produce more saliva occurs through the increase in local circulation due to vasodilatation, the induction of the proliferation of glandular cells and cell respiration/ATP synthesis as well as the release of growth factors and cytokines to stimulate protein exocytosis.28,29 Regarding an increase in salivary IgA, low-level laser intensifies the activation of B lymphocytes, which differentiate into plasma cells, thereby contributing to the increase in immunoglobulin levels.30

It is important to study the impact of malnutrition in children because its long-term consequences could be reduced which implies promoting positive effects on oral and general healthy.

The primary aim of this trial is to evaluate the effect of low-level laser on the saliva of children aged 1 to 5 years with energy-protein malnutrition. Secondary outcomes are to establish the degree of malnutrition in children aged 1 to 5 years and to analyze the saliva of children aged 1 to 5 years with energy-protein malnutrition.

2. Methods/Design

2.1. Type of study

An experimental cross-sectional study is proposed, which will be conducted at the Center for Educational and Nutrition Recovery (CREN) in the city of Maceió, state of Alagoas, Brazil.

2.2. Trial registration

Clinicaltrials.gov as NCT03355313, first received in 21 November 2017, https://clinicaltrials.gov/ct2/show/NCT03355313.

2.3. Sample

2.3.1. Characteristics and size. This cross-sectional study will use a sample of children aged 12 to 71 months of the 7th Administrative Region of Maceió. This region is one of the poorest region in Maceió. The children’s parents/guardians will be interviewed at the CREN in the city of Maceió, state of...
Alagoas, Brazil. The sample size was estimated considering a prevalence of malnutrition of about 10% in children under 6 years old, with statistical power of 80% and 95% significance, resulting in a sample of 50 children.

2.4. Allocation mechanism

The allocation sequence will be generated by CREN. The number/symbol of each child will be sent by the computer by the registration model in CREN which is based on the distribution by learning classes. Assessments regarding saliva and nutritional status will be binding for trial participants and data analysts.

2.5. Inclusion criteria

Children aged 1 to 5 years enrolled at the CREN in the city of Maceió whose parent/guardians signed as statement of informed consent agreeing to the participation of the children.

2.6. Exclusion criteria

Children aged 1 to 5 years not enrolled at the CREN in the city of Maceió and children whose parents/guardians did not sign a statement of informed consent, teachers, caregivers, health professionals, and voluntary interns.

2.7. Interventions

2.7.1. Evaluation of nutritional status. The children will be weighed on a duly calibrated anthropometric scale (capacity: 150 kg; precision: 100 g) barefoot and wearing light clothing in the presence of the mother or caregiver. Height will be determined using a non-flexible metric tape measuring 2 m in length (precision: 0.1 cm). The measurement will be performed twice and the mean will be used for the calculation of the indices. Using these measures, height for age and weight for age will be determined using the nutritional reference standards recommended by the World Health Organization, with a cutoff point (Z score) between +1 and –1 standard deviations (SD) of the median indicative of the ideal range, Z between –1 and –2 SD indicative of mild malnutrition, Z between –2 and –3 SD indicative of moderate malnutrition and Z >–3 indicative of severe malnutrition.[29]

2.8. Saliva collection and analysis

Saliva will be collected at the dental sector of the CREN in the city of Maceió with the child seated in a dental chair under conventional light. Total resting saliva will be collected between 9 and 11 am (to avoid the influence of circadian rhythm) using the drainage method. The children will have not ingested food or beverages (except water) 1 hour before the procedure and will not have performed oral hygiene within 2 hours before the procedure. The child will be instructed to swallow prior to the collection and then instructed not to swallow, allowing the saliva to drain between the lips (which will be separated) into a test tube (aspirator) connected to a 15-mL Flacon tube positioned near the mouth.[30] Collection time will be 5 minutes. The volume of saliva will be measured and the salivary flow rate will be determined (mL/min). Salivary flow values will be analyzed based on the values below[31] (Table 1).

One aliquot (1 mL) of saliva will be transferred immediately to a small tube (capacity: 5 mL) for titration with hydrochloric acid 0.005 N. The tube will be shaken for 15 seconds and buffering capacity will be measured using a glass electrode (Orion) coupled to a pH meter (Procyon 720 A, Procyon, São Paulo, Brazil). Titration under partial carbon dioxide gas is considered the standard method for determining the buffering capacity of saliva. The reading of this test is performed based on the following parameters: pH 3.0 to 4.0 = very low to low buffering capacity; pH 4.5 to 5.0 = intermediate buffering capacity; and pH ≥5.5 = normal/good buffering capacity.[32]

The remaining saliva will be stored in Eppendorf tubes and frozen at –20°C until the analysis of salivary IgA. Concentrations of salivary IgA in all samples will be measured using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Diametra IgA Saliva Kit, Italy) (Fig. 1). Salivary IgA (µg/mL) in each sample will be calculated using a standard curve obtained from the calibrators in the kit[19] and will be analyzed[33] (Table 2). The data will be analyzed using Analysis of Variance (ANOVA) and Pearson’s correlation test (α = 0.05). The Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) version 15.0 will be used for all analyses.

2.9. Administration of low level laser

Low-level laser will be administered using the Photon Lase III device (DMC Equipamentos LTDA, São Paulo, Brazil). Laser will be administered for 10 seconds on 4 intraoral points and 4 extraoral points in the region of the parotid glands bilaterally as well as 1 intraoral point and 1 extraoral point in the regions of the submandibular and sublingual glands. The first session will occur after the collection of saliva. The second and third sessions will be performed 7 and 14 days after the first session, respectively. The final saliva collection will be performed after the third laser session.[34,35]

The laser will be adjusted according to the following parameters: Central wavelength (nm) = 808; spectral band width (FWHM) = 2 nm; operational mode = continuous; mean radiant power (mW) = 100; polarization = randomized; aperture diameter (cm) = 0.2; irradiation at aperture (mW/cm²) = 2500; beam profile = multimodal; beam spot on target (cm²) = 0.04; irradiation at target (mW/cm²) = 2500; exposure time (s) = 40; radiant exposure (J/cm²) = 100.0; radiant energy (J) = 42; number of points irradiated = 10; irradiated area (cm²) = 0.40; application method = Contact; number of treatment sessions = 3; frequency of treatment sessions per week = 1; Total radiant energy (J) = 40.

The salivary flow rate and salivary IgA will be compared before and after of each laser application (after 7 and 14 days) and correlation with the malnutrition will be established. The results will be analyzed using ANOVA and Tukey’s contrast test. The level of significance will be 5%.

3. Conclusion

The study of salivary aspects in malnourished children and possible treatments that can be used to improve salivary quality...
and quantity in these children has significant social relevance, as saliva is one of the main mechanisms against infection and participates in essential functions of life, such as swallowing and the maintenance of oral health.

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Author contributions

Conceive and design the study: Karlla Almeida Vieira, Alessandro Melo Deana, Sandra Kalil Bussadori. Will perform the experiment: Karlla Almeida Vieira, Clarissa Moraes Bastos, Marilya Gabriella Correia Vitor, Karlla Almeida Vieira, Maria Fernanda Setúbal Destro Rodrigues, Sandra Kali Bussadori. Will analyze the data: Karlla Almeida Vieira, Vanessa Christina Santos Pavesi, Maria Fernanda Setúbal Destro Rodrigues, Kristianne Porta Santos Fernandes. Will perform the statistical analysis: Alessandro Melo Deana, Sandra Kalil Bussadori. Write the paper: Karlla Almeida Vieira, Alessandro Melo Deana, Kristianne Porta Santos Fernandes, Sandra Kalil Bussadori. Conceptualization: Karlla Almeida Vieira, Alessandro Melo Deana, Sandra Kalil Bussadori. Data curation: Karlla Almeida Vieira, Kristianne Porta Santos Fernandes, Sandra Kalil Bussadori. Formal analysis: Karlla Almeida Vieira, Alessandro Melo Deana, Maria Fernanda Setúbal Destro Rodrigues, Vanessa Cristina Santos Pavesi, Sandra Kalil Bussadori. Investigation: Karlla Almeida Vieira, Maria Fernanda Setúbal Destro Rodrigues. Methodology: Karlla Almeida Vieira, Clarissa Moraes Bastos, Marilya Gabriella Correia Vitor, Alessandro Melo Deana, Maria Fernanda Setúbal Destro Rodrigues, Sandra Kalil Bussadori. Project administration: Karlla Almeida Vieira, Sandra Kalil Bussadori. Supervision: Karlla Almeida Vieira, Sandra Kalil Bussadori. Validation: Karlla Almeida Vieira. Writing – original draft: Karlla Almeida Vieira, Alessandro Melo Deana, Sandra Kalil Bussadori. Writing – review and editing: Karlla Almeida Vieira, Alessandro Melo Deana, Kristianne Porta Santos Fernandes, Sandra Kalil Bussadori.

### Table 2

| Age        | n   | IgA * (mg/dL) |
|------------|-----|---------------|
| <1 mo      | 7   | N.D.          |
| 1 to 6 mo  | 50  | 3.1±2.0      |
| 7 to 11 mo | 12  | 2.0±0.9      |
| 12 to 23 mo| 8   | 2.3±1.5      |
| 24 to 71 mo| 16  | 4.1±2.4      |
| >72 mo     | 32  | 5.5±2.9      |

Source: SOLÉ et al. (1987).[33]

IgA = Immunoglobulin A, N.D. = not detectable, n = number of patients evaluated.

Figure 1. Schedule of enrolment, interventions, and assessments.
References

[1] Ribeiro CCC, Silva MCB, Machado CMP, et al. Is the severity of caries associated with malnutrition in preschool children? Gênc saúde coletiva 2014;19:937–63.

[2] FAO, IFAD, WFP. The State of Food Insecurity in the World 2015. Meeting the 2015 International hunger targets: taking stock of uneven progress. Rome, FAO. Available at: http://www.fao.org/3/a-i4646e.pdf. Accessed May 15, 2017.

[3] Sawaya AL, Solymos GMB, Florencio TMMT, et al. The two Brazils: who they are, where they are and how the poor Brazilians live. Estud av 2003;17:21–87.

[4] Cabral MJ, Vieira KA, Sawaya AL, et al. Socioeconomic, nutritional, and food intake profiles of beneficiaries of the Social Family Program in Brazil. Estud av 2013;27:71–87.

[5] Ferreira Hda S, Cesar JA, Assunção ML, et al. Time trends (1992–2003;17:21–87). Acessed May 15, 2017.

[6] Brasil, Instituto Brasileiro de Geografia e Estatística (IBGE). Synthesis of Social Indicators 2014 - an analysis of the living conditions of the Brazilian population. 2015; IBGE, Rio de Janeiro:250.

[7] Costa DP, Mota ACM, Bruno GB, et al. Protein-energy malnutrition and early childhood caries. Rev Nutr 2010;23:119–26.

[8] Borsos MA. Nutrition and Dentrics: The Basics. 14th ed. 2001; Senac São Paulo, São Paulo:96 p.

[9] Fragaa JAA, Varela DDS. The relationship between malnutrition and child development. Rev Assoc Bras Nutr 2012;4:59–62.

[10] Black RE, Allen LH, Bhutta ZA, et al. Maternal, Child Undernutrition - Meeting the 2015 International hunger targets: taking stock of uneven progress. Rome, FAO. Available at: http://www.fao.org/3/a-i4646e.pdf. Accessed May 15, 2017.

[11] Silva MC, Silva ACD. Functional performance of children with chronic malnutrition aged 1 to 3 years old. Cad Ter Ocup UFSCar 2014;22:327–34.

[12] Batista LRV, Moreira EAM, Corso ACT. Food, nutritional status and oral condition of the child. Rev Nutr 2007;20:191–99.

[13] Sokal-Gutierrez K, Bathsheba JT, Husby H, et al. Early childhood caries. Rev Nutr 2010;23:119–26.

[14] Johansson I, Saellström AK, Rajan BP, et al. Saliva as a matrix for human biomonitoring in occupational and environmental medicine. Int Arch Occup Environ Health 2015;88:1–44.

[15] Michalka B, Rossbach B, Göen T, et al. Saliva as a matrix for human biomonitoring in occupational and environmental medicine. Int Arch Occup Environ Health 2015;88:1–44.

[16] Rodríguez PN, Martínez Reinoso J, Gamba CA, et al. Association among dental caries and stimulated salivary IgA with tobacco smoking. Aust Dent J 2013; 58:219–23.

[17] Kaur M, Shah S. A study of analytical indicators of saliva. Ann Essences Dent 2012;4:9–18.

[18] Reddy V, Raghuramu N, Bhaskaram C. Secretory IgA in protein-calorie malnutrition. Arch Dis Child 1976;51:871–4.

[19] Golpusand Hagh L, Zakavi F, Aminfar S, et al. Association of dental caries and salivary IgA with tobacco smoking. Aust Dent J 2013; 58:219–23.

[20] Michalka B, Rossbach B, Göen T, et al. Saliva as a matrix for human biomonitoring in occupational and environmental medicine. Int Arch Occup Environ Health 2015;88:1–44.

[21] Ruiz S, Mira JG, Kantor Junier O. IgA deficiency. J Pediatr (Rio J) 1998;74:433–40.

[22] Heremans JP, Mestecky J, Lawton AR. The IgA system in connection with local and systemic immunity. The Immunoglobulin a System. Boston, MA:Springer; 1974.

[23] Silva RP, Natali AJ, Oliveira PS, et al. Salivary immunoglobulin A (s-IgA) and exercise: relevance of its control in athletes and methodological implications. Rev Bras Med Esporte 2009;15:459–66.

[24] Psoter WJ, Spielman AL, Gebran B, et al. Effect of childhood malnutrition on salivary flow and pH. Arch Oral Biol 2008;53:231–7.

[25] Simões A, Campos L, Souza DN, et al. Laser phototherapy as topical prophylaxis against radiation-induced xerostomia. Photomed Laser Surg 2010;28:357–63.

[26] Vidovic Juras D, Lukac J, Cekić-Arambasin A, et al. Effects of low-level laser treatment on mouth dryness. Coll Antropol 2010;34:1039–43.

[27] Lončar B, Stipetić MM, Baričević M, et al. The effect of low-level laser therapy on salivary glands in patients with xerostomia. Photomed Laser Surg 2011;29:171–5.

[28] Kucerová H, Dostálová T, Himmlova L, et al. Low-level laser therapy after molar extraction. J Clin Laser Med Surg 2000;18:309–15.

[29] WHO (WORLD HEALTH ORGANIZATION). Child Growth Standards; 2006. Available at: <http://www.who.int/growthstandards/Technical_report.pdf>. Accessed May 15, 2017.

[30] Navazesh M, Christensen CM. A comparison of whole mouth resting and stimulated salivary measurement procedures. J Dent Res 1982;61:1158–62.

[31] Lagerléf F, Tenovuo J. Saliva. In: Thystrup A, Fejerskov O. (eds.). Cariesologia Clinica. 2nd ed. Copenhagen: Munksgaard; 1994. 17–43.

[32] García LB, Bulla JR, Kotaka CR, et al. Bacteriologial and salivary tests for evaluation of caries risk. Rev bras anal Clin 2009;41:69–76.

[33] Solé D, Zahu MM, Leser PG, et al. Levels of IgA in saliva of normal and atopic individuals, determined by secretory anti-IgA antibodies and serum antigen. Rev Bras Alerg Immunol 1987;10:120–5.

[34] Simões A, Nicolau J, de Souza DN, et al. Effect of defocussed infrared diode laser on salivary flow rate and some salivary parameters of rats. Clin Oral Investig 2008;12:23–30.

[35] Terlević Dabić D, Jurisić S, Vučićević Boras V, et al. The effectiveness of low-level laser therapy in patients with drug-induced hyposalivation: a pilot study. Photomed Laser Surg 2016;34:389–93.