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JULIANA XIMENES DAMASCENO

ESTUDO LONGITUDINAL DO PERFIL SALIVAR PROTEICO DE CRIANÇAS NOS PRIMEIROS MESES DE VIDA.

FORTALEZA
2015

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Tese submetida à Coordenação do Curso de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito parcial para obtenção do grau de Doutora em Odontologia.

Área da concentração: Clínica Odontológica.

Orientadora: Profa. Dra. Cristiane Sá Roriz Fonteles.

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Aprovada em: ___/___/___

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Dedico este trabalho em primeiro lugar a
Deus, à minha mãe Saskia, e ao meu pai, Jeovani.

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“Sobre o amor muitos sabem falar, mas poucos sabem sentir.”

(Alice de Castro Damasceno)

RESUMO

Estudos sobre o desenvolvimento da composição salivar no primeiro ano de vida podem promover informações a cerca da maturação imunológica em crianças. A saliva é o fluido humano mais disponível e de fácil acesso, o que faz dela uma das ferramentas mais pesquisadas no campo de diagnóstico e biomarcadores. Nesse contexto, essa tese, constituída de 1 artigo objetivou caracterizar proteínas salivares nos primeiros meses de vida, utilizando eletroforese unidimensional. Nesse estudo, saliva total estimulada e foram obtidas de 79 bebês de ambos os gêneros no primeiro e terceiro meses de vida. Os sobrenadantes foram analisados, o fluxo salivar foi calculado (mL/min) para cada criança e a concentração de proteínas totais foi determinada pelo Método do Ácido Bicinconínico (BCA). Proteínas foram caracterizadas de acordo com o peso molecular através de eletroforese unidimensional. Os resultados demonstraram fluxos salivares significativamente diferentes entre o primeiro e terceiro meses de vida ($p = 0,000$), sendo o fluxo salivar no terceiro mês maior (Mediana = 0,10; Min-Max = 0,02-0,20 mL/ min) do que no primeiro mês (Mediana = 0,02; Min-Max = 0,02-0,06 mL/min).O peso em kg mostrou diferença significante ($p = 0,000$) entre o primeiro mês (Mediana: 3,32; Min-Max: 2,02-4,03) e o terceiro (Mediana: 6,26; Min-Max: 4,49-9,30). O presente trabalho também demonstrou diferença estatisticamente significante entre as concentrações de proteínas totais do primeiro e terceiro meses de vida ($p < 0,001$), onde a concentração de proteínas totais foi maior na saliva total do primeiro mês (Mediana = 1,29; Min-Max = 0,43-3,93 μg/mL) que no terceiro (Mediana: 1,293; Min-Max: 0,427-3,931 μg/mL). Bandas com 150 ($p=0,004$), 100 ($p=0,000$), 42 ($p=0,001$), 30 ($p=0,039$), 25 ($p=0,001$), 20 ($p=0,009$), 15 ($p=0,011$) e 13 kDa ($p=0,002$) apresentaram maior intensidade na saliva total durante o primeiro mês. A banda com 218 kDa foi observada exclusivamente durante o terceiro mês de vida. O Apgar no primeiro minuto se correlacionou positivamente com o número total de bandas expressas no primeiro ($p = 0,019$) e terceiro mês ($p = 0,014$). Em conclusão, a concentração de proteínas totais salivares reduz entre o primeiro e o terceiro meses de vida, e a expressão de proteínas salivares muda em função da idade, onde a expressão de determinadas bandas proteicas é específica a determinados períodos de desenvolvimento durante os primeiros 3 meses de vida.

Palavras chave: Criança. Saliva. Proteínas. Eletroforese.

ABSTRACT

Studies on the development of salivary composition in the first year of life can promote the information about the immune maturation in children. Saliva is the most easily available and accessible body fluid, which makes it one of the most sought after tools in diagnostic and biomarks. In this context, this thesis, constituted by 01 article aimed to characterize salivary proteins in the first months of life using unidimensional electrophoresis. On this study, stimulated whole saliva was obtained from 79 babies, both genders at first and third months of life. Supernatants were analyzed, salivary flow rate (mL/min) was calculated for each child and total protein concentration determined by the Bicinchoninic Acid Protein (BCA) method. Proteins were characterized according to their molecular weights within the unidimensional electrophoresis. The results showed salivary flow rates significantly different ($p = 0.000$) between the first and third months of life, being the flow rate of whole saliva at third month (Median: 0.10, min-max: 0.02-0.2 mL/ min) higher than at first month (Median: 0.02, min-max: 0.02-0.06mL/min). The weight in Kg showed significant differences ($p = 0.000$) between the first month (Median: 3.32, Min-Max: 2.02-4.03) and third months (Median: 6.26, Min-Max: 4.49-9.30).This study also showed difference concerning to total protein concentration between saliva of the first and third months ($p = 0.000$), being higher concentrations found in first (Median: 1.293, min-max: 0.427-3.931 μg/mL) than in third (Median: 0.720, min-max: 0.164-2.244 μg/mL). Bands with 150 ($p=0.004$), 100 ($p=0.000$), 42 ($p=0.001$), 30 ($p=0.039$), 25 ($p=0.001$), 20 ($p=0.009$), 15 ($p=0.011$) and 13 kDa ($p=0.002$) presented higher intensity in whole saliva of the first month. A band with 218 kDa was expressed exclusively during the third month of life. Apgar at 1 min was positively correlated with the total number of bands expressed in the first ($p = 0.019$) and third months ($p = 0.014$). In conclusion, the concentration of total salivary proteins decreases between the first and third months, and the expression of the salivary proteins changes depending on the age, where the expression of specific protein bands is specific to certain developmental stages during the first 3 months.

Key words: Child. Proteins. Saliva. Electrophoresis.

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

BCA	Ácido bicinconínico
FFOE	Faculdade de Farmácia, Odontologia e Enfermagem
COMEPE	Comitê de ética em pesquisa
SDS PAGE	Poliacrilamida Duodecil Sulfato de Sódio
STH	Saliva Total Humana
ATC	Ácido Tricloroacético
UECE	Universidade Estadual do Ceará
UFC	Universidade Federal do Ceará
VMN	Valor Máximo de Normalidade

SUMÁRIO

1 INTRODUÇÃO GERAL

Atualmente, há a compreensão de que quase tudo que se pode medir no sangue é mensurável em saliva (WONG, 2006). Nos últimos dez anos, o uso da saliva como método de diagnóstico avançou rapidamente, por tratar-se de uma técnica não invasiva, relativamente fácil de ser realizada, apresentando vantagens tanto para o profissional da saúde quanto para o paciente (STRECKFUS; BIGLER, 2002). A exploração dos componentes salivares é de extrema relevância, podendo levar ao desenvolvimento de técnicas de amplificação ou redução na expressão de suas moléculas, utilização desses componentes de forma terapêutica, avaliações de risco ou monitoramento no caso de doenças sistêmicas (STRECKFUS; BIGLER, 2002). Estudos prévios descreveram a presença de um grande número de dados analíticos diagnósticos em saliva, incluindo hormônios esteroides (FORDE *et al*., 2006), anticorpos (VYSE *et al.*, 1999; OLIVEIRA *et al.*, 2000), vírus (MALAMUD, 1992), neoplasias malignas (STRECKFUS *et al.*, 2000; BIGLER *et al.*,2002; FRANZMANN *et al*., 2005; MBULAITEYE *et al.*, 2006) e doenças autoimunes (STUCHELL *et al.*, 1984; TISHLER *et al.*, 1999).

A saliva total humana consiste em uma secreção exócrina complexa de fundamental importância para a manutenção da homeostase da cavidade bucal (EDGAR, 1990), originada a partir da secreção das glândulas salivares maiores (parótida, submandibular e sublingual), com limitadas contribuições das glândulas salivares menores, do fluido crevicular gengival, bem como de outras fontes (GUSMAN *et al.*, 2004). Em sua composição existe uma imensa variedade de proteínas que são exclusivas desse fluido e que apresentam funções biológicas de extrema importância para a saúde oral (GUSMAN *et al.*,2004). Alguns estudos envolvendo protêomica mostram a complexidade da saliva ao evidenciar a presença de mais de 1400 peptídeos e proteínas diferentes em sua composição, onde uma parte desses componentes pode ser agrupada em poucas famílias como proteínas ricas em prolinas, amilase, albumina, imunoglobulinas, lisozimas, lactoferrinas, lactoperoxidase, histatinas (grupo de proteínas que exerce atividade antimicrobiana), estaterinas que particpam da homeostase do cálcio, mucinas para lubificação e cisteínas como inibidoras de proteases. (YAO *et al*., 2003; HUANG *et al*., 2004). A maioria das imunoglobulinas salivares (>85%) pertencem a subclasse IgA e uma menor quantidade à subclasse IgG, que juntas representam em torno de 5-15% de todas as proteínas salivares (NIEUW AMERONGEN; VEERMAN, 2002). Essas famílias de proteínas salivares caracterizam-se pelo elevado polimorfismo genético como também pelas

várias modificações pós- translacional, pré e pós-secretórias como glicosilação, fosforilação, transglutaminação, sulfatação e clivagens proteolíticas (CABRAS *et al*., 2009)

As glândulas salivares humanas são consideradas morfologicamente completas no útero, e o seu crescimento continua através da proliferação de células diferenciadas (BEN-ARYEH *et al*, 1990). Suas secreções mostram-se importantes para a saúde oral uma vez que realizam a limpeza mecânica da cavidade bucal e apresentam funções de proteção através de uma série de mecanismos fisiológicos e bioquímicos (DEZAN *et al.*, 2002). As superfícies da mucosa oral da criança recém-nascida constitui a porta de entrada para a maioria dos microorganismos patogênicos no primeiro dia de vida (SEIDEL *et al.*, 2001), sendo protegidas por um sistema imune complexo, interdependente, que compreende fatores imunológicos específicos e inespecíficos. Quase imediatamente após o nascimento, as mucosas são colonizadas por microorganismos comensais que constituem a flora normal nestas superfícies (FITZSIMMONS, 1994). A capacidade do hospedeiro para resistir à infecção ou para modificar os padrões de colonização de microrganismos que entram na cavidade oral é, em parte, dependente da presença de um bom funcionamento do sistema imune da mucosa (SMITH *et al.*, 1989).

A imunidade humoral específica é mediada primeiramente pelas imunoglobulinas que constam predominantemente da imunoglobulina secretora IgA e quantidades menores de imunoglobulinas séricas IgG e IgM, que são transportadas através do sulco gengival (SMITH *et al.*, 1993; SEIDEL *et al.*, 2000). Embora o papel das imunoglobulinas salivares em relação à flora indígena, cárie dentária e doença periodontal ainda esteja indefinido, suas funções geralmente se encontram no campo da defesa do organismo (TENOVUO, 1998; WAN *et al.*, 2003). Na cavidade oral, a imunoglobulina IgA secretora pode limitar a aderência microbiana às superfícies epiteliais e dentárias; bem como neutralizar enzimas, toxinas e vírus. Ela também atua sinergicamente com outros fatores salivares antibacterianos como a lisozima, lactoferrina, peroxidase salivar e mucinas. A imunoglobulina sérica IgG pode aumentar a fagocitose e destruir os microorganismos orais através da ativação do sistema complemento ou da opsonização, enquanto a imunoglobulina IgM, de grande importância nos primeiros dias de resposta imune primária, pode aglutinar bactérias e fixar o complemento (SMITH *et al.*, 1993).

Durante o primeiro ano de vida, a criança sofre um rápido desenvolvimento que é acompanhado por mudanças no ambiente bucal. Pode-se encontrar alterações significativas na concentração de todos os componentes salivares ao longo da infância. Essas alterações indicam o processo de crescimento e maturação das glândulas salivares, como parte do

desenvolvimento geral dos sistemas do corpo. Na primeira infância, o desenvolvimento do sistema nervoso é dominante, posteriormente, a maturação do sistema hormonal e imunológico pode se refletir na composição salivar. Também, as mudanças na composição sérica, o crescimento da massa das glândulas salivares, as diferenças na alimentação e na atitude emocional das crianças, que mudam em função da idade, podem ser refletidas na saliva (BEN-ARYEH *et al.*, 1990). Algumas dessas mudanças também podem estar relacionadas com o surgimento do primeiro dente na cavidade oral, pois a presença de dentes facilita a mudança na ingestão de alimentos fluidos para sólidos e concomitantemente a comunidade microbiológica oral sofre mudanças uma vez que os dentes promovem novos nichos ecológicos para colonização bacteriana (RUHL *et al.*, 2005). Como as proteínas salivares desempenham importante papel na mastigação e digestão dos alimentos, na manutenção da integridade dos tecidos orais e adesão das bactérias orais, é de fundamental importância observar se as mudanças ocorridas durante o desenvolvimento da criança são acompanhadas por alterações na composição dessas proteínas.

Com o interesse crescente no uso da saliva para o diagnóstico, pela possibilidade de identificação de um grande número de moléculas salivares através de nanotecnologias e a comparação dos níveis e expressões dessas substâncias com níveis sanguíneos , a necessidade de identificação e de padronização dos valores normais dos componentes salivares torna-se mais evidente para que o papel funcional dessas importantes moléculas seja elucidado e bem avaliado. Com isso, torna-se essencial determinar o perfil dessas substâncias fisiológicas nas amostras salivares nos primeiros meses de vida. A obtenção de um perfil padronizado de proteínas representa um dos maiores obstáculos técnicos ao estudo das proteínas salivares, o que refletem as grandes variações de concentrações relatadas em poucos estudos prévios publicados sobre o desenvolvimento do sistema imune oral (WAN, 2003) e da composição protéica salivar no primeiro ano de vida (RUHL *et al*., 2005; MORZEL *et al.*, 2011). Apesar da significância desses achados, há ainda a necessidade de se conduzir estudos mais detalhados acerca das proteínas salivares, especialmente em crianças no primeiro trimestre de vida.

2 PROPOSIÇÃO

Os objetivos do presente trabalho foram:

2.1 Objetivo Geral

Avaliar o perfil de proteínas salivares em crianças no primeiro trimestre de vida a partir da análise das proteínas presentes em saliva total humana (STH).

2.2 Objetivos Específicos

1. Quantificar e comparar as concentrações de proteínas totais durante o primeiro e terceiro mês de vida;
2. Mensurar e comparar o fluxo salivar durante o primeiro e terceiro mês de vida;
3. Identificar o perfil de proteínas da saliva total humana (STH) durante o primeiro e terceiro mês de vida;
4. Relacionar a concentração de proteínas totais, o fluxo salivar e o perfil proteômico com o APGAR, a massa corpórea, o gênero e o padrão alimentar da criança.

3 CAPÍTULOS

Esta tese está baseada no Artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisa envolvendo seres humanos, ou parte deles, o projeto de pesquisa foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovado sob o protocolo COMEPE nº 507.838, conforme o ofício eletrônico de 08 de janeiro de 2014 (Anexo A). Assim sendo, esta tese é composta de um capítulo, contendo artigo redigido de acordo com a revista científica escolhida, conforme descrito abaixo:

"Longitudinal evaluation of salivary protein profile in babies during the first trimester of life."

Autores: Juliana X. Damasceno, Thyciana R. Ribeiro, Bianca P. Toscano, Francisco César M.C. Filho, Johnata K. Marinho, Cláudia F. Santos, Cristiane S. R. Fonteles.

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3.1 Capítulo 1

LONGITUDINAL EVALUATION OF SALIVARY PROTEIN PROFILE IN BABIES DURING THE FIRST TRIMESTER OF LIFE

Abstract

Objective(s): The aim of this longitudinal study was to describe the changes in the protein profile in whole saliva of babies in the first trimester of infancy using unidimensional electrophoresis.

Study Design: Seventy-nine healthy infants, both genders, participated in this study. After medical history interview and oral examination, stimulated whole saliva was collected from each child, during two different moments (1st and 3rd months of life). Salivary collections were performed for 05 min, and after centrifugation at 4ºC supernatants were stored at -80ºC for later protein analysis. Total protein concentration was determined by the bicinchoninic acid protein (BCA) method. Proteins were characterized according to their molecular weights within the unidimensional electrophoresis.

Results: Total protein concentrations differed in the saliva of the first and third months (p = 0.000), being higher concentrations found in the first (Median: 1.29, min-max: 0.43-3.93 μg/mL) than in third month (Median: 0.72, min-max: 0.16-2.24 μg/mL). Flow rates differed between the first (Median: 0.02, min-max: 0.02-0.06 mL/min) and third months (Median: 0.10, min-max: 0.02-0.2 mL/ min) (p = 0.000). Unidimensional electrophoresis showed 23 bands in whole saliva. A band with 218 kDa was present exclusively in saliva during the third month. Bands with 150 (p=0.004), 100 (p=0.000), 42 (p=0.001), 30 (p=0.039), 25 (p=0.001), 20 (p=0.009), 15 (p=0.011) and 13 kDa (p=0.002) presented higher intensity in whole saliva of the first month.

Conclusion(s): Salivary protein profile expressed differences during the first month compared to the third month, including differences in total protein concentration, flow rate and band intensity.

Key words: infants, proteins, saliva, electrophoresis

Introduction

Whole saliva represents a complex mixture of different contributions originating from the major and minor salivary glands, crevicular fluid, serum, epithelial cells, bacteria and food debris. Besides water and salt, other components including proteins, peptides, hormones, lipids and sugar constitute whole saliva[1]. Among the predominant proteins and peptides are amylase, mucins, proline-rich proteins, histatins and sthaterin[1]. This large array of peptides and proteins covers different molecular weight ranges, futher increasing in complexity by the presence of posttranslational modifications and interactions with high molecular glycoproteins[1].

The saliva protein composition during the human infant development, particularly in relation to the first trimester is rather poorly documented. The first months of an infant's life are marked by major changes affecting the oral cavity, particularly the initial mucosae colonization by commensal microorganisms and the introduction of important dietary changes[2]. Intake of foods introduces a wider variety of antigens to the oral cavity with possible consequences on salivary components of the adaptative immune system. Common sense would dictate that such events might affect the expression of specific proteins.

Proteins can be detected by a number of methodologies. Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate, has proven highly useful for resolving and characterizing a mixture of protein components in both uni and bidimensional systems[3,4]. In order to futher document the saliva protein composition in infancy, we followed longitudinally a group of infants during the first trimester of life and monitored their saliva electrophoretic SDS-PAGE (Polyacrylamide Duodecil Sodium Sulfate) profiles at 1 and 3 months of age.

Materials and Methods

Subjects

Seventy-nine (43 girls and 36 boys) healthy individuals, both genders, were recruited at birth from the Maternity School Assis Chateaubriand (Brazil). Were excluded from the study pre-mature babies, babies borned from smoking mothers, elitist and / or drug users, perinatal complications, presence of systemic, genetic or congenital diseases identified during the interview, babies or mothers who were making chronic drug use and babies whose parents or legal guardians refused to sign the informed consent form. They were examined at the Federal University of Ceara in the first and third months by one examiner. Intraoral examination of the children was performed using a dental mirror under standard dental lighting. Birth details and neonatal medical history were verified from hospital records. Information regarding demography, medical health, feeding and diet, and oral hygiene habits were obtained by personal interview with the mother, and were reconfirmed at each recall visit. The study was approved by the Ethics Committee of the Federal University of Ceará Medical School, Brazil (protocol #507.838). Consent forms were signed by parents or legal guardians prior to patient enrollment in the study.

Saliva collection

Dental examination was performed prior to saliva collection to evaluate the general condition of the oral cavity. Stimulated whole saliva was collected from each infant using a standard sterile plastic pipette placed passively on the floor of the mouth. This method of collection was acceptable to most infants and crying was rarely induced. Salivary collection was performed for 5 min, so that the initial flow rate could be calculated. A protease inhibitor cocktail (Sigma, P2714) was added to samples soon after the collection. Then, after centrifugation at 4^{o}C, 12.000g, for 10 min (Eppendorf Centrifuge 5804 R, Germany), supernatants were separated, and stored at -80^{o}C for posterior analysis. Saliva samples were

collected 1 h after feeding between 8 and 11:30 am. The oral hygiene was made 1 h before the collection.

Determination of total protein concentration

The samples were homogenized with a mechanical agitator (Vortex AP-56, Phoenix, São Paulo, Brazil). Total protein concentration was determined by the Bicinchoninic Acid Protein (BCA) method using a BCA assay kit (BCA-1, Thermo Scientific, Pierce). All samples were analyzed in duplicates. The BCA working reagent was prepared by mixing 50 parts of Reagent A with 1 part of Reagent B until the color of the solution became light green. Four 96 well plate assays were prepared with a blank, BSA (bovine seric albumin) protein standards and saliva samples; and incubated at 37,5^{o}C. An absorbance at 562nm was recorded and the protein concentration was determined by comparison to a standard curve.

SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)

After the determination of total protein concentration, the saliva samples were treated with ketone. All reconstituted samples (whole saliva) were briefly vortexed and 50µg aliquots of protein were loaded on 12.5% polyacrylamide gels (12 x 14 cm). Proteins were separated according to their molecular weight within the electrophoresis apparatus (IPGphor and Hoefer SE600; Amersham Pharmacia Biotech, Cambridge, UK). At the end of the running session, the gel was stained with Coomassie blue for overnight and distained with distaining solution containing 10% acetic acid, 25% methanol and 65 % H2O.

Image Acquisition and Analysis

The gels were digitalized (Chemic Doc XRSF; Bio-Rad, Hercules; CA, USA), and the images were processed with ImageLab software (Bio- Rad, USA). Molecular weights of the proteins were estimated based on comparisons to prestained broad-range protein standards (Bio-Rad).

Statistical analysis was performed using Shapiro-Wilk test, Wilcoxon Signed Ranks test, Spearman Correlation and Chi-Square tests. Data were expressed in median with percentiles 25 and 75. Results were considered statistically significant when $p < 0.05$. Statistical analysis was performed with SPSS 20.0 program.

Results

The study population consisted of 79 infants (43 girls and 36 boys). Teeth eruption was no reported in this population. Regarding the feeding, they were still on a milk diet at the third months. Data were available for all the infants at first (53 breastfed, 1 formula fed and 24 receiving breast milk and formula) and third (41 breastfed, 17 formula fed and 21 receiving breast milk and formula) months of life.

There was a significant positive correlation between the 1st and 2nd measurements of total protein obtained in the first (p=0.000) and third (p=0.000) months of life, demonstrating reliability of the saliva collection and analysis performed. A statistically significant positive correlation was observed between the Apgar scores at 1 and 5 minutes (Spearman's correlation, p = 0.000). Weights on 1st and 3rd months also correlated positively (p = 0.000) (Table 1).

Total protein concentration in saliva differed between the first and third months (p = 0.000), with higher protein levels in the first month of life (Median: 1.29; Min-Max: 0.42-3.93 µg/mL) when compared with the third month (Median: 0.72, Min-Max: 0.16-2.24 µg/mL). The flow rates differed significantly (p = 0.000) between the first (Median: 0.02, Min-Max: 0.02-0.06mL/min) and third months (Median: 0.10, Min-Max: 0.02-0.2 mL/ min). The weight in Kg showed significant differences (p = 0.000) between the first month (Median: 3.32, Min-Max: 2.02-4.03) and third months (Median: 6.26, Min-Max: 4.49-9.30) (Table 2).

Protein profile unidimensional electrophoresis showed the presence of 23 bands (10, 13, 15, 17, 20, 25, 27, 30, 35, 37, 42, 45, 50, 55, 60, 69, 88, 100,135,150, 189, 218 and 250 kDa) in whole saliva. The total number of expressed bands differed significantly between the first and third months of life (p=0.001). The band with 218 kDa was present exclusively during the third month. All the other bands were present during the first and third months of life. The protein profile expressed through electrophoresis showed diferences in the intensity

of protein bands (Fig. 1). Bands with 150 (p=0.004), 100 (p=0.000), 42 (p=0.001), 30 (p=0.039), 25 (p=0.001), 20 (p=0.009), 15 (p=0.011) and 13 kDa (p=0.002) presented higher intensity in the whole saliva of babies during the first month only.

In the first month of life, the total number of expressed bands positively correlated with the 1st and 2nd measurements of total protein and mean total protein concentrations (p = 0.030; p = 0.010 and p = 0.019); however, this correlation was not observed during the third month. Apgar at 1 min was positively correlated with the total number of bands expressed in the first (p = 0.019) and third months (p = 0.014). There was a statistically significant positive correlation between the total number of expressed bands on the 1st and 3rd months of life (p = 0.000) (Table 2).

The bands of 25 kDa (girls: n=36; boys: n=21, p=032) and 17 kDa (girls: n=22; boys: n=8, p=014) demonstrated a statistically significant association with gender during the first month of life. In addition, the expression of bands of 42 kDa (bottle: n=5; breast: n=28; bottle and breast: n=13, p=000) during the third month and 10 kDa during the first month (bottle: n=1; breast: n=2; bottle and breast: n=2, p=000) significantly associated with the type of feeding.

In the 1st month of life, presence of the 10 kDa-band significant associated with the presence of the following bands: 27 kDa (p = 0.011), 30 kDa (p = 0.011), 37 kDa (p = 0.000), 189 kDa (p = 0.002). During the third month, the 17 kDa-band significantly associated with the band of 69 kDa (p = 0.05). The 42 kDa-band showed an associoation with bands of 17 kDa (p = 0.018), 20 kDa (p = 0.040), 25 kDa (p = 0.000), 27 kDa (p = 0.007), 50 kDa (p = 0.029), 55 kDa (p = 0.014) 88 kDa (p = 0.044) and 189 kDa (p = 0.021) during the third month of life.

Discussion

The interest in saliva has increased in the last few years due to its potential to diagnose viral, bacterial and systemic diseases [5]. This fluid is a promising option for diagnosing certain disorders and monitoring the evolution of specific pathologies or the dosage of medicines or drugs; and the advantages of saliva as a diagnostic tool include the non-invasive aspect of the sampling technique and the positive correlation between many parameters in serum and saliva[6]. Hence, the present study was performed to study longitudinally salivary protein profile in the first and third months of life in a large sample of healthy babies. To our knowledge, only a few previous studies have focused on salivary research during the first trimester of infancy. Little is known about changes in the composition of proteins during the first year of an infant´s life. Since this is a period of intrinsic devepmental adaptation, considerable growth and substantial changes in feeding habits, with a concomitant shift in the bacterial oral ecology, we believe this is a matter of considerable interest that should be further investigated.

During infancy, salivary composition undergoes changes that reflect the development and maturation of the salivary glands [7]. The ability of saliva to exert protection against infectious microorganisms is directly linked to the amount and quality of the daily production of this fluid. Thus, measurement of the salivary flow rate of patients is one of the parameters used for risk assessment of installation and development of oral infectious diseases. According to Serratine and Silva [8] there is a shortage of studies on this subject, especially with children, due to the difficulty in obtaining cooperation. According to Ben-Aryeh et al.[9], in infants under the age of 3 days, saliva could not be drawn into a serynge; in infants under 1 month, salivary secretion was low and the saliva highly viscous. In addition, saliva tends to flow at a much freer rate in infants over 1 month of age. Working with children during the first year of life, Collares et al.[10] found that their salivary flow rates were higher between 90

and 180 days after birth, a period preceding the eruption of the primary dentition. In the present study, standardizing the conditions for saliva collection by using the same suction device for all participants, lead to a flow rate at the third month (Median: 0.10, Min-Max: 0.02-0.2 mL/ min) significantly higher (p= 0.000) than the observed rates (Median: 0.02, Min-Max: 0.02-0.06mL/min) in the first month of life. Many studies have shown gender differences in salivary flow rates, although not always to the levels of statistical significance [11,12,13,14]. In agreement with our findings, Dezan et al. [15], using non stimulated saliva, with children at 18, 30 and 42 months observed no gender differences in the initial flow rates.

In addition to the salivary flow rate, the analysis of total salivary proteins is also an important parameter for salivary analysis. In the present study the BCA method was used, which is considered an easy, sensitive and rapid method to measure the total concentration of salivary proteins (Zaia et al. 1998). Total protein concentrations in whole saliva showed an increase when comparing the results from the third (Median: 0.72, Min-Max: 0.16-2.24 μg/mL) and first (Median: 1.29, Min-Max: 0.43-3.93 μg/mL) months. Other studies have described similar results when investigating salivary total protein levels. Bellavia et al. [16] reported a protein concentration of 1.9 mg/ml for 3- to 15-day-old infants; Ben-Aryeh et al. [9] found concentrations varying from 3.60 mg/ml for children aged 2–3 months to 1.19 mg/ml for children aged 7–12 months; Hyyppä et al. [17] observed concentrations varying from 0.71 to 0.78 mg/ml in children aged 2– 19 months. It must be stated that none of these studies carried out a longitudinal design; hence, the comparisons were done between different children at different age groups. However, the present study consisted of a 3-months follow-up of 30-day old babies, where an increase in salivary protein concentration was a reality to these children over time.

The present results showed that while the overall quantity of salivary proteins increased during the first tree months of life, unidimensional electrophoresis allowed the

detection of differences in the expression of protein bands between the first and third months, with the respective expression of 22 and 23 bands in the first and third months. The 218kDa band was present exclusively in saliva during the third month. In addition, protein profile presented through electrophoresis differed in the expression of protein bands' intensity, in which bands with 150 kDa, 100 kDa, 42 kDa, 30 kDa, 25 kDa, 20 kDa, 15 kDa and 13 kDa presented higher intensity in whole saliva of the first month. As the intensity of staining and "thickness" of protein bands are indicative of their relative abundance, [18] these proteins were more abundant in the first month. The lower concentration of these proteins in the third months could be associated with the increase of the flow rate. Morzel et al [2], studying 73 infants longitudinally at 3 and 6 months of age, found that out of 21 bands, 13 significantly differed between the 3rd and 6th months.

The expression of 25 (p=0.32) and 17 kDa bands (p=0.14) was associated with gender, during the first month of life. Specific bands associated with feeding during the first (10 kDa p=0.00) and third (42 kDa p=0.00) months. Based on previus reports on saliva composition[2,18], it is very likely that complement c1q precursor and cystatin S are the most abundant proteins in bands 25 kDa and 17 kDa, respectively. On the other hand, leukocyte elastase inhibitor and cystatin A are the most abundant proteins in bands 42 kDa and 10 kDa, respectively. This probably occurred due to the fact that feeding, for example, introduces a variety of antigens to the oral cavity with possible regulation of antibacterial salivary proteins and they may be developmentally up or down regulated during the first months of life.

The salivary proteome consists of components specifically secreted by major and minor salivary glands and components originating from other sources, such as the gingival crevicular fluid, desquamated ephitelial cells, plasma exudation and host oral flora. Cabras et al.[19] evidenced this complexity, disclosing the presence in human saliva of more than 1400 different peptides and proteins. The major components distinctive of human saliva, presently

secreted by the salivary glands can be grouped into a few families: histatins, statherins, proline-rich peptides, S-type cystatins, acidic proline rich phosphoproteins, basic salivary proline rich proteins, basic glycosylated proline rich proteins, amylase and mucins. These salivary protein families are characterized by elevated genetic polymorphism as well as several pre and post- translational modifications, such as glycosylation, phosphorylation, transgutamination, sulfation and proteolytic cleavages. Hence, future studies should focus in identifying these proteins, and evaluating the salivary concentration of these molecules.

The results of the present study are particulary important to achieve the goal of using saliva as a diagnostic and prognostic fluid. Indeed, quantitative and qualitative modifications in salivary secretions can be used to assess developmental variabilities throughout time. In the present study healthy infants demonstrated important variations in protein expression with age, gender and type of feeding, contributing to our understanding of the physiological variability occurring in human saliva. In brief, infants in the first month of life showed a remarkably higher protein levels and the amount of expressed bands differed significantly between the first and third months of life in babies.

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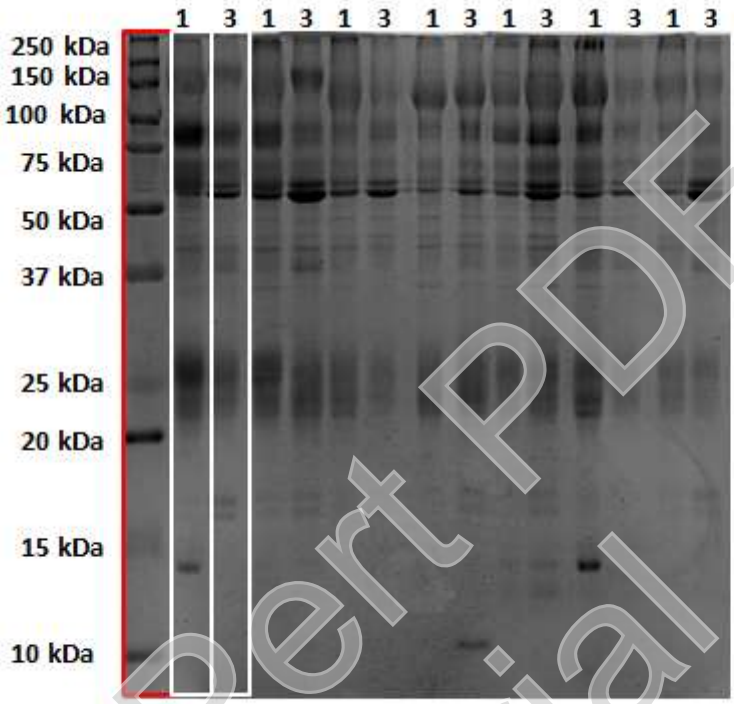
Figure:

Figure 1: Unidimensional electrophoresis gels representing protein bands found in fisrt and third months.



Tables:

Table 1**:** Correlation between Apgar scores at 1 and 5 minutes, child's weight in Kg, total protein concentrations and total number of bands, using Spearman correlation test ($p \leq 0.05$).

		Apgar value at 1 min	Apgar value at 5 mins	Weight in Kg at 1mon	TotalProtein Concentration (mg/mL) 1st duplicate (1st mon)	TotalProtein Concentration (mg/mL) 2nd duplicate (1st mon)	TotalProtein Concentration (mg/mL) Mean (1st mon)	TotalProtein Concentration (mg/mL) 1st duplicate (3rd mon)	TotalProtein Concentration (mg/mL) 2nd duplicate (3rd mon)	TotalProtein Concentration (mg/mL) Mean(3rd mon)	Total Number of Bands (1st month)	Total Number of Bands (3rd month)
Apgar value at 1 min	Correlation Coefficient	1.000	.547**	-.009	.156	.132**	.146	-.050	.002**	-.039	-.263	-.277**
	p- value	.	.000	.934	.171	.246	.198	.661	.983	.734	.019	.014
	N	79	79	79	79	79	79	79	79	79	79	79
Apgar value at 5 min	Correlation Coefficient	.547**	1.000	.068	-.056**	-.018	-.041	-.076**	.014	-.060	.017**	-.046
	p- value	.000	.	.550	.626	.873	.721	.503	.901	.598	.880	.686
	N	79	79	79	79	79	79	79	79	79	79	79
Weight in Kg at 3mon	Correlation Coefficient	-.065	.134	.406**	.017	.071	.052**	.045	.048	.049**	.055	.116
	p- value	.567	.238	.000	.881	.532	.646	.696	.674	.667	.628	.307
	N	79	79	79	79	79	79	79	79	79	79	79
TotalProtein Concentration (mg/mL) 1st duplicate (1st mon)	Correlation Coefficient	.156	-.056	.096	1.000	.924	.979	.154	.212	.200	-.244	-.181
	p- value	.171	.626	.401	.	.000	.000	.175	.061	.077	.030	.111
	N	79	79	79	79	79	79	79	79	79	79	79
TotalProtein Concentration (mg/mL) 2nd duplicate (1st mon)	Correlation Coefficient	.132	-.018	.090	.924	1.000	.979	.150	.202	.193	-.287	-.214
	p- value	.246	.873	.432	.000	.	.000	.188	.075	.089	.010	.059
	N	79	79	79	79	79	79	79	79	79	79	79
TotalProtein Concentration (mg/mL) Mean (1st mon)	Correlation Coefficient	.146	-.041	.097	.979	.979	1.000	.162	.214	.205	-.263	-.199
	p- value	.198	.721	.395	.000	.000	.	.153	.058	.069	.019	.079
	N	79	79	79	79	79	79	79	79	79	79	79

TotalProtein Concentration (mg/mL) 1st duplicate (3rd mon)	Correlation Coefficient	-.050	-.076	.132	.154	.150	.162	1.000	.849	.957	-.113	-.058
	p- value	.661	.503	.248	.175	.188	.153	.	.000	.000	.323	.614
	N	79	79	79	79	79	79	79	79	79	79	79
TotalProtein Concentration (mg/mL) 2nd duplicate (3rd mon)	Correlation Coefficient	.002	.014	.117	.212	.202	.214	.849	1.000	.955	-.009	.061
	p- value	.983	.901	.303	.061	.075	.058	.000	.	.000	.939	.595
	N	79	79	79	79	79	79	79	79	79	79	79
TotalProtein Concentration (mg/mL) Mean(3rd mon)	Correlation Coefficient	-.039	-.060	.111	.200	.193	.205	.957	.955	1.000	-.082	.010
	p- value	.734	.598	.330	.077	.089	.069	.000	.000	.	.472	.929
	N	79	79	79	79	79	79	79	79	79	79	79
Total Number of Bands (1st month)	Correlation Coefficient	-.263*	.017	.143	-.244*	-.287	-.263	-.113*	-.009	-.082	1.000*	.576
	p- value	.019	.880	.207	.030	.010	.019	.323	.939	.472	.	.000
	N	79	79	79	79	79	79	79	79	79	79	79

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 2**:** Analysis of comparation between measurements in weight in Kg, total protein concentration, densiometry of bands, salivary flow rates and total of bands ($p \leq 0.05$).

	TotalProtein Concentration (mg/mL) 2nd duplicate (1st mon) versus TotalProtein Concentration (mg/mL) (1st duplicate (1st mon)	TotalProtein Concentration (mg/mL) mean (3rd mon) versus TotalProtein Concentration (mg/mL) mean (1st mon)	Densitometry of Band150kDa (3rd mon) versus Densitometry of Band150kDa (1st mon)	Densitometry of Band100kDa (3rd mon) versus Densitometry of Band100kDa (1st mon)	Densitometry of Band42kDa (3rdmon) versus Densitometry of Band42kDa (1st mon)	Densitometry of Band 27kDa (3rd mon) versus Densitometry of Band 30kDa (1st mon)
Z	-3.414[b]	-5.627[c]	-2.896[b]	-3.637[b]	-3.471[b]	-2.059[b]
p-value	.001	.000	.004	.000	.001	.039
	Densitometry of Band 25kDa (3rd mon) versus Densitometry of Band 25kDa (1st mon)	Densitometry of Band 20kDa (3rd mon) versus Densitometry of Band 20kDa (1st mon)	Densitometry of Band 15kDa (3rd mon) versus Densitometry of Band 15kDa (1st mon)	Densitometry of Band 13kDa (3rd mon) versus Densitometry of Band 13kDa (1st mon)	Total Bands (3rd mon) versus Total Bands (1st mon)	Weight in Kg at 3mon versus Weight in Kg at 1mon
Z	-3.177[c]	-2.600[b]	-2.535[b]	-3.137[b]	-3.321[c]	-7.722[b]
p-value	.001	.009	.011	.002	.001	.000
	Salivary Flow Rate(mL/min)(3rd mon) - Salivary Flow Rate(mL/min) (1st mon)					
Z	-7.836[b]					
p-value	.000					

4 CONSIDERAÇÕES FINAIS

A partir dos resultados obtidos pôde-se concluir que:

1. Analisando os pesos e os fluxos salivares, houve diferença entre os grupos estudados, sendo o peso e o fluxo salivar das crianças no terceiro mês maior quando comparado com o fluxo salivar das crianças no primeiro mês. No entanto, a concentração de proteínas totais foi maior na saliva total das crianças no primeiro mês de vida.

2 . O perfil eletroforético da saliva dos bebês demonstrou 22 bandas em saliva total das crianças no primeiro mês de vida e 23 bandas na saliva total das crianças durante o terceiro mês, sendo a banda com 218 kDa de expressão exclusiva da saliva total do terceiro mês. As bandas com 150 kDa, 100 kDa, 42 kDa, 30 kDa, 25 kDa, 20 kDa, 15 kDa e 13 kDa apresentaram maior intensidade durante o primeiro mês de vida.

3. Houve uma correlação entre o Apgar no primeiro minuto e o número total de bandas expressas no primeiro e no terceiro mês de vida.

4. Houve uma associação entre os gêneros e a expressão das bandas com 25 kDa e 17 kDa no primeiro mês de vida.

5. O tipo de alimentação (leite materno) associou-se com a expressão das bandas 10 kDa no primeiro mês e 42 kDa aos 3 meses de vida.

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APÊNDICE A - TERMO DE CONSENTIMENTO INFORMADO

"Estudo longitudinal do perfil salivar protéico e de imunoglobulinas de crianças no primeiro ano de vida."

Seu filho ou filha está sendo convidado a participar de um projeto de pesquisa. Sua participação é importante, porém, ele (a) não deve participar contra a sua vontade. Leia com atenção as informações abaixo, sentindo-se livre para fazer qualquer pergunta que desejar, para que não haja dúvida alguma sobre os procedimentos a serem realizados.

Ao assinar este termo que consta de seu nome, nome de seu filho ou filha, idade, e número do prontuário, você estará declarando que por meio de livre e espontânea vontade sua e de seu filho ou filha, ele (a) estará participando como voluntário do projeto de pesquisa citado acima, de responsabilidade da Cirurgiã-Dentista Juliana Ximenes Damasceno da Faculdade de Odontologia, da Universidade Federal do Ceará. O abaixo-assinado estará ciente que:

a) O objetivo da pesquisa é verificar quais modificações ocorrem na saliva de uma criança durante o primeiro ano de vida.
b) Durante o estudo você deverá fornecer informação sobre o estado geral de saúde do seu filho ou filha.
c) A participação neste estudo consistirá de um exame clínico de seu filho ou filha para verificar a gengiva, os dentes presentes na boca e o tipo de cárie que ele (a) possa ter, e da coleta de saliva.
d) Nem a coleta de saliva, nem o exame odontológico ocasionarão DOR ao seu filho ou filha.
e) Uma amostra de saliva será colhida conforme segue:
    - Na amostra, haverá coleta da saliva já presente na boca do seu filho ou filha com o uso de uma pequena cânula (semelhante a um pequeno pedaço de borracha), enquanto se encontra em repouso no seu colo.
    - Para que seja feita a coleta é preciso que seu filho ou filha, esteja em jejum por no mínimo 1 hora, e que tenha higienizado a cavidade bucal ou escovado os dentes uma hora antes da consulta.
f) Seu filho ou filha NÃO RECEBERÁ INJEÇÃO de anestésico local.
g) Essa saliva após recolhida será analisada para que se possa verificar as defesas e as proteínas presentes.
h) Você tem a liberdade de desistir ou interromper a participação do seu filho ou filha neste estudo no momento que desejar, sem necessidade de qualquer explicação. Isso não vai lhe trazer qualquer penalidade ou prejuízo.
i) Os resultados obtidos durante este estudo serão mantidos em sigilo. Não haverá identificação por ocasião da exposição e/ou publicação dos mesmos.
j) As informações conseguidas através de sua participação não permitirão a identificação da sua pessoa, exceto à responsável pela pesquisa. A divulgação das mencionadas informações só será feita entre os profissionais estudiosos no assunto.
k) É condição indispensável para participação no estudo que seu filho ou filha não tenha nenhuma doença crônica e, portanto, não esteja no momento sob tratamento médico ou fazendo uso crônico de drogas ou medicações.

l) O surgimento de resfriados ou viroses, com conseqüente uso de medicações por período de tempo limitado, não exclui seu filho ou filha do estudo.

Endereço da responsável pela pesquisa:
Nome: Juliana Ximenes Damasceno
Instituição: Universidade Federal do Ceará / Curso de Odontologia
Endereço: Rua Monsenhor Furtado, s/n, Rodolfo Teófilo
Telefones para contato: 3366 8408 / 8854 0254

ATENÇÃO: Para informar qualquer questionamento durante a sua participação no estudo, dirija-se ao:
Comitê de Ética em Pesquisa da Universidade Federal do Ceará
Rua Coronel Nunes de Melo, 1127 Rodolfo Teófilo
Telefone 3366 8338.

O abaixo assinado, _____, _____anos, RG nº_____ declara que é de livre e espontânea vontade que está participando como voluntário da pesquisa. Eu declaro que li cuidadosamente este Termo de Consentimento Livre e Esclarecido e que, após sua leitura livre tive oportunidade de fazer perguntas sobre o conteúdo do mesmo, como também sobre a pesquisa e recebi explicações que responderam por completo minhas dúvidas. E declaro ainda estar recebendo uma cópia assinada deste termo.

Fortaleza___/___/_____

Nome do voluntário	Data	Assinatura
Nome do pesquisador	Data	Assinatura
Nome da testemunha (se o voluntário não souber ler)	Data	Assinatura
Nome do profissional que aplicou o T.C.L.E.	Data	Assinatura

APÊNDICE B - FICHA DE ANAMNESE

DADOS PESSOAIS

NOME _____

IDADE _____ _____DATA DE NASCIMENTO _____

NOME DO PAI _____

NOME DA MÃE _____

RESPONSÁVEL LEGAL _____

ENDEREÇO _____

TELEFONE PARA CONTATO _____

ESTADO DE SAÚDE GERAL DA MÃE

FAVOR LER E RESPONDER COM ATENÇÃO.

1) Você se encontra sob tratamento médico? SIM NÃO

Para que? Caso a sua resposta tenha sido SIM._____

2) Você tem alguma doença crônica? SIM NÃO

Qual? Caso a sua resposta tenha sido SIM. _____

3) Você está tomando algum remédio? SIM NÃO

Quais? Caso a sua resposta tenha sido SIM. _____

4) Você tem algum tipo de alergia? SIM NÃO

A que? Caso a sua resposta tenha sido SIM. _____

5) Você esteve recentemente hospitalizado? SIM NÃO

Para que? Caso a sua resposta tenha sido SIM. _____

6) Você fuma, consume bebidas alcoólicas ou é usuário de drogas? SIM NÃO

Frequência/Tempo? Caso a sua resposta tenha sido SIM. _____

ESTADO DE SAÚDE GERAL DA CRIANÇA

FAVOR LER E RESPONDER COM ATENÇÃO.

1) O seu filho ou filha se encontra sob tratamento médico? SIM NÃO
Para que? Caso a sua resposta tenha sido SIM._____

2) O seu filho ou filha tem alguma doença crônica? SIM NÃO
Qual? Caso a sua resposta tenha sido SIM. _____

3) O seu filho ou filha está tomando algum remédio? SIM NÃO
Quais? Caso a sua resposta tenha sido SIM. _____

4) O seu filho ou filha tem algum tipo de alergia? SIM NÃO
A que? Caso a sua resposta tenha sido SIM. _____

5) O seu filho ou filha esteve recentemente hospitalizado? SIM NÃO
Para que? Caso a sua resposta tenha sido SIM. _____

INFORMAÇÕES GERAIS

1. Idade da mãe _____
2. Peso da mãe_____
3. Número de partos_____
4. Idade gestacional_____
5. Índice de Apgar _____
6. Peso _____
7. Comprimento _____
8. Circunferência da cabeça _____
9. Tipo de parto _____
10. Tipo de alimentação _____
11. Hábitos de higiene oral _____

COMENTÁRIOS: _____

Afirmo que as informações acima são verdadeiras.

Data:_____

Assinatura:_____

APÊNDICE C - FICHA DE EXAME DA SAÚDE BUCAL

NOME DA CRIANÇA _____

IDADE _____DATA DE NASCIMENTO _____

DATA _____

EXAME EXTRA-ORAL

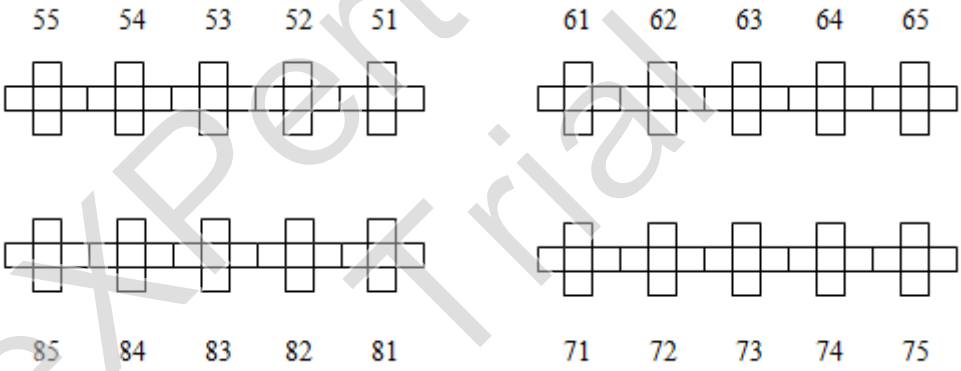
LINFADENOPATIA: PRESENTE ☐ AUSENTE ☐

ASSIMETRIA FACIAL POR INFECÇÃO: PRESENTE ☐ AUSENTE ☐

EXAME INTRA-ORAL

TECIDOS MOLES: NORMAIS ☐ PATOLÓGICOS ☐

EXAME DENTÁRIO



- Cor vermelha – corresponde a superfícies cariadas
- Cor azul – corresponde a superfícies restauradas
- X – corresponde a superfícies ausentes devido à cárie

COMENTÁRIOS:

_____

ANEXO A - Aprovação do Comitê de Ética

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ESTUDO LONGITUDINAL DO PERFIL SALIVAR PROTEICO E DE IMUNOGLOBULINAS DE CRIANÇAS NO PRIMEIRO ANO DE VIDA.

Pesquisador: Juliana Ximenes Damasceno
Área Temática:
Versão: 2
CAAE: 22512013.5.0000.5054
Instituição Proponente: Departamento de Clínica Odontológica
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 507.838
Data da Relatoria: 08/01/2014

Apresentação do Projeto:
Trabalho de mestrado da aluna Juliana Ximenes Damasceno orientada pela Profa. Dra. Cristiane Sá Roriz Fonteles baseado no estudo do perfil salivar proteico e de imunoglobulinas (IgA, IgE, IgG e IgM) de crianças no primeiro ano de vida. A amostra será de conveniência composta por 100 crianças nascidas no período de Dezembro/2013 na Maternidade Escola Assis Chateubriand (MEAC). Após anamnese e coleta de dados sobre características orais, duas amostras de saliva total serão coletadas de todos os participantes, sendo mensurados inicialmente o pH, fluxo salivar e a capacidade tampão. A concentração total de proteínas será determinada pelo método de Bradford e a análise proteômica salivar será realizada por meio de eletroforese bidimensional. ELISA será utilizado para quantificação dos níveis de imunoglobulinas nas amostras de saliva. Após obtenção dos resultados, a análise estatística será realizada por meio dos testes t de student e ANOVA para dados que se encaixarem em uma curva de normalidade e através dos testes de Mann-Whitney e Kruskal-Wallis para dados que não obedecerem a uma distribuição normal.

Objetivo da Pesquisa:
Avaliar os níveis de imunoglobulinas presentes na saliva de crianças durante o primeiro ano de vida e o perfil de proteínas salivares dessas crianças a partir da análise de proteínas presentes em saliva total humana (STH).

Endereço: Rua Cel. Nunes de Melo, 1127
Bairro: Rodolfo Teófilo **CEP:** 60.430-270
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Avaliação dos Riscos e Benefícios:

A pesquisa representa risco mínimo visto que a coleta de saliva será feita por meio de uma cânula de borracha, não havendo nenhum procedimento invasivo. O estudo pode trazer informações importantes sobre a composição da saliva no primeira ano de vida, podendo orientar ações de promoção de saúde bucal da criança, diminuindo assim a incidência de cárie dental.

Comentários e Considerações sobre a Pesquisa:

Atualmente, há a compreensão de que quase tudo que se pode medir no sangue é mensurável em saliva. Esta técnica é não invasiva, relativamente fácil de ser realizada e apresenta vantagens tanto para o profissional da saúde quanto para o paciente. Neste contexto, esta pesquisa é relevante visto o crescente interesse no uso de saliva para o diagnóstico, sendo necessária a identificação dos seus componentes e de padronização dos valores normais numa dada população.

Considerações sobre os Termos de apresentação obrigatória:

A pesquisadora apresentou ao CEP: folha de rosto devidamente preenchida e assinada pela chefe do departamento de Clínica Odontológica, TCLE, autorizações dos laboratórios e da maternidade escola, anuência da pesquisadora principal, cronograma e orçamento incluídos no corpo do projeto.

Recomendações:

Não se aplica.

Conclusões ou Pendências e Lista de Inadequações:

A pesquisadora incluiu: currículo lattes, anuência da orientadora e carta de encaminhamento a este comitê.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

ANEXO B - Lista de proteínas cuja massa aparente teve destaque no estudo

CÓDIGO DE ACESSO	PESO MOLECULAR	PROTEÍNA
1433S_HUMAN	27.8 kDa	P31947 14-3-3 protein sigma (Stratifin) (Epithelial cell marker protein 1).
1433T_HUMAN	27.8 kDa	P27348 14-3-3 protein theta (14-3-3 protein tau) (14-3-3 protein T-cell) (HS1 protein).
1433Z_HUMAN	27.7 kDa	P63104 14-3-3 protein zeta/delta (Protein kinase C inhibitor protein-1) (KCIP-1).
6PGL_HUMAN	27.5 kDa	O95336 6-phosphogluconolactonase (EC 3.1.1.31) (6PGL).
ACTA_HUMAN	42.0 kDa	"P62736 Actin, aortic smooth muscle (Alpha-actin 2)."
ACTC_HUMAN	42.0 kDa	"P68032 Actin, alpha cardiac (Alpha-cardiac actin)."
ACTS_HUMAN	42.1 kDa	"P68133 Actin, alpha skeletal muscle (Alpha-actin 1)."
ACTY_HUMAN	42.3 kDa	P42025 Beta-centractin (Actin-related protein 1B) (ARP1B).
ACTZ_HUMAN	42.6 kDa	P61163 Alpha-centractin (Centractin) (Centrosome-associated actin homolog) (Actin-RPV) (ARP1).
AFAM_HUMAN	69.1 kDa	P43652 Afamin precursor (Alpha-albumin) (Alpha-Alb).
AGR2_HUMAN	20.0 kDa	O95994 Anterior gradient protein 2 homolog precursor (Secreted cement gland protein XAG-2 homolog) (AG-2 protein) (hAG-2) (HPC8).
AINX_HUMAN	55.4 kDa	Q16352 Alpha-internexin (Alpha-Inx) (66 kDa neurofilament protein) (Neurofilament-66) (NF-66).
AL3A1_HUMAN	50.4 kDa	"P30838 Aldehyde dehydrogenase, dimeric NADP-preferring (EC 1.2.1.5) (ALDH class 3) (ALDHIII)."
ALBU_HUMAN	69.4 kDa	P02768 Serum albumin precursor.
APOA1_HUMAN	30.8 kDa	P02647 Apolipoprotein A-I precursor (Apo-AI) (ApoA-I) [Contains: Apolipoprotein A-I(1-242)].
APOC3_HUMAN	10.9 kDa	P02656 Apolipoprotein C-III precursor (Apo-CIII) (ApoC-III).
ARF1_HUMAN	20.6 kDa	P84077 ADP-ribosylation factor 1.
ARF3_HUMAN	20.5 kDa	P61204 ADP-ribosylation factor 3.
ARF4_HUMAN	20.4 kDa	P18085 ADP-ribosylation factor 4.
ARF5_HUMAN	20.4 kDa	P84085 ADP-ribosylation factor 5.
ARF6_HUMAN	20.0 kDa	P62330 ADP-ribosylation factor 6.
ARPC3_HUMAN	20.4 kDa	O15145 Actin-related protein 2/3 complex subunit 3 (ARP2/3 complex 21 kDa subunit)

		(p21-ARC).
ASPG_HUMAN	37.2 kDa	P20933 N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase precursor (EC 3.5.1.26) (Glycosylasparaginase) (Aspartylglucosaminidase) (N4-(N- acetyl-beta-glucosaminyl)-L-asparagine amidase) (AGA) [Contains: Glycosylasparaginase alpha chain; Glycosylasparaginase
ATPK_HUMAN	10.8 kDa	"P56134 ATP synthase f chain, mitochondrial (EC 3.6.3.14)."
B2MG_HUMAN	13.7 kDa	P61769 Beta-2-microglobulin precursor.
BAP31_HUMAN	27.9 kDa	P51572 B-cell receptor-associated protein 31 (BCR-associated protein Bap31) (p28 Bap31) (CDM protein) (6C6-AG tumor-associated antigen) (DXS1357E).
C1QC_HUMAN	25.8 kDa	"P02747 Complement C1q subcomponent, C chain precursor."
C1QT3_HUMAN	27.0 kDa	Q9BXJ4 Complement C1q tumor necrosis factor-related protein 3 precursor (Secretory protein CORS26).
CALL5_HUMAN	15.9 kDa	Q9NZT1 Calmodulin-like protein 5 (Calmodulin-like skin protein).
CALU_HUMAN	37.1 kDa	O43852 Calumenin precursor (Crocalbin) (IEF SSP 9302).
CATB_HUMAN	37.8 kDa	P07858 Cathepsin B precursor (EC 3.4.22.1) (Cathepsin B1) (APP secretase) (APPS).
CD81_HUMAN	25.8 kDa	P60033 CD81 antigen (26 kDa cell surface protein TAPA-1) (Target of the antiproliferative antibody 1) (Tetraspanin-28) (Tspan-28).
CD9_HUMAN	25.3 kDa	P21926 CD9 antigen (p24) (Leukocyte antigen MIC3) (Motility-related protein) (MRP-1) (Tetraspanin-29) (Tspan-29).
CENB2_HUMAN	88.0 kDa	Q15057 Centaurin beta 2 (Cnt-b2).
CH10_HUMAN	10.8 kDa	"P61604 10 kDa heat shock protein, mitochondrial (Hsp10) (10 kDa chaperonin) (CPN10) (Early-pregnancy factor) (EPF)."
CLCB_HUMAN	25.2 kDa	P09497 Clathrin light chain B (Lcb).
COMT_HUMAN	30.0 kDa	P21964 Catechol O-methyltransferase (EC 2.1.1.6).
COX2_HUMAN	25.6 kDa	P00403 Cytochrome c oxidase subunit 2 (EC 1.9.3.1) (Cytochrome c oxidase polypeptide II).
COX5B_HUMAN	13.7 kDa	"P10606 Cytochrome c oxidase polypeptide Vb, mitochondrial precursor (EC 1.9.3.1)."
COX6B_HUMAN	10.1 kDa	P14854 Cytochrome c oxidase polypeptide VIb (EC 1.9.3.1) (Cytochrome c oxidase subunit AED).
CP2S1_HUMAN	55.8 kDa	Q96SQ9 Cytochrome P450 2S1 (EC 1.14.14.1) (CYPIIS1).
CRIS2_HUMAN	27.3 kDa	P16562 Cysteine-rich secretory protein-2 precursor (CRISP-2) (Testis-specific protein TPX-1).
CRIS3_HUMAN	27.6 kDa	P54108 Cysteine-rich secretory protein-3 precursor (CRISP-3) (SGP28 protein).
CRYAB_HUMAN	20.2 kDa	P02511 Alpha crystallin B chain (Alpha(B)-crystallin) (Rosenthal fiber component) (Heat-shock protein beta-5) (HspB5).
CSRP1_HUMAN	20.4 kDa	P21291 Cysteine and glycine-rich protein 1 (Cysteine-rich protein 1) (CRP1) (CRP).

CT178_HUMAN	25.0 kDa	Q9H444 Protein C20orf178.
CTN1_HUMAN	100.1 kDa	P35221 Alpha-1 catenin (Cadherin-associated protein) (Alpha E-catenin).
CUL3_HUMAN	88.9 kDa	Q13618 Cullin-3 (CUL-3).
CYTC_HUMAN	15.8 kDa	P01034 Cystatin C precursor (Neuroendocrine basic polypeptide) (Gamma-trace) (Post-gamma-globulin).
DCXR_HUMAN	25.9 kDa	Q7Z4W1 L-xylulose reductase (EC 1.1.1.10) (XR) (Dicarbonyl/L-xylulose reductase) (Kidney dicarbonyl reductase) (kiDCR) (Carbonyl reductase II) (Sperm surface protein P34H).
DEF1_HUMAN	10.2 kDa	"P59665 Neutrophil defensin 1 precursor (HNP-1) (HP-1) (HP1) (Defensin, alpha 1) [Contains: HP 1-56; Neutrophil defensin 2 (HNP-2) (HP-2) (HP2)]."
DEF3_HUMAN	10.2 kDa	"P59666 Neutrophil defensin 3 precursor (HNP-3) (HP-3) (HP3) (Defensin, alpha 3) [Contains: HP 3-56; Neutrophil defensin 2 (HNP-2) (HP-2) (HP2)]."
DHC3_HUMAN	30.7 kDa	O75828 Carbonyl reductase [NADPH] 3 (EC 1.1.1.184) (NADPH-dependent carbonyl reductase 3).
DHCA_HUMAN	30.2 kDa	P16152 Carbonyl reductase [NADPH] 1 (EC 1.1.1.184) (NADPH-dependent carbonyl reductase 1) (Prostaglandin-E(2) 9-reductase) (EC 1.1.1.189) (P
DLC2A_HUMAN	10.8 kDa	"Q9NP97 Dynein light chain 2A, cytoplasmic (Dynein-associated protein Km23) (Bithoraxoid-like protein) (BLP)."
DLC2B_HUMAN	10.9 kDa	"Q8TF09 Dynein light chain 2B, cytoplasmic."
DNJB1_HUMAN	37.9 kDa	P25685 DnaJ homolog subfamily B member 1 (Heat shock 40 kDa protein 1) (Heat shock protein 40) (HSP40) (DnaJ protein homolog 1) (HDJ-1).
DPP4_HUMAN	88.3 kDa	P27487 Dipeptidyl peptidase 4 (EC 3.4.14.5) (Dipeptidyl peptidase IV) (DPP IV) (T-cell activation antigen CD26) (TP103) (Adenosine deaminase complexing protein-2) (ADABP) [Contains: Dipeptidyl peptidase 4 membrane form (Dipeptidyl peptidase IV membrane fo
HBB_HUMAN	15.9 kDa	P68871 Hemoglobin beta chain.
HBD_HUMAN	15.9 kDa	P02042 Hemoglobin delta chain.
SH3L3_HUMAN	10.4 kDa	Q9H299 SH3 domain-binding glutamic acid-rich-like protein 3 (SH3 domain- binding protein SH3BP-1) (P1725).
SMD1_HUMAN	13.3 kDa	P62314 Small nuclear ribonucleoprotein Sm D1 (snRNP core protein D1) (Sm-D1) (Sm-D autoantigen).
SMT3B_HUMAN	10.9 kDa	P61956 Ubiquitin-like protein SMT3B precursor (Sentrin-2) (Ubiquitin-related protein SUMO-3) (HSMT3).
SODC_HUMAN	15.8 kDa	P00441 Superoxide dismutase [Cu-Zn] (EC 1.15.1.1).
SPB6_HUMAN	42.6 kDa	P35237 Placental thrombin inhibitor (Cytoplasmic antiproteinase) (CAP) (Protease inhibitor 6) (PI-6) (Serpin B6).
SPLC2_HUMAN	27.0 kDa	"Q96DR5 Short palate, lung and nasal epithelium carcinoma associated protein 2 precursor (Parotid secretory protein) (PSP)."
SQRD_HUMAN	50.0 kDa	"Q9Y6N5 Sulfide:quinone oxidoreductase, mitochondrial precursor (EC 1.-.-.-)."
SSB_HUMAN	17.3 kDa	"Q04837 Single-stranded DNA-binding protein, mitochondrial precursor (Mt-SSB) (MtSSB) (PWP1-interacting protein 17)."
STAT3_HUMAN	88.1 kDa	P40763 Signal transducer and activator of transcription 3 (Acute-phase response factor).

SUCB1_HUMAN	50.3 kDa	"Q9P2R7 Succinyl-CoA ligase [ADP-forming] beta-chain, mitochondrial precursor (EC 6.2.1.5) (Succinyl-CoA synthetase, betaA chain) (SCS-betaA) (ATP- specific succinyl-CoA synthetase beta subunit)."
TALDO_HUMAN	37.5 kDa	P37837 Transaldolase (EC 2.2.1.2).
TBA2_HUMAN	50.0 kDa	Q13748 Tubulin alpha-2 chain (Alpha-tubulin 2).
TBA3_HUMAN	50.1 kDa	Q71U36 Tubulin alpha-3 chain (Alpha-tubulin 3) (Tubulin B-alpha-1).
TBA8_HUMAN	50.1 kDa	Q9NY65 Tubulin alpha-8 chain (Alpha-tubulin 8).
TBAK_HUMAN	50.2 kDa	P68363 Tubulin alpha-ubiquitous chain (Alpha-tubulin ubiquitous) (Tubulin K- alpha-1).
TBB1_HUMAN	50.3 kDa	Q9H4B7 Tubulin beta-1 chain.
TBB3_HUMAN	50.4 kDa	Q13509 Tubulin beta-3 chain (Tubulin beta-III) (Tubulin beta-4).
TMED4_HUMAN	25.9 kDa	Q7Z7H5 Transmembrane emp24 domain containing protein 4 precursor.
TMED9_HUMAN	25.1 kDa	Q9BVK6 Transmembrane emp24 domain containing protein 9 precursor (Glycoprotein 25L2).
TMP21_HUMAN	25.0 kDa	P49755 Transmembrane protein Tmp21 precursor (21 kDa transmembrane trafficking protein) (p24delta) (S31III125) (S31I125) (Tmp-21-I).
TOLIP_HUMAN	30.3 kDa	Q9H0E2 Toll-interacting protein.
TPPC3_HUMAN	20.3 kDa	O43617 Trafficking protein particle complex subunit 3 (BET3 homolog).
TTHY_HUMAN	15.9 kDa	P02766 Transthyretin precursor (Prealbumin) (TBPA) (TTR) (ATTR).
TWF1_HUMAN	42.2 kDa	Q12792 Twinfilin-1 (A6 protein) (Protein tyrosine kinase 9).
TYPH_HUMAN	50.0 kDa	P19971 Thymidine phosphorylase precursor (EC 2.4.2.4) (TdRPase) (TP) (Platelet-derived endothelial cell growth factor) (PD-ECGF) (Gliostatin).
UBE2N_HUMAN	17.1 kDa	P61088 Ubiquitin-conjugating enzyme E2 N (EC 6.3.2.19) (Ubiquitin-protein ligase N) (Ubiquitin carrier protein N) (Ubc13) (Bendless-like ubiquitin conjugating enzyme).
UCRH_HUMAN	10.7 kDa	"P07919 Ubiquinol-cytochrome c reductase complex 11 kDa protein, mitochondrial precursor (EC 1.10.2.2) (Mitochondrial hinge protein) (Cytochrome C1, nonheme 11 kDa protein) (Complex III subunit VIII)."
UGDH_HUMAN	55.0 kDa	O60701 UDP-glucose 6-dehydrogenase (EC 1.1.1.22) (UDP-Glc dehydrogenase) (UDP-GlcDH) (UDPGDH).
UGRP2_HUMAN	10.1 kDa	Q96QR1 Uteroglobin-related protein 2 precursor (Cytokine HIN-1) (High in normal-1) (Secretoglobin family 3A member 1) (Pneumo secretory protein 2) (PnSP-2).
VAPA_HUMAN	27.3 kDa	Q9P0L0 Vesicle-associated membrane protein-associated protein A (VAMP- associated protein A) (VAMP-A) (VAP-A) (33 kDa Vamp-associated protein) (VAP-33).
VATH_HUMAN	55.9 kDa	Q9UI12 Vacuolar ATP synthase subunit H (EC 3.6.3.14) (V-ATPase H subunit) (Vacuolar proton pump H subunit) (V-ATPase 50/57 kDa subunits) (Vacuolar proton pump subunit SFD) (VMA13) (Nef binding protein 1) (NBP1).
WFDC2_HUMA	13.0 kDa	Q14508 WAP four-disulfide core domain protein 2 precursor (Major epididymis- specific

N		protein E4) (Epididymal secretory protein E4) (Putative protease inhibitor WAP5).
VPS29_HUMAN	20.5 kDa	Q9UBQ0 Vacuolar protein sorting 29 (Vesicle protein sorting 29) (hVPS29) (PEP11).
VDAC1_HUMAN	30.6 kDa	P21796 Voltage-dependent anion-selective channel protein 1 (VDAC-1) (hVDAC1) (Outer mitochondrial membrane protein porin 1) (Plasmalemmal porin) (Porin 31HL) (Porin 31HM).

Fonte: FANG, X. et al. Comparison of electrokinetics-based multidimensional separations coupled with electrospray ionization-tandem mass spectrometry for characterization of human salivary proteins. , v. 79, n. 15, p. 5785-5792, Aug 2007.

ANEXO C - Instruções para autores do "The Journal of Pediatrics"

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Virginia Law Foundation. The medical and legal implications of AIDS. Charlottesville (VA): The Foundation; 1987.

For chapters in books
Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. The metabolic and molecular bases of inherited diseases. New York: McGraw-Hill; 2001. p. 3421-52.

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Article Types

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Full-length manuscripts for the Original Articles section of The Journal of Pediatrics must include a structured abstract of less than 250 words, to appear after the title page, with the following headings: Objective(s), Study design, Results, and Conclusion(s). The Objective(s) should put the study in context with the current literature (i.e., what is new, not textbook background information) and reflect the purpose of the study, that is, the hypothesis that is being tested or the question being asked (e.g., "To assessâ€¦," "To evaluateâ€¦"). The Study design should include the study methodology, the setting for the study, the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The Results section should include the outcome of the study and statistical significance, if appropriate. The Conclusion(s) states the significance of the results and limitations of the study.Original research articles should be approximately 18 double-spaced, numbered pages, including the title page, references, figures, and tables. Failure to comply with length restrictions may result in a delay in the processing of your paper. The following length targets are recommended for Original Articles:

Structured Abstract: less than 250 words
Introduction: 1 page
Methods: 2-3 pages
Results: 2-3 pages
Discussion: 3-5 pages
Combined total of 4 Tables and Figures

Clinical and Laboratory Observations

Clinical and Laboratory Observations (CLOs) are either: (1) "case reports" that provide novel insight into pathophysiology, diagnosis, or treatment of an entity that does not represent a coincidental association; (2) small series of diagnostic or therapeutic interventions; or (3) brief, focused studies related to a topic of interest to pediatricians. Please note that CLOs are not designed to present information that is generally available in textbooks, even if the reported entity is novel. CLOs are designed to provide readers with new information and stimulate new approaches to diagnosis, clinical management, or research. CLOs should be approximately 9 double-spaced, numbered manuscript pages, including the title page, references, figures, and tables; the text should be less than 1000 words with a brief, unstructured abstract of less than 50 words. A combined total of 2 Tables and Figures is recommended.

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