FERNANDA YUKIE KOBAYASHI

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FACULDADE DE ODONTOLOGIA DE PIRACICABA

FERNANDA YUKIE KOBAYASHI

"EVALUATION OF OROFACIAL FUNCTION, TEMPOROMANDIBULAR DISORDERS, BITE FORCE AND SALIVARES LEVELS OF HYDROCORTISONE AND ALPHA-AMYLASE IN CHILDREN AND ADOLESCENTS"

Orientadora: Profa. Dra. Maria Beatriz Duarte Gavião

Co-orientadora: Profa. Dra. Paula Midori Castelo Ferrua

"AVALIAÇÃO DA FUNÇÃO OROFACIAL, DISFUNÇÃO TEMPOROMANDIBULAR, FORÇA DE MORDIDA E NÍVEIS SALIVARES DE CORTISOL E ALFA-AMILASE EM CRIANÇAS E ADOLESCENTES"

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia de Piracicaba da UNICAMP para obtenção do título de mestra em odontologia na área de odontopediatria.

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"A mente que se abre a uma nova idéia jamais voltará
ao seu tamanho original."
Albert Einstein

RESUMO

O objetivo desta dissertação foi avaliar as variáveis morfológicas e fisiológicas em crianças e adolescentes diagnosticados com desordens temporomandibulares (DTMs). Dois estudos foram conduzidos. O primeiro teve como objetivo avaliar a força de mordida (FM) e as funções orofaciais nas diferentes fases da dentição (dentição mista inicial, intermediária e final e dentição permanente) em crianças e adolescentes com diagnóstico de DTMs. Participaram 290 indivíduos de 8 a 14 anos; 47 formaram o Grupo DTMS e 243 o Grupo controle. A DTM foi avaliada por meio do *Research Diagnostic Criteria for Tempormandibular Disorders (RDC/TMD),* eixo I, e diagnosticada conforme os critérios estabelecidos. As funções orofaciais foram avaliadas com a versão brasileira do Nordic Orofacial Test – Screening (NOT-S) (entrevista e exame clínico). Para mensuração da FM utilizou-se um gnatodinamômetro digital. Computou-se a idade e o índice de massa corporal (IMC). Os dados foram analisados pela estatística descritiva, teste t de Student, correlação de Pearson ou Spearman, qui-quadrado, teste binomial ou exato de Fisher e regressão logística (α=0,05). Observou-se que a prevalência de DTM foi mais alta em meninas na dentição permanente (p=0,014) e em meninos na dentição mista intermediária (p=0,006). No grupo DTM, os escores do NOT-S entrevista (p=0,026) e NOT-S total (p=0,0063) foram maiores em relação ao controle. Não houve diferença na FM entre gêneros e grupos (p>0,05). As variáveis incluídas na regressão logística múltipla foram o IMC e o NOT-S (entrevista, exame e total). A função sensorial da entrevista foi o domínio que determinou diferença significativa na proporção de indivíduos entre grupos (p=0,021). Observou-se número significativamente maior de indivíduos com DTMs com alteração no domínio face em repouso no exame clínico do NOT-S. Concluiu-se que as fases das dentições e a FM não foram associadas à DTM. A idade correlacionou-se positivamente com a FM e IMC na dentição permanente. As disfunções orofaciais foram consideradas a variável preditiva da DTM, mas a característica transversal do estudo infere que esta associação pode ser bidirecional. O segundo estudo teve como objetivo a quantificação dos biomarcadores salivares de estresse, cortisol e alfa-amilase, em crianças e adolescentes com DTM, na faixa etária de 7 a 14 anos. Trinta e oito indivíduos compuseram o Grupo DTM e

38 o Grupo controle, pareados pela idade, gênero e presença de bruxismo. A saliva foi coletada em domicílio durante dois dias alternados, ao acordar, após 30 e 60 minutos e às 20h00. O cortisol foi quantificado pela técnica de enzimaimunoensaio e a alfa-amilase pelo método enzimático automatizado. Os dados foram analisados pelo teste de Shapiro-Wilks, estatística descritiva, teste de Mann-Whitney e coeficiente de Spearman (α=0.05). A área sob a curva das concentrações de cortisol e alfa-amilase foi calculada pelo método trapezoidal. Não houve diferenças entre o cortisol salivar e alfa-amilase entre os grupos. As correlações entre os biomarcadores não foram significativas para os grupos DTM e controle (r=0.03 e r=0.17 respectivamente). Concluiu-se que os níveis de cortisol e alfa-amilase salivar não se apresentaram alterados em crianças e adolescentes com DTM.

Palavras chave: Transtornos da articulação temporomandibular, sistema estomatognático, dentição, força de mordida, hidrocortisona, alfa-amilase, saliva.

ABSTRACT

The aim of this work was to evaluate the morphological and physiological variables in children and adolescents with temporomandibular disorders (TMDs). Two studies were carried out. The first aimed to evaluate the bite force (BF) and the orofacial functions in the different dentition phases (early, intermediate and final mixed dentition and permanent dentition) in children and adolescents with TMD diagnosis. Two-hundred ninety subjects participated; 47 composed the TMD group ad 243 the Control group. The TMD was diagnosed using Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), axis I. The orofacial functions were evaluated using the Nordic Orofacial Test – Screening (NOT-S) (interview and clinical examination). FM was measured using a digital gnathodynamometer. The age and body mass index (BMI) were considered. The data were analyzed by descriptive statistic, Student or Mann-Whitney test, Pearson or Spearman correlation, chi-square, binomial test or Fisher exact test and logistic regression (α=0.05). The prevalence of TMDs was higher for girls in permanent dentition (p=0.014) and for boys in intermediated mixed dentition (p=0.006). For TMD group the scores of NOT-S interview and the NOT-S total were higher than the Control group (p=0.026 and p=0.0063, respectively). There was not differences in BF between genders and groups (p>0.05). The variables included in the multivariate logistic regression were BMI and NOT-S (interview, exam and total). Sensory function of the interview was the domain that determined the significant difference in the proportions of subjects between groups (p=0.021). It was observed a greater number of boys and girls with alterations in face at rest domain in NOT-S exam. Concluding, the dentition phases and BF were not associated with TMD. The BF was correlated with age and BMI. The orofacial dysfunction was considered the predictor for TMD, but the cross-sectional design of the study infers that this association may be bidirectional. The second study aimed to quantify the stress biomarkers, cortisol and alpha-amylase, in children and adolescents with TMD, diagnosed using RDC/TMD, axis I. Thirty six subjects, aged from 7 to 14 years composed the TMD group and 36 the Control group, matched by gender, age and presence of bruxism. The saliva was collected at home during two alternate days, in the morning at awakening while lying in bed, 30 and 60 minutes after awakening (fasting), at night, at 8 pm. The salivary cortisol was assayed by enzyme

immunoassay and the alpha-amylase by enzymatic automated method. The data were analyzed by Shapiro-Wilks test, descriptive statistics, Mann-Whitney test and Spearman coefficient (α=0.05). The area under the curve (AUCG) of salivary cortisol and amylase concentrations against time was calculated by trapezoid method respective to the ground level. There was no difference for salivary cortisol and sAA AUCG, neither for BMI between groups. The correlations between the two biomarkers were not significant for both groups. It was concluded that the levels of salivary cortisol and alpha-amylase were not altered in children and adolescents with TMD.

Key words: Temporomandibular joint, oral function, dentition, bite force, cortisol, alpha-amylase, saliva

SUMÁRIO

INTRODUÇÃO

O sistema estomatognático consiste em um conjunto de estruturas, sendo que as principais são as articulações temporomandibulares (ATMs), dentes, ossos e músculos relacionados. A ação conjunta e harmoniosa destas estruturas é responsável por desencadear as funções orofaciais (Lund *et al.,* 1991; Zhao *et al.*, 2007) como mastigação, respiração, deglutição e fonação, estas atuando como base para a interação social, promovendo habilidades como a fala, aparência e a expressão facial (Bakke *et al*., 2007; Bergendal *et al.,* 2009Strini *et al.,* 2011). O desequilíbrio na harmonia das estruturas do sistema estomatognático pode influenciar negativamente as respectivas funções e, consequentemente, o crescimento e desenvolvimento do sistema estomatognático. Além disso, o comprometimento nas condições orais pode influenciar negativamente a qualidade de vida do indivíduo (Barbosa *et al.,* 2011; Strini *et al.,* 2011).

As desordens temporomandibulares (DTM) são sinais e sintomas clínicos que também advém deste desequilíbrio (Okeson *et al*., 1996, Thilander *et al*., 2002, Pereira *et al*., 2009;) e que dependem de tempo para se estabelecer. Estudos indicam a presença desses sinais e sintomas em crianças e adolescentes (Sonmez *et al*., 2001; Barbosa *et al*., 2008) com grande variabilidade na prevalência de acordo com a literatura. Tecco *et al.* (2011) verificaram que na faixa etária de 5 a 11 anos a prevalência foi de 22,58%, não diferindo da faixa etária de 12 a 15 anos de 28,11%. Vierlola *et al.* (2012) observaram que 35% das crianças avaliadas com idade entre 6 a 8 anos apresentaram pelo menos um sinal clínico de DTM. O estudo de Emodi-Perlman *et al.* (2012) em crianças de 5 a 12 anos reportou a prevalência de 43%, sendo 53% para os meninos e 27% para as meninas. No Brasil, Pizolato et al. (2011) verificou uma prevalência de 26,32% em uma amostra que apresentava uma faixa etária de 9,73 anos (±1,38) Os sinais e sintomas mais frequentemente encontrados em crianças e adolescentes são limitação na abertura bucal, estalo e crepitação por deslocamento de disco articular e dor muscular (Pereira *et al.,* 2007; Barbosa *et al.,* 2008; Pereira *et al.,* 2010; Tecco *et al.,* 2011). De acordo com os resultados encontrados na literatura, as DTMs em crianças e adolescentes podem ser associadas com

distúrbios musculares, mas percebeu-se pouca evidência sobre a respectiva associação com distúrbios não dolorosos articulares, como os deslocamentos de disco e patologias comuns (Barbosa *et al.,* 2008*)*. Neste sentido, a força de mordida constitui uma variável quantitativa importante na avaliação dos músculos mastigatórios, tanto em indivíduos com DTM e com outras alterações do sistema estomatognático, como maloclusões, reabilitações protéticas e antes e após cirurgias ortognáticas (Gupta *et al.,* 2012; Barrera-Mora *et al.,* 2012; Rodrigues-Garcia *et al.* 2005, Iwase *et al.* 2006). A força de mordida tem sido utilizada na avaliação dos músculos mastigatórios tanto em adultos quanto em crianças (Kobayashi *et al.,* 2012; Marquezin *et al,* 2012; Sathyanarayana *et al,* 2012).

A literatura tem apontado a relação das variáveis acima com quadros de estresse. Estudos mostram que há a associação entre os sinais e sintomas de DTM, depressão e sintomas de ansiedade, sendo considerados fatores de risco para as desordens (Kindler *et al*., 2012). Além disso, há o agravamento dos sintomas quando expostos a um agente estressor ambiental (Gallagher *et al*., 1991; Korszun *et al*., 1996. Quadros de depressão foram associados especificamente com dores articulares, enquanto que os sintomas de ansiedade com dores musculares (Kindler *et al*., 2012). Baad & Jaqtap (2012) observaram o papel do estresse em crianças e adolescente, entre 5 a 15 anos, com distúrbios de comportamento e lesões orofaciais. Indivíduos com deficiência nas condições orais relatam impacto no bem-estar social e emocional quando expostos a situações estressantes, que, fisiologicamente, leva ao aumento nos níveis de cortisol (Strini *et al*., 2011; Barbosa *et al*., 2012).

O cortisol é um hormônio ativado pelo eixo hipotálamo-hipófise-adrenal (HHA) em resposta a um agente estressor físico ou psicológico que, quando exposto repetidas vezes a uma situação de estresse, pode desencadear a secreção em excesso, levando a efeitos prejudiciais à saúde (Kupper *et al*., 2005).

Concentrações distintas de cortisol podem ser observadas durante as primeiras horas após acordar. O pico ocorre após 30-45 min pós-despertar e as concentrações decrescem até o período noturno (Clow *et al*., 2004), obedecendo assim o ciclo circadiano.

Vários métodos são indicados para a quantificação deste hormônio. O mais usual é determinação do cortisol plasmático, procedimento este invasivo, pois requer coleta de sangue que pode gerar estresse em alguns indivíduos e, consequentemente, alterar os resultados de níveis de cortisol. Assim, técnicas não invasivas têm sido desenvolvidas de forma a minimizar a interferência do estresse, como o cortisol capilar (Russell *et al*., 2011; Manenschijn *et al*., 2012), na urina (van Soelen *et al*., 2012.) e na saliva (Nagakura *et al*., 2012).

Os estudos com a saliva agregaram outra forma de quantificação dos níveis salivares de alfa-amilase (Nater *et al.*, 2007). A alfa-amilase salivar (AAS) é uma enzima produzida localmente na mucosa oral. Estudos de Chatterton *et al.* (1997) e Skosnik *et al.* (2000) apontam que os níveis de AAS são indicados pela atividade do sistema nervoso simpático e aumentam sob condições estressantes, sendo confirmado com um aumento das catecolaminas plasmáticas.

A abordagem dessas variáveis durante o crescimento e desenvolvimento do indivíduo é de extrema importância. Além da constante modificação nas estruturas relacionadas fisicamente, o indivíduo passa por diversas experiências novas. Essas situações são dadas como fatores ambientais que afetam o comportamento e podem levar ao comprometimento na qualidade de vida (Barbosa *et al.,* 2011). Além disso, observando desde o estabelecimento da harmonia das estruturas ao término do crescimento e desenvolvimento, podemos detectar a presença ou ausência dos sinais e sintomas das patologias citados anteriormente e atuar de forma preventiva.

Dessa forma, o objetivo geral da presente dissertação foi avaliar as variáveis morfológicas e fisiológicas em crianças e adolescentes diagnosticados com desordens temporomandibulares (DTMs). Os objetivos específicos foram:

- Avaliar a força de mordida e as funções orofaciais nas diferentes fases da dentição em crianças e adolescentes com diagnóstico de DTM;

- Quantificar os biomarcadores salivares de estresse, cortisol e alfa-amilase, em crianças com diagnóstico de DTM.

Esta Dissertação está baseada na Resolução CCPG UNICAMP/002/06 que regulamento o formato alternativo para Dissertações de Mestrado e Teses de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato.

Por se tratar de pesquisa envolvendo seres humanos, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tenso sido aprovado (Anexo 1).

CAPÍTULO 1

Associations between bite force, orofacial function, and temporomandibular disorders in children and adolescents

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Running title: Bite force, orofacial function, and temporomandibular disorders

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Abstract

Objective: To evaluate bite force (BF) and orofacial functions at different dentition phases (initial-mixed, intermediated-mixed, final-mixed, and permanent dentition) in children and adolescents, diagnosed with temporomandibular disorders (TMDs), selected from four public schools in Piracicaba, São Paulo, Brazil, and were 8–14 years old. **Methods**: Of the 290 participants recruited, 47 were placed into the TMDs groups. TMDs was diagnosed using Axis I of the Research Diagnostic Criteria for Temporomandibular Disorders. Orofacial functions were evaluated using the Brazilian Portuguese Version Nordic Orofacial Test-Screening (NOT-S), which involves both an interview and a clinical

examination. BF was measured using a digital gnathodynamometer. Age and body-mass index (BMI) were also considered. **Results**: For the TMD group, scores associated with NOT-S interview and NOT-S total were higher than for the control group ($p = 0.026$ and $p = 0.0063$, respectively). We detected no differences in BF between genders or groups ($p > 0.05$). Variables included in the multivariate logistic regression were BMI and NOT-S (interview, exam, and total). Based on this analysis, NOT-S interview scores was associated with TMDs. Reported sensory function was the specific domain within NOT-S interview that established the significant difference between the groups ($p = 0.021$). The TMD group also had a greater number of alterations in the face-at-rest domain of the NOT-S exam. **Conclusion**: There was no association between TMDs and either dentition phase or BF. Instead, BF correlated with age and BMI. Orofacial dysfunction, specifically the sensory function evaluated in NOT-S interview, was associated with TMD in the studied sample, but this association may be bidirectional, requiring further researches.

Key words: Bite force, dentition, stomatognathic system, temporomandibular disorders

Introduction

The main structures of the stomatognathic system include temporomandibular joints, teeth, bones, and masticatory muscles. These structures interact with one another to carry out orofacial functions, such as mastication, respiration, and swallowing (1). Orofacial functions are also critical for social interaction, as they are involved in speech and facial expressions (2-4). Some pathologies, traumas, and parafunctional habits can affect these functions, causing orofacial dysfunction (2-4), or temporomandibular disorders (TMDs) (5, 6).

TMDs consist of clinical signs and symptoms that involve imbalance between structures of the stomatognathic system (7). TMDs are generally chronic conditions that mainly affect adults, but epidemiological studies have revealed signs and symptoms in children and adolescents (5, 8). Etiologies of these disorders are generally multifactorial,

and contributing factors include oral parafunctions, trauma to the mandible or temporomandibular joints (9), and psychosocial factors (10). TMDs are also associated with myofunctional alterations (11, 12), which often lead to compensatory muscle behaviors (13). Individuals with TMD, therefore, often exhibit abnormal muscle strengths (14-16). Bite-force (BF) measurements can assess muscle efficiency and are an important tool for analyzing the functional state of the masticatory system (17). These measurements have been used to evaluate oral function in patients having a number of conditions, such as malocclusion, TMD, neuromuscular diseases, and parafunctional habits (18, 19). Moreover, it is important to characterize orofacial functions because abnormalities may be reflected in the growth and development of the stomatognathic system.

Development of both the masticatory system and oral function are related to dentition stages. Owais *et al*. (20) observed that BF increases significantly from the initial-mixed to the final-mixed dentition stage, while others (21,22) showed that dental maturation leads to gains in masticatory ability. It is important to analyze factors that affect the functional output of the stomatognathic system, especially those involved in physiological oral functions during growth and development. The aim of the present study, therefore, was to characterize the relationship between TMD, BF, and orofacial functions during different dentition stages.

Materials and methods

The study was approved by the Research Ethics Committee of the Piracicaba Dental School, University of Campinas, Brazil (nº 004/2010). All children and their parents or guardians gave verbal and written permission to participate in the research (protocol 004/2010). This was a cross-sectional study that included 295 children or adolescents of both genders. Participants were selected from four public schools in Piracicaba, São Paulo, Brazil, and were 8–14 years old. Fifty-two individuals were diagnosed with TMD in accordance with Research Diagnostic Criteria for TMD (RDC/TMD). Five children were excluded because of obesity or a discrepancy between dentition phase and their chronologic age. The final sample, therefore, was composed of 290 children, 47 within the TMD group (18 boys and 29 girls), and 243 within the control group (111 boys and 132 girls). A

parent(s) or guardian(s) provided written responses to a pre-structured questionnaire concerning demographic characteristics.

Clinical Examination

Clinical examinations were performed at the schools under well-lit conditions using a light clinical mirror with LED (Lumin RG–Septodont) and a millimeter probe (Duflex SS White, Rio de Janeiro, Brazil). The examiners used personal protective equipment. Two dentists (FYK and MCSM) were chosen for these examinations based on previously performed evaluations (i.e., calibration evaluations). For these calibration tests, 18–25 randomly selected children (who were not included in this study) were subjected to two identical examinations conducted 15 d apart.

The inclusion criteria were the presence of mixed or permanent dentition and the absence of pain of dental origin, premature tooth loss, abnormalities in shape, number, or structure that might compromise mesiodistal dimensions, occlusal-cervical tooth decay or trauma, and soft-tissue abnormalities. The exclusion criteria were uncooperative behavior, general systemic disturbances (e.g., cerebral palsy, movement disorders, speech and language disorders, and behavioral/cognitive syndromes), current use of medications (e.g., antidepressives, muscle relaxants, narcotics, or non-steroidal anti-inflammatories), facial traumas, neurological or psychiatric disorders, use of dental prostheses, previous or present orthodontic treatment, and other orofacial pain conditions that could interfere with TMD diagnoses such as, pain of dento-alveolar origin, migraine, pain referred from eyes or ears.

Body weight and height were determined using an anthropometric scale. Body mass index (BMI) was calculated from these measurements (weight/height^2).

TMD diagnosis

The calibrated examiner FYK assessed clinical signs of TMD using the dual-axis classification system, RDC/TMD (23). Axis I of RDC/TMD involves physical

findings, and Axis II involves pain-related disability and psychosocial status. Axis II evaluations were not performed in this study because they require the examiner to ask questions that are inappropriate for children (24).

The Axis I assessment includes: reported orofacial pain, the presence or absence of deviation associated with opening the mouth, temporomandibular joint sounds, and pain associated with muscle palpation. After the Axis I examination, subjects were diagnosed using the criteria presented in charter 1.

According to the RDC/TMD criteria, subjects were assigned to groups that either exhibited (I and II) or did not exhibit (group 0) signs of TMD. Arthralgia and arthritis (Group III) were not diagnosed.

Chart 1-RDC/TMD diagnostic criteria

GROUP 0	Control group: Subjects with no signs and symptoms of TMD.
GROUP I	Muscular disorders • Myofacial pain: pain in at least 3 of 20 sites on the same side of the face. • Myofacial pain with limited opening: pain in at least 3 of 20 sites on the same side of the face, and mouth opening < 40 mm.
GROUP II	Disc displacement: • With reduction: reciprocal temporomandibular joint clicking (three times during opening and closing movements, and two times during lateral mandibular excursions). • Without reduction and limited opening: maximal opening ≤ 35 mm (increased 4 mm with assistance), contralateral excursions < 7 mm and/or a deviation without correction to the ipsilateral side during opening, and the presence of joint sounds inconsistent with the criteria for disc displacement with reduction. • Without reduction, and without limited opening: maximal opening without assistance > 35 mm (increased > 5 mm with assistance), contralateral excursion ≥ 7 mm, and the presence of joint sounds inconsistent with the criteria for disc displacement with reduction.
GROUP III	Arthralgia and arthritis: Diagnosis required the subject to report pain that was not compatible with the subject's age over an extended period. For patients with arthritis, diagnosis also required images to support the diagnosis.

Evaluation of orofacial function

The calibrated examiner MCSM evaluated orofacial function using the Nordic Orofacial Test-Screening (NOT-S) protocol, which was adapted to the Portuguese (Brazilian) language and validated by Leme et al. 2011. The exam consists of 12 topics related to orofacial dysfunction; 6 assessments were collected during a structured interview, whereas the remaining 6 assessments were performed during the clinical examination. The interview assessments included: (I) sensory function, (II) breathing, (III) oral habits, (IV) chewing and swallowing, (V) drooling, and (VI) dryness of the mouth. The clinical examination was used to assess: (I) the face at rest, (II) nose breathing, (III) facial expression, (IV) masticatory muscle and jaw function, (V) oral motor function, and (VI) speech. Each assessed domain contained one to five items, reflecting the complexity of the specific function.

NOT-S interviews were carried out by asking the questions contained in the ‘‘Screening form’’. To assess orofacial dysfunction during the clinical examination, each subject was asked to perform a task related to each item. Clinical examinations were carried out in conjunction with an illustrated manual (www.mun-h-center.se). Each item had criteria for a respective function. A positive response or a task performance that qualified as impaired resulted in a score of one, thus indicating dysfunction in the scored domain. A negative response or a satisfactory task performance resulted in a score of zero. The total score was the sum of all the domain scores, and could range from 0 to 12 (2).

Dentition stage

Dentition was categorized as follows:

- Initial-mixed dentition (Initial MD): permanent incisors and first permanent molars in eruption.
- Intermediated mixed dentition (IntMD): permanent incisors and first permanent molars erupted and in occlusion.

- Final-mixed dentition (FMD): permanent incisors and first permanent molars erupted, and premolars in eruption.
- Permanent dentition (PD): all permanent teeth in occlusion, except for the third molars.

Evaluation of maximal bite force

Maximal BF was measured using a digital gnathodynamometer with fork strength of 10 mm (Digital Dynamometer Model DDK Kratos, Kratos Equipamentos Industriais Ltda., Cotia, Brazil). This digital device provides the unilateral maximal BF in Newtons (N). Subjects were seated erect in a chair with their head fixed, keeping the Frankfort plane approximately parallel to the floor. The fork was placed unilaterally over the first permanent molars. The examiner FYK instructed the children to bite on the fork as forcefully as possible for 5 sec. Measurements were performed on each side three times, with a 2-min interval. The final value was the mean of the measurements, with an accuracy of 0.01 N.

Measurement errors

The error for measurement of each of the studied variables was OR Method errors for each of the studied variables were assessed by subjecting 25 subjects (who were not included in the study) to two identical examinations conducted 15 apart. The intra-class correlation coefficient (ICC) was used to assess measurement errors associated with weight (ICC = 0.85), height (ICC = 0.91), and right bite force (ICC = 0.76). The Spearman correlation coefficient was used to assess NOT-S scores (interview $r_s = 0.65$, $p < 0.01$; exam $r_s = 0.72$, $p < 0.001$). Finally, the Kappa test was used to assess TMD diagnoses (mouth opening = 0.92, pain on palpation = 0.67).

Statistical analysis

Statistical analysis was performed using v. 15 of the statistical package for social sciences (SPSS, IMB, Chicago, USA) and BioEstat v. 5.3 (Mamirauá, Belém, PA, Brazil) with a 5% significance level. The D'Agostino-Pearson test was used to determine

whether the distribution of some variables deviated from normality (BioEstat). For these values and for categorical variables, non-parametric test were applied.

Descriptive statistics were used for all variables, and the percentages, means, standard deviations, medians, and inter-quartile ranges were considered. Comparisons between TMD and control groups were performed using the unpaired Student's t-test. Spearman and Pearson coefficients were determined between variables. To compare NOT-S results between groups, we used either the chi-square or binomial test, as indicated (BioEstat). Univariate logistic regression was used to assess the relationship between TMD and individual variables (SPSS). Analyses were adjusted for gender, dentition phase, BMI, NOT-S, and BF. Results presented are adjusted odds ratios, 95% confidence intervals, and significance levels. Factors associated with TMD using univariate analysis ($p < 0.2$) were included in a multivariable model (SPSS). The significance level was set at 5%. All statistical tests were two-sided.

Results

Table 1 shows the distribution of the TMDs group according to the RDC/TMD diagnosis. The most common diagnosis was TMDs of muscular origin. Characteristics of arthrogenious TMDs were detected at both FMD and PD stages. When all participants were analyzed together, the occurrence of TMDs was not significantly different between genders ($p = 0.258$). For the PD stage, however, TMDs was more prevalent in girls than boys ($p = 0.014$). Conversely, TMDs prevalence were highest in boys when IntMD stage was evaluated ($p = 0.006$). No significant gender differences were detected for the other two dentition phases.

Table 1–RDC/TMD diagnosis according to dentition phase

RDC/TMD	IA		IB		IIA		IIB		IIC		Total		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	*p*-value*
Initial MD	2	2	-	-	-	-	-	-	-	-	2	2	-
IntMD	5	3	3	-	-	-	-	-	-	-	8	3	0.006
FMD	-	7	2	1	-	-	-	-	1	-	3	8	0.074
PD	4	12	-	1	1	3	-	-	-	-	5	16	0.014

RDC/TMD groups: IA – myofacial pain; IB - myofacial pain with limited opening; IIA – disc displacement with reduction; IIB - disc displacement without reduction IIC - disc displacement without reduction, but without limited opening.
Dentition states: Initial MD - Initial mixed dentition; IntMD - Intermediated mixed dentition; FMD - Final mixed dentition; PD - Permanent dentition.
♂ male gender; ♀ female gender.
*Qui-square or Fisher exact tests

Concerning age and BMI, there were no significant differences between genders and groups in any of the dentition phases (Table 2). Furthermore, BF was similar between the TMD and control groups. On the other hand, comparisons between groups showed that NOT-S scores, total (p=0.026) and interview (p=0.0063) were significantly higher in the TMD group than in the control group (p = 0.026 and p = 0.0063 for interview and total, respectively). The other variables did not differ significantly between groups (Table 2).

Table 2 – Descriptive statistics for analyzed variables associated with both the TMDs and control groups

	Dentition		N	Age Mean±SD	BMI Mean±SD	Interview NOT-S Median±IQR	Exam NOT-S Median±IQR	TOTAL NOTS Median±IQR	Right BF Mean±SD	Left BF Mean±SD	FM Média D/E Mean±SD
TMDs group	Initial MD	♂	2	7 (0)	15.23 (1.14)	2.5 (1.5)	0.5 (0.5)	3 (2)	236.15 (1.39)	273.03 (24.69)	254.59 (13.04)
		♀	2	8 (0)	17.56 (3.49)	3 (1)	0.5 (0.5)	3.5 (1.5)	392.28 (52.43)	311.07(15.81)	351.67 (18.31)
	IntMD	♂	8	11.94 (1.18)	19.77 (5.39)	2 (2)	1 (1)	3 (2.25)	340.36 (98.27)	347.76 (112.63)	344.06 (97.15)
		♀	3	9.67 (0.58)	20.62 (3.07)	3 (0.5)	0 (0)	3 (0.5)	363.88 (118.11)	331.01 (77.13)	347.45 (97.28)
	FMD	♂	3	11.00 (1)	19.83 (4.34)	3 (1)	2 (1.5)	4 (2)	365.60 (80.86)	357.30 (80.12)	361.45 (79.64)
		♀	8	11.00 (0.53)	20.14 (5.13)	2.5 (2)	1 (0.5)	3 (1.25)	340.20 (73.73)	348.61 (88.39)	344.41 (78.44)
	PD	♂	5	12.00 (1)	20.21 (5.49)	2 (2)	1 (1)	3	368.01 (148.68)	354.03 (137.80)	361.02 (136.84)
		♀	16	11.94 (1.18)	19.77 (5.39)	2 (2)	1 (1)	3 (2.25)	340.36 (98.27)	347.76 (112.63)	344.06 (97.15)
Control group	Initial MD	♂	5	7.33 (0.52)	16.85 (1.81)	2 (0.75)	0 (0)	2 (0.75)	265.21 (104.05)	259.10 (69.66)	262.15 (79.29)
		♀	9	7.22 (0.83)	15.36 (1.94)	2 (2)	1 (1)	4 (2)	313.42 (125.58)	283.51 (91.39)	298.47 (107.77)
	IntMD	♂	22	8.50 (1.44)	16.81 (3.47)	1 (0.75)	1 (1)	2 (1)	350.27 (84.66)	346.52 (98.94)	348.40 (86.31)
		♀	38	7.92 (1.15)	17.57 (3.28)	2 (2)	0 (1)	2.5 (1)	297.36 (66.45)	278.59 (76.25)	287.97 (64.37)
	FMD	♂	38	10.95 (1.01)	18.51 (3.41)	2 (1)	1 (1)	2 (1)	391.22 (110.50)	383.73 (96.25)	387.47 (95.39)
		♀	35	10.54 (1.31)	18.68 (3.45)	2 (2)	0 (1)	2.5 (1)	334.46 (101.57)	344.89 (89.32)	339.67 (88.20)
	PD	♂	45	11.93 (1.03)	19.75 (4.87)	2 (2)	1 (1)	3 (2)	394.47 (137.51)	378.61 (121.82)	386.54 (121.57)
		♀	50	11.90 (1.20)	19.95 (3.96)	2 (2)	1 (1)	3 (2)	355.08 (96.24)	384.90 (92.04)	369.99 (88.07)

TMDs – temporomandibular disorders; SD – standard deviation; NOT-S – Nordic orofacial test screening; IQR – Interquartile range; BF – bite force; Dentition states:

Initial MD - Initial mixed dentition; IntMD - Intermediated mixed dentition; FMD - Final mixed dentition; PD - Permanent dentition.

♂ male gender; ♀ female gender.

Unpaired t-test or Mann- Whitney

Correlation analyses were performed between age, BMI, NOT-S scores, and BF (Table 3). Age was significantly correlated with both BMI and BF ($p<0.0001$). The other variables did not show significant coefficients, in both groups.

Table 3–Correlations between variables (only significant coefficients are shown, $p< 0.0001$)

Independent variable	Dependent variables	r
Age	BMI	0.273**
	BF right	0.299**
	BF left	0.378**
	BF mean sides	0.361**

Spearrman correlation test

Univariate logistic regression (Table 4) revealed that variables potentially associated with TMD were BMI, NOT-S interview, NOT-S examination, and NOT-S total. Since NOT-S total is the sum of NOT-S interview and NOT-S examination, it was excluded from the multivariate model. Based on the respective results, an association between NOT-S interview and TMD was identified (Table 4). This association was considered weak, since the OR was 1.40 (1.04-1.87).

Table 4–Associations between TMD and analyzed variables using univariate and multivariate logistic regressions (n total =290).

Dependent variable (TMD)	Independent variable	Coef.	*P*-value	OR	95% CI
Univariate	Gender	0.30	0.352	1.35	0.71-2.57
	Dentition phase	0.02	0.885	1.02	0.74-1.42
	Age	0.09	0.264	1.10	0.93-1.29
	BMI	**0.06**	**0.122**	**1.06**	**0.99-1.13**
	NOT-S Interview	**0.33**	**0.026**	**1.40**	**1.04-1.87**
	NOT-S Examination	**0.28**	**0.094**	**1.32**	**0.95-1.84**
	NOT-S total	**0.29**	**0.007**	**1.34**	**1.08-1.66**
	BF right	0.00	0.797	1.00	1.00-1.00
	BF left	0.00	0.973	1.00	1.00-1.00
	BF mean (right/left)	0.00	0.874	1.00	1.00-1.00
Multivariate	NOT-S Interview	0.32	0.05	1.34	1.0-1.81

Bold fonts: variables with p<0.2 (to enter in multivariate logistic regression)

Sample distributions with respect to the NOT-S domains demonstrated that the proportion of children with abnormal sensory function (both reported during the NOT-S interview and observed during the face-at-rest portion of the NOT-S exam) was significantly higher for the TMD group than for the control group (Table 5). Based on these findings we analyzed specific items within these domains. This verified that, compared with the control group, the TMD group had a higher proportion of facial asymmetries, deviant-lip positions, and gag reflexes during tooth brushing (Table 5).

Table 5–Sample distributions according to NOT-S domains

	TMD group (n=47)	Control (n=243)	*P*-value
NOT-S domains	n	n	
Interview			
(I) Sensory function	**10**	**21**	**0.021**
(II) Breathing	10	35	0.331
(III) Habits	27	142	0.972
(IV) Chewing and swallowing	39	179	0.243
(V) Drooling	0	0	-
(VI) Dry mouth	24	101	0.297
Examination			
(1) Face at rest	**12**	**25**	**0.009**
(2) Nose breathing	10	25	0.061
(3) Facial expression	9	43	0.976
(4) Masticatory muscle and jaw function	14	60	0.582
(5) Oral motor function*	3	20	0.668
(6) Speech*	1	12	0.394

Chi-square test and *binomial test for two proportions

Table 6. Sample distribution for the domains that presented different proportions between TMD and Control groups

Domain	Questions	TMD	Control	
I) Interview		(n=10)	(n=21)	*p*-value
Sensory function	A. Does brushing your teeth elicit a gag reflex?	8 (80%)	12 (57,1%)	0.018
	B. Do you put so much food in your mouth that it becomes difficult to chew?	3 (30%)	10 (47,6%)	0.492
1) Exam		(n=12)	(n=25)	
Face at rest	*A.* Asymmetry	7 (58,3%)	11 (44%)	0.007
	B. Deviant lip position	6 (50%)	11 (44%)	0.028
	C. Deviant tongue position	0	0	-
	D. Involuntary movements*	0	4 (16%)	0.615

Binomial test for two proportions and * Fisher exact test

Some children presented positive answers for more than one question

Discussion

Epidemiologic studies have shown that all age groups exhibit signs and symptoms of TMD (8, 26). Prevalence among children is increasing, so it is important that we understand associated factors. In the present study, we evaluated 290 children and diagnosed 47 (16%) with TMD according to RDC/TMD criteria. These criteria provide good-to-excellent reliability when clinically examining children and adolescents (5), but we sought to improve this diagnostic capacity. Here the most prevalent diagnosis was TMD of muscular origin. Artrogenious TMD was diagnosed for one boy in the FMD group and for five children (one boy and four girl) in the PD group. The number of children with TMD was higher in the PD group, but proportions were similar for the other dentition phases. The initial MD group was not evaluated because of the low number of children with TMD in this stage. Moreover, for the PD group TMD was diagnosed more frequently in girls than in

boys. This agrees with Pereira *et al*. (27), who showed that females are more likely to experience TMD than males.

Significant correlations between age and both BMI and BF were detected, which agrees with previous studies (28, 29) and demonstrates that when a child grows these variables increase. Indeed, the jaw-closing force increases with both age and growth, staying fairly constant between ages ~20 to 50 years, and declining thereafter (30). Palinkas *et al*. (29) obtained a similar result when measuring muscle thickness, which increases between 7 and 12 years of age. Another factor that may influence BF is dental status. Dental fillings, dentures, teeth position, and the number of teeth all affect BF (31).

Using univariate logistic regression, we identified associations between TMD and NOT-S interview and NOT-S total ($p = 0.026$ and $p = 0.007$, respectively, Table 4). We then built a model of multivariate logistic regression that included variables identified by the univariate analyses ($p < 0.2$). In addition to NOT-S interview and NOT-S total, therefore, BMI and NOT-S exam were included. The multivariate logistic regression identified NOT-S interview as the main predictive variable for TMD although the odds ratio was low (1.34). Distribution of the subjects according to NOT-S domains is shown in Table 6. The largest effect within NOT-S interview was measured for the sensory-function domain, which identified a significant difference between groups. When specific items within this domain were compared, individuals within the TMD group were more likely to experience gag reflexes in response to tooth brushing ($p = 0.018$, Table 6). The gag reflex can be an expression of emotional distress (32), and patients with this reflex are often dentally anxious (33). As TMD may have emotional and/or psychological underpinnings (34, 35), the observed relationship between TMD and the gag reflex could be a manifestation of stress related to the events in oral cavity, although this possibility warrants further research.

Although the NOT-S exam was not predictive of TMD, children within the TMD group were significantly different from controls in the face-at-rest domain ($p = 0.009$, Table 5). During the NOT-S exam, this domain is evaluated for one minute while the child

watches a picture. Asymmetry is then verified by both skeletal and soft-tissue factors. In addition, a deviant lip position is recorded when the child presents an open mouth or another deviation for more than 40 sec of this observation period. The facial asymmetries that we found are supported by Yamada *et al*. (36), who observed that patients with TMD have morphological facial disharmony. Nevertheless, we did not consider occlusion in this study because it has been demonstrated that morphological or functional occlusal factors are not significantly associated with TMD (37). Furthermore, the occurrence of a deviant lip was over-represented in the TMD group. This aspect is related to an open mouth, as mentioned above, and Pizolato *et al.* (12) found that open lips are associated with TMD. This suggests that myofunctional alterations may compromise balance within the stomatognathic system, corroborating previous studies that found associations between oromyofunctional disorders and TMD (11).

Based on high scores for both NOT-S interview and NOT-S total, the TMD group had orofacial functional impairment. It must be taken into account, however, that the duration of TMD may influence orofacial function because TMD signs and symptoms often affect orofacial function over time; i.e., individuals with TMD typically exhibit adaptations or compensations that involve muscles and stomatognathic functions (11). It was not possible for us to establish, therefore, whether orofacial dysfunction began before or after TMD onset. As such, we cannot draw definitive conclusions about causal effects. This is similar to Ohrbach *et al.* (9) concerning the use of clinical findings as risk factors for chronic TMD.

We must also take into account limitations of the present study. We performed a cross-sectional evaluation, and although the sample consisted of a large number of participants, it was not representative of the general population. As such, our findings cannot be readily extrapolated to the general population. Moreover, fluctuations of TMD signs and symptoms require longitudinal studies to establish onset. These limitations influence our ability to interpret causality from the observed associations, i.e., they may represent bidirectional relationships. Despite these concerns, it is very important to recognize predispositions for disorders of the stomatognathic system within children and

adolescents, using proper TMDs diagnosis criteria and precise instruments to evaluate functions related to stomatognathic system, as employed in the present study.

Conclusion

In the present study, dentition phases and BF were not associated with TMD, but significant correlations between BF and both age and BMI were identified. Orofacial dysfunction, specifically the sensory function evaluated in NOT-S interview, was associated with TMD in the studied sample. Because of the cross-sectional design of this study, however, this identified association may involve a bidirectional relationship, requiring further researches..

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CAPÍTULO 2

Salivary cortisol and alpha-amylase levels of children with temporomandibular disorders: a case-control study

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Abstract

This study aimed to evaluate salivary biomarkers of sympathetic nervous system and hypothalamus–pituitary–adrenal axis (alpha-amylase and cortisol, respectively) in children and adolescents with signs and symptoms of temporomandibular dysfunction (TMD). Thirty eight subjects, aged from 7 to 14 years composed the TMD group and 38 the Control group, matched by gender, age and presence of bruxism. TMD was diagnosed according to the *Research Diagnostic Criteria*, axis I. Four saliva samples were collected at home using *salivettes* in two alternate week days: in the morning while lying in bed, 30 and 60 minutes after awakening (fasting), and at night (8 pm). Salivary cortisol was assayed by enzyme immunoassay, while salivary alpha-amylase (sAA) was analyzed using enzymatic automated method. Data were evaluated using descriptive statistics, Shapiro-

Wilk, Mann-Whitney and Spearman correlation tests (α=0.05). The area under the curve (AUC_G) of salivary cortisol and sAA concentrations against time was calculated by trapezoid method respective to the ground level. The results did not show significant difference for salivary cortisol and sAA AUC_G, neither for BMI between groups. In both groups, correlations between the two biomarkers were not significant. According to the results found in this case-control study, salivary cortisol and sAA levels as markers of hypothalamus–pituitary–adrenal axis and sympathetic activity, respectively, did not differ between children and adolescents with and without TMD. Moreover, a significant correlation between both stress biomarkers was not observed.

Key words: Alpha-amylase, Hydrocortisone, Temporomandibular joint disorders, Saliva

Introduction

Temporomandibular disorders (TMD) are clinical signs and symptoms involving imbalance of anatomical structures, such as temporomandibular joints (TMJ), masticatory muscles, bones and associated tissues (Barbosa *et al.,* 2008). The most prevalent clinical signs reported in adults are TMJ sounds upon palpation, limitation of mandibular movements, TMJ and muscle tenderness. Symptoms, such as headache, TMJ sounds, bruxism, difficulty in opening the mouth, jaw pain and facial pain have been reported (Pereira *et al.,* 2009; Melo *et al.,* 2012). In children, limited mouth opening, clicking and crepitation, and TMJ and muscle pain are the signs and symptoms most frequent (Barbosa *et al.,* 2008, Tecco *et al.,* 2011).

One of the most controversial issues in clinical dentistry is the etiology of TMD, since these disorders are considered a heterogeneous group of psychophysiological disturbers, but the relative importance of individual factors is still controversial. Parafunctional habits, which are common in children as bruxism, nail biting, nonnutritive sucking and others, are considered contributory factors for TMD manifestation (Pizolato *et al.,* 2011). Also, previous studies show that symptoms of depression and anxiety symptoms are considered risk factors for TMD disorders (Kindler *et al.,* 2012), and there is a

worsening of symptoms when the patient is exposed to an environmental stressor (Gallagher *et al.,* 1991; Korszun *et al.,* 1996). Moreover, individuals with such disabilities may present impacts on social and emotional welfare, which physiologically lead to activation of stress systems (Strini *et al.,* 2011, Barbosa *et al.,* 2012).

Cortisol, the main glucocorticoid in humans, is released by the adrenal cortex in a pulsatile manner under circadian rhythm. Repeated exposure to stressful situations can trigger over activation of hypothalamus–pituitary–adrenal (HPA) axis, which may lead to detrimental effects on health (Kupper *et al.,* 2005). Circadian variability is observed in all adrenal hormones; the peak of cortisol is reached 30–45 min after awakening with a decreasing pattern thereafter, being very low at nadir (Clow *et al.,* 2004). Past studies has shown an increase in cortisol levels in womens with TMD, when compared with controls (Korszun *et al.,* 2002; Da Silva Andrade *et al.,* 2008).

Although there is no consensus regarding the percentage of TMD patients in who psychological factors play a role, it is clear that such factors need to be taken into account, along with structural indicators, to properly diagnose and plan management strategies (Barbosa *et al.,* 2008). Little is known about the relationship between stress biomarkers and TMD in children and adolescents. Thus, the main objective of this study was to compare the levels of salivary cortisol and sAA between young subjects with and without TMD. Moreover, the correlation between levels of salivary cortisol and sAA was also evaluated.

Material and Methods

Sample

This study was approved by the Research Ethics Committee of the Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil (Protocol No. 004/2010). After obtaining informed consent from all subjects' parents/guardians, 316 children and adolescents between six and 17 years were screened in public schools of Piracicaba, Brazil.

Sample size was calculated using previous findings of studies which compared salivary biomarkers between groups with and without specific diseases, taking into account a power of the test of 80% and alpha level of 0.05. According to the results, it would be necessary 19-31 subjects on each group for evaluation of salivary cortisol levels on independent samples (Koray *et al*., 2003; Barbosa *et al.,* 2012) and 17 subjects on each group for the evaluation of sAA levels (Inagaki *et al*., 2010).

After anamnesis and clinical and physical examinations, 76 young subjects aged between seven and 14 years were selected for this case-control study, being 38 subjects (24 girls and 14 boys) on each group: TMD and controls. Control group was matched for age, gender and presence of sleep bruxism, since they are known potential factors for salivary cortisol and sAA levels (Strahler *et al.,* 2010; Castelo *et al.,* 2012).

The inclusion criteria were the presence of mixed or permanent dentition. The exclusion criteria were the presence of pain from dental origin, premature tooth loss, anomalies of shape, number, structure and/or changes that might compromise the mesiodistal dimensions, occlusal-cervical tooth decay and/or trauma, and soft tissue abnormalities, uncooperative behavior, systemic disturbances in general, current use of medications (e.g., antidepressive, muscle relaxant, narcotic or non-steroidal anti-inflammatory), facial traumatism, neurological or psychiatric disorders, use of dental prostheses, previous or present orthodontic treatment and other orofacial pain conditions, which could interfere with TMD diagnoses.

Clinical and physical examination

The clinical examination was performed at public schools, in a reserved room, using a mirror with artificial light and probe. The clinical signs and symptoms of TMD were assessed using the Research Diagnosis Criteria (RDC/TMD) by a trained examiner (FYK). The RDC/TMD is based on a series of protocoled clinical procedures and on strict diagnostic criteria applied to the most common types of TMD (Dworkin and LeResche, 1992; Pereira Júnior *et al.,* 2004). Two diagnostic axes are contemplated: Axis I

establishes a diagnosis based on clinical variables, while axis II establishes a diagnosis based on psychological variables. Due to the sample age, only Axis I was used in the present evaluation. Figure 1 describes the diagnoses of the subjects: myofascial pain (IA / n = 28), disc displacement with reduction (IIA / n = 4); myofacial pain with limited opening (IB /n = 5) and disc displacement without reduction (IIC/ n = 1).

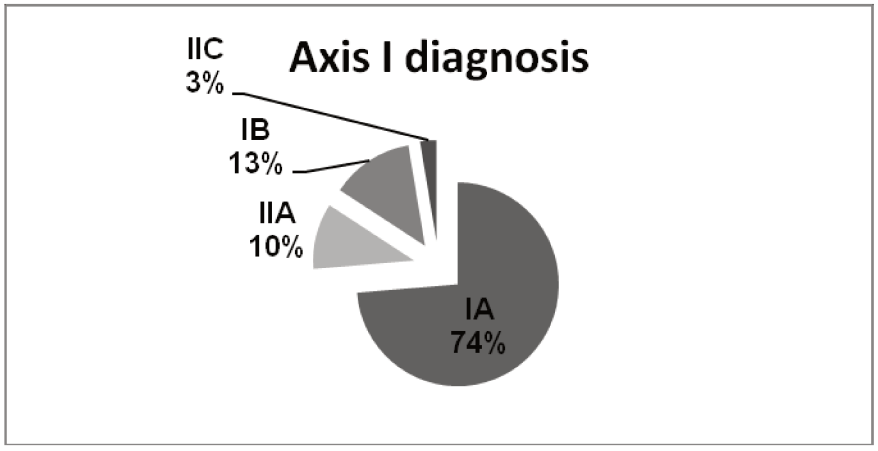

Figure 1. Diagnoses of the studied group

Body weight and height were determined using an anthropometric scale and body mass index (BMI = Kg/m^2) was calculated.

Salivary assessment

Home stimulated saliva samples were collected on two alternate days (week days) by chewing cotton rolls for 3-4 minutes, until they were soaked with saliva (Salivettes, Sarstedt, Numbrecht, Germany). Subjects and their parents were instructed to wake at 7 am. The first sample was taken while lying in bed, the second sample was taken 30 minutes after awakening (fasting), the third sample was taken 1 hour after awakening (fasting) and the last sample was taken at night (at 8 pm), on each day. The samples were stored into a refrigerator and delivered to the researcher in the following day at the schools. Samples were transported on ice to the laboratory on the same day, centrifuged (at 3500 rpm for 5 min) and stored at -80˚C until analysis, resulting in a clear supernatant of low viscosity.

Subjects were instructed not perform physical exercises or ingestion of caffeinated beverages a day earlier. They should also abstain from food, beverages and brushing teeth at the time of sampling allowing only water intake (Larsson *et al.,* 2009). If there were visible signs of blood in the samples, they would be discarded due to possible contamination of plasma cortisol.

Salivary cortisol analyses

Salivary cortisol was assayed using a commercial, highly sensitive enzyme immunoassay kit (Salimetrics™, State College, PA, USA). Whole saliva (25 µl) was added to each well of the microtiter plate and read at 450 nm in a microplate reader (Stat Fax 2100, Awareness Tech. Inc., Palm City, FL, USA). The Elisa was performed following the manufacturer's instructions. The minimal concentration of cortisol that can be distinguished from 0 is 0.003 µg/dL. For more details, see Castelo *et al.,* (2012).

Salivary alpha-amylase analyses

Amylase levels were analyzed using Flexor E6002-190 Automated Clinical Chemistry Analyser automated technique (Vital Scientific, Dieren, Switzerland), at the Clinical Analyses Laboratory of ABC Medical School (Santo André, SP, Brazil). Salivary amylase concentrations were measured using enzymatic method in diluted saliva (1:25) (ELI Tech, Seppim S.A., SEES, France). Samples of known concentrations provided by the Brazilian Society of Clinical Pathology and Laboratory Medicine were used as the standard to calibrate the automated system, and the parameters adopted were: serum amylase level = 61.3 / range 50.3-72.3 U/mL.

Measurements errors

For assessment of method error of the clinical variables (TMD signs and wear facets of sleep bruxism), Kappa test was used on data collected from subjects not included in the studied sample, in two separate occasions at an interval of 14 days. The strength of the intra-examiner agreement was based on the following standards: Kappa

values above 0.8 were considered excellent, from 0.61 to 0.8 good, 0.41 to 0.6 acceptable, 0.21 to 0.40 regular and below 0.20 fair (Cohen *et al.,* 1960).

Statistical analysis

Statistical analysis was performed using BioEstat 5.3 (Mamirauá, Belém, PA, Brazil) with a 5% significance level. Shapiro-Wilk test showed that the distributions of the studied variables deviated from normality. The characteristics of the studied variables were evaluated using descriptive statistics, and they consisted of means, standard deviations, medians and interquartile ranges.

The area under the curve (AUC_G) of salivary cortisol and amylase concentrations against time was calculated by trapezoid method respective to the ground level (Pruessner *et al.,* 2003) for each day, and the final value consisted of the mean of both. Data for AUC_G were not normally distributed; thus, a Mann–Whitney test was used to test the differences in salivary cortisol and amylase AUC_G between children with and without TMD. Also, differences in BMI were tested between groups using Mann–Whitney test. The correlation between salivary cortisol and sAA AUC_G was evaluated by means of Spearman correlation test.

Results

The evaluation of the method error showed that for *mouth opening*, the kappa coefficient obtained was considered excellent (0.92). Signs of sleep bruxism (wear facets) presented high level of reliability, with a kappa coefficient of 0.77 (good agreement). Kappa value for pain palpation on right masseter was 0.67, indicating good agreement (for more details, please see Marquezin *et al.,* 2012).

The characteristics of the sample in accordance with clinical groups (with TMD and controls) are shown in Table 1. Salivary cortisol profiles for TMD and control groups are described in Figure 2 and sAA profiles are shown in Figure 3.

Statistical analyses showed that clinical groups did not differ for salivary cortisol and sAA AUC_G, neither for BMI. Moreover, the coefficients obtained in the correlation between salivary cortisol and sAA AUC_G were not significant for TMD group (r=-0.03), neither for control group (r=0.17).

Table 1. Characteristics of the sample in accordance with clinical groups

	With TMD	Without TMD	*p*-value*
N	38	38	NA
Age (y)			
Mean (SD)	10.63 (1.68)	10.63 (1.68)	NA
Median (IQR)	11.00 (1.00)	11.00 (1.00)	NA
Age range	7 - 14	7 - 14	NA
Gender (n)	24♀ 14♂	24♀ 14♂	NA
BMI (Kg/m^2)			
Mean (SD)	19.41 (4.90)	19.36 (4.41)	-
Median (IQR)	18.28 (6.35)	18.35 (5.71)	0.79
Presence of sleep bruxism	7(yes) 31(no)	7(yes) 31(no)	NA
Salivary amylase AUC_G (U/mL/min)			
Mean (SD)	2544.52 (2142.00)	2054.53 (1046.89)	-
Median (IQR)	1873.94 (1575.83)	2044.03 (1183.60)	1.00
Salivary cortisol AUC_G (µg/dL/min)			
Mean (SD)	90.22 (63.36)	94.21 (63.13)	-
Median (IQR)	76.95 (82.18)	82.96 (67.71)	0.66

NA, not applicable; NS, not significant; SD, standard deviation; BMI, body mass index; AUC_G, area under the curve with respect to ground.
*Mann-Whitney test.

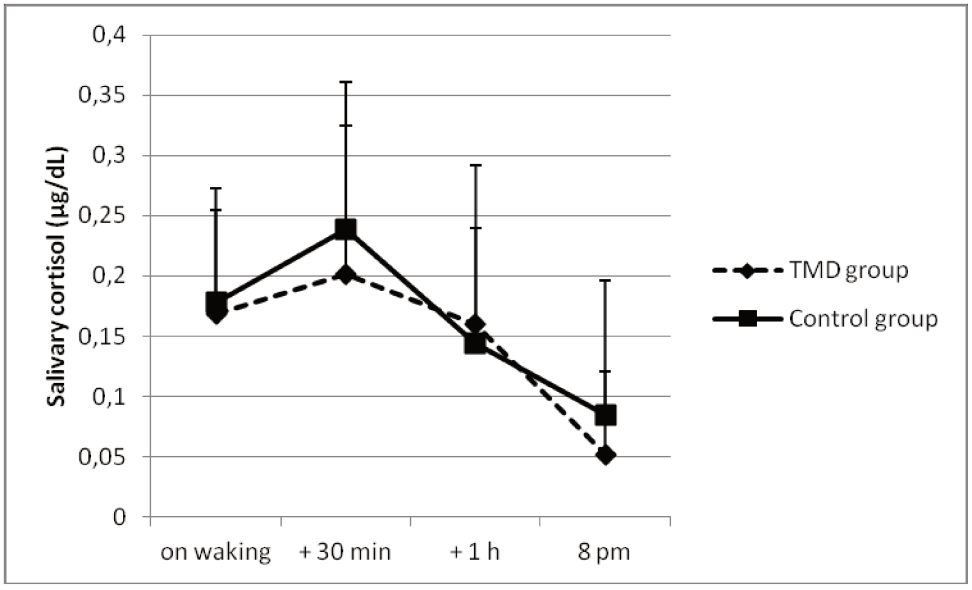

Figure 2. Salivary cortisol profiles in TMD and control groups. Graph shows means and standard deviations.

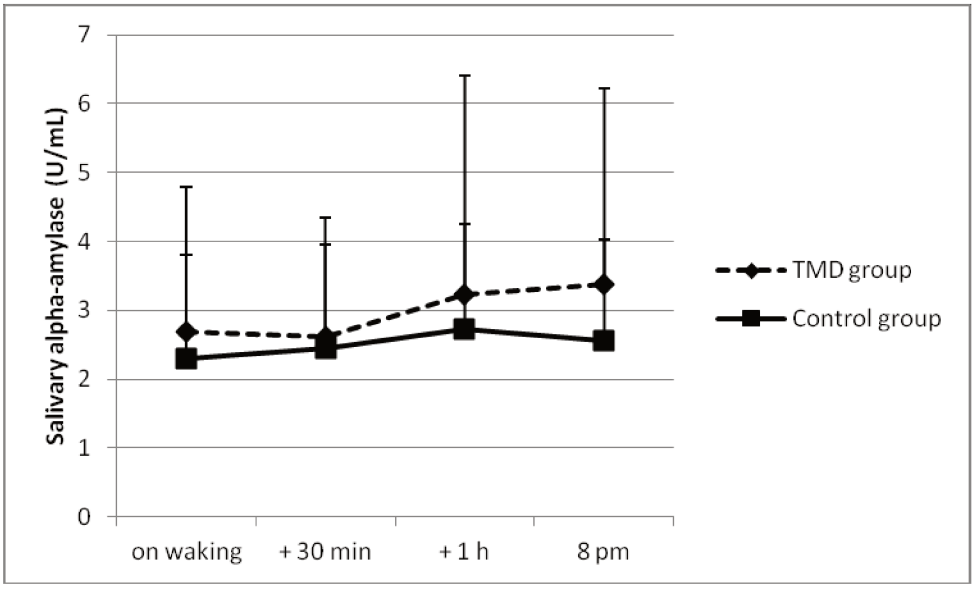

Figure 3. Salivary alpha-amylase profiles in TMD and control groups. Graph shows means and standard deviations.

Discussion

Clinically, TMD is a relatively common disorder attributed or associated to the presence of parafunctional habits (bruxism, nail biting and nonnutritive sucking) and others, such as genetics, gender, malocclusion, anxiety and depression and psychosocial stressors (Barbosa *et al.,* 2008; Da Silva Andrade *et al.,* 2008; Tecco *et al.,* 2011). Little was found in the literature about the relationship between salivary stress biomarkers and signs and symptoms of TMD, especially in children and adolescents. Thus, this case-control study aimed to examine salivary cortisol and amylase levels in young subjects with and without TMD.

For this goal, four saliva samples were obtained on two different days in order to examine the diurnal profiles of cortisol and sAA levels. The cortisol profile is characterized by a marked increase in its concentration following awakening, with a peak after about 30-45 min (awakening response), and a subsequent decline over the day, similar with the results found. The awakening cortisol response is a discrete and dynamic part of the circadian cortisol secretory cycle, and literature has suggested associations with stress, diseases and psychological disorders (Clow *et al.,* 2002; Petrelluzzi *et al.,* 2008).

Both groups showed sAA secretion profiles with an increase towards the evening, although control group has shown a slight drop at 8 pm. This pattern corroborates the study of Nater *et al.,* (2007), who found that sAA levels has a pronounced and distinct diurnal rhythm influenced by age, whereas cortisol showed a distinct circadian release pattern.

A significant difference in salivary cortisol levels was not observed between young subjects with and without TMD. A previous study observed that measures of plasma cortisol in 15 women with well-defined TMD showed increased levels 30% to 50% higher than those of controls (Korszun *et al.,* 2002). According to the authors, this increased activation of the HPA axis may be due to the conscious perception of pain in the facial region, worse than pain elsewhere in the body. Likewise, the study done by Da Silva Andrade *et al.,* (2008) observed significant difference in morning salivary cortisol levels

between females with and without TMD, the former also presenting higher scores of depression and somatization; although such difference must be taken with caution due to the small sample size included.

In children and preadolescents with TMD, a worse perception of the impacts of oral health on their overall well-being and higher diurnal decline of salivary cortisol levels than their controls were found previously (Barbosa *et al.,* 2012). The study of Nilsson and Dahlström (2010) observed that, irrespective of RDC/TMD diagnosis, women patients with TMDs appeared to be more psychologically distressed than controls, although they did not observe significant difference in salivary cortisol levels on waking between groups. A possible explanation for their result would be the use of a single collection to examine the difference in cortisol levels between groups, since TMD seen to be a heterogeneous group of subjects when taking into account cortisol secretion (Jones *et al.,* 1997).

sAA levels were examined as a marker of autonomous activity and, also, no difference was found between subjects with and without TMD in the studied sample. In adults, TMD may involve dysregulation of sympathetic activity; a previous study observed that TMD patients showed lower plasma epinephrine and norepinephine at baseline and under challenges than healthy women of similar ages, but a difference in plasma cortisol was not observed between groups (Light *et al.,* 2009). According to authors, such alterations may compromise cardiovascular and catecholamine responses to stressors in those subjects over the time. But the study done by Nater *et al.* (2007) observed that chronic stress and stress reactivity were associated with higher activity of sAA over the day. TMD may be a consequence of one or more risk factors and if its signs and symptoms are associated with higher or lower activation of sympathetic activity and, therefore, with secretion of sAA, that should be better examined in the future.

A significant correlation between salivary cortisol and sAA levels was not observed, which is in line with the results obtained by previous studies (Nater *et al.,* 2007). This finding suggests a clear distinction between sAA and cortisol representing the

activation of different stress systems, e.g. the sympathetic activity and the HPA axis, respectively.

BMI did not differ between TMD and control groups. The study of Nater *et al.* (2007) showed a decrease in average sAA levels by 3.4% with each point on the BMI scale; thus, BMI is a potential confounding factor that should be controlled when biomarkers are being measured. However, some limitation of the present study should be cited. Salivary collection was made using *salivettes* on home sampling and, thus, subjects may have chew on the cotton roll producing stimulated saliva; according to DeCaro (2008), there is a possible alteration in sAA activity. However, those responsible were informed verbally and in writing about collection procedures, and a phone or email contact was made for any questions.

Although not seen a difference in stress markers between children with and without TMD, it is important to emphasize that the present study included only young subjects with at least one sign of TMD, and the literature has shown that this condition may worsen over the time into adulthood (Magnusson *et al.,* 1985; Tecco *et al.,* 2011), when a clear relationship between TMD and stress biomarkers secretion could be seen.

Conclusion

According to the results found in this case-control study, salivary cortisol and alpha-amylase levels as biomarkers of hypothalamus–pituitary–adrenal axis and sympathetic activity, respectively, did not differ between children and adolescents with and without TMD. Moreover, a significant correlation between both stress biomarkers was not observed.

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The authors declare that they have no conflict of interest.

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CONCLUSÃO

De acordo com os resultados encontrados conclui-se que:

- A prevalência de DTM foi maior em meninas na fase de dentição permanente;
- Não houve associação da DTM com as fases das dentições e a força de mordida;
- A idade correlacionou-se positivamente com a força de mordida e o índice de massa corporal;
- Houve associação entre escores de disfunção orofacial e DTM;
- Os níveis de cortisol e alfa-amilase salivar não diferiram entre crianças e adolescentes com DTM.

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APÊNDICE 1

Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

As informações que possui este documento o convidam a autorizar, por escrito, a participação do menor _____, com o conhecimento dos procedimentos e riscos a pelos quais o menor passará, por vontade própria e sem qualquer intimidação.

1. Título do trabalho experimental

"Avaliação das disfunções orofaciais e temporomandibulares, parâmetros mastigatórios e níveis salivares de cortisol e alfa-amilase em crianças e adolescentes".

2. Responsáveis pela pesquisa

Profa. Dra. Maria Beatriz D. Gavião (responsável), Profa. Dra. Paula Midori Castelo (co-orientadora dos trabalhos), Maria Carolina Salomé Marquezin e Fernanda Yukie Kobayashi (apresentadoras deste termo) do Depto. de Odontologia Infantil da Faculdade de Odontologia de Piracicaba – UNICAMP.

3. Objetivos

O objetivo deste estudo será avaliar clinicamente a presença de sinais e sintomas de disfunção orofacial e temporomandibular e sua relação com alterações na morfologia e na função mastigatória em crianças e adolescentes. Além disso, os níveis de marcadores salivares do estresse serão verificados.

4. Justificativa

Estudos sugerem que a prevalência de sinais e sintomas de dor e disfunção da articulação temporomandibular (da mandíbula) é grande e, muitas vezes, desconhecida pelos pais e ignorada pelos profissionais de saúde. Daí a importância da avaliação da presença e do comprometimento das funções orais (comer, falar, engolir) que possam vir a acontecer.

5. Procedimentos do experimento

Todos os procedimentos da pesquisa serão realizados pelas mesmas pesquisadoras: Maria Carolina Salomé Marquezin e Fernanda Yukie Kobayashi. Seleção da amostra – serão selecionados 200 crianças e adolescentes de ambos os sexos, do ensino fundamental e médio, as quais serão selecionadas na Faculdade de Odontologia de Piracicaba, junto ao Departamento de Odontologia Infantil – Área de Odontopediatria (após a devida concordância da criança em participar da pesquisa e autorizada pelo responsável), de acordo com os seguintes procedimentos:

Anamnese – por meio de entrevista com o responsável, verificando-se: histórico pré-natal, natal e pós-natal, histórico dentário, hábitos de sucção (dedos, chupeta, lábios), ranger dos dentes, tipo e tempo de aleitamento e uso de medicamentos.

Exame clínico intrabucal e extrabucal – o material utilizado será o de uso rotineiro da clínica (pinça, sonda exploradora e espelho bucal), além de equipamentos de proteção individual (gorro, máscara, jaleco e luvas). Serão verificadas as condições dos lábios, gengiva, língua, palato, freios labial e lingual, número de dentes e oclusão dentária. Além disso, serão realizadas as medições de peso e altura.

Avaliação das disfunções orofaciais – cada criança será avaliada por meio de exame físico segundo um roteiro específico (The Nordic Orofacial Test – Screening).

Avaliação das disfunções temporomandibulares - Os sinais e sintomas de DTM serão avaliados por meio de exame clínico e questionário específico.

Avaliação cefalométrica em norma lateral e frontal - Serão realizadas telerradiografias da cabeça em norma lateral e frontal (radiografia póstero-anterior) nas crianças que apresentarem disfunção orofacial e/ou temporomandibular.

Performance Mastigatória – a avaliação da capacidade mastigatória individual será verificada pela fragmentação de um alimento teste artificial denominado Optocal plus, confeccionado com materail não-tóxico que não será deglutido (engolido) e, sim, será eliminado, cuspindo em uma peneira para posterior avaliação.

Avaliação da máxima força de mordida - A máxima força de mordida será medida por meio de um aparelho chamado gnatodinamômetro digital adaptado para as condições orais de indivíduos jovens. Trata-se de técnica indolor, que não provoca nenhum dano físico a criança.

Coleta salivar – A saliva será coletada em dois dias em casa, pelo pai/mãe/cuidador, por meio de técnica não-invasiva (algodão).

6. Possibilidade de inclusão em grupo controle/placebo

Todas as crianças serão avaliadas e receberão os mesmos procedimentos diagnósticos; portanto, não haverá grupo placebo.

7. Métodos alternativos de diagnóstico ou tratamento da condição

Os métodos conhecidos e já estudados serão utilizados na pesquisa. Não será objetivo da pesquisa o tratamento da condição, mas será garantido à criança e ao responsável o esclarecimento sobre sua condição, os riscos à sua integridade física e o encaminhamento à Clínica de Especialização em Odontopediatria ou de Graduação em Odontologia da Faculdade de Odontologia de Piracicaba (UNICAMP).

8. Riscos previsíveis

Os procedimentos realizados não oferecem riscos, pois os exames clínicos intra e extra-bucal seguem os passos da rotina clínica, utilizando-se instrumental e material adequados e esterilizados. Os exames de performance mastigatória e de quantificação salivar de hormônios serão realizados sob a supervisão da pesquisadora; os mesmos constituem técnicas indolores, não-invasivas, que não oferecem riscos à criança, pois utilizam materiais que não são perigosos a vida e seguem as regras de assepsia e limpeza preconizadas pela Faculdade de Odontologia de Piracicaba – UNICAMP.

9. Benefícios e vantagens

O tratamento preventivo e/ou curativo (restaurador) necessário estará assegurado à criança, seja realizado pela Cirurgiã Dentista responsável (aluna de pós-graduação em Odontopediatria), ou pelas próprias pesquisadoras, no caso da criança ainda não estar em atendimento na clínica. No caso de presença e/ou persistência de hábitos parafuncionais e disfunções orofaciais, os responsáveis receberão os devidos esclarecimentos para que procurem orientação fonoaudiológica na rede particular ou pública de atendimento. Na presença de maloclusão (problemas ortodônticos) ou alteração da função mastigatória, os responsáveis serão alertados, bem como a Cirurgiã Dentista responsável; no caso da criança ainda não se encontrar em tratamento, o encaminhamento às estagiárias e alunas de pós-graduação do Departamento será viabilizado, assim como o

encaminhamento à clínica de graduação da Faculdade. No entanto, será alertada ao responsável a possível demora deste procedimento devido a grande quantidade de pacientes cadastrados, podendo ele, se possível, buscar tratamento na rede particular ou pública.

10. Acompanhamento e assistência ao sujeito

O responsável pelo sujeito tem a garantia de ser esclarecido sobre a condição da criança, que deverá receber assistência e acompanhamento odontológicos preventivos e/ou curativos adequados pela Cirurgiã Dentista responsável pela criança ou pelas pesquisadoras, dentro de suas atribuições, durante o período de duração da pesquisa, bem como, se necessário, os esclarecimentos para que procure atendimento por profissionais de outras áreas de saúde, como psicólogos, fonoaudiólogos, etc.

11. Garantia de esclarecimentos

O responsável pelo menor tem a garantia de que receberá respostas a qualquer pergunta ou esclarecimento sobre qualquer dúvida referente aos procedimentos, riscos e benefícios empregados neste documento e outros relacionados à pesquisa, em qualquer momento.

12. Garantia de ressarcimento/indenização/reparação de dano

Não há previsão de ressarcimento ou indenização por dano, pois a participação na pesquisa não trará riscos, nem causará despesas ao voluntário. Caso sessões complementares forem necessárias para obtenção de dados, os gastos de transporte serão de responsabilidade dos pesquisadores.

13. Garantia de sigilo

Haverá sigilo e anonimato quanto aos dados confidenciais obtidos.

14. Retirada do consentimento

O responsável pelo menor tem a liberdade de retirar seu consentimento a qualquer momento e deixar de participar do estudo, sem qualquer prejuízo ao atendimento odontológico a que a criança esteja sendo ou será submetida na Clínica de Especialização em Odontopediatria desta Faculdade.

15. Garantia de entrega de cópia

Este termo de consentimento compõe-se de duas cópias idênticas, sendo uma entregue ao responsável pelo menor e outra que será arquivada pelo Departamento.

16. Consentimento pós-informação

Eu,_____
___, responsável pelo menor _____, certifico que li as informações acima, fui suficientemente esclarecido (a) de todos os itens e estou plenamente de acordo com a realização do experimento e autorizo a execução do trabalho de pesquisa exposto.

Piracicaba, _____ de _____ de _____.

Nome (legível): _____

RG: _____ CPF: _____

Endereço: _____ tel: _____

Assinatura: _____

Atenção: A sua participação em qualquer tipo de pesquisa é voluntária. Em caso de dúvida quanto aos seus direitos, escreva para o Comitê de Ética em Pesquisa da FOP-UNICAMP. Endereço: Av. Limeira, 901 – Piracicaba – SP – cep 13414-900 ou pelo telefone: 19 - 21065349. E-mails: mariacarol_bariri@hotmail.com e fernandaykobayashi@gmail.com. Telefone para contato: Fernanda Yukie Kobayashi (11) 7371-1555 , Maria CarolinaSalomé Marquezin (14) 8134-4933

APÊNDICE 2

Ficha de anamnese

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE ODONTOPEDIATRIA – MESTRADO

As alunas de mestrado em Odontopediatria da Faculdade de Odontologia de Piracicaba (FOP-UNICAMP) Ana Bheatriz Marangoni Montes, Fernanda Yukie Kobayashi e Maria Carolina Salomé Marquezin, estão realizando a pesquisa chamada "*Avaliação das disfunções orofaciais e temporomandibulares, força de mordida e níveis salivares de cortisol e alfa-amilase em crianças e adolescentes",* orientada pela Profª Drª Paula Midori Castelo e Co-orientada pela Profª Drª Maria Beatriz Duarte Gavião.

Como visto e acordado no Termo de Conscentimento Livre e Esclarecido enviado anteriormente, gostaríamos de fazer algumas perguntas de interesse científico e clínico, que devem ser preenchidas e entregues pelos seus filhos na própria escola, local onde as pesquisadoras irão retirar este questionário.

Dúvidas, esclarecimento ou sugestões que gostariam de fazer às pesquisadoras, entrar em contato com as pesquisadoras. Agradecemos desde já a colaboração dos senhores responsáveis.

1. IDENTIFICAÇÃO

Nome_____
Data de nascimento _____/_____/_____ Idade_____anos Sexo: (F) (M)
Endereço_____
Bairro_____Cidade_____CEP_____
Telefone_____ Recado_____ Cel._____
Pai _____Idade _____anos
Estado civil: ( ) solteiro ( ) casado ( ) divorciado ( ) viúvo ( ) outros
Grau de instrução: ( ) sem escolaridade ( ) 1º grau ( ) 2º grau ( ) superior
Profissão_____

Mãe _____ Idade ___ anos
Estado civil: ( ) solteira ( ) casada ( ) divorciada ( ) viúva ( ) outros
Grau de instrução: ( ) sem escolaridade ( ) 1º grau ( ) 2º grau ( ) superior
Profissão_____
End.comercial_____ Fone_____
Irmãos_____ Idades_____
Renda familiar total_____
Pediatra ou clínico responsável_____

2. HÁBITOS FREQUÊNCIA

TIPO	SIM	NÃO	ESPORÁDICO	NOITE	CONTÍNUO
Sucção (chupar) dedos					
Sucção (chupar) chupeta*					
Sucção (chupar) lábios					
Mordedura dos lábios					
Roer unhas					
Ranger dentes					
Respiração pela boca					
Ronco					
Asma					
Bronquite					
Sonambulismo					
Enurese noturna (xixi na cama) – últimos 6 meses					

*MARCA OU TIPO DE CHUPETA_____
INÍCIO (hábito)_____ FINAL (retirada do hábito)_____
MÉTODOS USADOS PARA DOMINAR OS HÁBITOS_____

CARACTERÍSTICAS COMPORTAMENTAIS:

- ❑ agitado ❑ irritado ❑ triste
- ❑ calmo ❑ ansioso ❑ desanimado
- ❑ alegre ❑ desatento ❑ atento
- ❑ normal ❑ outros

As pesquisadoras agradecem a compreensão e colaboração com a pesquisa e ficamos à disposição para quaisquer esclarecimentos.
Muito obrigada!

APÊNDICE 3
Ficha Clínica

EXAME CLÍNICO MESTRADO 2011 DATA:____ /____ /_____

Pesquisadoras: Ana Bheatriz M. Montes, Fernanda Y. Kobayashi e Maria Carolina S. Marquezin

IDENTIFICAÇÃO

<table>
<tr><td colspan="2">Código paciente:_____

Escola:</td><td colspan="2">Estadual( ) Pública( )</td><td>Série:</td></tr>
<tr><td colspan="5">Nome:</td></tr>
<tr><td colspan="2">Sexo (F) (M)</td><td colspan="2">Idade:</td><td>Data de nascimento:</td></tr>
<tr><td colspan="5">Nome da mãe:</td></tr>
<tr><td colspan="5">Nome do pai:</td></tr>
<tr><td colspan="5">Endereço:</td></tr>
<tr><td colspan="5">E-mail:</td></tr>
<tr><td colspan="3">Telefone:</td><td colspan="2">Responsável:</td></tr>
<tr><td>Variáveis corporais</td><td>Peso:</td><td>Altura:</td><td colspan="2">I M C :</td></tr>
</table>

A criança usa algum medicamento como benzodiazepínicos, AINES, corticosteróides ou antidepressivos?

_____

_____

Há presença de cáries ou perdas? (CRITÉRIO DE EXCLUSÃO) (S) (N)

ANEXO 1

Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Avaliação das disfunções orofaciais e temporomandibulares, parâmetros mastigatórios e níveis salivares de cortisol e alfa-amilase em crianças e adolescentes"**, protocolo nº 004/2010, dos pesquisadores Maria Beatriz Duarte Gavião, Fernanda Yukie Kobayashi, Maria Carolina Salomé Marquezin e Paula Midori Castelo, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 31/03/2010.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Orofacial and temporomandibular dysfunction evaluation, masticatory parameters and salivary cortisol and amylase levels in children and adolescents"**, register number 004/2010, of Maria Beatriz Duarte Gavião, Fernanda Yukie Kobayashi, Maria Carolina Salomé Marquezin and Paula Midori Castelo, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 03/31/2010.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2

RESEARCH DIAGNOSTIC CRITERIA (RDC) – EIXO I

Formulário de Exame

Raça

Italiano 1 □

Asiático ou Insulano Pacífico 2 □

Negro 3 □

Branco 4 □

Outro 5 □

1. Você tem dor no lado direito do rosto, lado esquerdo ou ambos os lados?

nenhum 0 □ direito 1 □ esquerdo 2 □ ambos 3 □

2. Você poderia apontar as áreas aonde você sente dor ?

Direito		**Esquerdo**	
Nenhuma	0	Nenhuma	0
Articulação	1	Articulação	1
Músculos	2	Músculos	2
Ambos	3	Ambos	3

Examinador apalpa a área apontada pelo paciente, caso não esteja claro se é dor muscular ou articular

3. Padrão de Abertura

Reto 0

Desvio lateral direito (não corrigido) 1

Desvio lateral direito corrigido (“S”) 2

Desvio lateral esquerdo (não corrigido) 3

Desvio lateral corrigido (“S”) 4

Outro 5

Tipo _____(especifique)

4. Extensão de movimento vertical incisivos maxilares utilizados 11

21

a. Abertura passiva sem dor __ __ mm

b. Abertura máxima passiva __ __ mm

c. Abertura máxima ativa __ __ mm

d. Transpasse incisal vertical __ __ mm

Tabela abaixo: Para os itens "b" e "c" somente

DOR MUSCULAR				**DOR ARTICULAR**			
nenhuma	**direito**	**esquerdo**	**ambos**	**nenhuma**	**Direito**	**esquerdo**	**ambos**
0	1	2	3	0	1	2	3
0	1	2	3	0	1	2	3

5. Ruídos articulares (palpação)

a. abertura

	Direito	**Esquerdo**
Nenhum	0	0
Estalido	1	1
Crepitação grosseira	2	2
Crepitação fina	3	3

Medida do estalido na abertura __ __ mm __ __ mm

b. Fechamento

	Direito	**Esquerdo**
Nenhum	0	0
Estalido	1	1
Crepitação grosseira	2	2
Crepitação fina	3	3

Medida do estalido de fechamento __ __ mm __ __ mm

c. Estalido recíproco eliminado durante abertura protrusiva

	Direito	Esquerdo
Sim	0	0
Não	1	1
NA	8	8

6. Excursões

a. Excursão lateral direita __ __ mm

b. Excursão lateral esquerda __ __ mm

c. Protrusão __ __ mm

Tabela abaixo: Para os itens "a" , "b" e "c"

DOR MUSCULAR				**DOR ARTICULAR**			
nenhuma	**direito**	**esquerdo**	**ambos**	**nenhuma**	**direito**	**esquerdo**	**ambos**
0	1	2	3	0	1	2	3
0	1	2	3	0	1	2	3
0	1	2	3	0	1	2	3

d. Desvio de linha média __ __ mm

direito	**esquerdo**	**NA**
1	2	8

7. Ruídos articulares nas excursões

Ruído direito

	nenhum	**estalido**	**Crepitação grosseira**	**Crepitação leve**
Excursão Direita	0	1	2	3
Excursão Esquerda	0	1	2	3
Protrusão	0	1	2	3

Ruído esquerdo

	Nenhuma	estalido	Crepitação grosseira	Crepitação leve
Excursão Direita	0	1	2	3
Excursão Esquerda	0	1	2	3
Protrusão	0	1	2	3

INSTRUÇÕES, ÍTENS 8-10

O examinador irá palpar (tocando) diferentes áreas da sua face, cabeça e pescoço. Nós gostaríamos que você indicasse se você não sente dor ou apenas sente pressão (0), ou dor (1-3). Por favor, classifique o quanto de dor você sente para cada uma das palpações de acordo com a escala abaixo. Circule o número que corresponde a quantidade de dor que você sente. Nós gostaríamos que você fizesse uma classificação separada para as palpações direita e esquerda.

0 = Sem dor / somente pressão

1 = dor leve

2 = dor moderada

3 = dor severa

8. Dor muscular extra-oral com palpação

	DIREITO	**ESQUERDO**
a. Temporal (posterior)	0 1 2 3	0 1 2 3
"parte de trás da têmpora"		
b. Temporal (médio)	0 1 2 3	0 1 2 3
"meio da têmpora"		
c. Temporal (anterior)	0 1 2 3	0 1 2 3
"parte anterior da têmpora"		
d. Masseter (superior)	0 1 2 3	0 1 2 3
"bochecha/abaixo do zigoma"		
e. Masseter (médio)	0 1 2 3	0 1 2 3
"bochecha/lado da face"		
f. Masseter (inferior)	0 1 2 3	0 1 2 3
"bochecha/linha da mandíbula"		

g. Região mandibular posterior 0 1 2 3 0 1 2 3

(estilo-hióide/região posterior do digástrico)

"mandíbula/região da garganta"

h. Região submandibular 0 1 2 3 0 1 2 3

(pterigoide medial/supra-hióide/região

anterior do digástrico) "abaixo do queixo"

9. Dor articular com palpação

	DIREITO	**ESQUERDO**
a. Polo lateral	0 1 2 3	0 1 2 3
"por fora"		
b. Ligamento posterior	0 1 2 3	0 1 2 3
"dentro do ouvido"		

10. Dor muscular intraoral com palpação.

	DIREITO	**ESQUERDO**
a. Área do pterigoide lateral	0 1 2 3	0 1 2 3
"atrás dos molares superiores"		
b. Tendão do temporal	0 1 2 3	0 1 2 3
"tendão"		

ANEXO 3

Nordic Orofacial Test – Screening NOT-S

O NOT-S é usado quando um paciente tem dificuldade para falar, mastigar ou engolir.

A seção de anamnese é conduzida como uma entrevista estruturada. O examinador faz a pergunta, explica, e faz perguntas adicionais quando necessário, interpreta a resposta e preenche o questionário.

A entrevista do NOT-S contém seis sessões : Função Sensorial, Respiração, Hábitos, Mastigando e Engolindo, Salivação e Secura da Boca (I-VI).

O exame do NOT-S contém seis sessões: Face em Repouso, Respiração Nasal, Expressão Facial, Músculos Mastigatórios e Função Mandibular, Função motora oral e Fala (1-6).

O manual ilustrado deve ser utilizado durante o exame.

País _____

	Fonoaudiólogo	Dentista	Médico	Fisioterapeuta	Outros
Examinador _____	☐	☐	☐	☐	☐

Data do exame ____/____/_____

Data de nascimento ____/____/_____ ♀ ☐ ♂ ☐

Nome:_____

Primeiro Diagnóstico Médico (especificar somente um): _____

Código de diagnóstico (ICD-10):

_____

Posição durante o exame

☐ Sentado

☐ Deitado

Posição da cabeça quando sentado

☐ Normal (reta e vertical)

☐ Outra

☐ Respostas com ajuda de outra pessoa

CÓDIGO PARA AVALIAÇÃO: O ESCORE TOTAL DO NOT-S PODE VARIAR DE 0 A 12	X = SIM 0 = NÃO - = NÃO AVALIADO	SE EM UMA SESSÃO HOUVER UMA OU MAIS RESPOSTAS X, COLOQUE O ESCORE 1 NA CAIXA DA COLUNA À DIREITA

NOT-S	ESCORE TOTAL	☐☐

ENTREVISTA NOT-S

Pontuação		
I	**Função Sensorial** **A**- Escovar seus dentes faz você ter ânsia de vômito? Isso acontece muitas vezes? ..... Desconforto óbvio como enjôo, vômito, ou refluxo – aumento de sensibilidade. **B**- Você coloca tanta comida na boca que fica difícil de mastigar? Isso acontece todo dia? ..... Não consegue perceber quando a boca está cheia – diminuição da sensibilidade.	☐ ☐ ☐
II	**Respiração** **A**- Você respira normalmente ou usa algum suporte para respirar? CPAP, Oxigênio, respirador, outros. **B**- Você ronca muito quando dorme? Isso acontece toda noite? ..... Ronco ou apnéia; não se aplica a sintomas de asma ou alergias.	☐ ☐ ☐
III	**Hábitos** **A**- Você roe as unhas, ou chupa os dedos ou outros objetos todos os dias? Hábito de sucção de chupeta e dedos não é avaliado abaixo dos 5 anos. **B**- Você chupa ou morde seus lábios, língua ou bochechas todos os dias? **C**- Você aperta forte seus dentes ou range eles durante o dia?	☐ ☐ ☐ ☐
IV	**Mastigando e Engolindo** **A**- Não come com a boca	☐

	Tubo nasogástrico, gastrostomia, outros – pular perguntas B-E	
	B- Você acha difícil comer alimentos com certa consistência (mais duros)?	☐
	Excluir alergias e dietas especiais como vegetarianismo e intolerância ao glúten	
	C- Você demora mais do que 30 minutos para comer uma refeição completa?	☐
	D- Você engole grandes pedaços sem mastigar?	☐
	E- Você costuma tossir durante as refeições?	☐
	Acontece em quase todas as refeições.	
V	**Salivação**	
	A - Você fica com saliva no canto da boca ou escorre saliva para o queixo	☐
	todos os dias?	
	Tem que limpar a boca, não se aplica enquanto dorme.	☐
VI	**Secura da boca**	
	A- Você precisa beber algum tipo de líquido para conseguir comer uma torrada?	☐
	B- Você sente dor na mucosa (pele) da boca ou na língua?	☐
	Dor recorrente ou sensação de formigamento pelo menos uma vez na semana; não se aplica a dor de dente ou vesículas (lesões bolhosas) na boca.	☐
Nome:	ENTREVISTA NOT-S	Soma:

EXAME NOT-S

Pontuação		
1	**Face em repouso** **Observe a figura por um minuto, começando agora.** Observação de um minuto. Avalie A-D Figura 1 **A**- Assimetria ..... (considerar tanto osso quanto tecidos moles) **B**- Desvio da posição dos lábios ..... (boca aberta ou outros desvios em mais de 2/3 do tempo) **C**-Desvio da posição da língua ..... (ponta da língua visivelmente entre os dentes em mais de 2/3 do tempo) **D**- Movimentos involuntários ..... (repetidos movimentos involuntários da face)	☐ ☐ ☐ ☐ ☐
2	**Respiração nasal** Figura 2 **A**- Feche a boca e faça 5 profundas inspirações pelo nariz (cheire) Não consegue fazer 5 inspirações sucessivas pelo nariz. Se o paciente não consegue fechar os lábios, o paciente ou o examinador pode, manualmente ajudar a manter os lábios fechados. Não avaliar se o paciente estiver resfriado.	☐ ☐
3	**Expressão facial** Figura 3 **A**- Feche os olhos bem forte ..... Os músculos faciais não estão ativados, esteticamente, em simetria. Figura 4 **B**- Mostre seus dentes ..... Os lábios e os músculos faciais não são simetricamente ativados então os dentes são facilmente visíveis. Figura 5 **C**- Tente assobiar/assoprar ..... Não consegue fazer biquinho com os lábios simetricamente.	☐ ☐ ☐ ☐
4	**Músculos mastigatórios e função mandibular**	☐

	Figura 6 **A**- Morda forte com seus dentes do fundo ..... Não se pode registrar atividade simétrica quando dois dedos ficam pressionando os músculos mandibulares (m. masseter dos dois lados). Figura 7 **B**- Abra a boca o máximo que conseguir ..... Não consegue abrir a boca numa distância correspondente à largura do dedo indicador e do dedo do meio da mão esquerda do paciente. Se os dentes anteriores estiverem ausentes, use a largura de três dedos (indicador, dedo do meio e anelar) como medida.	☐ ☐
5	**Função motora oral** Figura 8 **A**- Ponha sua língua para fora o quanto puder ..... Não consegue alcançar a borda do vermelhão dos lábios com a ponta da língua. Figura 9 **B**- Lamba os seus lábios ..... Não consegue usar a ponta da língua para molhar os lábios e não consegue alcançar os cantos da boca. Figura 10 **C**- Encha sua boca de ar e segure por pelo menos 3 segundos ... Não consegue encher a boca de ar sem vazamento de ar ou sem fazer barulhos. Figura 11 **D**- Abra a boca bem grande e diga ah-ah-ah! ..... Não se nota elevação da úvula e o palato mole é observado.	☐ ☐ ☐ ☐ ☐
6	**Fala** **A**- Não fala ..... Pular perguntas B-C. Figura 12 **B**- Conte alto até 10 ..... A fala não é clara com um ou mais sons indistinguíveis ou nasalidade anormal. Abaixo de 5 anos de idade exclua sons de R, S da avaliação. Figura 13 **C**- Diga PATAKA, PATAKA, PATAKA..... Não avalie este item em crianças menores de 5 anos de idade.	☐ ☐ ☐ ☐
Nome:	EXAME NOT-S	Soma:

Anexo 4

z

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February 1, 2013

Editor, Journal of Oral Rehabilitation

Subject: Note from BiomEditor to JOR editor regarding revision of a manuscript to be submitted (or previously submitted) to JOR

JOR manuscript reference number (as communicated by the paper's authors): Unknown

Article title (as originally sent to us): Temporomandibular disorder, bite force and orofacial function in young subjects

Corresponding author as indicated in the manuscript: Maria Beatriz Duarte Gavião
Author who corresponded with BiomEditor: Maria Beatriz Duarte Gavião
BiomEditor internal reference number: 16781

Dear JOR editor:

I am writing at the request of the authors ("client") of the above-noted manuscript submitted to your journal. The client indicated that JOR requests that a note from me be sent to you regarding the revision of their manuscript by our company, BiomEditor.

I confirm that we have revised the above-noted manuscript with respect to scientific English and returned the revised file(s) to the client. I note that our revision of the manuscript was rather extensive.

Important: Indeed, the client may have updated their manuscript appropriately so that it will be acceptable for publication. At the time of this writing, however, the client had not asked us to review their final version of the paper that they may soon send (or have already sent) to you, and thus I cannot attest to the final content of their paper or the accuracy with which the client further revised the paper based on our recommendations.

Thank you. My sincere regards,

Timothy C. Taylor

Timothy C. Taylor, Ph.D.
Chief Science Officer

Anexo 5
Comprovante de submissão à revista Journal Oral of Rehabilitation

07-Feb-2013

Dear Prof. Maria Beatriz Gavião,

Thank you for submitting your manuscript entitled "Associations between bite force, orofacial function, and temporomandibular disorders in young subjects" to the Journal of Oral Rehabilitation.

Your manuscript will now be screened by the Editor in Chief of JOR. If your manuscript is of interest to JOR, it will immediately be sent for review.

If your manuscript has been screened and found relevant for the journal, but does not comply with the guidelines for JOR, it will be unsubmitted and returned to your Author Centre. You will be asked to correct it and continue with your submission. When we have received the corrected version, your manuscript will be sent for review.

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