UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Avaliação das disfunções orofaciais e temporomandibulares, ansiedade e níveis de cortisol salivar em crianças e adolescentes com bruxismo do sono

Dissertação de Mestrado apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Mestre em Odontologia,na área de Odontopediatria.

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"É do buscar e não do achar que nasce o que eu não conhecia."

Clarice Lispector

RESUMO

O bruxismo do sono (BS) é uma parafunção caracterizada pelo ranger ou apertar dos dentes, pode causar desgaste dentário e dor, apresenta alta prevalência na infância e é considerado uma resposta física ao estresse, embora esta relação seja controversa. O objetivo deste estudo, composto por dois capítulos, foi avaliar fatores morfológicos, funcionais e fisiológicos em crianças e adolescentes de 7 a 17 anos com sinais e sintomas de BS. O primeiro capítulo, refere-se a um estudo do tipo caso-controle, intitulado *"Is sleeping bruxism related to temporomandibular disorders, malocclusion and orofacial dysfunction?"*, que comparou a presença de disfunção temporomandibular (DTM), maloclusão e disfunção orofacial em indivíduos com e sem BS. Foram avaliados 316 indivíduos, dos quais 52 apresentaram BS, de acordo com os critérios de diagnóstico estabelecidos pela Classificação Internacional de Distúrbios do Sono. O grupo controle foi formado por 104 indivíduos pareados de acordo com a idade, gênero e variáveis corporais. Deste modo, a amostra considerada para este estudo foi composta por 156 indivíduos, com idade média de 10,86±2,32 anos. A DTM foi avaliada pelo *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMD) (Eixo I). Para a maloclusão utilizou-se o Índice de Necessidade de Tratamento Ortodôntico (IOTN) e para as disfunções orofaciais o instrumento *The Nordic Orofacial Test Screnning* (NOT-s). Os dados foram submetidos à estatística descritiva, teste de Shapiro- Wilks, testes *t* de Student para amostras independentes, Mahn-Whitney, Chi-quadrado ou exato de Fisher, quando indicados, com nível de significância de α=0,05. Os resultados mostraram que a prevalência de BS foi maior no gênero masculino. A presença maloclusão e DTM foi similar em ambos os grupos. No entanto, os escores de NOT-S foram significativamente maiores no Grupo Caso e no gênero feminino. Hábitos, problemas na mastigação, na deglutição e nos músculos mastigatórios foram as alterações mais prevalentes da disfunção orofacial em indivíduos com BS. Concluiu-se que a disfunção orofacial foi mais severa e prevalente em crianças e adolescentes com BS; maloclusão e DTM não apresentaram relação com BS. O segundo estudo, intitulado **"***Evaluation of sleep bruxism, temporomandibular disorder, anxiety and salivary cortisol levels in children and adolescents,"* teve como objetivo verificar se havia associação entre BS e fatores emocionais, subjetivamente pela avaliação de sintomas de

ansiedade e objetivamente pela quantificação dos níveis de cortisol salivar. Verificou-se a influência de características antropométricas, gênero e DTM. O estudo envolveu 316 indivíduos com idade de 10,64±2,24 anos. Os sintomas de ansiedade foram avaliados com a Escala Multidimensional de Ansiedade para Crianças (MASC). Para avaliação do cortisol salivar coletou-se amostras de saliva estimulada em dois dias alternados, ao acordar, 30 minutos e uma hora após e antes de dormir. A quantificação do cortisol foi realizada pela técnica de enzimaimunoensaio. Para as características antropométricas determinou-se o índice de massa muscular (IMC=peso/altura^2). A DTM foi avaliada pelo RDC/TMD. Os resultados foram analisados por meio de estatística descritiva, análise de variância e regressão logística múltipla. Não houve associação significativa entre BS e idade, IMC, DTM, ansiedade e níveis de cortisol salivar. O gênero masculino apresentou maior probabilidade de apresentar BS. Os escores de ansiedade foram significativamente mais altos tanto para o gênero feminino quanto para indivíduos com DTM. Conclui-se que crianças e adolescentes com DTM apresentaram maiores escores de ansiedade em relação aquelas com bruxismo e do grupo controle. Escores de ansiedade foram maiores em meninas, porém meninos foram mais propensos a apresentar BS. O BS não apresentou associação com ansiedade, níveis de cortisol salivar, IMC e DTM.

Palavras-chave: Bruxismo do sono, Disfunção da Articulação Temporomandibular, disfunção orofacial, ansiedade, Cortisol

ABSTRACT

Sleeping bruxism (SB) is a parafunction characterized by grinding or clenching of teeth that can cause tooth wear and pain, shows high prevalent in children, and is considered a physical response to stress. The aim of this study, a composite of two chapters, was to evaluate morphologic, functional, and physiological factors in children and adolescents age 7 to 17 years with signs and symptoms of SB. The first chapter refers to a case-control study titled "Is sleeping bruxism related to temporomandibular disorders, malocclusion, and orofacial dysfunction?" It compared the presence temporomandibular disorders (TMD), malocclusion, and orofacial dysfunction in individuals with and without SB. Three hundred and sixteen subjects were evaluated, of whom 52 presented BS, according to the diagnostic criteria established by The International Classification of Sleep Disorders. The control group was composed of 104 individuals matched by gender, age, and body variables. Thus, the considered sample consisted of 156 subjects, with mean age of 10.86±2.32 years. The TMD was assessed by the *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMD) (Axis I). For malocclusion the study used The Dental Health Component of the Index of Orthodontic Treatment Need (IOTN), and for orofacial dysfunction *The Nordic Orofacial Test Screening* (NOT-S) was employed. Data were submitted to descriptive statistics, Shapiro-Wilks, Student *t* test for independent variables, Mahn-Whitney, Chi-square, and Fisher's exact tests, when indicated, with a significance level of α=0.05. The results showed that the prevalence of SB was higher in the male gender. The presence of malocclusion and TMD was similar in both groups. However, the NOT-S scores were significantly higher for the case group than the control groups, as well as for females. Habits, impairment in chewing, swallowing, and masticatory muscles were the most frequent conditions of orofacial dysfunction in subjects with bruxism. It was concluded that the orofacial dysfunction was more severe and prevalent in children and adolescents with SB; malocclusion and TMD had no relation to SB. The second chapter, "Evaluation of sleep bruxism, temporomandibular disorders, anxiety, and salivary cortisol levels in children and adolescents," aimed to verify if there was an association between SB and emotional factors, both subjectively, by evaluating anxiety symptoms, and objectively, by determining the salivary cortisol levels. The influence of the

anthropometric characteristic, gender, and TMD was verified. The study involved 316 individual with mean age of 10.64±2.24 years. Anxiety symptoms were assessed with the Multidimensional Anxiety Scale for Children (MASC). For assessment of salivary cortisol, salivary samples were collected on two alternate days: upon waking, 30 minutes and 1 hour after waking, and before sleep. Quantification of cortisol was performed by enzyme immunoassay. For anthropometric characteristics, the body mass index was calculated (BMI=weight/height^2). The TMD was evaluated by the RDC/TMD. The results were analyzed using descriptive statistics, analysis of variance, and multiple logistic regression. No significant relation was found between bruxism, age, BMI, TMD, anxiety, and salivary cortisol levels. Males had a higher probability of having SB. Anxiety scores were higher in females and subjects with TMD. It was concluded that children and adolescents with TMD had higher anxiety scores compared to those in the control group and those with bruxism. Anxiety scores were higher in girls, but boys were more prone to BS. The BS was not associated with anxiety, salivary cortisol levels, BMI, and TMD.

Keywords: Sleep Bruxism, TMJ dysfunction, orofacial dysfunction, anxiety, cortisol

SUMÁRIO

INTRODUÇÃO

O bruxismo é uma atividade parafuncional que pode se manifestar com o indivíduo acordado (vigília) ou durante o sono. O bruxismo do sono (SB) é caracterizado pelo o ranger e/ou apertar dos dentes (Makino *et al*., 2009) e é amplamente considerado como uma resposta física ao estresse emocional, embora essa relação ainda seja controversa (Tahara *et al*., 2007). A prevalência em crianças pode atingir até 38% (Cheifetz *et al*., 2005), sendo mais alta que a encontrada em adultos. O BS pode representar uma ameaça para a integridade das estruturas do sistema mastigatório, se a magnitude e a direção das forças exercidas exceder a capacidade adaptativa do sistema (de la Hoz-Aizpurua *et al*., 2011), causando desgaste dentário, dor (Barbosa *et al.*, 2008).

Estudos sobre a prevalência de bruxismo em crianças são complexos devidos a variação e persistência ao longo do tempo (caráter cíclico) e pelas próprias limitações nos critérios diagnósticos. Em geral, as crianças não têm consciência da parafunção e não são capazes de relatar dor e/ou cansaço muscular decorrentes da parafunção, nem a presença do apertar dos dentes enquanto estão acordadas.

Na tentativa de padronização, a Academia Americana de Medicina do Sono (American Academy of Sleep Medicine - AASM, 2001) propôs critérios de diagnóstico para o bruxismo incluindo indicadores clínicos e anamnésticos que têm servido de base para pesquisadores e clínicos, sendo considerados como sinais e sintomas de bruxismo do sono em crianças o relato de sons de ranger dos dentes por irmãos ou pais/responsáveis e a presença de facetas de desgaste dentário brilhantes e polidas.

O desgaste dentário deve ser avaliado com cautela, uma vez que é um sinal cumulativo. Além disso, a confirmação mais acurada da presença do hábito é somente possível utilizando-se ferramentas como exames eletromiográfico e de áudio-vídeo (Harada *et al*., 2006); no entanto, esses exames são dispendiosos e difíceis de serem utilizados em estudos com grande número de sujeitos. Portanto, a observação de sinais e sintomas relacionados ao bruxismo em conjunto com as queixas apresentadas pelos responsáveis ou familiares ainda é a forma mais acessível e apropriada de diagnóstico (Koyano *et al*., 2008).

A hipótese multifatorial da gênese e patogênese de bruxismo do sono (SB) inclui a genética, a estrutura do sono (micro-despertar), meio ambiente, estresse emocional, ansiedade e outros fatores psicológicos, níveis de catecolaminérgicas no Sistema Nervoso Central (SNC), uso de drogas (ecstasy, álcool, cafeína, tabaco) e medicamentos (inibidores seletivos da recaptação da serotonina, benzodiazepínicos, drogas dopaminérgicas) (de la Hoz-Aizpurua *et al*., 2011). Oclusão (Demir *et al.*, 2004) e hábitos orais (Carra *et al.*, 2011) tem sido descritos como fatores desencadeantes do BS. Até mesmo a estrutura familiar das crianças pode ser importante e deve ser avaliada (Castelo *et al*., 2010).

Fatores relacionados à morfologia dos arcos dentários, anatomia e oclusão podem ser influenciadores do bruxismo, principalmente desvios entre a posição de contato retruída e a posição de intercuspidação, os quais seriam uma das causas mais comuns desta parafunção (Sujimoto *et al*., 2011). Segundo Behr *et al.* (2011), a maloclusão pode gerar redução da tonicidade dos músculos mastigatórios e na ausência de equilíbrio oclusal, a atividade neuromotora dos músculos é acionada por receptores periodontais que interpretam contatos prematuros como uma necessidade de retrair a posição da mandíbula, porém como não há estabilidade dos contatos oclusais, o movimento neuromotor é acionado constantemente para buscar a posição de repouso. Sendo assim, fatores morfológicos podem interferir nos aspectos funcionais do sistema mastigatório, mas a influência no bruxismo permanece controversa.

Estes aspectos funcionais incluem a respiração, a mastigação, a deglutição, a postura muscular, como postura dos lábios e língua (Stahl *et al*., 2007 e Grabowski *et al*., 2007) e são vitais ao organismo e consideradas funções orofaciais (Stahl *et al*., 2007), as quais são resultantes de atividades integradas ao sistema nervoso central e ao sistema neuromuscular (Lund, 1991) e atuam como base para a interação social relativa à fala, à comunicação emocional, à expressão facial e à aparência (Bakke *et al*., 2007). A alteração das respectivas funções pode causar desequilíbrio do sistema mastigatório em consequência, por exemplo, de hábitos parafuncionais (Stalh *et al*., 2007).

Atualmente os fatores psicológicos e patofisiológicos têm sido considerados como os principais envolvidos na ocorrência de bruxismo (Negoro *et al*., 1998, Lavigne *et al*.,2008; Serra-Negra *et al*., 2009). Por meio do relato dos pais, Cheifetz *et al*. (2005) observaram

que a presença de bruxismo em crianças estava relacionada à ansiedade e à história familiar da parafunção. A ansiedade tem tanto correlatos fisiológicos quanto psicológicos (Prins *et al*., 2011); as manifestações fisiológicas mais comuns são: aceleração da frequência cardíaca e respiratória, tremor, sudorese, diarréia e tensão muscular. A avaliação da ansiedade em crianças geralmente é feita por questionários de auto-relato, relato de pais / professores ou entrevistas padronizadas.

No entanto, os estudos não deixam clara a importância de cada um dos fatores (centrais e periféricos) no desenvolvimento do bruxismo, pela diversidade de formas de avaliação clínica e psicológica (Lobbezoo e Naeije, 2001; Marbach *et al*., 2003). Sob condições de estresse, existem evidências relativas à produção de tensão muscular aumentada ou hiperatividade muscular e estado de mioespasmo da musculatura estomatognática (Manns e Rocabado, 1998; Alamoudi, 2001).

Indivíduos expostos a situações estressantes apresentaram aumentos nos níveis de cortisol e catecolaminas, sendo que estas respostas visam preparar o corpo para enfrentar uma ameaça física ou psicológica, desviando o uso de glicose para o SNC, aumentando o débito cardíaco e suprimindo funções periféricas, não essenciais para o momento, como a função digestiva, imunológica e reprodutora (Lueken e Lemery, 2004). Tais reações são desencadeadas pela ativação do eixo hipotálamo-hipófise-adrenal e do sistema simpático adrenomedular que são responsáveis entre outras funções pela liberação de cortisol e catecolaminas, respectivamente (Hewig *et al*., 2008). Neste contexto, os biomarcadores salivares, tais como o cortisol, têm sido utilizados para avaliar reações de estresse frente a desafios físicos e/ou psicológicos (Takai *et al*., 2004; Nater *et al*., 2006).

Apesar de o bruxismo ser considerado um fenômeno temporário ou flutuante, a sua associação com sinais e sintomas de disfunção temporomandibular (DTM), assim como com outros hábitos parafuncionais, tem sido relatada na literatura (Barbosa *et al*., 2008; Pereira *et al*., 2009A). O bruxismo do sono e os hábitos parafuncionais que afetem o sistema mastigatório foram considerados fatores predisponentes que podem aumentar o risco de desenvolver DTM (Bonjardim *et al*., 2005).

O termo DTM é uma designação coletiva que envolve uma série de sinais e sintomas que afetam a musculatura mastigatória, a articulação temporomandibular e estruturas associadas. É uma desordem relativamente comum, mas pouco diagnosticada precocemente, de etiologia é multifatorial, e quando um indivíduo é exposto a dois ou mais fatores, ocorre um efeito sinérgico (Vanderas e Papagiannoulis, 2002). No entanto, o estabelecimento de indicadores de riscos para DTM permanece contraditório, especialmente na infância (Pereira *et al*., 2009A; Pereira *et al*., 2010).

Um dos possíveis mecanismos pelo qual o bruxismo poderia relacionar-se com o aparecimento de sinais e sintomas de DTM em crianças baseia-se na sobrecarga funcional do sistema estomatognático. O contato forçado involuntário entre as superfícies oclusais dos dentes durante movimentos não funcionais mandibulares poderia exceder a tolerância fisiológica das estruturas relacionadas, causando desgaste dentário, dor e, consequentemente, disfunção (Barbosa *et al*., 2008). Além disso, a presença de sinais e sintomas DTM também tem sido associada à ansiedade (Bonjardim *et al.*, 2005).

Considerando que a infância e adolescência são períodos dinâmicos no desenvolvimento, torna-se de importância a avaliação e diagnóstico de alterações que possam acometer indivíduos jovens. Sendo assim, os objetivos deste estudo foram avaliar as seguintes variáveis em crianças e adolescentes com bruxismo do sono:

- Características morfofuncionais da oclusão, funções orofaciais e DTM;

- Verificar a possível associação entre o bruxismo do sono, sintomas de ansiedade e níveis salivares de cortisol;

- A influência de características antropométricas, gênero e disfunção temporomandibular.

Esta Dissertação está baseada na Resolução CCPG UNICAMP/002/06 que regulamento o formato alternative 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). Sendo assim, esta Dissertação é composta de dois capítulos, conforme consta a seguir.

CAPÍTULO 1

Is sleeping bruxism related to temporomandibular disorders, malocclusion and orofacial function?

Submetido ao periódico Pediatric Dentistry em 15/02/2012

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Keywords: Disability evaluation; Malocclusion; Sleep Bruxism, Temporomandibular Joint Disorders

ABSTRACT

Purpose: This is a case-control study that aims to evaluate temporomandibular disorders (TMD), malocclusion, and orofacial dysfunction in subjects with sleep bruxism (SB), comparing them to subjects without SB. **Methods:** SB was considered according to the parents' report and the presence of dental wear facets. The case and control groups were comprised of 52 and 104 individuals, respectively, considering gender, age, and dentition phase. TMD was evaluated using the Research Diagnostic Criteria for Temporomandibular Disorders. The Dental Health Component of the Index of Orthodontic Treatment Need was used for malocclusion diagnostic. Orofacial dysfunctions were assessed using the Nordic Orofacial Test-Screening (NOT-S), which consists of an interview and a clinical examination. Data were analyzed using descriptive statistics, *t* independent tests, Mann-Whitney, and Chi-square/Fisher exact tests. **Results:** The prevalence of SB was higher in boys than girls ($P<0.05$). The presence of malocclusion and TMD was similar in both groups ($P>0.05$). Children with SB presented higher NOT-S scores than controls, as did girls in relation to boys. **Conclusion:** Malocclusion and TMD had no effect in BS. Orofacial dysfunction was more severe and prevalent in children and adolescents with SB, as habits and alterations in chewing, swallowing, and in masticatory muscles were the factors more related with SB.

INTRODUCTION

Sleep bruxism (SB) is a parafunctional oromotor habit that sometimes can pose a threat to the integrity of the structures of the masticatory system if the magnitude and direction of the forces exerted exceed the system's adaptive capacity.[1] This alteration may cause tooth wear and/or pain and other structural damage of the masticatory system, and can be considered as pathology.[2] The prevalence of bruxism varies in the literature, ranging from 5% to 38% in children and adolescents.[3-6]

The self-perception of this parafunction is poor, especially in children. A multifactorial etiology has been attributed to bruxism. Morphological disorders are considered a peripheral cause of bruxism.[7] When the occlusion is not equilibrated, motor neuron activity of masticatory muscles is triggered by periodontal receptors.[8] In cases of

bruxism, central[9] and influencing factors act as stimulants to the central nervous system, causing an alteration in the neurotransmission of dopamine,[10] resulting in tooth clenching or grinding.

In general, the influencing factors can be listed as temporomandibular disorders (TMD),[11] oral habits,[3,12] anxiety,[13] and stress.[14] One of the mechanisms by which bruxism may be related to signs and symptoms of TMD in children is based on the functional overload of the stomatognathic system. Malocclusion has been considered a peripheral cause of bruxism because it can reduce masticatory muscle tone. Moreover, in younger children, bruxism may be a consequence of masticatory neuromuscular system immaturity.[15] In addition, orofacial function is the result of complex activities of the central nervous system and the neuromuscular system;[16] consequently, the respective dysfunction can cause an imbalance of the stomatognathic system, due to, for example, parafunctional habits,[17] some diseases, and trauma.[18] Furthermore, TMD has been associated with psychological factors,[13] whereas oral parafunctions are commonplace among children and adolescents under the influence of stressful life events.[12]

Considering that symptoms of bruxism can persist into adulthood[2] and cause harmful effects in permanent dentition when bruxism develops early,[19] we consider it relevant to investigate the influence of TMD, malocclusion, and orofacial function on SB, which is the aim of the present study.

METHODS

The study was approved by the Ethics Committee of the Piracicaba Dental School, University of Campinas, Brazil. All children and their parents/guardians gave verbal and written permission for their children to participate in the research (protocol n. 034/2010).

The survey was conducted at four public schools in Piracicaba, SP, Brazil. The sample size was calculated by Epi info version 6.0.1 software. A standard error of 5%, a 95% confidence interval level, and a 20% prevalence of SB[20] were used for the calculation. The minimum sample size to satisfy the requirements of SB prevalence was estimated at 246 subjects. Three hundred and sixteen children and adolescents of both genders (138

boys and 178 girls) age 7 to 17 years old (mean age±SD: 10.64±2.24 yr) were evaluated in order to guarantee the minimal sample size. Fifty-six children and adolescents, 32 boys and 24 girls, were diagnosed with SB.

Study design

The design consisted of a case-control study to identify if malocclusion, TMD, and orofacial dysfunction are influencing factors on sleep bruxism. Children presenting SB were placed in the case group. The control group was composed of gender, age, and dentition phase-matched children, chosen from the 316 children initially evaluated but without SB, with a respective proportion of 1:2. In order to match the case and control groups, four individuals with SB were excluded due to the following causes: one girl presented severe obesity (14yr-old, IMC=37) and two girls presented a dentition phase discrepant to their chronologic age; consequently, those 3 girls did not have matched controls. In addition, one boy did not present the body variables data. Thus, 52 children and adolescents comprised the case group and 104 matched ones comprised the control group, totaling 156 children and adolescents (mean age 10.86±2.32 yr).

The inclusion criteria included at least the presence of erupted permanent incisors and first permanent molars. The exclusion criteria were extensive dental caries, early tooth loss, history of dental trauma, previous orthodontic treatment, systemic and/or mental developmental disorders (including diseases of the endocrine and metabolic systems), sleep disorders (obstructive apnea syndrome), and use of medications that could interfere with the central nervous system, as well as inappropriate behavior and/or refusing to cooperate with dental procedures and evaluation of the proposed variables.

Body weight and height were determined by a calibrated examiner with the use of an anthropometric scale and body mass index (BMI=weight/height^2).

Anamnesis and clinical examination

The information was obtained using a prestructured questionnaire sent to parents/caregivers with questions about parafunctional habits (nail biting, digital sucking, sleep bruxism, and mouth breathing).

Clinical examination was carried out in the schools and in well lit, using a light clinical mirror with LED (Lumin RG–Septodont) and millimeter probe; the examiners used adequate protection products. Three dentists (ABMM, FYK, and MCSM) were previously calibrated to perform the evaluations; two repeated measurements at an interval of 15 days were taken using 18 to 25 randomly selected children not included in this study.

Bruxism

The signs and symptoms of SB were recorded (ABMM) taking into account the following parameters, according to recommendations of the International Classification of Sleep Disorders:[20,21,22]

1. Sibling or parental report of grinding sounds (at least three times a week);

2. Presence of shiny and polish facets on incisors and/or first permanent molars (based primarily on palatal surface and incisal edges and working cusps, respectively, observed in clinical examination).

Temporomandibular disorders diagnosis

The clinical signs were assessed using the Research Diagnosis Criteria (RDC/TMD) Axis I (FYK),[23,24] including: pain on palpation, mandibular range of motion (mm), associated pain (e.g., jaw opening pattern, unassisted opening, maximum assisted opening, and mandibular excursive and protrusive movements), sounds from TMJ, and tenderness induced by muscle and joint palpation.

Occlusion

The Index of Orthodontic Treatment Need (IOTN)[25] was used to evaluate the dental components of the malocclusion (ABMM). The IOTN records the need for treatment based on the Dental Health Component (DHC), consisting in a scale with five scores of severity: 1 (no or little need for treatment) to 5 (great need for treatment). The malocclusion was observed by five characteristics: tooth loss, overjet, crossbite, displacement of the contact point, and overbite.[25,26,27]

Orofacial function

Orofacial function was evaluated using the Nordic Orofacial Test-Screening (NOT-S),[18] translated and culturally adapted to Brazilian Portuguese language by Leme et al.[28] This exam consists of a structured interview and clinical examination, each part with six domains. The domains assessed by interview are: (I) Sensory Function, (II) Breathing, (III) Habits, (IV) Chewing and Swallowing, (V) Drooling, and (IV) Dryness of the mouth. In the examination, the following functions are assessed: (1) Face at Rest, (2) Nose Breathing, (3) Facial Expression, (4) Masticatory Muscle and Jaw Function, (5) Oral Motor Function and (6) Speech. Each domain contains one to five items, reflecting the complexity of the specific function.

NOT-S was individually applied by the same researcher (MCSM). The NOT-S interview was performed by asking the questions in the screening form. To assess orofacial dysfunction in the clinical examination, the subjects were requested to perform tasks for each item. The clinical examination was carried out in conjunction with the illustrated manual (www.mun-h-centre.se). Each item has criteria for the respective function. An answer of YES or a task that met the criteria for impaired function resulted in a score of 1, indicating a dysfunction in the scored domain. An answer of NO or a task that did not meet the criteria resulted in a score of 0. The total score was the sum of the score for each domain and ranged from 0 to 12.

Measurements errors

The consistency of intraexaminer reproducibility was assessed using intraclass correlation coefficient (ICC) for body measurements, Kappa statistics for TMD signs, clinical diagnosis of sleep bruxism, Pearson correlation coefficient for IOTN, and Spearman correlation coefficient for NOT-S interview, examination, and total scores (Chart 1). Assessment of method error was calculated in 18 to 25 randomly selected children, who were not included in the studied sample. For this, the measurements were repeated at interval of 15 days (Chart 1).

For assessment of method error of the clinical variables, the intraclass correlation coefficient (ICC) (body measures), Kappa test (TMD signs and sleep bruxism clinical

diagnosis), Pearson correlation coefficient (IOTN), and Spearman correlation coefficient (NOT-S interview, clinical, and total score) were calculated from subjects not included in the studied sample in two separate occasions at an interval of 14 days (BioEstat 5.0; Mamirauá, Belém, PA, Brazil) (Chart 1).

Chart 1. Method error of the clinical variables assessed by means of intraclass correlation coefficients (ICC) and Kappa test from subjects not included in the studied sample

Variable	Subjects (n)	ICC	Kappa values (interpretation)	Pearson coefficient (*p*-value)	Spearman coefficient (*p*-value)
Weight	25	0.85	-	-	-
Height	25	0.91	-	-	-
Mouth opening	25	-	0.92 (excellent)	-	-
Pain on palpation (right masseter)	25	-	0.67 (good)	-	-
Sleep bruxism (wear facets)	18	-	0.77 (good)	-	-
IOTN	25	-	-	0.69 (<.001)	-
NOT-S interview score	25	-	-	-	0.65 (<.001)
NOT-S clinical examination score	25	-	-	-	0.72 (<.001)
NOT-S total score	25	-	-	-	0.75 (<.001)

Statistical analysis

The data normality was checked by D'Agostino-Pearson test. Descriptive statistics were performed considering range values, median, means, standard deviations, and standard errors of the means. For comparing values between groups, *t* independent tests or Mann-Whitney tests were applied when indicated. For comparing proportions between groups and genders, Chi-square or Fisher's exact tests were used. All statistical tests were

carried out using SPSS 19.0 software (SPSS, Inc, Chicago, IL) at a significance level of 5%.

RESULTS

Sample characteristics

The descriptive data are presented in Table 1. From 52 children and adolescents diagnosed with sleep bruxism, 60% were boys. Girls were significantly heavier than boys in both groups ($p=0.04$), but the height was similar; consequently the BMI was significantly greater for girls ($p<0.05$).

The TMD prevalence was similar for both genders and groups (Fisher exact and Chi-square tests, $p>0.05$), and no differences between groups and genders for IOTN classification were found, which suggests that there is no association between SB and TMD or malocclusion, respectively. However, NOT-S scores presented significant differences between genders in both group, with higher values for girls in the control group for total NOT-S scores ($p=0.01$) and for interview domain scores in both groups ($p=0.01$).

Table 1. Descriptive data for age, body variables, TMD, IOTN, NOT-S considering statistical differences between genders

		Case group			Control group		
		Boys (n=31)	Girls (n=21)		Boys (n=62)	Girls (n=42)	
Age	Mean±SD	10.45±2.14	11.29±2.39	*P*=.19*	10.56±2.14	11.38±2.60	*P*=.26*
	Median	11	11		11	11	
	Range	7-16	7-16		7-16	7-16	
Weight	Mean±SD	**38.11±12.06**	**46.03±15.72**	*P*=.04*	**39.34±14.3**	**45.06±13.87**	*P*=.04*
	Median	34.4	45.1		35.95	44.85	
	Range	24.9-72.9	20.6-83.2		21.9-98.7	24-84.2	
Height	Mean±SD	1.44±0.14	1.48±0.12	*P*=.35*	1.44±0.13	1.49±0.12	*P*=.06*
	Median	1.4	1.49		1.45	1.49	
	Range	1.26-1.71	1.64		1.21-1.78	1.22-1.68	
BMI	Mean±SD	**17.94±3.12**	**20.68±5.35**	*P*=.03†	**18.47±4.19**	**19.95±3.96**	*P*=.007†
	Median	16.83	21.45		16.74	18.90	
	Range	14.32-27.44	13.18-33.33		14.23-31.15	13.69-30.93	
With TMD		n=4	n=4		n=9	n=9	
IOTN	Mean±SD	2.29±1.30	2.76±0.10	*P*=.10†	2.53±1.02	2.67±1.05	*P*=.57†
	Median	2	2		2	2.5	
	Range	1-5	1-4		1-4	1-5	
NOT-S Interview	Mean±SD	**1.87±1.09**	**2.62±1.02**	*P*=.01†	**1.65±0.96**	**2.14±1.05**	*P*=.01†
	Median	2	2		2	2	
	Range	0-4	1-5		0-5	0-4	
NOT-S Examination	Mean±SD	1.10±0.98	1.10±1.18	*P*=.77†	0.69±0.74	0.67±0.72	*P*=.87†
	Median	1	1		1	1	
	Range	0-4	0-4		0-3	0-3	
NOT-S total	Mean±SD	2.97±1.38	3.71±1.71	*P*=.24†	**2.34±1.38**	**2.81±1.02**	*P*=.01†
	Median	3	3		2	3	
	Range	1-6	2-7		0-7	1-5	

BMI, body mass index; TMD, temporomandibular disorder; IOTN, index of orthodontic treatment need; NOT-S, The Nordic Orofacial Test-Screaning.
Values in bold mean statistical significant difference between genders within each group
* t-test † Mann Whitney

Furthermore, NOT-S scores were compared between groups and genders. It was observed that boys in the case group presented higher scores in their NOT-S examinations than those in the control group (p=0.03); this finding was not seen in girls. However, in NOT-S total scores boys and girls in the case group had values significantly higher than their controls (p<0.05). Pooling genders, subjects with bruxism presented higher scores for all NOT-S categories than the controls (Table 2).

Table 2. Descriptive analyses for NOT-S scores considering differences between case group and control group

Group	Case	Control	Case	Control	Case	Control
	Boys	Boys	girls	girls	n=52	n=104
NOT-S	n=31	n=62	n=21	n=42		
Interview	1.87±1.09	1.65±0.96	2.62±1.02	2.14±1.05	**2.17±1.06**	**1.85±1.02**
	P=.30		*P*=.16		*P*=.04	
Examination	**1.10±0.98**	**0.69±0.74**	1.10±1.18	0.67±0.72	**1.10±0.91**	**0.68±0.73**
	P=.03		*P*=.25		*P*=.01	
Total	**2.97±1.38**	**2.34±1.38**	**3.71±1.71**	**2.81±1.02**	**3.27±1.35**	**2.53±1.26**
	P=.01		*P*=.03		*P*=.002	

NOT-S, The Nordic Orofacial Test-Screaning; NOT-S I-VI, interview; NOT-S 1-6, exam
Values in bold mean statistical significant difference between case and control groups

Since there was a difference in NOT-S scores between groups, different scores for both interview and examination domains are presented in Table 3. For both groups the item "Chewing and swallowing" in the interview was significantly more prevalent for girls; in the control group the item "Masticatory muscle and jaw function" in the examination was also more prevalent for girls. In both groups the domains "Habits," "Chewing and swallowing," and "Dryness of the mouth" in the interview were the most prevalent, and proportions in the case group were higher than the control group for "Habits," "Chewing and swallowing," and "Dryness of the mouth." Examination scores varied in the two groups and only the score for "Masticatory muscle and jaw function" was more prevalent in case group than the control one.

Table 3 – Distribution of children (%) for NOT-S scores in accordance with groups and genders

	Group	Case			Control			Case	Control	
		Boys n=31	Girls n=21		Boys n=62	Girls n=42		Total n=52	Total n=104	
Interview	Sensory function	3(10)	4(19)†	*P*>.05	4(6)	1(2)†	† *P*=.03	7(13)	5 (5)	*P*>.05
	Breathing	4(13)	4(19)	*P*>.05	8(13)	2(5)	*P*>.05	8(15)	10 (10)	*P*>.05
	Habits	19(61)	14(67)†	*P*>.05	32(52)	15(36)†	† *P=.04*	33(63)‡	47(45)‡	‡*P*=.04
	Chewing and swallowing	20(65)*	19(90)*†	**P*=.049	41(66)*	16(38)*†	* *P*=.008 † *P*=.001	39 (75)‡	57(55)‡	‡*P*=.02
	Drooling	0	0		0	0		0	0	
	Dryness of the mouth	12(39)	14(67)†	*P*<.05	17(27)	14(33)†	† *P* =.003	26 (50)‡	31(30)‡	‡*P*=.02
Examination	Face at rest	4(13)	5(24)†	*P*>.05	8(13)	2(5)†	† *P*=.03	9(17)	10 (10)	*P*>.05
	Nose breathing	8(26)	2(10)	*P*>.05	6(10)	3(7)	*P*>.05	10(19)	9 (9)	*P*>.05
	Facial expression	6(19)	8(38)	*P*>.05	8(13)	7(17)	*P*>.05	14(27)	15 (14)	*P*>.05
	Masticatory muscle and jaw function	10(32)	6(29)†	*P*>.05	12(19)*	2(5)*†	**P*=.04 † *P*=.013	16(31)‡	14(13)‡	‡*P*=.018
	Oral motor function	619)	1(5)	*P*>.05	5(8)	0	*P*>.05	7 (13)	5 (5)	*P*>.05
	Speech	0	1(5)	*P*>.05	4(6)	0	*P*>.05	1(2)	4 (4)	*P*>.05

† means significant difference between girls of Case and Control groups (there was no significant differences between boys of both groups)

* means significant difference between boys and girls within each group

‡ means significant difference between Case and Control groups

DISCUSSION

Sleep bruxism has a multifactorial etiology, including pathophysiologic, psychological, and morphologic factors.[15] The diagnostic of bruxism has been considered reliable when parents report teeth-grinding or teeth-clenching during sleep[20] as a minimal criterion. We attempted to use this indication, since the parents answered the questionnaire about parafunctional habits. Moreover, the episodes of bruxism should be occurring three times a week and confirmed by permanent tooth wear, since the latter is a cumulative sign, guaranteeing the precise diagnosis by two criteria, as previously performed[22,29,30] and recommended by The American Academy of Sleep Medicine.[20]

The sample was selected randomly, and from 316 children and adolescents who met the inclusion and exclusion criteria, 52 presented a diagnosis of sleep bruxism, with a higher prevalence of boys (60%). These results are in agreement with Lam et al.,[6] who observed that bruxism was more prevalent among Chinese boys in an epidemiologic study. Serra-Negra et al.[5] found a prevalence of 56.5% for girls, whereas in adults homogeneous gender distributions have been considered by Shetty et al.[31] in a literature review. These controversial results show that the multifactorial aspect of bruxism can play a role in study designs, considering different methodologies for dealing with this condition.

The occurrence of TMD was very low in both case and control groups, showing a similar proportion between them. For the diagnosis of this condition RDC/TMD was used; it presents good to excellent reliability for the clinical examination of children and adolescents.[15] Usually, the association of bruxism and TMD has been investigated with signs and symptoms of TMD. Using RDC/TMD, a more precise diagnosis can be achieved. Because of this, the TMD prevalence in the present study was low, considering the sample age and the implied multifactorial aspects.

In this context, an increase in the prevalence of TMD symptoms has been noted over a 20-year period by Köhler et al.[32] Thus, different diagnosis criteria for TMD and bruxism have been influential in comparing different studies that have difficulty finding reliable evidence.[15] In the present study there was no significant relationship between TMD and SB. Investigations based in only one criterion of a bruxism diagnosis showed a positive

association with TMD;[4,33] however, when the studies have been taken into account more quantitative and specific methods of diagnosis, the association with SB and TMD was lower.[34]

In relation to IONT scores, no significant differences were found between groups and gender, showing that malocclusion was not an influencing factor. Similar results were found by Demir et al.[29] in 7-to-19-year-old Turkish subjects. Agreeing with findings of the present study, the lack of a relationship between bruxism and characteristics of occlusion was also found by Cheng et al.[35] in children during mixed dentition, despite bruxism having been diagnosed only by questionnaires given to parents. These authors observed that occlusion and mixed dentition do not increase the incidence of bruxism in children. The fact that this is a cross-sectional study could explain the results about the influence of malocclusion upon bruxism because the evaluation was done at a determined time, suggesting the need for longitudinal studies to confirm the respective influence.

It must also be taken into account that the present study's sample was randomly selected (i.e., the subjects were not seeing treatment for a specific oral condition, such as bruxism or malocclusion); in addition the IOTN scores were low, showing slight malocclusion severity. In a 7-to-17-yr-old population seeking orthodontic treatment, Carra et al.[11] recommend that the clinical assessment of sleep parafunctions must be taken into account during the planning of orthodontic treatment, since parafunctions were associated with signs and symptoms suggestive of TMD.

Despite the present result and controversies in the literature about peripheral influential factors on bruxism, the occlusion characteristics must not be discarded, because they determine the localization of biomechanical transmission, and functional patterns of the masticatory muscles are regulated via the receptors of the periodontal apparatus.[7] During malocclusion, premature and one-sided contact can be registered. Receptors may interpret this contact in such a way that the mandible needs to be retracted to the resting position by muscular activity. If assuming a final closing position is not possible because of malocclusion, movement patterns in the motor cortex are constantly triggered in an attempt to achieve the resting position.[36]

According to the present findings, orofacial dysfunction was the only variable involved with bruxism, because NOT-S scores presented significant differences between groups. The most prevalent items in the interview domain were "Habits," "Chewing and swallowing," and "Dryness of the mouth," with higher proportions in the case group. These findings showed that habits are frequent in the studied sample, as is difficulty eating hard foods. The last one was confirmed during the examination, when it a higher prevalence of problems related to masticatory muscle and jaw function was observed. Moreover, for evaluation of dryness of the mouth, most children answered yes to the question, "Do you have to drink to eat a cracker?" This confirmed difficulty in eating hard foods. The high prevalence of "Habits" and "Chewing and swallowing" is in agreement with Strini et al.[37] Bakke et al.[18] also observed a high frequency of item "Habits" in healthy subjects, but "Chewing and swallowing" was the most prevalent for the clinically referred sample. The high prevalence of habits in children could be related to emotional imbalances, considering the sample age, and probably could be a influential factor in the establishment of bruxism, since there was significantly higher prevalence of habits in the case group.

Despite that, the respective scores were low, ranging from 2.5 in the control group to 3.3 in the case group, which agrees with Gustavsson et al.[38] and Strini et al.,[37] who verified low scores among different age groups. It is important to point out that individuals with oral disabilities reported impacts on emotional and social welfare from being exposed to stressful situations,[12,37,39] which can influence the development of bruxism, since it is regulated centrally and influenced peripherally. On the other hand, the NOT-S values were lower than the ones found by Bake et al.[18] The respective difference can be due to the sample characteristics—i.e., our sample was comprised of healthy individuals,[37,39] whereas in the Bake et al.[18] study the sample came from centers for specialized dental care and clinics for speech pathology.

It is also important to consider that the NOT-S interview is a subjective evaluation, and the questions about habits and breathing must be performed by specially trained professionals in order to reach a specific diagnosis. However, NOT-S is specific for epidemiological studies and shows good validity and reliability when assessing orofacial function.[18] Thus, these limitations must be considered to avoid overestimating the results.

Differences between genders for NOT-S scores (Tables 1 and 2) showed greater values for boys, which can be attributed to a higher respective bruxism prevalence. Other studies that used NOT-S protocol did not show a difference between genders.[37,38] It was observed that the proportion of girls who presented several compromised NOT-S domains was significantly higher than their controls. This finding was not observed for boys in both groups. As only the domain "Chewing and swallowing" showed a significant proportion for girls in the case group, it is possible to infer that despite the lower prevalence of bruxism for girls found in the present study, orofacial dysfunction can be a large contributor to the presence of bruxism in the female gender.

In addition, individuals with impaired oral conditions reported damage in situations of emotional stress,[12] which may influence bruxism development. The timing of the attrition, habits, and signs and symptoms of TMD are also important because there is a risk of recording no alterations when the subjects have recently begun a habit and may not yet show signs. The same risk exists if habits have stopped but a sign is observed. Long-term studies may be necessary to determine the association between childhood parafunctional habits and the development of future TMD.

CONCLUSIONS

- SB was not related to malocclusion and TMD.

- SB was related to orofacial function, and the respective dysfunction was more severe and prevalent in children and adolescents with SB. Oral habits, alterations in chewing, swallowing, and in masticatory muscles were the influencing orofacial factors related to SB.

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CAPÍTULO 2

"*Evaluation of sleep bruxism, temporomandibular disorder, anxiety and salivary cortisol levels in children and adolescents*"

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ABSTRACT

Sleep bruxism (SB) is a parafunction of the masticatory system characterized by grinding or clenching of the teeth. The literature suggests the influence of psychological factors on the etiology of this parafunction; however, this relationship still remains inconclusive. Moreover, a causal relationship between SB and temporomandibular disorders (TMD) has been suggested. **Objectives:** This study aims to evaluate the associations between SB and psychological factors (symptoms of anxiety and salivary cortisol level as a biomarker of stress), TMD, gender, and anthropometric characteristics in 308 Brazilian children and adolescents age 7 to 17 years. **Methods:** SB diagnosis was confirmed by both the parental report and the presence of tooth wear. The Research Diagnostic Criteria (RDC/TMD) was used to evaluate signs and symptoms of TMD. Symptoms of anxiety were assessed using the Brazilian version of the Multidimensional Anxiety Scale for Children (MASC). Saliva samples were collected four times a day in two alternate days, and the area under the curve was determined (AUC). Data were analyzed using Chi-square, Mann–Whitney/Kruskal-Wallis, and Spearman correlation tests. Cronbach's alpha (α) and intraclass correlation coefficient (ICC) were calculated to test the reliability of MASC. Multiple logistic regression was used to verify the associations between SB and other variables (gender, age, body mass index, MASC scores, TMD diagnosis, and values of AUC salivary cortisol). **Results:** MASC showed good internal (α=0.88) and excellent external (ICC=0.86) reliability. Participants with TMD presented higher MASC total scores compared to those with SB and the controls, proving MASC validity. Girls presented higher MASC total and domain scores ($p<0.05$ and $p<0.001$) and AUC salivary cortisol ($p<0.01$) than boys. There were negative correlations between age and MASC total scores ($r=-0.17$; $p<0.01$). Values of AUC salivary cortisol did not differ between bruxists and controls ($p>0.05$), and this parafunction was just associated with the male gender (OR=0.49; $p<0.05$). **Conclusions:** Children and adolescents with TMD presented higher anxiety levels than those with SB and controls. Moreover, females and younger children also reported more symptoms of anxiety. Salivary cortisol levels did not differ between bruxists and controls, but were significantly higher in older females than males. On the other hand, males were more likely to present SB.

Keywords: Anxiety**,** Hydrocortisone**,** Saliva**,** Sleep bruxism, Temporomandibular Joint Disorders

INTRODUCTION

Sleep bruxism (SB) is a very common parafunction of the masticatory system and is defined as "an oral parafunction characterized by grinding or clenching of the teeth during sleep that is associated with an excessive (intense) sleep arousal activity" (American Academy of Sleep Medicine, 2001). A multifactorial hypothesis of the genesis and pathogenesis of SB has been proposed in the literature, which includes genetics, sleep structure (micro-arousals), psychological factors (e.g., emotional distress and anxiety), the catecholaminergic balance of the central nervous system (CNS), drugs (e.g., ecstasy, alcohol, caffeine, tobacco), and medications (e.g., selective serotonin reuptake inhibitors, benzodiazepines, dopaminergic drugs) (de la Hoz-Aizpura et al., 2011). Also, malocclusions and respiratory disorders have been described as triggering factors of SB (Monaco et al., 2002; Seraidarian et al., 2009), as well as children´s family structure (Castelo et al., 2010).

The influence of psychological factors on SB development is still controversial in the literature. Carra et al. (2011) found associations between SB and some behavioral complaints, such as manifestation of specific psychosocial factors, or personality traits, such as thought, conduct, and antisocial disorders. Other personality traits, such as neuroticism, perfectionism, aggressiveness, higher sensitivity to stress, and maladaptative coping strategies also seem to be related to an increased risk of developing SB (Restrepo et al., 2008; Serra-Negra et al., 2009). Associations between anxiety and signs and symptoms of SB in children have also been observed (Manfredini et al., 2004; Cheifetz et al., 2005; Gungormus et al., 2009; Serra-Negra et al., 2009). Anxiety is a common mental disorder that causes considerable emotional and physical suffering, often resulting in severe disability (Prins et al., 2011). Anxiety has both physiological and psychological correlates; common physiological manifestations are acceleration of heart and breathing rates, tremors, sweating, diarrhea, and muscle tension. The feelings may be accompanied or entirely replaced by physiological sensations. The methods recommended for measuring anxiety in children are self-reporting questionnaires, parent/teacher reporting, or standardized

interviews. Self-reporting may be the first option that can be eventually complemented by proxy reports (Barbosa and Gavião, 2008).

SB is also considered a physical response to emotional stress. However, this relationship still remains inconclusive in the literature; while some studies found associations between stressful life events and SB (Tahara et al., 2007), others found no associations (Grechi et al., 2008; Emodi-Perlman et al., 2012; Castelo et al., 2010). Recently, salivary biomarkers such as cortisol have been used to assess stress reactions (Takai et al., 2004; Nater et al., 2006). Individuals exposed to stressful situations showed increases in cortisol and cathecolamines, which prepare the body to face the physical or psychological threats, deviate glucose to the central nervous system, increase the cardiac output, and suppress nonessential peripheral functions, such as the as digestive, immune, and reproductive systems (Lueken and Lemery, 2004). Such reactions are triggered by the activation of hypothalamic–pituitary–adrenal axis and sympatho-adrenomedullary (SAM) system, which are responsible among other functions for the release of cortisol and catecholamines, respectively. Makino et al. (2009) found a negative correlation between SB and salivary stress biomarkers (chromogranin A – CgA). Tahara et al. (2007) also observed this relationship while evaluating salivary cortisol levels.

Salivary cortisol (SC) is measured by a noninvasive and easy procedure (being collected even at home) and can be obtained many times a day in order to provide information for physiological and diagnostic studies (Castro et al., 2000). Changes in salivary cortisol concentrations can be observed during the first hours after waking, and the peak is reached 30–45 minutes post-awakening, with decreasing concentrations thereafter, and is very low in the evening and at night (Strini et al., 2011). Cortisol has a predictable pattern of circadian fluctuations and demonstrates diurnal (light and dark) variations in adults and children (Hershel et al., 1998). Salivary cortisol levels may vary in response to a number of factors, including genetic differences, age/developmental stage/pubertal stage, sex, weight, temperament, coping style, social competence, and pain sensitivity (McCarthy et al., 2009)

Furthermore, SB and psychological conditions that could affect the masticatory system are considered contributory factors for temporomandibular disorders (TMD), since

they may increase the risk of developing signs and symptoms of TMD (Bonjardim et al., 2005; Barbosa et al., 2008). The presence of TMD has a multifactorial etiology, and when an individual is exposed to two or more factors, a synergistic effect occurs (Vanderas and Papagiannoulis, 2002). However, the correlation between risk indicators for TMD remains contradictory, especially in childhood (Pereira et al., 2009; Pereira et al., 2010). Bonjardim et al. (2009) found a relationship between TMD and anxiety in young adults. However, there are few studies covering the relationship between TMD and anxiety in childhood. Barbosa et al. (2008) suggested that prevention of parafunctional habits in young children is important to avoid related TMD problems.

Therefore, the aim of this study was to assess the relationship between SB and TMD, symptoms of anxiety, and salivary cortisol levels as a biomarker of stress. Moreover, the association with covariables, such as anthropometric characteristics, age, and gender was also evaluated.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of the Dental School of Piracicaba, State University of Campinas, Brazil, and all children and their parents/guardians gave verbal and written permission to carry out the research (protocol n. 034/2010).

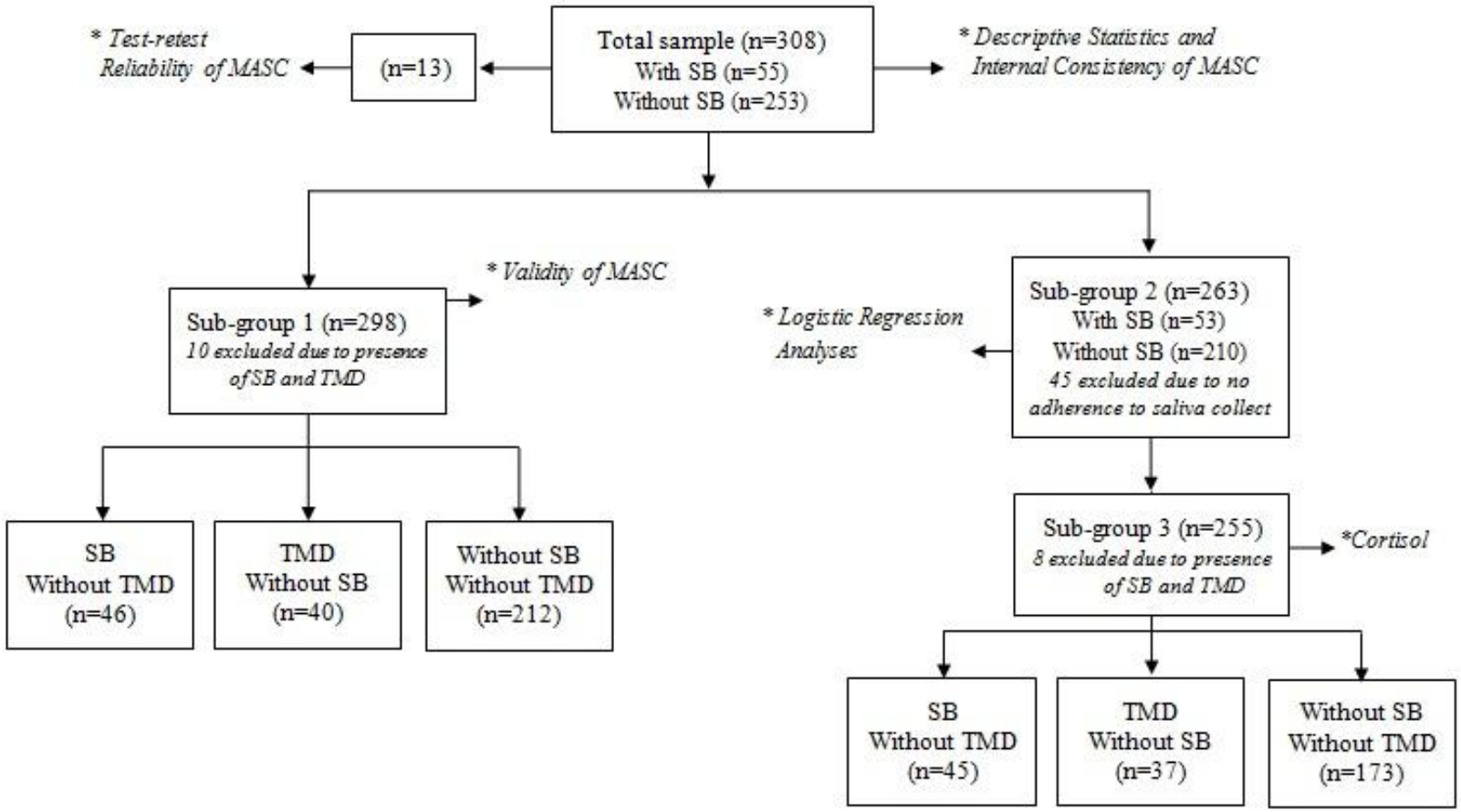
Study design

A cross-sectional study with students in public schools in Piracicaba, Brazil, was developed. Piracicaba has 50,187 students enrolled in the elementary school system (www.ibge.gov.br). The sample size was calculated by Epi info version 6.0.1 software. A standard error of 5%, a 95% confidence interval level, and a 20% prevalence of SB (American Academy of Sleep Medicine, 2001) were used for the calculation. The minimum sample size to satisfy the requirements was estimated at 246 subjects. Three hundred and eight children and adolescents of both genders (137 boys and 171 girls) age 7 to 17 years old (mean age ± SD: 10.64 ± 2.23 yr) were evaluated in order to guarantee the minimal sample size. Fifty-five were diagnosed as sleeping bruxists (31 boys and 24 girls). From the

total sample (n=308), subgroups were formed in accordance with the number of individuals who filled the criteria for data analysis of each variable (Figure 1). Comparisons related to anxiety and salivary cortisol were performed considering two age groups: 7-11 years and 12-17 years.

The inclusion criteria were the presence of erupted permanent incisors and first permanent molars. The exclusion criteria were extensive dental caries, early tooth loss, history of dental trauma, and previous orthodontic treatment, systemic and/or mental developmental disorders (including diseases of the endocrine and metabolic systems), and use of medications that could interfere with the central nervous system (e.g., antidepressants, muscle relaxants, narcotics, or non-steroidal anti-inflammatory drugs), as well inappropriate behavior and/or refusing to cooperate in dental procedures and evaluations of proposed variables.

Body weight and height were determined by a calibrated examiner with the use of an anthropometric scale, and body mass index (BMI=weight/heigth^2) was calculated.



*means the analyzed variable in each group
SB, sleep bruxism; TMD, temporomandibular disorder

Figure 1 – Sample distribution in accordance with data analysis

Anamnesis and clinical examination

The interviews were conducted using a pre-structured questionnaire sent to parents/caregivers with questions about parafunctional habits (e.g., nail biting, digital sucking, sleep bruxism, mouth breathing, and enuresis nocturna) and behavioral characteristics (e.g., agitation, irritation, calm, anxiety, attention, happiness, and sadness).

Clinical examination was carried out in the schools in well lit, using light clinical mirrors with LED (Lumin RG–Septodont) and millimeter probes, while the examiner used adequate protection products. All evaluations were performed by calibrated dentists.

Sleep bruxism

The signs and symptoms of SB were recorded (A.B.M.M.) taking into account the following parameters (Koyano et al., 2008; Castelo et al., 2010):

1. Sibling or parental report of grinding sounds (at least three times a week);

2. Presence of shiny and polished facets on incisors and/or first permanent molars (based primarily on palatal surface and incisal edges and working cusps, respectively, observed in clinical examination).

The presence of SB was confirmed by both the parental report and the presence of tooth wear, since the latter is a cumulative sign.

Temporomandibular disorders diagnosis

The clinical signs were assessed using the Research Diagnosis Criteria (RDC/TMD) Axis I (FYK) (Dworkin and Leresche, 1992; Pereira et al., 2004), including: pain on palpation, mandibular range of motion (mm), associated pain (jaw opening pattern, unassisted opening, maximum assisted opening, and mandibular excursive and protrusive movements), sounds from the TMJ, and tenderness induced by muscle and joint palpation.

Anxiety questionnaire

Anxiety symptoms were evaluated using the Portuguese version of the Multidimensional Anxiety Scale for Children (MASC) (Nunes, 2004; Vianna, 2008), which was originally developed by March et al. (1997). It is a 39-item 4-point Likert-style self-reporting scale ranging from "never true" (score 0) to "often true" (score 3) that has undergone extensive psychometric evaluation. Four scales were derived empirically through principal components analysis: Social Anxiety (9 items), Separation Anxiety (9 items), Harm Avoidance (9 items), and Physical Symptoms (12 items) (appendix 4).

Salivary cortisol

Saliva collection

Home-stimulated saliva samples were collected 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 a.m. on a weekday. 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, and the last sample was taken at night (at 8 p.m.). The samples were stored in a refrigerator and delivered to the researcher the following day at the schools. Samples were transported on ice to the laboratory on the same day and were centrifuged (at 3500 rpm for 5 minutes) and stored in a freezer at –40˚C until analysis.

Subjects were instructed not perform physical exercise or ingest any caffeinated beverages a day earlier. They were also told to abstain from food, beverages, and their brushing teeth at the time of sampling. They were only allowed 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 (Grajeda and Pérez Escamilla, 2002).

Salivary cortisol analysis

The salivary cortisol was assayed using a commercial, highly sensitive enzyme immunoassay kit (product no. 1-1102; Salimetrics, State College, PA, USA) according to the manufacturer's directions. Were pipette 25 µl of standards, controls, and whole saliva in the microtiter plate in the sequence (Raff et al., 2003; Kunz-Ebrecht et al., 2004).

Afterward, reagent solutions were pipette in sequence, following the incubation time of each one. After the whole process, a yellow color was obtained read the absorbance of the solution at 450 nm in a microplate reader (Stat Fax 2100, Awareness Tech. Inc., Palm City, FL, USA). Cortisol levels were expressed as the area under the curve (AUC) in relation to the ground (time).

Measurements errors

For assessment of method error of the clinical variables, the intraclass correlation coefficient (ICC) (body measures) and Kappa test (TMD signs and sleep bruxism clinical diagnosis) were calculated from subjects not included in the studied sample in two separate occasions at an interval of 14 days (BioEstat 5.0; Mamirauá, Belém, PA, Brazil) (Table 1).

The ICCs for weight and height were 0.84 and 0.91, both indicating excellent agreement. A high level of reliability was also found for clinical sign of SB, with a Kappa value of 0.77 (good agreement). Kappa values for clinical signs of TMD ranged from 0.67 for pain and palpation to 0.92 for mouth opening, indicating good to excellent agreement, respectively.

Table 1. Method error of the clinical variables assessed by means of intraclass correlation coefficients (ICC) and Kappa test from subjects not included in the studied sample.

Variable	Subjects included (n)	ICC	Kappa values (interpretation)
Weight	25	0.85	-
Height	25	0.91	-
Mouth opening	25	-	0.92 (excellent)
Pain on palpation (right masseter)	25	-	0.67 (good)
Sleep bruxism (wear facets)	18	-	0.77 (good)

Statistical analysis

Statistical analysis was performed using Sigma Stat 3.1 (Sigma Stat Software Inc., Richmond, CA, USA), BioEstat 5.0 (Mamirauá, Belém, PA, Brazil), and SPSS 9.0 (SPSS, Chicago, IL, USA) with a 5% significance level. Normality was assessed using the

Kolmogorov-Smirnov and Shapiro-Wilk tests. Because the score distributions were asymmetrical, non-parametric tests were used in the analyses performed. Percentages, means, standard deviations, medians, and interquartile ranges were calculated for descriptive statistics. Chi-square and Mann-Whitney tests were applied to compare proportions and means between groups, respectively.

The area under the curve (AUC) of salivary cortisol concentrations against time was calculated by the trapezoid method respective to the ground level (Pruessner et al., 2003). Data for AUC salivary cortisol were not normally distributed and though log-transformations were applied, normality was not achieved; thus, a Kruskal-Wallis test was used to test the differences in values of AUC salivary cortisol between children with SB and without TMD (n=45), with TMD and without SB (n=37), and without SB and TMD (n=173). Differences in AUC salivary cortisol values were also assessed between genders using the Mann-Whitney test.

Psychometric properties of MASC

Psychometric properties of MASC were assessed by validity and reliability tests. Data used to test internal reliability were obtained from all participants (n=316), and 13 answered a second copy of the questionnaire two weeks later to assess the external reliability. To evaluate the validity of MASC, participants were divided in the following clinical groups: with SB and without TMD (n=47), with TMD and without SB (n=44), and without SB and TMD (n=216).

MASC total scores for each participant were calculated by summing the item codes, whereas the subscale scores were obtained by summing the codes for questions within the four health domains. The validity of a questionnaire represents the degree to which it measures what it is meant to measure. Discriminant construct validity was evaluated by comparing the mean scale scores between the two clinical groups (TMD and bruxism) and between the clinical and control groups using the Kruskal-Wallis test. MASC total and domain scores were also compared between genders using the Mann-Whitney test. Moreover, correlations between MASC scores and age were assessed using the Spearman coefficient.

Reliability can be defined as a measure of the internal consistency or homogeneity of the items. Two measures were used for the analysis of internal reliability: the corrected item total correlation and the Cronbach's alpha coefficient (Cronbach, 1951). Values above 0.2 for the former and 0.7 for the latter are acceptable (Streiner and Norman, 1991). Alphas were also calculated with each item deleted. Test-retest reliability was assessed by means of ICC, calculated by the one-way analysis of variance random effects parallel model (Shrout and Fleiss, 1979).

Logistic regression analysis

A backward stepwise logistic regression model was used to determine the relationship between the presence of signs and symptoms of SB as the dependent variable and the following independent variables: gender (male=0; female=1), age (in years), BMI (continuous variable), TMD diagnosis (no=0; yes=1), MASC scores (≤ median=0; > median=1), and values of AUC salivary cortisol (≤ median=0; > median=1).

RESULTS

Sample characteristics

As demonstrated in Figure 1, 31 boys (56.0%) and 24 girls (44.0%) presented SB, but the respective proportion between genders was not statistically significant ($p>0.05$, Chi-square test). In the entire sample, there were no significant differences between subjects with or without SB in relation to age and BMI ($p>0.05$, Mann-Whitney test) (Table 2). Also, the number of individuals with TMD did not differ between groups ($p>0.05$, Chi-square test).

Table 2 - Demographic characteristics between groups with and without sleep bruxism (n=308).

Clinical groups	n	Gender (♂/♀)	Age (y) Mean (SD)	BMI (Kg/m^2) Mean (SD)	With TMD n (%)
With SB	55	31/24	10.8 (2.3)	19.3 (4.9)	9 (16.4)
Without SB	253	106/147	10.6 (2.2)	19.0 (4.3)	41 (16.2)

SB, sleep bruxism; BMI, body mass index; SD, standard deviation; TMD, temporomandibular disorder
p>0.05

MASC Feasibility, Measurement Sensitivity and Reliability

The total scale score ranged from 0 to 116, with a mean of 49.5±19.1, indicating that the MASC detected substantial variability in anxiety levels (Table 3). There were two children with floor effect (score=0) and no participant with ceiling effect (score=117). The domain scores also showed substantial variability that is, there were children with zero and maximum scores, except for physical symptoms that present no ceiling effect (score=36).

Cronbach's alpha for MASC, on the whole, was good (0.88). For the domains, the coefficients ranged from 0.60 for harm avoidance to 0.82 for physical symptoms, indicating questionable to good levels of internal consistency. For testing the external reliability of the MASC, 13 individuals answered a second copy of the questionnaire. The ICC (external reliability) was 0.86 for the total MASC scores, indicating excellent agreement, and for the domains it ranged from 0.37 to 0.90, indicating fair to excellent agreement.

Table 3 - Descriptive and reliability statistics for the MASC total and domain scores (n=308).

	No of items	Mean±SD	Range	Floor effect*		Ceiling effect†		Cronbach's Alpha	ICC (n=13)
				n	%	n	%		
Total scale (0-117)‡	39	49.5±19.1	0-116	2	0.6	0	0.0	0.88	0.86
Domains									
Social Anxiety (0–27)‡	9	11.6±6.7	0-27	12	3.9	2	0.6	0.79	0.90
Separation Anxiety (0–27)‡	9	14.1±5.5	0-27	3	1.0	2	0.6	0.67	0.37
Harm Avoidance (0–27)‡	9	13.7±5.0	0-27	3	1.0	3	1.0	0.60	0.56
Physical Symptoms (0–36)‡	12	10.1±7.2	0-35	14	4.5	0	0.0	0.82	0.68

MASC, multidimensional anxiety scale for children; SD, standard deviation

* Percentage of children with 0 score

† Percentage of children with maximum scores

( ) ‡=range of possible values

Due to the great MASC scores variability as well as the age range of the sample, correlations between them were performed (Table 4). The domains "Separation anxiety" ($r=-0.32$; $p<0.0001$) and "Harm avoidance" ($r=-0.19$; $p<0.001$) were negatively correlated with age. Similar result was observed for MASC total scores, showing that younger children presented with higher anxiety levels ($r=-0.13$; $p<0.05$).

Table 4. Correlation between MASC total and domain scores and age (n=308).

	MASC total	MASC domains			
		Social Anxiety	Separation Anxiety	Harm Avoidance	Physical Symptoms
Spearman coefficient	-0.13	0.05	-0.32	-0.19	-0.03
P-value	0.019	0.383	< 0.0001	< 0.001	0.586

MASC, multidimensional anxiety scale for children

In Table 5 the differences between genders in relation to MASC scores and salivary cortisol levels are demonstrated according to age group. Girls had significantly higher mean MASC total scores than boys ($p<0.01$). The mean scores for girls 7-11 years

old were also higher than boys in the "Separation anxiety" and "Harm avoidance" domains ($p<0.001$); in the 12-17-year-old group, girls had significantly higher means than boys in the "Social anxiety," "Harm avoidance," and "Physical symptoms" domains ($p<0.05$, $p<0.001$ and $p<0.01$). It was also observed that girls had higher values of salivary cortisol than boys did in the 12-17 age group ($p<0.001$).

Table 5 - Differences in means (SD) of MASC scores and AUC salivary cortisol by age group and gender

Age groups and genders	MASC scores (n=308)							AUC salivary cortisol (n=263)
	n	Total	Social Anxiety	Separation Anxiety	Harm Avoidance	Physical Symptoms	n	
7-11[a]	195	11.9±6.5[*]	15.1±5.7	14.3±5.1[†]	10.2±7.3[**]	51.0±19.3	170	101.7±69.2
♂[b]	84	10.5±6.5[**]	13.3±5.5	13.2±5.0[†]	8.9±6.1[*]	45.9±16.8	71	94.1±71.0
♀[b]	111	12.0±7.1[**]	16.5±5.5	15.2±5.0[†]	11.3±8.0[*]	54.9±20.2	99	107.2±67.8
12-17[a]	113	11.4±6.8[*]	12.3±4.7	12.7±4.8[†]	9.9±7.1[**]	46.8±18.4	93	96.4±52.3
♂[c]	53	10.4±6.4[†]	11.3±4.2[*]	10.9±3.7	8.1±7.4[†]	40.7±17.0[**]	43	77.7±41.9[†]
♀[c]	60	13.26.3[†]	13.24.9[*]	14.35.2	11.56.6[†]	52.218.0[**]	50	112.6±55.2[†]

MASC, multidimensional anxiety scale for children; SD, standard deviation; AUC, area under the curve

[a]Comparisons between age groups; [b]Comparisons between genders in 7-11 yr-old group; [c]Comparisons between genders in 12-17 yr-old group

*$P<0.05$; **$P<0.01$; [†]$P<0.001$ (differences between lines, Mann-Whitney Test)

MASC Discriminant Construct Validity and AUC salivary cortisol

Children with TMD had significantly higher mean MASC total scores compared to those with bruxism ($p<0.05$) and controls ($p<0.01$) (Table 6). The mean scores for the TMD group were also higher than bruxists and controls in the social anxiety and physical domains. No significant differences in the other domain scores were observed between the clinical and control groups. Moreover, the values of salivary cortisol were similar among the respective groups.

Table 6 - Differences in means±SD of MASC scores and AUC salivary cortisol by clinical groups

MASC scores	With bruxism (n=46)	With TMD (n=40)	Control (n=212)
Total scale [0-117]	49.1±15.6[a*]	58.8±22.6[b]	47.4±18.8[a**]
Domains			
Social Anxiety [0–27]	11.0±5.5[a*]	14.9±6.5[b]	11.0±6.9[a†]
Separation Anxiety [0–27]	14.2±5.0	15.0±6.0	13.8±5.6
Harm Avoidance [0–27]	14.1±4.3	14.7±5.8	13.4±5.0
Physical Symptoms [0–36]	9.7±6.7[a*]	14.3±8.5[b]	9.2±6.6[a†]
AUC salivary cortisol	109.6±59.3	89.3±63.8	100.1±65.4

MASC, multidimensional anxiety scale for children; SD, standard deviation; AUC, area under the curve

Values in square brackets indicate range of possible scores of MASC

Means followed by different superscript letters in lines differ significantly (differences between columns, Kruskal-Wallis test) *P<0.05; **P<0.01; †P<0.001

Logistic regression analysis

Backward stepwise logistic regression revealed that male gender was significantly associated (OR=0.49; p<0.05) with the presence of SB in the studied sample (n=263) (Table 7). Other variables, such as age, BMI, TMD, MASC scores, and values of AUC salivary cortisol were not associated with SB.

Table 7 - Backward stepwise logistic regression to test the association of independent variables with sleep bruxism in the studied sample (n=263). Independent variables eliminated from the model are not shown.

Dependent variable	Independent variables	Coef.	*P*-value	OR	95% CI	Significance of the model		
						R	*P*-value	Power (α=0.05)
Bruxism	constant	-1.673	-	-	-	6.593	0.036	0.798
	Gender	-0.703	0.034	0.495	0.26-0.95			

Coef., Coefficient; OR, odds ratio; CI, confidence interval.

DISCUSSION

This study evaluated the relationship between signs and symptoms of bruxism and TMD, symptoms of anxiety, and salivary cortisol levels in children and adolescents. Also, anthropometric characteristics (weight and height), age, and gender were verified. Evaluation of tooth wear for bruxism activity is still controversial (Pergamalian et al., 2003). Nevertheless, there are to date no definitively reliable methods for assessing bruxism in the clinic that have reasonable diagnostic validity, technical validity, effects on therapeutic decisions, and cost-effectiveness. The clinical diagnostic criteria used in the present study seem to be useful among the diagnostic tools that have been reported to date, and their clinical validity might be improved with modifications (Koyano et al., 2008).

Considering the minimum acceptable level for agreement at 0.40 for Kappa tests, 0.70 for group comparisons, and 0.90–0.95 for individual comparisons (Scientific Advisory Committee of the Medical Outcomes Trust, 2002), overall reliability results for clinical variables were still good. Despite good muscle reliability found in the present study (k=0.67), a low reproducibility for pain scores is not unusual (List et al., 2006) because pain intensity may vary over short periods of time (Ohrbach et al., 2010) partly due to poor memory recall for pain (Jamison et al., 1989). Moreover, reliability results for wear facets (k=0.77) are in accordance with other studies that have demonstrated a positive relationship between tooth wear and bruxism (Carlsson et al., 2003). However, tooth wear is a cumulative record of both functional and parafunctional wear, and has multiple factors associated with it, such as age, gender, occlusal condition, diet, and drink (Koyano et al., 2008).

In relation to psychometrical properties of MASC, the reliability coefficients for both total and subscales exceeded standards for group- and individual-level comparisons (Streiner and Norman, 1996), except for the “Harm avoidance” and “Separation anxiety” domains, which were slightly lower at 0.60 and 0.67, respectively. However, these values are acceptable, as they are far greater than 0.50, an indicative level for non-homogeneous scales (Bowling, 1997). According to Gherunpong et al. (2004), alpha is not a perfect indicator of reliability, as it tends to underestimate the reliability of multidimensional scales, and because lower values can be expected from health-related measures. In addition,

the results of this study suggest that MASC has good test-retest reliability, except for the separation anxiety domain, for which the reliability coefficient was regular at 0.37. An alternative explanation for this outcome is that enjoying contact with people might be an inherently unstable construct to children, and varies over time (Masalu and Astrom, 2003).

A higher prevalence of signs and symptoms of SB was observed in boys than girls (56.0 vs. 44.0%); however, it did not quite reach statistical significance. The influence of gender on SB was still controversial in the literature. While some studies found a higher prevalence of SB in males (Demir et al. 2004; Cheifetz et al., 2005; Serra-Negra, 2010; Fonseca et al., 2011), others found it in females (Manfredini et al., 2004). These contradictory outcomes may be explained by the substantial variability of SB symptoms over time and the limitations of clinical diagnostic criteria. The prevalence of TMD in children and adolescents with SB was about 16%, corroborating previous studies (Thilander et al., 2002; Emodi-Pearlman, 2012). However, this proportion was not significant in the evaluated population, as was also observed by Cheifetz et al. (2005). Carlsson et al. (2003) observed an association between tooth clenching and TMD in a 20-year follow-up study. TMD signs and symptoms in growing individuals can fluctuate, and the prediction of single TMD signs for the development of a severe disorder late in life is unclear (Bonjardim et al., 2005; Pizolato et al., 2011).

A prevailing theory indicates anxiety and stress as primary contributing factors to bruxism behavior in children (Monaco et al., 2002). Considering the total evaluated sample, the overall anxiety level was similar to that found by Anderson et al. (2009), who evaluated American children using the MASC instrument. However, these results differed in relation to the domains more affected; and while the present study found higher scores for the "Social anxiety" and "Separation anxiety" domains, Anderson et al. (2009) found higher scores for "Physical symptoms" and "Harm avoidance." On the other hand, studies performed in other countries, such as Iceland (Olason et al., 2004) and Taiwan (Yen et al., 2010), observed lower MASC scores than those found in the present study. When considering the comparative analyses between clinical groups, children and adolescents diagnosed with TMD presented higher MASC total and domain scores than bruxists and controls (supporting the discriminate construct validity of the MASC). These results were

consistent with the Bonjardim et al.'s (2005) study, which found associations between symptoms of anxiety and clinical signs of TMD, primarily muscle tenderness. Similarly, previous studies have suggested that children and adolescents suffering from TMD pain seem to be more sensitive and somatically focused than their healthy peers (Wahlund et al., 2005; Hirsh and Türp, 2010).

Significant negative correlations were observed between age and MASC total scores, as well as the "Separation anxiety" and "Harm avoidance" domains, suggesting that younger participants presented higher anxiety levels. This may have occurred due to overprotection or rejection in terms of separation anxiety (e.g., "I try to stay near Mom and Dad") that may be relevant in young individuals. Also harm avoidance can be expected for individuals living in an urban environments, and manifests as fear of dangerous events (e.g., "I keep my eyes open for danger"); moreover, several of the items from this subscale are related to perfectionism (e.g., "I try to do everything exactly right" and "I try to do things other people will like"). Fincham et al. (2008) evaluated a sample of 14-19-year-old South Africans and found higher means of MASC scores were probably due to factors associated with higher anxiety, such as political instability, high unemployment, low per capita income, widespread crime, violence, and poverty, despite the disintegration of oppressive laws implemented by the apartheid regime in South Africa.

Girls presented significantly more symptoms of anxiety than boys, corroborating previous studies (Fincham et al., 2008; Yen et al., 2010). According to Fincham et al. (2008), differences in genders may occur due to hormonal factors, such as hypothalamic-pituitary-adrenal axis (HPA) dysregulation. In agreement, the present study found higher values of AUC salivary cortisol in older girls compared to boys, corroborating Netherton et al. (2004). When considering the results of multivariate analysis, male gender was the only variable associated with the presence of SB, which supports the higher prevalence of this parafunction in boys (56.0%). On the other hand, the absence of association between SB and psychological factors (symptoms of anxiety and AUC salivary cortisol) may support the fact that girls have presented more symptoms of anxiety and salivary cortisol levels than boys. The relationship between psychological factors and SB is still controversial in the literature; while some studies found associations between these variables (Manfredini et

al.,2004; Cheifetz et al., 2005; Gungormus and Erciyas, 2009; Serra-Negra et al., 2009; Carra et al., 2011), others did not (Grechi et al., 2008). Tahara et al. (2007) evaluated the effects of chewing and clenching during stress loading (arithmetic exercises) on salivary cortisol levels of adults and verified that chewing and clenching (signs of bruxism) brought about a reduction in stress, as evidenced by a reduction in salivary cortisol levels. Sato et al. (2010) suggested that the manifestation of stress-elicited emotions (e.g., bruxism behavior) is beneficial because it reduces sympathetic arousal and restores autonomic balance, and the suppression of these manifestations leads to the simultaneous activation of the sympathetic and parasympathetic nervous system, which may result in tension and organic diseases. These contradictory outcomes may be explained by the characteristics of the evaluated sample in the present and the above-mentioned studies, such as younger vs. older subjects, absence vs. presence of sleep-disordered breathing, and general vs. patient population, respectively. Moreover, comparisons with previous studies may be interpreted with caution due to different methodologies used, as salivary collection was carried by chew wax before and after a stress situation.

It is also important to consider that the answers of NOT-S interview have subjective insight, and the definite diagnosis about some dysfunctions, such as mouth breathing, must be obtained by specific exams and professionals. This observation can be considered as a limitation of the present study. However, NOT-S has shown good validity and reliability,[18] and has been demonstrated as useful for assessing orofacial function in epidemiological studies.

No significant associations were also found between SB and BMI, corroborating with Netherthon et al.'s (2004) study. Moreover, there was no association between SB and age, which was also observed by Carra et al. (2011), who found a higher prevalence of SB in children when compared to adolescents. The low prevalence in adolescence may be explained by the fact that parents may check their children's sleep during the night less frequently, contributing to parents underreporting signs and symptoms of SB in this age range. In adults, SB diagnose is based on self-reporting and prevalence is lower than 10% (Kato et al., 2011). Environmental and developmental factors may influence the occurrence

of SB during the stages of development of dentition. However, Carlsson et al. (2003), in a 20-year follow-up, suggested that bruxism may be a persistent trait in many subjects.

Considering the limitations of this study, additional research involving a follow-up clinical population is required to confirm our findings with respect to its clinical significance. Furthermore, to better understand the etiology of SB in children and adolescents, longitudinal studies are necessary, as they allow theoretical models to be investigated without the limitations of cross-sectional study designs.

CONCLUSIONS

Children and adolescents with TMD presented higher anxiety levels than those with SB and controls. Moreover, females and children also reported more symptoms of anxiety. Salivary cortisol levels did not differ between bruxists and controls, but were higher in adolescent females. On the other hand, males were more likely to present SB.

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CONCLUSÕES GERAIS

Os resultados encontrados na amostra estudada mostraram que:

O bruxismo do sono apresentou relação significativa com disfunção orofacial, pois crianças com bruxismo do sono apresentaram maiores escores de NOT-S.

As alterações orofaciais observadas com maior freqüência foram relacionadas a hábitos, mastigação e deglutição e músculos mastigatórios.

Em relação à ansiedade, o gênero feminino apresentou escores maiores de ansiedade que o gênero masculino e indivíduos DTM apresentaram sintomas de ansiedade maiores que indivíduos com bruxismo do sono e do grupo controle.

O gênero masculino apresentou associação ao bruxismo do sono.

No entanto não foi encontrada relação entre bruxismo do sono e DTM, idade, peso e altura corporal, índice de massa corporal, maloclusão, ansiedade e níveis de cortisol em crianças e adolescentes.

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ANEXO 1

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, níveis salivares de cortisol e ultrassonografia das articulações temporomandibulares em crianças e adolescentes com necessidade de tratamento ortodôntico"**, protocolo nº 034/2010, dos pesquisadores Maria Beatriz Duarte Gavião e Ana Bheatriz Marangoni Montes, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 08/07/2010.

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, salivary cortisol levels and ultrasound of temporomandibular joints in children and adolescent"**, register number 034/2010, of Maria Beatriz Duarte Gavião and Ana Bheatriz Marangoni Montes, 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 07/08/2010.

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

Ruídos direito

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

Ruídos 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 intra-oral 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

"MASC" – Escala Multidimensional de Ansiedade para Crianças

Validada pela Psic. Michelle Moreira Nunes

Nome: Idade:

Sexo: Mas. Fem. (circule um)

Data: ___/___/_____ Série escolar:

Este questionário pergunta a você como você vem se sentindo, o que você tem pensado, tem sentido ou como tem agido recentemente. Para cada item, por favor, faça um círculo ao redor do número que indica com que frequência a afirmativa é verdadeira para você. Se o que a sentença diz é verdade sobre você muitas vezes, circule 3. Se ela é verdade sobre você algumas vezes, circule 2. Se a sentença é verdade sobre você uma vez ou outra, circule 1. Se dificilmente ou nunca a sentença é verdade sobre você, circule 0. Lembre-se que não há respostas certas ou erradas, responda apenas como você vem se sentindo recentemente.

Aqui estão dois exemplos para lhe mostrar como completar o questionário. No exemplo A, se você muito poucas vezes tem medo de cachorro, você deve circular 1, significando que a afirmativa raramente é verdadeira sobre você. No exemplo B, se às vezes os trovões o perturbam, você deve circular 2, significando que a afirmativa é às vezes verdade sobre você.

	Nunca é verdade sobre mim	Raramente é verdade sobre mim	Às vezes é verdade sobre mim	Frequentemente é verdade sobre mim
Exemplo A: Eu tenho medo de cachorros	0	1	2	3
Exemplo B: Trovões me perturbam	0	1	2	3

Agora tente esses itens você mesmo:

1.	Eu me sinto tenso ou nervoso.	1	2	3	4
2.	Eu costumo pedir permissão para fazer as coisas.	1	2	3	4
3.	Eu me preocupo que as pessoas dêem risada de mim.	1	2	3	4
4.	Eu fico com medo quando meus pais saem.	1	2	3	4
5.	Sinto falta de ar.	1	2	3	4
6.	Eu fico atento se há algum perigo.	1	2	3	4
7.	A idéia de ficar longe de casa me assusta.	1	2	3	4
8.	Eu fico tremendo ou inquieto.	1	2	3	4
9.	Eu me esforço para obedecer meus pais e professores.	1	2	3	4
10.	Eu tenho medo que os outros meninos (ou meninas) gozem de mim.	1	2	3	4
11.	Eu tento ficar perto da minha mãe ou pai.	1	2	3	4
12.	Eu tenho tontura ou sensação de desmaio.	1	2	3	4
13.	Eu verifico as coisas antes de fazê-las.	1	2	3	4
14.	Eu me preocupo em ser chamado na classe.	1	2	3	4
15.	Eu me sinto desassossegado (sobressaltado).	1	2	3	4
16.	Eu tenho medo que os outros achem que eu sou bobo.	1	2	3	4
17.	Eu deixo as luzes acesas à noite.	1	2	3	4

18.	Eu sinto dores no peito.	1	2	3	4
19.	Eu evito sair sem minha família.	1	2	3	4
20.	Eu me sinto estranho, esquisito, ou fora da realidade.	1	2	3	4
21.	Eu tento fazer coisas que vão agradar aos outros.	1	2	3	4
22.	Eu me preocupo com o que os outros pensam de mim.	1	2	3	4
23.	Eu evito assistir filmes ou programas de TV que assustam.	1	2	3	4
24.	Meu coração dispara ou "falha".	1	2	3	4
25.	Eu evito as coisas que me aborrecem.	1	2	3	4
26.	Eu durmo junto de alguém da minha família.	1	2	3	4
27.	Eu me sinto inquieto e nervoso.	1	2	3	4
28.	Eu tento fazer tudo exatamente do jeito certo.	1	2	3	4
29.	Eu me preocupo em fazer alguma coisa boba ou que me deixe sem graça.	1	2	3	4
30.	Eu fico com medo quando ando de carro ou de ônibus.	1	2	3	4
31.	Eu sinto mal estar no estômago.	1	2	3	4
32.	Se eu fico aborrecido ou com medo, eu conto logo para alguém.	1	2	3	4
33.	Eu fico nervoso se eu tenho que fazer alguma coisa em público.	1	2	3	4
34	Tenho medo de tempo tuim, escuridão, altura, animais ou insetos.	1	2	3	4
35.	Minhas mãos tremem.	1	2	3	4
36.	Eu preciso ter certeza que as coisas estão seguras.	1	2	3	4
37.	Eu tenho dificuldade em chamar outros meninos (ou meninas) para brincar comigo.	1	2	3	4
38.	Minhas mãos ficam suadas ou frias.	1	2	3	4
39.	Eu sinto vergonha.	1	2	3	4

ANEXO 5

PEDIATRIC DENTISTRY

Submission Confirmation

Thank you for submitting your manuscript to *Pediatric Dentistry*.

Manuscript ID: PediaD-2012-02-2128

Title: Is sleeping bruxism related to temporomandibular disorders, malocclusion and orofacial function?

Authors: Manfredini, Ana Beatriz
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Date Submitted: 15-Feb-2012

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