

Morning salivary cortisol with regard to gender in individuals with perceived facial pain

Cortisol salivar matutino em relação ao sexo em indivíduos com sintomas de dor facial

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ABSTRACT

BACKGROUND AND OBJECTIVES: Facial pain seems to be related to physiological responses to stress and sexual dimorphism. However, the relationship among facial pain, cortisol secretion and gender has been poorly investigated. This study aimed to investigate differences in morning salivary cortisol profile between males and females either with or without perceived facial pain symptoms.

METHODS: Participated in the study 39 individuals reporting facial pain and 33 painless controls of both genders. Facial pain symptoms were evaluated with Axis II Research Diagnostic Criteria for Temporomandibular Disorders, which has supplied chronic pain scores. Saliva was collected in the morning to obtain cortisol peaks, being stored for further use. Salivary cortisol levels were evaluated by immunosorbent assay. Statistical analysis has included hypotheses tests and ANOVA with significance level of 5% and a binary logistic regression, which has tested the association between gender, cortisol and each facial pain symptom.

RESULTS: There has been no association between facial pain and gender. Cortisol levels were similar among individuals with and without facial pain, regardless of gender. The adjusted model has shown that most symptoms were not associated to gender, regardless of cortisol levels.

CONCLUSION: In individuals with and without facial pain symptoms, morning salivary cortisol levels regulation has been similar for both genders.

Keywords: Chronic pain, Comorbidity, Cortisol, Facial pain, Gender, Psychological stress.

RESUMO

JUSTIFICATIVA E OBJETIVOS: A percepção da dor facial parece estar relacionada com respostas fisiológicas ao estresse e com o dimorfismo sexual. No entanto, a relação entre dor facial, secreção de cortisol e o sexo ainda foi pouco investigada. O objetivo deste estudo foi investigar as diferenças nos perfis de cortisol salivar matutino em homens e mulheres com ou sem sintomas de dor facial.

MÉTODOS: Trinta e nove indivíduos que relataram dor facial e 33 controles sem dor, de ambos os sexos, participaram deste estudo. Os sintomas de dor facial foram avaliados utilizando o Eixo II do Critério de Diagnóstico para Pesquisa das Disfunções Temporomandibulares, que forneceu os escores de dor crônica. A saliva foi coletada dos participantes no turno matutino a fim de obter os picos de cortisol, sendo armazenada até utilização posterior. Os níveis salivares de cortisol foram avaliados por ensaio imunoenzimático. A análise estatística incluiu testes de hipóteses e ANOVA com nível de significância de 5%, e uma regressão logística binária que testou a associação entre o sexo, cortisol, e cada sintoma de dor facial.

RESULTADOS: Não foi observada associação entre dor facial e o sexo. Os níveis de cortisol foram semelhantes entre indivíduos com ou sem dor facial, independentemente do sexo. O modelo ajustado mostrou que a maioria dos sintomas não teve associação com o sexo, independentemente dos níveis de cortisol.

CONCLUSÃO: Nos indivíduos com e sem dor facial, a regulação dos níveis de cortisol salivar matutino ocorreu de forma semelhante em ambos os sexos.

Descritores: Comorbidade, Cortisol, Dimorfismo sexual, Dor Crônica, Dor facial, Estresse psicológico.

INTRODUCTION

Facial pain has been commonly associated with a multifactorial scenario that includes musculoskeletal disorders, biomechanical unbalances of the temporomandibular joint and the masticatory system, or pre-existing pain conditions triggering several orofacial symptoms¹⁻⁴. Neuropsychological comorbidities are often present, including neuroendocrine abnormalities, sleep disturbance, pain amplification, and psychological distress due to hormonal response to stress or to excitation of the sympathetic nervous system⁵⁻⁸.

Moreover, neuroendocrine, psychosocial and psychological factors have been suggested as predisposing females to the devel-

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opment of facial pain, the severity of which has been shown to be greater in women than in men⁹⁻¹¹. Although previous investigations have demonstrated higher prevalence of psychological comorbidities in females, data regarding sex differences in facial pain prevalence require further investigation^{4,9,12}. Interestingly, females, either with or without chronic pain, exhibit higher responsiveness to stress^{13,14}, and the gender was shown to contribute significantly to the relationship between distress, muscle tension and facial pain¹⁵. However, the impact of facial pain on cortisol secretion in both genders remains unclear, deserving greater attention.

Cortisol is considered to be a stress biomarker⁶, and temporomandibular joint disorder (TMD) has been linked to increased levels of cortisol in women¹⁶. Nevertheless, recent but limited data found no association between distress, psychological factors and cortisol levels in women with TMD and facial pain^{17,18}. Accordingly, since most research that analyzed the connection between facial pain and cortisol levels included only female participants, evidences of the effects of both facial pain and gender on cortisol secretion are scarce.

Although psychoneuroendocrine factors involved in facial pain pathogenesis support its association with cortisol secretion and gender, a potential relationship remains controversial. Hence, this study was aimed at investigating whether morning salivary cortisol profiles would be affected by sexual dimorphism in individuals reporting facial pain symptomatology.

METHODS

This study was advertised throughout a Brazilian University (São Luis, Brazil), and interested volunteers contacted the research team for further information. Those who agreed to participate in the study received no financial compensation. Volunteers undergoing exams or other stressful activities were not included. Eighty-five individuals were initially enrolled, including undergraduate or graduate students. However, 11 participants were identified with history of chronic daily headache or migraine in the past 12 months, being then excluded from this study for not meeting the selection criteria. Yet, 2 voluntary participants were excluded due to inadequate saliva samples. Finally, a total of 72 individuals (46 females and 26 males) aged 18-39 years were included in the study. The following exclusion criteria were applied: presence of diabetes mellitus; history of hyper- or hypocortisolism, mood disorders, sleep apnea or other neurologic disorders; pregnant or climacteric women; premenopausal women during the menstrual cycle; individuals taking antibiotics, nonsteroidal anti-inflammatory drugs, anxiolytics, antidepressants, contraceptives, and immunosuppressants; individuals with any musculoskeletal or craniofacial disorder, and those under treatment for such dysfunctions; history of neuropathic or dental pain, cluster or chronic daily headache, or migraine¹⁹ in the past twelve months, and all participants signed an informed consent form after being informed about the aims, potential risks, and benefits of the study.

Individuals were firstly evaluated in terms of presence or absence of perceived facial pain by answering questions on the Axis II of the Research Diagnostic Criteria for Temporomandibular Dis-

orders (RDC/TMD)^{20,21}. The RDC/TMD Axis II was applied to investigate disabilities related to facial pain and psychological condition. Axis II main criteria included pain intensity, psychological stress, sleep quality, limitations related to mandibular function, among others²⁰.

These data allowed investigators to assign scores to determine each patient's chronic pain-related disability through the scores of the Graded Chronic Pain Scale (GCPS). GCPS scores ranged from 0 to IV. GCPS 0 indicates no disability in the past 6 months. GCPS I indicates low disability and low pain intensity. GCPS II indicates low disability and high pain intensity. GCPS III indicates high disability and moderately limiting. GCPS IV indicates high disability and severely limiting²⁰.

After data collection, study participants were separated into two groups: facial pain (n=39; 25 females and 14 males), which included patients with GCPS scores ranging I-IV, and control (n=33; 21 females and 12 males), including individuals with GCPS 0. To model the relationship between perceived facial pain symptomatology, cortisol and gender, the status of facial pain symptoms was labeled as present or absent. For the comparison of cortisol levels according to symptoms associated with sleep quality, these symptoms were classified according to their intensity as following: "no", indicating the absence of the symptom, and "mild", "moderate", "severe", or "very severe" symptomatology²⁰.

After the collection of clinical data, participants underwent saliva collection for the evaluation of salivary cortisol levels. Individuals of both groups (facial pain and control) had non-stimulated saliva collected in the morning shift between 9 and 10 am. Samples were stored in sterile graduated flasks and kept in ice to be used for the evaluation of cortisol levels by the immunoenzymatic test. These saliva samples were then centrifuged (11,900 rpm, 10 min). Then, supernatants received a protease inhibitor (phenylmethylsulfonyl fluoride; final concentration of 1 mM), and were stored at -20°C for posterior utilization. Salivary concentrations of cortisol were determined by Enzyme Linked Immunosorbent Assay (ELISA) using a commercially available specific kit (Cortisol Parameter Kit, R&D Systems, Inc., Minneapolis, MN, USA).

Sample size was calculated using the 11 PASS program (NCSS, LLC, Kaysville, UT, USA). A prevalence of 65.5% of at least one episode of facial pain as reported by Slade et al.²² was considered to produce a power of 80%.

Statistical analyses

Chi-square test of independence was applied to investigate associations between facial pain symptomatology and gender. Shapiro-Wilk test was used in the comparison of numerical variables between groups, followed by Student *t* test, which was used in the comparison of cortisol levels between genders. When comparing more than one group, one-way ANOVA was utilized, followed by Bonferroni test. A standard binary logistic regression approach was developed to test the association between gender, cortisol and each perceived symptom of facial pain. The level of significance considered for all statistical analyses was 5%. Data obtained were analyzed using the SAS 9.4 software (SAS Insti-

tute Inc., Cary, NC, USA). A Human Research Ethics Committee (Protocol #633/11, Brazil) approved this study that was conducted according to the W.M.A. Declaration of Helsinki.

RESULTS

In the male group, 46% of patients presented with GCPS 0, 27% with GCPS I, 19% with GCPS II, 4% with GCPS III, and 4% with GCPS IV. In the female group, 46% of patients presented with GCPS 0, 19.5% with GCPS I, 15% with GCPS II, 13% with GCPS III, and 6.5% with GCPS IV. Similar GCPS scores were observed in male and female participants with perceived facial pain (p=0.72). The risk of having some level of facial pain (GCPS scores > 0) was also found to be similar in both men and women (odds ratio [OR] = 1.02, 95% confidence interval [CI] = 0.38–2.67). Still, when the relationship between facial pain symptoms and gender was established, as shown in table 1, no association between facial pain and the gender was found (p>0.05). Figure 1A shows the values of salivary cortisol in male and female participants considering only the facial pain group. Statistically similar values were observed for men and women, regardless of

the presence of pain. When considering both facial pain and the control group, although there has been a trend for higher cortisol means in female as compared to male participants, the difference did not reach significance (p=0.05). Figure 1B shows the comparison of cortisol values between participants with orofacial symptoms and controls, respectively, in men and women separately. For both situations, no statistically significant difference was obtained (p>0.05). Yet, the comparison of cortisol levels according to GCPS scores in each gender showed similar levels of morning salivary cortisol in both men (p=0.74) and women (p=0.65). Table 2 shows the results of a standard binary logistic regression developed for modeling the relationship between facial pain symptomatology and gender. Adjusted ORs (95% CI) were obtained taking cortisol levels into consideration. Gender was found to be associated with “difficulty falling asleep” (p<0.05). Figure 2 shows the distribution of means and standard deviation of salivary cortisol, according to scores of disturbed sleep symptoms in both men and women, men only, and women only. In all these situations, no statistically significant difference was demonstrated between groups (p>0.05), similarly to the comparison according to other evaluated symptoms (data not shown).

Table 1. Association between perceived symptoms of facial pain reported by participants, gender and scores of the Graded Chronic Pain Scale obtained from the Research Diagnostic Criteria for Temporomandibular Disorders, Axis II

Facial pain symptom	Gender		Facial pain symptom	Gender	
	OR (95% CI)	p value		OR (95% CI)	p value
Difficulty opening the mouth	0.3 (0.03 – 2.9)	0.27	Malocclusion sensation	1.0 (0.2 – 4.2)	0.99
Difficulty in lateralization	1.4 (0.2 – 7.6)	0.57	Perceived stress	0.9 (—)	0.46
Chewing pain	1.5 (0.4 – 5.8)	0.79	Awake bruxism	0.7 (0.2 – 2.7)	0.61
Frequent headache	2.5 (0.6 – 10.2)	0.09	Sleep bruxism	0.3 (0.07 – 1.3)	0.10
Neck pain	0.9 (0.3 – 3.6)	0.87	Night-awake	1.7 (0.5 – 6.4)	0.68
Earache or TMJ pain	1.5 (0.4 – 5.8)	0.68	Restless sleep	1.6 (0.4 – 6.3)	0.09
TMJ noise	1.3 (0.3 – 4.8)	0.20	Difficulty falling asleep	3.2 (0.8 – 12.9)	0.58
Parafunctional habit	0.8 (0.2 – 3.7)	0.70	Pain (> 1 year)	0.3 (0.07 – 1.3)	0.10

CI = confidence interval; OR = odds ratio; TMJ = temporomandibular joint; *p<0.05, according to Chi-square test of independence.

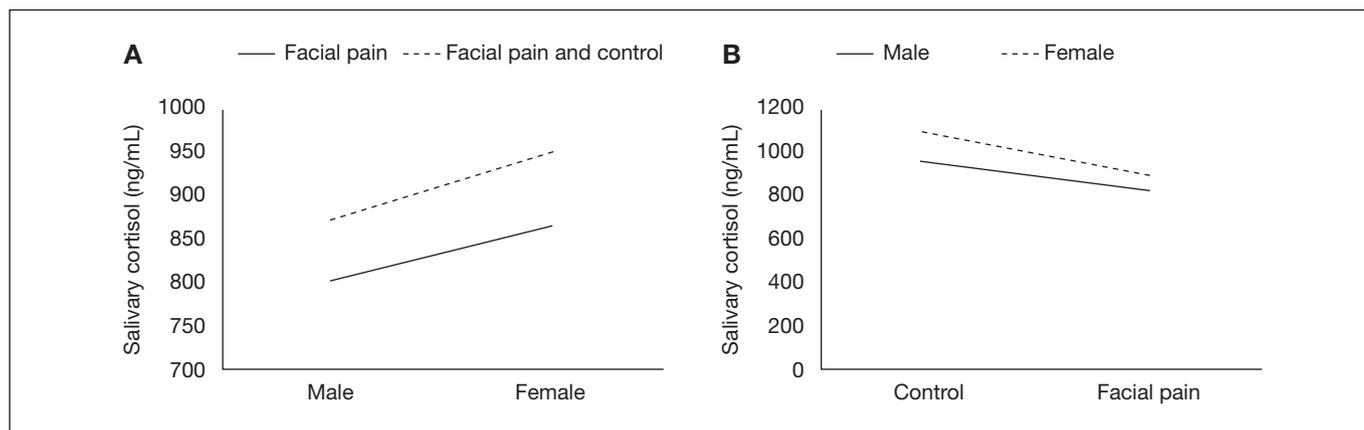


Figure 1. Comparison of values of salivary cortisol concentration according to gender or presence of perceived facial pain **A** – Comparison of cortisol means ± standard deviation between male (801.8 ± 415.6) and female (863.3 ± 403.6) participants, considering the facial pain group isolated (p=0.69), and then males (949.1 ± 432.5) and females (873.3 ± 425.9), considering both facial pain and the control group (p=0.50); **B** – Comparison of cortisol means between controls and participants with facial pain in males (932.9 ± 443.2; 801.8 ± 415.7; p=0.48) and females (1058.6 ± 454.6; 863.3 ± 403.6; p=0.15); Student t test.

Table 2. Standard binary logistic regression testing the effect of gender on each symptom reported. The adjusted model included the level of morning salivary cortisol as a covariate. The presence or absence of facial pain symptoms was obtained from the Research Diagnostic Criteria for Temporomandibular Disorders, Axis II

Facial pain symptom	Variable	Adjusted OR (95% CI)	p value	Facial pain symptom	Variable	Adjusted OR (95% CI)	p value
Difficulty opening the mouth	Cortisol		0.81	Malocclusion sensation	Cortisol		0.21
	Gender	— (—)	0.99		Gender	0.602 (0.09–3.73)	0.58
Difficulty in lateralization	Cortisol		0.84	Perceived stress	Cortisol		0.14
	Gender	0.93 (0.07–12.08)	0.96		Gender	— (—)	0.99
Chewing pain	Cortisol		0.96	Awake bruxism	Cortisol		0.45
	Gender	1.88 (0.41–8.52)	0.41		Gender	0.75 (0.16–3.45)	0.71
Frequent headache	Cortisol		0.79	Sleep bruxism	Cortisol		0.82
	Gender	3.62 (0.62–20.95)	0.15		Gender	0.39 (0.08–1.93)	0.25
Neck pain	Cortisol		0.33	Night-awake	Cortisol		0.94
	Gender	0.91 (0.19–4.27)	0.90		Gender	1.94 (0.42–8.82)	0.39
Earache or TMJ pain	Cortisol		0.33	Restless sleep	Cortisol		0.97
	Gender	1.32 (0.28–6.29)	0.72		Gender	3.67 (0.63–21.26)	0.14
TMJ noise	Cortisol		0.07	Difficulty falling asleep	Cortisol		0.18
	Gender	0.87 (0.16–4.69)	0.87		Gender	14.60 (1.43–149.02)	0.02*
Parafuncional habit	Cortisol		0.42	Pain (> 1 year)	Cortisol		0.82
	Gender	0.61 (0.11–3.34)	0.65		Gender	0.39 (0.08–1.93)	0.25

CI = confidence interval; OR = odds ratio; TMJ = temporomandibular joint; *p<0.05.

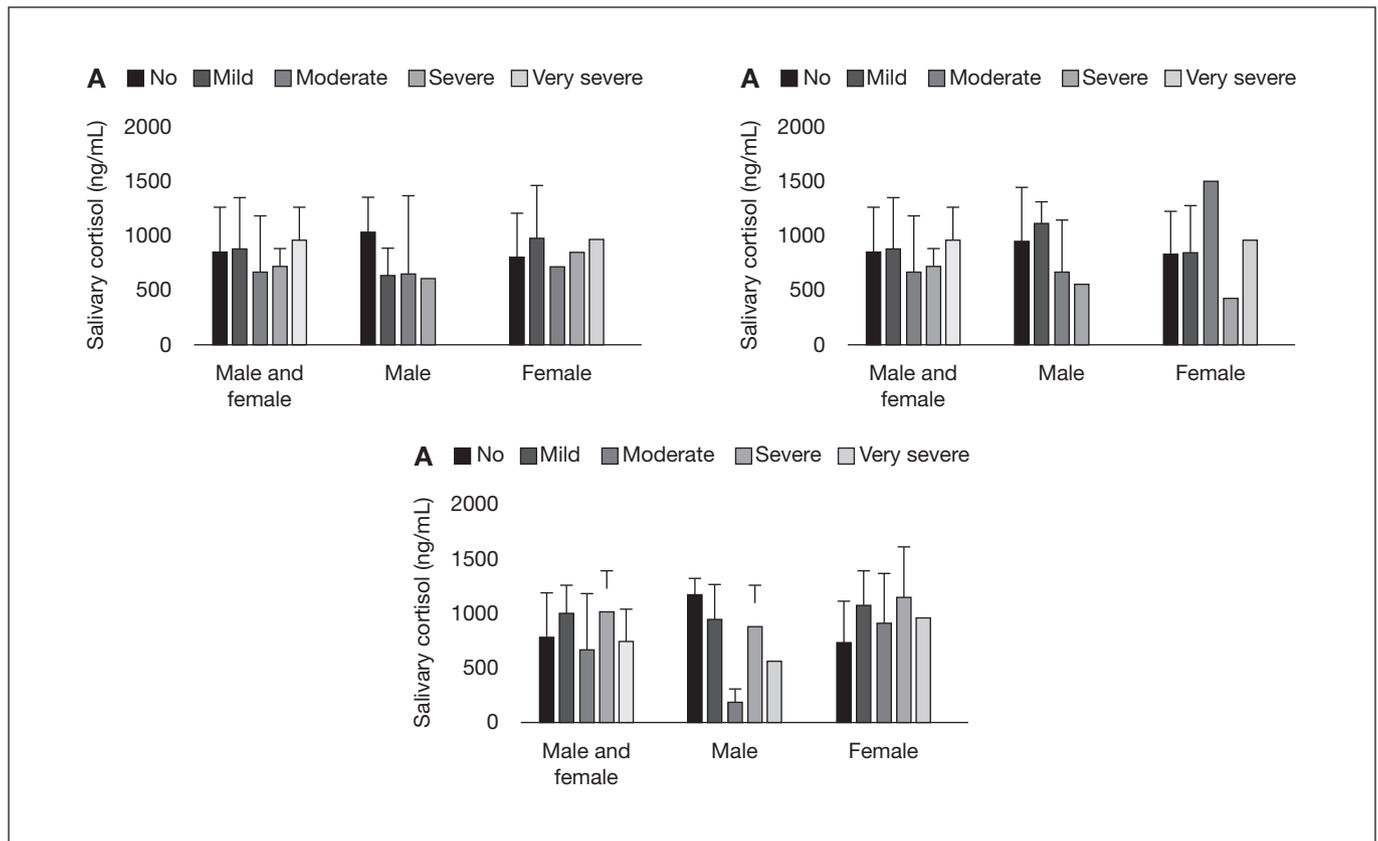


Figure 2. Comparison of means and standard deviation of salivary cortisol in both genders, in the male group only and, lastly, in the female group, according to the scores of symptoms related to disturbed sleep (“no” indicates absence, and the intensity of the symptom ranged from “mild” to “very severe”)

A – Comparison according to the scores of “night-awake” (p>0.05); **B** – Comparison according to the scores of “restless sleep” (p>0.05). **C** – Comparison according to the scores of “difficulty falling asleep” (p>0.05); one-way ANOVA, followed by Bonferroni test.

DISCUSSION

Facial pain, as most chronic conditions, is unlikely to be characterized by a homogenous and unmixed disease picture. In the present study, no perceived symptom of facial pain was significantly associated with gender, and no relationship was found between facial pain, gender and morning salivary cortisol levels. This suggests that the regulation of morning salivary cortisol might occur similarly in both genders in individuals with or without perceived facial pain.

The severity of facial pain has been reported to be elevated in women, mainly due to psychoneuroendocrine factors, as well as the role of estrogen in temporomandibular joint inflammation. Estrogen replacement therapy was associated with high prevalence of TMD, and serum estradiol levels during the luteal phase or in synovial fluid are higher in TMD women than in controls^{10,11}. This indicates that sex hormones, particularly estrogens, may be involved in facial pain, reason by which women during menstrual cycle, pregnancy and climacteric phase were excluded from our sample population. Conversely, the involvement of psychological distress in pain perception is becoming clearer as higher stress levels were found to enhance hyperalgesia, regardless of gender⁸.

Moreover, this study failed to observe changes in morning salivary cortisol levels when comparing male versus female participants, with and without perceived facial pain. Recent data have also found no difference in cortisol levels between women with facial pain and controls; however, sex differences were not taken into account as men were excluded from such studies^{17,18}. High correlation between salivary and blood levels of cortisol have been reported, and differences between measures during the circadian cycle with peaks occurring soon after awakening were shown⁶. Moreover, about 30% of free cortisol is enzymatically converted to cortisone and about 14% is bound to corticosteroid-binding globulin in saliva. Thus, one can imply that saliva contains lower levels of free cortisol when compared to plasma²³. Nevertheless, the salivary measure includes important advantages, such as stress-free collection and processing⁶.

Importantly, the differences between each gender in cortisol and distress levels might be impacted by social interactions and by the scenario where data were collected²⁴. Strini et al.²⁵ demonstrated that patients of both genders with different scores of orofacial dysfunction, including pain, were found to experience similar cortisol responses. Still, while most of the studies have reported women to be more susceptible to changes in HPA-axis function related to gender-oriented pain sensitivity^{14,26,27}, the relationship between facial pain and HPA-axis activity requires further exploration. In addition, the present study had some limitations, as it was not possible to develop a case-matched control design.

Persistent facial pain shows several aspects associated with a multisystem deregulation in sensory, autonomic, inflammatory, and psychological domains²⁸. In the present investigation, frequent headache was not found to be associated with variations in morning salivary cortisol levels between male and female participants. This is in agreement with previous data, which showed that sexual dimorphism do not influence salivary cortisol levels in patients with headache²⁹.

It is important not to confuse “frequent headache” with “chronic daily headache”. One major criteria to define chronic headache is the frequency of headache symptoms, which must appear at least fifteen days per month¹⁹. Hence, we considered frequent headache as that experienced less than fifteen days per month, without characteristics of migraine or cluster headache syndromes.

Furthermore, both sleep and awake bruxism were not found to be associated with either gender or morning salivary cortisol levels in the group of individuals with facial pain evaluated here. A previous report observed reduced levels of morning cortisol in patients with sleep bruxism in comparison to controls; however, no effect of gender on sleep bruxism was detected³⁰. It is important to emphasize that their population sample was composed by children, whereas we focused on young adults. Thus, despite the relevance of such preliminary findings, it can be difficult to draw conclusions based solely on them, particularly due to the impact of age on psychoneuroendocrine activity²³.

Notably, in the population studied, disturbed sleep was found to be frequent in patients of both genders with perceived facial pain. The relationship between pain and sleep has been a topic of wide discussion since sleep fragmentation was significantly associated with decreased levels of conditioned pain modulation in patients with facial pain^{31,32}. Additionally, sleep quality is also thought to be involved in HPA-axis activity, probably acting through distinct pathways in men and women in terms of capability to recover from sleep deprivation³³. Therefore, efforts to treat sleep disturbance early in the course of a pain condition should be kept in mind towards reducing the severity and impact of clinical pain and psychological stress^{7,32,33}.

CONCLUSION

Considering the data obtained, our results suggest that the regulation of morning salivary cortisol levels may occur similarly in individuals of both genders, either with or without facial pain symptoms. Such preliminary findings definitely require further investigation, considering that the exposition to facial pain symptomatology and comorbidity can dramatically impair patients' quality of life.

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