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Influence of the presence of Temporomandibular Disorders on postural balance in the elderly

Keywords

Temporomandibular Joint Disorder
Temporomandibular Joint
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ABSTRACT

Purpose: To investigate the influence of the presence of Temporomandibular Disorders (TMD) on postural balance in elderly individuals. **Methods:** The study sample consisted of 150 elderly: 103 women (67.7 \pm 5.0 years) and 47 men (69.3 \pm 5.5 years). Evaluation of the presence and severity of TMD included an anamnesis questionnaire, an evaluation of the temporomandibular joint (TMJ), and a muscular examination, which allowed the division of the elderly into 2 groups: G1 (experimental, n=95), with TMD; G2 (control, n=55), without TMD. Postural balance was assessed by means of the one-leg stance test (OLST) on a force platform (BIOMEC400), thus permitting the following measurements: center of foot pressure (COP); mean sway velocity (MV) and frequency (MF) of COP in both the anteroposterior (V_{AP} and F_{AP}) and medial/lateral (V_{ML} and F_{ML}) directions. The statistical analysis of data was performed using independent t-test, Variance Analysis, and Chi Square test (α =5%). **Results:** Presence of TMD was observed in 63.3% of the individuals (Group 2), with different degrees of severity (mild: 42.7%, moderate: 14.7%, severe: 6%). Significantly higher TMD was observed for women (73.8%) compared with men (40.4%) (p=0.0002). No significant difference was found in between the groups for all balance parameters, e.g., presence and severity of TMD, presence of pain to palpation of TMJ and masticatory and cervical muscles did not alter the variables related to postural balance in this population.

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INTRODUCTION

One of the main factors that affect the quality of life of the elderly is postural imbalance, which cannot be attributed to a specific cause, but to different extrinsic (environment, temperature, use of medicine) and intrinsic (muscular weakness, limited mobility, sensorial-motor deficit) factors that can disturb the postural balance system⁽¹⁾. According to Bittar et al.⁽²⁾, more than 50% of the cases of imbalance in the tertiary period of life originate between 65 and 75 years of age. In fact, the main consequences that stand out are falls, which account for 70% of accidental deaths in persons over 75 years of age⁽³⁾. Even when the falls cause minor lesions, they can seriously affect this population, and lead to fear of falling, with consequent restriction of activities, of mobility, diminished physical activity, and social isolation or depression⁽⁴⁾.

Temporomandibular Dysfunctions (TMD) include pathologies associated with masticatory muscles, the temporomandibular joint (TMJ), or with both, in that etiology is regarded as multifactorial and nonspecific⁽⁵⁾. Considering that the TMJ is directly related to the cervical and scapular regions, by means of a common neuromuscular system, the presence of TMD could alter body balance, and later lead to muscular imbalance, impaired motor coordination, muscular fatigue, and to symptoms of pain, with subsequent physical incapacity when not treated⁽⁵⁾.

The relationship between head posture, cervical posture, the stomatognathic system, and TMD has been widely studied and discussed. Patients with TMD present more alterations in the head position and in the cervical spine than patients without TMD⁽⁶⁾. Farias et al.⁽⁶⁾ compared the craniocervical posture between patients with and without TMD within the age bracket of 18 and 30 years. The diagnosis of TMD was based on the RDC (Research Dental Criteria) questionnaire, associated with a report of the symptoms by the patients, whereas radiographic exams afforded the evaluation of the cervical region. The results showed that symptomatic individuals regarding TMD showed a greater tendency to flexion of the first cervical vertebra and forward (hyperlordosis) the cervical spine (C2-C7). The authors, however, point out that it is not possible to assert whether TMD was a determining factor for the origin of these alterations, or vice versa. Some studies^(7,8) have reported association between TMD and posture, for TMD patients present greater shifts in the gravity center of their body, with their head thrust forward, which could lead to the shortening of some muscles. An anterior position of the head would presumably influence the gravity center of the head, thereby indicating the relationship between body posture and TMD. Likewise, postural alterations of the cervical region could cause TMD, modify the orientation of the head, and thus the position of the jaw. Hypothetically, this phenomenon of biomechanical alterations associated with the gravity center and mass distribution, together with TMD, could affect the maintenance of postural balance and, consequently, increase the chance of falls, mainly in the elderly.

However, other studies indicate no relationship between TMD and posture^(9,10). Nevertheless, most studies focus only on the postural aspect of the head, and not on balance, thus

limiting the establishment of a relationship between TMD and balance deficits.

Although the parameters obtained from a force platform are the most precise measures to quantify balance deficits⁽¹¹⁾, few studies have used this methodology. One of such studies is that by Okubo et al.⁽¹²⁾, which investigated 34 elderly individuals (12 males and 22 females) concerning the influence of the use of complete dentures on postural control when standing and walking. Aiming to evaluate body sway, the elderly were instructed to remain standing on a platform with their eyes closed for 60 seconds, with three attempts for each condition (wearing dentures and not wearing dentures). The authors observed that stability was affected when the patients were evaluated without complete dentures, and concluded that the use of dentures could efficiently help maintain and improve postural balance in the elderly.

Notwithstanding, there are no studies evaluating the relationship between TMJ and postural balance in the elderly using a force platform, which could justify the divergence present in the literature on this theme. Therefore, the aim of this study was to investigate the influence of the presence of TMD on postural balance in the elderly using a force platform.

METHODS

This study was approved by the Research Ethics Committee of the "Universidade Norte do Paraná" – UNOPAR (Process no. PP/0070/09).

During the screening process, volunteers were duly informed by the research team as to the objectives of the study and all the procedures of the clinical evaluation. The elderly participants signed an Informed Consent Form prior to study commencement.

Sample

The study sample consisted of 150 physically independent elderly, in that 103 were women with mean age of 67.7 years (SD=5.0) and 47 were men with mean age of 69.3 years (SD=5.5).

As criteria for inclusion in the study, the elderly should have natural teeth or prostheses with acceptable functional occlusion. Individuals who were toothless and not duly rehabilitated by prostheses were excluded from the study, in addition to those with history of fracture in the inferior limbs; serious dysfunctions of the neurological, musculoskeletal, respiratory, and cardiac systems; surgeries of the locomotion system; reported falls in the past year; recurrent primary headaches; and diagnosis of fibromyalgia.

Methods

- Examiner Training and Calibration

Two examiners performed the evaluations of this research: one conducted the examination of TMD and one the postural balance assessment. For the TMD examination, a standard examiner in theoretical-practical training activities conducted the calibration process. Towards the end of the training, the

examiner's calibration was verified by the Kappa test, where the results of both evaluations were considered within the same group of 20 elderly individuals. Substantial agreement was obtained (kappa=0.71). For variables related to balance, the examiner was trained following an experimental protocol, and for each laboratorial session, the force platform system was calibrated.

- Evaluation of the presence and severity of TMD

The questionnaire first proposed by Helkimo⁽¹³⁾ was applied to patients with no interference of the examiner, so as not to create expectations and possibly lead to deviation from the clinical examination to be accomplished. The patients responded to 10 questions related to TMD symptoms, which allowed classification of each subject according to the presence and severity of such dysfunctions. This questionnaire was modified, which proved effective in obtaining a diagnosis through the anamnesis, as previously published⁽⁵⁾.

Anamnesis questionnaire:

- 1. Do you have difficulty in opening your mouth?
- 2. Do you have difficulty in moving your jaw sideways?
- 3. Do you feel discomfort or muscular pain when chewing?
- 4. Do you often have headaches?
- 5. Do you feel pain in your neck and/or shoulders?
- 6. Do you feel earaches or pain near your ears?
- 7. Do you notice any noise in your TMJ?
- 8. Do you regard your bite as "normal"?
- 9. Do you use only one side of your mouth to chew?
- 10. Do you feel any pain in your face when you wake up?

Three options of response were offered for the anamnesis questionnaire: "yes", "no", or "sometimes". Value "2" was attributed to every answer indicating the presence of the symptom; value "0" for the absence thereof; and value "1" for the "sometimes" answer. The total sum of the values obtained allowed the classification of the sample as regards TMD, as a TMD index.

Values of 0 to 3: free of TMD

Values of 4 to 8: mild TMD

Values of 9 to 14: moderate TMD

Values above 14: severe TMD

The presence of joint noises based on right and left TMJ inspection was also evaluated. This evaluation was conducted by placing the pointer fingers lightly on the region corresponding to the lateral pole of the condyle, facing the external acoustic meatus, while the patient performed movements of mandibular opening and closing.

The evaluation of the presence of TMJ symptoms was accomplished first by orienting patients as to the difference between pressure and discomfort, to ensure more trustworthiness

to their answers. This exam was performed through bilateral digital palpation, with the examiner's pointer fingers placed 10 to 20 mm before the external auditory conduct. The lateral aspect of the TMJ was palpated with patients keeping their mouths closed, whereas the posterior aspect was palpated with open-mouthed patients. These regions were pressed delicately and continuously, with approximately 450 to 900 grams of force⁽⁵⁾.

For the exam of muscular palpation, patients were given the same orientation in order to differentiate pain from discomfort. Palpation of masticatory muscles (anterior, medial and posterior temporal; origin, body and insertion of superficial masseter; deep masseter) was conducted with bilateral digital pressure, with constant pressure of 1,500 grams⁽⁵⁾. The presence of pain was checked through the eyelid reflex and/or by questioning the patients. Cervical muscles (posterior digastric, sternocleidomastoid, and superior trapezius) were palpated by clipping one's fingers (like pincers) on both sides. At least three sites of pain were necessary for pain to be regarded as present with the palpation of masticatory muscles, whereas at least one site of pain was necessary for cervical muscles.

The entire mouth cavity was inspected in an attempt to verify the absence of teeth, vertical and horizontal trespass, maxilomandibular relationships, presence and type of lateral and anterior guidance, interferences in the non-work side, and number of dental contacts in Maximum Intercuspal Position (MIP). The measurement of horizontal and vertical trespasses was performed with the help of a millimetric ruler. Patients were requested to make lateral movements of the jaw to evaluate the presence and type of lateral conductor. Patients were requested to protrude their jaw to an incisal edge position to verify the presence of anterior guidance. The number of MIP contacts was obtained by interposing an Accu-Film leaf between the teeth, while patients opened and closed their mouths. These exams checked the presence of acceptable functional occlusion.

Based on the results for presence and severity of TMD, the elderly were divided into two groups: Group 1 (experimental, n=95), patients with TMD; Group 2 (control, n=55), participants who did not suffer from TMD.

- Evaluation of postural balance

In order to assess the postural balance of the elderly, the two groups performed three trials of the one-leg stance test (OLST) on a force platform (BIOMEC400, EMG System do Brasil Ltda), with three attempts of 30 seconds each, and brief intervals of rest (Figure 1).

The platform, with dimensions of 50.8 × 46.4 cm, consists of sensors based on load cells located on each of its edges, and allows a simultaneous measurement of three force components (Fx, Fy, and Fz) and three moment of force components (Mx, My, and Mz) acting on the platform (where x, y, and z are the anteroposterior, medial-lateral, and vertical directions, respectively). Data of the center of pressure (COP) area refer to measurements of position as defined by two coordinates — the Anteroposterior and Medial/Lateral directions — on the platform surface, and they are characterized by locating the force vector of vertical reaction to the feet⁽¹⁴⁾.



Figure 1. One-leg stance test (OLST) on a force platform

The participants were instructed to remain barefoot on one leg on the force platform, with their arms hanging down along their bodies, and gaze fixed (open eyes) on a target set at the horizontal height of their eyes (Figure 1). The average of the trials was obtained by stabilometric analysis using the system software to calculate all variables of postural balance: 95% confidence ellipse of COP (COP) in square centimeters (cm²), mean sway velocity (MV) of COP in both Anteroposterior (V_{AP}) and Medial/Lateral (V_{ML}) directions in centimeters/second (cm/s), and mean sway frequency (MF) of COP in both Anteroposterior (F_{AP}) and Medial-Lateral (F_{ML}) directions in hertz (Hz).

Statistical analysis

The results obtained were statistically analyzed using the Statistical Package for Social Sciences (SPSS) program, version 15.0. The data were tested for normal distribution through the application of the Shapiro-Wilk test. Considering the normal distribution of main variables, the data were described by parameters of mean and standard deviation, and parametric tests (independent t test and ANOVA) and Chi Square Test

were used with the purpose of verifying whether there were differences between groups 1 and 2 for the variables of postural balance (COP, V_{AP} , V_{ML} , F_{AP} , and F_{ML}).

RESULTS

Presence of TMD was observed in 63.3% of the study sample with different degrees of severity (mild: 42.7%, moderate: 14.7%, severe: 6%), in that they were significantly higher for women (73.8%) compared with men (40.4%) (p=0.0002) (Tables 1 and 2).

No significant difference was found for the variables related to balance considering the presence (Table 3) and severity (Table 4) of TMD and presence of pain to palpation of the TMJ and of masticatory and cervical muscles (Table 5).

Table 1. Distribution of the presence and severity of Temporomandibular Disorders (TMD)

		TMD		
Free		Present n (%)		Total n (%)
n (%)	Mild	Moderate	Severe	
55 (36.7%)	64 (42.7%)	22 (14.7%)	9 (6.0%)	150 (100.0%)
55 (36.7%)		95 (63.3%)		150 (100.0%)

Table 2. Distribution of the presence of Temporomandibular Disorders according to gender

		TMD			
		Free n (%)	Present n (%)	Total n (%)	p
	Female	27 (26.2%)	76 (73.8%)	103 (100.0%)	0.0002*
Gender	Male	28 (59.6%)	19 (40.4%)	47 (100.0%)	0.0002
	Total	55 (36.7%)	95 (63.3%)	150 (100.0%)	

Qui Square: 15.46, and p: 0.0002*

*Statistically significant

Table 3. Balance variables (COP, V_{AP} , V_{ML} , F_{AP} and F_{ML}) related to the presence of TMD

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Balance Variables	Presence of TMD	М	SD	р
COD (am²)	Free	25.5	26.2	0.060
COP (cm²)	Present	20.4	27.5	0.269
V (em/e)	Free	4.8	2.2	- 0.106
V _{AP} (cm/s)	Present	4.1	2.6	0.106
V (om/o)	Free	4.9	1.6	- 0.558
V _{ML} (cm/s)	Present	4.5	3.8	0.006
E (U-)	Free	0.9	0.3	- 0.240
F _{AP} (Hz)	Present	0.9	0.3	0.240
E (U-)	Free	1.0	0.2	- 0.160
F _{ML} (Hz)	Present	1.0	0.3	0.100

Caption: COP = center of pressure; V_{ap} = anteroposterior sway velocity; V_{ml} = medial/lateral sway velocity; F_{ap} = anteroposterior sway frequency; F_{ml} = medial/lateral sway frequency. Mean (M), Standard Deviation (SD), and independent t test (p)

Table 4. Balance variables (COP, V_{AP} , V_{ML} , F_{AP} and F_{ML}) related to the severity of TMD

Balance Variables	Presence of TMD	М	SD	р
	Free	25.52	26.27	
COD (am²)	Mild	21.81	30.76	0.440
COP (cm²)	Moderate	14.48	11.14	0.440
	Severe	25.07	31.74	
_	Free	4.83	2.23	
\/ (om/o)	Mild	4.47	3.07	0.000
V _{AP} (cm/s)	Moderate	3.24	1.11	0.089
	Severe	3.97	1.74	
	Free	4.90	1.69	
\/ (om/o)	Mild	4.95	4.60	0.249
V _{ML} (cm/s)	Moderate	3.58	0.82	0.348
	Severe	4.43	1.47	
_	Free	0.99	0.32	
- (U-)	Mild	0.94	0.43	0.050
F _{AP} (Hz)	Moderate	0.82	0.24	0.356
	Severe	0.96	0.45	
	Free	0.99	0.32	0.356
E (U=)	Mild	0.94	0.43	
F _{ML} (Hz)	Moderate	0.82	0.24	
	Severe	0.96	0.45	

Caption: COP = center of pressure; V_{ap} = anteroposterior sway velocity; V_{ml} = medial/lateral sway velocity; F_{ap} = anteroposterior sway frequency; F_{ml} = medial/lateral sway frequency. Mean (M), Standard Deviation (SD), and ANOVA (p)

DISCUSSION

In the present study, the prevalence of TMD among the elderly was high – 63.3% (Table 1), a datum that corroborates those of other studies that used a similar methodology, such as Johansson el al.⁽¹⁵⁾. (71%), Almeida et al.⁽¹⁶⁾ (60%), and Camacho et al.⁽⁵⁾ (61%). In contrast, Abud et al.⁽¹⁷⁾ observed low prevalence of TMD, considering that 78.4% and 61.9%, respectively, were free of symptoms in the two groups of elderly evaluated.

Despite the high prevalence of TMD observed in this sample, few individuals presented greater severity (14.7% moderate and 6% severe) (Table 1). These data agree with those of the study by Almeida et al.⁽¹⁶⁾, who observed a smaller percentage of individuals with moderate (21.9%) and severe (7.3%) TMD. In contrast, in a study by Pow et al.⁽¹⁸⁾, higher percentage of severe TMD (18.3%) was observed for the group over 55 years of age. Moreover, it is worth mentioning that there is a trend towards diminished signs and symptoms of TMD with the advancement of age – as verified by Schmitter et al.⁽¹⁹⁾. These authors compared two groups – one of elderly persons (68 to 96 years of age) and another of adults (18 to 45 years of age) – and obtained higher sensitivity to pain with palpation of the TMJ and masticatory

Table 5. Balance variables (COP, V_{AP} , V_{ML} , F_{AP} and F_{ML}) according to the presence of pain during palpation of the temporomandibular joint (TMJ) and masticatory and cervical muscles

Balance Variables	TMJ Palpation	M	SD	р	
COP (cm²)	Painless	25.52	26.37	0.459	
COP (CIII-)	Painful	21.28	27.56	0.409	
V _{AP} (cm/s)	Painless	5.02	2.52	0.065	
	Painful	4.15	2.52		
V (om/o)	Painless	5.09	1.69	0.350	
V _{ML} (cm/s)	Painful	4.53	3.63	0.330	
E (U-)	Painless	1.00	0.35	0.044	
F _{AP} (Hz)	Painful	0.92	0.37	0.244	
E (U-)	Painless	1.15	0.30	0.013*	
F _{ML} (Hz)	Painful	1.01	0.29	0.013	
Balance Variables	Palpation of masticatory muscles	М	SD	р	
COP (cm²)	Painless	20.79	21.05	0.395	
COP (CIII-)	Painful	24.68	34.71	0.395	
V (om/o)	Painless	4.46	2.22	0.672	
V _{AP} (cm/s)	Painful	4.28	2.98		
V (am/a)	Painless	4.62	1.56	0.690	
V _{ML} (cm/s)	Painful	4.83	4.82		
F (U-)	Painless	0.95	0.37	0.792	
F _{AP} (Hz)	Painful	0.93	0.37		
E (U-)	Painless	1.08	0.32	0.007	
F _{ML} (Hz)	Painful	0.99	0.25	0.067	
Balance Variables	Palpation of cervical muscles	М	SD	р	
COP (cm²)	Painless	22.73	22.17	0.840	
COP (CIII-)	Painful	21.83	31.66		
V _{AP} (cm/s)	Painless	4.56	2.26	0.415	
	Painful	4.22	2.80		
V _{ML} (cm/s)	Painless	4.87	1.60	0.499	
	Painful	4.52	4.33		
F _{AP} (Hz)	Painless	0.93	0.34	0.700	
	Painful	0.95	0.40	- 0.793	
F (11-)	Painless	1.06	0.26	0.711	
F _{ML} (Hz)	Painful	1.04	0.33	- 0.711	

^{*}Statistically significant (p<0.05)

Caption: \overrightarrow{COP} = center of pressure; \overrightarrow{V}_{ap} = anteroposterior sway velocity; \overrightarrow{V}_{ml} = medial/lateral sway velocity; \overrightarrow{F}_{ap} = anteroposterior sway frequency; \overrightarrow{F}_{ml} = medial/lateral sway frequency. Mean (M), Standard Deviation (SD), and independent t test (p)

muscles for the group of adults (16 and 25%) compared with the group of elderly (0 to 12%).

Regarding gender, significantly higher prevalence was observed for females (p=0.0002) (Table 2), as reported by other studies(15,17,20,21). Likewise, Mundt et al. (21) correlated individuals of different age brackets (between 35 and 74 years of age) with gender and observed sensitivity or pain in at least one muscle in 18.5% of the females and 9.5% of the males. In addition, they noticed sensitivity or pain to palpation of the TMJ in 7.3% of the women and 3.4% of the men. Dao and LeResche(20) were not able to determine one single factor that could explain the greater prevalence of pain in the female gender. The authors⁽²⁰⁾, however, suggested some probable causes, such as sexual differences in pain mechanisms, factors not vet identified of the craniofacial system, psychosocial and hormonal differences, and environmental factors. In contrast, Pow et al. (18) did not find differences between genders. Furthermore, these authors(18) stated that these differences could exist in populations in Western countries, but not in Asiatic countries. Alternatively, they affirmed that these divergences could be related to cultural differences in the perception and communication of symptoms.

Moreover, the results of the present showed no significant difference between the variables related to balance, e.g., presence (Table 3) and severity (Table 4) of TMD; presence of pain to palpation of the TMJ and masticatory and cervical muscles (Table 5). Therefore, the results found in this study showed that the presence of TMD did not alter the variables related to balance in the elderly population assessed. In addition, similarity between groups 1 and 2 was observed even when the different degrees of severity of TMD (mild, moderate, or severe) (Table 5) were considered for the experimental group. Furthermore, comparison between individuals was performed after excluding from group 2 individuals with mild TMD who did not need intervention, only orientation. However, even excluding the elderly with mild TMD, no significant difference was found for the variables related to balance.

The relationship between TMD and postural balance is not well defined, inasmuch as few studies have evaluated this condition with adequate instrumentation (force platform) as it was done the present study. Most studies have centered mainly on the relationship of the position of the head and the cervical spine by means of angular measures in patients with TMD^(6,9,22-24), and did not objectively quantify parameters related to mechanisms of postural balance, such as COP measures and velocity, which are regarded today as the two most accurate and reliable variables to quantify balance deficits^(11,25). These measures were addressed in the present study and support the reliability of the results observed by the authors for the population under study.

According to Perinetti and Contardo⁽²⁶⁾, important attention has been given to this theme in the literature, although information regarding TMD and posture^(7,8) is not yet conclusive probably due to lack of efficient methodology. Perinetti⁽²⁷⁾ (2009), in an editorial on the study by Cuccia and Caradonna⁽²⁸⁾, commented that although association between TMD and postural control has been observed in some studies, this does not mean that clinical relevance will follow. In fact, the importance of conducting

controlled clinical research work must be emphasized in order to establish a real relationship between presence of TMD and posture.

One of the limitations of this study was the absence of evaluation of cervical posture in these individuals. Nevertheless, it should be noted that the maintenance of balance is a necessary condition for day-to-day functional activities. To the authors' knowledge, this is the first study that investigates the effects of TMD on balance in an elderly population. It must be emphasized that growing old is significantly progressive, and the neuromuscular alterations resulting from this process are increasingly present⁽²⁹⁾. Unfortunately, dysfunction of the neuroskeletal system added to neuromuscular and functional losses with the advancement of age contribute to a faster state of incapacity. Currently, falls among the elderly are regarded as one of the main problems of public health, inasmuch as they cause incapacity and, in some cases, even death⁽³⁰⁾. Postural imbalance is regarded as one of the main factors leading to falls, and the study of this outcome (postural control) in its relation with TMD is fundamental for a better acquaintance with the theme and, above all, for ascertaining that elderly persons with TMD are more prone to falling owing to their balance state (good or bad). The present study has shown that the presence of TMD does not alter balance measures.

However, it is important to emphasize that these results cannot be widespread to all elderly individuals, who may suffer from other alterations because of their age or muscular-skeletal system. Additionally, although the presence and severity of TMJ do not alter the variables related to postural balance, it is important to verify other complaints related to TMD that can lead to changes in postural balance, such as vertigo and problems in the cervical region. From the characteristics of each patient and their diversity of complaints, it is possible determine the interdisciplinary treatment procedures and therapy. Thus it is important to conduct additional studies in order to inform this population about the characteristics of TMD and possible treatment outcomes.

CONCLUSION

Based on the results herein obtained, we conclude that the presence and severity of TMD, in addition to the presence of pain to palpation of the TMJ and masticatory and cervical muscles do not alter the variables related to postural balance (COP, V_{AP} , V_{ML} , F_{AP} , and F_{ML}) studied in this population. Complementary studies should be developed, including a larger sample of patients suffering from moderate and severe TMD, aiming to identify the influence of this dysfunction in a more severe degree on postural balance.

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Author contributions

PVPON was responsible for the conception and execution of the study; MTY was responsible for the data collection; RAS was responsible for the conception of experiments; ACCFC was responsible for the interpretation of the data; RLN was responsible for the interpretation of the data; LLMM was responsible for the interpretation of the data; KBPF was responsible for data analysis.