Test–retest reliability of electromyographic variables of masseter and temporal muscles in patients with cerebral palsy

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A B S T R A C T

Introduction: The aim of this study was to evaluate the reliability of surface electromyography of the masticatory muscles in patients with cerebral palsy.
Methods: Surface electromyography was performed over the masseter and temporal muscles in 15 patients with cerebral palsy with the mandible at rest and during maximum clenching effort in two sessions. The data were analyzed using the root mean square amplitude, mean frequency, median frequency, zero crossings and approximate entropy.
Results: In the within-day evaluations, intraclass correlation coefficients were higher (0.80–0.98) for the all electromyography variables and muscles during maximum clenching effort. In the resting position, the coefficients revealed good to excellent reliability (0.61–0.95) for root mean square, mean frequency, median frequency and zero crossings and fair to good reliability (0.53–0.74) for approximate entropy. In the between-day evaluations, the coefficients revealed good to excellent reliability (0.69–0.86) for mean frequency, median frequency, zero crossings and approximate entropy. In the resting position, the coefficients revealed poor to fair reliability (0.23–0.57) for all electromyography variables studied. The root mean square had the highest standard errors during maximum clenching effort (2.37–5.91) and at rest (1.47–6.86).
Conclusion: Mean frequency, median frequency and approximate entropy are the most reliable variables of surface electromyography signals of the masseter and temporal muscles during maximum clenching effort in individuals with cerebral palsy. These measures can be used to evaluate the function and behaviour of the masticatory muscles in this population following oral rehabilitation and surgical oral procedures as well as for the study the physiology of these muscles.

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1. Introduction

The masticatory muscles participate in complex physiological actions, such as chewing, swallowing, speech and other movements that depend on a precise balance between force, velocity and amplitude. Each muscle has a specific form and function for effective interaction with other muscles of the stomatognathic system. However, these functions are compromised in individuals with cerebral palsy (CP).

CP is a neuromotor disability stemming from damage to a specific region of the brain during the prenatal, perinatal or postnatal period and is associated with sensory and motor dysfunctions of the orofacial region, such as dysphagia, dysarthria, drooling and difficulty chewing. This irreversible condition ranges from mild to severe. Spasticity is the most frequent manifestation of CP and impairment can extend to oromotor function in the form of difficulty chewing, swallowing and speaking. Moreover, CP is often accompanied by involuntary movements that compromise the bite and jaw-opening reflexes, resulting in weakness of the masticatory muscles and difficulty coordinating these muscles. Due the lack of motor control, individuals with CP are more likely to exhibit temporomandibular disorder (TMD) and parafunctional oral habits, such as finger/thumb sucking and bruxism (grinding/clenching one’s teeth). Previous studies report that the prevalence rate of signs and symptoms of TMD ranges from 13.3% to 67.6% in individuals with CP.

Surface electromyography (EMG) has been extensively and safely used to evaluate chewing function and assess the efficacy of different therapies, such as global postural re-education, fixed implant support rehabilitation and the use of botulinum toxin-A. However, intrinsic and extrinsic factors can influence the interpretation of EMG signals. Intrinsic neurophysiological and anatomical factors include the pH level in muscle fibres, blood flow, number of active motor units during muscle contraction, shape of the intracellular action potential, distribution of motor unit discharge rates, motor unit synchronization and muscle fibre geometry. Extrinsic factors include the size and shape of the electrodes and placement on the skin overlying the muscle (inter-electrode distance, location and the orientation of detection surfaces relative to the muscle fibres). Thus, in clinical studies assessing changes within the same subject over time (inter-session evaluations), small changes in electrode placement in relation to previous positions may lead to measurement errors, since different electrode locations over the same muscle provide signals with significantly different features.

Different EMG variables are used to quantify muscle activity. The time and frequency domains have been employed with linear methods for the evaluation of EMG signals of the masticatory muscles. However, EMG signals are highly complex and the mechanisms underlying the generation of such signals seem to be nonlinear or even chaotic in nature. Indeed, a number of papers have found that nonlinear methods of EMG analysis are more sensitive to changes in myoelectrical signals in comparison to the linear methods. In clinical practice, nonlinear time-series analyses reveal the inherent complexity of normal variability, indicating features of motor control that are important for physiotherapists to measure. Information entropy has been proposed as a measure of irregularity (nonlinear behaviour) in biological signals. The measurement of entropy is reported to be a reliable method for characterizing neuromuscular alterations and approximate entropy (ApEn) provides a general understanding of the complexity of EMG data.

In statistics, ApEn is used to establish the uncertainty or variability of a system. ApEn calculated from EMG signals is dependent on the variability of both amplitude and frequency and better represents this aspect than the amplitude or frequency alone. Thus, ApEn can be used to quantify the irregularity or complexity of EMG signals of the masseter and temporal muscles in patients with CP. The combination of linear and nonlinear measures for the characterization and classification of EMG signals of the masticatory muscles in such individuals may be important to the quantification and understanding of the neurophysiological conditions of these muscles.

The selection of a measure for research or clinical use is motivated by several factors, including reliability. A number of studies have substantiated the reliability and reproducibility of EMG in the evaluation of the masticatory muscles in healthy individuals. However, as EMG signals are influenced by anatomic and neurophysiological factors, it is not possible to state whether the reproducibility found in healthy individuals holds true for patients with CP due to the oromotor abnormalities in these patients. Thus, reliability studies are particularly important for the masticatory muscles in this population due to the potential variation in measurements (intrinsic factors) that can affect the interpretation of the findings. To the best of our knowledge, no studies have addressed the reliability of surface EMG of the masticatory muscles in adults with CP or the reproducibility of nonlinear EMG variables of the masticatory muscles. It is therefore important to determine the variation in EMG measurements (within-day and between-day reliability) before proposing the use of EMG as a tool for evaluating the efficacy of therapies administered to improve the function of the masticatory muscles in this population.

The aim of the present study was to evaluate within-day and between-day reliability of EMG variables of the masseter and temporal muscles in patients with CP.

2. Methods

2.1. Subjects

Twenty-three adult patients with CP were evaluated at the Oral Special Care Clinic of the Institute of Science and Technology – Campus São José dos Campos/UNESP (Brazil). Only 15 individuals with spastic diparetic CP (8 males and 7 females) met the eligibility criteria. The sample was classified using the Gross Motor Functional Classification Scale (GMFCS): two individuals were classified on level I; two were classified on level II; four were classified on level III; and seven were classified on level IV. The inclusion criteria were spastic diparetic CP, partially preserved cognitive function (ability to respond to verbal commands, such as “open your mouth”, “close your mouth” and “clench your teeth”) and a statement
of informed consent signed by the participant or legal guardian agreeing to voluntary participation in the study. The exclusion criteria were having undergone orthodontic or orthopaedic treatment of the jaws or therapies to reduce spasticity (e.g., botulin toxin) in the 6 months prior to the study.

This study received approval from the local ethics committee (process number: 25000.058696/2010-74) and the Brazilian National Human Research Ethics Committee (CONEP number: 007/2011). All participants/guardians were properly informed regarding the objectives and procedures and signed a statement of informed consent prior to testing.

2.2. Electromyography

The EMG signals were captured using an eight-channel module (EMG System do Brasil Ltda) consisting of a conditioner with a band pass filter with cutoff frequencies at 20–500 Hz, an amplifier gain of 1000 and a common mode rejection ratio >120 dB. All data were acquired and processed using a 16-bit analogue-to-digital converter (EMG System do Brasil Ltda), with a sampling frequency 2 kHz per channel. Active bipolar electrodes with a pre-amplification gain of 20 times were used.

2.3. Procedure

The subjects visited the laboratory on two different occasions, with a 1-week interval between visits. During the sessions, the participants were instructed to remain seated in a chair, feet apart, shoulders relaxed and hands resting on thighs. The subjects were seated in a well-illuminated, silent recording room in a comfortable upright position with eyes open and without head support. A short training period was conducted prior to beginning the tests to prepare the subjects for the activities. Explanations concerning the procedures and electrode placement were given and the subjects were trained to bite as hard as possible (maximum clenching effort [MCE]).

Pre-gelled, self-adhesive, bipolar, silver–silver chloride electrodes (MediTrace) were positioned over the right masseter (RM), left masseter (LM), right temporal (RT) and left temporal (LT) muscles, with an inter-electrode distance of 20 mm. The sites for the electrodes were cleaned with a cotton ball soaked in 70% alcohol to diminish impedance. Surface electrodes were bilaterally placed according to anatomical references and the procedures were guided by the direction of muscle fibres at two points: anterior temporal muscle – 2 to 3 cm superoposterior distant to the lateral corner of the eyes in the region of greatest evidence of muscle mass, no hair, parallel to the muscle fibres, but with the sensing surface perpendicularly oriented; the superficial portion of masseter – 1 to 2 cm above the gonial angle of the mandible in the region of greatest evidence of muscle mass, with muscle fibres parallel to the surface. A rectangular metallic electrode measuring 3 cm by 2 cm coated with Lectron II conductive gel (Pharmaceutical Innovations) to increase the conduction capacity and avoid interference from external noise was attached to the left wrist of the volunteers for reference. The evaluations were carried out at rest and during MCE. In session 1 (test), three readings were performed in the resting position with a 2-min interval between readings. After an additional 3 min, three readings were performed during MCE, with a 5-min interval between readings. The signals were recorded for 10 s each under each condition. The same procedures (resting position and MCE) were repeated after a 1-week interval in session 2 (retest).

2.4. Data processing

The EMG signals were processed using specific routines carried out in the Matlab program, version 7.1 (The MathWorks Inc., Natick, MA, USA). For MCE, a three-second period was selected through a visual inspection of the raw data (Fig. 1). A moving window was used to select the EMG signals of the RT, RM, LT and LM muscles based on the greatest amplitude and regularity of the four muscles simultaneously. For the resting position, the entire 10-s period of the EMG signal was used in the analysis.

The EMG signals were analyzed using traditional linear analyses (amplitude and frequency domain). The complexity of the EMG signal was analyzed using approximate entropy (ApEn) (nonlinear analysis). The amplitude of the raw EMG signal was defined as the root mean square (RMSraw) calculated using a 200-ms moving window. The mean amplitude during the 3-s and 10-s trials recorded during MCE and the resting position, respectively, was used for analysis. The amplitude of the EMG signal during MCE (RMSraw – MCE) was normalized by the mean amplitude of the three EMG signals recorded during 10 s in the resting position (RMSraw – rest) as follows: 

\[
\text{RMS}_{\text{MCE}} = \frac{\text{RMS}_{\text{MCE}}}{\text{RMS}_{\text{MCE}} - \text{RMS}_{\text{rest}}}
\]

This normalization procedure is an alternative for patients with neurological disorders. The amplitude of the signal in the resting position was expressed as the percentage of the mean RMSraw – MCE recorded in the three readings, as follows:

\[
\text{RMS}_{\text{rest}} = \left(\frac{\text{RMS}_{\text{raw}} - \text{MCE}}{\text{RMS}_{\text{raw}} - \text{MCE}}\right) \times 100
\]

In the analysis of the frequency domain, the power spectral density of the EMG signal was calculated using Welch’s averaged periodogram with a Hamming window length of 2048 points. Overlap was 50% of the window length. Mean frequency (MNF) and median frequency (MDF) of the power

![Fig. 1 – EMG signal of the right masseter (RM), right temporal (RT), left masseter (LM) and left temporal (LT) muscle during maximum clenching effort. The grey band shows three-second period selected after visual inspection (Patient with cerebral palsy – age: 23 years; body mass: 51.4 kg). Three-second period selected through a visual inspection of the raw data.](image)
spectrum were calculated. The number of zero crossings (ZCs) was also analyzed. The ZC rate of the signals is defined as half the number of ZCs per second.\textsuperscript{34}

ApEn was calculated to quantify the irregularity or complexity of the EMG signals. This analysis returns a value between 0 and 2, with higher values reflecting greater irregularity within the time series.\textsuperscript{34–36} Given N points and tolerance r, ApEn \((m, r,N)\) is approximately equal to the negative mean natural logarithm of the conditional probability that two sequences similar for m points within the tolerance remain similar at the next point. The embedding dimension \(m\) and tolerance distance \(r\) were set to \(m = 2\) and \(r = 0.2\) of the standard deviation \((\pm SD)\) of the data sequence, as suggested by Pincus (1991).

For a better understanding of the use of this index in the analysis of the EMG signal, a number of studies have compared entropy values between subjects with and without illness/dysfunction\textsuperscript{37–39} and higher entropy values have been found among nondisabled subjects. These findings suggest that the absence of physiological complexity is related to pathology. In other words, values near 0 indicate the presence of illness or dysfunction and values near 2 indicate the absence of illness or dysfunction.

### 2.5. Data analysis

The Shapiro–Wilk test demonstrated that the data were normally distributed. Data on the EMG readings obtained in two sessions (test-retest) were expressed as mean and standard deviation (SD). The reliability of each EMG measure was quantified using intraclass correlation coefficients (ICCs) and the standard error of the mean (SEM). ICCs were calculated using the ICC\(_{1,1}\) model\textsuperscript{40} and SEM was estimated by subtracting the ICC value from 1, taking the square root of this value and multiplying by the SD (SEM = SD/\(\sqrt{1-ICC}\)).\textsuperscript{41} For the purposes of the present study, ICCs were interpreted using the following criteria: 0.00–0.39 = poor; 0.40 to 0.59 = fair; 0.60–0.74 = good; and 0.75–1.00 = excellent.\textsuperscript{42} The SEM was used to express reliability in absolute values, with higher values indicating a high level of error and implying non-reproducibility of the tested values. All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0.

### 3. Results and discussion

Fifteen individuals with spastic diparetic CP (8 males and 7 females; mean age: 26.9 ± 5.8 years; body mass: 54.4 ± 10.9 kg; height: 160.0 ± 9.0 cm; body mass index: 22 ± 3.63 kg/m\(^2\)) were included in the study. The data were analyzed to determine the reliability of the EMG variables in this population. Tables 1 and 2 respectively display the mean ICCs and SEM for each EMG reading of each muscle analyzed with the mandible at rest and during MCE.

<table>
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<th>Table 1 - Within-day (test and retest) and between-day (test-retest) reliability of EMG signal readings during MCE.</th>
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<td><strong>Mean (SD)</strong></td>
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<td><strong>RMS(_{SCE}) ((\mu)V)</strong></td>
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ICC, intraclass correlation coefficient; SEM, standard error of mean; RMS\(_{SCE}\), root mean square (MCE: maximum clenching effort); MDF, median frequency; MNF, mean frequency; ZC, zero crossing; ApEn, approximate entropy; RT, right temporal; RM, right masseter; LT, left temporal; LM, left masseter.
3.1. Intra-session reliability

During MCE, the ICCs for the three trials recorded in the two sessions (test and retest) revealed excellent intra-session reliability (range: 0.80–0.98) for all EMG variables in all muscles (Table 1). The RMS<sub>rest</sub> was the variable with the highest SEM (range: 2.37–5.91). In the resting position (Table 2), the ICCs revealed good to excellent reliability (range: 0.61–0.95) for RMS<sub>rest</sub>, mean and median frequencies (MNF, MDF) and zero crossings (ZC). However, the approximate entropy (ApEn) analysis revealed fair to good reliability (range: 53–74). The SEM was highest for RMS<sub>rest</sub> (range: 1.47–6.86).

3.2. Inter-session reliability

Inter-session reliability was calculated between the test and retest sessions with three readings under each condition. During MCE, the ICCs revealed good to excellent reliability (range: 0.60–0.86) mean and median frequencies (MNF, MDF) and zero crossings (ZC) and approximate entropy (ApEn). ICCs values for RMS<sub>ace</sub> were fair to good (range: 0.56–0.72) and the SEM was the highest for this variable (range: 6.98–11.93). In the resting position, the ICCs revealed poor to fair reliability (range: 0.23–0.57) for all EMG variables studied. The aim of the present study was to determine the reliability of linear and nonlinear measures of EMG activity in the masticatory muscles of individuals with CP during intra-session and inter-session analyses. The findings reveal that several variables are reliable and precise in the reading of EMG signals during MCE.

Regarding the intra-session analysis, all EMG variables exhibited good to excellent reliability at rest and during MCE. However, lesser reliability was found regarding the RMS, especially when the muscles were at rest. This lower degree of reliability may be related to the manner by which the amplitude of the EMG signal was normalized. In individuals without cognitive impairment, the indication is to normalize the amplitude of the signal by MCE. However, considerable variability was found in the EMG readings during MCE in the present study, indicating that the subjects had difficulty in maintaining MCE in a constant fashion (Fig. 1) and the decision was made to normalize the EMG signal based on the rest position, as suggested by Soderberg and Knutson (2000). Therefore, the amplitude values of the EMG signals should be interpreted with caution. With regard to the signal captured with the muscles at rest, the normalization process was not responsible for the low degree of inter-session reliability (test–retest), since low reliability was found for all EMG variables (MDF, MNF, ZC and ApEn).

Approximate entropy (ApEn) exhibited good to excellent intra-session and inter-session reliability during MCE. Moreover, the standard error of the mean (SEM) was low in both analyses. In the intra-session analysis with the muscles at rest, good reliability was found in the ApEn in the first session (test), but weak reliability was found for the right temporalis (RT) and left masseter (LM) muscles in the second session (retest). Nonetheless, the SEM was low. In the inter-session analysis, poor reliability was found for ApEn with the muscles at rest.
The variables extracted from the analysis of the frequency of the EMG signal exhibited better inter-session reliability (good to excellent) during MCE. The ICCs and SEM were similar for mean and median frequencies (MNF, MDF). Zero crossings (ZC) exhibited lower ICCs than those found for MDF and MNF, but the values remained within the same range of reliability and the SEM was low. The ZC rate has properties close to MDF and MNF, but is highly dependent on the signal-to-noise ratio in EMG signals and is also very sensitive to deviations in the amplitude of the signal. Thus, the use of this variable in the analysis of EMG signals in individuals with CP could be unnecessary when MDF and MNF are employed.

The better reliability of the frequency domain in relationship to amplitude domain during MCE may be related to the weakness of the masticatory muscles and difficulty coordinating these muscles in this group of patients. These problems are reflected in the fluctuation of the amplitude of the EMG signal, as demonstrated in Fig. 1. The amplitude of the signal is related to the recruitment and the discharge rates of active motor units. The results of computer simulation studies suggest that motor unit synchronization can alter the amplitude of the EMG signal due to the summation of motor unit potentials at the surface of the muscle. As motor unit synchronization reflects connections within the central nervous system, the fluctuation in the amplitude of EMG signal in the present sample may stem from abnormalities in the synchronization, recruitment and discharge rates of the active motor units caused by CP. Thus, the variation in the amplitude of the EMG signal stemming from these abnormalities and the method employed for the normalization of the amplitude of the signal may explain the lower degree of reliability regarding the RMS.

In relation to the mean and median frequencies (MNF, MDF), it has been suggested that shifts in these indices reflect the recruitment of progressively larger and faster motor units. Thus, the 3-s range used to analyze the EMG signal in MCE may not have been sufficient for possible alterations in the order of motor unit recruitment to be directly reflected in the MDF and MNF, which may explain the greater reliability of these variables.

Studies involving healthy individuals report that the masseter and temporal muscles exhibit a high degree of reliability (determined by the intraclass correlation coefficient), as found in the present study for individuals with CP. However, the studies cited did not determine reproducibility with the muscles at rest, which hinders comparisons with the present findings under this condition.

4. Conclusions

The present findings demonstrate that mean frequency, median frequency and approximate entropy are the most reliable variables of surface electromyography signals of the masseter and temporal muscles during maximum clenching effort in individuals with cerebral palsy. These measures can be used to evaluate the function and behaviour of the masticatory muscles in this population following oral rehabilitation and surgical oral procedures as well as for the study the physiology of these muscles. However, the ICCs of the EMG data recorded in the resting position demonstrate poor reliability in the inter-session analysis of the following EMG variables: RMS, MDF, MNF, ZC and ApEn.

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None.

Competing interests

The authors declare that there are no conflicts of interest.

Ethical approval

Not required.

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