

Accepted Manuscript

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PII: S1050-6411(17)30079-2

DOI: <http://dx.doi.org/10.1016/j.jelekin.2017.02.008>

Reference: JJEK 2060

To appear in: *Journal of Electromyography and Kinesiology*

Received Date: 2 March 2016

Revised Date: 8 December 2016

Accepted Date: 22 February 2017



Please cite this article as: F. Musto, R. Rosati, C. Sforza, M. Toma, C. Dellavia, Standardised surface electromyography allows effective submental muscles assessment, *Journal of Electromyography and Kinesiology* (2017), doi: <http://dx.doi.org/10.1016/j.jelekin.2017.02.008>

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STANDARDISED SURFACE ELECTROMYOGRAPHY

ALLOWS EFFECTIVE SUBMENTAL MUSCLES ASSESSMENT

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Abstract

The aims of this pilot study were to evaluate: i) the reproducibility and variability of an electromyographical protocol developed for the assessment of submental muscles (SM) ii) to apply the new protocol to maximal teeth clenching, a simple and largely studied static task in order to quantify the relative contribution of submental muscles. In 20 healthy subjects, aged 19-35 years, surface electromyography of SM, masseter (MM) and anterior temporalis (TA) muscles was performed during maximal voluntary clenching (MVC) with and without cotton rolls and the pushing of the tongue against the palate. Clenching on cotton rolls and pushing the tongue against the palate were used to standardise respectively MM and TA, and SM muscular potentials. The exercises were repeated in two appointments (T1-T2); submental muscles standardisation was also repeated twice (A-B) in each session to assess repeatability. Symmetry and activity were calculated for each couple of muscles. A two-way analysis of variance was computed for SM: no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found. SM recruitment was 31% of the maximal activity, with symmetry values larger than 80%. In conclusion, standardised electromyography allows a reliable assessment of Submental muscles activity.

Key words: surface electromyography, teeth clenching, submental muscles.

The authors certify that there is no financial support for this research and that there is no conflict of interest.

Introduction

Stomatognathic system and neck muscles often act as co-workers in many functions. Oral functions such as eating and swallowing are complex sensorimotor organizations that incorporate activities from multiple muscle groups in head and neck areas. Anatomical and functional correlations have been assessed between the trigeminal and the cervical system with reciprocal coactivations and inhibitions of the jaw, shoulder and even upper limb muscles during the performance of selected tasks [Ferrario et al, 2003]. A convergence of afferent neurons from the upper cervical nerve roots (C1–C3) and the trigeminal nerve into the trigemino-cervical nucleus caudalis has been assessed [Florencio et al, 2015].

Neurophysiological data shown the existence of trigemino-cervical reflexes in healthy subjects [Serrao et al, 2003; 2010]. Electrical stimulation of the trigeminal nerve induces early and late reflex responses in the neck muscles, termed trigemino-cervical reflexes. Such stimulation induces reflex responses in the neck muscles, including an early disynaptic, non-nociceptive reflex and a late polysynaptic, nociceptive reflex that also involves the proximal arm muscles (e.g. biceps brachii). The latter response has a nocifensive function and may be considered the electrophysiological counterpart of the reflex responses, known as head retraction reflexes, that protect against potential injury to the face and head [Serrao et al, 2003; 2010]. Wall and Taub (1962) proposed that the trigeminal system is necessary to provide a rapid pathway to the neck muscles for some physiologic reactions such as sucking-orienting reflex, reaction to pain and some head protective reflexes. Also suprahyoid muscles have a strictly relation with masticatory muscles coordination for the correct management of swallowing [Palmer et al, 2008; Takeda and Saitoh, 2016].

Among these muscles, the submental group directly participates to all the oral functions due to its insertion on the mandibular bone. For this reason, the role of submental muscles during different oral tasks can be of valuable interest in dentistry. Neck muscles maintain head position and support mandible movements and stability. Free neck movements are a prerequisite for mandibular movements, due to the close link of the mandible with suprahyoid and airway structures [Eriksson et al, 1998].

Functional and anatomical coupling between different components of head and neck muscular system has been assessed using non-invasive techniques. For example, maximal voluntary (MV) teeth clenching induced an overall symmetric contraction in the upper trapezius muscle [Sforza et al, 2011]; when young healthy subjects with a normal occlusion clench on an asymmetrical occlusal interference, they have an altered left-right side pattern of contraction of their sternocleidomastoid muscle: in almost all subjects, a previously symmetrical pattern became asymmetrical [Ferrario et al, 2003]. In clinics, the concurrent evaluation of jaw and neck muscles may help in a better patient assessment and treatment monitoring, since neck muscles alterations could be related with oral function [Yano et al, 2015]. Muscle activity associated with mastication, clenching and swallowing may be evaluated with surface electromyographical (sEMG) technique. sEMG is a simple, non-invasive, low cost method currently used in both research and clinical practice for the quantitative and qualitative analysis of superficial muscles. There have been many controversial opinions on the clinical application of sEMG, in fact many technical (electrodes type and position, cross-talk with other muscles etc.) and biological (tissues thickness, differences in muscular contraction etc.) factors influence the registered signal and make comparative and longitudinal evaluations difficult to be conducted [Suvinen et al, 2009]. However, the use of standardised and repeatable protocols eliminates such factors and makes qualitative and quantitative analysis possible to be carried on as well as clinically useful [Suvinen et al, 2009;

Sforza et al, 2011]. Previous investigations assessed the reproducibility of various protocols for the standardisation of sEMG signals, for different kind of muscles, such as Masseter, Anterior Temporalis [Ferrario et al, 2000] , Trapezius [Sforza et al, 2011] and Sternocleidomastoid muscles [Ferrario et al, 2003]; however no standardised surface electromyography (ssEMG) reliability has been demonstrated for the assessment of the submental muscles activity.

The aims of the present study were:

- i) to evaluate the reproducibility and variability of an ssEMG protocol developed for the assessment of submental area muscles.
- ii) to apply the new protocol during maximal teeth clenching, a simple and largely studied static task in order to quantify the relative contribution of submental muscles.

Material and methods

Study design

The present study can be considered as a pilot study. Standardised cutaneous myoelectric activity of Temporalis, Masseter and Submental muscles was recorded during teeth clenching. Data were obtained during two examination sessions (T1 and T2) with a 2 weeks interval. Statistical instruments were applied to evaluate test reliability and define healthy subjects mean values.

Subjects

Twenty healthy students attending the second year of our Dental degree course (10 males, 10 females, age range 19–35 years, mean 25, SD 5), volunteered for the study after a detailed explanation of the experimental protocol and possible risks involved. The study protocol was approved by the local ethic committee (Ethic Committee from Università degli Studi di Milano – Code: DG-EMG-2016). The subjects were visited by a dentist, expert in sEMG acquisition (MF); all

the subjects had not systemic diseases, were free from stomatognathic apparatus and neck pathologies and were selected for the study according to the following inclusion criteria: a minimum of 28 permanent teeth, no periodontal problems, no craniofacial and cervical trauma and surgery, no temporomandibular and craniocervical disorders, no current orthodontic treatment and no painful sensation when clenching their teeth. The subjects were excluded from the study if they had neurological problems that could interfere with the experimental procedure, or if they were taking drugs that could affect the musculoskeletal system, such as anti-inflammatory or pain relief drugs.

During the sEMG recording, the environment was quiet and with low light. The subjects sat in a comfortable office type chair, with erect posture with the feet flat on the floor and with arms resting on their legs.

Electrode type and positioning

The anterior Temporalis (TA), Masseter (MM) and submental area muscles (SM) of both sides (left and right) were examined. Disposable pre-gelled silver/silver chloride bipolar surface electrodes (rectangular shape, 21x41 mm, 20 mm inter-electrode distance) (F3010, Fiab, Firenze, Italy) were positioned according to the recommendations of SENIAM (Surface EMG for Non-Invasive Assessment of Muscles) [Hermens et al, 2000]. On each muscle a bipolar electrode was positioned on the muscular bellies parallel to muscular fibres as follows:

- MM: the operator, standing in front of the seated subject, palpated the muscular belly while the subject clenched his/her teeth. The electrodes were fixed parallel to the exocanthion-gonion line and with the upper pole of the electrode under the tragus-labial commissural line.

- TA: the muscular belly was palpated during tooth clenching and the electrodes were fixed vertically along the anterior margin of the muscle (corresponding to the fronto-parietal suture).
- SM: each electrode was placed parallel to the anterior digastric belly, paramedian to the midline and lightly diverging, 1 cm posterior to the mental symphysis.

A disposable reference electrode was applied to the forehead or on the earlobe (Figure 1). To reduce skin impedance, the skin was carefully cleaned prior to electrode placement, and recordings were performed 5 min later, allowing the conductive paste to adequately moisten the skin.

Instrumentation

Surface EMG activity was recorded using a computerised instrument (Easymyo, 3 Technology S.r.l., Udine, Italy). The analogue sEMG signal was amplified (gain 100, bandwidth 0–1000 Hz, peak-to-peak input range from 0 to 3600 mVpp) using a differential amplifier with a high common mode rejection ratio (CMRR 100 dB in the range 0–60 Hz, input impedance 100Gohm), digitised (24 bit resolution, 4000 Hz A/D sampling frequency), and digitally filtered (high-pass filter set at 30 Hz, low-pass filter set at 400Hz, band-stop for common 50–60 Hz noise). The signals were averaged over 25 ms, with muscle activity assessed as the root mean square (RMS) of the amplitude (μV). sEMG signals were recorded for further analysis. Before acquisition session, the subjects were properly trained to elicit true maximal voluntary contraction using an on-time sEMG signal visualization.

Measurements

Each appointment (T1 and T2, with a 2 weeks interval between them), was composed by three acquisition steps:

- Masticatory muscles standardisation procedures: two 10-mm thick cotton rolls were positioned on the mandibular second premolars/first molars of each subject, and a 5-s maximum voluntary contraction (MVC) was recorded to standardise TA and MM sEMG signal. The mean sEMG potential obtained in the first acquisition was set at 100%, and all further sEMG potentials were expressed as a percentage of this value ($\mu\text{V}/\mu\text{V}\times 100$) [Ferrario et al, 2000].
- Submental muscles standardisation procedures: the subjects were invited to push their tongue at their best (without teeth clenching) against the palate, and a 5-s sEMG SM activity was recorded. All further SM sEMG potentials were expressed as a percentage of this value ($\mu\text{V}/\mu\text{V}\times 100$). This test was repeated twice (A and B) in each appointment, in order to assess SM muscles standardisation procedures repeatability.
- MV teeth clenching: TA, MM and SM sEMG activity was recorded during a 5-s MVC test in intercuspal position (IP); as “the complete intercuspatation of the opposing teeth independent of condylar position, sometimes referred to as the best fit of the teeth regardless of the condylar position” (The Academy of Prosthodontics, 2005). The subjects were invited to clench as hard as possible and to maintain the same level of contraction during the entire test.

For each acquisition the best performance 3s intervals were isolated and analysed. During the tests, the subjects were asked to perform at their best, to avoid head and neck movements and maintain a relaxed facial expression to reduce cross-talks. Within subject, test order was randomised by a computer random number generator and 1 minute rest period was allowed. All acquisitions were made by the same operator.

sEMG data analysis

Using the instrument software tools, for each acquisition session, sEMG waves were analysed computing two standardised indexes as follow:

- Muscles symmetry was estimated calculating the percentage overlapping coefficient (POC, %) [Ferrario et al, 1999; 2000], an index of symmetric muscular contraction ranging between 0% and 100%. When two paired muscles contract with perfect symmetry, a POC of 100% is obtained. Temporalis, Masseter and submental muscles POCs were obtained for each subject for each acquisition session.
- Muscles activity was estimated calculating the standardised activity index (Impact, %). It quantifies the total muscular recruitment during MVC relative to the standardisation test computing the mean total muscle activities as the integrated areas of the sEMG potentials over time [Ferrario et al, 2002]. The index was obtained for each muscle and for each acquisition session.

Submental muscles indexes were computed twice in each appointment since standardisation procedures were repeated (T1A-T1B; T2A-T2B).

Statistical Evaluations

Descriptive statistics were computed for all sEMG indexes. To evaluate SM muscles ssEMG protocol reliability, "Factor 1" (F1-appointment, T1 vs T2), "Factor 2" (F2- standardisation session, A vs B) and F1 X F2 systematic effects on SM indexes were estimated using two-way factorial analysis of variance.

Mean absolute difference (MAD), technical error of measurement (TEM), and relative error magnitude (REM) were calculated for SM indexes to quantify the reliability of the proposed ssEMG protocol. MAD is the average of absolute differences between the values of 2 sets of

measurements; TEM or Dahlberg's error was used to evaluate the random error and was computed as

$$TEM = \sqrt{(\sum D^2) / 2n}$$

where D is the difference between each couple of replicate measurements and n is the number of couples [Rosati et al, 2010].

The REM was obtained by dividing the MAD for a variable by the grand mean for that variable and multiplying the result by 100; it represents an estimate of error magnitude relative to the size of the measurement.

To evaluate the effect of sex and the possible differences among masticatory and submental muscles in muscle symmetry and recruitment during teeth clenching, a set of analyses of variance were made. For both POC and Impact indexes, a two-way ANOVA (factor 1: muscles, Temporalis, Masseter, and Submental; factor 2: sex) was made. For all statistical tests, significance was set at 5% ($P < 0.05$), with a beta error (Type II) larger than 0.95 [Sforza et al, 2011].

Results

The ssEMG assessment of SM muscles was reliable, as shown in Table 1. For both POC and Impact indexes, no Factor 1 (T1 vs T2) or Factor 2 (A vs B) or F1 X F2 significant effects were found, with small MAD and TEM values. In contrast, the REM value of impact index was larger than 30%. Since SM indexes were repeatable, mean values were computed for the subsequent comparisons (between males and females and among muscles).

Data about masticatory and submental area muscles symmetry and recruitment during teeth clenching are reported in Table 2; the values of the symmetry index POC were larger than 80% for all couples of muscles. Regarding recruitment values, Temporalis and Masseter indexes resulted approximately the same as the ones recorded during the standardisation acquisition, while

submental muscles developed on average 31% of the activity expressed during the standardisation test. The recruitment of submental area muscles resulted significantly lower than that of Masseter and Temporalis muscles in both sexes ($P < 0.001$). Side-related differences were not evaluated due to the high POC values; for Impact index comparisons we considered a mean between the Impact of the right and the left side.

Discussion

The standardised protocol proposed in the present study demonstrated a high repeatability of the EMG indexes of all the analysed muscles both intra and inter-appointment. Both symmetry and recruitment of submental area muscles were found to be reliable during a basic oral function such as MVC. ssEMG protocols during clenching have been largely studied and accepted by the scientific literature as a useful tool to evaluate the superficial masticatory muscles activity and therefore they recently entered in the daily clinical practice [Ferrario et al, 2006; Suvinen et al, 2009]. On the contrary, surface EMG analysis of dynamic oral functions, such as mastication and swallowing, is still an open field of research and further scientific approval is needed before clinical applications. Several studies have concluded that the activity of the mouth floor muscles during swallowing is highly variable in terms of quantitative and qualitative data [Vaiman et al, 2004A;2004B;2004C]. The purpose of the current study was therefore to develop a standardised protocol for the assessment of the submental muscles, which is mandatory to ensure reliable data during oral functions analysis.

In the present study, a static analysis, such as MVC, was preferred as it represents the simplest and best described oral task. No significant differences were found for symmetry and recruitment indexes of the submental muscles neither within the single appointment nor between the two

recording sessions. However the symmetry showed a low variability, while the recruitment index was highly variable (REM about 37%). One possible explanation for this large value can be found in the biomechanics of the analysed muscles. During maximal voluntary clenching in intercuspal position, the elevator muscles (among which Masseter and Temporalis) perform their activities between relatively fixed bone ends, although the mandible undergoes a non-negligible deformation caused by elevator muscles stress during clenching (Jiang and Ai, 2002). Submental muscles have a fixed origin on the mandible which is solidarized to the skull by the contraction of elevator muscles, and an insertion on the hyoid bone whose stability is directly linked to mutual coordination with infrahyoid muscles. The elevator muscles must clench as hard as possible; submental muscles instead must "negotiate" their performance with infrahyoid muscles to ensure the stability of the whole pharyngo-laryngeal complex. It is plausible that the larger variability of the submental muscles during teeth clenching may be due to this "dynamism in coordination" to assure hyoid positioning.

Regarding muscle recruitment in healthy subjects, we can emphasize that the Masseter and Temporalis muscles perform similarly when clenching either with cotton rolls or in maximal intercuspidation (as amply demonstrated in previous studies) [Botelho et al, 2009; Forrester et al, 2011; Frongia et al, 2013].

As expected the floor of the mouth showed a significantly lower activation than masseter and temporalis muscles, since it works mainly as a jaw depressor. However, in our recordings the submental muscles were recruited with an average value of about 31% of the standardising exercise, which let hypothesise a not negligible co-working role in clenching. Our results are slightly higher than those (about 24%) reported by a previous study [van Willigen et al, 1997]. The differences in the values could be due to different standardisation exercises (in the previous study the subject was asked to activate the mouth opening muscles with a blocked jaw). This quite high

co-activation of the mouth floor during teeth clenching has been proposed as the result of a motor pattern established with the occlusal overload protection purpose in the event of sudden mechanical failure [van Willigen et al, 1997].

Regarding muscles symmetry in healthy subjects, our data confirm those of previous studies that reported mean values of asymmetry lower than 17% for all muscles. [Ferrario et al, 2000].

The major limitation of the present investigation is the standardisation procedure (pushing the tongue against the hard palate) that did not assure to obtain the maximal muscles activity, but it allowed us to identify a repeatable exercise involving a high activity level of submental muscles which can be used as a reference for subsequent tasks analysis. Another limitation is the reduced number of recruited subjects, since the research was designed as a pilot study aiming at the definition of a new protocol that should be further verified in a larger sample, thus obtaining mean values of the submental muscles activity in the population. After the validation, the protocol might be applied to the study of other oral functions, in which submental muscles are supposed to be more recruited, such as swallowing and chewing. In the future quantitative parameters could thus be at a disposal of both the clinician and the researcher during diagnosis, treatment planning and monitoring of patients with altered oral functions.

Conclusions

Within the limitations of the present study, we could conclude that the proposed protocol allowed a reliable assessment of submental muscles during MVC in healthy subjects. The muscles had a symmetrical pattern of activation reaching approximately one third of their maximum recorded activity.

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Table 1: Standardised surface electromyography (ssEMG) indexes –POC (%) and Impact (%) mean and standard deviation (SD) for Submental Muscles and their reliability, evaluated computing a two-way analysis of variance: F1 (T1 vs T2) and F2 (A vs B). No F1 X F2 significant differences. N.S. not significant ($p>0.05$). Mean Absolute Difference (MAD); Technical Error of Measurement (TEM) and Relative Error Magnitude (REM).

Submental ssEMG reliability during teeth clenching										
		T1		T2		T1 vs T2	A vs B	MAD	TEM	REM
		A	B	A	B					
POC (%)	mean	85.79	85.92	85.37	85.46	N.S.	N.S.	5.13	4.72	6
	SD	7.66	7.04	4.21	4.14					
IMPACT (%)	mean	31.99	32.11	28.67	29.79	N.S.	N.S.	11.48	10.80	37
	SD	14.52	12.12	16.54	12.48					

Table 2: Masticatory muscles (Anterior Temporalis, Masseter, Submental muscles) ssEMG during teeth clenching. Symmetry estimated by the Percentage Overlapping Coefficient index (POC%) and recruitment by the Impact (%) index. Comparisons were performed by 2-way ANOVAs. N.S. not significant ($p>0.05$). Mean with different superscripts (A and B) differ at *post hoc* test.

	POC (%)				IMPACT (%)			
	Men		Women		Men		Women	
	Mean	SD	Mean	SD	mean	SD	mean	SD
Temporalis	84.17	1.93	82.81	2.18	104.6 ^A	16.5	108.2 ^A	19.5
Masseter	82.93	2.79	83.14	2.03	109.85 ^A	23.7	99.52 ^A	17
Submental	84.75	3.86	81.46	8.57	31.74 ^B	12.84	29.54 ^B	14.86
Comparison	muscle	N.S.			muscle	$p<0.001$		
	sex	N.S.			sex	N.S.		
	muscle x sex	N.S.			muscle x sex	N.S.		

Figure 1: Electrodes positioning for Masseter muscles, Temporalis Muscles, Submental area muscles. Reference electrode was applied on the earlobe.

