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ACCEPTED MANUSCRIPT

Reorganization of muscle activity in patients with chronic temporomandibular

disorders

Reorganization of muscle activity on TMD

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1

Highlights

- We examined reorganization of masticatory muscle activity in chronic TMD patients.
- A functional index of EMG for maximal clenching and gum chewing was introduced.
- TMD patients have reduced cooperation and coordination between masticatory muscles.
- TMD patients recruited the balancing side more than the control group.
- Worse functional index was associated with more severe symptomatology.

Abstract

Objective To investigate whether reorganization of muscle activity occurs in patients with chronic temporomandibular disorders (TMD) and, if so, how it is affected by symptomatology severity.

Methods Surface electromyography (sEMG) of masticatory muscles was made in 30 chronic TMD patients, diagnosed with disc displacement with reduction (DDR) and pain. Two 15-patient subgroups, with moderate (TMDmo) and severe (TMDse) signs and symptoms, were compared with a control group of 15 healthy subjects matched by age. The experimental tasks were: a 5s inter-arch maximum voluntary clench (MVC); right and left 15s unilateral gum chewing tests. Standardized sEMG indices characterizing masseter and temporalis muscles activity were calculated, and a comprehensive functional index (FI) was introduced to quantitatively summarize subjects' overall performance. Mastication was also clinically evaluated.

Results During MVC, TMDse patients had a significantly larger asymmetry of temporalis muscles contraction. Both TMD groups showed reduced coordination

activity distribution shifted significantly from masseter to temporalis muscles. During

chewing, TMDse patients recruited the balancing side muscles proportionally more than

controls, specifically the masseter muscle. When comparing right and left side chewing,

the muscles' recruitment pattern resulted less symmetric in TMD patients, especially in

TMDse. Overall, the functional index of both TMDmo and TMDse patients was

significantly lower than that obtained by controls.

Conclusions Chronic TMD patients, specifically those with severe symptomatology,

showed a reorganized activity, mainly resulting in worse functional performances.

Keywords Temporomandibular Disorders, Electromyography, Jaw Muscles,

Mastication, Pain, Motor Control

Introduction

Temporomandibular disorder (TMD) is a comprehensive term for musculoskeletal

disorders in the jaw muscles and/or the temporomandibular joint (TMJ). A common

subgroup of TMD is the disc displacement with reduction (DDR), manifesting with

TMJ noise during jaw movement or function, with a prevalence ranging from 18% to

35% of the population. (Naeije, Te Veldhuis, Te Veldhuis, Visscher & Lobbezoo, 2013;

Schiffman et al., 2014). TMD patients with both DDR diagnosis and pain as well as

complaints of mandibular function impairment are common among those that seek

treatment (Ferreira et al., 2014; Santana-Mora et al. 2014; Tartaglia, Lodetti, Paiva, De

Felicio & Sforza, 2011).

3

Relevant information about patients and their functional limitations may be obtained by specific questionnaires for measurement of symptoms (Helkimo, 1974; De Felício, Melchior & Da Silva, 2009; Gonzalez et al., 2011; Ohrbach et al., 2011; 2013); and clinical evaluations (Dworkin & LeResche, 1992; De Felício et al., 2012; Ferreira et al., 2014). Whereas, functional analyses, like surface electromyography (EMG), have been employed in order to obtain a better understanding of TMD. EMG analysis is useful to elucidate the masticatory muscles function and adaptation in patients with TMD, using indices of muscular dominance and asymmetry as those proposed by Naeije, McCarroll and Weijs (1989). to analyze maximum voluntary contraction (MVC) (Santana-Mora et al., 2009; 2014) and with the assessment of the concomitant co-ordination and cooperation of paired temporalis and masseter muscles during mastication (Kumai, 1993). Additionally, EMG has the potential to contribute to therapy plan (De Felício, Mapelli, Sidequersky, Tartaglia & Sforza, 2013; Ferreira et al., 2014; Lodetti et al., 2014; Ries et al., 2014; Santana-Mora et al. 2014).

Previous studies showed decreased raw EMG activity of jaw muscles in TMD patients compared to control subjects, association between decreased activity and increased severity (Ardizone et al., 2010), and asymmetry between affected side and non-affected side in unilateral TMD patients (Santana-Mora et al., 2009). To distinguish real changes from biological and instrumental noise, standardized EMG has been strongly recommended (Ferrario, Tartaglia, Maglione, Simion & Sforza, 2004; Ferrario, Tartaglia, Galletta, Grassi & Sforza, 2006), mainly for inter-individual comparison. Particularly in chronic TMD (present for at least 6 months), impaired coordination in the masticatory muscles has been observed during MVC (De Felício et al., 2012; Lodetti et al., 2014; Santana-Mora et al., 2014; Tartaglia et al., 2011) and during chewing (Ferreira et al., 2014); these changes were not evident in non-chronic TMD of

mild-moderate severity symptomatology (De Felício et al., 2013). Thus, both duration and severity that negatively influence the TMD prognostic (Rollman, Visscher, Gorter & Naeije, 2013; Von Korff & Dunn, 2008) may be associated with the orofacial motor function (De Felício et al., 2013; Ries et al., 2014).

Despite the previous findings and the near consensus about association between unilateral chewing preference and TMD (Casanova-Rosado et al., 2006; Ferreira et al., 2014; Ratnasari et al., 2011; Santana-Mora et al., 2009) the relationship between the masticatory muscles in chronic TMD exhibiting different grades of severity is not trivial. Therefore, researchers have investigated the relationship between orofacial motor function and pain by means of EMG analysis, in standardized settings, without the interference of confounding factors. Based on these studies (Sae-Lee et al., 2008; Shimada, Baad-Hansen & Svensson, 2015; Shimada, Hara & Svensson, 2013) and theories (Hodges & Tucker, 2011; Hodges & Smeets, 2015; Peck, Murray & Gerzina, 2008) it has been proposed that pain in jaw muscles, rather than a stereotyped change, may involve differential effects as increased, decreased, or redistributed activity with reorganization of activity occurring within and between muscles. Authors (Sae-Lee et al., 2008; Shimada et al. 2013; 2015) have suggested that investigations with patients are required.

The objective of the current cross-sectional study was to investigate whether reorganization of muscle activity occurs in chronic TMD patients with clinically detected DDR and, if so, how it is affected by symptomatology severity. New fit-for-purpose EMG indices were introduced, a global functional index (FI, %) was elaborated and its ability to represent comprehensive functionality of the masticatory muscles was tested.

Materials and methods

Subjects

The study was approved by the institutional Ethics Committee and all subjects gave written informed consent to participate.

Thirty chronic TMD patients (pain duration > 6 months, mean±SD 59.2±52.7, range 7-240 months) with DDR (disc displacement with reduction) were selected among consecutive patients who came to our institution for treatment of orofacial pain and TMD. Fifteen healthy subjects, paired for age and sex, were recruited for the control group (C, 14 women and 1 man).

The inclusion criteria for the patient group were to present chronic TMD pain (myalgia and/or arthralgia) with DDR diagnosis, based on history and clinical examination; for C group were good general health and absence of TMD history. Subjects with tooth absence (except the third molars), dental pain or periodontal problems, denture use, dentofacial deformities, crossbite, open bite, pacemaker use, neurological or cognitive deficits, previous or current tumors or traumas in the head and neck region, pregnancy, current or previous orthodontic, orofacial myofunctional or TMD treatments, current use of analgesic, anti-inflammatory and psychiatric drugs were excluded from the study.

History and clinical examination

Participants were interviewed and examined by independent experienced examiners, one for each protocol, blinded to the outcome of the other ones. Individuals' TMD symptomatology was screened and measured with a validated self-report questionnaire about TMD signs and symptoms and orofacial functional status (ProTMDmulti) (De

6

Felicio et al. 2009; 2012). The total ProTMDmulti score ranges from 0 (no pain referred) to 400 (worst pain perception), and the median of the 30 patients' scores (109) was used to classify patients into two groups: TMDmo (moderate, subjects with score<109) and TMDse (severe, subjects with score>109).

Clinical examination was performed in accordance with the Research Diagnostic Criteria for TMD (RDC/TMD), axis I (http://www.rdc-tmdinternational.org/) (Dworkin & LeResche, 1992). A clinical examination protocol was also adopted. (Ohrbach, Gonzalez, List, Michelotti & Schiffman, 2014). The diagnostic procedures were based on the "Diagnostic Criteria for the Most Common Pain-Related Temporomandibular Disorders" – DC/TMD (Schiffman et al., 2014).

The demographic of each group and the patients' distribution of TMD severity and diagnosis are shown in Table 1.

sEMG recordings and measurement instrumentation

The masseter and anterior temporalis muscles of both sides (left and right) were examined. EMG activity was recorded using a wireless EMG system (FreeEMG, BTS S.p.A., Garbagnate Milanese, Italy), with light probes (weight, 5 g) clipped to the electrodes. Paired disposable Ag/AgCl pre-gelled electrodes (sensor area, 3.14 cm²; inter-electrode distance, 2 cm; Kendall, Covidien, Mansfield, Canada) were placed on the skin along the main direction of the muscular fibers, detected by palpation during MVC. Before electrode placement, the skin was scrubbed with an alcohol soaked gauze pad. Men were kindly requested to attend clean-shaven, to facilitate electrode placement. For each subject, the electrodes were positioned at the beginning of the experimental session, and all trials were performed without any modification of the electrodes and/or of their position. The analog EMG signal was amplified and digitized

(gain 150, resolution 16 bit, sensitivity 1 mV, temporal resolution 1 ms) using a differential amplifier with a high common mode rejection ratio (CMRR>110 dB in the range 0-50 Hz, input impedance>10 G Ω). All the recorded EMG signals were digitally band-pass filtered between 80 and 400 Hz with a 2nd order Butterworth filter, and rectified by calculating the root mean square (RMS) in temporal windows of 25 ms.

The subject, who sat on a chair with his/her head in a natural erect position, was asked to perform two experimental tasks: a 5-s inter-arch maximum voluntary clench (MVC) and two 15-s unilateral (right and left) gum chewing tests.

To standardize the EMG potentials of the four analyzed muscles, two 10 mm thick cotton rolls were positioned on the mandibular second premolar/first molars of each subject, and a further 5-s MVC (COT) was recorded (Ferrario et al., 2004). The 3-s period with the most stable signals was automatically detected and the corresponding mean value of each muscle's RMS sequence was referred to as 100% of amplitude.

To avoid any fatigue effect, a rest period of at least 3 min was allowed between standardization and functional recordings.

Maximum voluntary clench

The subject was invited to clench his/her teeth in maximum contact (inter-cuspal position) as hard as possible, and to maintain the same level of contraction for 5 s. The best 3-s period (that with the most constant EMG signals) was automatically selected, its four 120-RMS samples standardized, and used for the computation of the percentage overlapping coefficient (POC) indices (Ferrario et al., 2006). These coefficients quantify the temporal equilibrium (co-ordination) of the standardized activities and are calculated as the residual to 100% of the percentage ratio of the non-superimposed area between two RMS sequences over their total sum, ranging between 0% (no equilibrium)

and 100% (perfect co-ordination). For each subject, temporalis (POC_T) and masseter

(POC_M) muscles indices assessed the degree of right-left asymmetry; the torsion

coefficient (POC_{TORS}) quantified the potential force momentum (Ferrario et al., 2006).

Another index assessed the degree of co-operation between bilateral temporal and

masseter muscles' activities (POC_{TM}). The Asynergy index [deriving from the Activity

index introduced by Naeije et al. (1989) but herein operating on normalized signals

was computed as the percentage ratio of the difference between the two mean masseter

and the two mean temporalis standardized potentials and the sum of all (range: $\pm 100\%$).

Besides these standardized parameters, also the absolute mean RMS activities (unit,

μV) of masseter (COT_M) and temporalis (COT_T) muscles performed in COT trials were

analyzed.

Mastication: Unilateral gum chewing

The second task was the 15-s unilateral, left and right, chewing of a pre-softened

sugarless gum (1.5 g; Trident, Kraft Foods, São Paulo, Brazil).

EMG signals of the four muscles were standardized on COT trial as detailed for

MVC, the masticatory cycles were automatically detected (Ferrario et al., 2004) and the

chewing frequency computed (unit, Hz).

A bivariate analysis was performed on the simultaneous differential right-left

masseter (rM-lM, x-coordinate) and temporalis (rT-lT, y-coordinate) standardized

activity (Lissajous's Cartesian plot) (Kumai, 1993; Ferrario et al., 2004). Variability in

the pattern of contraction of masticatory muscles was estimated by the 90%-standard

ellipse area (unit, $\%^2$): small ellipses correspond to highly repeatable muscular patterns,

while wider ellipses indicate a larger variability for the same task (Kumai, 1993;

Ferrario et al., 2004).

9

To directly compare right- and left-side chewing ellipses, the latter was mirrored, making the ΔM and ΔT coordinates represent the differentials between the working side muscles and the balancing side ones; positive values of the two thus indicated a prevalence of the working side muscular activities (Figure 1). Then, the coordinates of each ellipse's center, ΔM _center and ΔT _center, were calculated (unit, %).

To assess if the two unilateral side chewing tasks were performed with symmetrical muscular patterns, from the centers of the two ellipses (left and right-side chewing), the symmetrical mastication index (SMI, %) was computed (Ferrario et al., 2004). SMI ranges between 0% (asymmetrical muscular pattern) and 100% (symmetrical muscular pattern).

The total (right and left masseter and temporalis muscles) standardized activity during 15-s chewing was computed as the sum of the four integrated areas of the EMG potentials over time (Global activity, %s). Also, the mean activity of a single chewing cycle (Activity/cycle, %s) and the percentage of the activity referred to the working side muscles (wActivity, %) were calculated (Ferreira et al., 2014).

Currently, for each subject's chewing index other than SMI, the mean between right and left chewing side values was calculated and further considered for the inter-group comparison. Besides, the inter-side differences were quantified by indices of symmetry (SIs), calculated as the ratio between the lower side value and the higher of the two. Only for the wActivity, the SI was calculated as the residual to 100% of the absolute difference between right and left chewing side values. For all SI variables 100% indicated the best symmetry condition.

Functional Index

A comprehensive functional index (FI, %) was introduced to quantitatively summarize each subject's overall performance. At first, the tolerance interval for a normal distribution, covering at least a proportion of 95% of the control population (TI_{95%}), was computed for each standardized parameter, based on control group scores, which were all normally distributed (Kolmogorov-Smirnov test, non-significant). One-sided or two-sided 95%-tolerance intervals were arbitrarily chosen depending on the characteristics of the indices (i.e. how their formulas were devised), as follows:

- one-sided TI95% with upper limit for: Global activity, Activity/cycle;
- one-sided TI_{95%} with lower limit for: POC_M, POC_T, POC_{TORS}, POC_{TM}, SMI, Δ M center, Δ T center, wActivity and the SI indices;
- two-sided TI_{95%} with symmetric bilateral limits for: Asynergy index and Chewing frequency.

Then, each subject's FI was calculated as the ratio of the number of parameters with scores within the relevant TI_{95%} over the total 15 assessed parameters; non-repeatable (see below) and non-normalized EMG indices were excluded. The index ranges from 0% (no patient's value is inside the relevant TI_{95%}) to 100% (all 15 patient's values are inside the relevant TI_{95%}).

Clinical evaluation of the masticatory type

Masticatory type was assessed using the orofacial myofunctional evaluations with scores. The subjects were instructed to chew in a usual manner (free chewing) a chocolate-flavoured stuffed Bono cookie® (Nestlé, São Paulo, Brazil). During chewing test, video images were registered. Subsequently, masticatory type was analyzed, classified and scored, according with masticatory strokes localization as follows: bilateral alternate (score, 4); bilateral simultaneous (score, 3); unilateral preference

(with the masticatory strokes occurring on the same side of the oral cavity from 66 to 94% of times; score 2); chronic unilateral (when the masticatory strokes occurred on the same side in 95-100% of the times; score 1); or anterior (when the masticatory strokes occurred in the region of the incisors and canines; score 1) (De Felício et al., 2012).

Measurement reliability

EMG measurement repeatability of MVC had been already assessed by repeated analyses of seven normal adult subjects chosen at random; for all MVC variables the intra-class correlation coefficient (ICC) ranged between 0.63 and 0.98, without significant differences among the measurement sessions (Ferrario et al., 2006). Good repeatability and reproducibility of the MVC parameters have been confirmed by De Felício et al. (2012).

EMG measurement repeatability of the 15 mastication parameters was assessed comparing two consecutive repetitions of 15-s unilateral gum chewing in eight randomly chosen subjects (4 control, 4 TMD). The paired Student's t-test was applied to check for the presence of a systematic change in the mean, and the coefficient of variation (CV) of the typical error of measurement (TEM) was used to quantify the mean random variation of the recording. Besides, the ICC was computed to test the inter-repetition correlation.

Statistical analysis

For each group, median and interquartile range (IQR) were calculated for all the EMG indices, as well as for the demographic data and ProTMDmulti score. Because several variables had no homogeneity of variances, the Kruskal-Wallis test was applied to assess the difference of medians among the three groups; in case of significant

differences, the post-hoc Median tests were made for all pairs of groups (two-sided significance levels with Bonferroni's adjustment).

Fisher exact test was applied to compare inter-group sex distribution and masticatory type frequencies.

The level of significance was set at *P*<0.05. All statistical calculations were made using the software STATISTICA 12 (StatSoft Inc., Tulsa, OK, USA).

Results

Data repeatability

In general, the repeatability of the masticatory EMG parameters was good, except for the ΔT _center_SI (statistical difference between test and retest), SIs of Frequency and Global activity (ICC<0.6) and ΔT _center_SI, 90%-standard ellipse area and its SI (TEM_CV>15%) (Table 2). These non-repeatable parameters were excluded from the analysis.

Maximum voluntary clench

Significant inter-group differences were found for the two non-normalized maximal muscular activities COT_T and COT_M, and for the normalized indices POC_T, POC_{TM} and Asynergy (Table 3). The post-hoc tests showed that TMDse group had a significantly lower maximal activity of the temporalis (COT_T P=0.039) and masseter muscles (COT_M P=0.025) and larger asymmetry on temporalis muscles (POC_T, P=0.021) than C group. Also, both patient groups showed smaller co-ordination between the pairs of masseter and temporalis muscles when compared to C group (POC_{TM}: TMDmo, P=0.038; TMDse, P=0.008) and larger temporalis muscle prevalence (Asynergy index: TMDmo, P=0.026; TMDse, P=0.026).

Both POC_M and POC_{TORS} showed a decreasing trend with increasing TMD severity,

but the differences were not statistically significant.

Mastication: Unilateral gum chewing

During chewing, significant inter-group differences were found for ΔM center,

wActivity and SMI (Table 3). Compared to C group, the TMDse had significantly lower

median working side masseter prevalence (lower differential working-balancing side

activity of masseter muscle) (P=0.042), that induced also a lower wActivity (P=0.026),

together with a worse degree of symmetry (SMI) between chewing performed on right

and left sides (P=0.012). No differences were observed between TMDmo group and the

other two groups during the chewing test.

No statistically significant differences were found for SIs values and normalized

activities (Global Activity and Activity/cycle).

Functional Index

The functional index (FI), calculated on the ensemble of the analyzed variables (MVC

and chewing), decreased significantly from C group to the TMDmo (P=0.017) and the

TMDse (*P*<0.001) groups (Table 3).

Clinical evaluation of the masticatory type

Unilateral mastication was the predominant pattern in TMD patients (TMDse, 80%;

TMDmo, 74%). In contrast, the majority of controls (87%) performed a bilateral pattern

of mastication (Figure 2). Mean and SD of the associated masticatory type scores were

1.80±1.01 for the TMDse patients, 2.47±0.99 for the TMDmo group and 3.53±0.74 for

the controls (Fisher exact test, C x TMDmo, P=0.003; C x TMDse, P=0.001).

14

Discussion

The main findings in this study were: (1) chronic TMD patients with DDR, specifically those with severe symptomatology (TMDse), showed the greatest alterations of masticatory muscles activation and co-ordination; (2) the proposed functional index (FI), which summarizes the degree of normality of the EMG variables, was significantly lower for both TMDmo and TMDse groups than for the control group.

Methodological considerations

In this study, TMD diagnostic was based on clinical examination and associated history according to the DC/TMD (Schiffman et al., 2014) because, in addition to its relevance, it is consistent with the real practice in TMD clinic. Although, the lack of magnetic resonance imaging (MRI) to confirm the DDR diagnosis, as recommended (Schiffman et al., 2014), is a limitation of the study.

The ProTMDmulti protocol (De Felício et al. 2009, 2012) like other questionnaires/ scales (Helkimo, 1974; Gonzalez et al., 2011; Ohrbach et al., 2011; 2013), includes questions based on patients' complaints of pain and discomfort that form part of the case-classification for TMD. Studies have shown that self-reported facial pain, inability to open the mouth widely and TMJ noises are reliable predictors of TMD (Ohrbach et al., 2013). Moreover, when TMJ noises are prominent features in the clinical history, it is common that substantial muscular dysfunction and pain are also present in chronic TMD (Ohrbach et al., 2011).

The protocols of standardized EMG (MVC and chewing) employed in this study had been previously developed and applied to assess the influence of dental occlusion on neuromuscular coordination (Ferrario et al., 2004; 2006). These methods have also shown to be useful in the study of the jaw motor behavior associated with hindered orofacial functions, pain and other symptoms affecting the stomatognathic system (De Felício et al., 2012; 2013; Ferreira et al., 2014; Ries et al., 2014). Thus, because the relationship between chronic TMD severity and oral motor function was the focus of interest in the present study, care was taken regarding dental (e.g. caries, periodontitis, number of elements) and occlusion condition (e.g. cross-bite) in the selection of the subjects, patients or control, to exclude possible confounders in the analyses.

Additionally, new parameters for mastication analysis were included, in order to provide new insight on masticatory behavior and make its understanding easier. The indices ΔM _center and ΔT _center of the ellipses were introduced to replace the mean differential analysis of right-left temporalis and masseter peak cycle activity by differential analysis between working and balancing masseter and temporalis muscles. This approach allowed the direct comparison of both unilateral chewing. Also, the use of the Cartesian coordinates to describe the position of the ellipses centers, instead of the previous polar coordinates, amplitude and phase (Ferrario et al., 2004; Ferreira et al., 2014; Kumai, 1993), permitted to highlight the contribution of the two muscles (masseter and temporalis) in the symmetry index of the "Lissajous figure". Furthermore, instead of separate assessment of each chewing side as previously proposed (Ferrario et al., 2004), the mean value between left and right sides, and the corresponding symmetry index (SI), were calculated for all bilateral parameters.

During the MVC test, the chronic TMDse group showed significantly lower raw (non-standardized) activity, in both temporalis and masseter muscles (Santana-Mora et al., 2009; 2014). Even when induced experimentally in healthy subjects, pain produces decrease of activity in painful masseter muscle at 100% of MVC, but also in non-painful jaw-closing muscles (Shimada et al., 2013). Ardizone et al. (2010) also verified that patients with the greatest dysfunction exhibited a more pronounced activity decrease, although neither information about TMD diagnostic (if muscular, articular or both) nor symptoms duration were reported in their study.

Also, TMDse showed significantly lower symmetry in the temporalis muscles' contraction (POC_T) than controls, as already observed in chronic arthrogenous TMD (Tartaglia et al., 2011), with a good discriminant performance for arthrogenous TMD related to MRI (Lodetti et al., 2014), but not in short lasting TMD of mild-moderate severity (Felício et al., 2013).

During teeth clenching, both TMD groups had a significant decrease of temporal coordination (co-operation) between the standardized muscular activities of masseter and temporalis muscles (POC_{TM}), relative to the control group. Additionally, their muscular activity distribution shifted significantly from masseter to temporalis muscles (Asynergy index). This result is similar to the findings by Santana-Mora et al. (2014).

Mastication

During chewing, when normalized on the maximal activity exploited during MVC, Global activity and Activity/cycle values were not different between groups. This means that the relative energy employed to chew was similar among the groups, as also observed in patients with non-chronic TMD of mild-moderate severity (Felício et al., 2013). Indeed, considering that TMDse group had significantly reduced non-normalized

maximal muscular activities (COT_T and COT_M, Table 3), the actual energy used for chewing was reduced. This may be one of the factors of the reported difficulties in chewing (ProTMDmulti questionnaire, Table 1). Therefore, the normalized chewing parameters seem unaffected by duration or dysfunction severity: the patients seem to avoid to increase their chewing forces, probably to reduce exercise-induced TMJ and muscular pain.

Moreover, the TMDse group had significantly lower differential working-balancing side activity of masseter muscle (ΔM _center) than control subjects, as well as higher proportional participation of the balancing side muscles on the whole, while the working side remained the most active (wActivity index>50%); the healthy subjects showed a better ability to differentiate the muscles recruitment in favor of the working side (Ferreira et al., 2014).

When comparing right and left side chewing (SMI), the muscles' recruitment pattern, on average, resulted less symmetric in TMD patients, especially in the more severe ones. However, the overall activity distribution into working-balancing side muscles was similar between the two sides of mastication, with the wActivity-SI>86% for all groups. The latter finding, that was expected for healthy subjects, was observed also in TMD groups probably because, despite the prevalence of unilateral DDR, most patients had bilateral pain, myalgia and/or arthralgia (93% of the patients in TMDse group and 87% in TMDmo), with function exacerbating pain as showed by ProTMDmulti.

Another study reported a reduced symmetry in temporalis muscle activity for patients with TMD and pain (Ries et al., 2014); however, the duration and severity information of the disorders were not described.

These results, taken together, add information about masticatory function in patients with chronic TMD. Even with the confirmation, in the current study, of habitual side-preference during natural chewing (Casanova-Rosado et al., 2006; Ferreira et al., 2014; Ratnasari et al., 2011; Santana-Mora et al., 2009) by clinical examination, all patients were able to perform the task on right and left sides with quite the same energy expenditure. However, independently of side imposed for mastication and chewing preference, they recruited the balancing side more than the control group, specifically the masseter muscle, whose contribution increased as TMD symptomatology worsened.

Authors suggest that in a normal process the increased ratio of the working side over balancing side activity, due to decreased balancing side activity, may be a protective mechanism that prevents dental contact or reduces loads if it occurs during chewing, protecting also the TMJ (Morneburg, Döhla, Wichmann & Pröschel, 2014). On the contrary, the increased relative activity in balancing side during chewing, as observed, may represent a hindered function, or in other words, a suboptimal motor behavior as a precursor to pain (Hodges & Smeets, 2015), but also a compensatory mechanism with increased recruitment level of synergist (Herring, 2007) or of contralateral side muscles to maintain the overall force and to preserve the functional demands (Shimada, et al., 2013), if muscles and/or TMJs are painful during function. Re-organization/ distribution of activity occurring within and between muscles (Sae-Lee et al., 2008) has short-term benefit, but potential long-term consequences (Hodges & Smeets, 2015; Peck et al., 2008; Hodges & Tucker, 2011;).

Masticatory frequency, an additional information to the EMG indices that has been related to texture and size of food (Shimada, et al., 2015), did not show differences between groups with and without TMD when a standardized chewing gum is used (De Felício et al., 2013; Ferreira et al., 2014), as in the present study.

Thus, both TMDmo and TMDse groups had DDR and significantly different motor behavior compared to the control group (POCTM, Asynergy, and FI). Although, as symptomatology severity that includes pain increased, the median difference between TMD and control also increased in several parameters, as POCT, ΔT_center, wActivity, SMI and the non-normalized maximal muscular activities (COTT and COTM). Therefore, reorganization seems to reflect DDR-related articular impairment, but also as symptomatology severity is perceived by patients since the groups were classified by ProTMDmulti protocol.

Results of the EMG during MVC and during chewing, taken together, confirm that pain in masticatory system may involve differential effects dependent of the task, among other factors (Sae-Lee et al., 2008; Hodges & Smeets, 2015; Peck et al., 2008; Shimada, et al., 2013; 2015). Therefore, the nervous system seems, in fact, to have a range of options to achieve the goal of protection (Hodges & Tucker, 2011). Morphological and functional differences between orofacial muscles, particularly the jaw-elevator muscles, allow meeting several functional demands, and may explain the more pronounced change in the temporalis muscle during clenching (MVC) and in the masseter muscle during chewing. Temporalis and masseter muscles are active during both tasks, clenching and chewing; the temporalis is the first to contract during jaw closing and it has a stabilization action, while the masseter is a more powerful muscle, performing the main clench activity and chewing performance (Herring, 2007; Cecílio et al., 2010).

Both TMDmo and TMDse groups had DDR and significantly different motor behavior compared to the control group (POC_{TM}, Asynergy, and FI). Although, as symptomatology severity that includes pain increased, the median difference between TMD and control also increased in several parameters, as POC_T, ΔT center, wActivity,

SMI and the non-normalized maximal muscular activities (COT_T and COT_M). Therefore, reorganization seems to reflect DDR-related articular impairment, but also as symptomatology severity is perceived by patients since the groups were classified by ProTMDmulti protocol.

The FI Index was devised to quantitatively summarize each subject's overall performance, computing the rate of normal scores of MVC and chewing indices, whose tolerance limits were calculated considering the normal distribution of intra-group data of the control, reference group. The choice of unilateral (upper/lower) or bilateral limits of tolerance, for each parameter, depended on the possibility to identify single or dual distribution tails whose values were considered anomalous/pathologic by the authors (Ferrario et al., 2004; 2006; Kumai, 1993). The FI index substantially confirmed the alterations found in several TMD parameters, showing a worsening median performance from C group to both TMDmo and TMDse groups.

Conclusions

Patients with chronic TMD showed functional alterations in their masticatory muscles, with a re-organized activity, mainly resulting in worse co-ordination during MVC and increased participation of balancing side muscles during mastication. The symptomatology severity influenced the performance: the larger it is, the larger the functional alteration in the masticatory muscles.

The Functional Index (FI), which shows the overall degree of normality of each subject's EMG variables (MVC and chewing tasks), well summarizes the global functional condition of the masticatory system in the single patient.

In future studies the analyses should be made separately for patients with only either myofascial TMD pain or degenerative disorders. Psychological factors should also be examined beside symptomatology severity.

Conflict of interest

The authors report no conflict of interest.

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Figure captions

Figure 1.Lissajous's plot of differential working-balancing (w-b) masseter (ΔM) and temporalis (ΔT) muscles activities. Two unilateral (R, right; L, left) 90%-standard ellipses are illustrated as example (being the left one already mirrored), together with the corresponding center vectors (r and l), whose Cartesian coordinates are the ΔM _center and ΔT _center. The ellipses of the example are not overlapped for illustration clarity.

Figure 2. Distribution of masticatory type in the control group (C) and TMD groups, moderate (TMDmo) and severe (TMDse) (Y-axis; number of subjects).

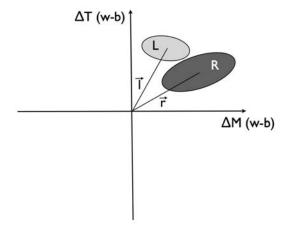


Fig 1

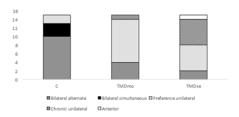


Fig 2

Table 1. Demographic characteristics, TMD severity and TMD diagnosis.

		Control	TMDmo	TMDse	
		n=15	n=15	n=15	P
Female	%	93	87	93	NS*
Age (years)	Median (IQR)	27 (26:36)	29 (25:34)	32 (29:40)	NS
ProTMDmulti score	Median (IQR)	0 ^a (0:2)	83 ^b (60:101)	157°(123:207)	< 0.001
Primary Diagnostic	Pain side	··(0/)	n(0/)	·· (0/)	
DDR side	(myalgia/arthralgia)	n(%)	n(%)	n(%)	
Bilateral	Bilateral		4(26.6)	7(46.7)	
	Unilateral		1(6.7)		
Unilateral	Bilateral		9(60.0)	7(46.7)	
	Unilateral		1(6.7)	1(6.6)	

P, probability of Fisher exact* or Kruskal-Wallis test. NS, non-significant difference.

Medians with different superscript (a, b, c) differ at post-hoc Median test. IQR, interquartile range.

DDR, disc displacement with reduction; TMD, temporomandibular disorder group (mo, moderate; se, severe).

 Table 2. Repeatability indices for the mastication parameters.

Measure	P (paired t-test)	ICC	TEM	CV_TEM
SMI	NS	0.795	11.1 %	15 %
ΔM_center	NS	0.989	5.3 %	7 %
ΔM_center_SI	NS	0.829	9.1 %	13 %
ΔT _center	NS	0.971	7.2 %	15 %
ΔT_center_SI*	0.020	0.885	16.2 %	29 %
90%-standard ellipse area*	NS	0.949	5183.4 %2	34 %
90%-standard ellipse area_SI*	NS	0.679	18.1 %	30 %
Global activity	NS	0.996	32.8 %·s	4 %
Global activity_SI*	NS	-0.374	11.5 %	14 %
Activity/cycle	NS	0.980	3.5 %·s	8 %
Activity/cycle_SI	NS	0.725	7.6 %	9 %
wActivity	NS	0.910	2.1 %	3 %
wActivity_SI	NS	0.876	3.3 %	4 %
Frequency	NS	0.891	0.1 Hz	7 %
Frequency_SI*	NS	-0.046	7.9 %	9 %

NS, non-significant. *=non-repeatable indices.

ICC, intra-class correlation coefficient; TEM, typical error of measurements; CV_TEM, coefficient of variation of TEM; SI, symmetry index.

Table 3.Electromyographic parameters of Maximum Voluntary Clench (MVC) and unilateral gum chewing. All values are expressed as medians (IQR).

		Control	TMDmo	TMDse	
Measure	unit	n=15	n=15	n=15	P
MVC					
COT _T *	μV	198a(129:263)	176 ^{a,b} (136:206)	121 ^b (72:194)	0.030
COT _M *	μV	240a(183:299)	196 ^{a,b} (115:288)	147 ^b (69:215)	0.030
POC_T	%	88.5 ^a (87.1:89.5)	85.8 ^{a,b} (82.0:90.1)	85.5 ^b (82.3:88.4)	0.017
POC_{M}	%	87.4(85.5:89.2)	87.1(84.5:89.3)	85.0(72.8:88.7)	NS
POC _{TORS}	%	91.6(90.8:92.9)	91.0(89.9:92.0)	90.2(86.7:92.1)	NS
POC_{TM}	%	91.4a(88.7:91.9)	85.7 ^b (81.0:90.2)	86.2 ^b (78.7:89.6)	0.008
Asynergy index	%	-4.9 ^a (-7.5:-1.8)	-13.4 ^b (-18.7:-5.2)	-11.1 ^b (-21.3:-4.6)	0.016
Chewing					
Frequency	Hz	1.23(1.11:1.34)	1.23 (1.15:1.42)	1.33 (1.17:1.36)	NS
ΔM_center	%	87a(62:102)	62 ^{a,b} (36:95)	54 ^b (28:75)	0.046
$\Delta M_center-SI$	%	80(63:92)	67(57:71)	65(42:87)	NS
ΔT _center	%	56(39:88)	47(27:63)	41(19:63)	NS
Global activity	%·S	698(574:906)	841(534:1169)	773(498:1448)	NS
Activity/cycle	%·S	38(29:54)	42(29:63)	46(30:72)	NS
Activity/cycle-SI	%	81(77:87)	86(70:92)	83(64:94)	NS
wActivity	%	70 ^a (65:76)	64 ^{a,b} (60:70)	63 ^b (60:68)	0.033
wActivity-SI	%	97(87:99)	87(79:100)	90(84:97)	NS
SMI	%	85 ^a (66:90)	70 ^{a,b} (43:81)	63 ^b (37:74)	0.012
FI	%	100 ^a (86:100)	73 ^b (67:87)	73 ^b (47:87)	0.001

P, probability of Kruskal-Wallis test; NS, non-significant.

Medians with different superscript (a,b, c) differ at post-hoc Median test. IQR, interquartile range.

TMD, temporomandibular disorder group (mo, moderate; se, severe); MVC, maximum voluntary clench; COT, MVC with cotton rolls; POC, percentage overlapping coefficient; M, masseter muscle; T, temporalis muscle; TORS, torsion; TM, co-operation between temporal and masseter muscles; SI, symmetry index; SMI, symmetrical mastication index; FI, functional index.

^{*=}not used for FI calculation. Non-repeatable indices are not reported.