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TITLE

DIAGNOSTIC DEVICES FOR THE EVALUATION OF MASTICATORY MUSCLES: ELECTROMYOGRAPHY AND ALGOMETER

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OVERVIEW

The issue of masticatory muscles impairment diagnosis in the field of the evaluation of temporomandibular disorders (TMD) represents one of the most controversial theme among dentists (McNeill, 1997).

According to previous studies analyzing the diagnostic efficiency for TMD, an accurate anamnesis and the clinical examination of the dysfunctional patient seems to be the gold standard especially for muscular pathologies (AADR, 2010). As showed by different systematic reviews (Lund et al., 1995; Petersson, 2010), the electronic devices can give only additional information for the assessment of masticatory muscles because of the areas not well understood such as the biological variation, the grade of adaptability of each muscles (Lund et al., 1995). However, the evaluation of masticatory muscles activation in terms of asymmetry and/or bite force could be useful in growing patients because of the possible onset of skeletal asymmetries and TMD related to a pathological condition affecting temporomandibular joint or to a malocclusion (Iodice et al., 2013; Iodice et al., 2016; Michelotti et al., 2016).

The activity of muscles is commonly investigated by using surface electromyography (sEMG) in order to identify possible muscular impairment. However, the assessment of jaw muscles activity by means of sEMG has been questioned for its low reliability (Al-Saleh et al., 2012). Indeed, biological variations, low repeatability in electrode placement, and artefacts can account for possible discrepancies between studies and conflicting results (Castroflorio et al., 2006; Castroflorio et al. 2008). To overcome this limit, EMG protocols using standardized EMG signals or indices were introduced (Ferrario et al., 2000).

Masticatory muscles evaluation can include measurements of muscle tenderness, pain detection thresholds, pain tolerance thresholds, pain response to suprathreshold stimuli and temporal summation (Bezov et al., 2011). The study of pain perception could be the key in understanding that sensitization of central nociceptive pathways is a relevant mechanism involved in the physiopathology of muscular pain (Bezov et al., 2011).

Pain perception can be assessed by pain pressure threshold (PPT) measurement. Pressure algometry is a useful technique to quantify the mechanical sensitivity of a muscle, in which pressure is registered through an algometer (Ylinen et al., 2007). PPT has been shown to be a valid method to measure mechanical pain threshold in human muscles, including craniofacial ones (Bezov et al., 2011; Chesterton et al., 2007; Farella et al., 2000).

The chapter 1 reports the standardized electromyographic protocol used in all reported experimental studies. Chapter 2 analyzes the jaw muscle activity in patients affected by TMD chronic myalgia. In the chapter 3 is reported the EMG evaluation of masticatory muscles in children affected by cross bite malocclusion. The chapter 4 explains the use of EMG in patients affected by a chronic autoimmune disease, i.e. Juvenile Idiopathic Arthritis (JIA). Chapter 5 includes the PPT evaluation in patients suffering from JIA. In the chapter 6 are reported the general conclusions about the use of the reported diagnostic instruments.

The work contained in this thesis has led to the following publication:

Michelotti A, Rongo R, Valentino R, et al. Evaluation of masticatory muscle activity in patients with unilateral posterior crossbite before and after rapid maxillary expansion. Eur J Orthod. 2018 Apr. doi: 10.1093/ejo/cjy019.

CHAPTER 1

EMG in the evaluation of masticatory muscles: a standardized protocol

To overcome the limits related to electromyography, different standardized protocols were proposed. The EMG protocol (Ferrario et al., 2000) proposed by Ferrario et al. has been largely used (De Felício et al., 2012; Ferrario et al., 2007; Michelotti et al., 2018; Santana-Mora et al., 2009; Tartaglia et al., 2008; Tartaglia et al. 2011;). This protocol allows computing indices of jaw muscles activity by using standardized EMG signals recorded during maximum voluntary contraction in maximal intercuspation and on cotton rolls. This method reduces biological and technical noise, and allows comparing the activity of paired jaw muscles by providing indices of asymmetric jaw muscles activation during function. In all reported experimental studies, we referred to a standardized protocol reported below.

The electrical activity of the right and left AT and MM muscles was recorded simultaneously during standardized tasks via sEMG. Silver-silver chloride bipolar surface pre-gelled electrodes (Kendall, Mansfield, MA, USA) with a diameter of 24 mm were placed on the skin along the main direction of the muscular fibres. To minimize electrode impedance, the skin was thoroughly cleaned with an abrasive preparation gel (Everi, Spes Medica, Genova, Italy) before electrode placement. For the TA, electrodes were placed vertically over the anterior border of the muscle, on the area corresponding to the fronto-parietal suture; for the MM, the upper pole of the electrode was placed at the intersection between the tragus-labial commissure and the exocanthion-gonion (mandibular angle) lines (Figure 1.1).

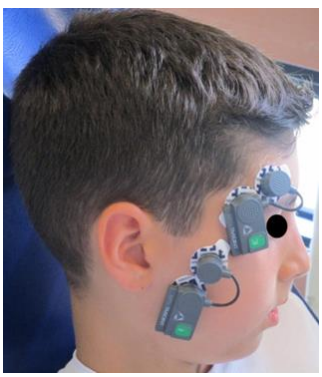

Figure 1.1. A patient with electrodes in place.

Recordings were performed at least 5–6 minutes after the application of the electrode to allow the conductive gel to adequately moisten the skin surface. All subjects sat in a dental chair. The position of the seatback was fixed, while the vertical excursion of the dental chair could be adjusted by the operator. The EMG analysis was performed using a wireless EMG device (TMJOINT, BTS SpA, Garbagnate Milanese, Italy). The EMG signals were acquired at 1KHZ, amplified (gain 150) and filtered via hardware (low-pass filter 500Hz; high-pass 10Hz). A software program (Dental Contact Analyser, BTS SpA) processed the raw electrical signals and generated root mean square (RMS) values. Thereafter, RMS values were processed by an algorithm to generate indices of muscle activity and asymmetry.

The EMG protocol included two static and two dynamic tests. The static tests included the following:

1. Maximum voluntary contraction (MVC) in intercuspal position (CLENCH)—subjects clenched their teeth as hard as possible for 5 seconds;

2. MVC in intercuspal position on cotton rolls (COT)—subjects clenched as hard as possible for 5 seconds on 10 mm thick cotton rolls (Intermedical, Terlano, Bolzano, Italy) positioned from the mandibular first molar to the canine on both sides.

For the 5-second static tests, two hundred 25 msec RMS samples were collected. The 120 samples, corresponding to 3 second, with the highest RMS values were used to compute the indices. The EMG waves of each muscle (120 samples) with and without cotton rolls were superimposed sample by sample, and the ratio between the superimposed areas and the total areas was computed automatically via software. Hence, for each subject, the EMG potentials recorded during the MVC were expressed as percentage of the mean RMS potential recorded during the MVC on the cotton rolls (EMG standardized potentials).
The dynamic tests included the following:
1. Chewing gum (Air Action Vigorsol, Lainate, Italy) on the right side for 15 seconds.
2. Chewing on the left side for 15 seconds.

Between the static and the dynamic tests, participants were asked to rest for 3 minutes.

The following standardized EMG indices were calculated via software:

Computed indices (static tests).

1. POC (percentage of overlapping coefficient). The standardized EMG waves of the left and right AT and MM were compared by computing a percentage overlapping coefficient (POC, unit: %, range: 0–100 per cent, norm values 85 per cent ≤ POC ≤ 100 per cent) (11, 13, 24). If the muscles contract with perfect symmetry, a POC of 100 per cent (perfect symmetry) is expected. Conversely, a value corresponding to 0 per cent indicates the absence of concurrent activation of paired muscles (no symmetry). Three indices were computed for each subject (POC AT, POC MM and POC medium).
2. TC (torque coefficient). This index is obtained by measuring the overlapping activity (standardized EMG waves) between the left MM and right AT and the right MM and left AT. The higher muscular activity of one couple (i.e. left MM and right AT) over the other (i.e. right MM and left AT) results in a torqueing effect on the lower jaw. TC ranges between 0 per cent (no symmetric activation of the couples, greatest torqueing effect) and 100 per cent (perfect symmetric activation of the couples, no torqueing effect). Normal values are 90 per cent ≤ TC ≤ 100 per cent (De Felício et al., 2009; Ferrario et al., 2000; Ferrario et al., 2006).
3. IMPACT (total standardized muscle activity). This index is computed as the integrated area of the EMG standardized potentials of both MM and AT over time (5 seconds MVC). Norm values are 85 per cent ≤ IC ≤ 115 per cent (Ferrario et al., 2000). Lower values indicate that the EMG standardized potentials were reduced during the clenching tasks, and that the maximal EMG activity could not be expressed.
4. ASIM (asymmetry index). This index is calculated by comparing the activity of the right couple (right AT and right MM) to the left couple (left AT and left MM). ASIM ranges from −100 per cent and +100 per cent; a value of 0 per cent depicts a perfect symmetric activation of the two couples. A negative value indicates greater activity of the left couple; conversely, a positive value indicates a greater activity of the right couple. Norm values are −10 per cent ≤ ASIM ≤ +10 per cent (Botelho et al., 2010).

Computed indices (dynamic tests).

1. SMI (symmetrical mastication index) was computed to assess whether the left- and the right-side chewing tests were performed with symmetrical muscular patterns. It indicates the distance between the centre of the chart and the centre of the ellipse in a graph that describes the prevalence of one side over the other during mastication (Figure 1.2). SMI ranges between 0 per cent (no symmetry) and 100 per cent

(symmetrical muscular pattern). Normal values are 70 per cent ≤ SMI ≤ 100 per cent (Ferrario et al., 1999) (Figure 1.2).

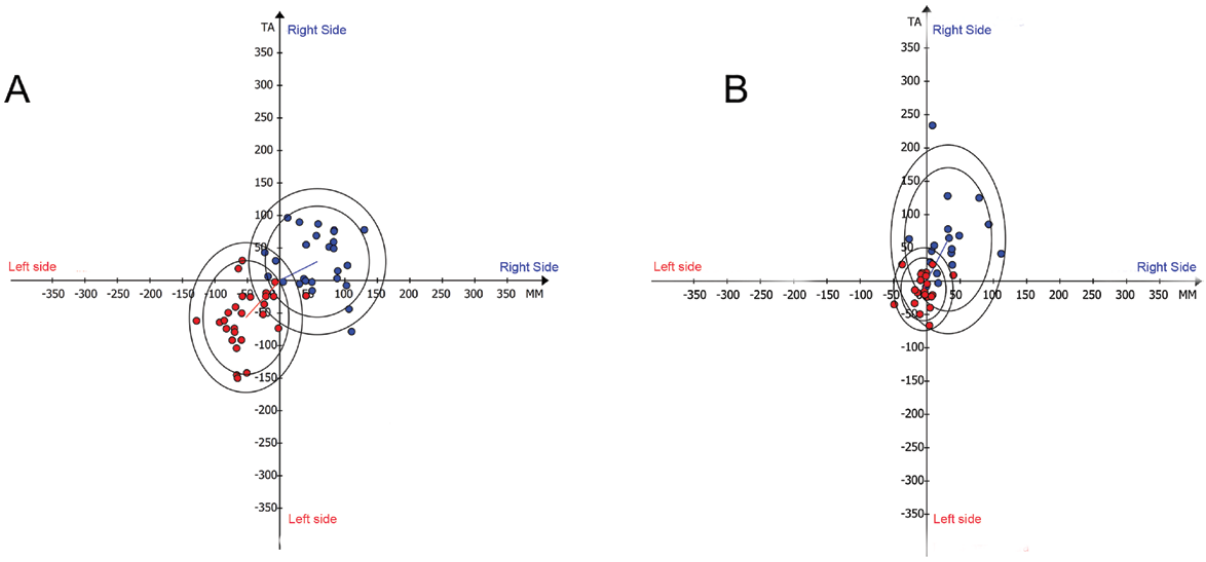

Figure 1.2. Determination of the symmetrical mastication index (SMI). *x*-axis: differential masseter (left (-) versus right); y-axis: differential temporal (left (-) versus right) (µV). Red dots and the corresponding ellipse depict data recorded during the task 'chewing on the left side'. Blue dots and the corresponding ellipse depict data recorded during the task 'chewing on the right side'. In an ideal condition (complete symmetric activity between right and left sides during chewing), the centre of the ellipse describing the task 'chewing on the right side' will be located in the first quadrant (top left) and the centre of the ellipse describing the task 'chewing on the left side' in the third quadrant (bottom right). The symmetrical mastication index (SMI, %) is calculated using the distance between the centres of the two confidence ellipses and the origin of the axes. If the right and left chewing tasks are symmetric, the right and left ellipses will have the same distance from the origin of the axes, and a 180 degree difference between phase angles (angle between the *x*-axis and the segment connecting the centre of the ellipse and the axis origin). Symmetric patient (A). Similar distances between the system origin and the centre of the ellipse, and similar phase angle between the two tasks. Asymmetric patient (B). The blue ellipse has a bigger distance respect to the centre and the difference between the angles is lower than 180 degree.

2. SPM (side of prevalent mastication) in case of SMI values lower than 70 per cent, the dominant side of mastication was identified as side of prevalent mastication (Figure 1.2). Three categories could be identified, that is right, left and symmetric.
3. FREQ (frequency index) measures the frequency of masticatory cycles during the chewing experimental tasks and was reported in hertz (Hz: bites per second).

The EMG protocol and the algorithm used for the standardization of the EMG signals and the computation of the indices have been used and described in several research studies (De Felício et al., 2009; Ferrario et al., 1999; Ferrario et al., 2000; Ferrario et al., 2006).

CHAPTER 2

Jaw muscle activity patterns in patients with chronic TMD myalgia during standardized clenching and chewing tasks

Temporomandibular disorders (TMD) include a set of different pathological conditions affecting masticatory muscles and/or temporomandibular joint with a multifactorial etiology (Slade et al., 2016). Previous studies have reported that jaw muscle activity during rest and functional tasks is different between patients with TMD and healthy subjects (De Felício et al., 2012; Ferrario et al., 2007; Santana-Mora et al., 2009; Tartaglia et al., 2008). A recent study has revealed that patients affected by TMD have an average greater electromyographic (EMG) activity of masticatory muscles compared to individuals without pain because of the high frequency of oral parafunctions (Cioffi et al., 2017). On the other hand, other studies have shown that jaw muscle activity is decreased in TMD patients (Kogawa et al., 2006; Santana-Mora et al., 2009; Tartaglia et al., 2008), in agreement with the pain adaptation model, which suggests that muscular activity decreases to limit movements and protect the sensory-motor system from further muscle tissue injury (Lund et al., 1991; Peck et al., 2008; Tucker et al., 2009).

The extent of jaw muscles asymmetry during maximum contraction of elevator muscles has been measured in normo-occlusion subjects suffering from TMD (De Felício et al., 2012; Ferrario et al., 2007; Tartaglia et al., 2008). Some studies found a larger asymmetry of temporalis and masseter muscles activity in TMD subjects as compared to TMD free controls as result from increased temporalis or relatively reduced masseter activity (Santana Mora et al., 2009; Tartaglia et al., 2011). On the other hand, a recent study by Da Silva and co-workers (da Silva et al., 2017) has shown that TMD patients do not present a greater masticatory muscles asymmetry than healthy controls.

This experiment aimed at examining jaw-muscles activity patterns of patients with chronic TMD myalgia using standardized EMG indices, which evaluate jaw muscles asymmetry during function, muscular work, and chewing rate to determine whether asymmetric or abnormal activation of paired muscles is associated with chronic TMD pain. It was hypothesized that patients with chronic TMD myalgia have more asymmetric muscular activity and increased muscular work during experimental isometric clenching, and abnormal chewing frequency.

2.1 Materials and methods

2.1.1 Study sample

Adults seeking for a TMD consultation at the Department of Neuroscience, Section of Orthodontics and Temporomandibular Disorders of the University of Naples Federico II, Italy were examined using the Diagnostic Criteria for TMD (DC/TMD) clinical examination protocol (Schiffman et al., 2014). Those with a diagnosis of TMD myalgia with self-reports of pain from at least 6 months were recruited.
One hundred subjects contacted at the Hospital, including employees and students, were invited to fill in the TMD pain screener (Gonzalez et al., 2011). Individuals reporting pain in the jaws and/or temples in the last 30 days were excluded. For both groups exclusion criteria were neurological disorders, craniofacial syndromes, and current orthodontic or dental treatment.
The total sample included fifty-two individuals: twenty-nine patients with TMD myalgia (mTMD, 2 males, 27 females, mean age ± standard deviation: 37.8±12.6 years) and twenty-three pain-free subjects (Ctr, 5 males, 18 females, mean age ± standard deviation: 34.4±11.9 years).
The Research Ethics Board at University of Naples "Federico II" approved the research protocol (protocol 22616). The research was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All subjects gave written informed consent to participate before entering the experiment.

2.1.2 Questionnaires

Patients with mTMD filled in the Graded Chronic Pain Scale included in the DC/TMD instrument (Schiffman et al., 2014). All subjects filled out the following questionnaires:

- The Oral Behavior Checklist (OBC) questionnaire was used to identify and quantify the frequency of self-reported oral behaviors (Markiewicz et al., 2006). The OBC investigates nocturnal bruxism (2 items) and different wake-

time oral behaviors (19 items). Each participant chooses among five response options: “none of the time,” “a little of the time,” “some of the time,” “most of the time” and “all of the time,” which are scored from 0 to 4. In this study, authors analyzed a reduced 6-items version (OBC-6). It includes items 3 (“grind teeth together during waking hours”), 4 (“clench teeth together during waking hours”), 5 (“press, touch or hold teeth together other than while eating –that is, contact between upper and lower teeth”), 10 (“bite, chew, or play with your tongue, cheeks, or lips”), 12 (“hold between the teeth or bite objects such as hair, pipe, pencils, pens, fingers) and 13 (“use chewing gum”) (Cioffi et al., 2016; Cioffi et al. 2017; Michelotti et al., 2012).

- The State Trait Anxiety Inventory (STAI X1- STAI X2) was used to assess the state-trait anxiety of individuals. Twenty items assess the state anxiety and 20 assess the trait anxiety. Trait anxiety includes constructs such as “I fell inadequate”, “I lack self-confidence”, and “I have disturbing thoughts”. Individuals selects one of the following options: “almost never”, “sometimes”, “often”, or “almost always”. Each answer is graded as a score from 1 to 4 (Spielberger et al., 1983).
- The Somatosensory Amplification Scale (SSAS) questionnaire was used to assess the degree of somatic awareness. It encloses ten claims used to analyze individual sensitivity to bodily sensations such as “I hate to be too hot or too cold”, “I have a low tolerance for pain” and “I am often aware of various things happening within my body”. Each participant chooses among the following options: “not at all,” “a little,” moderately,” “quite a bit,” or “extremely”. Each answer is graded as a score from 0 to 4 (Barsky et al., 1990).

2.1.3 EMG assessment

The activity of masseter (MM) and temporalis anterior (TA) muscles of both sides (left and right) was recorded during standardized tasks by means of sEMG according to the EMG protocol previously described.

2.1.4 Statistical analysis

All data were analyzed by using SPSS 21.0 software. Independent Student T-Test was used to assess difference between groups. Statistical significance was set at $p<0.05$. A sample including 21 subjects per group was sufficient to detect 5 %(SD = 4.55 per cent) (Ferrario et al., 2000) between-group differences in POC medium index ($\alpha= 0.05$ and $1-\beta= 0.9$).

2.2 Results

2.2.1 Questionnaires

TMD participants reported a mean pain intensity in the last 30 days of 50.5 ± 30.4 mm on a 100 mm Visual Analogue Scale (left endpoint: no pain; right endpoint: "the worst pain I can imagine"). Eleven patients had GCPS grade II and 18 grade III. A significant difference for self-reported oral behaviors was found between groups: mTMD group reported a higher frequency of oral parafunctions than CTR group (p=0.001). No differences were found both for the somatosensory amplification and for the anxiety between groups (Table 2.1).

	TMD Group	CTR Group	p value
OBC	9.52±5.86	3.88±3.33	**p=0.001**
SSAS	12.58±4.54	11.29±4.92	p=0.395
STAI X1	24.22±6.13	22.82±4.07	p=0.411
STAI X2	21.74±6.97	20.06±4.68	p=0.386

Table 2.1. Comparisons for OBC, SSAS, STAI scores. Bold type: statistically significant.

2.2.2 EMG indices

Both groups showed a similar asymmetric contraction pattern comparing right and left TA muscles (POC TA, Table 2.2, p value= 0.136) and right and left MM muscles (POC MM, Table 2.2, p value=0.311). No significant differences between right and left sides for both groups were found for the POC medium index (POC medium, Table 2.2, p value= 0.302). Tc index was similar between mTMD patients and healthy subjects (Tc, Table 2.2, p value= 0.289).
When comparing TA and MM muscles activities, a significantly higher MM muscles activity in mTMD patients compared to healthy subjects was found (ATTIV, Table 2.2, p value= 0.023).

Muscular work was significantly different between groups with a higher muscular work (mV/sec) in mTMD group compared to CTR group during experimental clenching (IMPACT, Table 2.2, p value= 0.004).

The frequency of chewing did not differ between groups both for right and left side (FREQ right side, Table 2.2, p value=0.116; FREQ left side, Table 2.2, p value= 0.244).

	TMD Group	CTR Group	p value
POC TA	74.26±20.48	81.48±11.38	p=0.136
POC MM	76.72±20.10	81.31±11.93	p=0.311
POC medium	78.27±11.68	81.40±9.42	p=0.302
Tc	84.26±9.62	86.88±7.98	p=0.289
ATTIV	-3.98±21.5	-17.21±18.69	**p=0.023**
IMPACT	139.38±82.77	92.04±52.46	**p=0.004**
FREQ right side	1.30±0.26	1.40±0.193	p=0.116
FREQ left side	1.37±0.27	1.47±0.29	p=0.244

Table 2.2. Comparisons for EMG measurements. Bold type: statistically significant.

2.3 Discussion

The present study demonstrated that both individuals with TMD myalgia and healthy controls present a slight asymmetric activation of jaw muscles during clenching, as compared to reference values (Ferrario et al., 2000). However, there were no significant differences between the study groups. Masseter muscles were significantly more active than temporalis muscles in TMD myalgia patients than controls. Furthermore, patients with TMD myalgia had a greater muscular work and self-reported frequency of oral parafunctions (OBC-6 scores) than controls. The lack of a significant difference in masticatory muscles asymmetry between mTMD patients and CTR reported in our study is consistent with Tartaglia et al (Tartaglia et al., 2008). Authors showed that only arthrogenous patients (group II and III according to RDC/TMD) were significantly asymmetric as compared to controls (Tartaglia et al., 2008), whereas the degree of asymmetry during activation was similar between myogenous patients (group I according to RDC/TMD) and controls (Tartaglia et al., 2008). In contrast, other studies found higher asymmetric activity both for temporalis and masseter muscles activity in both patients and healthy subjects (De Felício et al., 2012; Ferrario et al., 2007). In the current experiment, the activation of the couples right temporal-left masseter and left temporal-right masseter was not difference between groups (Tc index). An altered Tc index value may suggest the presence of a fulcrum that is the expression of unbalanced masticatory muscle activity in search of occlusal stability (Tartaglia et al., 2008). Hence, it is possible to hypothesize that both patients and controls had good occlusal stability, i.e. a proper intercuspation between the upper and lower dental arches. This result is in agreement with other studies, which showed that the Tc coefficient did not differ between TMD group and the CTR one (Ferrario et al., 2007; Tartaglia et al., 2008; Tartaglia et al., 2011). However, De Felício and co-workers found a significant larger unbalanced activity in TMD group compared to healthy subjects (De Felício et al., 2012). These inconsistent findings could be explained by the different criteria adopted for the selection of samples (De Felício et al., 2012). In the current study, the authors found a significant difference between the two groups for ATTIV index that compares the differential recruitment of temporalis and masseter muscles, with a negative value implying a greater differential recruitment of the temporal muscles. From the present study, a greater activation of masseter muscles than temporalis muscles was found in the mTMD group. These results were not in agreement with other studies reporting an increased activity of the temporalis muscles (Santana-Mora et al., 2014; Tartaglia et al, 2011). However, also in this case, discrepancies in diagnostic criteria may

explain the differences between the studies (Santana-Mora et al, 2014; Tartaglia et al., 2011). In order to assess the relative activation of MM and AT muscles as a function of different oral behaviors, Farella and co-workers evaluated the effects of induced non-functional oral tasks on the pattern of activity of the masticatory muscles of healthy subjects (Farella et al., 2008). Results reported a changing of the relative activity of each couple of muscles depending on the examined tasks (Farella et al., 2008). A rather symmetric activation was reported in most of oral task. However, masseter muscles showed a higher activation during tasks involving incisal biting, conversely the activity of anterior temporalis predominated when tasks in intercuspal position were performed (Farella et al., 2008). It could be supposed that in the current study mTMD patients showed a higher masseter activity and a relative anterior temporalis reduced one because of the muscle fatigue of AT related to a higher frequency of oral parafunctions performed in intercuspal position. Indeed, in the present experiment it was analyzed oral behavior by calculating OBC-6 score, which includes six items concerning intercuspal position tasks (Cioffi et al., 2016; Cioffi et al., 2017; Michelotti et al., 2012).

Finally, a significant difference was found for IMPACT index with higher values in mTMD patients compared to healthy subjects. IMPACT index expresses the muscular work represented geometrically by the area under the curve of electrical activity of the muscles examined over time. The authors can assume that in mTMD group, during a short lasting session (5 seconds) of clenching, there is a greater muscular work. This could be related to a greater tone of masticatory muscles in mTMD patients or it is likely that mTMD patients have an abnormal recruitment of muscular fibers as compared to healthy controls with a consequent alteration of muscular electrical activity. Clinical practice reports the feeling that the painful muscles commonly show an increased muscular hardness (Fricton, 1999; Murayama et al., 2012). A recent review analyzed the scientific evidence about the correlation between the hardness of masticatory muscles and myofascial TMD: some studies reported a significant difference in masseter muscles hardness between TMD patients and healthy subjects showing a higher elasticity index and higher elasticity modulus in TMD patients at resting position (Ariji et al., 2013; Takashima et al, 2017). It was suggested that the association between muscular pain and hardness could be related to a combination of sustained tonic contractions, tissue edema and metabolic alterations (Ashina et al., 1998; Sakai et al., 1995).

With respect to the frequency of chewing, the present study did not find any difference between groups. In addition, frequency for both sides was not different for mTMD patients compared to the CTR subjects. This finding suggests that mTMD did not limit jaw movement in the analyzed sample.

The STAI and SSAS were collected to account for the possible effect of psychological factors on outcome measures. Indeed, psychological factors may play a role in influencing the association between pain and motor activity (Brandini et al., 2011). However, both parameters were not different between mTMD and CTR groups; therefore, the authors did not adjust the comparisons using these values.

Although some studies reported that sEMG assessment of masticatory muscles gives additional value compared to clinical examination alone (Bodéré et al., 2005; Tartaglia et al., 2008), the evaluation of the asymmetry of the masticatory muscles activity in subjects affected by TMD showed controversial results reporting only in some cases differences between TMD patients and controls (De Felício et al., 2012; Ferrario et al., 2007). Manfredini and co-workers reported a low accuracy of the assessment both of the EMG activity at rest and ratio of EMG activity between paired muscles for the diagnosis of TMD pain patients (Manfredini et al., 2011). However, the outcome with the highest diagnostic accuracy for the identification of myalgia TMD patients, seems to be the evaluation of muscular force during clenching tasks (Manfredini et al., 2011; Tartaglia et al., 2008). Also in the current study, the main outcome discriminating mTMD patients and healthy subjects was the muscular work, which represents the electrical activation of muscular fibers during a clenching task.

Further longitudinal studies could be useful to understand better the role of muscular asymmetry as etiological factor in development of TMDs.

2.4 Conclusions

Myalgia TMD patients reported a greater muscular work. There was no association between asymmetric muscular activity and mTMD. Current findings suggest that an asymmetric pattern of contraction of the masticatory muscles during static and dynamic tasks is an intrinsic characteristic of each individual. So different activation patterns of masticatory muscles are not necessarily associated with a pathological condition.

CHAPTER 3

Evaluation of masticatory muscle activity in patients with unilateral posterior crossbite before and after rapid maxillary expansion

Posterior crossbite (PCB) is a common malocclusion, which affects 8–22 per cent of orthodontic patients in the primary and early mixed dentition (Shalish et al., 2013) and 5–15 per cent of the general population (Farella et al., 2007). Unilateral posterior crossbite (UPCB) with a functional shift of the mandible occurs in 71–84 per cent of individuals with PCB (Thilander et al., 2002). UPCB has been suggested to determine an asymmetrical activation of the masticatory muscles and therefore might contribute to the onset of skeletal asymmetries and temporomandibular joint disorders (TMD) (Iodice et al., 2013; Iodice et al., 2016; Michelotti et al., 2016). Based on these assumptions, early treatment of UPCB by maxillary expansion (Agostino et al., 2014; Bucci et al., 2016; Rongo et al., 2017) is commonly recommended to reduce the risk of developing craniofacial anomalies and TMD in adulthood (Iodice et al., 2013; Iodice et al., 2016).

The effects of the correction of UPCB on the activity of anterior temporalis (AT) and superficial masseter (MM) muscles have been evaluated using sEMG, with controversial findings (Andrade et al., 2010; Arat et al., 2008; De Rossi et al., 2009; Kecik et al., 2007; Martìn et al., 2012; Maffei et al., 2014; Piancino et al., 2016). One study (Martìn et al., 2012) concluded that the degree of asymmetry of masticatory muscles during function is not affected by the presence of crossbite. Others reported that the treatment of crossbite contributes to a more symmetric pattern of activation of the chewing muscles during function only to a slight extent (Kecik et al., 2007; Piancino et al., 2016). Finally, findings included in other reports may be questionable since they lack of untreated subjected acting as controls (De Rossi et al., 2009; Maffei et al., 2014). A recent review has reported that the treatment of crossbite contributes to increasing the activity of masticatory muscles, approaching levels similar to subjects with normal occlusion (Tsanidis et al., 2016). Differently from other

studies, this research has investigated the relationship between crossbite and asymmetry in the activity of the chewing muscles by using a standardized EMG protocol and indices.

The relationship between crossbite and asymmetric jaw muscle activity has been subject of debate in several studies. An early treatment of crossbite is commonly recommended to reduce the risk of developing skeletal asymmetries as a consequence of abnormalities in masticatory function between the right and left sides. A better understanding of both the possible relationship between UPCB and asymmetric muscular function and the effect of RPE on the extent of muscular asymmetry during function might contribute to clarifying whether an early treatment of UPCB with RPE should be recommended.

This study aimed at evaluating the AT and MM muscle activity of children with UPCB before and after RME by means of sEMG and a standardized EMG sampling protocol. The null hypotheses to be tested were: the UPCB patients do not present more asymmetric AT and MM muscle activity compared to UPCB-free controls during standardized tasks; and maxillary expansion does not determine a more symmetric activation of AT and MM muscles during functional tasks.

3.1 Materials and methods

3.1.1 Study sample

Twenty-nine children with UPCB (UPCB-group: 13 males, 16 females, mean age ± SD = 9.6 ± 1.6 years) and 40 UPCB-free controls (Control-group: 17 males, 23 females; mean age 10.5 ± 1.1 years) seeking an orthodontic consultation were recruited consecutively. For both groups, exclusion criteria were genetic or congenital abnormalities, craniofacial anomalies, systemic diseases affecting growth and development, clinical signs or symptoms of TMD (Schiffman et al., 2014), reporting of oral parafunctions (Schiffman et al., 2014), and previous or current orthodontic treatment. Inclusion criteria were an Angle Class I relationship, presence of the four first permanent molars, mixed dentition stage, and the absence of tooth mobility or decayed teeth. The experimental group included subjects with UPCB and lateral shift towards the UPCB side as assessed by Dawson's manoeuvre (Dawson, 1995).

The control group included subjects without UPCB. Parents or guardians received information about the research protocol and signed an informed consent. The research protocol was designed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was reviewed and approved by the Research Ethics Board (protocol 22616).

3.1.2 Rapid Maxillary Expansion

All subjects of the UPCB group were treated with a two-band palatal expander and rapid maxillary expansion (RME, Figure 3.1) (Martina et al., 2012). The appliance was banded to the maxillary first permanent molars and placed using glass ionometer cement (Multi-Cure Glass ionomer Cement; Unitek, Monrovia, CA, USA). The screw was initially turned eight times (2.0 mm) at chair side 2 hours after curing. Thereafter, the patients' parents were trained to turn the screw three times per day (0.75 mm). During the expansion phase, subjects were monitored once a week. The screw was activated until a 2-mm molar transverse overcorrection was achieved. After the active expansion phase, the screw was locked with light-cure flow composite resin (Premise Flowable; Kerr Corporation, Orange, CA, USA). The active treatment (expansion)

ranged between 10 and 16 days. The patients wore the appliance as fixed retainer for 6 months.

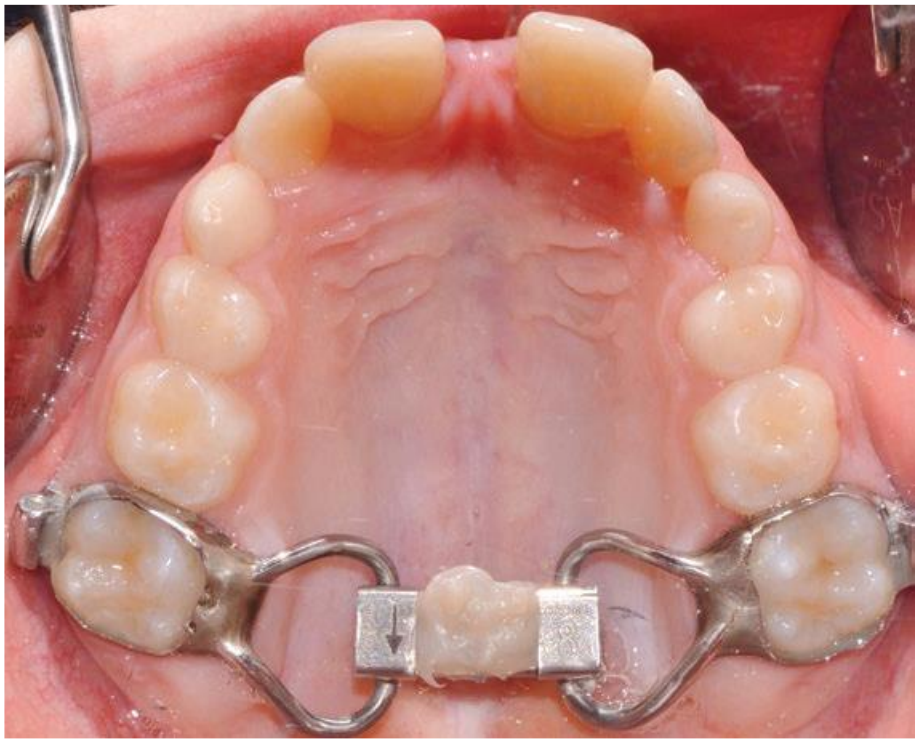

Figure 3.1. Two-band palatal expander at the end of the expansion phase

3.1.3 Data Collection

The EMG activity of the AT and MM of both sides (left end right) was recorded at baseline after recruitment (T0) for both the UPCB and Control-group. For the UPCB group, EMG activity was recorded also when the UPCB was corrected (T1), and 6 months after when the appliance was removed (T2). EMG indices for both static and dynamic tests were computed at each time point.

3.1.4 Sample size calculation and statistical analysis

A sample size calculation was performed before recruitment. The primary outcome measure of this study was the POC medium index. Based on a previous investigation (12), it was assumed that a difference in POC medium values of 5 per cent (SD = 4.55 per cent) between the UPCB and the Control-group could be considered of clinical

relevance. A sample including 21 subjects per group was sufficient to detect between-group differences in POC medium ($\alpha = 0.05$ and $1 - \beta = 0.9$).

The Shapiro–Wilk test was used to check whether data were normally distributed. Mean and standard deviation (SD) for data distributed normally, and median with first and third interquartile range for data not normally distributed were calculated. Between-group differences in standardized EMG indices, except ASIM and SPM, were tested by means of an unpaired t-test or Mann–Whitney U test for between-group comparisons. Repeated measures ANOVA or the Friedman test was used to test the effect of orthodontic treatment on EMG indices (POC AT, POC MM, Tc, IMPACT, SMI) and to detect differences between the time points in the UPCB group. The post hoc Tukey's test with Bonferroni's correction or the Wilcoxon signed-rank test was used. For FREQ, the differences between the crossbite side minus the non-crossbite side, in the UPCB group, and between the right side minus the left side in the Control-group, were calculated. This variable, normally distributed, was analyzed for each group and for each time point by means of a paired t-test for within-group comparisons and by means of an unpaired t-test for between-group comparisons. ASIM and SPM were reported as frequencies. ASIM and SPM values were used to categorize participants in two groups (symmetric and asymmetric) based on normative values (symmetric: -10 per cent $\leq$ ASIM $\leq +10$ per cent; asymmetric: ASIM $\geq +10$ per cent or ASIM ≤ -10 per cent; symmetric: SPM > 70 per cent; asymmetric: SPM $<$ 70 per cent). A chi-squared test was performed to examine whether the distribution of ASIM and SPM categories was similar between the study groups. Moreover, for patients that showed symmetrical jaw muscle activity, the prevalent side was recorded, and a chi-squared test was done to assess whether there was an association between the side of prevalent muscular activity and the side of the UPCB (UPCB group) at both T0 and T2.

Standard statistical software package (SPSS version 22.0, SPSS IBM, Armonk, NY, USA) was used for statistical analysis.

3.2 Results

3.2.1 EMG indices at baseline: within-group and between-group comparisons

All the indices of the static tests (POC, TC, IMPACT) did not differ between groups at T0 (Table 3.1). Based on the assessment of ASIM values, 20 out of 29 patients from the UPCB group and 29 out of 40 individuals presented symmetric EMG activity. The ASIM index was not associated with the presence of UPCB ($P = 0.749$). During the chewing tasks, SMI did not differ between the two groups (Table 3.1); moreover, 19 out of 29 patients in the UPCB group and 25 out of 40 individuals had a side of prevalent mastication (SPM). The chi-square test showed that SPM was independent from the presence of the UPCB ($P = 0.736$).

Finally, FREQ (T0) was not different both between sides (*UPCB*: UPCB side 1.6 ± 0.2 Hz; no UPCB side 1.6 ± 0.3 Hz—P=0.052; *Control-group:* Right 1.4 ± 0.3 Hz; Left 1.5 ± 0.3 Hz—$P = 0.072$) and between groups ($P = 0.614$).

Index	Control group	UPCB group	P value
POC AT	82.0 (9.3)	84.7 (5.9)	0.395
POC MM	84.5 (7.8)	83.0 (7.4)	0.092
POC medium	83.3 (7.7)	83.9 (4.9)	0.551
TC	88.3 (6.2)	88.3 (6.2)	0.738
IMPACT	117.5(89.5;152.5)	118.0 (102.0;146.0)	0.851
SMI	67.8 (22.8)	71.7 (17.4)	0.734

Table 3.1: Standardized EMG indices of control and UPCB group at T_0. Values are expressed in %, mean and standard deviation (SD), for data distributed normally and median with 1st and 3rd interquartile for data not distributed normally. For all variables, no significant differences were found.

3.2.2 EMG indices in the UPCB group before and after orthodontic treatment (T_0-T_2)

POC and TC values did not change significantly throughout the three time points of the study (All $P > 0.05$; Table 3.2). IMPACT changed significantly with time ($P = 0.040$); it decreased at T1 ($P = 0.007$), and returned to baseline values at T2 ($P = 0.424$).

	T0	T1	T2	P value	T0vsT1	T1vsT2	T0vsT2
POC AT	84.7 (5.9)	83.2(5.5)	83.6(10)	0.666			
POC MM	83.0 (7.4)	82.4(10.2)	84.9(5.6)	0.311			
POC medium	83.9 (4.9)	82.6(6.6)	84.4(5.3)	0.323			
TC	88.3 (6.2)	88(5.0)	87.3(6.8)	0.726			
IMPACT	118.0 (102.0;146.0)	97.0 (71.0;121.0)	122.3(97.0;124.0)	**0.040**	**0.007**	**0.036**	0.424
SMI	71.7 (17.4)	59.4(20.2)	67.1(20.8)	**0.040**	**0.027**	1	0.432

Table 2: Standardised EMG indices of UPCB group at T0, T1, T2. Values are expressed in %, mean and standard deviation (SD) (for data distributed normally) and median with 1st and 3rd interquartile (for data not distributed normally) are reported. Bold text indicates statistically significant differences between time points. Post hoc tests with Bonferroni's correction was used.

The ASIM categories varied considerably over the three time points (Figure 3.1).

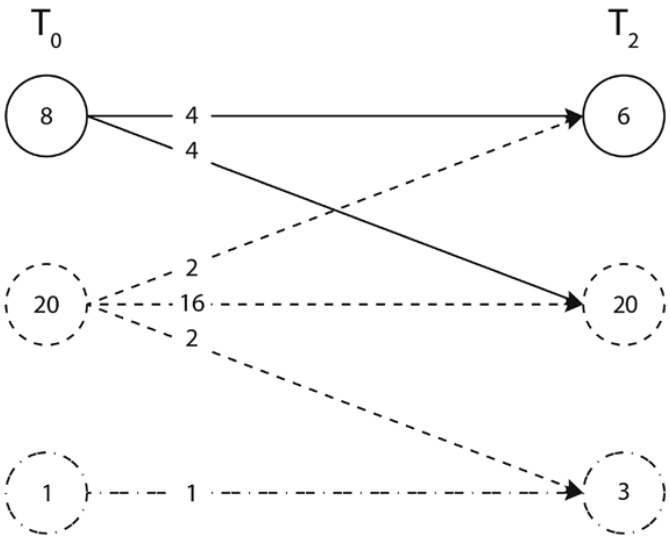

Figure 3.1. Changes in the asymmetry index (ASIM) 6 months after UPCB correction (T2). The numbers of subjects are reported. A solid line indicates an asymmetric muscular activity *coincident* with the side of the PCB. A dashed line indicates symmetric muscular activity. A dot-dash line indicates an asymmetric muscular activity *not coincident* with the side of the PCB.

SMI varied significantly across the time points ($P = 0.040$). It decreased immediately after the PCB correction (T1), indicating a greater asymmetry of the chewing pattern, and returned to values similar to the baseline at T2. SPM varied considerably across the time points in children with UPCB (Figure 3.2).

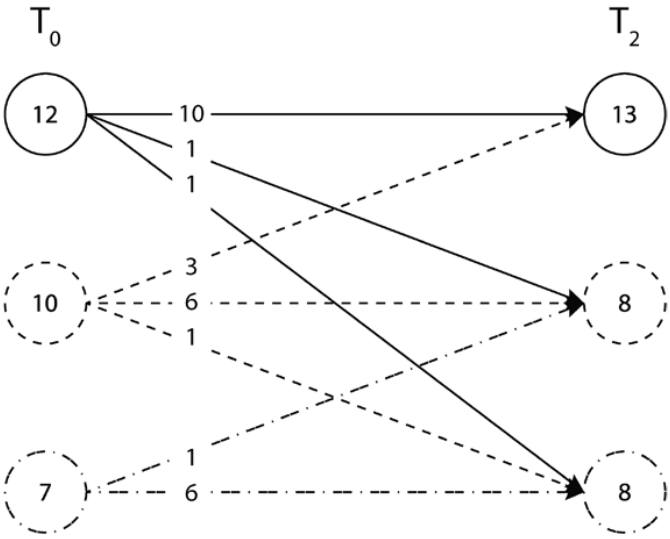

Figure 3.2. Changes in the side of prevalence mastication index (SPM) 6 months after UPCB correction (T2). The numbers of subjects are reported. A solid line indicates a prevalent side of mastication *coincident* with the side of the PCB. A dashed line indicates a symmetric mastication. A dot-dash line indicates a prevalent side of mastication *not coincident* with the side of the PCB.

FREQ did not differ significantly between the UPCB side and the no UPCB side (T0: UPCB side 1.6 ± 0.2 Hz; no UPCB side 1.6 ± 0.3 Hz—P=0.052; T1: UPCB side 1.5 ± 0.3 Hz; no UPCB side 1.5 ± 0.4 Hz—*P* = 0.773); T2: UPCB side 1.5 ± 0.3 Hz; no UPCB side 1.5 ± 0.2 Hz—*P* = 0.276) and between the time points (*P* = 0.255).

3.3 Discussion

The present study investigated whether individuals with UPCB have more asymmetric activity of the jaw muscles, and assessed whether the correction of UPCB contributes to more symmetric jaw muscle activity during standardized functional tasks. The findings of this study confirm the null hypotheses, that is patients with UPCB do not present more asymmetric AT and MM muscle activity as compared to UPCB-free controls, and that maxillary expansion does not determine a more symmetric activation of both AT and MM. In this study, an innovative EMG approach was used. This method, through the standardization of the EMG signals and normalizing the data as a percentage of the MVC effort on cotton rolls, reduces the biological noise, allows comparisons between subjects (Castroflorio et al., 2008) and is widely used and validated in normal subjects and in patients with TMD (Botelho et al., 2010; Castroflorio et al., 2008; De Felício et al., 2009; Ferrario et al., 1999; Rongo et al., 2017).
The data reveal that EMG indices (POC, TC, IMPACT and SMI) were similar between groups at baseline (T0). Also, the asymmetry of muscle contraction was not associated with the presence of UPCB (ASIM index and SPM index) both in static and dynamic tasks. This suggests that a crossbite does not contribute to more asymmetric activity of the masticatory muscles during functional tasks.
Hence, all the indices used to assess the symmetry in the activity of masticatory muscles showed consistent results, reinforcing the concept that the presence of a UPCB in children is not associated with asymmetric muscular activity during both clenching and chewing. The mean indices (POC, TC and SMI) measured in the current study for both groups were lower than the mean values reported in literature for healthy adults (i.e. POC = 86.6, Tc = 91 and SMI = 79.2) (De Felício et al., 2009; Ferrario et al., 1999; Ferrario et al., 2000; Ferrario et al., 2006), suggesting that adolescents (our sample included individuals younger than 13 years old) may have slightly more asymmetric activity of the masticatory muscles than adults. Developmental changes in musculotendinous structures and jaw muscle compartments may account for this slight discrepancy. Indeed, the adaptation of jaw muscles to functional and non-functional demands may be dependent on dental development and diet, which differ substantially between children and adults (Cioffi et al., 2012). However, further studies are needed to address this point. Our data reveal that the EMG indices (POC, TC, IMPACT and SMI) were similar between groups at baseline (T0). Also, the asymmetric activity of muscles during both static and dynamic tasks was not associated with the presence of UPCB. This suggest that a UPCB does not contribute to more asymmetric activity of the masticatory

muscles during functional tasks, and that a certain degree of asymmetric activation of jaw muscles during function has to be considered a physiological characteristic of the stomatognathic system (Ferrario et al., 1999; Ferrario et al., 2000; Ferrario et al., 2006).
Our indices cannot be compared with other studies, since they were never used before in children. Nonetheless, many studies evaluating the contraction pattern of masticatory muscles of children with and without PCB by using conventional EMG assessments reported inconsistent data with questionable clinical relevance (Alarcón et al., 2000; Kecik et al., 2007; Lenguas et al., 2012; Martìn et al, 2012; Piancino et al., 2016). Some studies concluded that children with UPCB have greater asymmetry in muscle activity than normocclusive children, finding differences of just 2 μV between groups (Kecik et al., 2007); or found one statistical significant difference among several statistical tests (Martìn et al, 2012); on the other hand some studies did not find any differences between the two groups (Alarcón et al., 2000; Lenguas et al., 2012).
Maxillary expansion did not significantly affect POC and TC indices. ASIM and SPM indices were highly variable across the time points. These results are in contrast with other studies analysing the effects of PCB correction on masticatory muscle activity (Andrade et al., 2010; Arat et al., 2008; De Rossi et al., 2009; Kecik et al., 2007; Maffei et al., 2014; Martìn et al, 2012; Piancino et al., 2016; Tsanidis et al., 2016).
A systematic review summarizing the functional changes occurring after an early treatment of UPCB has recently suggested that orthodontic treatment of UPCB could improve both occlusal contact quality and occlusal stability (Tsanidis et al., 2016). However, whether the correction of UPCB contributes to a more symmetric activation of jaw muscles during function is still questionable. Indeed, the increased symmetry of the muscle activity reported in some studies, which ranges between 20 and 50 μV (Andrade et al., 2010; Arat et al., 2008; De Rossi et al., 2009; Kecik et al., 2007; Maffei et al., 2014; Martìn et al, 2012; Piancino et al., 2016), although statistically significant, must be considered of limited clinical relevance because of discrepancies in research designs, inclusion criteria (i.e. bilateral PCB or no functional shift) (Arat et al. 2008; De Rossi et al., 2009; Maffei et al., 2014), treatment duration (De Rossi et al., 2009; Kecik et al., 2007; Maffei et al., 2014; Piancino et al., 2016), treatment protocol (De Rossi et al., 2009; Kecik et al., 2007; Martìn et al, 2012; Piancino et al., 2016) and EMG assessment (Andrade et al., 2010; Arat et al., 2008; De Rossi et al., 2009; Kecik et al., 2007; Maffei et al., 2014; Martìn et al, 2012; Piancino et al., 2016). In spite of this, in our study the EMG records were performed only after the expansion phase, without the interference of any other appliances (braces, retention plate), and the follow up of the

patients for just 6 months avoided any interference by the growth in the muscular function due to the brief period assessed (Sabashi et al., 2009). Finally, Di Palma *et al.* (Di Palma et al., 2017) used the same standardized indices in a group of 21 children with UPCB to evaluate the modifications of the RME on the AT and MM activity. They included only children that did not have an asymmetrical muscular activation and they found that 3 months after the correction achieved with the RME, patients did not show any significant change in the EMG activity. This study used a four bands hyrax that is more bulky than the two-band palatal expander used in our study, however, in the middle term there were not differences in the EMG activity due to the appliance design between the two studies. Immediately after UPCB correction (T1), IMPACT and SMI decreased significantly. The transient decrease in muscular recruitment (IMPACT) and the increased asymmetry during the chewing tasks (SMI) might be due to many factors, such as tooth soreness caused by the stimulation of the periodontium of the posterior teeth during expansion, the lack of adaptation of the neuromuscular system to the new occlusal condition, and the discomfort created by sudden changes in the maxilla-mandibular relationship (Dong et al., 2008). In fact, occlusal instability, modifications in dentition, and the repositioning of bones or skeletal configuration may cause transient effects on jaw muscles (Leung et al., 2001). The neuromuscular adaptation of the stomatognathic system to the new mandibular position does not occur immediately after treatment but only when a satisfactory occlusal engagement is achieved (Martín et al., 2012; Pirttiniemi, 1994; Sonnesen et al., 2007).

Our data suggest that an asymmetric activation of the jaw muscles during functional tasks is an ordinary aspect in children. It must be stressed that all body segments present with a certain degree of asymmetry, which should be regarded as a physiological characteristic of each individual. Healthy subjects should not be expected to have a perfect symmetric activation of masticatory muscles, which is a man-made construct, during normal function (Dong et al., 2008). This was shown in several studies analyzing healthy subjects without signs of dysfunction, in which the standardized indices POC and TC were never close to 100 per cent (Ferrario et al., 1999; Ferrario et al., 2000; Ferrario et al., 2006; De Felicio et al., 2009).

The aim of this study was testing the effect of cross bite with mandibular side shift on masticatory muscle asymmetry. This occlusal condition is characterized by a discrepancy between centric occlusion (CO) and centric relation (CR), which determines an asymmetrical position of the condyles in the glenoid fossa (Pinto et al., 2001). Hence, in the current study, the clinical manoeuvre described by Dawson (Dawson, 1995) was used to select study

participants. This clinical manoeuvre is commonly used to distinguish between functional and morphologic crossbite, and to detect the CR position and the discrepancy between CO and CR. In this study, all participants had a posterior unilateral crossbite in CO but not CR, with a shift CR–CO. This study has a few limitations. First, most of the EMG indices were computed using the MVC. MVC is dependent on the participant's compliance. Although all the participants were verbally encouraged during the experimental tasks, MVC values recorded may be slightly different across the time points. However, the RMS algorithm, used for the computation of the indices, analyzed the 3 seconds of the test with the highest EMG amplitude, providing a normalized estimate of the MVC. Therefore, it may be assumed that variations of MVC across the conditions did not significantly affect the outcome measures. Second, in this study, the dental contacts were not recorded, although interferences between the upper and dental arches were reported to influence the EMG indices (Augusti et al., 2015). Third, ASIM and SMI analysis were performed using adult normative values. This may raise questions concerning the validity of the analysis and the interpretation of the data. However, on the other hand there is no evidence suggesting that the threshold of muscular asymmetry is or should be different between adults and children.

3.4 Conclusions

In conclusion, the present study has shown that children with and without UPCB present slight asymmetric activity of AT and MM during functional tasks and these muscles of children with UPCB are not more asymmetric than healthy children without crossbite. Furthermore, the treatment of UPCB with RME does not reduce the asymmetry of MM and AT activity; hence, the symmetrization of the muscular activity cannot be an indication of maxillary expansion. Early treatment of UPCB by maxillary expansion should not be advocated to promote a more symmetric activation of the MM and AT in the short-medium term. Longitudinal studies with a long-term follow-up are still required to evaluate the long-term effects of the treatment.

CHAPTER 4

Evaluation of masticatory muscle activity in patients with juvenile idiopathic arthritis (JIA)

Juvenile idiopathic arthritis (JIA) represents the most common childhood rheumatic disease. It is characterized by the chronic inflammation of one or more joints, onset before 16 years and a minimum duration of 6 weeks (Petty et al., 2004). The prevalence is of 1 in 1000 children worldwide aged 0–15 years (Schneider and Passo, 2002). The pathogenesis is poorly understood, but interaction between environmental (infection, trauma, immunological phenomena) and genetic factors has been proposed (Calabro et al., 1976; Calabro, 1986). All joints may be affected, including the temporomandibular joint (TMJ), known as "the forgotten joint". It may be the first joint affected both uni- and bilaterally, or may be affected during the course of JIA (Ringold and Cron, 2009). The frequency of TMJ involvement varies from 17 to 87% depending on subtypes involved, diagnostic criteria reported and ethnicity (Cannizzaro et al., 2011; Kuseler et al., 1998; Pedersen et al., 2001; Ringold and Cron, 2009; Twilt et al., 2006; Weiss et al., 2008). JIA can interfere with the craniomandibular joint and muscle function (Karhulahti et al., 1993). JIA is characterized by destructive changes in the mandibular condyle that seem to lead to a change in mandibular position. A reduced vertical condylar growth leads to a posterior rotation of the mandible, mandibular retrognathia (Twilt et al., 2008), micrognathia (Arvidsson et al., 2010a, 2010b; Pearson and Ronning, 1996) and dental malocclusion with a significantly greater prevalence of anterior open bite (Kjellberg, 1995). Children with TMJ arthritis may have restricted condylar motion in the open-mouth position (Argyropoulou et al., 2009), and/or reduced condylar motion at maximally opened mouth (Stabrun et al., 1987). Furthermore, they could be affected by jaw pain, dysfunction, facial asymmetry, psychological disturbances and a reduced quality of life (Bakke et al., 2001; de Carvalho et al., 2012; Engstrom et al., 2007; Ringold and Cron, 2009). Muscle weakness may contribute to craniofacial growth deviations probably in association with poor function (Kreiborg et al., 1990; Lindehammar and Backman, 1995; Lindehammar and Sandstedt, 1998; Stabrun et al., 1987;Pearson and Ronning, 1996). Many children with JIA have reduced muscle

strength and they show structural and immunological changes in muscles such as the presence of inflammatory cells and expression of MHC class II on muscle fibers that may be a sign of inflammatory myopathy (Lindehammar and Lindvall, 2004). Muscle strength in JIA has been assessed in few studies with different methods, e.g., an isokinetic dynamometry, a hand-held dynamometry (HHD), ultrasound and a computerized dynamometer (Bröstrom et al., 2004; Giannini and Protas, 1993; Hedengren et al., 2001; Lindehammar and Backman, 1995; Vostrejs and Hollister, 1988). These studies have suggested that children with JIA have reduced muscle strength. On the other hand, Öberg et al., (Öberg et al., 2004) reported no difference in muscle strength between children with JIA and healthy controls. Muscle strength in children with JIA can be near to normal when the disease is not active (Saarinen et al., 2008). Among the studies that analyzed the masticatory muscle activity in JIA patients, there are no works evaluating the electromyographic (EMG) activity.

There are not studies using EMG standardized protocols to assess the activity of masticatory muscle in children with JIA but assessing the symmetry in muscle activity could give information on the growth pattern (Iodice et al., 2013). Hence, the aim of this study was to evaluate the muscular activity of masticatory muscles in subjects with JIA compared to subjects without JIA to evaluate any differences in terms of muscular asymmetry and/or the overall muscular activity. The null hypothesis is that patients with JIA do not present more asymmetric AT and MM muscle activity as compared to healthy controls.

4.1 Materials and methods

4.1.1 Study sample

This research protocol was approved by the Ethical Committee of University of Naples "Federico II" of Naples (protocol 16918), in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. In this study, 50 patients affected by JIA (JIA group: 10 males, 40 females, mean age±SD = 11.12±2.85 years) and 69 healthy subjects (CTR group: 30 males, 39 females, mean age±SD = 10.0±1.7 years) seeking an orthodontic consultation were recruited at the Department of Neuroscience, Section of Orthodontics and Temporomandibular Disorders of the University of Naples, Federico II.

The sample was divided in four subgroups:

1. patients with JIA with asymmetry (Symmetric JIA)
2. patients with JIA without asymmetry (Asymmetric JIA)
3. patients with posterior crossbite with functional sliding without JIA (UPCB)
4. healthy control patients without crossbite and without JIA (Healthy)

The inclusion criteria, for the first and second subgroups were:

- JIA diagnosis according to the ILAR criteria for study groups
- the age range between 8 to 16 years old

The exclusion criteria for all participants were:

- incomplete medical records
- presence of congenital/ acquired facial anomalies (eg, hemifacial microsomia, cleft lip/palate, Treacher-Collins syndrome, TMJ ankylosis)
- history of facial fractures
- previous intra-articular TMJ interventions (eg, steroid injections, operations)
- inability to verbalize or indicate pain or discomfort (eg, developmental delay)
- presence of medical comorbidities not allowing for a comprehensive clinical examination (eg, severe scoliosis limiting neck movement)
- orthodontic treatment in course or within the past 12 months.

Patients with JIA were recruited from the Clinic of Rheumatology of the Paediatric Department at the University of Naples "Federico II" and were diagnosed by a paediatric

rheumatologist according to the ILAR criteria. During the first visit, age, gender, subtype of JIA, disease onset date, year of diagnosis and administered systemic therapy were collected.

4.1.2 Anamnesis and clinical examination

A specialist in orofacial pain and temporomandibular disorders visited all patients according to the DC/TMD protocol (Schiffman et al., 2014) and collected all data.

The clinical examination according to the DC/TMD protocol included the following:

- Opening pattern: the examiner asked the patient to open slowly three times, observing from a position directly in front of the patient. The opening patterns were recorded as straight if there was no or minimally perceptible deviation (<2mm) during mouth opening; corrected deviation if the mandible exhibited a perceptible deviation (≥2mm) to the right or left but returned to the midline before or upon reaching the maximum unassisted opening; uncorrected deviation if the deviation of the mandible was of ≥2mm to either the right or the left from the midline with maximum unassisted opening.
- Opening movements: measurements were taken between the incisal edges of the maxillary and mandibular reference teeth. Pain free opening: the patient opened without feeling pain; maximum unassisted opening: the patient opened as wide as possible, even if feeling pain and the examiner asked the patient about presence and location of any pain produced by this procedure; maximum assisted opening: the patient opened maximally, and the examiner helped the patient to open wider, asking about any pain produced by this procedure. In paediatric patients the cut-off value for restricted mouth opening was ≤ 40 mm (Müller et al., 2013; Zwir et al., 2015).
- Lateral and protrusive movements: right and left lateral excursion, and protrusion. During these movements, the examiner asked the patient to move the mandible as far as possible in each direction, even if it was painful, and recorded presence and location of any reported pain.
- TMJ noises during jaw movements: using palpation the examiner recorded joint noises during opening, closing, lateral and protrusive movements. The

patient also reported whether they heard or felt a joint sound. Click: a distinct noise of brief and very limited duration, with a clear beginning and end. Crepitus: a noise that is continuous, over a longer period of jaw movement.

- Muscle and TMJ pain with palpation: Pain on palpation of the masseter and temporalis muscles and the TMJ were assessed. The examiner pressed on specific sites using the pad of one finger with standardized pressure: 1.0 kg force over the nine sites of each muscle and around the lateral pole of the TMJ, and 0.5 kg over the lateral pole of the TMJ.

The examiner evaluated molar and canine relationship, on the right and on the left side, overbite, overjet and presence of posterior and anterior crossbite.

Finally, an experienced orthodontist examined the patients using frontal facial photographs to evaluate the presence of chin deviation and level of asymmetry. A picture, with a ruler for the calibration on the background, was taken with the patient in the upright sitting posture with teeth in centric relation and lips at rest. For each patient, on the photograph, the facial midline (a perpendicular to the inter-pupillary plane passing for the glabella) was identified and the direction and severity of chin deviation was recorded. The orthodontist recorded a chin deviation when the chin shift from the facial midline was >2 mm to right or left side. The photographs did not present patient name and were recorded by an ID number, hence the observer did not know patient name and did not know the findings of the clinical examinations.

4.1.3 EMG assessment

The activity of masseter (MM) and temporalis anterior (TA) muscles of both sides (left and right) was recorded during standardized tasks by means of sEMG according to the EMG protocol previously described.

4.1.4 Sample size calculation and statistical analysis

A sample size calculation was performed before recruitment. The primary outcome measure of this study was the POC medium index. Based on a previous investigation (Ferrario et al., 2000), it was assumed that a difference in POC medium values of 5% (SD=4.55%) between two groups could be considered of clinical relevance. A sample

including 21 subjects per group was sufficient to detect between-group differences in POC medium (α=0.05 and 1-β=0.9).

The Shapiro Wilk test was used to check whether data were normally distributed. Mean and standard deviations (SD) for data distributed normally, and frequencies and percentages for nominal data were calculated.

Between-group differences in standardized EMG indices, except ASIM, ATTIV and TORS, were tested by means of an unpaired t test. Subgroup comparisons for the same EMG indices were performed by means of one-way analysis of variance (one-way ANOVA).

ASIM, ATTIV and TORS were reported as frequencies. ASIM and TORS, values were used to categorize participants in three groups (symmetric -10%≤ASIM/TORS≤+10%; prevalence of right activity ASIM/TORS≥+10%, prevalence of left activity ASIM/TORS≤-10%) based on normative values. Also, the ATTIV index was used to categorize patients in three groups (symmetric -10%≤ATTIV≤+10%; prevalence of masseter activity ATTIV≥+10%, prevalence of temporalis activity ATTIV≤-10%). A Chi squared test was performed to examine whether the distribution of ASIM, TORS and ATTIV categories was similar between the study groups.

Standard statistical software package (SPSS version 22.0, SPSS IBM, Armonk, NY, USA) was used for statistical analysis.

4.2 Results

Only the 50 with JIA were evaluated according to the DC/TMD protocol (Schiffman et al., 2014) and the results are reported in Table 4.1. The clinical examination showed that out of the 50 initial patients, 23 presented chin deviation and 18 had crepitus (Table 4.1).

			Mean	SD
Age			11.12	2.85
Maxiumum Unassisted Opening			45.1	6.2
Maxiumum Assisted Opening			48.3	6.2
Protrusion			4.4	1.8
Right laterotrusion			10	2.4
Left laterotrusion			10	2.3
Total number of patient			**N=50**	**%**
Opening pattern	Straight		38	76
	deviation	correct	5	10
		uncorrect	7	14
Ovb	< 0 mm		3	6
	0-4 mm		34	68
	> 4mm		13	26
Ovj	< 0 mm		0	0
	0-4 mm		39	78
	> 4mm		11	22
TMJ pain	No		34	68
	Yes		16	32
Muscular pain	No		26	52
	Yes		24	48
Pain during movements	No		48	96
	Yes		2	4
Pain during mouth opening	No		39	78
	Yes		11	22
Chin deviation	No		27	54
	Yes		23	46
TMJ crepitus	No		32	64
	Yes		18	36

Table 4.1. Clinical findings in JIA group.

4.2.1 EMG indices evaluation

No significant differences were found for analysed static indices between healthy control and JIA group (POC, TC and BAR). There was no difference for IMPACT index between groups so all subjects reported similar values of muscular work. During the chewing tests, SMI did not differ between the two groups (Table 4.2).

Index	JIA	95% CI	Healthy	95% CI	P value
POC AT	81.8 (11.9)	78.4-85.2	83.1 (8.1)	81.1-85.1	0.813
POC MM	82.7 (7.4)	80.6-84.8	83.9 (7.7)	82.1-85.8	0.166
POC medium	82.2 (7.2)	80.2-84.3	83.5 (6.6)	81.9-85.1	0.373
TC	86.4 (10.1)	83.6-89.3	88.3 (6.5)	86.8-89.8	0.688
BAR	80.9 (15.6)	76.5-85.4	82.0 (12.3)	79.3-85.3	0.966
IMPACT	117.8 (40.3)	106.4-129.3	138.1(106.3)	112.6-163.7	0.418
SMI	58 (30.6)	49.3-66.7	69.5 (20.7)	64.5-74.4	0.083

Table 4.2: Standardized EMG indices of CTR and JIA group. Values are expressed in mean and standard deviation (SD), for data distributed normally.

Based on the assessment of ASIM values, 33 out of 55 patients from the JIA group and 49 out of 69 individuals presented symmetric EMG activity. The ASIM index was not associated with the presence of JIA.

For ATTIV index, we did not find a significant difference between groups: in both groups, but in both group there was a higher activity of masseter muscles compared to temporalis (Table 4.3).

GROUP	TORS			ASIM			ATTIV		
	N	R	L	N	R	L	N	T	M
SYMMETRIC JIA (N= 28)	19	6	3	19	3	6	14	0	14
ASYMMETRIC JIA (N= 22)	18	1	3	14	4	4	9	4	9
JIA TOTAL (N= 50)	37	7	6	33	7	10	23	4	23
HEALTHY (N= 40)	27	5	8	29	5	6	18	4	18
UPCB (N= 29)	22	6	1	20	7	2	16	5	8
HEALTHY TOTAL (N=69)	49	11	9	49	12	8	34	9	26

Table 4.3. Number of patients according to TORS, ASIM and ATTIV indices.
(N symmetrical pattern of muscular activation; R number of patients with prevalent activation on the right side and L number of patients with prevalent activation on the left side. M number of patients with prevalent activation of masseter muscles and T number of patients with prevalent activation of temporalis muscles).

4.2.2 Comparison between all study subgroups

JIA patients were divided into 2 subgroups (Symmetric JIA and Asymmetric JIA) depending on the presence of a skeletal asymmetry to assess whether the presence of the latter could influence the EMG indices. Comparing all the subgroups included in this study, we did not find significant differences (Table 4.4).

The Chi-square test showed that indices values did not depend from the presence of JIA or the presence of functional and skeletal asymmetry. For all static and dynamic variables, no significant differences were found (Table 4.3).

Index	Asymmetric JIA	95% CI	Symmetric JIA	95% CI	UPCB	95% CI	Healthy	95% CI	P value
POC AT	82.4 (7.1)	79.0-85.6	81.3 (14.8)	75.5-87.0	84.7 (5.9)	82.5-87	82.0 (9.3)	79.0-85.00	0.307
POC MM	81.4 (7.1)	78.3-84.6	83.8 (7.6)	80.8-86.7	83.0 (7.4)	80.2-85.9	84.5 (7.9)	82.0-87.1	0.085
POC medium	8 1.8 (5.8)	79.2-84.4	82.6 (8.2)	79.4-85.7	83.9 (4.9)	82.0-85.8	83.3 (7.7)	80.8-85.7	0.358
TC	87.8 (5.3)	85.5-90.2	85.4 (12.7)	80.4-90.3	88.3 (6.2)	85.9-90.7	88.3 (6.2)	86.3-90.3	0.774
BAR	83.3 (7.8)	79.9-86.8	79.1(19.7)	71.4-86.7	82.4 (13.5)	77.3-87.6	82.2 (11.6)	78.5-85.9	0.961
IMPACT	121.0 (39.6)	103.5-138.6	115.3 (41.4)	99.2-131.4	129.6 (54.8)	108.8-150.5	144.3 (132.1)	102.0-186.5	0.717
SMI	51.8 (32.7)	37.3-66.3	62.9 (28.4)	51.8-73.9	71.7 (17.4)	66-78.3	67.8 (22.8)	60.5-75.1	0.206

Table 4.4: Standardized EMG indices of all four subgroups. Values are expressed in mean and standard deviation (SD) for data distributed normally.

4.3 Discussion

The present study investigated whether individuals with JIA have more asymmetric activity of the jaw muscles during standardized functional tasks. The findings of this study confirm the null hypotheses that patients with JIA do not present more asymmetric AT and MM muscle activity as compared to healthy controls.

Symmetric muscle activity was evaluated with a standardized EMG protocol. This method, that presents a high reproducibility and provides accurate records (Ferrario, 2000), is painless and non-invasive, and these are important factors for studies in children (Lenguas, 2012).

Our data reveal that the EMG indices (POC, TC, BAR and SMI) were similar between patients with or without JIA. Moreover, the asymmetric activity of muscles (ASIM, ATTIV, TORS) during static tasks was not associated with the presence of JIA. This suggest that JIA does not contribute to more asymmetric activity of the masticatory muscles during functional tasks, and that a certain degree of asymmetric activation of jaw muscles during function has to be considered a physiological characteristic of the stomatognathic system (Ferrario et al., 1999; Ferrario et al., 2000; Ferrario et al., 2006; Michelotti et al., 2018). A general consideration is that asymmetry is an ordinary aspect in humans, since both morphology and activity of paired structures is different in left and right sides of the body (Ferrario et al, 2000). It must be stressed that asymmetry of all body segments should be regarded as physiological variations of the ideal, which is a man-made construct. Asymmetry dominates in the human body: handedness and footedness is associated with neuromuscular asymmetry from lateralized muscular differences (Valderrabano et al., 2007), asymmetric excitability of the motor system (Triggs et al., 1994) and anatomical and functional cortical asymmetries (Hammond, 2006). Hence, although healthy, no subjects should be expected to have bilaterally symmetric activity close to 100% (Dong et al., 2008). This was shown in various studies on healthy subject without signs of temporomandibular disorders, in which the standardised indices assessed (POC and TC) were far from the perfect symmetry of the 100% (Ferrario et al., 2000). Our indices cannot be compared with other studies, since they were never used before in children with JIA. Other study in literature focused on modification in muscle structure and strength in-patient with JIA. According with our data, Öberg et al, (Öberg et al.,1994) reported no difference in muscle strength between JIA patients and healthy children; conversely, Lindehammar et al. (Lindehammar et al., 1998), and Saarinen et al. (Saarinen et al., 2008) that children affected by JIA have reduced muscle strength. Lindehamman and Lindvall (Lindehamman et al., 2004) analysed changes in leg muscle biopsies in children and teenagers with JIA and described the presence of inflammatory cells in the

muscle and/or an alteration of the expression of the major histocompatibility complex (MHC) II components in muscle fibres. These features can be considered as markers of inflammatory myopathy and reduced the muscles activity.

In this study, it was also evaluated if the presence of a skeletal asymmetry could influence the muscular activity. Hence, 22 children with JIA and skeletal asymmetry were compared with three other subgroups, JIA patients without skeletal asymmetry, patients without JIA but with unilateral posterior crossbite and healthy subjects. All the indices assessed did not show any statistically significant difference among the four subgroups, indicating that during the childhood skeletal asymmetry did not affect the symmetry in muscular activity. However, few studies analysed masticatory muscle activity in patients with skeletal asymmetry during adulthood. Akimoto et al. (Akimoto et al., 1994) reported a greater EMG activity of the masseter muscle in the non-shifted side in patients with facial asymmetry. Other studies have reported that the integrated EMG of the masseter muscle was larger in the midline shifted side in patients with facial asymmetry (Hirose, 1990; Kondoh, 1991; Haraguchi et al., 1994). Unfortunately, no consistent results have been reported, but it seems that in adulthood an asymmetry in the activity of masticatory muscles occurred independently by the concordance with the side of skeletal asymmetry. Muscular asymmetry in JIA patient has not been analysed in other studies, Michelotti et al. (Michelotti et al., 2018) used the same EMG standardized indices of masseter and anterior temporalis in patients with UPCB due to functional shift, showing that also the UPCB did not affect the masticatory muscle activity.

Muscular work (IMPACT index) was similar for all subgroups. Others reported that both isometric and isokinetic strength were significantly reduced in patient with JIA (Lindehamman and Lindvall, 2004).

This study has a few limitations, first, most of the EMG indices were computed using the MVC. MVC is dependent on the participant's compliance. However, the RMS algorithm, used for the computation of the indices, analysed the 3 seconds of the test with the highest EMG amplitude, providing a normalized estimate of the MVC. Second, in this study, the dental contacts were not recorded, although interferences between the upper and dental arches were reported to influence the EMG indices (Augusti et al., 2015). Third, ASIM, ATTIV and TORS analyses were performed using adult normative values. This may raise questions concerning the validity of the analysis and the interpretation of the data. However, on the other hand there is no evidence suggesting that the threshold of muscular asymmetry is or should be different between adults and children.

4.5 Conclusions

In conclusion, the present study has shown that children with and without JIA present slight asymmetric activity of AT and MM, with major activity of MM, during functional tasks and these muscles of children with JIA are not more asymmetric than healthy children without JIA.

CHAPTER 5

Evaluation of masticatory muscles and temporomandibular joint pressure pain threshold in patients with juvenile idiopathic arthritis compared to healthy subjects: a case control study

According to previous studies patients with JIA report pain, fatigue, decrease in muscle mass paralleled by an increase in fat mass (Bechtold et al., 2004; Roth et al., 2007), bone damage, decreased bone mineral composition and worse quality of life compared to healthy subjects (Burnham et al., 2008; Ganotti et al., 2007; Hedengren et al., 2001; Klepper et al., 2008; Long and Rouster-Stevens, 2010; Maggio et al., 2010; Takken et al., 2003; Saarinen et al., 2008; Sandstedt, 2013). Several studies analyzed muscular strength in patients affected from JIA and controversial results were reported (Lindehamman and Lindvall 2004; Öberg et al., 1994; Saarinen et al. 2008). JIA has been reported to increase the frequency of soreness of the masticatory muscles, and this could be a reflection of the central mechanics of the pathology (Alstergren et al., 2008; Bakke et al., 2001; Pedersen et al., 2008). In many cases, there are immunological changes in the muscles such as an increase in the number of inflammatory cells, and/or an alteration of the expression of the major histocompatibility complex (MHC) II components in muscle fibers. These features can be considered as markers of inflammatory myopathy.

Patients suffering from JIA have bone deficit due to the reduced area of the cross section of the muscle. Therefore, the bone defect is probably due to the co-presence of impaired bone growth and reduced muscle strength (Stagi et al., 2014).

TMJ is affected in 40% of cases (Stoll et al., 2012) as shown by magnetic resonance imaging (MRI) (Larheim et al., 2015). This technique is able to detect inflammation at joint level with high sensitivity. The identification of a new radiological algorithm in JIA is of paramount importance to allow rapid integration of imaging into the clinical workflow and decision-making process (Malattia et al., 2018). When the pathology persists for a long time, a damage of the cartilage as erosions/flattening/widening of the condyle and fossa/eminence in the subchondral bone could occur (Sheybani et al., 2013). This event may be particularly worrying during growth, because the mandibular growth zone is located below the fibrocartilage and the inflammation could cause severe growth disturbances (Kjellberg et al., 1995; Ronchezel et al., 1995; Weiss et al., 2008). A deficit in craniofacial growth can also occur because of muscle weakness and reduced functional ability (Kreiborg et al., 1990; Pearson and Ronning, 1996; Stabrun et al., 1987). Finally, TMJ involvement can lead to several functional disabilities, such as reduced mandibular mobility and masticatory muscles disorders.

Pain is the most common and distressing symptom experienced by patients with JIA (Schanberg et al., 2003). Pain perception can be assessed by pain pressure threshold (PPT) measurement. Pressure algometry is a useful technique to quantify the mechanical sensitivity of a muscle, in which pressure is registered through an algometer (Ylinen et al., 2007). PPT has been shown to be a valid method to measure mechanical pain threshold in human muscles, including craniofacial ones (Bezov et al., 2011; Chesterton et al., 2007; Farella et al., 2000).

Some studies evaluating PPT in the body areas related to the joint or bone in patients with active or quiescent JIA, reported reduced pain thresholds and tolerance both in areas affected or not affected. This suggests that JIA alters the perception of pain by reducing the pressure threshold (Cornelissen

et al., 2014; Leegaard et al., 2013). The lowered pain thresholds and tolerance are compatible with increased reported pain, suggesting a role for central sensitization and nociceptive pathway plasticity in JIA (Munro and Singh-Grewal, 2013).

No studies evaluating the pain pressure threshold of masticatory muscles and TMJ in patients affected by JIA are currently available in literature. Therefore, the aim of this study is to evaluate the PPT of craniofacial areas in subjects with JIA. The null hypothesis is that patients affected by JIA have a lower PPT compared to healthy subjects.

5.1 Materials and methods

5.1.1 Study sample

Fifty-two patients with a diagnosis of JIA (JIA-group: 10 males, 42 females, mean age±SD = 11.12±2.85 years) according to the ILAR (International League of Associations for Rheumatology) criteria were recruited from the Clinic of Rheumatology of the Paediatric Department. Fifty-two healthy subjects with no JIA and TMD (CTR group: 23 males, 29 females, mean age±SD = 11.58±2.12 years) seeking a dental consultation were recruited at the Dental Clinic.

For both groups, the exclusion criteria were genetic or congenital abnormalities, craniofacial anomalies, systemic diseases affecting growth and development, previous or current orthodontic treatment and comorbidities potentially associated with pain.

Parents or guardians received information about the research protocol and signed an informed consent. The research protocol was designed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was reviewed and approved by the Research Ethics Board (protocol 16918).

5.1.2 Anamnesis and clinical examination

Anamnestic information on patient's symptoms was collected using standardized questionnaires filled in by the patient and/or parents/guardians. Data were collected regarding subtype of JIA, disease onset date and the ongoing drug therapy. Patients were also asked about joint noises, pain in the last month in the orofacial region (masticatory muscles and/or TMJ), if the pain changed (got better or got worse) with movement, and filled in a pain drawing for the location of pain.

The clinical examination was carried out according to the standardized DC/TMD protocol (Schiffman et al., 2014;) by one calibrated examiner (RV) and included evaluation of opening pattern, mandibular movements, joint sounds (click or crepitus), joint and muscle

palpation. Finally, the presence of chin deviation and level of asymmetry were recorded when the chin shift from the facial midline was >2 mm to right or left side.

The diagnosis of temporomandibular joint (TMJ) damage was made following the evaluation of positive findings at MRI according to Kellenberger et al (Kellenberger et al., 2018) and the presence of one of the following criteria: positive crepitus, muscle pain during palpation, TMJ pain at palpation, deviated opening and the asymmetry. Based on the presence of TMJ damage we distinguished three different sub-groups in patients affected by JIA: JIA patients without TMJ damage (JIA-NoTMJ), JIA patients with unilateral TMJ damage (JIA-UniTMJ) and JIA patients with bilateral TMJ damage (JIA-BilTMJ).

5.1.3 Pressure Pain Threshold measurement

PPT was assessed by a commercially available digital algometer (Figure 5.1). In this study, Sense Box (manufactured by Somedic AB, Vallgaten 2A, SE-242 31 Hörby, Sweden in 2012) was used that shows high reliability (Bernhardt et al., 2007). The instrument consists of a gun shaped handle with a pressure sensitive strain gauge at the tip. The tip is coated with a rubber and has a surface of 1 cm^2; the rate of pressure increase was about 20kPa /s (Schoenen et al., 1991). This device is connected to the PC and through a software it is possible to read the PPT's value. The PPT has been defined as the point where the pressure stimulus applied on the site has passed from a feeling of pressure to one of pain. The patients were instructed to push a button to stop the pressure delivered from the algometer as soon as the pressure sensation became painful, whereby the pressure value (measured in kPa) froze on the digital display. A display indicated the rate of the pressure (kPa) and the rate of pressure increase (kPa/s). The test is performed in 6 points on the face and 2 on the hands, repeated 4 times.

Figure 5.1. Algometer

During the test, the subject sat in a dental chair and was asked to relax and keep the teeth apart during the recordings, the algometer was held perpendicular to the skin. The following sites are examined: anterior temporal (AT), masseter (MM), temporomandibular joint (TMJ) and thenar eminence (TH), both on the right and on the left. To ensure the precise transfer of these points in each session, a reproducible method was used. A single operator performed all measurements (GL). For the anterior temporalis, an imaginary line was drawn between the upper orbital margin to the upper point of the outer ear, 2 cm behind the anterior border of the muscle; this border was determined through palpation during forceful voluntary contraction. For the masseter muscle, the most prominent point of muscle was identified, through the palpation during the voluntary contraction. To ensure precise relocation of these sites in each session, a transparent pliable plastic template was aligned to the ear, to the labial margin, and to the eye, and the location of each site was marked (Michelotti et al., 1999). To perform the PPT at the TMJ level the algometer was placed on the tragus, in the pre-auricular region. Afterwards, on the muscle hold we analyzed the point that connects the longitudinal axis of the thumb and index finger. The sites were evaluated on a randomized order with a 5 second interval between them. We performed four PPT measurements for each site, with a 2-minute interval between sessions. The first measurement of the PPT of a session is generally higher than the following ones, and for this reason it was excluded when the mean was calculated; therefore the value of the PPT was determined as the mean of the following 3 measurements. The reliability of this method has been reported elsewhere (Jensen et al., 1986; Kosek et al., 1993; Pigg et al., 2010; Rolke et al., 2006).

5.1.4 Statistical analysis

All data were analyzed by means of non-parametric Wilcoxon, Mann-Whitney exact test and Kruskal-Wallis, where appropriate. Statistical analysis were performed using commercial statistical software (Statgraphics Plus 2.0, Manugistics Inc., Rockville, Maryland, USA) and statistical significance was accepted at $p < 0.05$.

5.2 Results

In JIA group the main JIA subtypes were oligoarticular (61%) polyarticular (35%) and systemic (4%).

Considering all JIA patients, the PPT was significantly lower among children with JIA compared to healthy subjects (p =0,00) for all analyzed sites (Table 5.1).

	JIA group (kPa)	**CTR group (kPa)**	**p-value**
TH muscle	383,92±133,27	680,09±270,95	**0,000**
MM muscle	270,55±84,75	384,71±139,53	**0,000**
AT muscle	285,65±86,49	403,20±158,89	**0,000**
TMJ	235,53±92,33	351,29±130,14	**0,000**

Table 5.1. Comparison between patients affected by JIA and healthy subjects.

For a further evaluation of results, TMJ damage was taken into account. Comparing healthy subjects with JIA patients with JIA-BilTMJ a significant difference in PPT values was found for all tested sites (Table 5.2).

	CTR Group (kPa)	**JIA-BilTMJ group (kPa)**	**p-value**
TH muscle	667,26±284,12	370,06±122,05	**0,000**
MM muscle	377,55±147,94	257,22±70,27	**0,000**
AT muscle	395,59±166,80	278,59±76,32	**0,000**
TMJ	344,66±137,62	217,83±93,03	**0,000**

Table 5.2. Comparison between healthy subjects and patients affected by JIA with bilateral involvement.

Comparing JIA-BilTMJ with JIA-NoTMJ it was not found any significant difference for all analyzed sites (Table 5.3).

	JIA-BilTMJ group (kPa)	**JIA-NoTMJ group (kPa)**	**p-value**
TH muscle	370,06±122,05	426,73±151,91	0,501
MM muscle	257,22±70,27	282,94±72,71	0,479
AT muscle	278,59±76,33	310,04±77,3	0,339
TMJ	217,83±93,03	258,64±80,56	0,322

Table 5.3. Comparison between patients affected by JIA with bilateral involvement and patients affected by JIA without bilateral involvement.

Afterwards, comparing healthy subjects with JIA-NoTMJ, significant differences were reported (Table 5.4).

	CTR Group (kPa)	**JIA-NoTMJ group (kPa)**	**pVALUE**
TH muscle	667,26±284,12	426,73±151,91	**0,001**
MM muscle	377,55±147,94	282,94±72,71	**0,008**
AT muscle	395,59±166,81	310,04±77,3	**0,02**
TMJ	344,66±137,62	258,64±80,56	**0,013**

Table 5.4. Comparison between healthy subjects and patients affected by JIA without bilateral involvement.

Means and significance of the comparison between patients affected by JIA-UniTMJ were reported in Table 5.5 comparing PPT values of the affected side with those of the healthy side. PPT on TMJ was significantly lower on the damaged site (p=0,035) whereas for muscular sites, it was not found any significant difference.

	PPT AFFECTED SIDE (kPa)	**PPT UNAFFECTED SIDE (kPa)**	**p-value**

TH muscle	379,99±165,55	405,66±156,47	0,179
MM muscle	289,15±117,96	274,51±103,51	0,607
AT muscle	303,15±132,62	280,86±117,32	0,29
TMJ	245,29±85,95	274,62±105,55	**0,035**

Table 5.5. Comparison between affected and not affected side in JIA patients with monolateral involvement.

5.3 Discussion

Previous studies analyzed PPT in patients affected by JIA testing no orofacial sites; all these studies reported a lower pressure pain threshold in this group of patients compared to healthy subjects (Cornalissen et al., 2014; Leegaard et al., 2013) exploring finger, wrist, knee and ankle points.

The current study first analyzed PPT in masticatory muscles and TMJ in patients affected by JIA: our results were in agreement with those showed when other body sites were analyzed. We found a reduced PPT in the cranio-facial district comparing JIA patients to healthy subjects. These findings could confirm a role for central sensitization and nociceptive pathway plasticity in JIA (Munro and Singh-Grewal, 2013).

In this study, we also analyzed the influence of TMJ damage. For patients with only one side involved we found a significant difference between affected side and healthy side only for TMJ area ($p=0.035$) suggesting a further role of the inflammatory process in pain perception. This result supports the theory of Arabshai and Cron (Arabshai and Cron, 2006) which, despite the lack of clinical data related to TMJ, have found a prevalence of the involvement of TMJ, in patients suffering from JIA, high enough, so much to consider "the forgotten joint" one of the most involved. For this reason, children diagnosed with JIA should be subjected to early screening for TMJ arthritis by enhanced MRI.
According to previous studies, systemic rather than local inflammatory mediators modulate PPT (Alstergren P et al., 2008). Patients affected by rheumatoid arthritis reported a TMJ pressure pain threshold lower than healthy individuals (Fredriksson L et al., 2003) so it could be supposed a similar mechanism for patients affected by JIA.
Also comparing healthy subjects and JIA patients with bilateral TMJ involvement, it was found a significant difference in all examined sites. Healthy subjects reported a higher PPT.

In the comparison between patients affected by JIA with bilateral TMJ involvement and patients without TMJ involvement, no significant differences were found. Conversely, comparing healthy subjects and JIA patients without TMJ involvement it was found a significant difference in all analyzed sites. This is in agreement with the statement that children with JIA have a lower pain threshold even in areas not usually affected by arthritis (Leegaard et al., 2013). These data confirms the hypothesis that JIA alter the pain perception with reduction of PPT both in patients with or without TMJ involvement. Once again, our results could confirm the hypothesis of an alteration of central sensitization and nociceptive pathway plasticity in JIA (Munro and Singh-Grewal, 2013) reducing the importance of the articular involvement. Our data are also in agreement with what was

stated by Cornelissen et al. (Cornelissen et al., 2014), which is that JIA is associated with an increased pain sensitivity both thermal and mechanical, even in absence of pain reports, or markers of disease activity.

5.4 Conclusions

In conclusion, the present study showed a lower PPT in the orofacial areas in JIA patients compared to healthy subjects regardless of TMJ involvement; we could assume that it is the pathology itself to produce a central hypersensitivity cause of a reduced pain tolerance.

Future research will have to be directed towards understanding the mechanisms of an alteration of central sensitization and nociceptive pathway plasticity induced by JIA.

CHAPTER 6

General conclusions

The activity of masticatory muscles can be well evaluated by using anamnesis and clinical examination. Diagnostic instruments could improve the quantitative and qualitative assessment of these muscles. The evaluation of the electromyographic activity and the measurement of the pressure pain threshold of masticatory muscles could be identified as some of these diagnostic tools.

For the EMG assessment, from this works we can conclude that the asymmetry is an intrinsic characteristic of each subjects. TMD patients reported an asymmetric activity of masticatory muscles not different from healthy subjects. Similarly, comparing UPCB patients with normocclusion children no differences were found in the masticatory muscles activity in terms of muscular asymmetry also after the correction of UPCB.

EMG evaluation of masticatory muscles in children suffering from JIA confirmed the presence of asymmetric muscular activity in both JIA and healthy subjects with no significant differences.

PPT evaluation of masticatory muscles could be used to better understand the pain perception in patients affected by systemic disease. According to our findings, patients suffering from JIA showed a lower pain tolerance compared to healthy subjects. This could be due to a central hypersensitivity.

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