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TITLE

ASSOCIATION BETWEEN AWAKE ORAL PARAFUNCTIONAL BEHAVIORS AND TEMPOROMANDIBULAR DISORDERS

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Chapter I. Temporomandibular disorders: definition and etiology

Temporomandibular disorders (TMD) are a set of conditions concerning the temporomandibular joints (TMJ), the masticatory muscles or both and involve musculoskeletal pain, disturbances in the mandibular movement patterns, and/or impairment in functional movement (Liu F et al., 2013; Janal MN et al. 2008). They represent a very common public health problem affecting 5% to 12% of the population and they are the second most common musculoskeletal condition (after chronic low back pain) resulting in pain and disability (Schiffman E et al., 2014).

TMD-related pain is the main symptom driving treatment-seeking, because it can strongly affect daily activities, the psychosocial domain, and quality of life (John MT et al., 2007; Cioffi et al., 2014).

The etiology of TMD is multifactorial and still debated. Recent or past trauma, individual anatomic and neuromuscular abnormalities, biopsychosocial and neurobiological factors, and bruxism may contribute to their establishment (Green CS, 2001; Manfredini et al., 2010; Michelotti A et al., 2010; Melis M et al., 2014).

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) have been the most widely employed diagnostic protocol for TMD research since its publication in 1992. This classification system was based on the biopsychosocial model of pain that included an Axis I physical assessment, using reliable and well-operationalized diagnostic criteria, and an Axis II assessment of psychosocial status and pain-related disability. The intent was to simultaneously provide a physical diagnosis and identify other relevant characteristics of the patient that could influence the expression and thus management of their TMD. Indeed, the longer the pain persists, the greater the potential for emergence and amplification of cognitive, psychosocial, and behavioral risk factors, with resultant enhanced pain sensitivity, greater likelihood of additional pain persistence, and reduced probability of success from standard treatments. The RDC have been modified, elaborated and simplified during the time, through a validation project, and today their name has been converted into DC/TMD, Diagnostic Criteria for Temporomandibular disorders (Schiffman et al., 2014).

The new DC/TMD protocol is intended for use within any clinical setting and supports the full range of diagnostic activities from screening to definitive evaluation and diagnosis. The new protocol provides a common language for all clinicians while providing the researcher with the methods for valid phenotyping of their subjects—especially for pain-related TMD. Moreover, although the validity data identifies the need for imaging to obtain a definitive TMJ-related diagnosis, imaging should not be used routinely but rather considered when it is important to a specific patient. The Axis II screeners provide the clinician with an easy method to screen for pain intensity, psychosocial distress, and pain-related disability for triaging, treatment planning, and estimating the patient's prognosis. The additional Axis II instruments, a core part of all TMD assessments, provide the clinician and researcher with current methods to further assess the status of the individual regarding multiple factors relevant to pain management. The new DC/TMD protocol is a necessary step toward the ultimate goal of developing a mechanism and etiology-based DC/TMD that will more accurately direct clinicians in providing personalized care for their patients. (Schiffman et al., 2014).

Chapter II. Oral parafunctions

II.1 Oral parafunctions: definition and diagnostic tools

The term "oral parafunctions" comprises a group of oral habits and jaw movements that differ from physiological functional needs such as mastication, communication, swallowing or breathing (Van der Meulen MJ et al., 2006; Ohrbach R et al., 2008; Lobbezoo F et al., 2013). The most commonly studied oral parafunction is bruxism. It consists of masticatory muscle activities that occur during sleep (characterized as rhythmic or non-rhythmic) and wakefulness (characterized by repetitive or sustained tooth contact and/or by bracing or thrusting of the mandible) respectively (Lobbezoo F et al., 2013). They include also other activities that encompass excursive positioning, gum chewing, /lip/cheek/objects biting, tongue and they could be considered as adverse behaviors because of their detrimental effects on teeth, temporomandibular joints and jaw muscles (Winocur E et al., 2006; Michelotti A et al., 2010; Koutris M et al., 2013). Detecting waking-state oral behaviors in the natural environment may be challenging because these behaviors are mostly unobservable and occur outside awareness. According to a recent international consensus, methods for assessing wake-time oral parafunctions can be distinguished as instrumental or non-instrumental (Lobbezoo F et al., 2018). Among instrumental approaches there are the surface electromyography (EMG) and ecological momentary assessment (EMA). Non-instrumental approaches include questionnaires and checklists completed from the patient.

II.2 Instrumental approaches

As mentioned in the previous paragraph, objective recordings to detect wake-time oral behaviors include instrumental approaches based on electromyography (EMG) (Ohrbach R et al., 2009; Cioffi I et al., 2017) and ecological momentary assessment (EMA) (Michelotti A et al., 2012; Chen et al., 2014; Glaros et al., 2014; Kaplan et al., 2016).

- Thanks to novel technical advances, **surface electromyography (sEMG)** has become an objective, reliable, and non-invasive technique for evaluating the extent and duration of muscle activity (Castroflorio T et al.,2008).

  In controlled experimental conditions, EMG has been shown to be a powerful tool for the clinical evaluation of the jaw elevators, to detect muscle hyper and hypo function, rest position, and fatigue (Hugger S et al.,1998), and to distinguish between functional and non-functional oral behaviors (Gallo LM et al.,1998).
- **The EMA (Ecological Momentary Assessment)** is an electronic diary method in order to acquire momentary self-reports of oral parafunctional behaviors (Schiffman S, 2000). Differently from retrospective self-reports that are collected during clinic visits, which are limited by recall bias and are not well suited to address how behavior changes over time and across contexts, the EMA involves repeated sampling of subjects' current behaviors and experiences in real time, in subjects' natural environments. EMA aims to minimize recall bias, maximize ecological validity, and allow study of microprocesses that influence

behavior in real-world contexts. Interestingly, EMA studies assess particular events in subjects' lives or assess subjects at periodic intervals, often by random time sampling, using technologies ranging from written diaries and telephones to electronic diaries and physiological sensors. Hence, this tool holds the promise to advance the science and practice of clinical psychology by shedding light on the dynamics of behavior in real-world settings (Schiffman S et al., 2008).

II.3 Non-instrumental approaches

Non-instrumental approaches to detect wake-time oral behaviors include the oral history, the clinical inspection (Svensson P et al., 2016) and the compilation of self-reports (questionnaires and checklists) assessing the frequency and the extent of self-reported oral behaviors (Michelotti A et al., 2012; Kaplan SE et al., 2016; Cioffi I et al., 2017).

- **Clinical features of oral parafunctions**, in particular both awake and sleep bruxism include the presence of masticatory muscle hypertrophy and indentations on the tongue or lip and/or a linea alba on the inner cheek. However, these signs can also be consequences of functional oromotor activity, such as swallowing (Takagi I et al., 2003). Damage to the dental hard tissues (eg, cracked teeth), repetitive failures of restorative work/prosthodontic constructions, or mechanical wear of the teeth (ie, attrition) may also be indicators of awake bruxism and sleep bruxism. However, although attrition may be indicative of (especially) sleep bruxism, it does not rule out past sleep bruxism without current activity.
- **Self-reported assessment** of sleep or awake oral parafunctions continues to be the primary tool in research and clinical practice. However, a noteworthy limitation is that the complex bruxism-psyche relationship could actually drive self-reporting of the condition and so self-report might reflect distress of the subject rather than actual masticatory muscle activity. Therefore, improvement

of self-reporting to enhance reliability and validity compared to instrumental measures should be conduced.

- Among self-reported checklists, the **Oral Behaviors Checklist (OBC)** (Markiewiecz MR et al., 2006) is an instrument widely used in research and clinical settings. It is a 21-item self-reporting questionnaire, quantifying the frequency of observable and non-observable oral behaviors (e.g. clenching, grinding, chewing gum, holding objects etc.). The frequency of these behaviors can be scored by asking to the patient of choosing 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. A total score of 0 suggests no reported parafunctions, scores ranging from 1 to 24 and from 25 to 84 denote low and high parafunctions respectively, as indicated by the scoring manual for self-Report Instruments of Diagnostic Criteria for Temporomandibular Disorders (DC/TMD).

  The construct validity of the original English version of the OBC has been successfully verified for most of its items against EMG (Markiewiecz MR et al., 2006; Ohrbach el al., 2008). Moreover, the OBC has been included in the newly recommended Diagnostic Criteria for TMD (DC/TMD) as a screening tool, because of the known contribution of oral behaviors to TMD (Schiffman E et al., 2014). A Dutch version of the OBC has also been validated recently (van der Meulen MJ et al., 2014).

- In addition to the OBC (total score), **a reduced 6-items version (OBC-6)** (Michelotti et al., 2012; Cioffi I et al., 2016; Cioffi, et al., 2017), has been analysed in several studies. The rationale for testing this scale is that it includes six items referring to tooth clenching related wake time oral behaviors implying pressure against soft tissues, objects, or teeth (tooth clenching), while all other items do not (e.g., sustained talking, yawning, hold telephone between the head and shoulders, etc.). The OBC-6 score is computed by summing the scores of 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, fingernails, etc"), and 13 ("use chewing gum"). A previous study found a correlation between oral activity episodes, observed through EMG, and those observed through the OBC-It 6, suggesting that it could be a sensitive tool to detect daytime tooth clenching episodes (Cioffi I, et al, 2017).

A translation of the OBC from the English source language to Italian has been perfomermed. The process involved forward-translation, back translation, review, and cultural adaptation into Italian language in accordance to the Guidelines for Translation and Culture Equivalency proposed by the INFORM (International Network of Orofacial Pain and Related Methodology), a scientific group of the International Association For Dental Research, IADR (Ohrbach R et al., 2017). The following study, published recently from our group (University Federico II of Naples) proved the reliability of the Italian version of this tool, defined as OBC-It (Total score) and OBC-It 6.

II.4 Analysis of the reliability of the Italian Version of the Oral Behaviors Checklist and the relationship between oral behaviors and trait anxiety in healthy individuals. *J Oral Rehabil; 2018.*

II.4.1 Abstract

Background: The Oral Behaviors checklist (OBC) is a valid 21-item instrument quantifying the self-reported frequency of oral behaviors. An Italian version (OBC-It) has been released recently. It is also known that anxiety and oral behaviors are known to be associated in individuals with orofacial pain due to temporomandibular disorders (TMD). However, information about this relationship in pain-free individuals is still limited.

Objectives: The aim of this study was to test the reliability of the OBC-It and its reduced version (OBC-It 6), focusing on tooth clenching related wake time oral behaviors, and the effect of patient instructions on reliability. A second aim was to test the association between trait anxiety and oral behaviors in pain-free individuals.

Methods: 282 TMD-free students, divided in two groups (Group A, n=139, mean age±SD = 22.6±5.4 years; group B, n=143, 23.7±4.2 years), filled in the State-Trait-Anxiety Inventory and the OBC-It. Group B received instructions about the OBC-It, while Group A did not. After two weeks (T1), both groups filled in the OBC-It again. However, group B was further divided in two subgroups, B1 and B2. The first received the same instructions again, while B2 did not.

Results: The test-retest reliability of the OBC-It (A: ICC=.87, B1: ICC=.94; B2: ICC=.95) and OBC-It 6 (A: ICC=.85, B1: ICC=.89, B2: ICC=.93) was excellent in all

groups. Trait anxiety was weakly associated with OBC-It only in females ($R2=.043$, $P=.021$).

Conclusions: The OBC-It is a reliable tool but further subjects' instructions may be needed. Trait anxiety has a limited effect on oral behaviors in TMD-free subjects.

II.4.2 Introduction

Oral parafunctional behaviors are adverse behaviors because of their detrimental effects on teeth, temporomandibular joints and jaw muscles. Among questionnaires, used for their detection, the OBC is a very commonly used self-reported checklist. However, assessing patients' oral behaviors in a reliable and valid way should be of great importance for clinicians in the management and reduction of those behaviors.

The primary aim of this study was to test the reliability of the Oral Behaviors Checklist Italian version (OBC-It) and its reduced version (OBC-It 6), which focuses on tooth clenching related wake time oral behaviors, and the effect of patient instructions on its reliability. A second aim was to test the association between trait anxiety and oral behaviors in TMD pain-free individuals.

II.4.3 Materials and methods

Study participants

The study sample included medical students recruited at the University of Naples, Federico II, Italy. Both a flyer and word of mouth were used to enroll participants.

Those willing to participate in this research signed an informed consent and were asked 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 since concurrent orofacial TMD-like pain has been reported to affect the frequency of oral parafunctional behaviors (Cioffi I et al., 2016). From an initial pool of 364 subjects screened, 82 were excluded because of concurrent pain in the jaw or temple area. The final sample included 282 subjects (156 males, 126 females; mean age ± SD = 23.1 ± 4.9 years).

Procedure

Participants were invited to fill in the OBC-It and the State-Trait-Anxiety Inventory (STAI; form Y) (Spielberger CD et al., 2004).

- **The OBC-It** includes two items regarding nocturnal bruxism and nineteen items investigating the frequency of different wake-time oral behaviors in the last 30 days. Each participant was asked to score the frequency of these behaviors by choosing among the five response options. In addition to the OBC-It (total score), the reduced 6-items version (OBC-It 6) (Michelotti et al., 2012; Cioffi I et al., 2016; Cioffi, Michelotti et al., 2016), was analyzed.
- **The State-Trait Anxiety Inventory (STAI, form Y)** is a self-administering 4-point scale, including two 20-item lists measuring state anxiety (S-Anxiety) and trait anxiety (T-Anxiety). S-Anxiety refers to transitory psychobiological emotional state of tension and nervousness that varies in intensity over time. T-

Anxiety refers to a relatively stable anxiety proneness as a personality trait. Total score ranges from 20 to 80 (Spielberger CD et al., 2004).

All participants (n=282) were allocated into two groups, i.e. group A including 139 subjects (81 males and 58 females, mean age ± SD = 22.6 ± 5.4 years) and group B including 143 subjects (74 males and 69 females, 23.7 ± 4.2 years). The allocation of participants to groups was randomly generated using a custom-made java script. At baseline (T0) both groups completed the STAI (T-Anxiety) and the OBC-It. Group A was asked to answer based only on his/her understanding of the OBC-It items, while group B received additional instructions about the constructs of the checklist by means of a computer based presentation and a standardized verbal explanation (provided by VD) before the completion. After two weeks (T1), group A was invited to fill in the OBC-It again, and group B was divided in two subgroups, B1 and B2. Group B1 (n=72) received again the same instructions, while group B2 (n=71) did not (Fig. 1).

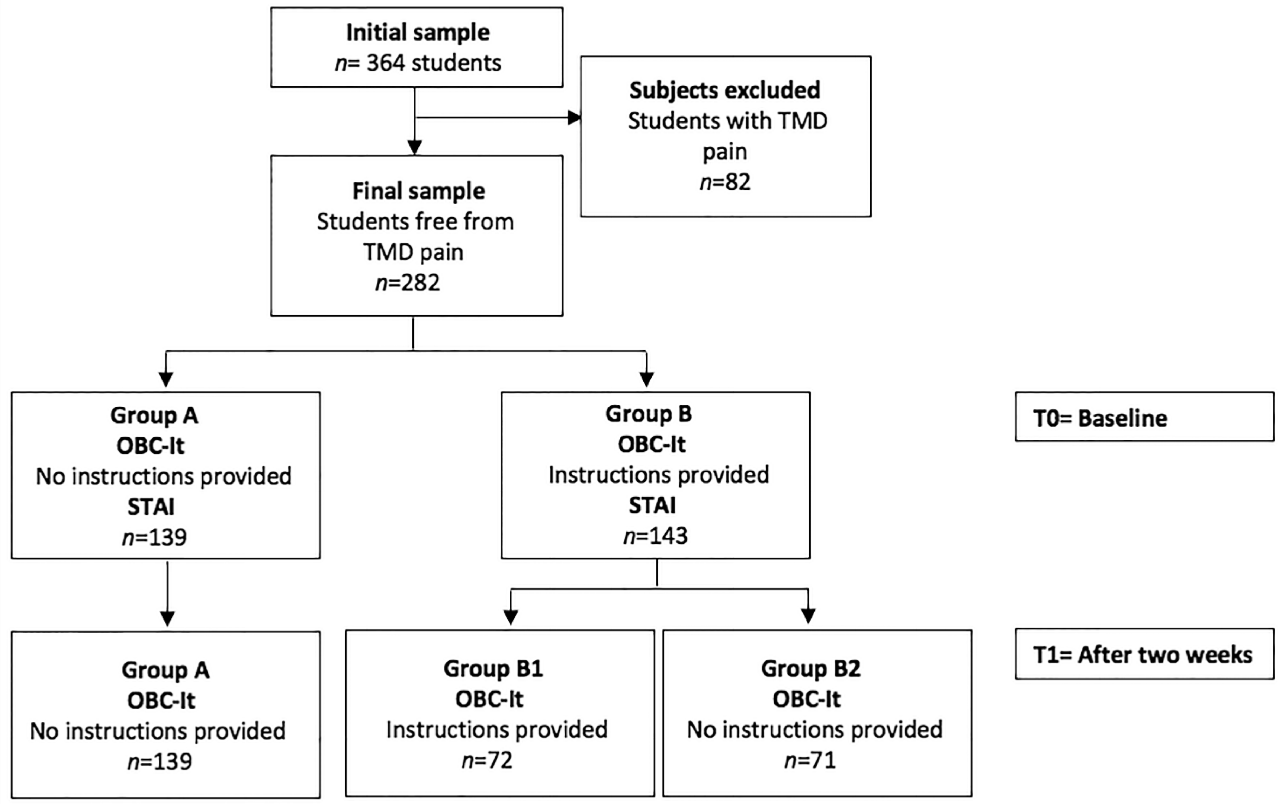

Figure 1. Study design: recruitment of the sample and allocation of participants to groups A, B1 and B2.

Study participants received no financial compensation for participation and were assured that they could leave the study at any time. They were informed about the scopes of the study and gave written consent before enrolment. 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 166/17).

Data analysis

Intra-class correlation coefficients (ICCs) were computed to measure the reliability of the single items included in the OBC-It, total OBC-It score and partial score (OBC-It 6) in all groups. The ICCs values were interpreted according to Fleiss (27): ICC <.40 = poor reliability; ICC ≥.40 but ≤.75 = fair to good reliability; ICC >.75 = excellent reliability. Pairwise between groups comparisons in ICCs were tested after Fisher's z transformation.

Normality of T-Anxiety and OBC-It and OBC-It 6 was tested using the Kolmogorov-Smirnov test. The Kruskall-Wallis test was used to test between groups differences in T-Anxiety and OBC-It 6 at T0-baseline (data not normally distributed, all P<.001), while ANOVA was used for OBC-It (data normally distributed, P=.200).

A linear regression statistical analysis was used to test the association between OBC-It and OBC-It 6 (dependent variables) and T-Anxiety and gender (independent variables). Data were analyzed with SPSS (IBM) Ver. 24. The statistical significance was set at P<.05.

II.4.4 Results

At baseline (T0), T-Anxiety was similar between groups (median value [IQR], Group A: 45.0 [5.0]; Group B1: 45.0 [6.0]; Group B2: 45.0 [5.0]; P=.929). This was the case also for OBC-It 6 (total score) (Group A: 14.0 [4.0]; Group B1: 14.0 [4.0]; Group B2:

14.0 [4.0]; P=.241) and OBC-It (total score) (mean ± SD; Group A: 50.7 ± 8.1; Group B1: 50.9 ± 8.7; Group B2: 49.0 ± 8.7; P=.315).

The computed ICCs for all groups are reported in table 1. In Group A, the ICC for the OBC-It (total score) was .87 (P<.001), showing an "excellent" reliability of the checklist. The ICCs for the single constructs ranged between .66 (Item 11, "Hold jaw in rigid or tense position, such as to brace or protect the jaw" and Item 20, "yawning") to .90 (Item 19, "singing"). All the ICCs showed "excellent" reliability of the constructs, with the exception of "Press, touch, or hold teeth together other than while eating (that is, contact between upper and lower teeth)" (item 5, ICC=.74), "Hold or put jaw forward or to the side" (item 7, ICC=.75), "Hold jaw in rigid or tense position, such as to brace or protect the jaw" (item 11, ICC=.66), "Lean with your hand on the jaw, such as cupping or resting the chin in the hand" (item 15, ICC=.68), and "yawning" (item 20, ICC=.66), which showed a "fair to good" reliability.

In group B1, the ICC for OBC -It (total score) was .94 (P<.001). Also in this case the reliability was "excellent" and slightly greater than group A (P=.006). The ICCs for the single constructs ranged between .61 (Item 11, "Hold jaw in rigid or tense position, such as to brace or protect the jaw") and .97 (item 14, "Play musical instrument that involves use of mouth or jaw (for example, woodwinds, brass, string instruments"). All the ICCs showed "excellent" reliability of the constructs, with the exception of "Hold or put jaw forward or to the side" (item 7, ICC=.74), "Hold jaw in rigid or tense position, such as to brace or protect the jaw" (item 11, ICC=.61), which showed "fair to good" reliability.

In Group B2, the ICC for OBC-It (total score) was .95 (P<.001) and was greater than group A (P<.001). The ICCs for the single constructs ranged between .67 (Item 15, “Lean with your hand on the jaw, such as cupping or resting the chin in the hand.”) and .95 (Item 1, “Clench or grind teeth when asleep, based on any information you may have”). All the ICCs showed “excellent” reliability of the constructs, with the exception of “Press, touch, or hold teeth together other than while eating (that is, contact between upper and lower teeth)” (item 5, ICC=.75) and “Lean with your hand on the jaw, such as cupping or resting the chin in the hand” (item 15, ICC=.67) which had a “fair to good” reliability. The ICCs for OBC-It 6 (total score) resulted “excellent” in all groups, and significantly greater in group B2 than A (P=.006).

Item	Group A	Group B1	Group B2
	ICC (T_0 vs T_1)* (95% CI)	ICC (T_0 vs T_1)* (95% CI)	ICC (T_0 vs T_1)* (95% CI)
Single items scores			
1 Clench or grind teeth when asleep, based on any information you may have	.87^{A} (.81-.90)	.89^{B} (.82-.93)	.95^{AB} (.79-.92)
2 Sleep in a position that puts pressure on the jaw (for example, on stomach, on the side)	.83^{A} (.75-.87)	.94 ^{AB} (.91-0.96)	.85^{B} (.75-.90)
3 Grind teeth together during waking hours	.80^{A} (.72-.85)	.95^{AB} (.92-.97)	.84^{B} (.73-.89)
4 Clench teeth together during waking hours	.85 (.79-.89)	.87 (.79-.92)	.91 (.86-.94)
5 Press, touch, or hold teeth together other than while eating (that is, contact between upper and lower teeth)	.74^{A} (.64-.81)	.87^{AB} (.79-.91)	.75^{B} (.60-.84)
6 Hold, tighten, or tense muscles without clenching or bringing teeth together	.88^{A} (.83-0.91)	.77^{A} (.63-.85)	.84 (.75-.90)
7 Hold or put jaw forward or to the side	.75 (.65-.82)	.74 (.58-.83)	.82 (.70-.88)
8 Press tongue forcibly against teeth	.85 (.79-.89)	.86 (.78-.91)	.87 (.79-.92)
9 Place tongue between teeth	.84 (.77-.88)	.89 (.81-.93)	.90 (.83-.93)
10 Bite, chew, or play with your tongue, cheeks or lips	.82 (.74-.87)	.87 (.79-.92)	.87 (.79-.92)
11 Hold jaw in rigid or tense position, such as to brace or protect the jaw	.66 (.49-.76)	.61^{A} (.37-.76)	.79^{A} (.65-.86)
12 Hold between the teeth or bite objects such as hair, pipe,	.82^{AB}	.90^{A}	.92$^{\mathbf{B}}$

pencil, pens, fingers, fingernails, etc	(.74-0.87)	(.83-0.93)	(.87-0.95)
13 Use chewing gum	0.88 (.83-.91)	.88 (.80-.92)	.88 (.81-0.92)
14 Play musical instrument that involves use of mouth or jaw (for example, woodwinds, brass, string instruments)	.79[A] (.70-.84)	.97[AB] (.94-.97)	.79[B] (.66-.87)
15 Lean with your hand on the jaw, such as cupping or resting the chin in the hand	.68 (.56-.77)	.80 (.68-.87)	.67 (.46-.79)
16 Chew food on one side only	.87 (.81-.90)	.82 (.71-.88)	.90 (.84-.93)
17 Eating between meals (i.e. food that requires chewing)	.76[AB] (.66-.82)	.86[A] (.78-.91)	.90[B] (.84-.93)
18 Sustained talking (for example, teaching, sales, customer service)	.83[A] (.75-.87)	.88[B] (.80-0.92)	.94[AB] (.89-.96)
19 Singing	.90 (.86-.93)	.90 (.84-.93)	.94 (.90-.96)
20 Yawning	.66[AB] (.52-.75)	.89[A] (.82-.93)	.83[B] (.72-.89)
21 Hold telephone between your head and shoulders	.88 (.83-.91)	.81 (.68-.87)	.85 (.75-.90)
Total scores			
OBC-It (total score)	.87[AB] (.81-.90)	.94[A] (.89-.96)	.95[B] (.91-.96)
OBC-It 6 (total score)	.85[A] (.79-0.89)	.89 (.81-0.92)	.93[A] (.88-.95)

Table 1. Computed ICCs between different time points, at baseline (T0) and after 2 weeks (T1) for group A (n=139), B1 (n=72) and B2 (n=71).

Trait anxiety was significantly but weakly associated with OBC-It (total score) [F(1, 123)=5.470, P=.021, R2=.043] in females. A unit increase in T-Anxiety predicted an increase in OBC-It (total score) of .420 (Unstandardized B coefficient). No significant association was found in males (P=.172). OBC-It 6 (total score) was not associated with T-Anxiety neither in females (P=.092) nor males (P=.128)

II.4.5 Discussion

The present study examined the reliability of the constructs of the Italian version of the Oral Behaviors Checklist. The reliability of the OBC-It (total score) was "excellent" (ICC=.87). The current findings are consistent with similar studies testing the English (ICC=.88) (15) and the Dutch version (ICC=.86) of the OBC (van der Meulen MJ et al., 2014). Moreover, the semantic validity of ten oral parafunctions listed in the English OBC, using EMG devices, was proved in previous studies (Markiewiecz MR et al., 2006; Ohrbach el al., 2008), suggesting that the OBC is well understood by English speakers. In this study, it was tested whether supplemental instructions about the OBC-It constructs could improve the reliability of the overall checklist score and its items, differently from other studies (Ohrbach el al., 2008; Kaplan et al., 2016; van der Meulen MJ et al., 2014). Interestingly, the ICCs of the items 1, 2, 3, 5, 12, 14, 17, 18, 20 were excellent and higher in the groups B1 and B2, who received standardized supplemental instructions, than group A (Table 1). This suggests the importance of giving instructions for the correct completion of the checklist in both clinical and research settings.

Minimal significant differences in ICCs results between group B1 and B2 (Table 1) were found, showing that a single explanation of the constructs may be sufficient to ensure a proper understanding of the OBC-It constructs.

Single constructs presented reliability values ranging from “fair to good” to “excellent” in all groups. The computed ICCs for the item 5 “Press, touch, or hold teeth together other than while eating (that is, contact between upper and lower teeth)” ranged from “fair to good” in group A and B2 to “excellent” in group B1, indicating a limited understanding of the construct and the need of a reinforced explanation to make its meaning clearer. On the contrary, the ICC of item 11 “Hold jaw in rigid or tense position, such as to brace or protect the jaw” was “excellent” in group B2 and decreased to “fair to good” in group B1, possibly showing a difficult understanding of the constructs, despite subjects were helped with additional instructions about its correct interpretation.

Also item 20 “yawning” showed variable ICCs results that cannot be attributed to a wrong interpretation of the construct but possibly to the fact that this behavior generally fluctuates across days. Differently, items 4, 7, 8, 9, 10, 13, 15, 16 and 19, showed “excellent” reliability values which did not differ between groups, suggesting these are clear constructs and are usually not misinterpreted.

The reduced 6-item version (OBC-It 6) of the checklist was also examined. A correlation between oral activity episodes, observed through EMG, and those observed through the OBC-It 6 was previously found, suggesting that it could be a sensitive tool to detect daytime tooth clenching episodes (Cioffi I, et al, 2017). Moreover, in the present study, the OBC-It 6 showed “excellent” ICCs values that were similar to those obtained for the OBC-It. Therefore, this shorter tool could be used in the future both in research and in clinical setting as a useful screening to detect wake time oral parafunctions.

Associations between oral parafunctional behaviors and trait anxiety have been observed in some investigations (Michelotti et al., 2010; Cioffi I, Michelotti A et al., 2016). Since OBC constructs do not depict a current state but assess the self-reported frequency of oral activities over the preceding month, we tested its association only with trait anxiety, which describes a stable personality characteristic rather than a temporary feeling. According to our previous findings, trait anxiety plays a role in influencing the intensity and frequency of clenching episodes, as measured by EMG (Cioffi et al., 2017). Similarly, our current findings showed that the frequency of parafunctions, as detected from both the OBC-It and T-Anxiety in the total sample, were weakly but significantly associated only in females. This may be in part related to the recruitment of a sample without pain. In fact, it is known that patients with chronic TMD pain generally report higher levels of mood disorders (e.g. depression) than healthy subjects who often refer lower levels of emotional distress (Manfredini et al., 2004). Moreover, oral behaviors with tooth contact and muscle tension occur in people with painful TMD at significantly higher frequency than in subjects without TMD pain (Glaros et al., 2005). In the light of our findings it could be interesting to evaluate the association between trait anxiety and oral behaviors in subjects affected from TMD pain and to compare them with a control group of pain-free subjects.

This study presents several strengths. First, the large sample size and the great number of questionnaires analyzed (282) ensure a good precision of the estimates. Second, the fact that participants were free from TMD pain prevented from any possible influence on the frequency of oral behaviors and on data analysis. Another strength is that the Italian translation of the OBC has been performed through published standards procedures (Ohrbach et al., 2017). Finally, this is the first attempt to test the effects of supplemental instructions on the reliability of OBC measurements.

The present study has also some limitations. First, as students of a medical field, participants were aware about the possible contributing effect of oral parafunctional activities to TMD. Also, due to their level of education, they could discretely interpret information written in a test. Hence, our sample and results may be not representative of the whole population. Second, we did not record any activity that may have affected masticatory muscles and parafunctional activities over time (e.g. medications, stress, exercising etc.). In addition, it might be argued that the OBC refers to oral behaviors performed during the month prior to the time of the completion of the checklist, therefore it could not take into account any fluctuation of the incidence of parafunctional activities. However, a direct and linear relationship between responses to the OBC and in field assessment, through EMA, has been showed for each of the constructs, therefore supporting the validity of this retrospective self-report assessment (Kaplan et al., 2016). Finally, while the OBC is a screening questionnaire included in the Axis II instruments of the DC/TMD (Schiffman et al., 2014), the STAI is not. Hence, it would be advisable for future studies evaluating anxiety disorders to use also other questionnaires, such as the General Anxiety Disorder questionnaire (GAD-7) (Löwe B et al., 2008), which is included in the DC/TMD as a measure of emotional functioning (Schiffman et al., 2014).

II.4.6 Conclusion

This study has shown that the OBC-It and its reduced version, OBC-It 6, are highly reliable. Moreover, the reliability is increased by giving supplemental information. Therefore, it would be advisable to develop standardized instructions that clinicians should provide to patients for a better comprehension of some items included in the checklist. Finally, the present study has found that trait anxiety is weakly related to the frequency of oral behaviors in pain-free individuals.

Chapter III. Relationship between awake oral parafunctional behaviors and temporomandibular disorders.

Daytime clenching, i.e., awake bruxism, continues to be the subject of intense discussions within the dental community for its possible relation with TMD pain (Christensen LV et al., 1981, Ohrbach et al., 2013). Experimental sustained low-level tooth clenching has been shown to induce soreness in elevator jaw muscles in healthy subjects (Farella M et al., 2010; Glaros AG et al., 2012). A significant association between daytime clenching and myofascial pain (MP) of the masticatory muscles was demonstrated by self-reports (Huang GJ et al., 2002; Ohrbach et al., 2008; Michelotti et al., 2010; Melis M et al., 2014) and by objective recordings (Chen CY et al., 2007; Michelotti A et al., 2012; Cioffi et al., 2015).

Finally, the contributing role of oral parafunctions to the onset of TMD has been further supported recently by a large-scale prospective cohort study (Ohrbach et al., 2013) and by the significant reduction of pain symptoms after habit reversal treatment (Glaros et al., 2007). Nonetheless, a number of studies have shown (Velly AM et al., 2003) the absence of clinically relevant relationships between different types of self-reported parafunctions, including daytime clenching, and TMD-pain complaints (van der Meulen et al., 2006) and the lack of a correlation with facial pain intensity (van der Meulen et al., 2014).

Also, other studies, using tooth wear as an indicator for longterm parafunctional behaviors, failed to find a clinically relevant dose–response relationship between clenching and TMD pain (Hirsch C et al., 2004; Schierz O et al., 2007). These controversial findings have mainly been related to the technical difficulty in identifying the presence of waking-state oral parafunctions in the natural environment because people are often unaware of their oral habits (Ohrbach et al., 2008). Hence, objective and more reliable measurements based on electromyographic assessments should be collected to confirm or deny the possible relation between daytime clenching and TMD pain. Therefore, we conduced with our group two researches to study more in detail this topic. The first one (III.1) aimed at investigating the relationship between oral behaviors and TMD through the use of objective methods (sEMG) and the second one through the use of a subjective method (III.2) for the assessment of oral behaviors, such as the compilation of a self-report, the Oral Behavior Checklist (OBC).

III.1 Frequency of daytime tooth clenching episodes in individuals affected by masticatory muscle pain and pain-free controls during standardized ability tasks; *Clin Oral Investig. 2017*.

III.1.1 Abstract

Objectives: Tooth clenching has been suggested to be related to temporomandibular pain. However, the electromyographic characteristics of daytime clenching episodes have been minimally investigated. This study aimed to analyze the frequency, amplitude, and duration of daytime clenching episodes in patients with masticatory muscle pain and pain-free individuals.

Methods: Fifteen women with masticatory muscles myalgia (MP group, mean ± SD age = 26.4 ± 7.6 years) matched for age to 18 pain-free women (CTR group, mean ± SD age = 25.3 ± 2.8 years) were submitted to three different ability tasks (filling out questionnaires for 40 min, reading for 20 min, and playing a videogame for 20 min). The electromyographic activity periods (AP) of the right masseter greater than 10 % (AP10), 20 % (AP20), and 30 % (AP30) of the maximum voluntary contraction were analyzed.

Results: The mean frequencies of AP10, AP20, and AP30 were greater in MP than in CTR individuals (all $p < 0.05$). The mean duration of AP10 was higher in MP group than CTR group only while filling out the questionnaires ($p = 0.0033$). CTR group had an increased frequency and duration of AP10 while playing the videogame than while reading a magazine. The ability tasks did not affect the muscle activity in the MP group.

Conclusions: Individuals with masticatory muscle pain have an increased frequency of both high and low-intense daytime clenching episodes. The type of ability task affects the frequency and the duration of clenching episodes only in painfree individuals.

Clinical relevance: Clinicians should recognize that the frequency and intensity of daytime clenching are noticeably increased in individuals with masticatory muscle pain in order to better tailor treatment.

III.1.2 Introduction

It is known that an association between diurnal parafunction and TMD development exists. However, objective and more reliable measurements based on electromyographic assessments should be collected to confirm or deny the possible relation between daytime clenching and TMD pain. Currently, quantitative and/or qualitative information about the characteristics of daytime clenching episodes are limited (Fujisawa M et al., 2013; Manfredini et al., 2014), and it is not known whether the characteristics of clenching episodes (e.g., frequency, amplitude, and duration) differ between healthy and TMD individuals. Also, it is not clear whether and how certain mental ability tasks affect the frequency of daytime clenching episodes.

The aim of the present study was to assess the frequency, amplitude, and duration of daytime clenching episodes in TMD patients affected with masticatory muscle pain and to compare them to a control group of pain-free individuals while performing standardized mental ability tasks. It was hypothesized that (1) the frequency, amplitude, and duration of daytime clenching episodes differ significantly between TMD and pain-free individuals and that (2) the ability tasks affect the frequency of daytime clenching in both the groups.

III.1.3 Materials and methods

Study participants

The target population was composed of women aged >18 years seeking for a TMD consultation at the Department of Neurosciences, section of Temporomandibular disorders, at the University of Naples, Federico II, Italy. A preliminary screening was performed according to a modified version of the questionnaire TMD-Pain screener (Gonzalez et al., 2011) (question #1—Bin the last 30 days, how long did any pain last in your jaw or temple area on either side? No pain, pain comes and goes, pain is always present) including a 0–100 mm visual analog scale (VAS) (Miller MD et al., 1993), where 0 is the lowest pain and 100 the worst pain ever. Individuals who reported to have current pain in the jaw or temple area ≥ 30mmwere considered eligible for the study. A preliminary TMD investigation of these subjects was performed by a single examiner (AM) according to the DC/TMD (Schiffman et al., 2014). Individuals who presented a DC group I diagnosis (myalgia, myofascial pain, myofascial pain with referral) were informed about the possibility of participating in the research, and that this could require about 2 h of their time. Those ones who were willing to participate were included in the study.

Exclusion criteria included wearing extended dental fixed or removable prostheses (equal or greater than three teeth), ongoing orthodontic or dental treatment, neurological disorders, habitual intake of drugs affecting the central nervous system or anti-inflammatory drugs, and/or migraine diagnosis at the moment of screening. Concurrent joint click was not considered as further exclusion criterion.

From an initial pool of 40 subjects screened, 18 women suffering from masticatory muscle pain (MP group) were recruited and matched for age to a control group composed of 18 TMD-free individuals (CTR group). The CTR group was recruited

in the same period from among individuals accompanying orthodontic patients. The inclusion criterion was the absence of TMD diagnosis according to DC/TMD (Schiffman et al., 2014).

Exclusion criteria were similar to those used for the MPgroup. Three subjects of the MP group dropped out due to technical reasons. Thus, the final study sample was made of 15 women suffering from masticatory muscle pain (MP group, mean ± SD age = 26.4 ± 7.6 years) matched for age to a control group composed of 18 pain-free individuals (CTR group, mean ± SD age = 25.3 ± 2.8 years). All patients recruited for the study were screened and examined before the experimental phase. Information concerning their diagnosis was immediately provided. They were also told that the treatment options were conservative. On the other hand, specific treatment modalities including strategies for reducing the frequency of oral habits were discussed only after the experimental phase.

Questionnaires

Each subject was asked to complete a set of questionnaires at the beginning of the study. Both groups filled in the Oral Behavior Checklist (Markiewicz MR et al., 2006), the State–Trait Anxiety Inventory (STAI) (Spielberg CD et al., 1970), and the Somatosensory Amplification Scale (SSAS) (Barsky AJ et al., 1988). The MP group was asked to fill in the DC/TMD symptom questionnaire (Schiffman et al., 2014). The CTR group was asked also to reply to an additional questionnaire concerning general health and employment status. For clinical purposes, patients were also asked to complete the Graded Chronic Pain (GCP) questionnaire of the Research Diagnostic Criteria for Temporomandibular disorders (RDC/ TMD) (Dworkin SF et al., 1992)

Pressure pain thresholds

Pressure pain thresholds (PPTs) were assessed with an electronic algometer (Somedic, Sweden) equipped with a rubber tip (surface area 1 cm2) in order to assess participants' sensitivity to pain. The device was positioned perpendicular to the skin at the selected site and the pressure was increased at 30 kPa/sec by using a visual feedback. The PPT was determined as the point at which the pressure stimulus changed from a sensation of pressure into a sensation of pain (Ohrbach R et al., 1989). The subject indicated the PPT by pressing a button, which froze the current pressure value on the digital display. The procedure was explained to the subject who was asked to keep the muscles relaxed during the measurements. PPTs were assessed by a single-blind examiner (VD). All measurements were taken at three locations on both right and left side. The measurement sites were selected on each muscle as follows. For the masseter muscle, the site was located midway between the origin and insertion, 1 cm posterior to its anterior boundary. For the temporalis muscle, the site was located on the line from the top edge of the eyebrow to the highest point of the pinna of the ear, 2 cm behind the anterior margin of the muscle as determined by palpating the muscle during voluntary contraction. For the thenar muscle, measurements were made on the skin of the palmar side, on the thenar eminence. The measurements were repeated for a total of four trials at each muscle, with a 1-min interval between trials. The order of measurements was randomized across subjects. While assessing the PPT at masticatory muscles locations, the subject's head was supported by counter pressure from the opposite hand of the operator. PPTs at thenars were measured with hands flat on the table (Fig.2).

Figure 2. Measurement of the Pressure Pain Thresholds at the temporalis muscle (left) and masseter (right)

Surface EMG recording

A portable EMG device (Deregibus A et al., 2014) (Bruxoff ®, Orthorizon, Torino, Italy) was used to acquire EMG signals at the right masseter muscle. The reference electrode was placed on the middle point of the clavicle. Disposable bipolar self-adhesive concentric electrodes (Code® 2.0, Spes Medica, Genova, Italy) with a radius of 2 cm and a silver/silver chloride surface were used. The concentric ring systems of the electrodes show higher spatial selectivity with respect to the traditional detection systems and reduce the problem of electrode location because they are insensitive to rotations and reduce EMG cross talk. The electrode was placed along a line going from the mandibular angle to the cantus, about 20 mm above the mandibular angle (Castroflorio T et al., 2005), and recording was performed 5–6 min later (Fig.3). Before electrode placement, the skin was cleaned and slightly abraded with an abrasive gel (Everi - Spes Medica, Genova, Italy) to diminish impedance, allowing the conductive paste to adequately moisten the skin surface. Maximum voluntary contraction (MVC) in maximum intercuspal position was recorded, asking the subject to clench as hard as possible and to maintain the same level of contraction for 3 s. This test was repeated three consecutive times, separated by 5 s interval. Verbal encouragement was given to the subject during the test. A trial lasting approximately 2 min was performed before starting the definitive recording, in order to assess the correct placement of the electrodes, that was followed by an 80-min EMG recording (see the experimental protocol).

The signals were sampled at 800 Hz, with eight-bit resolution, and stored in the storage drive of the Bruxoff. The EMG channels were filtered between 10 and 400 Hz. Root mean square (RMS) values were computed. The mean RMS value of the three MVC tests was used to calculate activity periods (AP) at 10 % (AP10), 20 % (AP20), and 30 % (AP30) of the MVC, which were considered as the threshold levels able to detect parafunctional activities. All activity periods (AP10, AP20, AP30) were identified and counted by a dedicated software (OTBiolab®, OT Biolettonica, Torino, Italy).

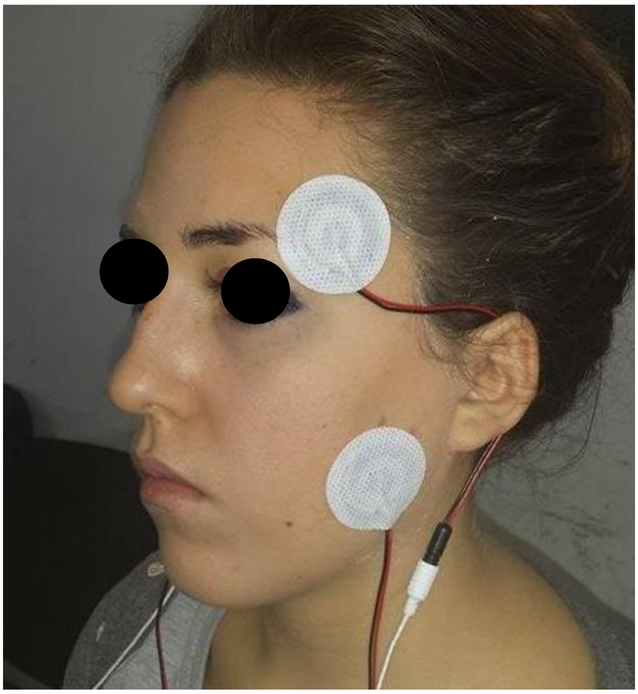

Figure 3. Location of the EMG electrodes

Experimental protocol

The EMG recording was performed in a silent and comfortable room in our clinic. The subject was invited to sit with the head unsupported and was asked to maintain a natural upright position. Only the subject and one investigator (DL) were present in the experimental room. Each subject was told that the EMG assessment had the purpose of monitoring the activity of the jaw muscles, and was asked to switch off her mobile phone, not to speak to the operator during whole experimental recording session, and not to touch electrodes. Chewing gum or candies were strictly forbidden. The EMG activity was recorded for 80 min during three different sessions in which the participant was invited to fill in the questionnaires (described above) for 40 min (task 1), then to read a general interest magazine for 20 min (task 2), and to a play a game session (Arkanoid, Taito, Japan) on a conventional laptop for 20 min (task 3) (Fig 4).

Figure 4. Recordings of the EMG tasks. From left: questionnaire, reading a magazine, videogame

Each participant was monitored by one investigator (DL) during the entire experimental phase. DL checked the time for each session. Before starting the EMG recordings, all participants were told that the time for completing the questionnaires was 40 min and received instructions to concentrate on the questions and not to rush. If they finished earlier, they had to check again their replies and start another set of the same questionnaires available on the desk (but in this case, the forms included questions in another order). Therefore, all participants were fully involved in a mental task for 40 min during this session. If they were not able to complete the questionnaire within the 40 min, they had to stop and they were allowed to answer the questionnaire after the EMG recordings. The order of the tasks was randomly assigned to each participant. The same procedure was used for the CTR group.

Data analysis

The psychophysical measurements were reduced at each time point by computing the mean of the 3 trials obtained at each PPT location, after discarding the first measurement.

The OBC score (OBC6) was computed by scoring items 3, 4, 5, 10, 12, and 13 of the OBC.

The mean RMS value of the three EMG MVC peaks was computed for each study participant. The individual value retrieved was used to calibrate and scale each participant's entire EMG signal in preparation for the following statistical analysis. Hence, in the calibrated EMG signal, the MVC was the reference unit (namely 100).

All the scaled EMG signals greater than 10 were identified as AP10 (>10 % of the MVC), those greater than 20 as AP20 (>20 % of the MVC), and those greater than 30 as AP30 (>30 % of the MVC). The AP10, AP20, and AP30 were identified and counted, and together with their computed duration, were used for the following statistical analysis.

EMG variables (AP10, AP20, and AP30 count and their computed durations) were tested for normality of distribution. When normality was not verified, between groups and within group well performed by non-parametric tests (Mann– Whitney and Kruskal–Wallis tests). Otherwise, the analysis of variance was used. P values were adjusted using the Bonferroni method. A mixed regression model was used to test the association between the independent psychological variables (SSA, Trait and State anxiety) and the primary outcomes, i.e., AP10, AP20, and AP30 count (number of events during the entire EMG session and during each of the three tasks), single duration (Dur—mean duration of single clenching episodes), and cumulative duration (CDur— sum of the duration of all clenching episodes) over the entire experimental phase using logarithmically transformed data. Interaction between study group (fixed factor) and SSA, trait and state Anxiety scores (covariates) were tested and retained in the model when statistically significant.

Between groups comparisons in SSAS and STAI (trait and state anxiety), OBC and OBC6 scores were calculated by using t test. The statistical significance was set at $p < 0.05$. SPSS software ver. 20 (IBM Corp, Armonk, NY, US.) was used for running the statistical analysis.

III.1.4 Results

The MP group had 6.1 ± 1.9 pain rated on a VAS scale. According to the graded chronic pain (GCP) classification of the RDC/TMD (Dworkin SF et al.,1992), patients had a characteristic pain intensity (CPI) of 51.4 ± 23.2. Four subjects had GCP grade I, eight grade II, and three grade III. Descriptive statistics and comparisons between groups (MP vs CTR) for PPT assessments are reported in Table 2.

	MP		CTR		p value
	Mean	SD	Mean	SD	
LEFT					
masseter	131.6	49.7	156.9	45.3	0.137
temporalis	146.8	45.1	197.4	46.8	**0.004**
RIGHT					
masseter	126.1	37.9	169.2	45.4	**0.007**
temporalis	154.8	43.6	196.2	42.9	**0.010**
thenar	303.6	104.7	283.5	71.7	0.521

Table 2. Descriptive statistics and between-group comparisons for PPT values (KPa).
Bold type: statistically significant

PPTwas significantly lower in MP than in CTR group for both left and right anterior temporalis muscles (p = 0.004 and p = 0.010 respectively) and for the right masseter (p = 0.007). No significant differences were found for the thenar muscle. Descriptive statistics and between groups comparisons for AP10, AP20, and AP30 count, Dur, and CDur for both groups are reported in Table 3.

	MP		CTR		p value
	Mean±SD	Median [IQR]	Mean±SD	Median [IQR]	
Count AP10	84.9±78.3	49.0 [103.0]	17.8±13.1	17.0 [21.5]	**0.001**
Count AP20	52.6±58.9	38.0 [78.0]	6.8±8.3	4.0 [8.7]	**0.002**
Count AP30	36.9±49.7	13.0 [64.0]	3.7±5.3	1.5 [6.0]	**0.002**
Dur AP10	1.0±0.3	1.0 [0.3]	0.8±0.2	0.7 [0.4]	0.064
Dur AP20	0.8±0.2	0.7 [0.5]	0.7±0.1	0.7 [0.3]	0.241
Dur AP30	0.7±0.2	0.7 [0.4]	0.7±0.3	0.6 [0.3]	0.852
CDur AP10	82.9±91.0	43.5 [103.5]	15.1±13.5	10.5 [23.6]	**0.002**
CDur AP20	45.2±54.2	27.5 [61.0]	5.1±6.7	2.5 [6.7]	**0.002**
CDur AP30	30.4±40.1	7.5 [49.5]	2.6±4.1	1.0 [3.7]	**0.004**

Table 3. Descriptive statistics and between-group comparisons for EMG outcomes over 80 minutes recordings for AP10, AP20 and AP30 count (number of events during the entire EMG recording – 80 minutes), single duration (Dur – mean duration of single clenching episodes), and cumulative duration (CDur – sum of the duration of all clenching episodes).
Bold type: statistically significant

The number of AP10, AP20, and AP30 was significantly greater in MP than CTR individuals (all $p < 0.05$). No significant differences were found between groups with respect to AP durations. The cumulative durations of AP10, AP20, and AP30 were significantly higher in MP than CTR group (all $p < 0.05$). The RMS of the masseter during the MVC was higher in the CTR group (62.3 ± 25.1) than in the MP group (33.8 ± 30.0, $p = 0.002$). The distribution of the dependent variables within the three tasks for each group and within and between groups comparisons are reported in Figs. 5, 6, and 7.

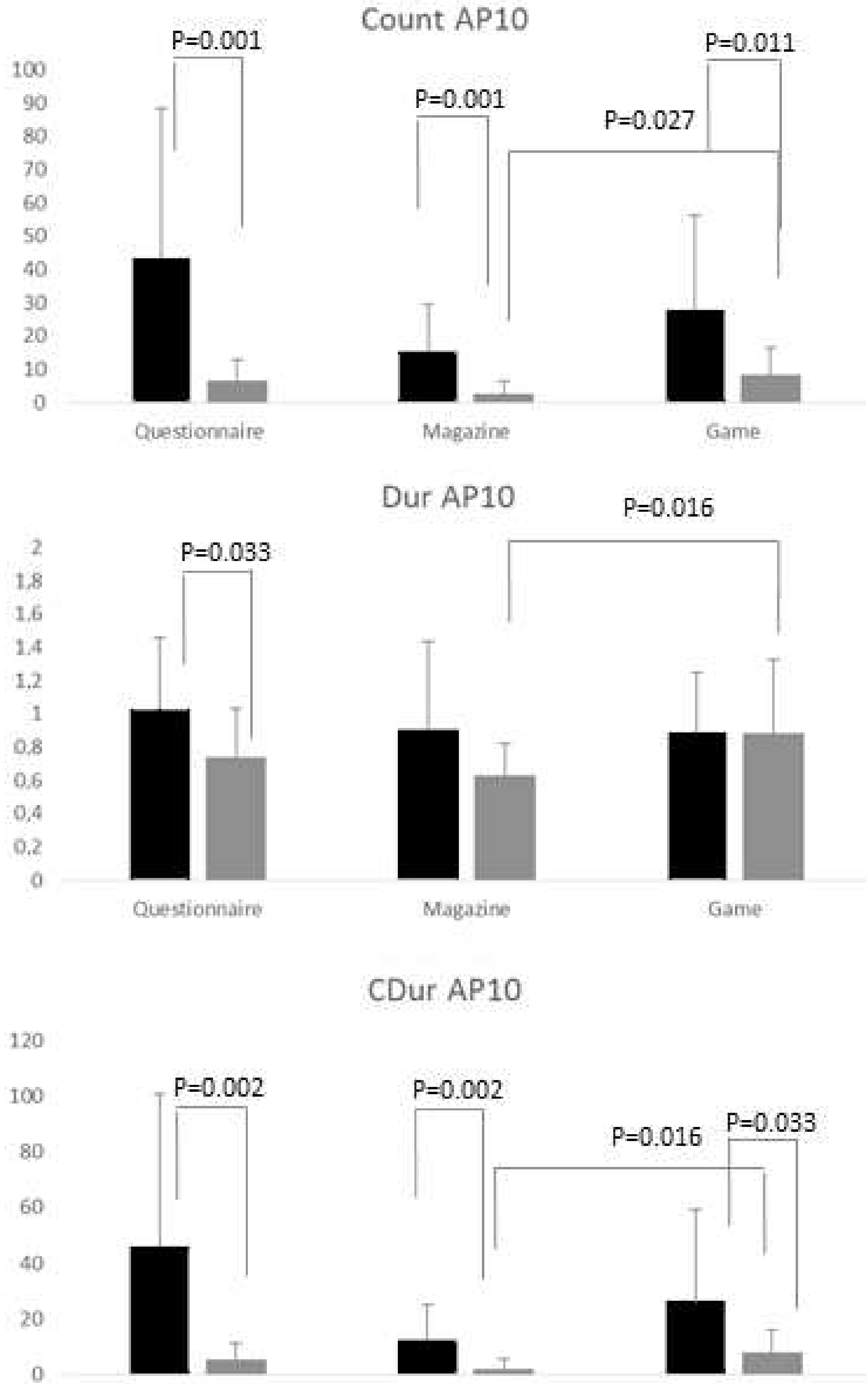

Fig. 5 Distribution of the dependent variables Count AP10 (number of episodes), Dur AP10 (seconds), and CDur AP10 (seconds) within the three tasks for each group (MP—black, CTR—gray) and within and between group comparisons. The lines above the bars indicate the standard deviation. Significant differences between and within groups are indicated by lines and corresponding p values.

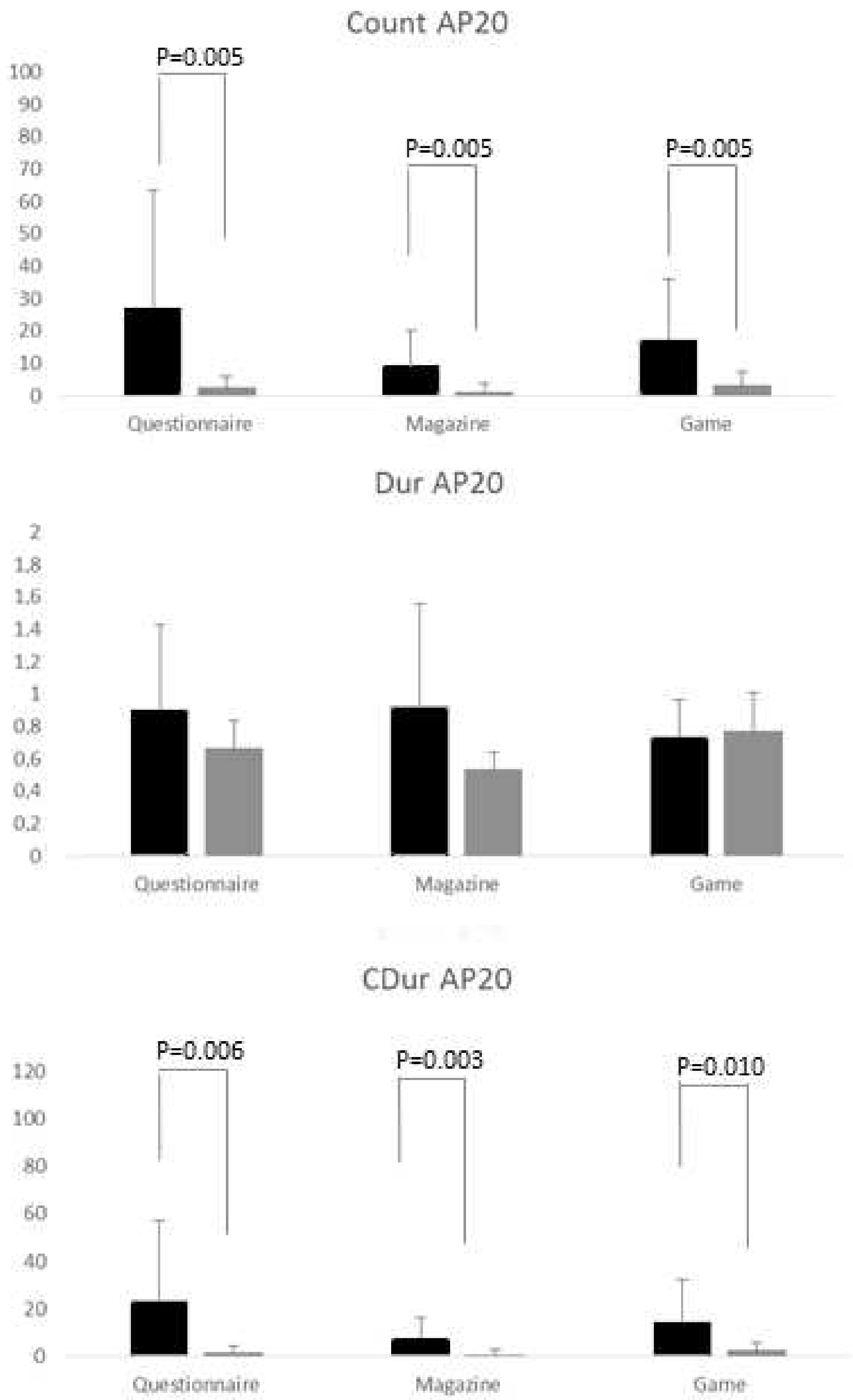

Fig.6 Distribution of the dependent variables Count AP20 (number of episodes), Dur AP20 (seconds) and CDur AP20 (seconds) within the three tasks for each group (MP – black, CTR – gray) and within and between group comparisons. The lines above the bars indicate the standard deviation. Significant differences between and within groups are indicated by lines and corresponding p values.

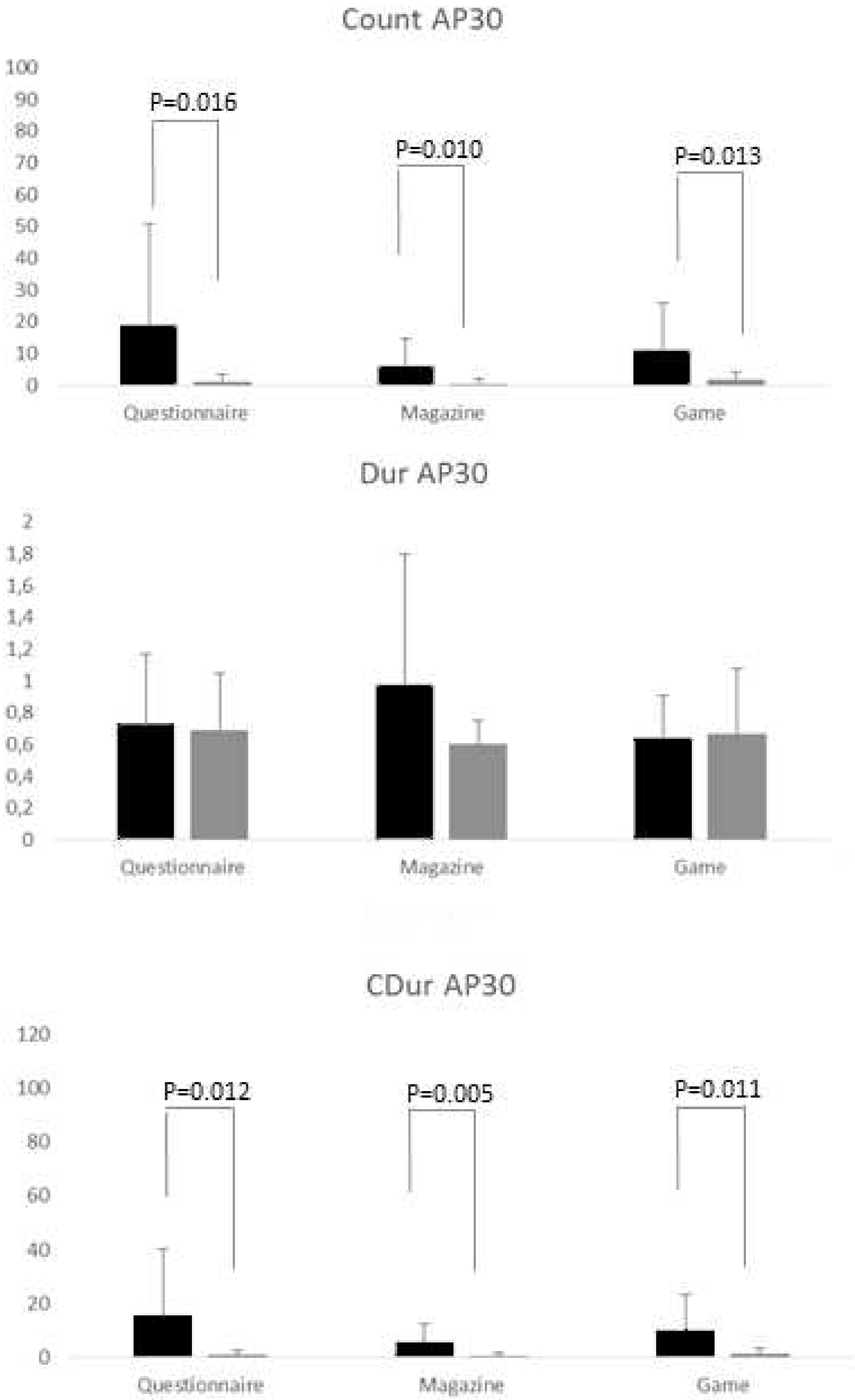

Fig.7 Distribution of the dependent variables Count AP30 (number of episodes), Dur AP30 (seconds) and CDur AP30 (seconds) within the three tasks for each group (MP – black, CTR – gray) and within and between group comparisons. The lines above the bars indicate the standard deviation. Significant differences between and within groups are indicated by lines and corresponding p values.

Count AP 10, Count AP20, Count AP30 and CDur AP10, CDur AP20, and CDur AP30 were significantly higher in MP than CTR group in all tasks (all $p < 0.05$). Dur AP10 was higher in MP group than CTR group only during the task including questionnaires ($p = 0.0033$). CTR group had a higher Count AP10, Dur AP10, and CDur AP10 while playing the videogame as compared to reading a magazine. Descriptive statistics and between group differences for STAI, SSAS, OBC, and OBC6 outcomes are reported in Table 4.

	Group	Mean	SD	p-value
STAI (State anxiety)	CTR	43.6	3.9	**0.027**
	MP	40.4	4.1	
STAI (Trait Anxiety)	CTR	44.2	3.8	0.774
	MP	44.6	4.2	
SSAS	CTR	11.5	6.4	0.089
	MP	15.2	5.8	
OBC	CTR	19.4	9.1	**0.001**
	MP	32.2	10.1	
OBC6	CTR	4.9	2.8	**0.004**
	MP	8.8	4.3	

Table 4 Descriptive statistics and between-group comparisons for questionnaires outcome. **Bold type:** statistically significant; STAI state and trait anxiety, SSAS somatosensory amplification scores, OBC oral behavior checklist—21 items, OBC6 oral behaviour checklist—6 items

Trait anxiety was associated to Count AP20 ($F = 4.63$; $p = 0.040$), Count AP30 ($F = 4.90$; $p = 0.035$), CDur AP10 ($F = 4.61$;$p = 0.040$), and CDur AP30 ($F = 4.44$;$p = 0.044$). State anxiety and SSAS were not associated to any of the dependent variables (all $p > 0.05$, data not shown).

OBC total scores were not correlated to the dependent variables, while OBC6 was positively correlated to CDur AP10 ($r = 0.351$, $p = 0.046$).

III.1.5 Discussion

This study has shown that the frequency of daytime clenching episodes is different between individuals suffering from myofascial pain of the masticatory muscles and healthy pain-free controls, and that certain ability tasks can affect the frequency of daytime clenching episodes only in pain-free individuals. Although daytime clenching is considered a risk for TMD (Michelotti A et al., 2010; Ohrbach R et al., 2013) little is known about the specific EMG characteristics of daytime clenching episodes, e.g., about their frequency, duration, and amplitude, and if these features differ between individuals suffering from myofascial pain of the masticatory muscles and healthy controls. A threshold of 30 mm on VAS scale was used to recruit patients with masticatory muscle pain because the smallest detectable difference for actual temporomandibular pain has been suggested to be 28 mm on VAS (Kropmans TJ et al., 1999). The possible relation between clenching and masticatory muscle pain has been tested in several studies, which showed that experimental low-level clenching tasks are associated with muscular soreness and fatigue, leading to TMD-pain like symptoms (Farella et al., 2010; Takeuchi T et al., 2015) that experimental high-level clenching is not related to long-lasting pain of the masticatory muscles (Svensson P et al., 1996; Farella et al., 2010) and that a delayed-onset of masticatory muscular soreness (DOMS) and a temporary diagnosis of myofascial pain occur in subjects performing bouts of eccentric and concentric jaw muscle contractions with different intensities (Koutris M et al., 2013).

The results of the present study reveal that individuals with myofascial pain of the masticatory muscles present higher counts of diurnal masseter activity periods (APs) than controls.

This result is in agreement with previous reports showing that the frequency of non-functional tooth contacts is higher in TMD than in TMD-free individuals (Chen CY et al., 2007; Fujisawa M et al., 2013; Funato M et al., 2014) and that daytime clenching and oral parafunctions are more frequent in subjects with MP diagnosis (Huang GJ et al., 2002; Michelotti A et al., 2010; Glaros AG et al., 2012; Ohrbach R et al., 2013). MP individuals had an about five times higher count of clenching episodes as compared to TMD-free individuals, when examining all the episodes greater than the 10% ofMVC. This ratio is even higher (amounting to approximately ten times) when considering very intense (greater than 30 % of MVC) clenching episodes. Interestingly, MP individuals showed approximately 4 % of the clenching episodes within 10–20 % of the maximum voluntary contraction, and the more intense clenching episodes (AP30) amounted to approximately 43 % of the recordings. While examining the CTR group, it was found that approximately 41%of the clenching episodes were within 10–20 % of the MVC, while the more intense clenching episodes (AP30) were only 21 % of the recordings.

Moreover, the cumulative duration of the AP episodes was higher in MP group than CTR for AP10 clenching episodes (2 % of the entire experiment in MP group and 0.3 % in the CTR group). All these data suggest that MP individuals show a high frequency of tooth clenching episodes of different intensities and support the hypothesis that MP might be a manifestation of muscle overload due to an alternate pattern of high- and low-level contraction episodes. It can be hypothesized that the metabolic demand of such muscular exercise may be not satisfied in MP patients, thus leading to muscular fatigue and pain. Indeed, it has been shown that low levels (5 %) of maximum voluntary contraction can produce a clear hemodynamic response in masticatory muscles (Kim YJ et al., 1999), but sufficiency of blood flow to maintain muscle fiber homeostasis is less when the rate of metabolic turnover is greater (Monteiro AA et al., 1989). In a recent study involving healthy subjects, delayed onset muscular soreness (DOMS) was determined following concentric and eccentric muscle contractions of different intensities (Koutris M et al., 2013). The authors suggested that pain probably resulted from of an accumulation of metabolites within the muscles because of an obstruction of the muscles' blood flow during the exercise. However, further studies with specific methodologies are needed to test whether these mechanisms might have contributed to the onset of masticatory muscle pain in the MP group. The greater extent of parafunctional behaviors present in the MP group than in the CTR group is further supported by the OBC6 and OBC scores, which were higher in the MP group than CTR. Interestingly, only the shortened version of OBC, namely OBC6, was correlated to the count of episodes, suggesting that OBC6 might be sensitive do detect daytime tooth clenching episodes.

For this study, participants were submitted to different ability tasks in which mental, practical, and both mental and practical abilities were needed. A significant effect of the ability task (questionnaire, reading a magazine, and playing a videogame) on daytime tooth clenching was found only in CTR group, in which it was found that the count, the duration, and the cumulative duration of episodes >10%MVC (AP10) were higher while playing a videogame. On the contrary, the frequency of clenching episodes in the MP group was not affected throughout all the experimental tasks. Hence, it is likely that the progress of time and a practical ability task (i.e., playing a videogame) affected the occurrence of clenching episodes and muscular activity of the CTR group but not of the MP group, who continued to show frequent clenching behaviors independently from the task, as reported previously (Cioffi I et al., 2015). The EMG response found in the CTR group (i.e., higher frequency and duration of clenching episodes while playing the videogame as compared to reading a magazine) is confirmed by some authors who report acute changes (i.e., relative increase) in myoelectric activity of masseter and temporalis muscle with stressful conditions (Nicholson RA et al.,2000; Tsai CM et al., 2002). It is somehow likely that clenching episodes in the CTR group were triggered by the videogame, which requires both a mental and practical ability. Contrary to a previous report (Michelotti A et al., 2012), we did not find between groups differences in trait anxiety. This is in agreement to Giannakopoulos and coworkers who reported that individuals with facial pain did not present increased anxiety as compared to the general population (Giannakopoulos NN et al., 2010). Nonetheless, the association found between trait anxiety and the dependent variables suggests that anxiety played a major role in influencing the intensity and the frequency of clenching episodes in some individuals. Also, although not significant, MP individuals had slightly higher levels of somatosensory amplification, a characteristic related to bodily and occlusal hypervigilance. The concept of somatosensory amplification has been applied to the chronic pain population to explain how maladaptive cognitions may lead to heightened pain perception (Feuerstein M et al., 1995). However, this factor was not associated with the dependent variables in the current experiment.

Interestingly, state anxiety was greater in the CTR group than in the MP group. Although both groups received similar that the EMG recording was felt more emotionally by painfree people, who were worried and nervous because not comfortable with medical and laboratory evaluations, while it is possible that the information received by the MP patients during the clinical examination contributed to decrease their state anxiety (Sjöling M et al., 2003) The PPT values were within the ranges previously found for TMD and TMD-free individuals. As expected, MP group had lower PPTs at the masseter and temporalis than subjects in the CTR group (Michelotti A et al., 2008; Al-Harthy M et al., 2016; Cioffi I et al, 2015). Nonetheless, the difference at the left masseter was not statistically significant. This is likely the result of a greater variation of the measurements at this site in the MP group. Moreover, the absence of between groups difference in PPT measurements at the thenar eminence let us hypothesize that central sensitization phenomena did not affect the current findings (Graven-Nielsen T et al., 2010; Ramalho D et al., 2015).

This study has some limitations. First, for this study, we recruited only individuals with actual masticatory muscle pain. Therefore, we cannot draw conclusions about individuals with a history of temporomandibular pain and/or with recurrent pain.

Second, the short duration of the experiment does not allow inferring about the relation between daytime clenching and the intensity of masticatory muscle pain. Third, the recordings obtained may be contaminated by artifacts (e.g., due to movements of the electrodes). Video recordings could have addressed this limitation. However, the distribution of these artifacts is likely to be similar between groups and across the conditions and therefore should not have influenced the results. In addition, it might be argued that the initial MVC trials may have acted as artificial stressors with a different impact on the EMG signal of both groups. But, since an interval of approximately 5 min was present between the MVC recordings and the experimental sessions, it can be hypothesized that the MVC trials had a minimal impact on the EMG recordings. Finally, the EMG recordings do not allow distinguishing functional (e.g., swallowing) from non-functional masseter contractions. To our knowledge, the frequency of swallowing might be affected by systemic, including hormonal, and oral conditions as well as by age (Tanaka N et al, 2013). In this study, both groups were not affected by medical conditions, were not using drugs affecting the frequency of swallowing and the salivary pattern, and were matched by age. Finally, it has been shown that the number of functional tooth contacts (including swallowing) does not differ between TMD patients and healthy subjects (Kato T et al., 2006; Chen CY et al., 2007; Katase-Akiyama S et al., 2009). Therefore, it could be assumed that swallowing did not affect the differences found between groups.

III.1.6 Conclusions

Individuals affected with masticatory muscle pain present a greater frequency of daytime clenching episodes than painfree individuals during standardized mental and practical ability tasks. The type of ability task does not affect the frequency and the duration of clenching episodes in myofascial pain patients. Conversely, pain-free individuals are more sensitive to the tasks in which both mental and practical skills are needed (playing a videogame) and increased the frequency of the clenching episodes.

III.2 Association between waking state oral behaviors and TMD Diagnostic subgroups (*In submission*)

III.2.1 Introduction

As widely described in the introduction, oral parafunctions are considered as adverse oral behaviors and risk factor for TMDs development.

In particular, specific awake oral parafunctions have been associated with TMDs diagnosis in different age groups (Miyake R et al., 2004; Fernandes G et al., 2016; Mejersjö C et al.,2016; Leketas M et al, 2017). However TMD is a collective term that embraces a variety of temporomandibular disorders and it has to be noted that many risk assessment studies on TMDs focus on one overall TMD Diagnosis and not to a specific TMD category (H. Karibe et al., 2015; Ü. Şermet Elbay et al., 2017).

Hence, doing a more detailed research as concerns TMD subgroups, it has been proved that parafunctions like clenching/grinding are a risk factor for the development of disc displacement (DD) (Michelotti A et al., 2010) and that sustained incisal clenching, like nail biting, determines a disc compression (Takenami Y et al., 1999); moreover, other authors showed that clenching/grinding (Svensson P et al., 2001; Huang GJ et al., 2002; Glaros AG et al., 2004; Michelotti et al., 2010; Fernandes G et al., 2016), chewing-gum, daily nail biting and having oral piercing (Mejersjö C et al., 2016) were associated with the development of myofascial pain (MP). Those data suggest that each oral parafunction has different effects on muscle and TMJ, therefore an association between specific parafunctions and different TMD subgroups might exist. Nevertheless, bruxism seems to remain the most studied parafunction. Therefore, further studies about the relationship between oral parafunctions, and TMD should include a wider group of oral activities and focus on specific TMD subgroups (Molina OF et al., 2001).

Moreover, oral parafunctions are significantly associated with psychological symptoms (Khawaja SN et al., 2015). Interestingly, daytime tooth clenching seems to be related to psychological distress, anxiety and depression (Manfredini et al., 2009; Endo H et al., 2011). However, controversial is the relationship between psychological symptoms and TMD diagnosis. According to some authors, patients affected from TMD express more often anxiety and depression symptoms than controls (Sharma S et al., 2011; Maixner W et al., 2011; Wieckiewicz M et al., 2014) and in the same way, the pain reduction often involves reducing the symptoms of anxiety and stress (Rollman GB et al., 2000). Probably anxiety and other psychological factors cause muscles hyperactivity and this muscle overload can determine the onset of TMD (Chisnoiu AM et al., 2015). However, a significant association has not always been found (Calixtre LB et al., 2014).

The first aim of this study was to assess the association between different types of awake oral parafunctions, using the Oral Behavior Checklist, and subgroups of TMD patients presenting dysfunction or pain. Secondly, we evaluated whether awake oral parafunctions are associated with anxiety.

III.2.2 Materials and Methods

Study participants

In this retrospective study, cases were selected among 785 consecutive patients referred to the Dental Clinic of the University of Naples "Federico II" for a TMD consultation, from May 2015 to May 2017. Temporomandibular disorders were diagnosed according to Diagnostic Criteria/TMD (DC/TMD). The clinical records were assessed by examiners who had been calibrated for DC /TMD. Subjects ≥18 years old, with a unique diagnosis of TMD, were included in the study. Patients with trigeminal neuralgia, fibromyalgia, burning mouth syndrome, atypical facial pain, atypical odontalgia, migraine, cervical and/or neuropathic pain were excluded. TMD group was matched for age and sex to a control (CTR) group recruited from among individuals accompanying patients of the Dental Clinic in a subsequent time period and including subjects ≥18 years old and free from TMD. The exclusion criteria used for patients were also used for the controls. Subjects willing to participate in the study signed an informed consent and were screened for signs and symptoms of TMD by means of a preliminary TMD-pain screening according to the questionnaire of Gonzalez et al (Gonzalez YM et al., 2011), and through questions 8-14 of the Axis I DC/TMD symptoms questionnaire regarding joint disease. Individuals who answered negatively to both questionnaires were considered free from TMD-pain and dysfunction and were included in the final sample. The local Ethics Committee approved the study protocol and each participant signed an informed consent.

Questionnaires

Assessment of pain intensity

TMD patients filled in the Graded Chronic Pain Scale (GCPS) (Schiffman E et al., 2014), a short, reliable and valid instrument for assessing pain intensity, numbers of days with interference and pain-related disability in the previous month. According to the GCPS, pain intensity has been measured through the "characteristic pain intensity (CPI) scale". CPI scores are obtained by calculating the mean score between 3 pain intensity questions (worst pain, average pain and pain at this moment). Each of these is scored on an 11-point numerical scale from 0 (no pain) to 10 (pain as bad as could be). The scores are multiplied by 10; thus, CPI score ranges from 0 to 100. Based on the CPI, the TMD group was divided in two subgroups: patients without pain and affected only by dysfunction (TMD-Dysfunction group, CPI=0) and patients with pain (Painful-TMD group, CPI>0).

Assessment of oral parafunctions

All groups filled in the Oral Behaviors Checklist (OBC). The Total score is an index of each participant's self-assessment of the severity of his/her awake oral parafunction and is obtained summing the frequencies of each of the 21 items. For the purpose of this investigation, the first two questions of the OBC, which assessed the sleep-related parafunctional activities, were excluded from the analysis. Therefore, the OBC-TS was measured as the sum of frequencies of remaining 19 items that are related to the awake oral parafunctions. Secondly, we grouped the OBC Items according to the factors obtained from principal component analysis (PCA) of the 19 Items.

Assessment of anxiety

Groups were assessed for anxiety according to the Generalized Anxiety Disorder Scale (GAD-7). It is a short self-report questionnaire, which includes seven questions and gives information about the severity of anxiety of a subject and its influence on the quality of life (Schiffman E et al., 2014). Its validity and reliability have been demonstrated (Spitzer RL et al., 2006; Löwe B et al., 2008). Possible responses to each item of the questionnaire are as follows: “not at all”, “several days”, “more than half the days”, “nearly every day” which are equivalent to scores of 0, 1, 2 and 3, respectively, yielding a maximum score of 21; scores ≥5, ≥10 and ≥15 represent a cut-point for respectively mild, moderate and severe anxiety grade.

Statistical analysis

Based on the calculation of the statistical power, the sample size had to include above 700 subjects including a TMD and a CTR group. Continuous variables are reported as means and standard deviations or median and interquartile range (IQR), depending on variables distribution. Shapiro-Wilk test was used to evaluate the normal distribution of data. Due to a not normal distribution, non parametric tests were used for the analysis.

Therefore, the OBC-TS was measured as the sum of frequencies of the 19 (from OBC-3 to OBC-21) items related to the awake oral parafunctions.

Secondly, these 19 items were examined using principal component analysis (PCA) with orthogonal varimax rotation, reducing the analyzed items to factor loadings. The proposed factors were measured and graphically controlled in order to assign each item to the respective factor (Fig 11). Each factor was subsequently analyzed based on clinical evaluation.

Differences in OBC-TS (Total score), OBC (Grouped Items) score and GAD-7 score among groups (TMD-Free, TMD Dysfunction and Painful-TMD) were estimated using the Kruskal-Wallis test (KW) followed by Dunn's post-hoc tests multiple comparisons. Spearman correlation analysis was used to assess the relationship between variables, when requested. Statistical significance was set at $p<0.05$

III.2.3 Results

Out of 785 consecutive patients screened, 416 were excluded because not affected from TMD/DC or because of incomplete questionnaires. The final TMD group included 369 patients diagnosed with exclusively TMD/DC (267 females and 102 males, mean age ± SD= 37.95±16.39 years): 70 subjects (51 females and 19 males, mean age ± SD= 40.2±16.4 years) were not affected from pain but only from dysfunction (TMD-dysfunction group, CPI=0) and 299 subjects were affected only from pain (Painful-TMD group, CPI>0). TMD group was matched for age and sex to a CTR group of 374 TMD-Free subjects (270 females and 104 males, mean age ± SD= 37.1± 15.9 years) (Fig.8).

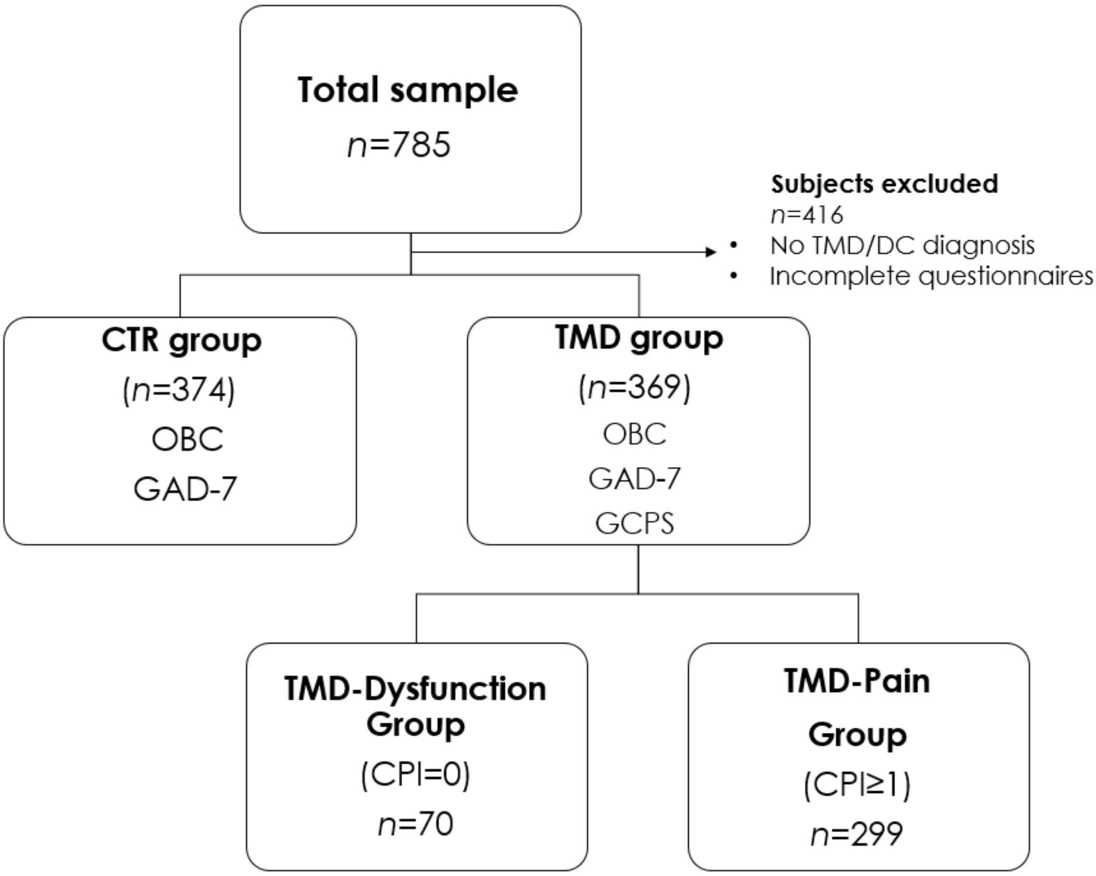

Fig 8. Sample recruitment and division in groups: CTR group, TMD group: TMD-Dysfunction group and TMD-Pain group.

The percentage of females in the TMD group resulted higher than males (72.4 %). Females showed a slightly higher OBC (Total score) with respect of males only in TMD group (*P* = 0.04). No gender differences as concerns the OBC (Total score) were found within the CTR group (*P* = 0.15). A negative correlation was found between OBC-TS and age in the total sample (rho= -0.3, *P* = 0) where a progressive reduction of the OBC-TS was observed with the increasing of the age in the TMD (rho= -0.2, *P* = 0) as well as in CTR group (rho= -0.4, *P* = 0 (Fig 9).

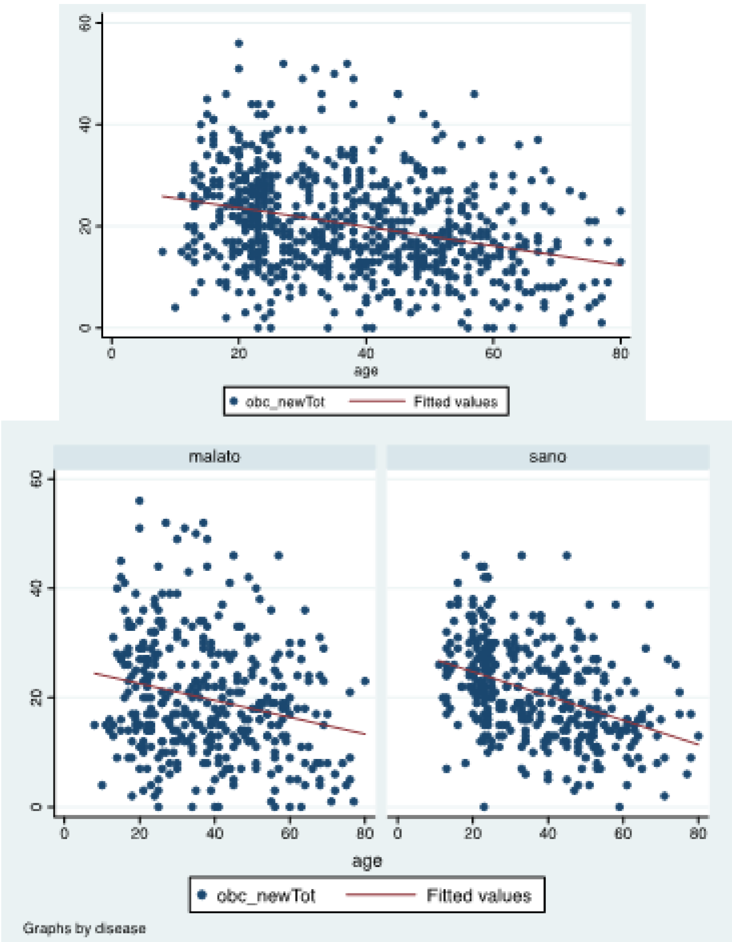

Fig.9 Comparison between different age groups for OBC (Tot score) outcome, in the total sample, TMD and CTR group.

OBC-TS differed across the three groups (TMD-Free, TMD-Dysfunction, and Painful-TMD) (KW test H(2)=30.9, p<0.001). Post-hoc pair-wise comparisons indicated that the median total score in both TMD-Free (20, IQR 15-27) and Painful-TMD participants (20, IQR 13-28) was significantly higher compared to the TMD-Dysfunction participants (13, IQR 8-19) (p<0.001) (Fig.10). The median OBC-TS did not differ between TMD-Free and Painful-TMD participants (p<0.001) (Fig 10).

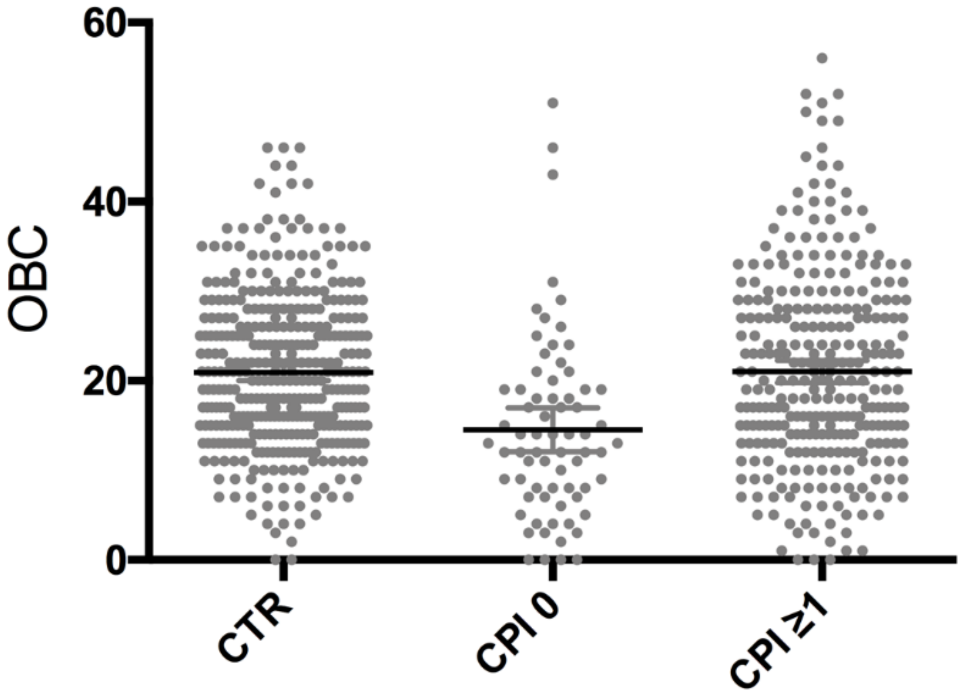

Fig. 10 Differences between groups for OBC (Tot score)

Based on the results of the principal component analysis, items (from OBC-3 to OBC-21) were assigned and grouped into 3 different factors loadings that, based on our clinical evaluation, we defined as SFA, UFA and OTHER group (Fig 11). Group of SFA (Static Functional Activities) included 6 items representing static oral activities (3, 4, 5, 6, 7, 11), UFA (Usual functional activities) included 9 items representing all usual functional activities such as chewing, talking or yawning (10, 12, 13, 15, 17, 18, 19, 20, 21), and other Items included the remaining 4 items (8, 9, 14, 16).

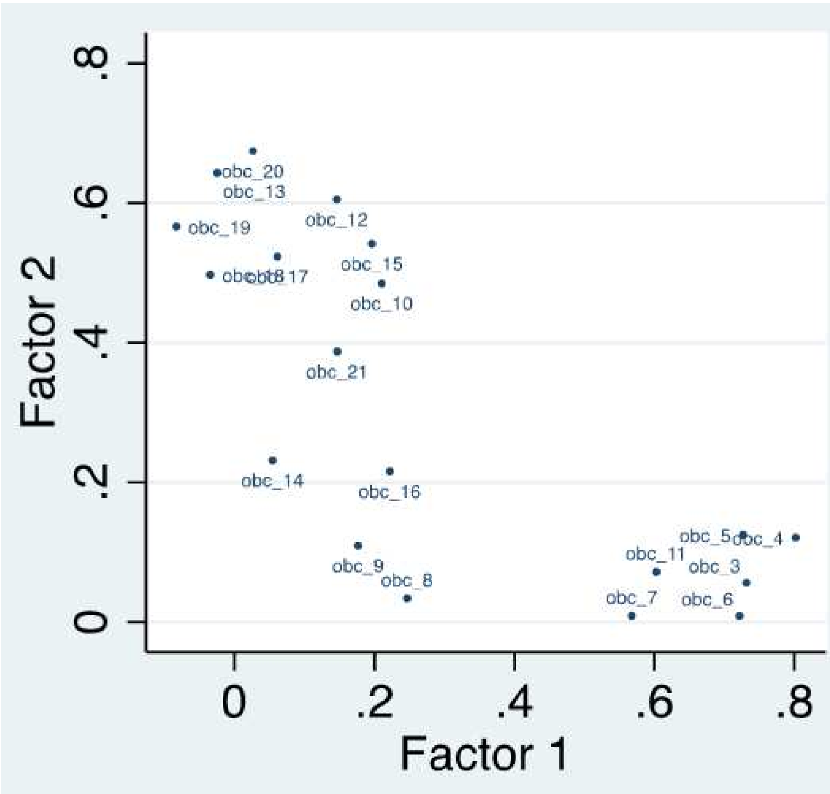

Fig.11 Principal component analysis (PCA) of the 19 diurnal oral parafunctions items with orthogonal varimax rotation, reducing the analyzed items to factor loadings. The proposed factors have been measured and graphically controlled in order to assign each item to the respective factor.

Differences between CTR group and TMD subgroups about the OBC score of grouped items were also evaluated. In particular, when considering the grouped items SFA the median total score in the Painful-TMD group (6, IQR 3-11) was higher compared to TMD-Free (4, IQR 1-6) and TMD-Dysfunction group (2.5, IQR 0-6)(KW test $H(2)=64.3$, $p<0.05$)(Fig.12).

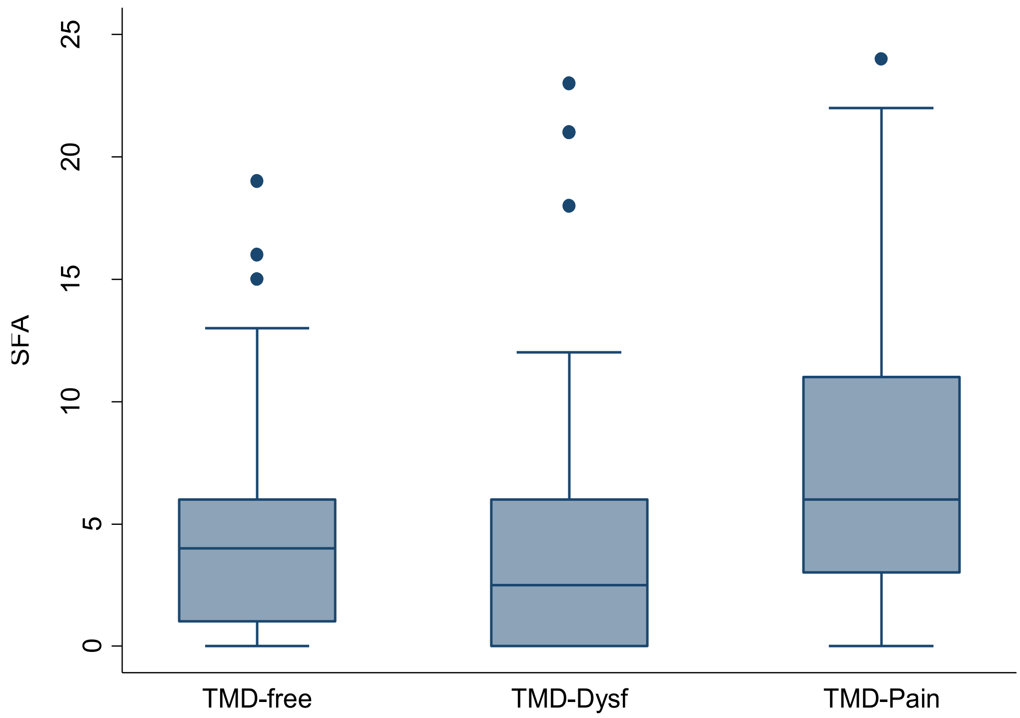

Fig.12 Frequency of grouped items SFA (Static Functional Activities) in the TMD Subgroups (TMD-Free, TMD-Dysfunction and TMD-Pain)

In contrast, grouped items UFA, characterizing mainly dynamic parafunctions, associated to a higher mandibular dynamic, such as chewing, talking or yawning, showed a median total score in both Painful-TMD (10, IQR 6-14) and TMD-Dysfunction group (7, IQR 4-11) lower than TMD-Free group (14, IQR 9-18) (KW test H(2)=92.2, $p<0.05$)(Fig.13).

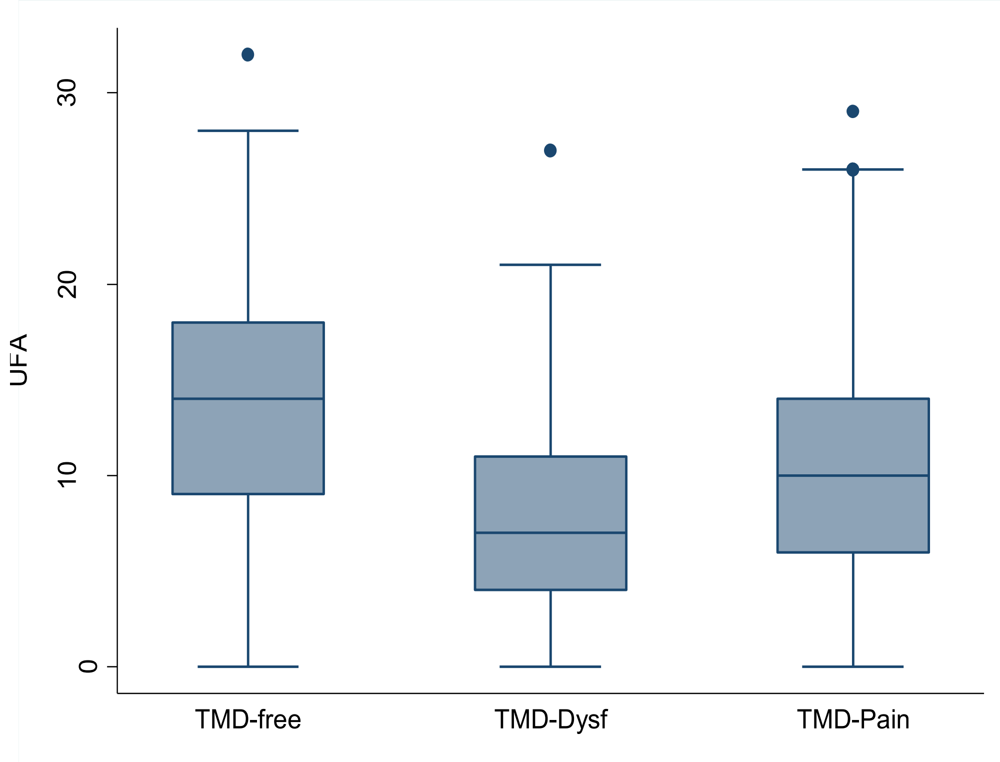

Fig.13 Frequency of grouped items UFA (Usual Functional Activities) in the TMD Subgroups (TMD-Free, TMD-Dysfunction and TMD-Pain)

Finally, no significant differences between Painful-TMD (3, IQR 1-4), TMD-Free (3, IQR 1-4) and TMD-Dysfunction (2, IQR 1-4) were shown when considering the Other grouped items (KW test H(2)=92.2, p<0.05) (Fig.14).

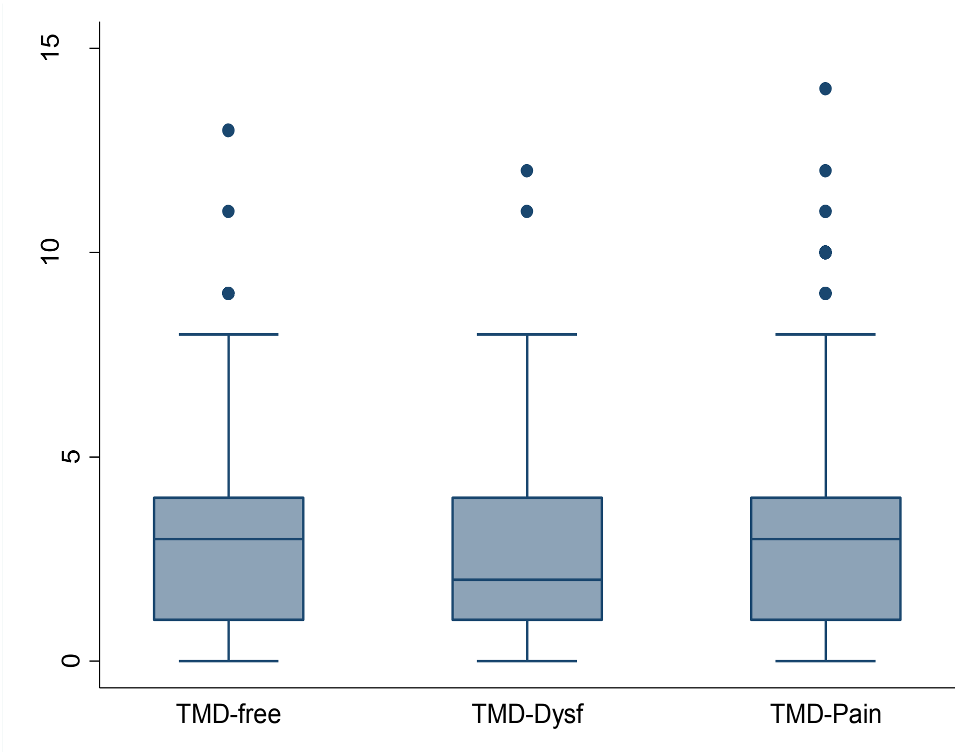

Fig.14 Frequency of grouped items Other in the TMD Subgroups (TMD-Free, TMD-Dysfunction and TMD-Pain)

No correlation was observed between the GAD-7 total score and age in the total sample (r = 0.06; P = 0.13) (Fig 15); GAD-7 was significantly higher in females than males (*P* =0.01).

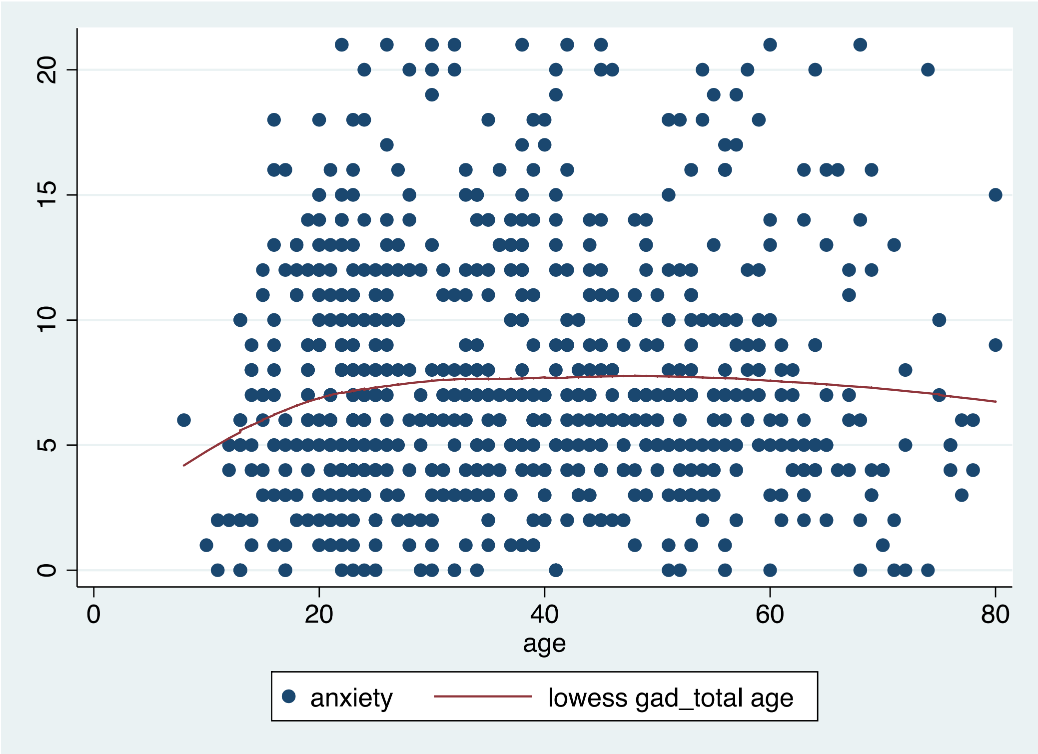

Fig. 15 . Correlation between GAD-7 score and age in the total sample.

Differences in GAD-7 score, determined using Kruskal-Wallis test, were significant when comparing CTR, Dysfunction and Pain groups [H(2) = 10.5, p < 0.01)]. In particular, In TMD-Pain group GAD-7 score was higher than in CTR group, however this difference was not significant (*P* = 0.16); on the contrary, GAD-7 score of TMD-Dysfunction group was significantly lower compared to CTR (*P* = 0.03) and TMD-Pain group (*P* = 0.002) (Fig 16).

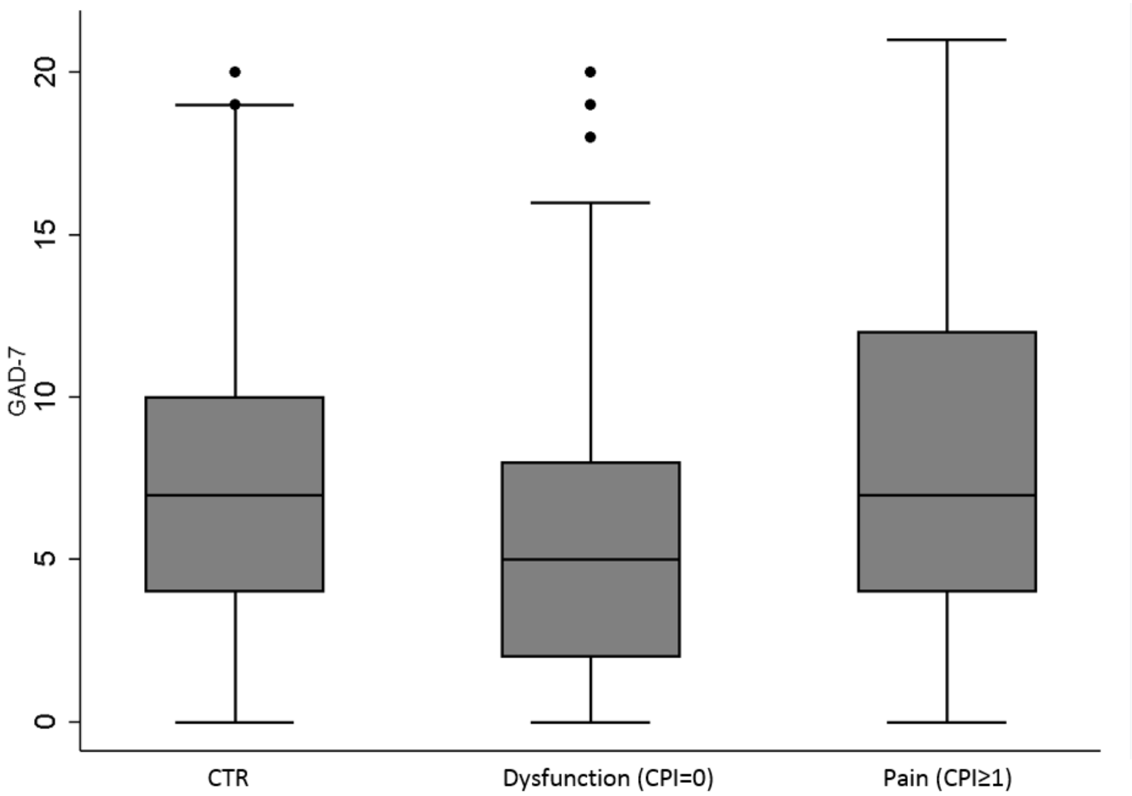

Fig. 16 Scores of GAD-7 in subgroups

Finally, a moderate correlation was found between GAD-7 and OBC-TS in all population (rho=0.36, $p<0.001$) and confirmed when divided by groups: CTR (rho=0.33, $p<0.001$), TMD-Dysfunction (rho=0.22, $p<0.06$) and TMD-Pain subjects (rho=0.43, $p<0.001$). (Fig 17)

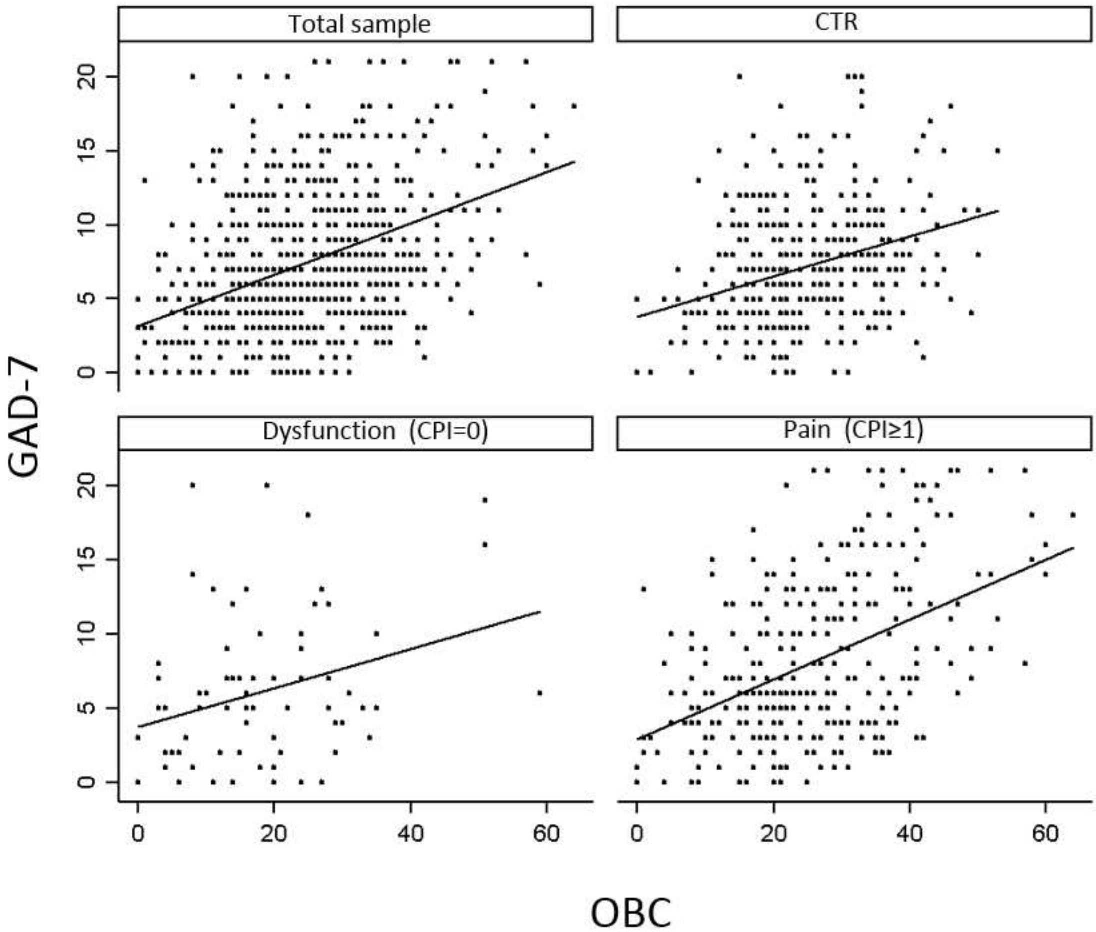

Fig 17. Correlation between OBC (Tot score) and GAD-7 score in each subgroup. CTR=control; CPI 0= TMD-Dysfunction; CPI ≥1= TMD-Pain.

III.2.4 Discussion

TMD group included more females than males (72.4 %); this is consistent with studies showing that TMD symptoms more often concern women ($P < 0.05$, 30) and that female gender is a risk factor for the development of TMD pain (Huang GJ et al., 2002; Velly et al., 2003; LeResche L et al., 2007; Michelotti A et al., 2010;). This may result from biological differences, including hormonal ones, and also psychosocial factors (Koidis P. T et al., 1993; LeResche L et al., 2003).

Oral parafunctions: OBC-TS

We found an association between parafunctions and gender only in TMD group, where female subjects showed higher oral parafunctions levels than males ($P = 0.04$). This is in agreement with previous studies conducted on children (Bayardo RE et al., 1996; Farsi NM et al., 2003) and adolescents (Winocur E et al., 2006), but in contrast with reports conducted on adults ($P = 0.965$) (Khawaja SN et al., 2015) and in a group of Dutch TMD patients ($Z = -1{,}49$, $P = 0.136$) (van der Meulen et al., 2014).

The main findings of this research are that the OBC-TS does not present differences between healthy subjects and TMD patients. Therefore, patients and healthy subjects presented a similar frequency of parafunctional habits. The lack of a significant difference between two groups is in agreement with previous researches where oral habits and bruxism were not found to be more frequent in the TMD group (Shiau YY et al., 1989) and with other researches which did not prove a consistent role of parafunctions in the etiology of TMD (Lobbezoo F et al, 1997). In contrast, other studies showed that extensive oral parafunctions were strong predictors of TMD incidence (Michelotti et al., 2010; Ohrbach R et al., 2013; Leketas et al., 2017).

However, when comparing CTR group and TMD subgroups, both TMD Free and Painful-TMD subjects presented an OBC-TS significantly higher than the TMD Dysfunction group (Fig 10). This is consistent with a recent study in which individuals with pain-related TMD and TMD-Free scored in the frequency of oral parafunctions, according to the OBC, significantly higher than non-painful TMD individuals (Khawaja SN et al., 2015) ($P \leq 0.001$). Moreover the same study showed a significant association between self-reported oral parafunctions and pain intensity, in particular TMD-High pain subjects had significantly higher mean OBC score than non-painful TMD diagnoses groups ($P \leq 0.001$) (Khawaja SN et al., 2015). On the contrary, Dutch authors found a not significant correlation of TMD pain neither with the OBC-TS, nor with any of the 21 items separately (van der Meulen et al., 2006; van der Meulen et al., 2014). Also, a previous research conducted from our group showed that individuals with masticatory muscle pain presented a greater frequency of daytime clenching episodes measured with sEMG, and higher OBC scores, than pain-free individuals (Cioffi I et al. 2017). However, the higher level of parafunctions found in the above-mentioned research with respect of the present study can be explained by the smaller sample including younger female subjects and with a higher pain intensity. Indeed, according to our study, this category of patients presents higher level of parafunctions.

We also observed that the total frequency of parafunctions, according to the OBC-TS, was negatively correlated to the age in the total sample and decreased with increasing age (Fig.9). In contrast, no relationship of the OBC mean score with age was found in a previous report (van der Meulen et al., 2014), although, the sample was not equivalent with a lower size and it included only TMD patients.

Oral parafunctions: OBC (grouped items)

A further analysis was performed considering the grouped items of the checklist. In fact the second main finding of the present study, was that, when considering the grouped items SFA, the median total score in the Painful-TMD group was higher compared to TMD-Free and TMD-Dysfunction group (Fig.12); this means that static parafunctions that are characterized by tooth clenching behaviors and reduced mouth movements, are referred mostly from patients with TMD pain with respect of healthy subjects. Similarly, in a previous study, painful-TMD patients, with myofascial pain and artarlgia, showed significantly more frequent and more intense teeth contact and tension in jaw and face than normal controls and subjects with disc displacement ($P \leq 0.01$) (Glaros AG et al., 2005). Also, a recent study conducted through the OBC, demonstrated that the very frequent expression of holding, tightening, or tense muscles and grinding teeth together during waking hours are associated with respectively 10.83 times ($P<0.05$) and 4.94 times ($P<0.05$) higher risk of TMD (Leketas M et al., 2017), however it did not analyzed TMD subgroups differences. In particular, parafunctions as clenching and grinding have been previously considered risk factors for the development of myofascial pain (OR: 4.9) (Huang GJ et al., 2002; Velly AM et al., 2003; Glaros AG et al., 2005);

On the contrary, in the present study, both painful-TMD and TMD-Dysfunction subjects scored lower than TMD-Free subjects in the Usual Functional Activities, movements carried out in everyday life that are related to the normal function of jaw and that often involve higher mandibular excursions, such as chewing, talking or yawning (Fig 13).

The "Pain Adaptation Model" might explain why painful TMD patients reported a lower frequency of UFA. This is because the presence of pain induces a reduced activity of agonist muscles and an activation of antagonist muscles determining a limitation of mouth movements (Lund JP et al., 1991).

On the other hand, dysfunctional patients could reduce the oral parafunctions including movements because afraid of worsening their health condition and this phenomenon goes under the name of kinesiofobia (Visscher CM et al., 2010). Also, clinicians typically recommend to dysfunctional individuals to avoid several oral activities with the aim of preventing jaw damage or trauma (De Boever JA et al., 2008). No significant differences between groups were shown when considering the other 4 parafunctions (Fig. 14).

Anxiety (GAD-7)

Anxiety was also evaluated. No correlation was observed between the GAD-7 score and age in the total sample (Fig 15). Gad-7 was significantly higher in females than males accordingly to previous researches (Stallman H et al., 2010).

CTR group resulted more anxious than TMD-dysfunction group and less anxious than TMD-Pain group. Nevertheless, the difference between CTR group and TMD-Pain group was not significant (Fig 16). On the contrary TMD-Pain group resulted significantly more anxious than TMD-Dysfunction group confirming an association of anxiety with TMD pain (Velly et al.,2003; Bonjardim LR et al.,2005; Casanova-Rosado JF et al., 2006; Manfredini D et al., 2009). In the present study, a moderate correlation has been showed between anxiety levels and frequency of awake oral parafunctions in the total sample as well as in healthy subjects and in each diagnostic TMD subgroup (Fig. 17) accordingly to what found in previous researches conducted on smaller samples of TMD patients (van der Meulen et al., 2014; Cioffi I et al., 2017; Khawaja SN et al., 2015; Manfredini et al., 2009) and healthy individuals (Cioffi et al., 2016). Interestingly, the correlation progressively increased going from CTR and TMD-Dysfunction ($r = 0.31$) to TMD-Pain subjects ($r = 0.47$). Hence, it can be stated that anxiety can affect parafunctional behaviors, particularly in TMD-pain patients. Also Restrepo et al reported that children with bruxism had a significantly higher-tension personality and were more prone to anxiety, as well as had more TMD signs and symptoms, than a control group (Retrespo CC et al., 2008).

Accordingly, it is known that nervous subjects present a high rate of hypervigilance and mood disorders (Manfredini et al., 2004) and parafunctional habits than controls (Glaros AG et al., 2005), this increases the activity of the masticatory muscles, which consequently results in TMD pain (Rugh J. D et al., 1975; Manfredini et al., 2004). It is generally accepted that certain affective and cognitive behavioral factors contribute to these differences in individual pain perception (Shaffer SM et al., 2014; Wieckiewicz M, et al., 2015). For instance, and specifically relevant to the medical and dental settings, pain perception is influenced by factors such as somatosensory amplification and anxiety (Manfredini D et al., 2010; Sharma S et al., 2011).

Probably because, in this kind of patients, psychological, and social factors may reduce the adaptive capacity of the masticatory system, thus resulting in TMDs (79-80). Therefore, as the psychosocial factors are related to both oral parafunctions and pain intensity, they should be the focus of research until, perhaps, more detailed and precise research projects will clarify the exact mechanisms involved in oral parafunctions.

Advantages and limits of the study

The strength of the present investigation is that the assessment of oral behaviors frequency and anxiety levels was conducted through validated self-report questionnaires like the OBC and the GAD-7, rarely used in previous works.

In addition, TMD diagnosis was obtained through a standardized clinical assessment. Moreover, the sample was large and heterogeneous. Finally, the analysis was conducted on a two well distinct population of adults (healthy and affected from TMD/DC diagnosis), and also analysed separately TMD pain and dysfunction subjects.

A limitation of this research is that, despite the validity of the OBC in the assessment of oral parafunctions (Kaplan et al., 2016), this tool is based on subjective information given by the patients who might not be always aware of their unconscious oral habits (Hathaway KM et al., 1995; Molina OF et al., 2000). Therefore, the OBC should be used in association with an objective instrument for the evaluation of muscles overuse like the EMG (Ohrbach R et al., 2008). In addition, the present study was conducted on a sample of subjects attending the Section of Temporomandibular disorders at the University of Naples and not representative of the whole population. Hence, to increase the strength of our results, it could be interesting to compare them with those obtained in other study population, for example comprising Italian subjects recruited in an environment different from the hospital structure, or even subjects belonging to other countries.

III.2.5 Conclusion

This report showed that Specific awake oral behaviors listed in the OBC are differently associated with painful and non-painful TMDs. Moreover, anxiety is correlated to the extent of oral parafunctions and it is more present in subjects affected from TMD-Pain. Hence, clinicians should focus their attention to specific waking-state oral habits and to psychological characteristics in the evaluation and treatment of diagnostic subgroups of TMD patients.

Chapter IV. General conclusions

The conclusion of all researches described in this thesis, is that a strong correlation between awake oral parafunctions and temporomandibular disorders exists, and in particular with Pain due to TMD, by confirming what widely explained in literature. The strength of those results is that they are based on standardized methods to make TMD diagnosis (DC/TMD) and valid objective (EMG) and subjective (checklists) ways for the assessment of awake oral behaviors. Moreover, we found that a self-report questionnaire like the OBC, also if not considered an objective tool, is highly reliable and valid in the detection of these behaviors and could be used more often in the clinical field of TMD diagnosis.

Based on this data, clinicians should focus on the reduction of awake parafunctional behaviors in the multifactorial treatment of patients affected from TMD. This might reduce, therefore, the overload and damage determined on temporomandibular joint and consequently the development of these disorders. Hence, clinicians should pay more attention and spend more time in the administering of the behavioral counselling that is fundamental to treat this typology of patients. However, more researches concerning this field, based on greater samples and standardized methods, should be conducted to expand and clarify the knowledge about this controversial topic.

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