Salivary oxidative stress markers' levels in patients with temporomandibular disorders

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Doctoral thesis / Disertacija

2020

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, School of Dental Medicine / Sveučilište u Zagrebu, Stomatološki fakultet**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:127:026845

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Zagreb, 2020

Sveučilište u Zagrebu

Stomatološki fakultet

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Vrijednosti salivarnih biljega oksidativnog stresa u ispitanika s temporomandibularnim poremećajima

DOKTORSKI RAD

Mentor:
prof. dr. sc. Iva Alajbeg

Zagreb, 2020.

This thesis has been conducted at The Department of Removable Prosthodontics, School of Dental Medicine, University of Zagreb. The work has been fully supported by the Croatian Science Foundation Project "The Role of Oxidative Stress and Opiorphin in Temporomandibular Disorders" (No. IP- 2014-09-3070; ethics approval 01-PA-26-6/15, item 3.2; project leader: Professor Iva Alajbeg), Operational Programme "Efficient Human Resources 2014-2020" and the European Social Fund within the "Young Researchers' Career Development Project – Training of Doctoral Students" under the mentorship of Professor Iva Alajbeg.

English language lector: David Rix, *writer, editor, proofreader, publishing*

Croatian language lector: Sanja Šterk, *mag. educ. philol. croat., mag. litt. comp.*

Commission for doctoral Thesis defence:

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Thesis defence date: 7 February 2020

The dissertation contains:
117 pages
29 figures
23 tables
1 CD

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Želim zahvaliti cijelom timu projekta “Uloga oksidativnog stresa i opiorfina u temporomandibularnim poremećajima”, a pritom bih izdvojila nekoliko imena. Veliko hvala Ivani Lapić na brzo napravljenim analizama uzoraka i pomoći pri metodologiji istraživanja, doc.dr.sc. Lei Vuletić na odličnim savjetima prilikom pisanja znanstvenih radova te prof.dr.sc. Ivanu Alajbegu na korisnim savjetima i podršci.

Hvala cijelom Zavodu za mobilnu protetiku, a osobito prof.dr.sc. Sonji Kraljević Šimunković na pomoći pri sakupljanju ispitanika.

Hvala izv.prof.dr.sc. Tomislavu Laucu koji mi je gotovo dvije godine izlazio u susret kako bih bila prisutna na svim obavezama projekta i doktorskog studija.

Hvala roditeljima, obitelji i prijateljima na podršci i savjetima te neiscrpnom izvoru optimizma i sreće.

Ipak, najveću zahvalnost dugujem svojoj mentorici, prof.dr.sc. Ivi Alajbeg, zbog toga što je vrhunski učitelj, velika podrška i sjajna osoba. Njezin odnos prema struci, znanosti i prenošenju znanja služit će mi kao inspiracija u daljnjem radu.

SUMMARY

Temporomandibular disorders (TMD) are associated with an altered oxidative status; however, the impact of treatment on changes in the oxidative status has not yet been clarified. With the aim of assessing their interaction with TMD, levels of selected salivary oxidative stress (OS) markers (glutathione peroxidase, superoxide dismutase (SOD), total antioxidant capacity (TAC), uric acid (UA), 8-hydroxydeoxyguanosine and malondialdehyde (MDA)) and salivary cortisol were compared between 34 chronic TMD patients and 33 healthy control subjects. Also, changes in the OS and clinical treatment outcomes (spontaneous pain, oral-health-related quality of life, self-perceived stress functional limitations, maximal and pain-free mouth opening, characteristic pain intensity) were followed during a 6-month treatment period where patients were randomly allocated to two treatment groups – stabilization splint group and placebo splint group. The changes were compared after the 1^{st}, 3^{rd} and 6^{th} months in the two treatment groups, as well as between the treatment groups when divided into diagnostic subgroups (myofascial pain and disc displacement) and when classified according to pain intensity (high-intensity pain and low-intensity pain).

Both the TAC and the individual OS markers (MDA and UA) were significantly higher in TMD patients compared to the control ($p<0.05$). This data suggests a compensatory increase in the antioxidant defence mechanism as a response to higher levels of stress. After 6 months of treatment, a significant reduction in the TAC was observed in both treatment groups ($p<0.05$). Moreover, a significant reduction was present for oxidant MDA levels and the oxidant (MDA) to antioxidant (SOD) ratio in patients with high-intensity pain ($p<0.05$). In patients treated with a stabilization splint, changes in the oxidative status were followed by a significant decrease of pain, improvement of health-related quality of life and functional limitations of the lower jaw. To sum up, a stabilization splint showed better treatment effectiveness during a 6-month period compared to a placebo splint. The oxidative status was altered in TMD patients compared to healthy controls and was demonstrated to be affected by splint therapy in favour of antioxidants. Both the intensity and source of the pain should be considered important factors in future investigations evaluating salivary OS markers in TMD patients.

Keywords: oxidative stress, temporomandibular disorders, orofacial pain, cortisol, stabilization splint, salivary diagnostics

SAŽETAK

VRIJEDNOSTI SALIVARNIH BILJEGA OKSIDATIVNOG STRESA U ISPITANIKA S TEMPOROMANDIBULARNIM POREMEĆAJIMA

Uvod

Temporomandibularni poremećaji (TMP) odnose se na skup problema koji se javljaju u području temporomandibularnih zglobova (TMZ), žvačnih mišića i okolnih struktura. Ako izuzmemo bolove uzrokovane dentalnim problemima, najčešći su uzrok bolova u orofacijalnom području. Simptomi zbog kojih pacijenti najčešće traže stručnu pomoć jesu bolovi žvačnih mišića i TMZ-a, ograničeno otvaranje usta te zvukovi u zglobu. Simptomi uzrokovani TMP-om često se javljaju kao kroničan problem gdje simptomatska akutna mikro- ili makroozljeda prelazi u dugotrajno stanje koje iziskuje posebnu, stručnu pomoć. Poremećajima su najčešće zahvaćene osobe ženskog spola stare između 20 i 40 godina. Temporomandibularni poremećaji smatraju se višečimbeničnim stanjem čija etiologija nije u potpunosti jasna. Upravo potonje određuje način dijagnostike i liječenja TMP-a, a nastojanja da se podloga u nastajanju i održavanju poremećaja razjasni, jedna je od glavnih tema znanstvenih istraživanja tog područja. Dijagnostika se uglavnom oslanja na kliničku interpretaciju simptoma pomoću validiranih upitnika, a ostale metode, primjerice radiološka dijagnostika, primjenjuju se kao pomoć pri nejasnim i složenim kliničkim slikama. Liječenje neinvazivnim i reverzibilnim metodama teži uklanjanju ili ublažavanju simptoma koji otežavaju pacijentovu svakodnevicu. Najčešće su korišteno terapijsko sredstvo za ublažavanje i uklanjanje simptoma TMP-a okluzijske udlage, od kojih se pak najčešće preporučuje stabilizacijska udlaga. Relativno nedavno javila su se nastojanja da se razjasni uloga stresa, koji se smatra jednim od rizičnih čimbenika ovih poremećaja. Osim subjektivne procjene stresa i povezivanja stresa s jačinom simptoma TMP-a (pomoću upitnika koje ispunjavaju sami pacijenti), s varijacijama u simptomima nastoje se povezati i promjene u oksidacijskom statusu, tj. prisutnost produkta neravnoteže između oksidansa i antioksidansa – oksidacijskog stresa (OS). Također, objektivni parametar za mjerenje stresa mogao bi biti i kortizol, hormon koji se često naziva indikatorom stresa. Istraživanjem spomenutih spojeva nastoji se razjasniti podloga TMP-a, ali i razviti objektivni dijagnostički alat koji bi služio za pronalazak i praćenje rizičnih pacijenata.

Ovo istraživanje sastojalo se od dvaju dijelova. U prvom dijelu, organiziranom kao istraživanje slučajeva i kontrola, cilj je bio izolirati i kvantificirati biljege OS-a i kortizol u slini pacijenata s kroničnim TMP-om te ih usporediti sa zdravom, kontrolnom skupinom.

U drugom su dijelu istraživanja, organiziranom kao nasumični klinički pokus, salivarni biljezi OS-a i ishodi liječenja u pacijenata s TMP-om praćeni tijekom 6 mjeseci. Pri tome su pacijenti slučajnim odabirom raspoređeni u dvije terapijske skupine [stabilizacijska udlaga (SU) i placebo-udlaga (PU)].

Materijali i postupci

Kliničko istraživanje provedeno je na Zavodu za mobilnu protetiku Stomatološkog fakulteta u Zagrebu od listopada 2016. godine do srpnja 2019. godine.

Kriteriji za uključivanje bili su: kronična bol koja je trajala dulje od 6 mjeseci, spontana bol veća od 30 mm na vizualno analognoj ljestvici (VAS), te dijagnoza miofascijalnog poremećaja ili pomaka zglobne pločice, postavljena uz pomoć dijagnostičkog kriterija za temporomandibularne poremećaje (DC/TMD). Pacijenti koji su zadovoljili uključne kriterije istraživanja bile su isključivo žene. Ispitanici su, prema preciznim uputama, sakupljali slinu dva puta dnevno: ujutro i poslijepodne. Iz sline su se izolirali biljezi OS [malondialdehid (MDA) i 8-hidroksideoksidgvanozin (8-OHdG)], antioksidacijski enzimi [superoksid dismutaza (SOD) i glutation peroksidaza (GPx), totalni antioksidacijski kapacitet (TAC)], kortizol (SC) te mokraćna kiselina (UA). U prvom dijelu istraživanja 34 pacijenta s TMP-om uspoređeno je s 33 kontrolna, zdrava ispitanika, koji su im odgovarali po dobi i spolu. U drugom dijelu istaživanja 34 pacijenta s TMP-om randomizirano je u dvije terapijske skupine – pacijenti liječeni stabilizacijskom udlagom (SS) i pacijenti liječeni placebo udlagom (PS) koji su praćeni tijekom 6 mjeseci s kontrolnim pregledima nakon prvog (T1), trećeg (T2) i šestog (T3) mjeseca liječenja. Uz biljege oksidacijskog stresa, pratili su se i sljedeći ishodi liječenja: iznos otvaranja usta [bezbolno otvaranje (MCO) i maksimalno otvaranje (MMO)], spontana bol prema VAS, kvaliteta života ovisna o oralnom zdravlju (OHIP-14 upitnik; engl. *Oral Health Impact Profile)*, samoprocjenjena razina stresa (PSS upitnik; engl. *Perceived Stress Scale*), specifični intenzitet boli (prema GCPS ljestvici stupnjevanja kronične boli; engl. G*raded Chronic Pain Scale*), te ograničenja funkcije donje čeljusti (JFLS engl. *Jaw Functional Limitation Scale*). Svi ispitivani parametri (biljezi oksidacijskog stresa i ishodi liječenja) bili su uspoređeni između dvije terapijske skupine, pri čemu su pacijenti bili podijeljeni u dijagnostičke podskupine TMP-a

[miofascijalna bol (eng. *myofascial pain MP*) i pomak zglobne pločice (eng. *disc displacement DD*)] te podskupine prema intenzitetu boli [visoki (VIB) i niski (NIB) intenzitet boli].

Za testiranje distribucije podataka korišten je Shapiro-Wilk test. Prije provođenja statističkih analiza svi podaci koji nisu slijedili normalnu distribuciju logaritamski su transformirani. U prvom dijelu istraživanja željelo se ispitati razlikuju li se ispitanici s TMP-om od kontrolne skupine prema mjerenim salivarnim parametrima (biljezi OS i SC). Studentov t-test korišten je za usporedbu dviju skupina (TMP u odnosu na kontrolu) dok je za ispitivanje razlika između tri skupine (kontrola u odnosu na TMP podskupine) korištena analiza varijance (ANOVA). Za procjenu povezanosti koncentracija salivarnog kortizola, razine percipiranog stresa i biljega OS korištena je Pearsonova korelacija.

U drugom dijelu istraživanja promjene u ishodima liječenja (VAS, OHIP-14, PSS, GCPS, MCO i MMO) i koncentracijama biljega OS i SC nakon 3 i 6 mjeseci liječenja u odnosu na početne vrijednosti unutar terapijskih skupina analizirane su koristeći jednosmjernu analizu varijance (within-subjects' ANOVA). Zatim su biljezi OS i SC, kao i ishodi liječenja (VAS, OHIP-14, PSS, GCPS, MCO i MMO) analizirani mješovitim modelom analize varijance ponovljenih mjerenja koristeći čimbenik "vrijeme" (T0, T1, T2, T3) kao izvor varijabilnosti unutar subjekata te čimbenike "vrsta terapije" (SU i PU), "dijagnostička podskupina" (MP i DD) i „intenzitet boli" (VIB i NIB) kao izvore varijabilnosti između subjekata, nakon čega su slijedili post hoc testovi. Eta kvadrat ($\eta 2$) služio je procjeni veličine efekta. Statistički značajnom smatrala se vrijednost $p < 0,05$.

Rezultati

Pacijenti s TMP-om i zdravi ispitanici nisu se značajno razlikovali po dobi, baš kao što se nisu statistički razlikovale niti mjere ishoda prije početka terapije između dvaju terapijskih skupina TMP-a ($p > 0,05$).

Usporedbom pacijenata s TMP-om i kontrolne skupine dobiveni su sljedeći rezultati: koncentracije jutarnjeg i popodnevnog TAC-a ($p=0,042$, $p=0,04$, respektivno), koncentracija jutarnje UA ($p=0,014$) i koncentracija popodnevnog MDA ($p=0,03$) bile su značajno više u skupini s TMP-om u usporedbi s kontrolnom skupinom. Usporedbom kontrolne skupine s dijagnostičkim podskupinama TMP bilo je vidljivo kako su SC ($p=0,003$) i UA ($p=0,04$) značajno viši, a popodnevni GPx značajno niži ($p=0,01$) u MP podskupini u usporedbi s

kontrolom. Također, značajno više koncentracije 8-OHdG-a bile su prisutne u DD podskupini (jutro: p=0,011; podne: p=0,009) u usporedbi s MP podskupinom. Usporedbom kontrolne skupine s TMP podskupinama s obzirom na intenzitet boli pokazalo se kako su jutarnji i popodnevni MDA (p=0,043, p=0,02, respektivno) te jutarnja UA (p=0,02) bili značajno viši u podskupini s VIB u usporedbi s kontrolom. Značajno niže koncentracije GPx-a (p=0,04) pronađene su u podskupini s NIB u usporedbi s kontrolom. Gledajući čitavu TMP skupinu, pronađena je značajna pozitivna korelacija između percipiranog stresa i salivarnog GPx-a te percipiranog stresa i MDA (r=0,425; r=0,472, respektivno). Više vrijednosti PSS-a bile su povezane i s višim vrijednostima MDA i GPx-a u DD podskupini (r=0,588; r=0,504 respektivno) i podskupini s VIB (r=0,545; r=0,655 respektivno). Jutarnji SC bio je pozitivno koreliran s GPx-om i UA u MP podskupini (r=0,643; r=0,592 respektivno) i podskupini s NIB (r=0,529; r=0,512 respektivno).

U obje terapijske skupine značajno se smanjila spontana bol, procijenjena prema vizualno analognoj ljestvici. Promjene u spontanoj boli značajno su se razlikovale između terapijskih skupina (interakcija vrijeme x terapijska skupina: Wilks Lambda=0,39, F=10,25; p=0,0002, veličina učinka=0,29) pri čemu su u skupini liječenoj SU prosječne vrijednosti VAS-a bile su značajno niže 1., 3. i 6. mjesec liječenja u odnosu na početnu vrijednost (p=0,0007, p=0,001 i p <0,0001 respektivno), dok je u skupini liječenoj PU značajna razlika bila prisutna samo između 6. mjeseca liječenja u usporedbi s početnim mjerenjem (p=0,004). Značajne razlike između dvije terapijske skupine pronađene su i za vrijednosti OHIP-14 upitnika (interakcija vrijeme x terapijska skupina: Wilks Lambda=0,56, F = 5,20; p=0,008, veličina učinka=0,18) i GCPS ljestvicu (interakcija vrijeme x terapijska skupina: Wilks Lambda=0,67, F=3,20, p=0,045, veličina učinka=0,17), pri čemu je poboljšanje kvalitete života ovisne o oralnom zdravlju i smanjenje karakterističnog intenziteta boli zabilježeno samo u SU skupini. Iako nisu pronađene razlike između terapijskih skupina za vrijednosti maksimalnog neasistiranog otvaranja usta, analiza unutar subjekata pokazala je značajno poboljšanje za SU skupinu (Wilks Lambda=0,53, F=4,04, p =0,029, veličina učinka=0,31), ali ne i za PU skupinu.

Koncentracije UA značajno su se mijenjale tijekom vremena (interakcija vrijeme x terapijska skupina x intenzitet boli: Wilks Lambda=0,55, F=5,32 p=0,007, veličina učinka=0,14) s većim padom koncentracija UA u SU skupini u usporedbi s PU skupinom; post-hoc analiza pokazala je da su koncentracije UA značajno niže u SS skupini 3. mjesec (HIP: p=0,015) i 6. mjesec

(LIP: p=0,007) u usporedbi s početnim mjerenjem. Tijekom perioda liječenja od 6 mjeseci došlo je do pada koncentracija TAC-a (ujutro: Wilks Lambda=0,59, F=4,57, p=0,013, veličina učinka=0,18; poslijepodne: Wilks Lambda=0,57, F=4,85, p=0,01, veličina učinka=0,15), međutim bez statistički značajne razlike između terapijskih skupina. Iako nisu pronađene razlike za vrijednosti salivarnog TAC-a između terapijskih skupina, analiza unutar subjekata pokazala je značajno smanjenje koncentracija jutarnjeg (ujutro: Wilks Lambda=0,49, F=4,72, p=0,017, veličina učinka=0,24) i popodnevnog TAC-a (poslijepodne: Wilks Lambda=0,32, F=9,64, p=0,001, veličina učinka=0,21) u skupini liječenoj SU, ali ne i u PU skupini. Koncentracije jutarnjeg MDA značajno su se smanjile u bolesnika s VIB u usporedbi s početnim mjerenjem (p=0,02); koncentracije popodnevnog MDA značajno su se smanjile u bolesnika s VIB liječenih SS u usporedbi s placebom (interakcija vrijeme x terapijska skupina x intenzitet boli: Wilks Lambda=0,56, F=4,87; p=0,011, veličina učinka=0,13). Koncentracije SC-a značajno su porasle u bolesnika s DD-om liječenih placebo udlagom (interakcija vrijeme x terapijska podskupina x dijagnostička podskupina: Wilks Lambda=0,46, F=7,75, p=0,001, veličina učinka=0,16).

Zaključak

U kroničnih pacijenata s TMP-om pronađene su značajno više koncentracije TAC-a, UA i MDA u usporedbi s kontrolnom skupinom. Više koncentracije antioksidansa u pacijenata s kroničnim bolnim poremećajima mogle bi se objasniti kao kompenzacijski porast i odgovor na dugoročno više koncentracije oksidansa. S obzirom na to da je u skupini liječenoj stabilizacijskom udlagom znatnije smanjena spontana bol, poboljšana kvaliteta života te poboljšana funkcija donje čeljusti u usporedbi sa skupinom liječenom placebo udlagom, možemo zaključiti da je stabilizacijska udlaga pokazala bolju učinkovitost tijekom šestomjesečnog liječenja. Iako bi placebo mogao biti dijelom odgovoran za poboljšanje simptoma TMP-a, vjerojatno ne može zadržati kontinuirani dugoročni pozitivni terapijski učinak. Tome u prilog idu i promjene u oksidacijskom statusu kroz terapijski period u obje terapijske skupine, s boljim odgovorom pojedinih biljega (salivarni UA) u SS terapijskoj podskupini. Dobiveni rezultati potvrđuju kako se u budućim istraživanjima u obzir trebaju uzeti izvor boli (TMP zglobnog ili mišićnog porijekla), intenzitet boli (visoki ili niski), kao i vrijeme prikupljanja sline.

Ključne riječi: oksidacijski stres, temporomandibularni poremećaji, orofacijalna bol, kortizol, stabilizacijska udlaga, salivarna dijagnostika

List of abbreviations

8-OHdG	8-hydroxydeoxyguanosine
AAOP	American academy of orofacial pain
CBCT	cone-beam computed tomography
CNS	central nervous system
CPI	chronic pain intensity
CT	computed tomography
DC/TMD	diagnostic criteria for TMD
DD	disc displacement
DDWR	disc displacement with reduction
DDWoR	disc displacement without reduction
GCPS	graded chronic pain scale
GPx	glutathione peroxidase
HIP	high-intensity pain
JFLS	jaw functional limitation scale
LIP	low-intensity pain
MCO	maximal comfortable mouth opening (pain-free opening)
MDA	malondialdehyde
MMO	maximal unassisted mouth opening
MP	myofascial pain
MRI	magnetic resonance imaging
NSAIDs	nonsteroidal anti-inflammatory drugs
OHIP-14	oral health impact profile-14
OS	oxidative stress
PS	placebo splint
RDC/TMD	research diagnostic criteria for TMD
ROS	reactive oxygen species
SC	salivary cortisol
Sens.	sensitivity
Spec.	specificity
SOD	superoxide dismutase
SS	stabilization splint
TAC	total antioxidant capacity
TENS	transcutaneous electric nerve stimulation
TMD	temporomandibular disorders
TMJ	temporomandibular joint
UA	uric acid
VAS	visual analogue scale

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1. INTRODUCTION

1.1. Temporomandibular disorders

1.1.1. Definition, aetiology, prevalence

Temporomandibular disorders (TMD) are the second most common cause of musculoskeletal pain and disability after lower back pain (1) and the most common cause of orofacial pain, not counting dental pain (2). It is a hypernym covering musculoskeletal and neuromuscular conditions that cause pain and dysfunction of the masticatory muscles, temporomandibular joints and associated structures (3, 4).

The principal symptoms that lead the patient to the clinician are (3):

A. pain in the joint, masticatory muscles and/or surrounding structures.
B. limitation of the lower jaw movement (usually reduced opening ability), and
C. joint sounds (clicking noises or crepitation).

The pain and the limitation can range from mild to intense, and sometimes can be so severe that they hamper normal daily routines and impact the patients' psychosocial functioning and quality of life (5). Other symptoms reported by patients are often a pain in the face, pain in the head and ear, tinnitus, ear fullness and vertigo (6).

The aetiology of TMD is still largely unresolved and to this day there is no unified opinion on what exactly causes the disorder. What is known is that TMD is of multifactorial origin associated with various risk factors including biological factors, behavioural factors, individual anatomy, injuries, stressors, pharmacotherapy, occlusal interferences and occlusal factors, neuroendocrine elements, genetics and systemic diseases (7). None of these factors are believed to be the primary cause of TMD, but TMD is rather a consequence of a person's susceptibility to various risk factor combinations. These factors are often clinically referred to as 'predisposing', 'initiating' and 'perpetuating' aspects of TMD (4, 8). In the past, occlusion was strongly related to TMD and to this day remains the most controversial risk factor for the initiation of TMD. Nowadays, occlusion is considered a potential cofactor with a much lower weight compared to previous beliefs (9). Some occlusal factors – skeletal class II, open bite, posterior crossbite, lowered vertical dimension of occlusion with high level of abrasion, lack of posterior teeth or improperly treated edentulism – are still mentioned as factors that, among

others, might have a modulatory role in existing TMD, but are not considered the main aetiological factors (10).

The prevalence of TMD is between 5% and 12% in the general population. Temporomandibular disorders affect both men and women of all ages with a greater occurrence in women with a female to male ratio of 2:1 in the general population and even greater prevalence in women in clinical settings. The annual incidence is around 2% with a peak at 20-40 years of age (2, 10). It has been estimated that TMD results in 17,800,000 lost workdays per year for every 100,000,000 working adults in the United States, therefore it carries a significant financial burden from loss of work. Because of the relatively high prevalence and the fact that they seriously affect one's psychosocial aspect of life, TMD represents a serious public health problem (10).

A systematic review including only studies adopting the Research Diagnostic Criteria for TMD reported a prevalence of up to 13% for masticatory muscle pain, up to 16% for disc derangement disorders and up to 9% for TMJ pain in the general population (11).

The result of the meta-analysis, conducted on 15 studies on TMD patients, showed that the overall prevalence was 45.3% (1400 patients out of 3091 for whom data were available) for group I diagnoses (muscle disorders), 41.1% (414/1006) for group II (disc displacements), and 30.1% (233/740) for group III (arthralgia, osteoarthritis, osteoarthrosis). Studies on general populations accounted for a total of 2491 subjects, with an overall 9.7% prevalence for group I, 11.4% for group IIa (disc displacement with reduction), and 2.6% for group IIIa diagnoses (joint pain) (11).

Most subjects with clinically detectable dysfunction are functioning adequately without significant symptoms. It seems that 3.6% to 7% of individuals with TMD are estimated to be in need of treatment (12, 13).

1.1.2. TMD-specific diagnostics subgroups

Temporomandibular disorders (TMD) encompass several diagnostic subgroups with different aetiologies. Disorders can be of intra-articular (joint) or extra-articular (muscle or surrounding structures) origin (2).

Various TMD classifications have been proposed in the literature and, therefore, the patient's examination protocols differed substantially in the past. Moreover, little data was available on the accuracy of most protocols and techniques for detecting signs and symptoms of TMD. Two leading associations the American Academy of Orofacial Pain (AAOP), and the International Research Diagnostic Criteria Consortium for Temporomandibular Disorders (RDC/TMD) have sought to equalize the classification systems for temporomandibular disorders to obtain equal criteria everywhere in the world (14, 15).

The AAOP uses the expanded taxonomy developed jointly with the International RDC-TMD Consortium Network According to the AAOP classification:

(i) masticatory muscle disorders are divided into:

A. muscle pain ((i) myalgia (local myalgia, myofascial pain with spreading, myofascial pain with referral), (ii) tendonitis, (iii) myositis and (iv) spasm)),
B. contracture ((i) muscle and (ii) tendon)
C. hypertrophy
D. neoplasm
E. movement disorders ((i) orofacial dyskinesia and (ii) oromandibular dystonia)
F. masticatory muscle pain attributed to systemic/central disorders ((i) centrally mediated myalgia and (ii) fibromyalgia).

(ii) temporomandibular joint disorders are divided into:

A. joint pain ((i) arthralgia and (ii) arthritis)
B. joint disorders ((i) disc-condyle complex disorders, (ii) other hypomobility disorders and (iii) hypermobility disorders)
C. joint diseases ((i) degenerative joint diseases, (ii) codylysis/idiopathic condylar resorption, (iii) osteochondritis dissecans, (iv) osteonecrosis, (v) systemic arthritides, (vi) neoplasm and (vii) synovial chondromatosis)
D. fractures
E. congenital/developmental disorders ((i) aplasia (ii) hypoplasia and (iii) hyperplasia) (14)

The Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) guidelines, established in 1992, provide standardized criteria for a two-axis diagnosis: physical diagnosis (axis I) and

psychosocial diagnosis (axis II). According to the RDC/TMD, clinical diagnoses are divided into three groups:

A. muscular disorders (myofascial pain; myofascial pain with limited opening),
B. disc displacement disorders (disc displacement with reduction; disk displacement without reduction with limited opening; disk displacement without reduction without limited opening),
C. arthralgia and other joint disorders (arthralgia; osteoarthritis; osteoarthrosis).

In 2014, a new diagnostic criterion was introduced. The DC/TMD is a dual-axis system based upon the biopsychosocial model of pain developed to simplify the use of protocols for clinical purposes. The validated Axis I DC/TMD diagnoses with its sensitivity (Sens.) and specificity (Spec.) values are (1, 11):

A. Pain-related temporomandibular disorders [myalgia (Sens. 0.90/ Spec. 0.99): myalgia, myofascial pain and myofascial pain with referral; arthralgia (Sens. 0.89/Spec. 0.98); headache attributed to TMD (Sens. 0.89/Spec. 0.87)],
B. Intra-articular temporomandibular disorders [disc displacement with reduction (Sens. 0.34/Spec. 0.92; disc displacement with reduction with intermittent locking (Sens. 0.38 Spec. 0.98); disc displacement without reduction with limited opening (Sens. 0.80/Spec. 0.97); disc displacement without reduction without limited opening (Sens. 0.54/Spec. 0.79); degenerative joint disease (Sens. 0.55/Spec. 0.61); subluxation (Sens. 0.98/Spec. 1.00)].

The acceptable sensitivity and specificity for a definitive diagnosis are considered to be sensitivity $\geq$ 70% and specificity $\geq$ 95%.

Considering the influence of the central nervous system on the perception and modulation of pain, Jeffrey P. Okeson proposed a classification system of masticatory muscle disorders that takes into account both physical and psychological axes in the mechanisms of a pain disorder (16). According to this classification, masticatory muscle disorders include protective co-contraction, local muscle soreness, myofascial pain, miospasm, centrally mediated myalgia, and fibromyalgia.

Regarding intra-articular disorder, this classification recognizes derangements of the condyle-disc complex (disc displacement, disc dislocation with reduction, and disc dislocation without

reduction). In addition, temporomandibular joint disorders include structural incompatibilities of the articular surfaces that may be present as deviations in form, adherences and adhesions, subluxation and spontaneous dislocations (luxation). The third in this classification are inflammatory disorders of the temporomandibular joint (synovitis/capsulitis, retrodiscitis and arthritis). Moreover, this classification recognizes inflammatory disorders of the adjacent structures, which include temporal tendonitis and inflammation of the stylomandibular ligament.

Research suggests that musculoskeletal conditions are the most common cause of TMD, and disc displacement is the most common intra-articular condition affecting the TMJ (17, 18).

1.1.2.1 Myalgia

Myalgia is defined as pain of muscular origin affected by jaw movement, function and/or parafunctional activities (i.e. sleep bruxism).

During clinical examination, the clinician can provoke pain in the patient's masticatory muscles using palpation. It is recommended that the force of the palpation should be 1 kg (1). Also, patients report pain during the movement of the lower jaw and mastication. Limitation of mandibular movements secondary to pain may be present. According to the DC/TMD, there are three subtypes of myalgia: local myalgia, myofascial pain and myofascial pain with referral. Local myalgia is defined as the pain the patient feels at the site of palpation. In myofascial pain, the pain is spreading beyond the location of the palpating fingers, however the pain is located within the boundary of the muscle being palpated. Myofascial pain with referral is pain that is spreading beyond the boundary of the palpated muscle and can be felt in surrounding structures of non-muscular origin (such as the ear, teeth or eyes).

To diagnose local myalgia, history must be positive for both:

- pain in the jaw, temple or in front of the ear in the last 30 days
- pain changing with jaw movement, function or parafunction

examination of the masseter and temporalis muscle must confirm both:

- pain location in the area of the masseter and temporalis muscle
- familiar muscle pain with palpation or during maximum unassisted or assisted opening

1.1.2.2. Temporomandibular joint disc displacements

In a normal joint, the thin intermediate zone of the disk is always interposed between the condyle and the temporal bone in both the closed-mouth and open-mouth positions. The disk is firmly attached to the medial and lateral poles of the mandibular condyle. This allows simultaneous movements of the disk and the condyle. In the closed mouth position, the condyle is centred in the glenoid fossa. The disk is interposed between the condyle inferiorly and the glenoid fossa superiorly and the articular eminence is anterior to the disk. When the mouth opens, the condyle moves anteriorly while the disc moves posteriorly on the condyle. The superior retrodiscal lamina lengthens, allowing the condyle-disc complex to translate out of the glenoid fossa. The disc maintains its position on the condyle during movement because of its morphology and interarticular pressure provided by the elevator muscles. The close relationship between the disc and the condyle prevents articular damage.

Destructive forces occurring in the joint can irreversibly alter the structure of the joint and, thus lead to disc-condyle complex abnormalities. Such abnormalities in the positioning of a disc in relation to the condyle are called disc displacements, equally known as internal derangements (19).

Disc displacement encompasses several stages of an abnormal relationship between the articular disc and the condyle.

Disc displacement (DD)

In the initial phase, the morphology of the disc is altered and the disc changes its position in relation to the condyle. The displacement usually occurs due to the elongation of the retrodiscal lamina and the collateral ligament (16, 20-22). Posterior and mediolateral displacements of the disc are described, but the most frequent changes are anterior disc displacements (23-27). Pain may be absent or may be experienced occasionally (16, 28, 29). The length of the discal ligaments and the thickness of the posterior border of the disc limit the forward movement of the disc in this initial phase. The superior lateral pterygoid muscle pulls the disc (not only forward but also medially on the condyle). If the pull of the superior lateral pterygoid muscle is prolonged, the posterior border of the disc may become thinner. Once the posterior border

becomes thinner, the disc can be displaced further in an anterior direction so that the condyle becomes positioned on the posterior border of the disc.

Disc displacement with reduction (DDWR)

Further deterioration of the condition results in the continued thinning of the posterior border of the disc. The disc is placed more to the anterior (and medially) due to superior lateral pterygoid muscle action, and the discal ligaments are further elongated. The condyle becomes positioned more posteriorly on the posterior border of the disc. In the closed mouth position, the disc is dislocated anteriorly, however, during opening, the condyle passes over the posterior border of the disc, thus reducing the dislocated disc and resulting in the clinically present sound – clicking. During closing, the normal disc position is maintained until the condyle returns to very near the closed position. In the last part of closing, the disc is again dislocated and a second clicking sound may occur (reciprocal clicking sound).

In order to diagnose disc displacement with reduction, the history must be positive for:

- joint noise in the last 30 days

the examination must confirm:

- a clicking, popping and snapping noise detected during opening and closing with palpation (during at least one of three repetitions)

Usually, when present without any additional symptom (pain or limited mouth opening), sounds in the joint represent the physiological adjustment to newly occurred state. Moreover, the clicking sound can be caused by an irregularity of the articulating surfaces, lack of synovial fluid or deformation of the disc. If the patient does not experience any other symptom, treatment is not required (30-34).

Disc displacement without reduction (DDWoR)

The next and the most severe stage of the disc displacement is a condition where the disc is permanently dislocated with no possibility for the condyle to recapture the disc. In the closed mouth position, the disc is in an anterior position relative to the condylar head, and the disc does not reduce with the opening of the mouth. Medial and lateral displacement of the disc may also be present. This disorder is usually associated with limited mandibular opening.

In order to diagnose disc displacement without reduction with limited opening, the history must be positive for both:

- jaw lock or catch so that the mouth will not open all the way
- limitation in jaw opening severe enough to interfere with the ability to eat

the examination must confirm that:

- the maximum assisted mouth opening is less than 40 mm (including vertical overlap)

The presence of an uncorrected deviation to the affected side (the mandible will turn to the side while opening the mouth without returning to the medial line when the mouth is fully opened) can help corroborate the diagnosis. The use of adequate therapy can reduce the feeling of pain and could eventually help the patient open his/her mouth near the physiological range (~36-70 mm) (35-37).

When the diagnosis needs to be confirmed, an MRI of the TMJ will reveal:

- the posterior border of the disc is located anterior to the 11:30 position and the intermediate zone of the disc is located anterior to the condylar head in the maximal intercuspal position
- in the fully opened position, the intermediate zone of the disc is located anterior to the condylar head

Even though it is believed that the disc will become more anteriorly displaced over time, it is not likely that all patients with a displaced disc would experience DDWoR, moreover the objective alterations present in the joint on the imaging exams may not be related to the severity of the clinical findings and symptoms (38).

1.1.3. Methods for TMD diagnosis

TMD diagnosis is hampered by the limited knowledge of the aetiology and natural progression of the disorders. The multifactorial origin of the disorders requires understandable and reliable diagnostic principles that could be used for both clinical and research purposes. Because TMD affects an area where various pain disorders can occur, it is important to exclude any condition that could mimic TMD (i.e. dental caries or abscess, oral lesions, maxillary sinusitis, salivary gland disorders, trigeminal neuralgia, postherpetic neuralgia, glossopharyngeal neuralgia, giant cell arteritis, primary headache syndrome and pain associated with cancer). Moreover, TMD can be secondary to a systemic disease as a consequence of, for instance, rheumatoid arthritis and juvenile rheumatoid arthritis or can be present as a comorbid pain condition (39, 40). It is of great importance to detect other pathognomonic symptoms if present.

In patients with TMD, special attention should be paid to the history. Through a detailed history, a clinician can find out whether the patient has experienced related trauma in the past, have parafunctions such as clenching of the teeth during the day and/or night or other oral and behavioural habits. The examination protocol, which follows the history, should combine physical and psychosocial aspects and lead the examiner, despite the heterogeneity of clinical findings, to the "correct" diagnosis.

A large number of diagnostic protocols and instruments have been in use over last three decades (Helkimo index, Hamburg protocol, M. Kleinrok protocol, RDC/TMD, DC/TMD). Mostly, all of them are based on a detailed clinical assessment (41).

Due to the significant influence of pain and disability on the daily functioning of patients, an important aspect of the TMD diagnostics is the behavioural approach. Many studies have shown that patients who suffer from TMD-related pain should be carefully assessed according to the biopsychosocial model. The Original Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) was the first step toward a uniformed TMD diagnosis and classification. However, the need to improve its validity and clinical efficiency emerged (1).

The latest evidence-based diagnostic protocol for assessing pain-related TMD and the differentiation of the most common pain-related TMD conditions follows the dual-axis Diagnostic Criteria for TMD (DC/TMD), with axis I being a self-report instrument used to assess the presence of any pain-related TMD and axis II being an instrument for the evaluation of psychosocial and behavioural functioning consisting of self-report screening and

comprehensive instruments: The Patient Health Questionnaire-4 (PHQ-4) assesses psychological distress, the Graded Chronic Pain Scale (GCPS) assesses pain-related disability and pain intensity, the Jaw Functioning Limitation Scale (JFLS) assesses disability and limitations of lower jaw movements, the Oral Behavioural Checklist (OBC) assesses the number and frequency of oral parafunctions, Generalized Anxiety Disorder-7 (GAD-7) assesses anxiety and the Patient Health Questionnaires PHQ-5 assesses depression and PHQ-15 assesses physical symptoms (1).

An ideal diagnostic instrument should allow detecting limitations in jaw motion and assessing the source of pain, and perhaps, if possible, give information about the possible causes of the signs and symptoms to provide the basis for differential diagnosis.

A ruler and the observation of the patterns of jaw movements are sufficient to assess a restriction in mouth opening (42). In the absence of a gold standard for pain rating, clinical evaluation remains the most reliable diagnostic approach (43). A clinical evaluation should be performed by a trained examiner.

Imaging techniques can offer additional information in the case of vague or equivocal symptomatology, for diagnosis confirmation of degenerative changes or disc displacement or for the purpose of scientific research. Frequently used diagnostic imaging methods are conventional radiography (panoramic radiography), computed tomography (CT), cone-beam CT (CBCT), magnetic resonance imaging (MRI) and ultrasonography. Magnetic resonance imaging is the first choice, representing the standard of reference for soft tissue assessment and it allows the evaluation of the disc position, the exact localization of joint effusions and structural abnormalities (44). CT or CBCT should be used for the most complex changes in bony structures (45). According to the present knowledge, there is no place for orthopantomography in the specialist phase of TMD diagnosis, except to exclude other possible causes of symptoms. Ultrasonography is a noninvasive and dynamic technique that may be useful for an assessment of joint effusion rather than disc displacement evaluation (46).

1.1.4. Pain modulation models and long-lasting pain effects

Chronic pain is an important public health problem. The understanding of pain modulation is mandatory for the clinicians who are dealing with painful disorders as different types of pain require different treatment approaches. Acute pain usually resolves within a reasonable amount of time. The assessment of chronic pain is more complex, however. The clinician must take into consideration the differences in the vulnerability of individuals to develop chronic pain after injury. Research suggests that a deficient descending inhibitory system is an important aspect in determining whether pain may become chronic. To comprehend this, it is important to understand the mechanisms of descending inhibition and the processes that can alter its function.

When peripheral neurons are stimulated by low pain intensity impulses, the impulses descend to the dorsal horn of the spinal cord, where corresponding painful stimuli are transmitted to the second-order neuron, which activates the descending inhibition. From that moment, the production of neurotransmitters starts, among which the most important mediators of pain elimination are serotonin and norepinephrine (47-50). Simultaneously, neurons of the second-order connect to the reticular formation, which then provides adaptations of motor and vascular behaviour. The whole process of pain inhibition, with signals being sent to the periphery, happens without the cortex being involved (51). Pain of low intensity will thus be solved at a subconscious level and will have no effect on the functioning of the cortex. If the descending inhibitory system does not work properly, the cortex and other parts of central nervous system (CNS) will become abnormally agitated and will experience non-painful stimuli as pain (16).

Another pain modulatory mechanism is called the "Gate Control" theory. The theory proposes that a non-painful input closes the "gate" to a painful input, which results in the avoidance of painful stimulations travelling to the CNS. This modulation is possible because the collaterals of the large sensory fibres (A alpha fibres) carrying cutaneous sensory input activate the inhibitory interneurons, which inhibit pain transmission information carried by the pain fibres (C fibres). The proposed theory is the underlying mechanism of pain management with transcutaneous electric nerve stimulation (TENS) (52).

With the presence of enhanced pain or long-lasting pain, the third-order neurons are stimulated. At that moment, the signals are being carried from the thalamus to the primary sensory cortex and increased inhibition occurs. The norepinephrine and serotonin pathways of pain elimination

are activated. They inhibit the transmission of painful stimuli to the first and second-order neurons. In the subnucleus caudalis or the dorsal horn of the spinal cord, it is mediated through endogenous opioids such as Gamma-Aminobutyric acid (GABA) and other inhibitory amino acids (53, 54). The recognition of these inhibitory amino acids is also enhanced in various stressful situations such as fear, depression and anxiety (55).

1.1.4.1. The underlying mechanisms of chronic pain, allodynia and hyperalgesia

Allodynia is a phenomenon that occurs when stimuli that do not usually cause pain, provoke a painful response. Hyperalgesia is an exaggerated response to low or mild intensity pain. Both pain phenomena are the result of central sensitization, which leads to an increased response from the neurons.

Prolonged and constant stimulation of the CNS may consequently lead to first peripheral and then central sensitization. The sensation of acute pain results from the activation of Aδ-fiber and C-fibre polymodal muscle nociceptors. The nociceptors are sensitized by the release of neuropeptides from the nerve endings. This may eventually lead to the central sensitization of dorsal horn second-order neurons manifested as prolonged neuronal discharges, increased responses to defined noxious stimuli, non-noxious stimuli and the extension of the receptive field (56). Lower thresholds of nociceptors caused by peripheral sensitization may likewise contribute to heightened pain complaints. After an initial burst of nociceptive stimuli, a small amount of peripheral stimulation is necessary for the maintenance of central sensitization (57).

In addition, it is suggested that genetic factors play a role in the aetiology of persistent pain conditions, presumably by modulating underlying processes such as nociceptive sensitivity, psychological well-being, inflammation and autonomic response (58).

Chronic craniofacial pain is generally described as a persistent pain that lasts at least 3 to 6 months and is associated with behavioural, psychological and psychosocial factors similar to those of other chronic pain conditions. It is very important to provide a comprehensive assessment for all patients with TMD based on the understanding of the biopsychosocial model in disease.

1.1.5. Current knowledge of therapeutic modalities for TMD

Management goals for TMD patients include decreased pain, decreased adverse loading, restoration of function and the resumption of normal daily activities. These management goals are best achieved through conservative, noninvasive (reversible) treatment such as behavioural therapy, self-management, physical therapy, pharmacotherapy (medications) and occlusal appliances.

The use of aggressive, invasive and irreversible treatments, such as surgery, arthroscopy, arthrocentesis and complex occlusal therapy should be avoided. The application of hyaluronic acid has a limited role in the treatment of TMD because of the invasiveness of its application (59, 60). However, treatment with botulinum toxin type A injection into the muscles showed a certain effectiveness in the treatment of myofascial pain but more studies on the subject need to be done (61).

Because most treatment approaches are reported to be similarly effective with little or no differences in treatment outcomes, conservative, noninvasive and reversible treatment modalities are preferred. Despite the increasing evidence that TMDs are best managed with conservative, reversible treatments, some clinicians continue to choose treatments based on personal biases rather than controlled scientific investigation. Research showed that the combination of non-invasive modalities provided better results than the use of just one treatment option (62-64), therefore the emphasis is on the multidisciplinary approach that often binds physical therapy, the behavioural approach and occlusal splint therapy with the use of pharmacotherapy when needed.

Often, due to the disorder's tendency to chronicity, treatment will be difficult and will require a professional and thorough approach. The differences between objective clinical manifestation and problems that patients are experiencing are creating an inconsistency between practitioners, which sometimes results in therapies that can mimic treatment success (65). Clinicians should aim for evidence-based treatment modalities that are successful in the management of TMD pain and disabilities.

1.1.5.1. Pharmacotherapy

Pharmacologic agents for the treatment of TMD range from short-term treatment, usually the treatment choice for the acute phase of the disorder (i.e. injuries), to the chronic administration of antidepressants and anticonvulsants usually prescribed in cases of persistent musculoskeletal pain and complex neuropathic pain. Although depression is often a comorbid feature found in chronic pain patients, some antidepressants have demonstrated efficacy in nondepressed individuals too, suggesting an additional mechanism of action besides their antidepressant activity. Research suggests that in patients who have chronic pain, the analgesic effect of antidepressants is often more rapid than their mood-altering effects (66).

Short-term treatment with non-steroidal anti-inflammatory drugs (NSAIDs) in combination with a muscle relaxant or benzodiazepine and physical therapy is usually the treatment of choice for pain of musculoskeletal origin (67). The use of NSAIDs leads to pain relief resulting in a reduction of peripheral sensitization by lessening the overload, fatigue and release of inflammatory mediators in the TMJ and masticatory muscles (60).

It is important to note that all drugs should be used with caution and require careful management and monitoring.

1.1.5.2. Physical Medicine

Physical therapy is a widely recommended treatment modality for musculoskeletal disorders. It encompasses ultrasound and thermal therapies, acupuncture, low-level laser therapy, TENS, pulsed electromagnetic fields and various combinations of these therapeutic options. Studies confirmed a certain benefit from the mentioned modalities. The problem was that studies compared different forms of therapies and many treatment regimens included several forms of intervention, thus the interpretation of these results are open to question. However, there seems to be good evidence considering the benefits of jaw manipulation and exercise because exercise and stretching can increase the range of movement of the lower jaw and some forms of manipulations and exercises can help in the relaxation of masticatory muscles (68, 69).

1.1.5.3. Behavioural therapy

A behavioural approach is considered beneficial for TMD patients and research suggests that self-management programs for TMD can have a long-term positive effect. A behavioural evaluation can reveal the behavioural, psychological and psychosocial information relevant to the patient's pain problem and, for that reason, must be equal to physically-oriented approaches. Michelotti et al. found that the key to achieving a good outcome in TMD management seems to be success in educating the patient about the disorder to enhance self-care (64).

Behavioural treatment approaches should include informative counselling, stress management and biofeedback. Biofeedback is a structured therapy based on the theory that when an individual receives information about a desired change and is supported in making the change, the change is more likely to occur. Biofeedback training uses equipment to measure biological activity – electrodes attached to the skin overlaying the muscle and connected to an amplifier. The equipment is designed with a feedback loop so that a patient can receive immediate feedback regarding performance. It produces visual or auditory signals that warn the patient about their activity.

Patients should be encouraged to exercise self-monitoring and to educate themselves about their condition. It is proposed that altering cognition, how and what people think, will alter behaviour and therefore lead to a reduction of symptoms.

Behavioural modification aimed at reducing the overuse of parafunctional behaviour is a central part of the overall treatment program for individuals with TMD (70).

1.1.5.4. Occlusal appliances

Occlusal appliances (often referred to as "occlusal splints"), the most widely used treatment modality for TMD, are removable devices usually made of hard acrylic. Various types of splints, with different indications and functions, are mentioned in the literature. Two of the most frequently used types are the stabilization splint and the anterior positioning splint (repositioning splint).

An anterior positioning splint positions the lower jaw in the position of protrusion. In the past, the general assumption was that with the help of an anterior positioning splint, it would be

possible to recapture the disc and eliminate joint sounds. During the last decades of the 20th century, many authors recommended the use of a protrusive repositioning splint for capturing the disc into a more backward, "normal" position. Studies have shown a high rate of disc prolapse and pain recurrence in these patients (71). Today, it is believed that this splint can help in certain painful conditions, mainly through the relief of retrodiscal tissues and insertions of selected masticatory muscles to the articular disc. The repositioning splints are no better than flat splints and rarely lead to a change in the condyle-disc relationship. Nevertheless, Ma et al. claim that a normal disc-condylar relationship could be maintained in the joints of patients in early puberty but the rate of unsuccessful treatment increases in late puberty. Because of the possible side effects, a repositioning splint should only be used when prescribed by trained TMD experts for a shorter period of time (72, 73).

The stabilization splint, named for its aim to contribute to occlusal stability, covers all the teeth in one arch – upper or lower. Upon mandibular closure, there are even and simultaneous contacts between the lower teeth and the appliance's flat surface. Slight protrusive and laterotrusive tooth-guided mandibular movements along the surface of the appliance are allowed. Cuspid rise during appliance-guided laterotrusion and the protrusion separation (disclusion) of all the remaining teeth is provided (74-76). The stabilization appliance is usually worn at night and, if necessary, it may be used for several hours a day during stressful periods of life.

Previously it was believed that the role of a stabilization splint was to provide stabilized and optimum centric relation occlusion creating an "ideal" state with no interferences. The "ideal" positioning of the mandible in relation to the maxilla would decrease abnormal muscle activity and ensure an orthopaedic position of the TMJ (16). Today, the role of the stabilization splint is considered debatable and there is no general agreement among researches on the effectiveness of splint therapy compared to alternative treatment options. Regardless, some evidence suggests that a stabilization splint can reduce TMD symptoms and they are considered better than no treatment at all (77, 78). Treatment success is considered to be due to the complex effects of different factors and changes in jaw activity patterns. A splint can provide a relaxing effect, an orthopaedic effect, a cognitive effect and even a placebo effect. It was previously believed that increasing the vertical dimension of occlusion led to muscle exertion resulting in muscle hyperactivity. On the contrary, EMG studies have demonstrated the opposite, showing that the elongation of the elevator muscles near or to the vertical dimension of the least EMG activity is effective in producing neuromuscular relaxation (79). Therefore, it has been proposed

that an increased vertical dimension of occlusion is possibly the most notable mechanism of action of occlusal splints. The underlying mechanism of the increased vertical dimension isn't fully understood but it is believed that it has an impact on neuromuscular patterns leading to plastic changes in the muscle fibres. Even so, the lack of well-controlled studies on the subjects is the reason for the debatable and scarce evidence of the connection between TMD symptoms and changes in the vertical dimension of occlusion (80, 81). The latter is probably the reason why some studies consider the placebo effect as an explanation of the efficacy of the occlusal splint, thus equating the effect of a stabilization splint with a soft splint, a non-occluding palatal splint and physical therapy (77, 78).

1.1.6. The role of stress in temporomandibular disorders

Various studies have attempted to understand the connection between stress and TMD, but it still remains unclear. Stress is defined as the ensemble of responses by an organism subjected to pressure or constraints from its environment, and these responses depend on the organism's ability to adapt. Stressors can be psychological, psychosomatic or biological (82). Our body's response to stress serves as a protection mechanism from potential danger, but when exposed to intense, long-lasting stressors, protection mechanisms that are constantly triggered can become insufficient and/or have an adverse effect on the body (83). TMD-related symptoms are frequently associated with individuals with pronounced anxiety, depression, somatization and various psychological distresses. It is important to note that depression and anxiety could be predisposing factors – the "cause", as well as the result of chronic and constant pain caused by TMD – "the consequence". With the acceptance of the biopsychosocial model as one of the factors contributing to the aetiology of TMD, clinicians are compelled to take into consideration not only the physical and anatomical aspects of the disorder, but also the psychosocial ones (84).

When observing the molecular aspects of stress, several studies have managed to correlate the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis with TMD. The stress activation of HPA leads to the secretion of cortisol (a glucocorticoid hormone) from the adrenal cortex. Cortisol increases in response to stressful events, thus is often referred to as a "stress indicator". There is the theory that cortisol has the potential to change the psychobiological resilience to oxidative stress damage (85). The question remains whether this dysregulation is the cause or an effect of TMD.

Painful TMD could be a consequence of predisposing factors such as psychological stress. On the other hand, the chronic pain in TMD *per se* could be considered a constant stressor that can alter the mechanisms of further pain control and regulation.

1.2. Oxidative stress

1.2.1. Oxidative stress: definition and its effects on the living organism

Normal cellular metabolism and environmental factors (smoking, pollution, radiation, medication) can both lead to the production of free radicals, highly reactive molecules that, when derived from oxygen, are often collectively referred to as reactive oxygen species (ROS). The structure of free radicals with an unpaired electron in the outer shell of the molecule allows them to be highly reactive with other molecules, causing large chain chemical reactions called oxidation (86).

The production of ROS is a normal state of cellular physiology and, when controlled by protective antioxidant mechanisms, cannot represent a serious danger to the normal functioning of the organism.

The balance between oxidants (endogenous and exogenous) and antioxidants (enzymatic and non-enzymatic) is the main factor in the defence against the oxidative damage. This defence is not always perfect mainly because antioxidants do not eliminate oxidants completely but rather control their levels (87, 88).

When the balance between the ROS and antioxidant mechanisms in the body is distorted, it can cause continuous and irreversible chemical modifications – therefore, damage to lipids, nucleic acids and proteins leading to the dysfunction of various systems in the body and resulting in specific diseases. This phenomenon is called oxidative stress (OS) and it represents an imbalance between oxidants and antioxidants in favour of oxidants due to either an excessive production of free radicals or the compromised effectiveness of antioxidant system (89).

Oxidative stress is considered to be the underlying mechanism of various chronic and degenerative diseases (88, 90-92). It is proposed that accelerated cellular ageing and the early onset of age-related disease result from accumulating excessive oxidative damage over time (93). Also, psychological stress has been associated with higher oxidative damage. Moreover, some research suggests that the glucocorticoid hormone cortisol might represent an important linkage between chronic stress, OS and OS-related pathologies (88).

1.2.2. Detecting the markers of oxidative stress

The interest in the role of free radicals in the etiopathogenesis of various diseases created a need for measuring techniques. Since free radicals are molecules that are highly reactive and have a very short half-life (it has been estimated that the lifetime of •OH, the most harmful ROS, is <1 ns) (94), it is difficult to measure them directly. Although highly sensitive direct chemical methods for detecting ROS have been developed, the methods used for quantifying oxidative stress in clinical research are mostly indirect, focusing on detecting damage mediated by ROS (95). A commonly used method measures the by-products that occur after oxidative damage in various processes within the organism. Those by-products are called markers of oxidative stress. The method's primary focus is on the use of antibodies against the specific 'footprints' of oxidative damage (96, 97).

There are various compounds that can signify oxidative damage. However, a product that can be referred to as a clinically useful biomarker must meet one of the following criteria: (i) show specificity for a certain disease (diagnostic), (ii) have prognostic value, and (iii) correlate with disease activity (98).

Products that are routinely measured as markers of oxidative damage are by-products of DNA oxidation and lipid peroxidation. Lipid peroxidation has been implicated in the pathogenesis of various diseases. Studies suggest that aldehyde products are bioactive molecules that can affect the pathological processes in the organism. Among the most toxic products are malondialdehyde (MDA), 4-hydroxynonenal and various 2-alkenals (99, 100).

Biological samples that are commonly used for assaying oxidative stress markers are blood, synovial fluid, urine and saliva.

1.2.2.1. 8--hydroxy--2'--deoxyguanosine (8-OHdG)

8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidized derivative of deoxyguanosine, is a widely used compound for the estimation of DNA damage and is considered a risk factor for many diseases. It is a specific marker for the estimation of DNA damage after the ROS attack and therefore, serves as a biomarker of oxidative stress in biological systems. Its activity is regulated by DNA repair enzyme activity and local antioxidant capacity (101, 102). 8-OHdG

can be measured with immunohistochemistry, by an enzyme-linked immunosorbent assay (ELISA) or high-pressure liquid chromatography, with mass spectrometric or electrochemical detection (HPLC-MS/MS; HPLC-EC) in serum, urine samples and saliva (103, 104).

1.2.2.2. Malondialdehyde (MDA)

Malondialdehyde (MDA) is one of the naturally occurring products of lipid peroxidation, polyunsaturated fatty acid peroxidation within cells. It is one of the many reactive electrophile species that cause toxic stress in cells and is considered mutagenic and carcinogenic (105). MDA is used as an oxidative stress indicator. In research, MDA is mentioned as a highly variable marker with a very wide range of concentrations intra- and inter-individually, as well as between various analytes, and is therefore mentioned as a marker whose level interpretation should be approached with increased caution (106). In research by Alajbeg et al. where OS biomarkers were followed during three consecutive days, malodialdehyde, despite being highly variable, showed better repeatability in the afternoon than in the morning measurements (89). Today, the method used for estimating aldehyde products by their ability to react with tiobarbituric acid is often replaced with new, modern methods (i.e. spectrofluorimetry, mass spectrometry or chemiluminescence) due to the old method's poor specificity for MDA (99).

1.2.3. The role of antioxidants and total antioxidant capacity (TAC)

Antioxidants are molecules that can donate an electron to a free radical resulting in the stabilization and neutralization of the chain reaction before vital molecules are damaged and thus invalidate the ill effects of oxidation (107). Protection against the effects of free radicals comes in two types of antioxidant defence: enzymatic and non-enzymatic.

Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase are part of an enzymatic antioxidant defence. SOD catalyses the dismutation of superoxide, GPx has the role of neutralizing hydrogen peroxide produced within the cell and catalase acts as a catalyst for the conversion of hydrogen peroxide to oxygen and water (108, 109).

The non-enzymatic antioxidants, with a role in scavenging free radicals, are vitamin C, vitamin E and albumin (109).

Uric acid (UA), often mentioned as a non-enzymatic antioxidant, is often a subject of controversy for its unclear role in the oxidant status of an organism. It is proposed that it can function as an antioxidant, primarily in plasma, as well as an oxidant, primarily within the cell (110).

The total antioxidant capacity (TAC) represents the total antioxidant response against free radicals. It is a measure of the amount of free radicals removed by the test solution and is commonly used to estimate the antioxidant capacity of biological samples (108, 111).

1.2.4. Oxidative stress and its connection to various human pathologies

Since exposure to oxidant molecules can generate free radicals that can alter proteins, DNA and membrane phospholipids, there has been increased interest in the role of oxidative stress in various human pathologies. The interest emerged mostly in diseases that are multifactorial or have an origin that is not yet fully understood (112, 113).

There has been a range of research into the topic proposing chronically altered oxidative status as a crucial factor in the etiopathophysiology of various diseases. Mostly, oxidative stress has been connected to carcinogenesis, neurodegenerative diseases such as Alzheimer's disease, liver diseases, insulin resistance and diabetes, cardiovascular diseases, psoriasis, periodontitis, lichen planus, orofacial pain etc. (95, 114).

It is also considered that multiple extrinsic risk factors such as tobacco and alcohol can increase oxidative damage and thus contribute to the overall increase in oxidative stress (115).

Studies hypothesize that in diseases, we would encounter higher levels of oxidative stress markers and lower levels of TAC and antioxidant enzymes. Such a state would point to insufficient defence mechanisms leading to pathologies (116, 117). However, some studies on oxidative stress of different pathologies have found higher TAC levels explaining it as a compensatory reaction to higher levels of ROS and distorted oxidative balance (118, 119).

The role of oxidative stress in pathological pain-related conditions has also been discussed. For example, hydrogen peroxide (H2O2) is a diffusible reactive oxygen species that contributes to the development of pathological pain conditions, not only by creating harmful reactive species but also by modulating synaptic plasticity (120). The presence of H_2O_2 apparently affects the release of intracellular calcium, leading to neuronal sensitization and pronociceptive patterns in interneurons in the spinal cord dorsal horn and therefore changes the response to painful stimuli (121).

1.2.5. The role of oxidative stress in chronic orofacial pain disorders and TMD-analysis of the literature

Recently, studies are attempting to connect TMD pathogenesis with an imbalance between oxidants and antioxidants. The explanation of the mechanism is no different to the mechanisms proposed in other painful pathologies. With the presence of pain conditions, there is an increased neural activity with an increased production of ROS. When the pain is chronic, the production of ROS can overcome the limitations of the protective mechanisms resulting in oxidative stress. It is believed that in TMD, the mechanical stress on the joint and on the masticatory muscles can generate free radicals, triggering a cascade of reactions that can exacerbate tissue damage, inflammation and pain (117).

So far, the evidence on the subject is scarce. Cai et al. reported that SOD activities may be connected in the pathogenesis of temporomandibular disorders. They found that both SOD and lipid peroxides levels were significantly higher in temporomandibular disorder patients than in healthy control subjects. The authors proposed the explanation that the overactivity of oxygen free radicals can lead to the overgeneration of antioxidant enzymes (122). Güven et al. observed that the activity of SOD seemed progressively reduced as the stage of the disease increased, which they explained as an insufficient scavenging capacity of free radicals (123). Nitzan et al. investigated the hypothesis that uncontrolled oxidative stress causes the collapse of the lubrication system. They analysed the synovial fluids by measuring their overall reducing power and found that the capacity to cope with oxidative stress is lower in joints with anchored disc phenomenon. They suggested that increased oxidative stress caused by free radicals in the TMJ could cause an imbalance of local antioxidant defences (124).

Richards et al. evaluated blood oxidative stress in individuals with temporomandibular dysfunction who also suffer from chronic fatigue syndrome and found out that jaw muscle pain and TMJ clicking and/or locking was associated with an increase in malondialdehyde levels (125). Rodríguez de Sotillo et al. reported increased OS products in TMD patients, with the oxidants MDA and 8-OHdG being statistically higher in patients with TMD compared to the control and a significant association between pain intensity and salivary OS markers. Moreover, significant differences in the MDA/total antioxidant status (TAS) and 8-OHdG/TAS ratios between patients with TMD and the controls indicate that oxidative stress plays a role in TMD pathophysiology (116). The total oxidant status is defined as the sum total of endogenous and food-derived antioxidants in the extracellular fluid of an individual, while the measurement of the serum TAC levels provides an integrated index, as opposed to one based on a simple summation of the measurable antioxidant (126, 127). De Almeida and Amenábar determined a lower TAC in patients with pain-related TMD but found no correlation between TAC and pain intensity (117). According to Etoz et al., TMD is strongly related to the antioxidant capacity of the TMJ since a lower TAC was observed in individuals who, besides pain, had articular disk displacement without reduction. Among other biomarkers, Basi et al. analysed F2-isoprostane levels and found that its levels were significantly reduced in masseter muscle samples from symptomatic TMD patients compared to controls. Moreover, the concentration of F2-isoprostane was associated with muscle pain intensity within the muscle and synovial compartments and with joint pain intensity within the muscle compartment, suggesting that oxidative stress contributes to pain in symptomatic TMD patients. Both Basi et al. and Etöz et al. support the role of OS in the intensity of pain in TMD but specimens other than saliva were used in these studies (128, 129). In the pilot study, Vrbanović et al. found that TAC was significantly higher in TMD patients than in the controls. Significant differences were also observed when the TAC levels between high-intensity pain patients and controls were compared. In addition, the TAC levels differed significantly between patients with disc displacement and the controls suggesting that the salivary oxidant status in chronic TMD is dependent on the intensity and source of pain (119).

A study that compared the treatment effect and outcomes in female TMD patients after a 3-month stabilization splint therapy showed a significant reduction in afternoon TAC and a significant reduction in afternoon MDA. A decrease in afternoon MDA to the superoxide dismutase ratio was present in high-intensity pain patients. The effect of treatment on the self-

perceived quality of life was more pronounced in MP patients while the reduction of spontaneous pain was significantly greater in high-intensity pain patients (130).

1.3. Saliva as a diagnostic media

Saliva is a complex fluid substance consisting of salivary gland secretion and the secretion of the gingival sulcus. Salivary gland secretion comes from the major salivary glands including the parotid gland, submandibular gland, sublingual gland and minor salivary glands, as well as from the Von Ebner's glands - posterior deep lingual glands.

Saliva plays a role in digestion, lubrication, the perception of oral sensations and the protection of the organism on both the local and systemic levels.

The composition of saliva is said to be complex due to the numerous compounds such as water, electrolytes, blood cells, proteins, antimicrobial agents and enzymes. The secretion of saliva is continuous and saliva as a biofluid can be characterized as clear, slightly acidic, hypotonic and mostly composed of water (95.5%). Other components present in saliva are inorganic ions, including sodium, chloride, potassium and calcium. Organic components include amino acids, proteins, antibodies, hormones, enzymes, lipids and cytokines. Recently, studies have found that among all the mentioned components, saliva additionally contains genomic, transcriptomic, proteomic, microbiologic and immunologic analytes thus making it possible to use salivary components as identification markers for various disorders (131).

With the advancement of technology in the field of diagnostics, saliva has become a desirable and highly usable biofluid that can serve as a diagnostic and monitoring tool in numerous fields of science, especially biomedicine. Advantages of salivary diagnostics include cost-effectiveness, non-invasiveness and easy and rapid sampling, which is why salivary diagnostics quickly becoming a desirable method in comparison with invasive methods such as blood and cerebrospinal fluid sampling (132).

Researchers developed methods for saliva sampling and validated methods for the assessment of miscellaneous salivary biomarkers that made the use of saliva specimens a reality. The advantages of salivary diagnostics quickly became recognized in various fields of medicine and dental medicine. Saliva became the sample for disease screening (such as HIV and hepatitis), the detection method for hormones (such as cortisol), and the tool for assessing various biomarkers (such as oxidative stress biomarkers) (133).

The assessment of salivary biomarkers is considered to have its benefits in diagnostics, disease monitoring, and predicting the severity of both systemic and oral diseases. The use of salivary

oxidative biomarkers for the early detection of oral cancer, for the prediction of disease progression and for a better understanding of complex, multifactorial oral diseases such as periodontitis, various oral lesions (i.e. aphthous stomatitis and lichen planus) has recently attracted a lot of interest. Furthermore, changes in biomarkers' levels in saliva can be used for monitoring treatment response and are thus considered a tool for treatment validation (134, 135).

Oxidative stress has begun to be considered a factor contributing to the initiation and progression of TMD, with salivary diagnostics being the simplest method for assessing oxidative stress biomarkers.

1.4. Aims and hypotheses of the research

This study aimed to quantify and compare OS biomarkers in TMD patients and a healthy control group. Moreover, the goal was to investigate the mechanism of stress response through the levels of salivary cortisol (SC) and to investigate whether there is a correlation between levels of SC and OS biomarkers.

The next aim was to compare the long-term effectiveness of stabilization splint (SS) with that of placebo splint (PS) in chronic TMD patients and to investigate differences in oxidative stress markers and treatment outcomes based on diagnostic subgroups [disc displacement (DD)/myofascial pain (MP)] and pain intensity [low-intensity pain (LIP)/ high-intensity pain (HIP)].

The hypotheses were:

(1) the levels of salivary markers of oxidative stress will be higher and/or salivary antioxidant levels will be lower in TMD patients compared to healthy controls,

(2) salivary OS markers would correlate with SC concentrations and

(3) there will be no difference in the oxidative stress marker levels and SC, as well as in the treatment outcomes, considering the type of therapy being applied (stabilization splint/placebo splint).

2. METHODS

This doctoral research was performed at the Department of Removable Prosthodontics, School of Dental Medicine, University of Zagreb from October 2016 to July 2019 with the approval of the Ethics Committee (05-PA-26-1/2017). The experimental procedures were conducted in accordance with the ethical standards of the Helsinki Declaration. All participants were informed about the study design and protocols and provided written consent.

2.1. Validation of a salivary diagnostics method

Diurnal variations and day-to-day fluctuations of salivary OS markers in healthy adult individuals were evaluated. Whole unstimulated saliva was collected 2 times a day over 3 consecutive days. No significant differences for salivary OS markers between men and women were present.

For all the examined OS markers, no significant day-to-day variations were demonstrated. Significant diurnal variations were found in salivary GPx, TAC and MDA levels. For SOD, TAC, GPx and UA, good-to-moderate variations were observed in more than 75% of the subjects. For MDA and 8-OHdG, significant intraindividual variations were observed in 60% and 40% of the subjects, respectively. Because of the significant diurnal variation found in the salivary markers' levels, in the present study, we chose to collect the subjects' saliva 2 times a day.

Details of the analytical performance (including intra- and inter-assay variability) are available in our previously published study of Alajbeg et al (89).

2.2. Selection of Participants

Power analysis, performed to estimate the sample size for the first part of the study, was based on data from the pilot study (116), which compared OS (as measured by biomarkers in saliva) between three groups (TMD patients with high and low pain intensity and a healthy control group). Accordingly, with a power set at 80% and a significance level of 5%, the projected sample size needed was 48 participants (16 per group). According to de Almeida et al (117), who compared OS (as measured by biomarkers in saliva) between two groups (subjects with TMD and pain with subjects with no TMD and no pain), the minimum difference in salivary

total antioxidant capacity (TAC) and total oxidative status (TOS) was estimated to be 0.134 μmol/L and 0.269 μmol/L, respectively, and the standard deviations of the TAC and TOS were expected to be 0.047 μmol/L and 0.418 μmol/L, respectively. With an alpha=.05 and power set at 80%, the projected sample size was approximately N =54 (26 per group). Taking into account the subjects' withdrawal of 20%, we adjusted the total sample size to the number of 66 participants (33 per group).

Power analysis, performed to estimate the sample size required for the second part of the study, was conducted with the following assumptions: a) the number of groups was 4 (MP-SS, MP-PS, DD-SS, DD-PS or HIP-SS, HIP-PS, LIP-SS, LIP-PS), b) the number of measurement was 4 (4 time periods T0, T1, T2, T3), c) a medium (0.07) effect size. The correlation between the repeated measure was set at 0.50. With the power set at 80% and a significance level of 5%, the minimal number of 32 participants was calculated.

Participants were selected from among the patients referred to the Department of Removable Prosthodontics due to reported pain and discomfort in the temporomandibular region as the primary problem. The clinician (IA), an expert in TMD diagnostics, conducted a clinical examination of patients and provided diagnosis according to the diagnostic criteria for TMD (DC/TMD) (1). The included subjects were over the age of 18, with chronic (lasting longer than 6 months) and spontaneous pain of >30 mm on a visually analogue scale (VAS). The inclusion criteria were the report of chronic pain, lasting more than 6 months and a diagnosis of myofascial pain (MP) or disc displacement (DD). Patients had to have their natural teeth and maintain good oral hygiene. Exclusion criteria were degenerative joint disease, inflammatory joint disease, orofacial pain unrelated to TMD, smoking, gum swelling, periodontitis, oral lesions, chronic systemic diseases (i.e. diabetes, cardiovascular diseases, cancer and autoimmune diseases), the use of supplements and medication known to affect oxidative status, pregnancy. Patients clinically diagnosed with both MP and DD and individuals who had already been under treatment for TMD were not included. During the research period, the only patients who met the inclusion criteria were women, therefore exclusively female participants were included in the study.

We recruited participants between October 2016 to January 2019. In that period 262 patients seeking treatment for orofacial pain were referred to our research team. During the recruitment

198 of them did not fully met the inclusion criteria, 25 of them refused to take part in the study due to travelling complications and obligations that prevented them from participating, and 5 dropped out due to other reasons. Finally, 34 patients were included and compared with 33 control subjects. The control group consisted of healthy individuals, mostly students and staff from the school of Dental Medicine, University of Zagreb, matched by age and gender with the TMD group. Also, 34 TMD patients were randomized in two groups – 19 participants were assigned to the stabilization splint group and 15 to placebo splint group. A diagram illustrating the flow of participants through each stage of the trial is shown in Figure 1.

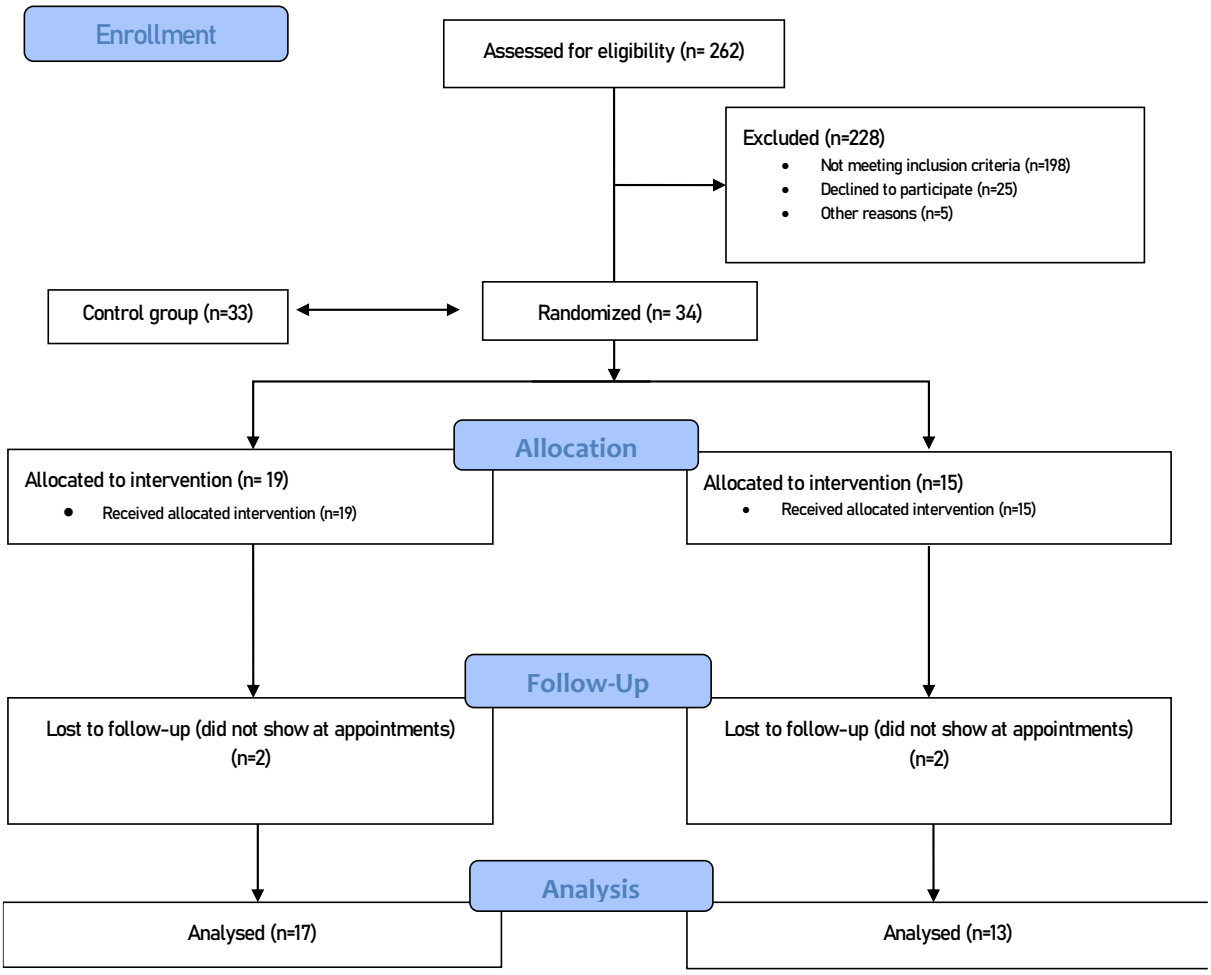

Figure 1. Flowchart illustrating the selection and distribution of the participants into the treatment groups

2.2.1. Grouping of patients related to pain intensity and diagnostic subgroups

In TMD patients, the characteristic pain intensity (CPI) was assessed using GCPS by computing the means of three items (current pain, worst pain, average pain) and multiplying them by 10. CPI < 50 was considered low-intensity pain (LIP), and CPI ≥ 50 was considered high-intensity pain (HIP). Subsequently, the division of TMD patients according to pain severity was formed (17 HIP and 17 LIP). According to DC/TMD, 16 patients were diagnosed with myofascial pain and 18 with DD.

2.2.2. Observer training

Ten randomly selected subjects, different from the ones included in the investigation underwent repeated clinical examinations by two experienced examiners to assess signs and symptoms for DC/TMD. No significant differenceswere noted between the first and the second measurements ($p = 0.86–0.89$, paired t-test). The weighted kappa statistics showed satisfactory agreement between the observers ($\kappa = 0.87–0.89$).

2.3. Study protocol

2.3.1. Study design

The study consisted of two phases. In the first part of the study levels of selected salivary OS markers (MDA, 8-OHdG, SOD, GPx and TAC), SC and UA were compared between subjects with chronic TMD and the healthy control group in order to determine whether there is a difference in the expression of biomarkers between these two groups. Also, the levels of salivary OS markers in non-TMD controls were compared to TMD patients with respect to two levels of pain intensity [high pain intensity (HIP) and low pain intensity (LIP)] and two different diagnostic subgroups [(MP and DD)].

In the second part of the study, TMD patients were randomized (RAND function, Microsoft Excel) into 2 groups and 2 types of interventions were conducted: one group was provided with stabilization splints (SS) and the other group was provided with a placebo splint (PS).

2.3.2. Intervention

2.3.2.1. Occlusal splint fabrication

Patients in the SS group received a hard acrylic (Resilit-S, Erkodent, Siemensstraße 3, 72285 Pfalzgrafenweiler, Germany) occlusal splint (stabilization splint) in the maxilla. The splint was made on a stone cast in an ARTEX articulator in the referent position of centric relation, with a thickness of 1.5 mm at the level of the first molar (Figure 2).

Patients in the PS group received a thin transparent film with a thickness of 0.5 mm (Erkodent). The splint was fabricated on the patient's maxillary stone cast. Contacts that interfered with maximal intercuspation were removed. The increase in the vertical dimension was less than 0.5 mm, thus thought to have a negligible influence on occlusion and condylar position (Figures 3 and 4).

The same dental technician made all the splints. Coverage of the labial surfaces and buccal surfaces of the maxillary teeth provided retention for the splints.

The clinician (IA) adjusted the splints so that opposing teeth occluded simultaneously with the splint surface. The same clinician adapted the splints during follow-up appointments if there was a need for it.

Both treatment groups were equally informed and counselled about their condition prior to the splint therapy in terms of explanation of the origin and prognosis of the disease. During the entire study, no participants received any other form of treatment.

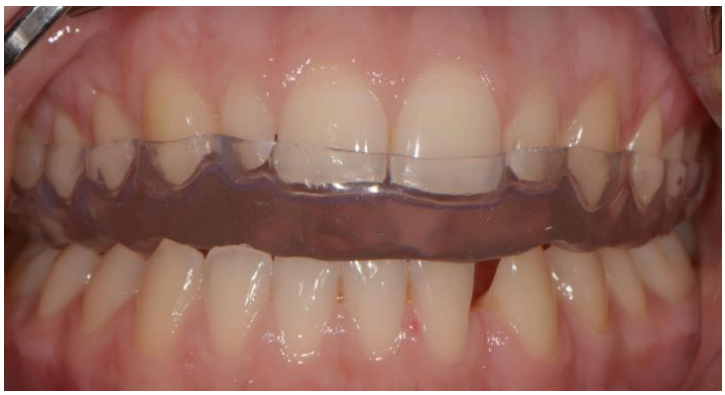

Figure 2. Stabilization splint in the mouth of the patient

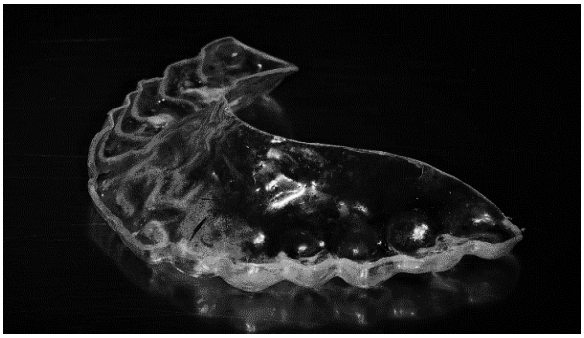

Figure 3. Thin transparent foil that served as a placebo splint

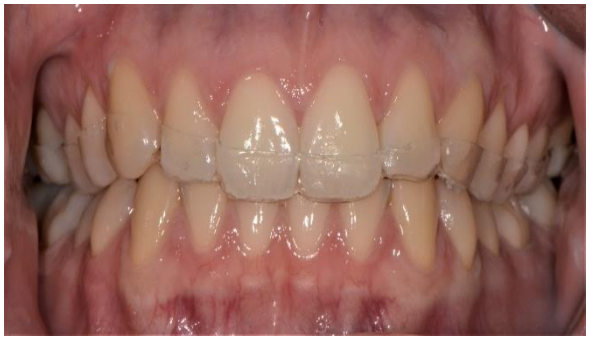

Figure 4. Placebo splint in the mouth of the patient

2.3.3. Study measures

All the participants provided information about their age, gender and demographic data.

At baseline TMD, patients had to fill in the following questionnaires: Patient Health Care Questionnaire-15 (PHQ-15), Patient Health Care Questionnaire-9, General Anxiety Disorder-7 (GAD-7), Oral Behaviour Checklist (OBC). Spontaneous pain was assessed with VAS, changes in oral health quality of life were assessed using the Oral health impact profile (OHIP-14), the level of perceived stress was assessed using Perceived stress scale (PSS), characteristic pain intensity was assessed with Graded chronic pain scale (GCPS) while functional limitations of the lower jaw were assessed with the Jaw Functional Limitation Scale (JFLS). The clinician (IA) conducting the baseline assessment measured the maximal comfortable mouth opening [pain-free mouth opening (MCO)] and maximal unassisted mouth opening (MMO) at the first appointment.

Treatment outcomes

Clinical treatment outcomes in the TMD group included spontaneous pain (according to VAS), self-perceived quality of life (assessed with OHIP-14), characteristic pain intensity (assessed with GCPS), level of perceived stress (assessed with PSS), and functional limitations of the lower jaw (assessed with JFLS). Moreover, the clinician conducting the follow-up assessments

(EV) measured the maximal comfortable mouth opening (MCO) and maximal unassisted mouth opening (MMO) at all follow-up appointments.

In the control group, the level of perceived stress was assessed using PSS.

Spontaneous pain — A 100 mm horizontal VAS scale was used to evaluate spontaneous pain from the temporomandibular joint and masticatory muscles. The left endpoint of the scale (0 mm) indicated "no pain" while the right endpoint of the scale (100 mm) represented "worst pain imaginable" (136).

Maximal comfortable mouth opening (MCO) — A pain-free or comfortable mouth opening was defined as the maximum distance the participant could open her mouth without experiencing any additional pain or discomfort. The maximal comfortable mouth opening was measured as the distance between the maxillary and mandibular central incisors (64).

Maximal unassisted mouth opening (MMO) — Maximal mouth opening was defined as the maximum distance the participant could open her mouth regardless of the pain they felt, measured as the distance between the maxillary and mandibular central incisors (137).

Stress perception — Patients expressed their stress perception in the PSS questionnaire, designed to indicate how erratic, disorderly and overloaded the respondents find their lives at a given moment. It consists of 10 questions and patients must choose 1 answer: 0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often. Possible scores ranged from 0–40, with higher numbers indicating higher levels of stress. The questionnaire was previously translated into and validated in Croatian by Hudek-Knežević et al. (138).

Oral-health-related quality of life — Short-form OHIP-14 questionnaire, which has been translated into and validated in Croatian (139), was used to show how oral outcomes (TMD-related pain and disability) impact the patients' quality of life. The patients expressed their status through 14 questions, choosing 1 of the 4 possible answers: 0 = never, 1 = hardly ever, 2 = sometimes, 3 = fairly often, and 4 = very often. Possible scores ranged from 0–56. The instrument was previously validated for the evaluation of TMD patients (140).

Characteristic pain intensity — The GCPS questionnaire was used to evaluate two dimensions of chronic pain severity: pain intensity and pain-related disability. Subscale scores for pain intensity and disability are combined to calculate a chronic pain grade that allows the classification of chronic pain patients into 5 categories: grades 0 (pain-free) to IV (high disability-severely limiting). All the items are scored on a scale, with responses ranging from 0–10. Scores are computed and divided into 3 subscales: the characteristic pain intensity score calculated as the mean intensity ratings for the reported current, worst and average pain; the disability score is calculated as the mean rating for difficulty performing every-day, social and work activities; and the disability points score is derived from a combination of ranked categories of the number of disability days and disability score (1, 141). In this study, we only followed the characteristic pain intensity scores' changes.

Jaw functional limitations caused by TMD — The JFLS questionnaire is a reliable and valid form that assesses global limitations caused by TMD. The questionnaire consists of 52 items grouped as follows: a) mastication (20 items), b) vertical jaw mobility (9 items), c) verbal and emotional expression (14 items), and miscellaneous (9 items) (142).

2.3.4. Saliva collection

Detailed instructions were given to participants on how to collect saliva. Five mL of whole unstimulated saliva were collected in a graduated tube (50Ml, self-standing graduated tubes, Ratiolab, Germany) twice a day, in the morning between 7 am and 8 am and in the afternoon between 5 pm and 6 pm. The subjects were instructed to fast before saliva collection in the morning and to not eat or drink anything but water at least 2 hours before sampling in the afternoon. All the samples were collected after rinsing the mouth with water. Tooth brushing was forbidden at least 2 hours prior to collection in order to avoid contamination of the saliva samples with blood. During collection, subjects were asked not to talk or think about food and to attempt not to generate saliva. The subjects in the control group collected samples only once and TMD patients collected samples at the baseline and at every follow-up appointment. All the samples collected at subjects' homes had to be stored in the deep freezer and transported in

such a way that saliva did not defrost. The saliva aliquots (1 mL) were stored at −80° C until analysed.

2.3.5. Biochemical salivary sample analysis

Biochemical salivary sample analysis was performed at Department of Laboratory Diagnostics, University Hospital Centre Zagreb by trained biochemist (IL). Saliva samples had been thawed and centrifuged prior to analysis (1000 ×g. 5 min).

Analysis of MDA — the MDA levels were measured using an MDA adduct competitive enzyme-linked immunosorbent assay (ELISA) kit (Kamiya Biomedical Company, Seattle, WA, USA). The test involves the addition of unknown samples to an MDA conjugate precoated ELISA plate, followed by the addition of an anti-MDA polyclonal antibody and a horseradish peroxidase (HRP) conjugate secondary antibody (Figure 5). The content of MDA protein adducts in the saliva samples was determined by measuring the absorbance of an enzyme conversion product after the addition of an enzyme substrate at a specified wavelength (450 nm). The MDA adduct level reflects the quantity of MDA that combines with proteins in the process of lipid peroxidation. The range of the assay is 6–1500 pmol/mL; thus, the minimum detectable concentration of MDA adducts that is less than the lowest standard is reported as <6 pmol/ml. The amount of MDA adduct in the standards used was predetermined by the manufacturer using a TBARS assay kit; the results obtained with this kit can be used to compare findings with those from other studies analysing MDA. The within-laboratory CV for the MDA adduct assay was determined by 10 replicate measurements of one saliva sample and the CV (%) obtained was 16.6% at 134 pmol/mL concentration.

Analysis of 8-OHdG — 8-OHdG levels were measured in a similar manner, using a highly sensitive ELISA competitive kit (Japan Institute for the Control of Aging, Shizuoka, Japan). This kit uses an 8-OHdG monoclonal antibody to bind, in a competitive manner, 8-OHdG in the analysed samples or 8-OHdG prebound to the wells of the microtiter plate. Immobilized 8-OHdG is detected with an HRP conjugated secondary antibody and tetramethylbenzidine as a chromogenic substrate, causing colour development measured at 450 nm. Intra-assay variation

was determined by measuring one saliva sample 10 times in a single batch, and the obtained CV (%) of the 8-OHdG assay was 13.9% at 1.19 ng/mL concentration.

Analysis of SOD, GPx and TAC — SOD, GPx, and TAC were measured utilizing the commercial colourimetric reagent kits RANSOD, RANSEL and TAS (Randox Laboratories Ltd., Crumlin, United Kingdom), respectively, and applied on a Cobas c501 biochemistry auto-analyser (Roche Diagnostics, Mannheim, Germany).

The RANSOD test measures SOD levels by employing xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which subsequently react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT), forming a red formazan dye. Because the role of SOD is to enhance the dismutation of superoxide radicals, its activity is easily measured by the degree of inhibition of this reaction. One unit of SOD corresponds to a 50% inhibition. The intra- and interassay variabilities for the RANSOD test were 4.6% and 7.1%, respectively, as declared by the manufacturer.

The RANSEL test measures GPx levels by determining the decrease in absorbance at 340 nm of NADPH, which is converted to NADP+ in the oxidation reaction of glutathione catalysed by glutathione peroxidase. The intra- and interassay variabilities for the RANSEL test were 4.9% and 7.3%, respectively, as determined by the manufacturer.

A TAS kit measures the TAC of the samples in a reaction catalysed by peroxidase, producing a radical cation, ABTS*+, whose absorbance is consequently measured at 600 nm. The antioxidant capacity is determined by the capability of antioxidants present in the analysed sample to suppress this reaction and the subsequent colour development. The intra-assay variability of this assay was 2.8% at 1.5 mmol/L TAC and was determined by 10 repeated measurements of saliva samples.

Analysis of uric acid — Uric acid was measured using the enzymatic uricase method, utilizing commercially available Roche Diagnostics reagents applied to a Cobas c501 Biochemistry analyser (Roche Diagnostics, Mannheim, Germany). The within-run and between-run coefficients of variation (CV%) of the uric acid assay were 1.4% and 1.8%, respectively, and were determined by 3 replicate measurements for five consecutive days of commercial control samples during method validation.

Analysis of SC — Free SC was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (Demeditec Diagnostics GmbH, Germany) according to the manufacturers' recommendations. This assay measures free SC based on the principle of the

competitive binding of cortisol from samples and a cortisol-enzyme conjugate to a polyclonal antibody that is pre-coated onto the microtiter wells. The resultant binding is measured spectrophotometrically and is inversely proportional to the concentration of free SC in the tested samples. The intra- and inter-assay variabilities of this assay kit are 5.8 and 6.4%, respectively, as stated by the manufacturer.

Analysis of the total proteins in saliva — The amount of the total proteins in the saliva samples was determined using a commercially available Roche Diagnostics automated turbidimetric urinary and cerebrospinal fluid (CSF) protein assay whose measuring range (0.02–2 g/L) and lower detection limit (0.02 g/L) covered the expected values and had satisfactory sensitivity for saliva samples. The within-run and between-run CVs of the assay were 0.9% and 1.0%, respectively. Analysis was performed on a Cobas c501 biochemistry analyser (Roche Diagnostics, Mannheim, Germany). All the OS marker values except the levels of SC were normalized to total protein concentration.

All measurements were performed using one reagent kit for each parameter.

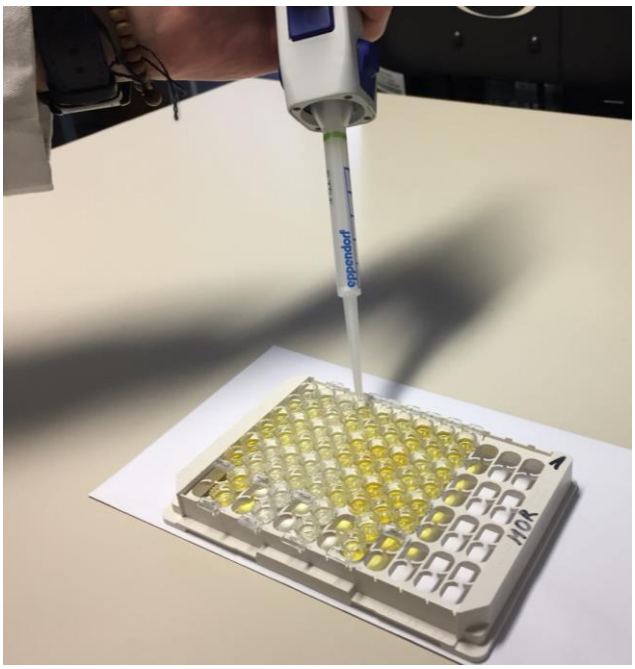

Figure 5. Enzyme Linked Immunosorbent Assay Test; photo courtesy of Ivana Lapić, Specialist in Medical Biochemistry and Laboratory Medicine, University Hospital Centre Zagreb

2.3.6. Monitoring oxidative stress markers, cortisol levels and treatment outcomes in TMD patients and the control group

In the control group, measurement of OS markers and SC, as well as the subjective stress level, was only performed at the first appointment.

In the TMD patients, measurement of the OS markers and SC was performed at the first appointment (T0), and at follow-up appointments in the 1st (T1), 3rd (T2) and 6th months (T3). The follow-up clinical evaluations in the 1st (T1), 3rd (T2) and 6th (T3) months were carried out by the clinician (EV), blinded for the type of treatment, each patient's initial CPI and specific diagnostic subgroup.

The parameters (OS markers and SC) were compared between the TMD patients and the control group (first appointment). Moreover, the OS markers and treatment outcomes were compared between two treatment groups, as well as between the treatment groups when divided into diagnostic subgroups (MP and DD) and when divided according to pain intensity (HIP and LIP). Two of the patients in each treatment group withdrew before the completion of the therapy, thus 17 patients in the SS group and 13 patients in the PS group finished the 6-month treatment (Figure 1).

2.4. Statistical analysis

Preliminary analyses consisted of descriptive statistics and normality tests. Descriptive statistics were performed to assess the mean, standard deviation (SD) and confidence intervals in each study group. Data distribution was tested using the Shapiro-Wilk test. Before performing the statistical analyses, a log transformation was used for all data that was non-normally distributed (OS markers and SC).

In the first part of the study, analyses were conducted to evaluate whether the TMD and control group significantly differ with respect to the OS marker levels and SC. Student's *t*-test and analysis of variance (ANOVA) were used to compare the means of the examined variables between pain-free control subjects and TMD patients, as well as between pain-free control subjects and TMD diagnostic subgroups. The Pearson correlation coefficient was used to assess the association between psychological stress, salivary OS markers and SC.

In the second part of the study, an independent samples *t*-test was used to compare treatment groups at baseline. Within-group changes of salivary OS markers and SC, as well as of treatment outcomes, were analysed in each treatment group with within-subject ANOVA and post hoc comparisons. The differences in the magnitude of the change of the measured parameters in the SS group vs. the PS group were tested using an independent samples *t*-test.

Next, OS markers and SC, as well as treatment outcomes (VAS, OHIP-14, PSS, GCPS, MCO and MMO), were analysed by means of a Mixed Between-Within Subjects repeated measures ANOVA using “time” (T0, T1, T2, T3) as the within factor and “treatment group” (SS and PS), “pain-intensity” (HIP and LIP) and "diagnostic subgroup" (MP and DD) as the between factors. Bonferroni corrected post hoc tests were applied to investigate the difference between the time points. The partial eta squared ($\eta 2$) was calculated for analyses as the indication of the effect sizes.

Analyses were performed using the Statistica 13.4.0 software package (1984-2018 TIBCO Software Inc.) and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Demographics and baseline data of the participants

The demographics and baseline data of the TMD patients and the control group are summarized in Table 1 and Table 2.

No statistically significant age differences were noted between the 34 TMD patients (36.08 ± 11.95) and 33 controls (34.24 ± 8.59) (t=0.72; p=0.47). Furthermore, no significant age differences between the two TMD diagnostic subgroups (MP 39.31 ± 11.56; DD 32.22 ± 11.87; t=1.51; p=0.14) or between the two TMD pain intensity subgroups (HIP 36.18 ± 13.98; LIP (36.00 ± 9.97; t=0.47, p =0.96) were found.

The mean CPI of the MP subjects (46.75 ± 16.50) and DD subjects (49.11 ± 20.9) were not statistically different (t = -0.36, p = 0.71). Nine MP and 8 DD subjects had LIP (39.66 and 33.37, respectively). The difference between the groups was non-significant (t = 0.76, p = 0.45). Similarly, no significant difference (t = -0.91, p = 0.37) was found between the 7 MP and 10 DD subjects with HIP (55.8 and 61.7, respectively).

Therefore, patients were pooled in the pain intensity subgroups regardless of the source. Conversely, patients were pooled in diagnostic subgroups regardless of the pain intensity.

The TMD patients' baseline data, depending on the diagnostic subgroup and intensity of pain, are presented in Table 3.

Table 1. Demographics and baseline data – TMD participants

Variable	Included (n=34)		Dropped out (n=4)	
	Mean (SD)	(95% CI)	Mean (SD)	(95% CI)
age	36.1 (11.95)	31.91 – 40.26	32.5(3.53)	0.73 – 64.26
OHIP-14	25.26 (10.29)	21.67 - 28.85	20.00 (8.48)	-56.23 - 96.23
PSS	18.15 (6.92)	15.73 - 20.56	20.50 (0.7)	14.14 - 26.85
VAS	6.10 (1.94)	5.4 – 6.8	5.50 (0.70)	-0.85 – 11.84
GCPS - CPI	48.00 (18.71)	41.47 – 54.52	49.5 (4.9)	5.00 – 93.97
PHQ-15	8.35 (4.36)	6.83 – 9.87	10.50 (4.94)	-33.9 – 54.97
PHQ-9	6.50 (4.88)	4.8 – 8.2	7.50 (7.8)	-62.4 – 77.4
GAD-7	6.56 (5.15)	4.76 – 8.35	8.5 (7.8)	-61.4 – 78.4
OBC	28.68 (10.86)	24.89 – 32.47	31.00 (12.73)	-83.35 – 145.35
JFLS *mastication*	3.46 (2.14)	2.71 – 4.21	3.05 (0.6)	-2.66 – 8.76
JFLS *jaw mobility*	4.47 (1.74)	3.85 -5.1	5.5 (1.41)	-7.2 -18.2
JFLS *verbal and emotional expression*	1.54 (1.5)	1.02 – 2.1	3.19 (2.91)	-23.1 – 29.39
MCO (mm)	27.74 (8.3)	24.82 – 30.64	42.5 (10.6)	-52.8 – 137.79
MMO (mm)	36.26 (7.9)	33.49 – 39.04	50.0 (0)	-

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale–chronic pain intensity; VAS, Visual Analogue Scale; JFLS, Jaw Functional Limitation Scale; PHQ-15, Physical Symptoms; PHQ-9, Patients Health Questionnaire; GAD-7, General Anxiety Disorder; OBC, Oral Behaviours Checklist; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; CI, Confidence Interval; n, number of participants; SD, Standard Deviation

Table 2. Demographics and baseline data – control group

Variable	Included (n=33)	
	Mean (SD)	(95% CI)
age	34.24 (8.59)	31.19 – 37.28
PSS	17.21 (4.76)	15.52 – 18.9

PSS, Perceived Stress Scale; CI, Confidence Interval; n, number of participants; SD, Standard Deviation

Table 3. Demographics and baseline data in TMD patients depending on the subgroups' division

Variable	Diagnostic subgroup			Pain intensity		
	MP n=16 Mean (SD)	DD n=18 Mean (SD)	p	HIP n=17 Mean (SD)	LIP n=17 Mean (SD)	p
age	39.31 (11.56)	33.22 (11.88)	0.141	36.18 (13.98)	36.00 (9.97)	0.966
OHIP-14	21.72 (7.51)	28.22 (11.58)	0.053	31.94 (8.81)	18.59 (6.80)	0.001
PSS	19.25 (5.18)	17.17 (8.18)	0.389	19.35 (7.75)	16.94 (5.95)	0.317
VAS	5.75 (1.98)	6.39 (1.91)	0.347	7.12 (1.69)	5.06 (1.64)	0.001
GCPS - CPI	46.75 (16.50)	49.11 (20.89)	0.719	59.29 (13.01)	36.71 (16.78)	0.000
PHQ-15	8.63 (3.69)	8.11 (4.98)	0.737	8.41 (5.05)	8.29 (3.70)	0.939
PHQ-9	5.94 (3.94)	7.00 (5.65)	0.534	6.12 (5.84)	6.88 (3.82)	0.655
GAD-7	5.88 (5.28)	7.17 (5.11)	0.474	6.94 (5.30)	6.18 (5.14)	0.672
OBC	27.94 (9.98)	29.33 (11.83)	0.714	27.47 (10.90)	29.88 (11.01)	0.526
JFLS *mastication*	2.88 (2.15)	3.98 (2.06)	0.138	4.32 (2.11)	2.59 (1.85)	0.016
JFLS *jaw mobility*	3.52 (1.84)	5.31 (1.15)	0.002	5.01 (1.42)	3.91 (1.90)	0.064
JFLS *verbal and emotional expression*	0.98 (1.46)	2.05 (1.40)	0.038	1.79 (1.51)	1.30 (1.51)	0.349
MCO (mm)	30.88 (8.62)	24.94 (7.21)	0.036	25.82 (7.82)	29.65 (8.63)	0.185
MMO (mm)	38.63 (8.60)	34.17 (6.88)	0.103	34.88 (8.37)	37.65 (7.49)	0.318

MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain; OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale–chronic pain intensity; VAS, Visual Analogue Scale; JFLS, Jaw Functional Limitation Scale; PHQ-15, Physical Symptoms; PHQ-9, Patients Health Questionnaire; GAD-7, General Anxiety Disorder; OBC, Oral Behaviours Checklist; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; CI, Confidence Interval; n, number of participants; SD, Standard Deviation

At the baseline, MP and DD patients differed significantly in two JFLS categories, as well in pain-free mouth opening. Significant differences in OHIP-14, VAS, GCPS-CPI scores and 1 JFLS category (mastication) were found between HIP and LIP patients (Table 3).

3.2. Pre-intervention comparison between TMD patients and controls

Table 4. Baseline data of salivary oxidative stress markers and salivary cortisol in TMD patients and the control group

	Marker	TMD			Controls		
		Mean	SD	(95% CI)	Mean	SD	(95% CI)
morning	GPx (U/g)	74.84	56.62	(54.76-94.91)	67.72	58.02	(47.47-87.96)
	SOD (U/g)	2636.51	1176.67	(2219.3- 3053.7)	3308.73	2350.72	(2488.5-4128.9)
	TAC (mmol/g)	2.53	1.25	(2.09- 2.97)	3.62	3.07	(2.55-4.69)
	UA (umol/g)	362.83	250.86	(273.9- 451.78)	605.35	501.54	(430.4-780.4)
	MDA (nmol/g)	423.29	457.46	(261.1- 585.51)	1333.65	2357.92	(510.9-2156.4)
	8-OHdG (ug/g)	2.12	2.38	(1.27 - 2.9)	1.77	2.44	(0.89-2.65)
	SC (ug)	7.45	2.83	(6.74- 8.74)	10.11	5.62	(8.151-12.07)
afternoon	GPx (U/g)	84.62	59.73	(63.44-105.80)	58.61	45.14	(47.47-87.96)
	SOD (U/g)	2029.56	1488.54	(1501.7-2557.4)	2648.47	2044.76	(42.87-74.36)
	TAC (mmol/g)	2.65	1.16	(2.24 - 3.07)	3.68	2.88	(1935.1-3361.9)
	UA (umol/g)	396.33	205.66	(323.41-469.25)	610.21	525.22	(2.67-4.68)
	MDA (nmol/g)	890.92	983.22	(542.3-1239.56)	2231.86	2735.46	(426.9-793.5)
	8-OHdG (ug/g)	1.71	1.73	(1.11 - 2.33)	1.91	2.13	(1277.4-3186.3)
	SC (ug)	2.13	1.49	(1.61-2.66)	2.06	1.46	(1.14-2.68)

TMD, temporomandibular disorders; GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol

Table 5. Comparison of salivary oxidative stress markers and salivary cortisol between TMD patients and the control group (Student t-test)

	Marker*	TMD		Controls		t-values	df	P
		Mean	SD	Mean	SD			
morning	GPx	1.69	0.4	1.76	0.35	-0.86	65	0.39
	SOD	3.32	0.67	3.38	0.2	-0.46	65	0.65
	TAC	0.47	0.25	0.35	0.22	2.13	65	0.04
	UA	2.66	0.34	2.47	0.3	2.51	65	0.01
	MDA	2.56	0.79	2.28	0.66	1.56	65	0.12
	8-OHdG	0.01	0.44	0.16	0.36	-1.63	65	0.11
	SC	0.94	0.25	0.86	0.18	1.45	65	0.15
afternoon	GPx	1.65	0.36	1.82	0.35	-2.03	65	0.05
	SOD	3.31	0.33	3.2	0.31	1.37	65	0.18
	TAC	0.49	0.24	0.38	0.2	2.01	65	0.04
	UA	2.66	0.35	2.54	0.25	1.58	65	0.12
	MDA	2.98	0.7	2.61	0.65	2.22	65	0.03
	8-OHdG	0.03	0.47	0.06	0.4	-0.25	65	0.4
	SC	0.24	0.25	0.24	0.28	-0.09	65	0.93

TMD, temporomandibular disorders; GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; *statistics performed on log transformed data

The mean levels of log TAC among the TMD patients were significantly higher than among the controls (am: t=2.13, p=0.042; pm: t=2.01, p=0.04). The mean levels of log UA (morning: t=2.51, p=0.014), as well as the mean levels of log MDA were significantly higher in the TMD patients than in the control group (afternoon: t=2.22, p=0.03) (Table 5).

The comparison between control subjects with TMD subgroups is shown in (Table 6).

Post hoc analysis showed that when comparing the control group with the TMD diagnostic subgroups, statistically significant differences were found between the control group and the MP patients for morning levels of log SC and morning levels of log UA, both being higher in the MP patients. The afternoon levels of log GPx were significantly lower in the MP patients than in the control group. Significantly higher levels of log 8-OHdG were found in DD compared to the MP patients, whereas the morning levels of log SC were significantly higher in MP patients (Table 7).

When comparing the control group with the TMD subgroups with respect to pain intensity, statistically significant differences were found between the control group and the HIP patients for morning and afternoon levels of log MDA and morning levels of log UA, with significantly higher values in the HIP patients. The afternoon levels of log GPx was significantly lower in LIP patients compared to the control group (Table 7).

Table 6. Comparison of salivary oxidative stress markers and salivary cortisol between the control group and the TMD subgroups – Analysis of variance

	Marker*		TMD diagnostic subgroups vs. controls		TMD pain intensity subgroups vs. controls
morning	GPx	F	0.75	F	1.1
		P	0.47	P	0.33
	SOD	F	0.1	F	0.69
		P	0.9	P	0.5
	TAC	F	2.25	F	2.54
		P	0.11	P	0.09
	UA	F	3.23	F	4.19
		P	0.04	P	0.02
	MDA	F	1.21	F	3.68
		P	0.31	P	0.03
	8-OHdG	F	6.29	F	2.66
		P	0.003	P	0.07
	SC	F	8.58	F	1.05
		P	0.004	P	0.36
afternoon	GPx	F	4.68	F	3.36
		P	0.01	P	0.04
	SOD	F	2.62	F	0.97
		P	0.08	P	0.38
	TAC	F	2.05	F	2.11
		P	0.14	P	0.13
	UA	F	1.96	F	1.24
		P	0.15	P	0.3
	MDA	F	2.55	F	4.17
		P	0.09	P	0.02
	8-OHdG	F	5.1	F	1.45
		P	0.008	P	0.24
	SC	F	0.23	F	0.005
		P	0.79	P	0.99

TMD, temporomandibular disorders; GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol, f-value; p-value; *performed on log transformed data

Table 7. Differences in salivary oxidative stress markers and salivary cortisol between the control group and TMD subgroups

		Controls (n=33)		TMD (n=34)							
				Diagnostic Subgroup				Pain Intensity			
				MP (n=16*)		DD (n=18*)		HIP (n=17**)		LIP (n=17**)	
	Marker	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
morning	GPx (U/g)	74.84	(54.76-94.91)	54.64	(26.91- 82.37)	79.33	(48.52 -110.16)	73.72	(45.13-102.32)	61.71	(30.13-93.29)
	SOD (U/g)	2636.51	(2219.3- 3053.7)	2992.15	(1669.1-4315.3)	3590.14	(2466.2-4714.1)	3383.01	(2026.9-4739.1)	3234.46	(2152.5-4316.5)
	TAC (mmol/g)	2.53	(2.09- 2.97)	3.67	(1.74-5.61)	3.58	(2.24-4.87)	4.17	(2.1-6.25)	3.07	(2.26-3.89)
	UA (umol/g)	362.83 [BC]	(273.9- 451.78)	633.72 [B]	(310.22- 957.24)	580.13	(380.21-780.05)	733.53 [C]	(408.99-1058.08)	477.17	(326.87-627.47)
	MDA (nmol/g)	423.29 [C]	(261.1- 585.51)	946.56	(260.8- 1632.41)	1677.71	(180.49-3174.92)	2084.6 [C]	(487.88-3681.45)	582.64	(176.7-988.59)
	8-OHdG (ug/g)	2.12 [B]	(1.27 - 2.9)	0.94 [AB]	(0.33 -1.55)	2.6 [A]	(0.96-4.24)	2.39	(0.68-4.09)	1.16	(0.567-1.75)
	SC (ug)	7.45 [B]	(6.74- 8.74)	13.18 [AB]	(10.24-16.12)	7.38 [A]	(5.29-9.46)	10.38	(7.38-13.38)	9.84	(6.98-12.70)
afternoon	GPx (U/g)	84.62 [BD]	(63.44-105.80)	39.75 [B]	(23.86-55.66)	75.37	(50.32-100.43)	69.94	(43.64-96.24)	47.28 [D]	(28.65-65.92)
	SOD (U/g)	2029.56	(1501.7-2557.4)	2227.1	(1152.9-3301.2)	3023.1	(2002.1-4044.1)	2679.54	(1507.74-3851.34)	2617.41	(1665.6-3569.2)
	TAC (mmol/g)	2.65	(2.24 - 3.07)	3.48	(2.06-4.91)	3.86	(2.31- 5.42)	3.81	(2.39-5.22)	3.56	(1.97-5.15)
	UA (umol/g)	396.33	(323.41-469.25)	655.68	(389.72-921.65)	569.79	(292.02-847.57)	640.06	(378.79-901.33)	580.36	(294.63-866.09)
	MDA (nmol/g)	890.92 [C]	(542.3-1239.56)	1752.73	(623.2-2882.27)	2657.75	(1073.2-4242.25)	3288.1 [C]	(1511.94-5064.3)	1175.60	(629.49-1721.71)
	8-OHdG (ug/g)	1.71	(1.11 - 2.33)	1.1 [A]	(0.36 -1.83)	2.72 [A]	(1.40-4.03)	2.25	(1.11-3.38)	1.57	(0.42-2.71)
	SC (ug)	2.13	(1.61-2.66)	1.9	(111-2.7)	2.19	(1.45-2.93)	2.01	(1.3-2.7)	2.11	(1.29-2.92)

*Pooled regardless of pain intensity; **pooled regardless of pathology source (diagnostic subgroup). TMD, temporomandibular disorders; MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain; GPx, glutathione peroxidase; SOD. superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; CI, Confidence Interval

[A] p- values between MP and DD: $p_{am\ 8\text{-}OHdG}$ =0.011. $p_{pm\ 8\text{-}OHdG}$ =0.009. $p_{am\ SC}$=0.001
[B] p- values between controls and MP: $p_{am\ UA}$ = 0.04. $p_{am\ SC}$=0.003. $p_{pm\ GPx}$ =0.014.
[C] p- values between controls and VIB: $p_{am\ MDA}$ = 0.043. $p_{pm\ MDA}$ = 0.02. $p_{am\ UA}$= 0.02
[D] p- values between controls and NIB: $p_{pm\ GPx}$ = 0.041
*statistics performed on log transformed data

Pearson's correlation was used to determine whether psychological stress, SC levels and OS markers were correlated in the TMD patients and controls. When all the TMD patients were considered, higher PSS scores were correlated with higher levels of GPx and MDA. When correlations were tested separately for each TMD diagnostic subgroups, significant positive correlations were present between the morning SC and OS markers (GPx and UA) in the MP patients. The same was observed when correlations were tested for each TMD pain intensity subgroup. In LIP, significant positive correlations were found between the morning SC and OS markers (GPx and UA). Higher PSS scores were correlated with higher OS marker levels (MDA and GPx) in the DD and HIP subgroups. In addition, higher PSS scores were correlated with lower levels of SOD when all the TMD patients were considered, as well as when correlations were tested for the DD and HIP subgroups separately (Table 9).

In the control group, higher PSS scores were correlated with lower levels of SOD. Significant positive correlations were present between the SC and UA (morning r=0.501, afternoon r=0.497) (Table 8).

Table 8. Correlations between the psychological stress, SC and OS markers in the control group

			PSS	GPx	SOD	TAC	UA	MDA	8-OHdG
controls (N=33)		PSS	-	-0.008	-0.431	-0.271	0.125	0.24	0.21
	SC	morning	-0.056	-0.014	0.123	0.006	0.501	0.166	0.167
		afternoon	0.047	-0.587	0.006	-0.036	0.497	0.129	0.014

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale

Table 9. Correlations between the psychological stress, SC and OS markers in the TMD patients

				PSS	GPx	SOD	TAC	UA	MDA	8-OHdG
TMD patients (N=34)			PSS	-	0.425	-0.431	-0.097	0.17	0.472	0.08
		SC	morning	-0.056	0.202	0.249	0.178	0.222	-0.054	-0.386
			afternoon	0.047	-0.096	0.125	0.266	0.19	0.133	0.163
Diagnostic subgroup Pooled regardless of pain intensity	MP (n=16)		PSS	-	0.311	0.354	0.182	0.155	0.24	-0.06
		SC	morning	0.029	0.643	0.191	0.466	0.592	0.18	-0.067
			afternoon	0.317	-0.18	0.283	0.385	-0.042	0.078	-0.025
	DD (n=18)		PSS		0.504	-0.665	-0.27	0.169	0.588	0.32
		SC	morning	-0.266	0.031	0.331	0.007	-0.027	-0.28	-0.321
			afternoon	-0.072	-0.185	-0.215	0.122	0.421	0.154	0.182
Pain intensity Pooled regardless of diagnostic subgroup	LIP (n=17)		PSS		0.168	-0.184	-0.309	0.056	0.387	-0.486
		SC	morning	0.429	0.529	-0.082	0.25	0.512	0.265	-0.362
			afternoon	-0.261	-0.072	0.053	0.429	0.237	0.261	0.256
	HIP (n=17)		PSS		0.655	-0.499	-0.022	0.217	0.545	0.412
		SC	morning	-0.349	-0.13	0.381	0.134	-0.011	-0.359	-0.373
			afternoon	0.313	-0.144	0.206	0.051	0.148	0.168	0.039

TMD, temporomandibular disorders; GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; MP, myofascial pain; DD, disc displacement; LIP, low-intensity pain; HIP, high-intensity pain

3.3. Intervention study in TMD patients

3.3.1. Comparison of the effectiveness of therapeutic interventions

3.3.1.1. Comparison of baseline data: treatment groups

The demographics and baseline data of two treatment groups are shown in Table 10. There were no differences between the treatment groups on any of the measured variables. Furthermore, no statistically significant age differences were noted between the two treatment groups (SU 38.8±11.8; PU 32.6±11.5; t=1.54, p=0.13).

Table 10. Comparison of pretreatment data between the treatment groups

Variable	Stabilization splint (n=19)	Placebo splint (n=15)	t	p
age	38.84 ± 11.84	32.66 ± 11.54	1.54	0.13
OHIP-14	26.31 ± 9.99	23.93 ± 10.85	0.66	0.51
PSS	17.63 ± 6.05	18.8 ± 8.04	-0.48	0.63
VAS	6.52 ± 2.03	5.53 ± 1.72	1.50	0.14
GCPS	49.42± 20.98	46.20 ± 15.89	0.49	0.62
PHQ-15	8.05 ± 4.47	8.73 ± 4.33	-0.44	0.65
PHQ-9	6.10 ± 4.85	7 ± 5.03	-0.52	0.60
GAD-7	6.37 ± 5.70	6.8 ± 4.54	-0.23	0.81
OBC	29.58 ± 10.99	27.53 ± 10.95	0.54	0.59
JFLS *mastication*	3.87 ± 2.34	2.93 ± 1.80	1.28	0.20
JFLS *jaw mobility*	4.85 ± 1.73	3.96 ± 1.67	1.50	0.14
JFLS *verbal and emotional expression*	1.75 ± 1.66	1.28 ± 1.28	0.90	0.37
MCO (mm)	27.57 ± 9.88	27.93 ± 6.18	-0.12	0.90
MMO (mm)	35.78 ± 9.12	36.86 ± 6.41	-0.38	0.70

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale-chronic pain intensity; VAS, Visual Analogue Scale; JFLS, Jaw Functional Limitation Scale; PHQ-15, Physical Symptoms; PHQ-9, Patients Health Questionnaire; GAD-7, General Anxiety Disorder; OBC, Oral Behaviours Checklist; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; n, number of participants

Table 11. Salivary oxidative stress markers and cortisol levels in the TMD treatment groups – baseline data

		Stabilization splint (n=19)		Placebo splint (n=15)	
	Marker	Mean	(95% CI)	Mean	(95% CI)
morning	GPx (U/g)	61.24	39.21-83.28	75.92	36.30-115.54
	SOD (U/g)	3592.90	2461.40-4724.41	2948.78	1627.68-4269.88
	TAC (mmol/g)	3.56	2.33-4.78	3.71	1.64-5.77
	UA (umol/g)	534.54	378.81-690.27	695.04	326.34-1063.75
	MDA (nmol/g)	541.24	199.01-883.48	2337.38	540.75-4134.00
	8-OHdG (ug/g)	0.90	0.56-1.25	2.89	0.97-4.82
	SC (ug)	9.52	7.01-12.02	10.87	7.43-14.31
afternoon	GPx (U/g)	59.93	34.31-85.56	56.94	38.06-75.83
	SOD (U/g)	2403.03	1645.87-3160.19	2959.36	1547.81-4370.91
	TAC (mmol/g)	3.66	2.25-5.07	3.71	2.09-5.33
	UA (umol/g)	597.14	343.47-850.81	626.77	326.72-926.83
	MDA (nmol/g)	1483.68	476.95-2490.41	3179.56	1401.70-4957.41
	8-OHdG (ug/g)	1.73	0.67-2.79	2.14	0.88-3.40
	SC (ug)	2.22	1.47-2.97	1.86	1.11-2.61

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; SS, stabilization splint; PS, placebo splint; CI, Coefficient of variability; n, number of participants

Table 12. Comparison of salivary OS markers and cortisol baseline levels between the treatment groups

	Marker*	Stabilization splint (n=19)		Placebo splint (n=15)		t-value	df	p
		Mean	SD	Mean	SD			
morning	GPx	1.61	0.47	1.75	0.33	-0.96	32	0.34
	SOD	3.45	0.34	3.16	0.93	1.23	32	0.23
	TAC	0.61	0.19	0.61	0.21	0.04	32	0.97
	UA	2.63	0.34	2.70	0.35	-0.64	32	0.53
	MDA	2.33	0.72	2.85	0.80	-1.99	32	0.06
	8-OHdG	0.26	0.14	0.47	0.32	-2.54	32	0.02
	SC	0.92	0.22	0.95	0.30	-0.33	32	0.74
afternoon	GPx	1.62	0.39	1.65	0.36	-0.22	32	0.83
	SOD	3.29	0.30	3.33	0.37	-0.34	32	0.74
	TAC	0.62	0.19	0.62	0.19	-0.08	32	0.94
	UA	2.66	0.32	2.65	0.39	0.13	32	0.90
	MDA	2.75	0.75	3.26	0.52	-2.22	32	0.03
	8-OHdG	0.34	0.27	0.42	0.26	-0.81	32	0.42
	SC	0.47	0.18	0.43	0.16	0.76	32	0.45

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; SS, stabilization splint; PS, placebo splint; n, number of participants *log transformed data

In the PS group, the levels of log 8-OHdG (morning: p=0.02), as well as the levels of log MDA (afternoon: p=0.03) were significantly higher compared to the SS group. The rest of the baseline data of salivary parameters did not differ significantly between the treatment groups (Table 12).

3.3.1.2. Changes in treatment outcomes within the treatment groups

Patients in the SS group exhibited a greater decrease in spontaneous pain (Wilks Lambda=0.08, F=51.32, p <0.0001, effect size = 0.77) than patients in the PS group (Wilks Lambda=0.42, F=4.59, p =0.028, effect size = 0.27). SS patients also showed significant improvements in perceived stress (Wilks Lambda=0.55, F=3.39, p =0.049, effect size = 0.21), quality of life (Wilks Lambda=0.24, F=14.48, p =0.0001, effect size = 0.55), characteristic pain intensity (Wilks Lambda=0.25, F=13.80, p=0.0001, effect size = 0.56), pain-free mouth opening (Wilks Lambda=0.54, F=3.39, p <0.045, effect size = 0.24) and maximal unassisted mouth opening (Wilks Lambda=0.53, F=4.04, p =0.029, effect size = 0.31). Post hoc comparisons of the treatment outcomes within the treatment groups are shown in Table 13 and Table 14.

Table 13. Pairwise comparisons of treatment outcomes between certain time-points within the stabilization splint group

Variable* (n=17)			Means		Mean Diff. (SE)	p
OHIP-14	baseline	vs. 3rd month	25.29	14.96	10.35 (2.15)	<0.0001
		vs. 6th month		10.41	14.88 (2.15)	<0.0001
PSS	baseline	vs. 3rd month	17.18	14	3.18 (1.29)	0.01
		vs. 6th month		13.18	4.0 (1.29)	0.001
VAS	baseline	vs. 3rd month	6.65	2.06	4.59 (0.54)	<0.0001
		vs. 6th month		0.94	5.71 (0.54)	<0.0001
GCPS-CPI	baseline	vs. 3rd month	49.18	21.92	27.26 (4.47)	0.001
		vs. 6th month		20.41	28.77 (4.47)	<0.0001
MCO (mm)	baseline	vs. 3rd month	28.94	34.35	-5.41 (1.67)	0.015
		vs. 6th month		34.76	-5.82 (1.67)	0.007
MMO (mm)	baseline	vs. 3rd month	35.82	40.47	-4.64 (1.47)	0.019
		vs. 6th month		42.47	-6.65 (1.47)	0.0003

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale – characteristic pain intensity; VAS, Visual Analogue Scale; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; p-value, statistical significance; t-value; SD, standard deviation; *log transformed data

Table 14. Pairwise comparisons of treatment outcomes between certain time-points within the placebo splint group

Variable* (n=13)			Means		Mean Diff. (SE)	p
OHIP-14	baseline	vs. 3rd month	22.08	15.69	6.38 (2.46)	0.13
		vs. 6th month		16.08	6 (2.46)	0.18
PSS	baseline	vs. 3rd month	19.15	19.15	0 (1.48)	1.0
		vs. 6th month		17.69	1.5 (1.48)	0.37
VAS	baseline	vs. 3rd month	5.38	3.62	1.77 (0.61)	0.096
		vs. 6th month		2.92	2.46 (0.61)	0.007
GCPS-CPI	baseline	vs. 3rd month	43.38	28.92	14.46 (5.12)	0.072
		vs. 6th month		31.72	11.67 (5.12)	0.23
MCO (mm)	baseline	vs. 3rd month	27.15	28.77	-1.62 (1.92)	1.0
		vs. 6th month		27.54	-0.38 (1.92)	1.0
MMO (mm)	baseline	vs. 3rd month	36.08	36.15	-0.08 (1.68)	1.0
		vs. 6th month		35.85	0.23 (1.68)	1.0

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale – characteristic pain intensity; VAS, Visual Analogue Scale; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; p-value, statistical significance; t-value; SD, standard deviation; *log transformed data

When compared with the baseline values, at the 3-month follow-up, patients in the SS group demonstrated a significant decrease in VAS, OHIP-14, PSS and CPI, as well as a significant increase of MCO and MMO. In the PS group, no significant changes were present after the 3rd month of treatment (Tables 13, 14).

At the 6-month follow-up, when compared with the baseline values, patients in the SS group demonstrated a significant decrease in VAS, OHIP-14, PSS and CPI, as well as a significant increase of MCO and MMO. In the PS group, a significant decrease in VAS scores was found.

All JFLS categories, except for emotional and verbal expression, decreased significantly in the 6th month (T3) compared to the baseline (T0) in the SS group (mastication t=4.92, p=0.00015; vertical jaw mobility t=4.82, p=0.00018; emotional and verbal expression t=1.82, p=0.086). No such changes in the JFLS categories were observed in the PS group (mastication t=0.24, p=0.81; vertical jaw mobility t=1.26, p=0.23; emotional and verbal expression t=-0.24, p=0.56) (Figures 6, 7).

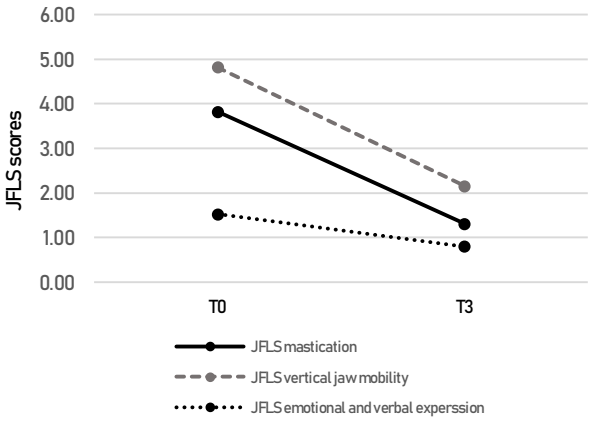

Figure 6. Changes in Jaw functional limitation scale (JFLS) categories between the baseline (T0) and 6-month follow-up (T3) – stabilization splint group (SS).

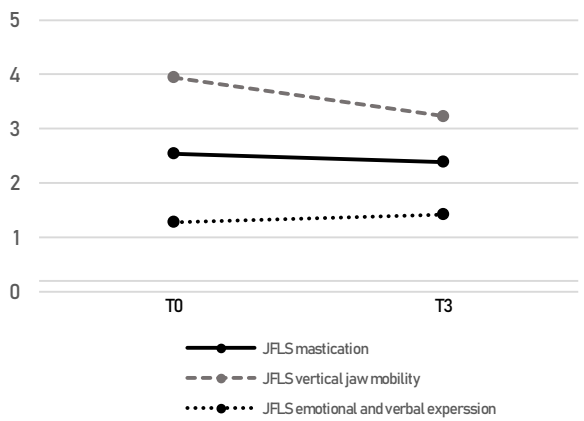

Figure 7. Changes in Jaw functional limitation scale (JFLS) categories between the baseline (T0) and 6-month follow-up (T3) – placebo splint group (PS).

3.3.1.3. Changes in OS markers and salivary cortisol within the treatment groups

Patients in the SS group showed a significant decrease in the levels of log TAC (morning: Wilks Lambda=0.49, F=4.72, p=0.017, effect size=0.24; afternoon Wilks Lambda=0.32, F=9.64, p = 0.001, effect size=0.21) and levels of log UA (morning: Wilks Lambda=0.56, F=3.94, p <0.049, effect size=0.15). No significant change of OS markers in the PS group was present.

Post hoc comparisons of OS markers and SC within the treatment groups are shown in Table 15 and Table 16.

Table 15. Pairwise comparisons of oxidative stress markers and salivary cortisol between certain time-points within the stabilization splint group

	Variable*	(n=17)		Means		Mean Diff. (SE)	p
morning	GPx	baseline	vs. 3rd month	1.62	1.50	0.11 (0.15)	0.46
			vs. 6th month		1.33	0.29 (0.15)	0.62
	SOD	baseline	vs. 3rd month	3.43	3.37	0.05 (0.17)	0.75
			vs. 6th month		3.36	0.07 (0.17)	0.68
	TAC	baseline	vs. 3rd month	0.49	0.32	0.17 (0.54)	0.002
			vs. 6th month		0.31	0.18 (0.054)	0.001
	UA	baseline	vs. 3rd month	2.69	2.51	0.18 (0.07)	0.019
			vs. 6th month		2.49	0.20 (0.07)	0.013
	MDA	baseline	vs. 3rd month	2.39	2.42	-0.03 (0.17)	1.0
			vs. 6th month		2.27	0.11 (0.17)	1.0
	8-OHdG	baseline	vs. 3rd month	-0.18	-0.044	-0.13 (0.09)	0.15
			vs. 6th month		-0.1	-0.08 (0.09)	0.41
	SC	baseline	vs. 3rd month	0.95	0.91	0.4 (0.09)	0.68
			vs. 6th month		0.99	-0.43 (0.09)	0.64
afternoon	GPx	baseline	vs. 3rd month	1.64	1.53	0.1 (0.16)	0.49
			vs. 6th month		1.38	0.26 (0.16)	0.10
	SOD	baseline	vs. 3rd month	3.27	3.38	-0.11 (0.12)	0.37
			vs. 6th month		3.30	-0.03 (0.12)	0.83
	TAC	baseline	vs. 3rd month	0.52	0.41	0.11 (0.05)	0.013
			vs. 6th month		0.37	0.15 (0.05)	0.001
	UA	baseline	vs. 3rd month	2.72	2.49	0.23 (0.087)	0.02
			vs. 6th month		2.61	0.11 (0.087)	0.28
	MDA	baseline	vs. 3rd month	2.79	2.62	0.17 (0.14)	0.23
			vs. 6th month		2.77	0.03 (0.14)	0.85
	8-OHdG	baseline	vs. 3rd month	-0.05	-0.11	0.06 (0.12)	0.62
			vs. 6th month		-0.02	-0.03 (0.12)	0.82
	SC	baseline	vs. 3rd month	0.29	0.26	0.3 (0.08)	0.72
			vs. 6th month		0.28	0.015 (0.08)	0.86

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale, *log transformed data

Table 16. Pairwise comparisons of oxidative stress markers and salivary cortisol between certain time-points within the placebo splint group

	Variable*	(n=13)		Means		Mean Diff. (SE)	p
morning	GPx	baseline	vs. 3rd month	1.70	1.45	0.25 (0.17)	0.16
			vs. 6th month		1.54	0.16 (0.17)	0.36
	SOD	baseline	vs. 3rd month	3.16	3.35	-0.19 (0.19)	0.33
			vs. 6th month		3.49	-0.33 (0.19)	0.084
	TAC	baseline	vs. 3rd month	0.49	0.40	0.08 (0.06)	0.20
			vs. 6th month		0.40	0.09 (0.06)	0.17
	UA	baseline	vs. 3rd month	2.70	2.58	0.12 (0.08)	0.16
			vs. 6th month		2.66	0.04 (0.08)	0.61
	MDA	baseline	vs. 3rd month	2.66	2.59	0.07 (0.20)	1.0
			vs. 6th month		2.74	-0.09 (0.20)	1.0
	8-OHdG	baseline	vs. 3rd month	0.16	0.07	0.13 (0.11)	0.25
			vs. 6th month		0.10	0.09 (0.11)	0.44
	SC	baseline	vs. 3rd month	0.99	0.78	0.22 (0.10)	0.108
			vs. 6th month		1.06	-0.06 (0.10)	0.54
afternoon	GPx	baseline	vs. 3rd month	1.62	1.51	0.10 (0.18)	0.56
			vs. 6th month		1.54	0.07 (0.18)	0.68
	SOD	baseline	vs. 3rd month	3.34	3.35	-0.01 (0.13)	0.92
			vs. 6th month		3.40	-0.06 (0.13)	0.66
	TAC	baseline	vs. 3rd month	0.51	0.41	0.099 (0.051)	0.06
			vs. 6th month		0.42	0.096 (0.051)	0.07
	UA	baseline	vs. 3rd month	2.66	2.64	0.02 (0.1)	0.84
			vs. 6th month		2.61	0.05 (0.1)	0.61
	MDA	baseline	vs. 3rd month	3.17	3.14	0.02 (0.17)	0.9
			vs. 6th month		3.30	-0.14 (0.17)	0.41
	8-OHdG	baseline	vs. 3rd month	0.04	0.01	-0.02 (0.15)	0.9
			vs. 6th month		0.13	-0.10 (0.15)	0.50
	SC	baseline	vs. 3rd month	0.21	0.28	-0.07 (0.09)	0.46
			vs. 6th month		0.37	-0.17 (0.09)	0.08

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; *log transformed data

When compared with the baseline values, at the 3-month follow-up, patients treated with SS exhibited a significant decrease in the levels of log TAC (morning: p=0.002, afternoon: p=0.013, respectively) and levels of log UA (morning: p=0.019, afternoon p=0.02, respectively) (Table 15). In patients treated with PS, no significant changes of the OS marker levels from the baseline to the 3rd month follow-up were found (p >0.05) (Table 16).

At the 6-month follow-up, when compared with the baseline values, patients treated with SS exhibited a significant decrease in the levels of log TAC (morning: p=0.001, afternoon: p=0.001, respectively) and levels of log UA (morning: p=0.013) (Table 15). In the PS group,

no significant changes of OS marker levels from the baseline to the 6th month follow-up were found (Table 16).

3.3.1.4. Differences in the percentage change of measured parameters: between-group comparison

Table 17. Comparison of percentage changes of treatment outcomes (VAS, MCO, MMO, OHIP-14, PSS) and TAC between treatment groups

	SS		PS				
	Mean	SD	Mean	SD	t-value	df	p
VAS reduction 3rd month (%)	69.41	28.28	21.13	69.49	2.61	28	0.014
VAS reduction 6th month (%)	86.92	12.56	42.58	40.30	4.29	28	0.000
Increase in MCO 3rd month (%)	24.60	39.97	8.60	24.75	1.27	28	0.216
Increase in MMO 3rd month (%)	16.64	24.72	1.44	16.24	1.92	28	0.065
Increase in MCO 6th month (%)	28.54	41.40	1.64	22.08	2.12	28	0.043
Increase in MMO 6th month (%)	23.82	32.11	0.73	18.01	2.32	28	0.028
TAC (am) reduction 3rd month (%)	20.59	46.49	12.29	32.24	0.55	28	0.587
TAC (pm) reduction 3rd month (%)	17.58	32.42	11.30	37.74	0.49	28	0.628
TAC (am) reduction 6th month (%)	28.10	28.72	5.95	46.09	1.62	28	0.117
TAC (pm) reduction 6th month (%)	26.57	25.28	7.69	55.14	1.25	28	0.220
OHIP reduction 3rd month (%)	43.89	32.28	24.43	47.81	1.33	28	0.190
OHIP reduction 6th month (%)	61.68	30.33	18.52	62.35	2.5	28	0.018
PSS reduction 3rd month (%)	15.68	35.75	-12.48	46.61	1.87	28	0.071
PSS reduction 6th month (%)	24.68	35.66	0.63	37.24	1.80	28	0.083

OHIP, Oral Health Impact Profile; PSS, Perceived Stress Scale; VAS, Visual Analogue Scale; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; TAC, total antioxidant capacity; p-value, statistical significance; t-value; SD, standard deviation; SS, stabilization splint, PS, placebo splint

Significantly greater VAS reduction was present after both the 3rd and 6th months of the SS treatment compared to the placebo. After the 6-month treatment period, a significantly greater reduction in OHIP-14 was present in the SS group compared to the placebo. Also, patients in the SS group had significantly greater changes in MCO and MMO compared to the PS group (Table 17; Figures 8, 9).

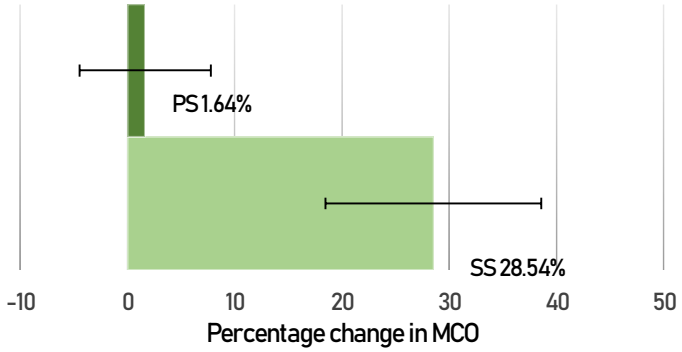

Figure 8. The effectiveness of therapeutic interventions - the percentage change in maximal comfortable mouth opening (MCO) following 6 months of treatment (the whiskers represent standard errors). *SS stabilization splint; PS placebo splint*

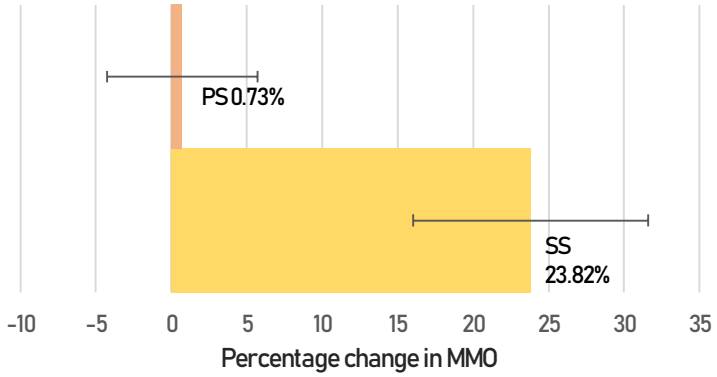

Figure 9. The effectiveness of therapeutic interventions - the percentage change in maximal mouth opening (MMO) following 6 months of treatment (the whiskers represent standard errors). *SS stabilization splint; PS placebo splint*

3.3.1.5. Correlations between the psychological stress, cortisol and oxidative stress markers after 3rd and 6th month of treatment

In patients treated with SS, at the 3rd month follow-up, a significant positive correlation between changes in perceived stress with the percentage change in the afternoon TAC (r=0.50, p=0.04), as well with the percentage change in the morning SC was found (r=0.64, p=0.005) (Figures 10 and 11).

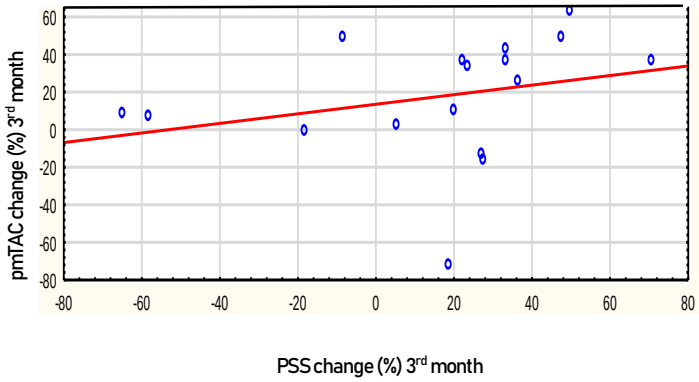

Figure 10. Correlation between the percentage change in total antioxidant capacity (TAC) and the percentage change in perceived stress (PSS) at the 3rd month follow-up in the SS group

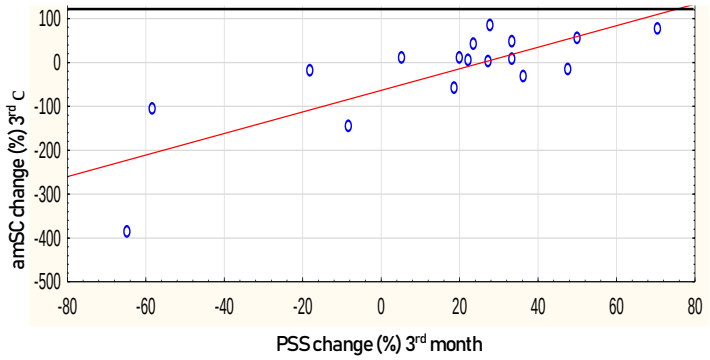

Figure 11. Correlation between the percentage change in morning salivary cortisol (SC) and the percentage change in perceived stress (PSS) at the 3rd month follow-up in the SS group

At the 6th month follow-up, no correlations between the measured parameters were found.

3.3.2. Comparison of therapeutic interventions between the TMD subgroups

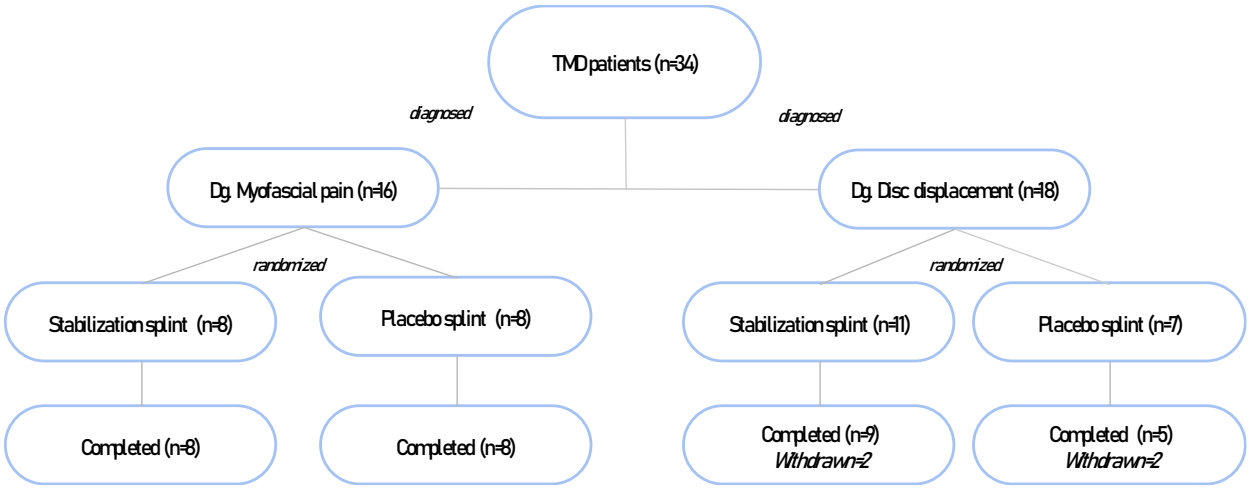

Figure 12. Distribution of the participants in the TMD diagnostic subgroups

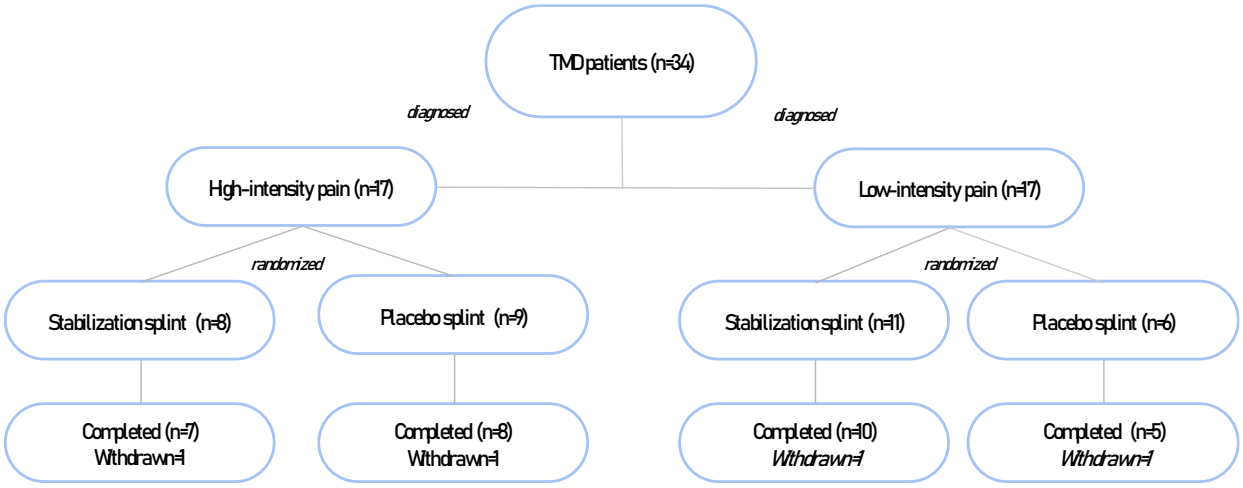

Figure 13. Distribution of the participants in the TMD pain intensity subgroups

3.3.2.1. Comparison of baseline data between TMD subgroups

No significant age difference was found between both treatment groups when divided into the diagnostic subgroups: MP (SU 42.25±10.1; PU 36.37±12.9; t=1.017, p=0.33), DD (SU 36.36±12.9; PS 28.28±8.81; t=1.45, p=0.16) (Table 18). There were no differences in other measured variables between the two treatment groups at baseline, both in MP and DD patients.

Table 18. Comparison of pretreatment data between the treatment groups – according to diagnostic subgroup

	MP (N=16)				DD (N=18)			
Variable	Stabilization splint (n=8)	Placebo splint (n=8)	t	p	Stabilization splint (n=11)	Placebo splint (n=7)	t	p
age	42.25	36.38	1.02	0.33	36.36	28.29	1.45	0.16
OHIP-14	23.38	20.50	0.73	0.47	28.45	27.86	0.10	0.91
PSS	20.88	17.63	1.28	0.22	15.27	20.14	-1.25	0.22
VAS	6.12	5.38	0.75	0.47	6.82	5.71	1.21	0.24
GCPS	46.25	47.25	-0.12	0.91	51.73	45	0.65	0.52
PHQ-15	8.13	9.13	-0.53	0.61	8	8.28	-0.11	0.91
PHQ-9	6.88	5.00	0.95	0.36	5.55	9.29	-1.40	0.17
GAD-7	6.75	5.00	0.65	0.53	6.09	8.86	-1.13	0.27
OBC	32.25	23.63	1.86	0.08	27.64	32	-0.75	0.46
JFLS *mastication*	3.02	2.73	0.26	0.79	4.49	3.16	1.38	0.18
JFLS *jaw mobility*	4.16	2.88	1.45	0.17	5.36	5.21	0.26	0.79
JFLS *verbal and emotional expression*	1.25	0.72	0.71	0.49	2.12	1.93	0.28	0.78
MCO (mm)	33.13	28.63	1.048	0.31	23.55	27.14	-1.03	0.31
MMO (mm)	39.38	37.88	0.34	0.74	33.18	35.71	-0.75	0.46

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale-chronic pain intensity; VAS, Visual Analogue Scale; JFLS, Jaw Functional Limitation Scale; PHQ-15, Physical Symptoms; PHQ-9, Patients Health Questionnaire; GAD-7, General Anxiety Disorder; OBC, Oral Behaviours Checklist; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; n, number of participants; MP, myofascial pain; DD, disc displacement

No significant age difference was found between treatment groups when divided into the pain intensity subgroups: HIP (SS 41.62±14.7; PS 31.32±12.1; t=1.58, p=0.13) and LIP (SS

36.81±9.5; PS 34.5±11.5; t=0.44, p=0.66) In LIP patients there were no differences in other measured variables between the two treatment groups at baseline.

In the HIP patients, however, differences between the two treatment groups at baseline existed for VAS, GCPS and the two JFLS categories, with significantly higher values in the SS group (Table 19).

Table 19. Comparison of pretreatment data between the treatment groups – according to pain intensity

	HIP (n=17)				LIP (n=17)			
Variable	Stabilization splint (n=8)	Placebo splint (n=9)	t	p	Stabilization splint (n=11)	Placebo splint (n=6)	t	p
age	41.62	31.33	1.58	0.13	36.81	34.50	0.44	0.66
OHIP-14	34.00	30.00	0.90	0.38	20.72	14.66	1.89	0.07
PSS	17.37	21.11	-0.99	0.33	17.81	15.33	0.81	0.42
VAS	8.25	6.11	3.31	0.004	5.27	4.66	0.71	0.48
GCPS	68.37	51.22	3.57	0.002	35.63	38.66	-0.34	0.73
PHQ-15	8.75	8.11	0.25	0.80	7.54	9.66	-1.13	0.27
PHQ-9	5.00	7.11	-0.73	0.47	6.90	6.83	0.03	0.97
GAD-7	6.37	7.44	-0.40	0.69	6.36	5.83	0.19	0.84
OBC	25.62	29.11	-0.64	0.52	32.45	25.16	1.33	0.20
JFLS *mastication*	5.49	3.27	2.49	0.024	2.69	2.41	0.29	0.77
JFLS *jaw mobility*	5.71	4.38	2.13	0.049	4.22	3.33	0.92	0.37
JFLS *verbal and emotional expression*	2.18	1.44	1.01	0.32	1.44	1.04	0.51	0.61
MCO (mm)	25.00	26.55	-0.39	0.69	29.45	30.00	-0.12	0.90
MMO (mm)	33.25	36.33	-0.74	0.46	37.63	37.667	-0.007	0.99

OHIP-14, Oral Health Impact Profile; PSS, Perceived Stress Scale; GCPS-CPI, Graded Chronic Pain Scale–chronic pain intensity; VAS, Visual Analogue Scale; JFLS, Jaw Functional Limitation Scale; PHQ-15, Physical Symptoms; PHQ-9, Patients Health Questionnaire; GAD-7, General Anxiety Disorder; OBC, Oral Behaviours Checklist; MCO, Maximal Comfortable Mouth Opening; MMO, Maximal Mouth Opening; n, number of participants; HIP, high-intensity pain; LIP, low-intensity pain

3.3.2.2. Changes in treatment outcomes during 6-month follow-up - mixed between-within subjects repeated measures ANOVA

During the 6-month period, no statistically significant changes were observed in the PSS scores, however, in the SS group, significantly lower values were observed compared to the PS group at all time points (F=5.21, p=0.032). No statistical significance in the PSS scores was present between the TMD diagnostic subgroups (Figure 14), or among the TMD groups with respect to pain intensity (p>0.05) (Figure 15).

The VAS scores for spontaneous pain showed a significant reduction over time (Wilks Lambda=0.15, F=35.58; p=0.0001, effect size=0.59). Changes in spontaneous pain differed significantly between the two treatment groups, with a greater reduction in the SS comparing to the PS group (interaction time x treatment group; Wilks Lambda = 0.39, F=10.25; p=0.0002, effect size=0.29). The post hoc analysis showed that in the SS group, the mean VAS scores were significantly lower in the 1^{st}, 3^{rd} and 6^{th} months of the treatment compared to the baseline (p=0.0007, p=0.001 and p<0.0001 respectively), while in the PS group, a significant difference in the mean VAS scores was only present between the 6^{th} month of treatment compared to the baseline (p=0.004). There were no significant differences in the VAS scores between the TMD diagnostic subgroups (Figure 16). Significant differences were present between the pain intensity subgroups (interaction time x pain intensity; Wilks Lambda=0.56, F=5.18, p=0.008). Differences considering pain-intensity were evident at the T0 and T1 time points, whereas at the T2 and T3 time point, no significant differences in pain intensity between the observed groups were found (Figure 17).

The OHIP-14 scores also showed a significant reduction over time (Wilks Lambda=0.34, F=12.78; p=0.0001, effect size=0.40). Changes in the OHIP-14 scores differed significantly between the two treatment groups, with reduced values only in the SS group during the 6-month period (interaction time x treatment group; Wilks Lambda=0.56, F=5.20; p=0.008, effect size=0.18). The post hoc analysis showed that in the SS group, the mean OHIP-14 score was significantly lower in the 1^{st}, 3^{rd} and 6^{th} months of the treatment compared to the baseline (p=0.045, p=0,0001 and p<0.0001 respectively), while in the PS group, no significant difference in the mean OHIP-14 score was present between baseline and follow-up appointments. There were no significant differences in the OHIP-14 scores between the TMD diagnostic subgroups (Figure 18). Significant differences were found between the pain intensity subgroups (interaction time x pain intensity; Wilks Lambda=0.66, F=3.41, p=0.037).

Differences were evident at the T0, T1 and T2 time points, whereas at the T3 time point, no significant differences in OHIP-14 scores between the observed groups were found (Figure 19).

The MCO values did not change significantly over the 6-month period, though pain-free maximal mouth opening differed significantly between the two treatment groups with greater overall values in the SS group compared to the PS group ($F=4.39$, $p=0.047$). Also, a significant difference was present when comparing the MP group to the DD group, with greater overall values in the MP patients ($F=5.18$, $p= 0.03$) (Figure 20 and 21).

The MMO values did not differ significantly between the treatment groups or between the TMD diagnostic and pain intensity subgroups ($p>0.05$), but changed significantly over time ($F=3.5$; $p=0.01$, effect size=0.13). Although the changes in MMO values did not differ significantly between the two treatment groups, a tendency to a constant increase of MMO was present in the SS group (Figures 22 and 23).

The GCPS scores decreased significantly over time (Wilks Lambda=0.42, $F=9.12$; $p=0.0005$, effect size=0.41). Changes in the GCPS scores differed significantly between the two treatment groups with a greater decrease in the SS group compared to the PS (interaction time x treatment group; Wilks Lambda=0.67, $F=3.20$, $p=0.045$, effect size: 0.17). The post hoc analysis showed that in the SS group, the mean GCPS scores were significantly lower at the 3rd and 6th month of treatment compared to the baseline ($p <0.0001$, $p <0.0001$ respectively), while in the PS group, no significant difference in the mean GCPS scores was present between the baseline and follow-up appointments. There were no significant differences in the GCPS values between the diagnostic subgroups (Figure 24), but differences existed with respect to pain intensity ($F=19.18$, $p=0.002$), with significantly higher values in HIP compared to LIP patients (Figure 25).

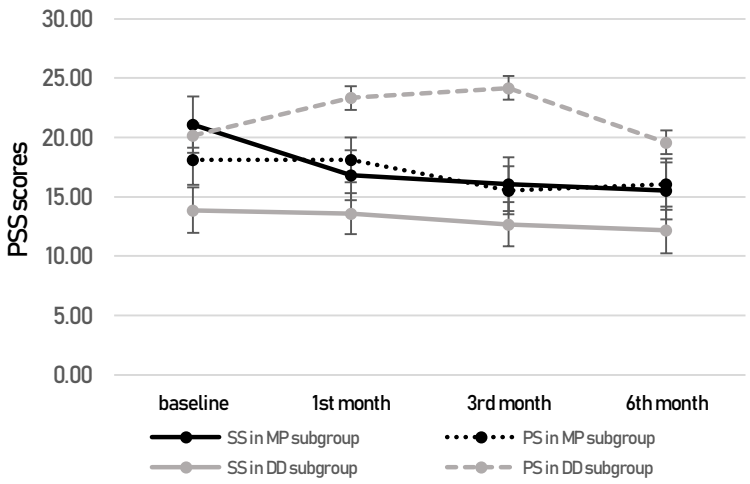

Figure 14. Changes in self-perceived stress (PSS) from the baseline to the 6th month of the therapy – TMD diagnostic subgroups *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement*

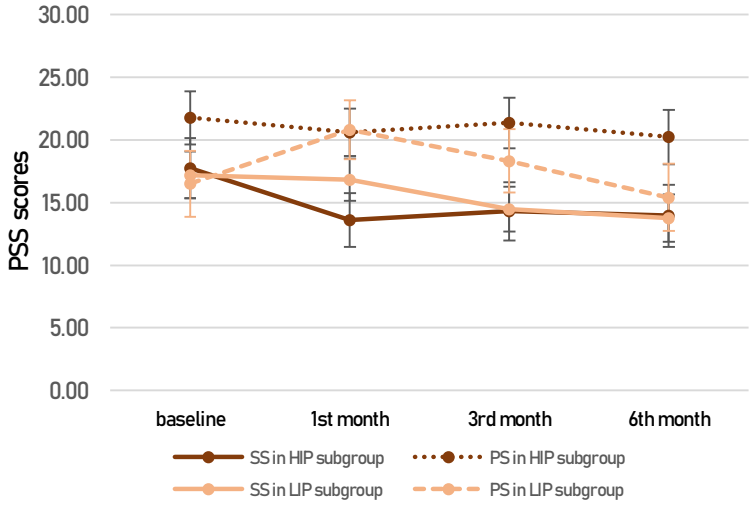

Figure 15. Changes in self-perceived stress (PSS) from the baseline to the 6th month of the therapy – TMD pain intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

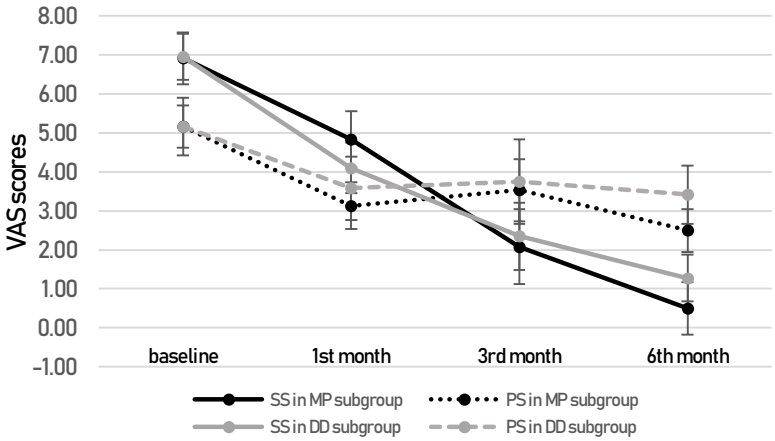

Figure 16. Changes in spontaneous pain (VAS) from the baseline to the 6th month of the therapy – TMD diagnostic subgroups *SS. stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement*

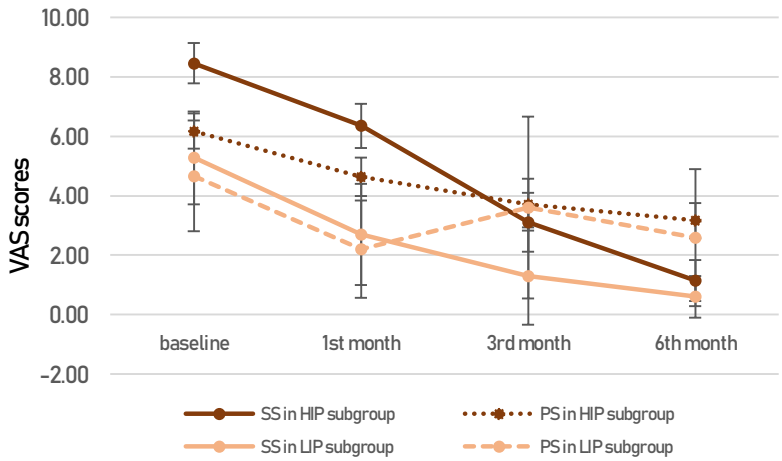

Figure 17. Changes in spontaneous pain (VAS) from the baseline to the 6th month of the therapy – TMD pain intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

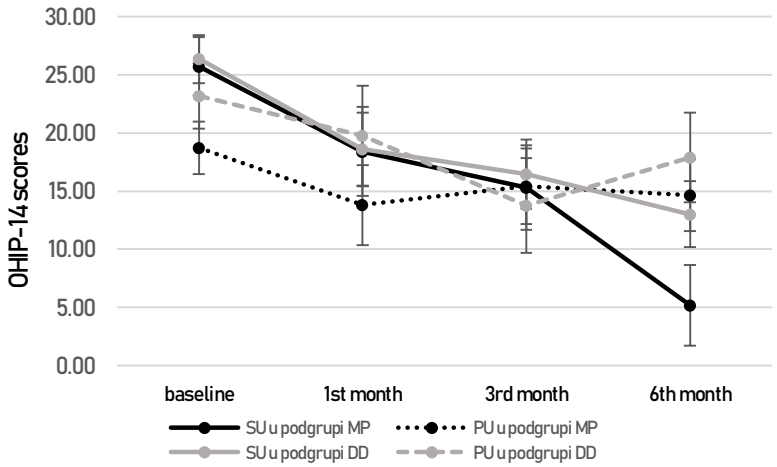

Figure 18. Changes in oral-health-related quality of life (OHIP-14) from the baseline to the 6th month of the therapy – TMD diagnostic subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement*

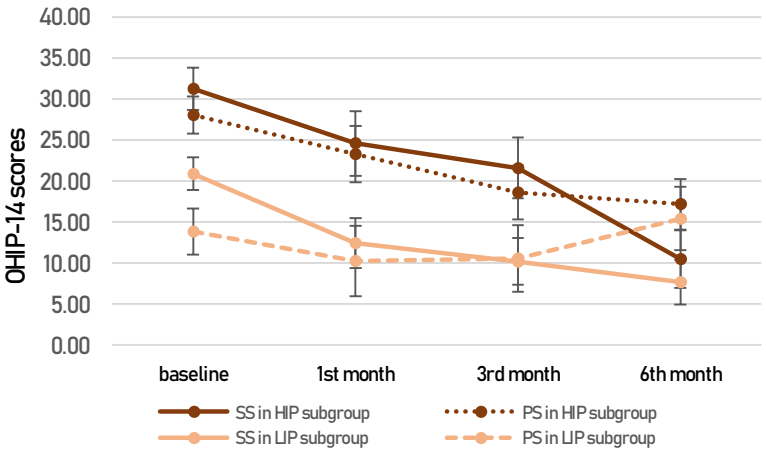

Figure 19. Changes in oral-health-related quality of life (OHIP-14) from the baseline to the 6th month of the therapy – TMD pain intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

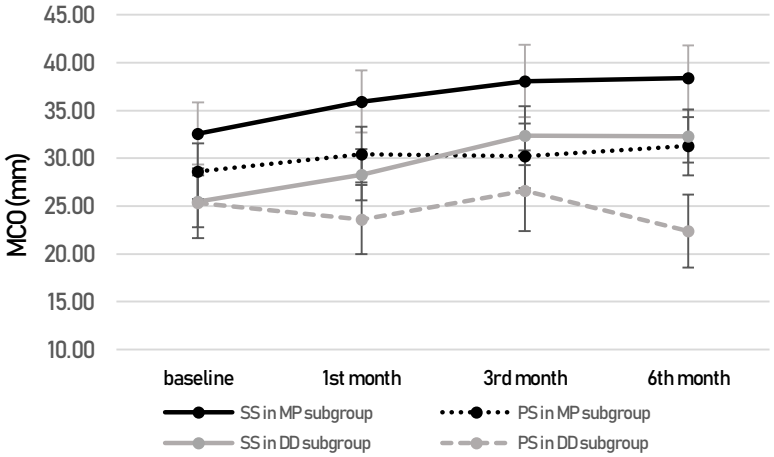

Figure 20. Changes in maximal comfortable mouth opening (MCO) from the baseline to the 6th month of the therapy – TMD diagnostic subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement*

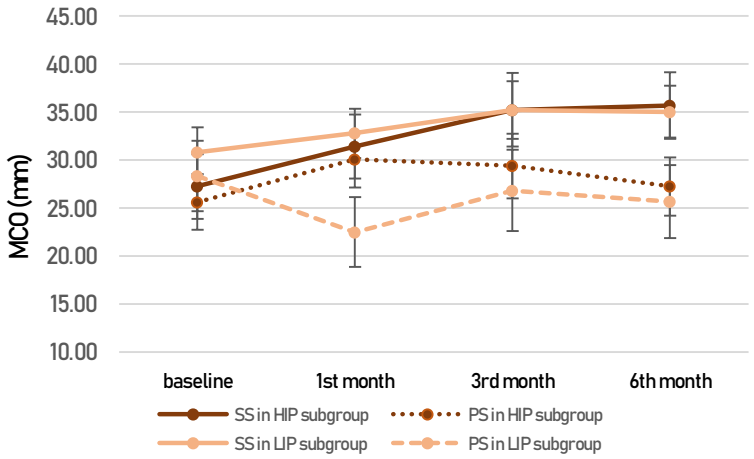

Figure 21. Changes in maximal comfortable mouth opening (MCO) from the baseline to the 6th month of the therapy – TMD pain-intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

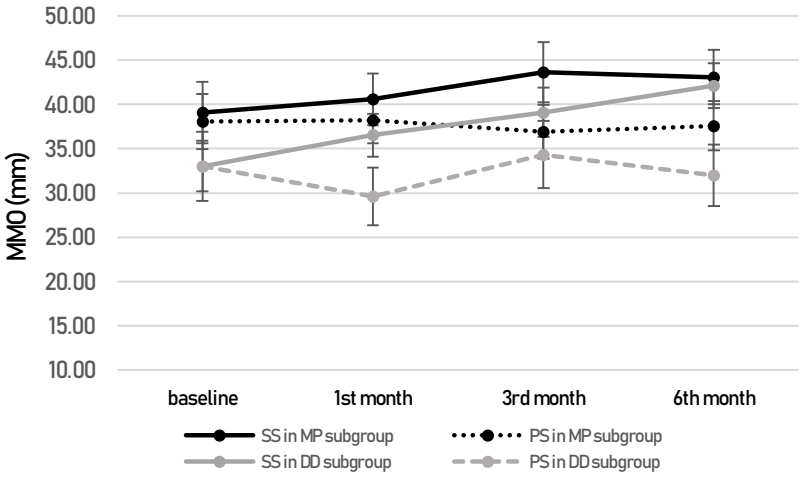

Figure 22. Changes in maximal unassisted mouth opening (MMO) from the baseline to the 6th month of the therapy – TMD pain-intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

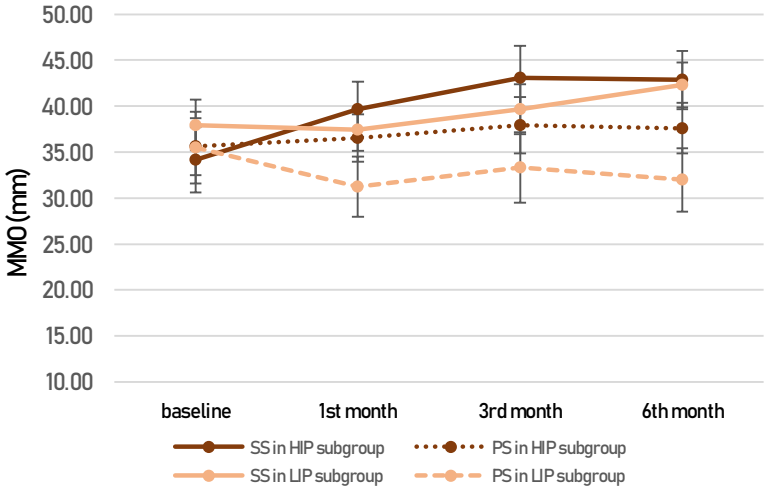

Figure 23. Changes in maximal unassisted mouth opening (MMO) from the baseline to the 6th month of the therapy – TMD pain-intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

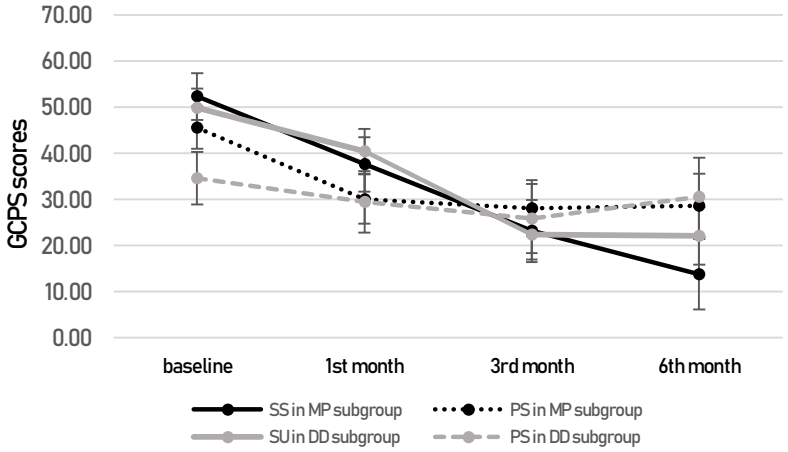

Figure 24. Changes in characteristic pain intensity (GCPS) from the baseline to the 6th month of the therapy – TMD diagnostic subgroups *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement*

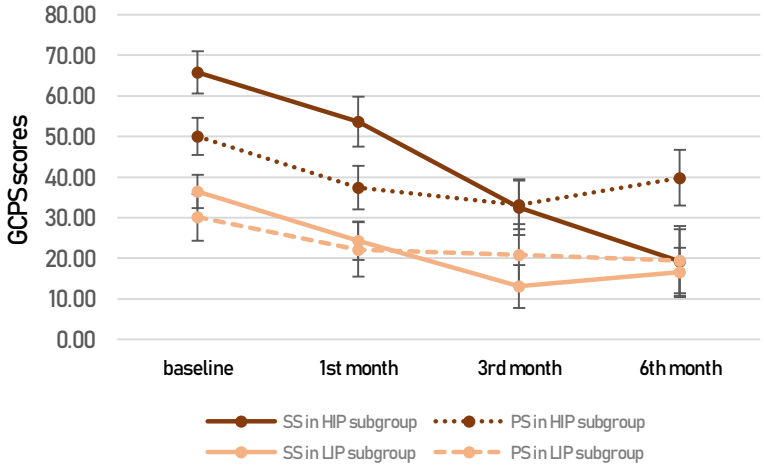

Figure 25. Changes in characteristic pain intensity (GCPS) from the baseline to the 6th month of the therapy – TMD pain intensity subgroups. *SS, stabilization splint; PS, placebo splint; HIP, high-intensity pain; LIP, low-intensity pain*

3.3.2.3. Baseline comparison of salivary oxidative stress markers and cortisol between the TMD subgroups

Table 20. Comparison of salivary OS markers and cortisol (baseline data) between the treatment groups – according to diagnostic subgroup

		MP (n=16)				DD (n=18)			
	Marker*	Stabilization splint (n=8)	Placebo splint (n=8)	t	p	Stabilization splint (N=11)	Placebo splint (N=7)	t	p
morning	GPx	1.54	1.68	-0.80	0.44	1.66	1.82	-0.71	0.49
	SOD	3.29	3.37	-0.40	0.70	3.56	2.93	1.57	0.14
	TAC	0.57	0.64	-0.65	0.53	0.64	0.57	0.72	0.48
	UA	2.64	2.75	-0.72	0.48	2.62	2.66	-0.18	0.86
	MDA	2.43	2.70	-0.76	0.46	2.25	3.01	-1.92	0.07
	8-OHdG	0.15	0.34	-2.37	0.03	0.34	0.64	-2.42	0.03
	SC	1.07	1.08	-0.06	0.95	0.81	0.81	0.06	0.95
afternoon	GPx	1.29	1.69	-3.26	0.01	1.86	1.60	1.48	0.16
	SOD	3.15	3.26	-0.62	0.55	3.40	3.41	-0.08	0.94
	TAC	0.55	0.67	-1.35	0.20	0.67	0.57	0.89	0.39
	UA	2.58	2.86	-2.21	0.04	2.72	2.40	1.83	0.09
	MDA	2.65	3.18	-1.60	0.13	2.83	3.35	-1.57	0.14
	8-OHdG	0.14	0.39	-2.75	0.02	0.51	0.46	0.30	0.77
	SC	0.48	0.38	1.13	0.28	0.47	0.47	-0.10	0.92

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; MP, myofascial pain; DD, disc displacement pain splint; n, number of participants, *log transformed data

In the MP patients before the start of treatment, significantly higher values of the log morning and afternoon 8-OHdG, as well as of the log afternoon GPx and UA, were observed in the PS group compared to the SS group. In the DD patients, significantly higher baseline values of log morning 8-OHdG were observed in the PS group (Table 20).

Table 21. Comparison of salivary OS markers and cortisol (baseline data) between treatment groups – according to pain intensity

		HIP (n=17)				LIP (n=17)			
	Marker*	Stabilization splint (n=8)	Placebo splint (n=9)	t	p	Stabilization splint (n=11)	Placebo splint (n=6)	t	p
morning	GPx	1.70	1.80	-0.52	0.61	1.54	1.68	-0.56	0.58
	SOD	3.56	2.93	1.49	0.16	3.36	3.51	-0.93	0.37
	TAC	0.66	0.61	0.43	0.67	0.57	0.60	-0.39	0.70
	UA	2.63	2.84	-1.31	0.21	2.63	2.50	0.79	0.44
	MDA	2.42	3.18	-2.52	0.02	2.27	2.34	-0.19	0.86
	8-OHdG	0.26	0.53	-1.96	0.07	0.26	0.36	-1.03	0.32
	SC	0.90	0.97	-0.51	0.62	0.94	0.92	0.13	0.90
afternoon	GPx	1.77	1.70	0.38	0.71	1.51	1.57	-0.27	0.79
	SOD	3.38	3.22	0.92	0.37	3.23	3.50	-1.91	0.08
	TAC	0.64	0.63	0.11	0.92	0.60	0.61	-0.12	0.90
	UA	2.62	2.70	-0.41	0.69	2.69	2.56	0.85	0.41
	MDA	2.84	3.49	-2.38	0.03	2.69	2.91	-0.60	0.56
	8-OHdG	0.38	0.48	-0.73	0.48	0.32	0.31	0.03	0.98
	SC	0.46	0.44	0.27	0.79	0.48	0.40	0.75	0.46

GPx, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; HIP, high-intensity pain; LIP, low-intensity pain; n, number of participants, *log transformed data

In the HIP patients, before the start of treatment, significantly higher values of log morning and afternoon MDA were observed in the PS group compared to the SS group, whereas in the LIP patients, there were no differences in the baseline values of oxidative stress markers and cortisol between the treatment groups (Table 21).

3.3.2.4. Changes in OS markers and salivary cortisol during 6-month follow-up - mixed between-within subjects repeated measures ANOVA

Changes in the TMD subgroups' salivary GPx; SOD; TAC, UA, MDA, 8-OHdG, and SC levels between the baseline and after the 1st, 3rd and 6th months of treatment are presented in Table 22. Concentrations are expressed in the log $_{10}$ scale.

Table 22. OS markers and salivary cortisol in the TMD subgroups across time-points

			Diagnosis								Pain Intensity											
			MP				DD				HIP				LIP					Interaction		
	Marker*	Time	SS		PS		SS		PS		SS		PS		SS		PS		P (time)	P (time * treatment)	P (time * diagnosis	P (time * intensity)
			Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se				
morning	GPx	T0	1.61	0.18	1.67	0.16	1.67	0.14	1.67	0.20	1.69	0.18	1.80	0.16	1.60	0.14	1.54	0.20	0.12	0.71	0.52	0.76
		T1	1.29	0.17	1.58	0.15	1.71	0.14	1.44	0.19	1.52	0.17	1.52	0.15	1.49	0.13	1.50	0.19				
		T2	1.52	0.21	1.26	0.19	1.49	0.17	1.70	0.24	1.44	0.22	1.57	0.19	1.58	0.17	1.39	0.24				
		T3	1.34	0.20	1.63	0.18	1.24	0.17	1.41	0.23	1.35	0.21	1.45	0.18	1.24	0.16	1.59	0.23				
	SOD	T0	3.33	0.28	3.40	0.25	3.54	0.23	2.97	0.31	3.51	0.29	2.78	0.25	3.37	0.22	3.58	0.31	0.38	0.18	0.39	0.11
		T1	3.31	0.12	3.52	0.10	3.46	0.10	3.52	0.13	3.46	0.12	3.42	0.10	3.31	0.09	3.61	0.13				
		T2	3.24	0.27	3.62	0.24	3.46	0.22	3.01	0.30	3.28	0.27	3.03	0.24	3.42	0.21	3.59	0.30				
		T3	3.29	0.11	3.47	0.10	3.40	0.09	3.55	0.13	3.38	0.12	3.49	0.10	3.30	0.09	3.52	0.13				
	TAC	T0	0.47	0.12	0.50	0.10	0.54	0.10	0.46	0.13	0.56	0.12	0.47	0.10	0.45	0.09	0.49	0.13	0.01	0.53	0.88	0.88
		T1	0.39	0.12	0.35	0.10	0.43	0.10	0.40	0.13	0.48	0.12	0.29	0.10	0.34	0.09	0.46	0.13				
		T2	0.25	0.12	0.38	0.11	0.40	0.10	0.40	0.13	0.29	0.12	0.42	0.11	0.36	0.09	0.36	0.13				
		T3	0.24	0.08	0.41	0.07	0.34	0.06	0.36	0.08	0.30	0.08	0.41	0.07	0.28	0.06	0.36	0.08				
	UA	T0	2.65	0.13	2.68	0.11	2.75	0.11	2.61	0.14	2.66	0.13	2.80	0.11	2.74	0.10	2.50	0.14	0.041	0.24	0.93	0.60
		T1	2.57	0.13	2.50	0.11	2.67	0.10	2.47	0.14	2.62	0.13	2.50	0.11	2.62	0.10	2.48	0.14				
		T2	2.36	0.18	2.58	0.16	2.62	0.15	2.48	0.20	2.35	0.18	2.68	0.16	2.64	0.14	2.38	0.20				
		T3	2.33	0.19	2.71	0.17	2.66	0.16	2.58	0.21	2.48	0.19	2.59	0.17	2.50	0.15	2.71	0.21				
	MDA	T0	2.47	0.30	2.61	0.27	2.35	0.25	2.57	0.37	2.42	0.31	3.03	0.31	2.40	0.24	2.15	0.34	0.12	0.18	0.29	0.046
		T1	1.59	0.38	2.52	0.34	2.14	0.31	2.57	0.46	1.69	0.39	2.55	0.39	2.05	0.30	2.54	0.42				
		T2	2.13	0.36	2.43	0.32	2.48	0.30	2.97	0.44	2.02	0.37	2.79	0.37	2.60	0.29	2.61	0.41				
		T3	1.80	0.35	2.73	0.32	2.38	0.29	2.74	0.43	1.89	0.36	3.08	0.36	2.29	0.28	2.39	0.40				
	8-OHdG	T0	-0.40	0.14	0.02	0.13	0.05	0.11	0.48	0.17	-0.19	0.14	0.47	0.14	-0.16	0.11	0.03	0.15	0.93	0.50	0.08	0.98
		T1	-0.22	0.17	0.08	0.16	-0.09	0.14	0.30	0.21	-0.13	0.18	0.41	0.18	-0.17	0.14	-0.02	0.19				
		T2	-0.24	0.17	-0.18	0.16	0.14	0.14	0.48	0.21	-0.20	0.18	0.47	0.19	0.10	0.14	-0.18	0.20				
		T3	-0.41	0.12	-0.09	0.11	0.14	0.10	0.38	0.14	-0.28	0.12	0.46	0.12	0.01	0.09	-0.17	0.13				
	SC	T0	1.10	0.09	1.04	0.08	0.84	0.08	0.89	0.11	0.99	0.10	0.97	0.08	0.95	0.07	0.96	0.11	0.006	0.42	0.23	0.57
		T1	0.83	0.06	0.89	0.06	0.91	0.05	0.76	0.07	0.79	0.06	0.90	0.06	0.95	0.05	0.75	0.07				
		T2	0.95	0.17	0.88	0.15	0.90	0.14	0.70	0.19	0.92	0.17	0.58	0.15	0.93	0.13	1.00	0.19				
		T3	1.15	0.11	0.90	0.10	0.91	0.09	1.33	0.12	1.04	0.11	1.08	0.10	1.03	0.09	1.15	0.12				
afternoon	GPx	T0	1.31	0.12	1.70	0.11	1.91	0.10	1.43	0.14	1.65	0.13	1.67	0.11	1.58	0.10	1.46	0.14	0.51	0.58	0.45	0.32
		T1	1.43	0.23	1.44	0.21	1.67	0.19	1.17	0.26	1.61	0.24	1.47	0.21	1.49	0.18	1.13	0.26				
		T2	1.58	0.18	1.73	0.16	1.53	0.15	1.30	0.20	1.62	0.18	1.28	0.16	1.49	0.14	1.75	0.20				
		T3	1.46	0.23	1.63	0.21	1.36	0.19	1.46	0.26	1.75	0.24	1.38	0.21	1.06	0.18	1.71	0.26				
	SOD	T0	3.15	0.14	3.31	0.13	3.38	0.12	3.49	0.16	3.31	0.15	3.23	0.13	3.21	0.11	3.57	0.16	0.16	0.41	0.68	0.84
		T1	3.12	0.29	2.73	0.26	3.39	0.24	3.29	0.32	3.25	0.30	3.11	0.26	3.26	0.23	2.92	0.32				
		T2	3.22	0.11	3.29	0.10	3.52	0.09	3.45	0.12	3.38	0.11	3.34	0.10	3.36	0.09	3.40	0.12				
		T3	3.22	0.09	3.37	0.08	3.40	0.07	3.46	0.10	3.31	0.09	3.38	0.08	3.31	0.07	3.45	0.10				
	TAC	T0	0.44	0.10	0.53	0.09	0.62	0.08	0.48	0.11	0.56	0.10	0.48	0.09	0.50	0.08	0.53	0.11	0.01	0.89	0.98	0.61
		T1	0.36	0.12	0.41	0.11	0.55	0.10	0.39	0.14	0.41	0.12	0.39	0.11	0.50	0.10	0.40	0.14				
		T2	0.34	0.10	0.41	0.09	0.48	0.08	0.41	0.11	0.45	0.10	0.40	0.09	0.37	0.08	0.41	0.11				
		T3	0.33	0.09	0.43	0.08	0.49	0.08	0.37	0.10	0.46	0.09	0.42	0.08	0.35	0.07	0.38	0.10				
	UA	T0	2.60	0.12	2.83	0.10	2.86	0.10	2.36	0.13	2.70	0.12	2.61	0.10	2.76	0.09	2.58	0.13	0.48	0.58	0.21	0.18
		T1	2.50	0.10	2.76	0.09	2.87	0.08	2.44	0.11	2.62	0.10	2.59	0.09	2.75	0.08	2.61	0.11				
		T2	2.48	0.20	2.71	0.18	2.49	0.16	2.47	0.22	2.59	0.20	2.67	0.18	2.37	0.16	2.52	0.22				
		T3	2.52	0.14	2.69	0.13	2.79	0.12	2.45	0.16	2.65	0.14	2.55	0.13	2.66	0.11	2.59	0.16				
	MDA	T0	2.66	0.30	3.08	0.27	2.92	0.24	3.13	0.36	2.81	0.31	3.32	0.31	2.77	0.24	2.90	0.33	0.056	0.48	0.48	0.47
		T1	2.32	0.33	3.17	0.29	2.77	0.27	3.03	0.40	2.39	0.33	3.19	0.33	2.70	0.26	3.01	0.36				
		T2	2.14	0.22	3.12	0.20	2.79	0.18	3.11	0.27	2.05	0.23	3.49	0.23	2.88	0.18	2.73	0.25				
		T3	2.75	0.25	3.20	0.23	2.73	0.21	3.39	0.31	2.61	0.26	3.59	0.26	2.87	0.20	2.99	0.29				
	8-OHdG	T0	-0.47	0.16	0.04	0.15	0.32	0.13	-0.02	0.20	-0.11	0.16	0.06	0.17	-0.04	0.13	-0.04	0.18	0.71	0.64	0.48	0.072
		T1	-0.36	0.16	-0.12	0.15	0.38	0.13	0.12	0.20	-0.03	0.17	-0.03	0.17	0.05	0.13	0.03	0.18				
		T2	-0.34	0.18	-0.14	0.16	0.13	0.14	0.36	0.21	-0.07	0.18	0.49	0.19	-0.13	0.14	-0.28	0.20				
		T3	-0.25	0.20	0.10	0.19	0.31	0.17	0.15	0.25	0.00	0.21	0.38	0.22	0.05	0.16	-0.13	0.23				
	SC	T0	0.34	0.09	0.11	0.08	0.33	0.08	0.35	0.10	0.34	0.10	0.21	0.08	0.33	0.07	0.24	0.10	0.53	0.55	0.014	0.071
		T1	0.41	0.10	0.28	0.09	0.28	0.08	0.48	0.11	0.40	0.10	0.20	0.09	0.29	0.08	0.57	0.11				
		T2	0.56	0.10	0.28	0.09	0.15	0.08	0.28	0.12	0.50	0.11	0.24	0.09	0.21	0.08	0.32	0.12				
		T3	0.46	0.14	0.23	0.13	0.23	0.12	0.59	0.16	0.40	0.14	0.39	0.13	0.29	0.11	0.43	0.16				

GPX, glutathione peroxidase; SOD, superoxide dismutase; TAC, total antioxidant capacity; UA, uric acid; 8-OHdG, 8-hydroxydeoxyguanosine; MDA, malondialdehyde; SC, salivary cortisol; PSS, perceived stress scale; MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain; p-value; SS, stabilization splint; PS, placebo splint, *log transformed data

The overall levels of morning and afternoon log TAC did not differ between the TMD subgroups, but decreased significantly over time (Figure 26) (morning: Wilks Lambda=0.59, F=4.57, p=0.013, effect size=0.18; afternoon: Wilks Lambda=0.57, F=4.85, p=0.01, effect size=0.15). The post hoc analysis showed that the levels of log TAC were significantly lower in the 3rd and 6th months of the treatment compared to the baseline.

The levels of morning log UA changed significantly over time (Wilks Lambda=0.66, F=3.30, p=0.041, effect size=0.11) (Figure 27). The analysis revealed a significant interaction between the time, treatment group and pain intensity (interaction time x treatment group x pain intensity; Wilks Lambda = 0.55, F=5.32 p=0.007, effect size=0.14). the levels of morning log UA differed significantly between the two treatment groups, with a greater decrease in the SS compared to the PS group. The post hoc analysis showed that the levels of log UA were significantly lower in the SS group in the 3rd (HIP: p=0.015) and 6th (LIP: p=0.007) month compared to the baseline. In the PS group, no significant differences were observed between the baseline and follow-up appointments.

The Levels of morning log SC changed significantly over time (Wilks Lambda=0.43, F=8.82, p=0.006, effect size=0.17). The analysis revealed a significant interaction between the time, treatment group and diagnostic subgroup (interaction time x treatment group x diagnostic subgroup; Wilks Lambda=0.46, F=7.75, p=0.001, effect size=0.16). The morning log SC differed significantly between the two treatment groups with a greater increase of log SC levels in the PS group. The post hoc analysis showed that the levels of log SC in the PS group were significantly higher in the 6th month of the treatment compared to all the earlier follow-up appointments (DD: $p<0.05$).
When evaluating the levels of afternoon log SC, a significant interaction between the time and diagnostic subgroup was found (interaction time x diagnostic subgroup; Wilks Lambda=0.59, F=4.49; p=0.014). In the 3rd month of treatment, the levels of afternoon log SC in the DD subgroup decreased compared to the baseline, while in the MP subgroup, the log SC levels increased.

When evaluating the levels of morning log MDA, the analysis revealed a significant interaction between time and pain intensity (interaction time x pain intensity; Wilks Lambda=0.67, F=3.22; p=0.045, effect size=0.12). The post hoc analysis showed that in HIP patients, the levels of morning log MDA were significantly lower in the 1st and the 3rd months of treatment compared

to the baseline (p=0.02). In the LIP patients, the levels of morning log MDA were higher at the follow-up appointments compared to the baseline (Figure 28c).

When evaluating the levels of afternoon log MDA, the analysis revealed a significant interaction between time, treatment group and pain intensity (interaction time x treatment group x pain intensity; Wilks Lambda=0.56, F=4.87; p=0.011, effect size=0.13). The levels of afternoon log MDA differed significantly between the two treatment groups, with a greater decrease in the SS compared to the PS treatment group. The post hoc analysis showed that in the HIP patients treated with SS, the levels of log MDA were significantly lower in the 3rd month of treatment compared to the baseline (p=0.005). In the HIP patients treated with PS, the levels of log MDA were significantly higher in the 6th month of treatment compared to the baseline (p=0.04). Moreover, in the HIP patients treated with PS, the levels of log MDA were significantly higher at all the follow-up appointments compared to the patients treated with SS. In the LIP patients, there were no significant differences in the morning log MDA values between the baseline and follow-up measurements (Figure 28d).

During the treatment period, no statistically significant changes in morning log 8-OHdG were found, however, significantly higher levels were found in the SS group compared to the PS group (F=6.33, p=0.02). A significant difference in the levels of morning log 8-OhdG was also found when comparing the MP group to the DD group with greater overall values in the DD group (F=11.15, p=0.003). The levels of afternoon log 8-OHdG did not change significantly over time, but were significantly higher in the DD compared to the MP diagnostic subgroup (F=10.31, p=0.004).

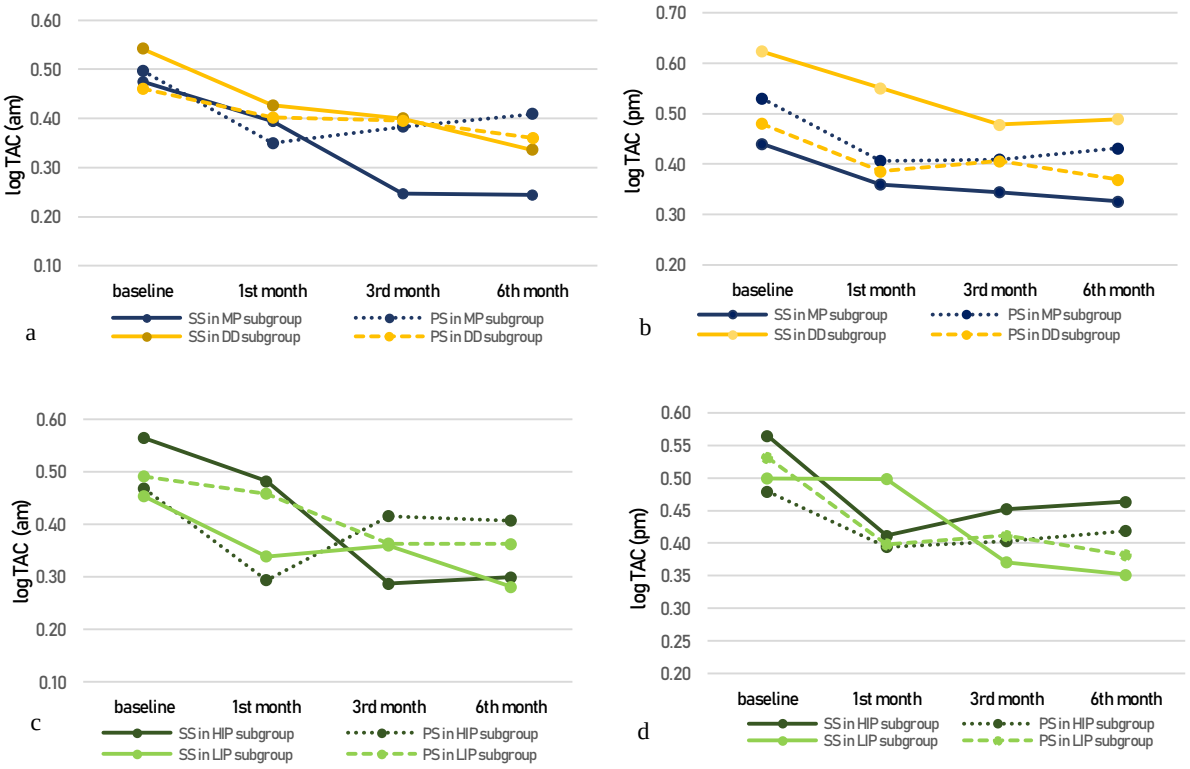

Figure 26. Changes in the morning (a, c) and afternoon (b, d) total antioxidant levels (TAC) in the 2 treatment groups from the baseline to 6th month of the therapy according to the diagnostic (a, b) and pain intensity (c, d) subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement; HIP, high intensity pain; LIP, low intensity pain*

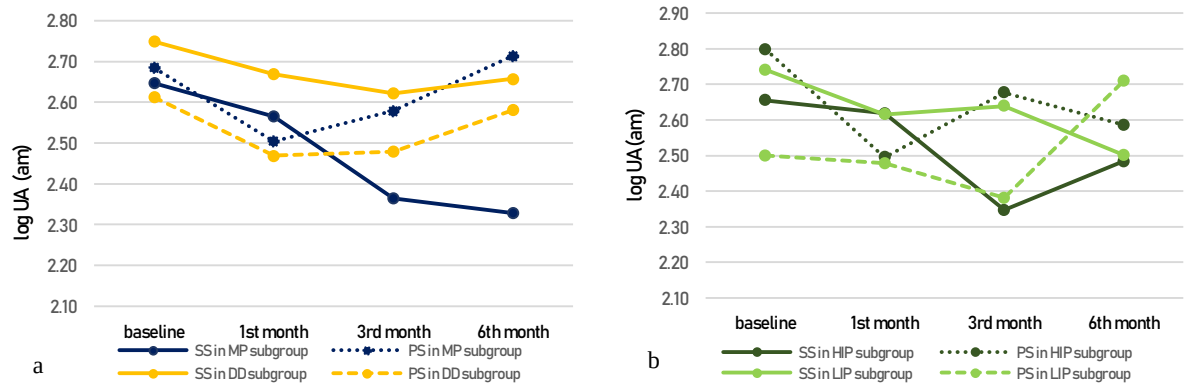

Figure 27. Changes in the morning uric acid levels (UA) in the 2 treatment groups from the baseline to the 6th month of the therapy according to the diagnostic (a) and pain intensity (b) subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain*

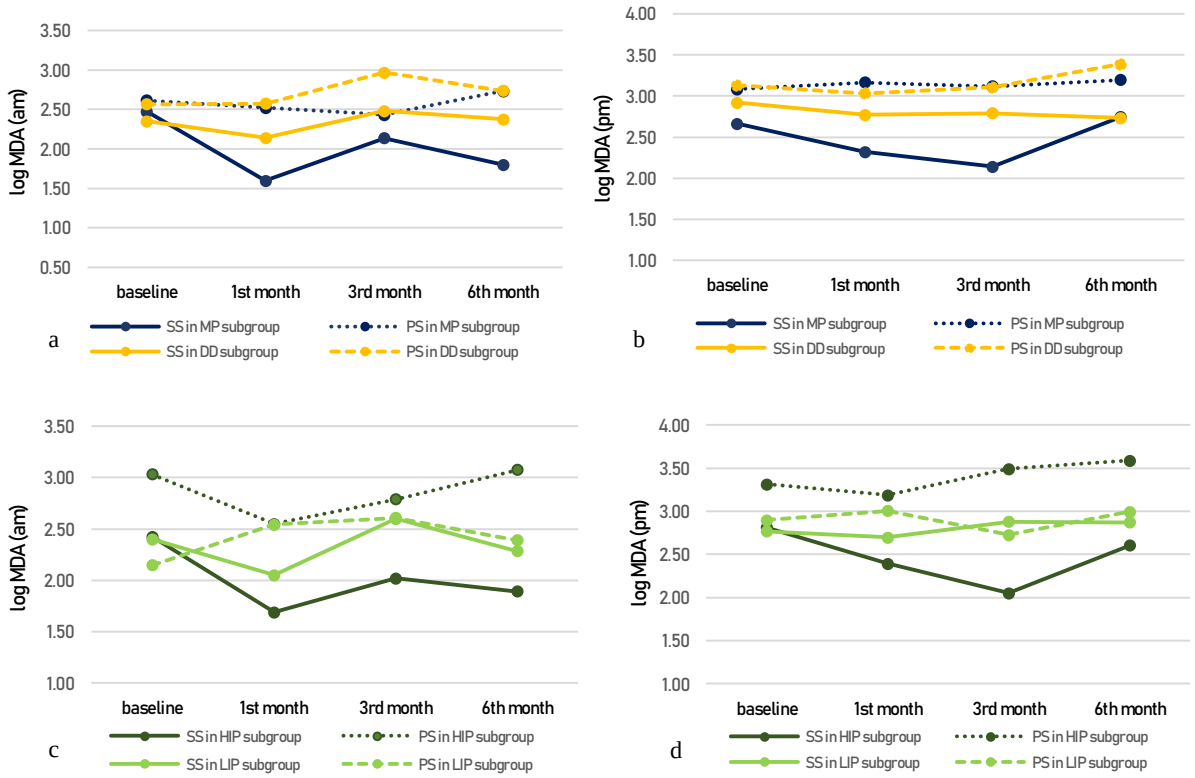

Figure 28. Changes in morning (a, c) and afternoon (b, d) malodialdehyde levels (MDA) in 2 treatment groups from the baseline to the 6th month of the therapy according to diagnostic (a, b) and pain intensity (c, d) subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain*

Table 23. Malondialdehyde to superoxide dismutase ratio (MDA/SOD) in TMD subgroups

		Diagnostic subgroup								Pain intensity subgroup											
		MP				DD				HIP				LIP				(time)		(time* treatment)		(time* diagnosis)		(time* pain intensity)	
		SS		PS		SS		PS		SS		PS		SS		PS							
MDA/SOD, means (SE) morning	T0	-0.86	0.35	-0.78	0.32	-1.20	0.29	-1.42	0.53	-1.08	0.36	-0.77	0.48	-0.97	0.28	-1.44	0.40	0.04		0.47		0.37		0.014	
	T1	-1.71	0.36	-1.00	0.32	-1.32	0.30	-1.69	0.54	-1.77	0.37	-1.62	0.49	-1.26	0.29	-1.07	0.41								
	T2	-1.11	0.37	-1.18	0.33	-0.98	0.30	-1.09	0.55	-1.26	0.38	-1.29	0.49	-0.82	0.29	-0.98	0.41								
	T3	-1.49	0.42	-0.74	0.38	-1.02	0.35	-1.20	0.63	-1.49	0.43	-0.81	0.57	-1.02	0.33	-1.13	0.47								
MDA/SOD, means(SE) afternoon	T0	-0.48	0.38	-0.33	0.39	-0.46	0.31	-0.45	0.46	-0.49	0.39	0.00	0.39	-0.44	0.30	-0.78	0.46	0.11		0.48		0.8		0.66	
	T1	-0.80	0.42	-0.30	0.43	-0.61	0.35	-0.27	0.52	-0.85	0.43	0.07	0.43	-0.56	0.33	-0.65	0.52								
	T2	-1.07	0.26	-0.44	0.27	-0.73	0.22	-0.35	0.32	-1.32	0.27	0.14	0.27	-0.48	0.21	-0.93	0.32								
	T3	-0.47	0.30	-0.42	0.31	-0.67	0.25	-0.04	0.37	-0.70	0.31	0.25	0.31	-0.44	0.24	-0.70	0.37								

MDA/SOD, malondialdehyde to superoxide dismutase ratio; MP, myofascial pain subgroup; DD, disc displacement subgroup; HIP, high-intensity pain; LIP, low-intensity pain, SS, stabilization splint; PS, placebo splint *log transformed data

The levels of the morning log MDA/SOD ratio changed significantly over time (Wilks lambda=0.64, F=3.34, p=0.041). The analysis revealed a significant interaction between time and pain intensity (interaction time x pain intensity; Wilks Lambda=0.56, F=4.63, p=0.01). The post hoc analysis showed that in the HIP subgroup, the levels of the log MDA/SOD ratio were significantly lower in the 1st (p=0.009) and 3rd (p=0.03) month compared to the baseline. In the LIP subgroup, there were no significant differences in the levels of the morning log MDA/SOD ratio between baseline and follow-up appointments (Table 23; Figure 29c).

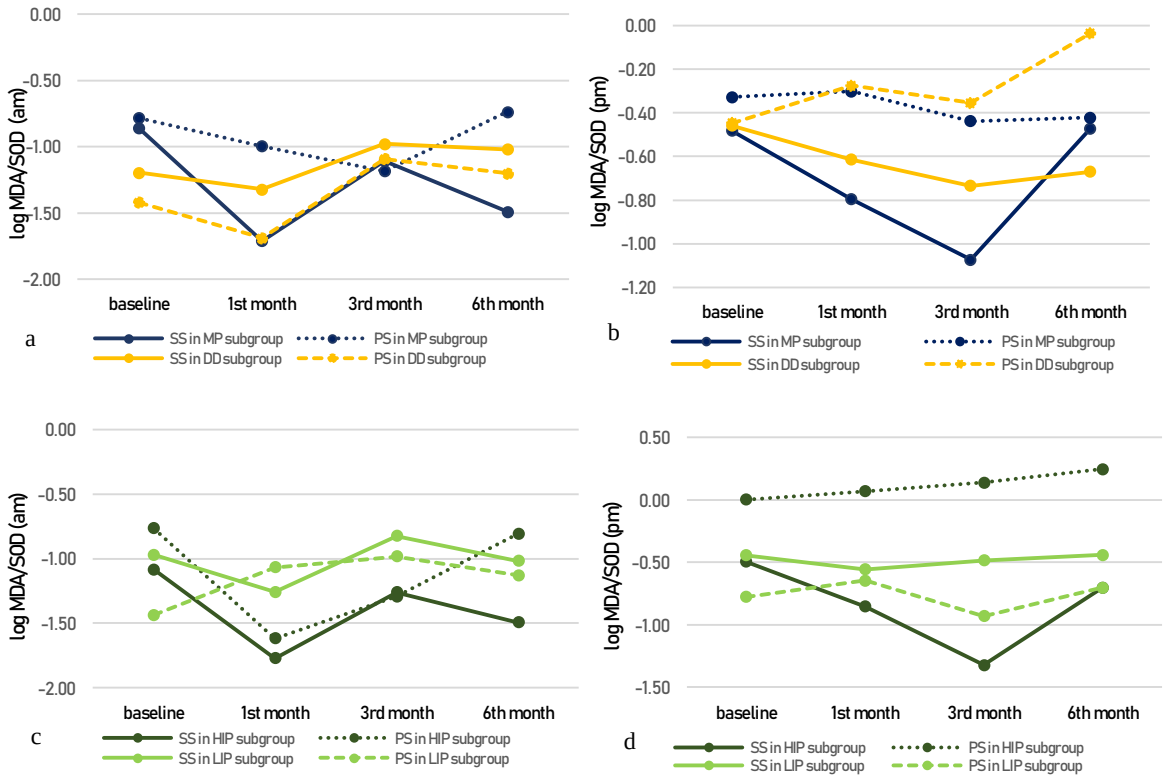

Figure 29. Changes in the morning (a, c) and afternoon (b, d) malodialdehyde to superoxide dismutase ratio (MDA/SOD) in the 2 treatment groups from the baseline to the 6th month of the therapy according to the diagnostic (a, b) and pain intensity (c, d) subgroups. *SS, stabilization splint; PS, placebo splint; MP, myofascial pain; DD, disc displacement; HIP, high-intensity pain; LIP, low-intensity pain*

The decrease in the levels of afternoon log MDA/SOD was also notable in the MP and DD subgroups in patients treated with SS, as well as in patients with HIP treated with the SS but those changes were not statistically significant (Table 23; Figure 29b and 29d).

4. DISCUSSION

Oxidative stress, as a term, has a markedly negative connotation in the world of medicine. It has been associated with various processes such as normally induced ageing processes but also processes that lead to the organism's function impairment and, subsequently, disease. Increasingly, attempts are being made to link certain diseases, especially multifactorial ones, to the negative effects of oxidative stress. There have already been attempts to connect the effects of oxidative damage with temporomandibular disorders, however not enough to definitively draw a strong and undeniable linkage. Uncertainty regarding TMD initiation processes is one of the reasons why studies on this subject draw attention and are of major importance. A better understanding of the disease could bring better screening, diagnostic and treatment tools, as was discussed during the International RDC/TMD Consortium Network 2009 (IADR) where the importance of studying TMD markers and genetic background in order to create the Axis III and IV diagnostic protocols as an addition to the current Axis I and II, was implied (15).

The mechanisms by which oxidative stress modulates the initiation, progression and severity of the symptoms in temporomandibular disorders are not known but are probably diverse. So far, research suggests that it probably has a role in the modulation of pain. Ray et al. showed that the production of lipoproteins modified with OS induces nociception and Medow et al. suggested direct alterations of local sensory nerve activity by certain reactive oxygen species as a mechanism of their influence on the initiation and perpetuation of pain-associated symptoms (143, 144). Also, some research tried to connect the objective parameters, such as the radiological severity of structural changes in the joint, with higher levels of OS markers mainly through the analysis of the joint's synovial fluid (145, 146).

The results of this research are promising and indicate the involvement of OS in mechanisms of TMD pathogenesis.

4.1. Oxidative stress markers between patients with TMD and the healthy control

Comparison between TMD patients and healthy controls results, obtained in the past, are prone to state that there might be a connection between oxidative stress and TMD and that oxidative stress has a role in the pathogenesis of the disorders (116, 117, 119).

The hypothesis set at the begging of this study was that the salivary oxidative stress marker levels will be higher and/or the salivary antioxidant levels will be lower in TMD patients compared to healthy controls.

This presumption was based on the previous research that encountered higher levels of oxidants and lower levels of TAC in TMD patients compared to the control (116, 117). However, the results of the present study did not confirm lower levels of salivary TAC in TMD patients as expected. On the contrary, the TAC was higher in TMD patients compared to the control group. Our research group first noticed the difference while undertaking pilot research that was published in 2018 (119). There, the phenomenon was interpreted as a compensatory answer to increased oxidative stress as a prerequisite for efficient defence (118). Since both the levels of TAC and levels of MDA, which is an oxidant, were higher in TMD patients compared to the control group, a compensatory model could have a role in explaining this result too.

These results are not an isolated case of increased oxidants in TMD patients. Cai et al. showed that both SOD and the oxidative stress indicator were significantly higher in temporomandibular disorder patients than in healthy control subjects. They explained the latter similarly to us stating that overactivity and the overproduction of oxygen free radicals can lead to the overgeneration of antioxidant enzymes, probably as a defence counteraction to an imbalanced oxidative status (122).

The different results, with lower levels of antioxidants in TMD patients, obtained by some of the studies can be explained as follows. De Almeida and Amenábar did not report the duration of the pain their patients experienced, and it is also not certain whether the patients in the study of Rodríguez de Sotillo et al. had chronic pain (116, 117). With the longer duration of the symptoms, the antioxidant capacity might regenerate if it previously decreased, or even increase its levels as a compensatory mechanism. The duration of the TMD pain might, in some ways, explain why the results from our study conflict with the previous studies. Moreover, the above-mentioned investigations did not report salivary biomarker levels normalized for the total protein concentration in saliva. The methodology, as well, could be the reason for the inconsistencies between our results and those of the previous studies. The normalization of salivary OS biomarkers for the total protein concentration in saliva minimizes the errors occurring due to variations in salivary proteins' concentrations as a response to stimulation or alterations of salivary flow (147). The main disadvantage of salivary research, especially

oxidative stress research, would probably be differences in methodology, thus general agreement would provide more comparable results (133, 134).

The uncertainty regarding uric acid's role as an oxidant or antioxidant (110) is not cleared up in this study; nonetheless, UA followed the same pattern as TAC throughout the study with higher levels in TMD patients. This could be either interpreted as uric acid being close to its antioxidant activity, but can also represent an oxidant that reduced its levels throughout the therapy. Probably the truth is closer to the first theory with uric acid being an antioxidant. After all, with concentrations of salivary UA linearly following the UA levels in plasma (148, 149), where uric acid is showed to act primarily as an antioxidant (110), the first theory is probably more accurate.

Since Rodriguez de Sotillo found significant differences in oxidative stress markers depending on pain intensity (116) and our research group previously found the same differences, as well as differences in oxidative stress markers depending on the diagnostic subgroups (119), we assumed that we would encounter different salivary oxidant levels in TMD patients depending on the source and intensity of pain.
Baseline differences between subgroups were present in the expected categories. For the diagnostic subgroups, differences in mouth opening and functional limitations are due to differences in the source of pain. Sore muscles can cause various levels of limited mobility, or limitations my even be missing, whereas limitations present due to anterior disc displacement will be predominantly limited closely to a hinge opening movement usually from 20-30 mm (150). Likewise, the differences in the high- and low-intensity pain subgroups were logical and existed only in the categories of spontaneous pain, characteristic pain, mastication, global jaw movements limitations, and consequently quality of life.

A particularly interesting finding is that the salivary cortisol levels tend to be greater in the myofascial pain subgroup when comparing to the control group and also when comparing to the disc displacement group. Myofascial pain is often related to enhanced stress reactivity, and coping with chronic pain may lead to a greater stress response (151). Moreover, the frequent experience of stress and anxiety may lead to the development of painful trigger points in muscles resulting in myofascial pain (152). Korszun et al. indicated a strong clinical association between muscular TMD, fibromyalgia and chronic fatigue syndrome, which relates to the disruption of the hypothalamic-pituitary-adrenal axis and subsequent hyperactivity to stress, which then manifests as one or more stress-related conditions (85). The higher salivary cortisol

levels in myofascial pain patients observed in our study might indicate that stress either has a role in the development of muscle-related TMD, or that painful TMD might produce additional stress on the body and further enhance cortisol secretion. The lack of significant differences of salivary cortisol levels in patients with disc displacement may be due to the fact that stress is less of a factor in disorders connected to the alteration of a joint's anatomy, though cannot be excluded as a contributing component.

Regarding the oxidative stress markers, MDA was showed to differ significantly according to pain intensity, being higher in the high-intensity pain subgroup. Moreover, the 8-OHdG levels were higher in TMD of joint origin. This is in accordance with the study of Rodríguez de Sotillo et al. who found significantly higher levels of 8-OHdG and MDA in TMD patients, and an association between higher levels of these OS markers with higher scores of pain intensity (116).

With the antioxidant GPX being lower in certain TMD subgroups, it can be speculated that regardless of the compensatory increase of TAC, individual antioxidants might show a lesser ability to adapt.

The second hypothesis was that the salivary OS markers would correlate with SC concentrations.

A heightened cortisol release occurs during the anticipation of stress, and increased metabolism alone generates free radicals where glucocorticoid hormones have been shown to play modulatory roles in the onset of oxidative stress. We predicted that higher levels of SC would correlate with higher PSS scores and higher levels of oxidative stress markers. No correlation was found between salivary cortisol levels and PSS; however higher PSS scores were correlated with higher levels of MDA. Significant positive correlations between salivary cortisol and UA and salivary cortisol and GPX in the myofascial subgroup once again showed that cortisol has a stronger connection to muscle-induced pain and one of the mechanisms by which cortisol operates might be through direct and indirect oxidative status alterations (153).

4.2. Salivary oxidative stress markers and treatment outcomes during the 6-month follow-up

We did not manage to confirm the third hypothesis that there will be no difference in the oxidative stress marker levels and salivary cortisol, as well as in the clinical treatment outcomes considering the type of therapy being applied (stabilization splint/placebo), but rather found significant differences between applied therapies.

Changes in the means between the TMD subgroups in different treatment groups were compared and with the obtained results, it may be said that the stabilization splint provided better continuous long-term therapeutic effect. The placebo splint performed very well in the reduction of spontaneous pain over time, but there was a difference between the treatment groups with the stabilization splint performing significantly better. A significant reduction of OHIP-14, GCPS and JFLS scores was found in the stabilization splint group across 6 months while no such change was present in the placebo splint group. It is interesting to note that a graphic representation of the placebo splint performance during the 6-month treatment in Figures 16, 17, 18, 19, 24 and 25 resemble the graphic representation of the stabilization splint but after the 3rd month it is followed by either a plateau or even a mild deterioration towards the higher values.

Regardless of the potentially negligible effect of the slightly increased vertical dimension of less than 0.5 mm, the design of the placebo device should be taken into consideration when interpreting these results since all the changes in the vertical positioning of the lower jaw might affect the treatment outcomes. It can be concluded that both the stabilization splint and the placebo splint were effective in the management of spontaneous pain. This could be attributed to the prior education of patients on the disorder (64) or the fact that even a slight increase in vertical dimension can facilitate muscle rearrangement leading to the relaxation of the elevator muscles and, consequently, the reduction of pain. The significant differences between the treatment groups in the improvement of pain and quality of life could be due to the fact that the stabilization splint was thicker (providing a greater increase in the vertical dimension of occlusion), as well as constructed to provide CR occlusion and a specific condylar position, thus contributing to the relief of the TMJ (76, 154).

An interesting discovery was that most of the mentioned parameters did not differ significantly between the TMD subgroups but differed significantly between the pain intensity subgroups.

The exception from the latter is MCO. Although the MCO scores only slightly increased over time without producing statistical significance, significantly better results were present in the myofascial pain group compared to the disc displacement subgroup. The difference observed between the myofascial pain patients and the disc displacement patients showed that, in terms of the MCO, female myofascial pain patients had a better response to occlusal splint therapy. The reason for the better improvement of the MCO results in the TMD of muscle origin when compared to the joint origin TMD probably lies in the fact that in the disc displacement group, the biological barrier (displaced disc) is blocking the condyle without the possibility of restoring its position, thus limiting the full range of opening (155).

Maximal and pain-free opening improved during the treatment period, but the improvement was not statistically significant. We need to take into consideration the clinical importance of these results because an increase of only a few millimetres in MMO and MCO can have an enormous impact on a patient's quality of life. In the current study, the MMO's and MCO's tendency to increase was a clear sign of improvement in TMD symptomatology, and it was naturally followed by an improvement in the patients' oral health-related quality of life.

The PSS scores differ significantly between treatment groups but did not change significantly over time. Lover values of PSS scores in the SS group showed that psychological stress, considered a predisposing factor for TMD (156), was positively affected by stabilization splint therapy.

Despite the previous claims that the placebo mainly shows little to no difference compared to the stabilization splint, the increased effectiveness of the stabilization splint was also found by Ekberg et al. They monitored patients over 6 and 12 months and recommended the stabilization splint appliance for further use in TMD management (157). Moreover, Alajbeg et al. found that, compared to placebo and amitriptyline therapy, the stabilization splint showed a significantly greater change in the MCO (158).

The differences obtained when the baseline values were compared with the 3rd and 6th time-point within the treatment groups certainly benefit the theory that supports the stabilization splint over the placebo. Namely, at the sixth-month time-point, in patients treated with a placebo splint, the only difference was present for VAS, meaning that the patients' perception of their pain perception improved, although the other symptoms and the objective signs, such as mouth opening, have not improved significantly. The results were presented within the treatment groups as a whole to demonstrate the real value of the stabilization splint, which was more

pronounced when the groups were compared with no subdivisions; however, subdivisions are necessary to show how certain salivary markers strongly depend on TMD subdiagnosis and pain intensity.

The placebo intervention is defined as a modality without medical effects, which benefits the health status because of the patient's belief that the modality is effective (159) and our results confirmed that with a thin thermoforming foil, we managed to create a sufficient placebo effect with patients believing that their status was better whilst, in reality, the results are showing differently. The general belief in the treatment of TMD is that there would be no difference between physical therapy, education and stabilization splint therapy, as well as with placebo splint therapy; however, there are some disagreements about those claims. Some studies are pointing out that the stabilization splint does not appear to produce a better clinical outcome than a soft splint, a non-occluding palatal splint and physical therapy (77, 78), while others claim that a hard stabilization appliance provides better clinical outcomes than the placebo (160). Latter claims are further enhanced with our clinical findings being followed by significant changes in some of the oxidative stress parameters that were pronounced in the stabilization splint group while in the placebo group, those differences were either lesser or lacking. Still, in studies with the goal of comparing TMD treatment options, patients have been monitored over different time periods, hence the inconsistent results can be attributed to different therapy duration. Furthermore, the design of occlusal or nonocclusal devices differs to a great extent and it is difficult to provide comparable results (157, 158, 161, 162).

The observation of oxidative stress parameter changes throughout the 6-month period showed that morning and afternoon TAC and morning UA decreased significantly over time. Uric acid levels showed a greater decrease in the stabilization splint group. The TAC, however, regardless of the greater decreasing trend in the stabilization splint group, did not show differences between the treatment groups. This could mean that regardless of better SS performance, PS can help to some extent throughout the reduction of spontaneous pain and in that way modulate the oxidative status.

MDA changes, once more revealed to be closely related to pain intensity, with morning MDA decreasing significantly in the high-intensity pain subgroup over time. Also, afternoon MDA in high-intensity pain patients was shown to decrease significantly in the group using the stabilization splint. In the placebo splint group, MDA showed significantly higher values after the 6^{th} month of treatment compared to the baseline, meaning that the placebo might even be

able to worsen the oxidative balance in favour of oxidants. However, it is important to accentuate the high inter- and intra-individual variability of MDA when interpreting the results, also the external and internal conditions that each patient was exposed to during therapy were not completely controllable and could vastly affect the oxidative balance without having any connections to TMD (89); nevertheless, this finding is certainly thought-provoking. Patients in the low-intensity pain subgroup showed no significance considering MDA. We may conclude that a decrease in TAC over time was followed by a significant reduction in the MDA levels of the HIP group. This may be due to the previously mentioned diminished need for antioxidative defence after the decrease in pro-oxidant activity, which is more detectable with higher pain intensity. In addition, the MDA to SOD ratio showed a significant decrease over time as well, with a greater decrease in the high-intensity pain subgroup compared to the low-intensity pain subgroup. This implies that antioxidant biomarkers prevail over oxidants, suggesting that patients with higher pain intensity have a stronger response to therapy. Higher pain intensity exposes patients to more stress and thus could be the main reason for these changes being more detectable. The connection could also be reversed with environmental factors and psychological factors (stress) provoking increased pain experience resulting in pain of higher intensity (163).

The results concerning 8-OHdG are not as clear. No significant changes in 8-OHdG levels over time were found; however, the oxidant tends to be higher in the group with joint origin TMD. 8-OHdG is highly variable and showed to be a marker whose baseline measurements differed significantly within the diagnostic subgroup subdivision, thus these results should be approached with caution.

In patients treated with a stabilization splint, significant positive correlations were found between the percentage reduction in PSS and the percentage reduction in TAC, as well as between the percentage reduction in PSS and the percentage reduction in salivary cortisol. These are important findings that are indicating the connection between stress perception and objective stress parameters. The major inconvenience is in the fact that despite both the TAC and SC decreased over time and correlated to stress perception, we cannot possibly know which happens to be the cause and which the consequence - the stress perceived by the patient (possibly because of TMD symptoms) that was followed by increased levels of TAC and SC in the saliva, or that TAC and SC, increased priory, tend to worsen the symptoms of TMD, which then results in stress perception.

At the beginning of the research, there were no similar studies that followed changes in oxidative stress markers throughout the therapy. To the best of our knowledge, only one similar study has been published to this day. Baş et al. conducted a study where they aimed to determine whether stabilization splint therapy reduces interleukin-6, MDA and 8-OHdG levels in synovial fluid in a 3-month treatment (164). They found no significant differences between the baseline and the third-month measurements; also, markers' levels did not differ depending on the type of therapy (stabilization splint/ no treatment). With the significant differences between the studies' methodologies, it could be said that the results are not likely to be comparable. Arthrocentesis, as additional stress for a patient, can interfere with the levels of the markers. Also, synovial fluid could only be relevant for the joint affected by TMD while in our study, we evaluated TMD of muscular origin as well. When comparing the results of similar research, especially when interpreting the results of such a delicate matter as oxidative stress, it is a substantial task to search for the differences in methodology so we know precisely to what extent the results are applicable.

The limitations of the study were as follows.

Firstly, this study had a small sample size in each subgroup, especially when considering the variability of some of the tested biomarkers. With a larger number of participants per subgroup, the effect of pain intensity or diagnostic TMD subgroups on OS status would be better investigated and more significant differences would certainly occur. Secondly, only female participants were suitable for inclusion. Because women are twice as likely to be affected by TMD (*National Institute of Dental and Craniofacial Research*), this limitation is often present in research on this subject. Also, in our previous study, which gave a detailed description of the methodology, no significant differences in the salivary OS markers between men and women were present (89), thus our results could probably be applied to the population usually affected by TMD.

It is important to bear in mind that the included patients were experiencing chronic, moderate to severe pain, whose response to the therapy may be different from those with mild pain. Furthermore, one of the aggravating circumstances was the researchers' inability to completely control conditions outside of the ambulance throughout the 6-month period.

All the parameters followed log-normal distribution and statistical analysis was performed on $\log_{10}$ transformed data. All significant differences and changes can only be relevant when the

log-transformation is taken into consideration. Careful interpretation is necessary when observing non-transformed data.

Nevertheless, the main advantage of the study was that we carefully selected and followed the subjects for a longer period of time with validated and widely accepted TMD diagnostic questionnaires and protocols. The samples were collected by means of a standardized collection procedure that was evaluated in our pilot study. Although our study population was limited in size, our findings suggest that dividing the rather heterogenic TMD population into diagnostic and pain-intensity subgroups, in similar research, is necessary. Also, we monitored the patients and, as far as possible, controlled additional actions or the intake of any substances and supplements that could interfere with the oxidative status.

Our results showed that the specific design of the stabilization splint contributes to the treatment of TMD not only through the improvement of clinical symptoms and signs but also through detectable changes in the oxidative status, thus probably helping the body's cleansing capacity to remove free radicals. Still, these results raise some questions on the origin of the changed oxidative status in TMD patients. It is still not clear whether oxidative stress is a predisposing state that leads to disease, the disease is the reason for a changed oxidative status or both. We strongly encourage further research into the mechanisms of oxidative stress involvement in the disease. A greater understanding of these mechanisms would possibly help target predisposed individuals, optimize the diagnostic protocols and treatments and provide a sufficient tool for assessment of the applied therapy. If these results are supported in the future with high-quality randomized and cohort studies with standardized methodology, care and understanding of TMD, as well as other pain-related conditions, could drastically improve.

5. CONCLUSIONS

Considering the limitations of this study, the conclusions are as follows:

- The mean levels of log TAC, as well as the mean levels of morning log UA and afternoon log MDA were significantly higher in TMD subjects compared to the control group ($p<0.05$).
- When the control group was compared to the TMD diagnostic subgroups, the morning levels of log SC and log UA were significantly higher and the afternoon levels of log GPx were significantly lower in MP patients compared to the control group ($p<0.05$). When the control group was compared to the TMD pain intensity subgroups, the mean levels of log MDA and mean levels of morning log UA were significantly higher in HIP patients compared to the control group ($p<0.05$).
- When all TMD patients were considered, a significant positive correlation was found between the perceived stress, measured by PSS scale, and GPx and MDA ($r=0.425$; $r=0.512$ respectively); higher PSS scores were negatively correlated to SOD ($r=-0.431$).
- When the TMD subgroups were considered, morning SC was positively correlated to GPx and UA in MP patients ($r=0.643$; $r=0.592$ respectively) and in patients with LIP ($r=0.529$; $r=0.512$ respectively); a significant positive correlation was noted between PSS and MDA and GPx in DD patients ($r=0.588$; $r=0.504$ respectively) and in patients with HIP ($r=0.545$; $r=0.655$ respectively).

- When compared with the baseline values, in the 3rd and 6th months of treatment, a significant decrease in VAS, OHIP-14, PSS, and CPI, as well as a significant increase of MCO and MMO, was found in the SS group ($p<0.05$), while in the PS group only a significant decrease of VAS between the baseline and the 6th month was present.
- When compared with the baseline values, in the 3rd and 6th month of treatment patients treated with SS exhibited a significant decrease of log TAC levels and log UA levels ($p<0.05$); in patients treated with PS no significant changes of the OS markers' levels from the baseline to follow-up appointments were found.

- Changes in VAS differed significantly between the two treatment groups (interaction time x treatment group; Wilks Lambda = 0.39, $p=0.0002$, effect size=0.29), with greater reduction in SS compared to the PS group; significant differences between the two treatment groups were found for the OHIP-14 (interaction time x treatment group; Wilks

Lambda=0.56, p=0.008, effect size=0.18) and GCPS scores (interaction time x treatment group; Wilks Lambda=0.67, p=0.045, effect size: 0.17), with reported improvement only in SS group.

- MMO values improved significantly over time (p=0.01, effect size = 0.13), but did not differ significantly between the treatment groups, nor subgroups (p >0.05).
- The levels of log UA changed significantly over time with a greater decrease in the SS compared to PS group (interaction time x treatment group x pain intensity; Wilks Lambda=0.55, p=0.007, effect size=0.14).
- The levels of afternoon log MDA decreased significantly in HIP patients treated with SS (interaction time x treatment group x pain intensity; Wilks Lambda=0.56, p=0.011, effect size=0.13) compared to the placebo.
- The MDA to SOD ratio changed significantly in the high-intensity pain subgroup across the treatment period (interaction time x pain intensity; Wilks Lambda=0.56, p=0.01).
- At the 6-month follow-up, the subjects treated with SS had a significantly greater reduction of VAS (86.92%) and OHIP-14 scores (61.68%) than the subjects treated with PS (42.58%, 18.52%, respectively); also, subjects in the SS group had a significantly greater increase in MCO (28.54%) and MMO (23.82%) compared to the PS group (1.64%. 0.73, respectively).
- A significant positive correlation between changes in perceived stress with the percentage change of the afternoon TAC (r=0.50, p=0.04) and the percentage change of the morning SC (r=0.64, p=0.005) was found only in patients treated with SS.

Due to the significant decrease in pain, improvement of health-related quality of life and functional limitations of the lower jaw in the group treated with the stabilization splint compared to the placebo, we may conclude that the stabilization splint showed better treatment effectiveness during a 6-month period. The oxidative status was shown to be altered in the TMD patients compared to the healthy controls and to some extent is affected by splint therapy in favour of antioxidants. The obtained results demonstrated that the intensity and source of the pain, as well as the time of saliva sampling, should be considered in future investigations, which are necessary for further clarification of the role of oxidative stress in both the initiation and progression of TMD.

6. LITERATURE

1. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. J Oral Facial Pain Headache. 2014;28(1):6–27.

2. Gauer RL, Semidey MJ. Diagnosis and Treatment of Temporomandibular Disorders. Am Fam Physician. 2015;91(6):378-386.

3. List, T, Jensen RH. Temporomandibular disorders: Old ideas and new concepts. Cephalalgia,.2017;37(7), 692–704.

4. Nidal G. Concepts of TMD Etiology: Effects on Diagnosis and Treatment. IOSR-JDMS. 2016;15(6):25-42.

5. Meyer RA. Temporomandibular Joint Pain. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 162. Available from: https://www.ncbi.nlm.nih.gov/books/NBK270/

6. Leeuw R. Dor orofacial: guia de avaliação, diagnóstico e tratamento. 4ª ed. São Paulo: Quintessence; 2010.

7. Chisnoiu AM, Picos AM, Popa S, Chisnoiu PD, Lascu L, Picos A, Chisnoiu R.. Factors involved in the etiology of temporomandibular disorders - a literature review. Clujul Med. 2015;88(4):473–478.

8. Laplanche O, Ehrmann E, Pedeutour P, Duminil G. TMD clinical diagnostic Classification (Temporomandibular Disorders). J Dentofacial Anom Orthod 2012;15:202

9. Pullinger AG, Seligman DA. Quantification and validation of predictive values of occlusal variables in temporomandibular disorders using a multifactorial analysis. J Prosthet Dent. 2000;83:66–75.

10. Dworkin SF, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord. 1992;6(4):301-55.

11. Manfredini D, Guarda-Nardini L, Winocur E, Piccotti F, Ahlberg J, Lobbezoo F. Research diagnostic criteria for temporomandibular disorders: a systematic review of axis I

epidemiologic findings. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;112:453-462.

12. Dworkin SF, Huggins KH, LeResche L, Von Korff M, Howard J, Truelove E, Sommers E. Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases and controls. J Am Dent Assoc. 1990;120(3):273-81.

13. Schiffman EL, Fricton JR, Haley DP, Shapiro BL. The prevalence and treatment needs of subjects with temporomandibular disorders. J Am Dent Assoc. 1990;120(3):295-303.

14. De Leeuw R, Klasser GD; American Academy of Orofacial Pain. Orofacial Pain: Guidelines for Assessment, Diagnosis, and Management. 5th ed. Chicago, Ill.: Quintessence Publ.; 2013.

15. Klasser, GD, Goulet, J-P, De Laat, A, & Manfredini, D. Classification of Orofacial Pain. Contemporary Oral Medicine. 2016;1–23.

16. Okeson JP. Temporomandibularni poremećaji i okluzija. 1. hrvatsko izdanje ed. Zagreb: Medicinska naklada Zagreb; 2008.

17. Reiter S, Goldsmith C, Emodi-Perlman A, et al. Masticatory muscle disorders diagnostic criteria: The American Academy of Orofacial Pain versus the research diagnostic criteria/temporomandibular disorders. J Oral Rehabil. 2012;39(12):941–947.

18. Stohler CS. Muscle-related temporomandibular disorders. J Orofac Pain. 1999;13(4):273–284.

19. Bag AK, Gaddikeri S, Singhal A, et al. Imaging of the temporomandibular joint: An update. World J Radiol. 2014;6(8):567–582.

20. Westesson PL, Bifano JA, Tallents RH, Hatala MP. Increased horizontal angle of the mandibular condyle in abnormal temporomandibular joints. A magnetic resonance imaging study. Oral Surg Oral Med Oral Pathol. 1991;72(3):359-63.

21. Nilner M, Petersson A. Clinical and radiological findings related to treatment outcome in patients with temporomandibular disorders. Dentomaxillofac Radiol. 1995;24(2):128-31.

22. Stegenga B, de Bont LG, Boering G, van Willigen JD. Tissue responses to degenerative changes in the temporomandibular joint: a review. J Oral Maxillofac Surg. 1991;49(10):1079-88.

23. Westesson PL, Larheim TA, Tanaka H. Posterior disc displacement in the temporomandibular joint. J Oral Maxillofac Surg. 1998;56(11):1266-73; discussion 73-4.

24. Westesson PL, Kurita K, Eriksson L, Katzberg RW. Cryosectional observations of functional anatomy of the temporomandibular joint. Oral Surg Oral Med Oral Pathol. 989;68(3):247-51.

25. Liedberg J, Westesson PL, Kurita K. Sideways and rotational displacement of the temporomandibular joint disk: diagnosis by arthrography and correlation to cryosectional morphology. Oral Surg Oral Med Oral Pathol. 1990;69(6):757-63.

26. Kurita K, Westesson PL, Tasaki M, Liedberg J. Temporomandibular joint: diagnosis of medial and lateral disk displacement with anteroposterior arthrography. Correlation with cryosections. Oral Surg Oral Med Oral Pathol. 1992;73(3):364-8.

27. Isberg-Holm AM, Westesson PL. Movement of disc and condyle in temporomandibular joints with clicking. An arthrographic and cineradiographic study on autopsy specimens. Acta odontologica Scandinavica. 1982;40(3):151-64.

28. Tallents RH, Hatala M, Katzberg RW, Westesson PL. Temporomandibular joint sounds in asymptomatic volunteers. J Prosthet Dent. 1993;69(3):298-304.

29. Davant TSt, Greene CS, Perry HT, Lautenschlager EP. A quantitative computer-assisted analysis of disc displacement in patients with internal derangement using sagittal view magnetic resonance imaging. J Oral Maxillofac Surg. 1993;51(9):974-9; discussion 9-81.

30. Stegenga B, de Bont LG, Boering G, van Willigen JD. Tissue responses to degenerative changes in the temporomandibular joint: a review. J Oral Maxillofac Surg. 1991;49(10):1079-88.

31. Scapino RP. The posterior attachment: its structure, function, and appearance in TMJ imaging studies. Part 2. J Craniomandib Disord. 1991;5(3):155-66.

32. Scapino RP. The posterior attachment: its structure, function, and appearance in TMJ imaging studies. Part 1. J Craniomandib Disord. 1991;5(2):83-95.

33. Stegenga B. Osteoarthritis of the temporomandibular joint organ and its relationship to disc displacement. J Orofac Pain. 2001;15(3):193-205.

34. Eversole LR, Machado L. Temporomandibular joint internal derangements and associated neuromuscular disorders. J Am Dent Assoc. 1985;110(1):69-79.

35. Stegenga B, de Bont LG, Dijkstra PU, Boering G. Short-term outcome of arthroscopic surgery of temporomandibular joint osteoarthrosis and internal derangement: a randomized controlled clinical trial. Br J Oral Maxillofac Surg. 1993;31(1):3-14.

36. Minakuchi H, Kuboki T, Matsuka Y, Maekawa K, Yatani H, Yamashita A. Randomized controlled evaluation of non-surgical treatments for temporomandibular joint anterior disk displacement without reduction. Journal of dental research. 2001;80(3):924-8.

37. Khare, N, Patil, SB, Kale, SM, Sumeet, J, Sonali, I, & Sumeet, B. Normal Mouth Opening in an Adult Indian Population. J Maxillofac Oral Surg. 2012;11(3):309–313.

38. Hu YK, Yang C, Xie QY. Changes in disc status in the reducing and nonreducing anterior disc displacement of temporomandibular joint: a longitudinal retrospective study. Sci Rep. 2016;6:34253.

39. Okeson JP, de Leeuw R. Differential diagnosis of temporomandibular disorders and other orofacial pain disorders. Dent Clin North Am. 2011;55(1):105–120.

40. Contreras EFR, Fernandes G, Ongaro PCJ, Campi LB, Gonçalves DAG. Systemic diseases and other painful conditions in patients with temporomandibular disorders and migraine. Braz Oral Res. 2018;23;32:e77.

41. Telishevska UD, Telishevska OD. Classifications of temporomandibular disorders and patients' examination protocols - comparative analysis by the convenience of their daily use in clinical practice. Wiad Lek. 2018;71(3 pt 2):738-745.

42. Baba K, Tsukiyama Y, Clark GT. Reliability, validity, and utility of various occlusal measurement methods and techniques. J Prosthet Dent. 2000;83(1):83-9.

43. John MT, Dworkin SF, Mancl LA. Reliability of clinical temporomandibular disorder diagnoses. Pain. 2005;118(1-2):61-9.

44. Lamot U, Strojan P, Šurlan Popovič K. Magnetic resonance imaging of temporomandibular joint dysfunction-correlation with clinical symptoms, age, and gender. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;116(2):258–263.

45. Tomas X, Pomes J, Berenguer J, Quinto L, Nicolau C, Mercader JM, Castro V. MR Imaging of Temporomandibular Joint Dysfunction: A Pictorial Review.RadioGraphics. 2006;26(3):765–781.

46. Blankstein A. Ultrasound in the diagnosis of clinical orthopedics: The orthopedic stethoscope. World J Orthop. 2011;2(2):13–24.

47. Glazer EJ, Steinbusch H, Verhofstad A, Basbaum AI. Serotonin neurons in nucleus raphe dorsalis and paragigantocellularis of the cat contain enkephalin. J Physiol (Paris). 1981;77(2-3):241-5.

48. Basbaum AI. Descending control of pain transmission: possible serotonergic-enkephalinergic interactions. Adv Exp Med Biol. 1981;133:177-89.

49. Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. Annu Rev Physiol. 1978;40:217-48.

50. Belcher G, Ryall RW, Schaffner R. The differential effects of 5-hydroxytryptamine, noradrenaline and raphe stimulation on nociceptive and non-nociceptive dorsal horn interneurones in the cat. Brain Res. 1978;151(2):307-21.

51. Benarroch EE. Pain-autonomic interactions. Neurol Sci. 2006;27 Suppl 2:S130-3.

52. Wall PD. The gate control theory of pain mechanisms. A re-examination and re-statement. Brain. 1978;101(1):1-18.

53. Fay RA, Norgren R. Identification of rat brainstem multisynaptic connections to the oral motor nuclei using pseudorabies virus. III. Lingual muscle motor systems. Brain Res Brain Res Rev. 1997;25(3):291-311.

54. Fay RA, Norgren R. Identification of rat brainstem multisynaptic connections to the oral motor nuclei in the rat using pseudorabies virus. II. Facial muscle motor systems. Brain Res Brain Res Rev. 1997;25(3):276-90.

55. Graeff FG, Guimaraes FS, De Andrade TG, Deakin JF. Role of 5-HT in stress, anxiety, and depression. Pharmacol Biochem Behav. 1996;54(1):129-41.

56. Graven-Nielsen T, Arendt-Nielsen L. Peripheral and central sensitization in musculoskeletal pain disorders: An experimental approach. Curr Rheumatol Rep. 2002;4(4):313-21.

57. Staud R, Smitherman ML. Peripheral and central sensitization in fibromyalgia: pathogenetic role. Curr Pain Headache Rep. 2002;6(4):259-66.

58. Smith SB, Maixner DW, Greenspan JD, Dubner R, Fillingim RB, Ohrbach R, Knott C, Slade GD, Bair E, Gibson DG, Zaykin DV, Weir BS, Maixner W, Diatchenko L. Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. J Pain. 2011;12(11):T92-101. doi: 10.1016/j.jpain.2011.08.005.

59. Manfredini D, Bonnini S, Arboretti R, Guarda-Nardini L. Temporomandibular joint osteoarthritis: an open label trial of 76 patients treated with arthrocentesis plus hyaluronic acid injections. Int J Oral Maxillofac Surg. 2009;38(8):827-34.

60. Abouelhuda AM, Khalifa AK, Kim YK, Hegazy SA. Non-invasive different modalities of treatment for temporomandibular disorders: review of literature. J Korean Assoc Oral Maxillofac Surg. 2018;44(2):43-51.

61. Schwartz M, Freund B. Treatment of Temporomandibular Disorders with Botulinum Toxin. The Clinical Journal of Pain. 18(6):S198–S203.

62. McNeill C. Management of temporomandibular disorders: concepts and controversies. J Prosthet Dent. 1997;77(5):510-22.

63. Gomes CA, El Hage Y, Amaral AP, Politti F, Biasotto-Gonzalez DA. Effects of massage therapy and occlusal splint therapy on electromyographic activity and the intensity of signs and symptoms in individuals with temporomandibular disorder and sleep bruxism: a randomized clinical trial. Chiropr Man Therap. 2014;22:43.

64. Michelotti A, Iodice G, Vollaro S, Steenks MH, Farella M. Evaluation of the short-term effectiveness of education versus an occlusal splint for the treatment of myofascial pain of the jaw muscles. J Am Dent Assoc. 2012;143:47–53.

65. Laskin DM., Greene CS, Hylander WL. Temporomandibular disorders: An evidence-based approach to diagnosis and treatment. Chicago: Quintessence Pub; 2006.

66. Hersh EV, Balasubramaniam R, Pinto A. Pharmacologic Management of Temporomandibular Disorders. Oral and Maxillofacial Surgery Clinics of North America, 2008;20(2),197–210.

67. Dionne RA. Pharmacologic Approaches. In Laskin DM (ed.). An evidence based approach to diagnosis and treatment. Chicago: Quintessence , 2006:347-357.

68. Feine JS, Thomason M. Physical Medicine. In Laskin DM (ed.). An evidence based approach to diagnosis and treatment. Chicago: Quintessence , 2006: 359-375.

69. Moraes Ada R, Sanches ML, Ribeiro EC, Guimarães AS. Therapeutic exercises for the control of temporomandibular disorders. Dental Press J O59.rthod. 2013;18(5):134-9.

70. Ohrbach R. Biobehavioral therapy. In. Laskin DM (ed). An evidence based approach to diagnosis and treatment. Chicago: Quintessence, 2006: 391-403.

71. Moloney F, Howard JA. Internal derangements of the temporomandibular joint. III. Anterior repositioning splint therapy. Aust Dent J. 1986;31(1):30-9.

72. Ohnuki T, Fukuda M, Nakata A, Nagai H, Takahashi T, Sasano T, Miyamoto Y. Evaluation of the position, mobility, and morphology of the disc by MRI before and after four different treatments for temporomandibular joint disorders. Dentomaxillofac Radiol. 2006;35(2):103-9.

73. Ma Z., Xie Q., Yang C. et al. Can anterior repositioning splint effectively treat temporomandibular joint disc displacement? Sci Rep. 2019;9(534).

74. Shah FK, Rajput G, Lall S, Bey A. Temporomandibular joint pain treated by occlusal appliance therapy. J Indian Prosthodont Soc 2009;9:112-115.

75. Zhang C, Wu JY, Deng DL, et al. Efficacy of splint therapy for the management of temporomandibular disorders: a meta-analysis. Oncotarget. 2016;7(51):84043–84053.

76. Al-Ani Z, Gray RJ, Davies SJ, Sloan P, Glenny AM. Stabilization splint therapy for the treatment of temporomandibular myofascial pain: a systematic review. J Dent Educ. 2005;69(11):1242-50.

77. Klasser GD, Greene CS. Oral appliances in the management of temporomandibular disorders. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(2):144-150.

78. Türp JC, Komine F, Hugger A. Efficacy of stabilization splints for the management of patients with masticatory muscle pain: a qualitative systematic review. Clin Oral Invest. 2004;8:179.

79. Manns A, Miralles R, Santander H, Valdivia J. Influence of the vertical dimension in the treatment of myofascial pain-dysfunction syndrome. J Prosthet Dent. 1983;50(5):700-709.

80. Suvinen TI, Kemppainen P. Review of clinical EMG studies related to muscle and occlusal factors in healthy and TMD subjects. J Oral Rehabil. 2007;34:631–644.

81. Moreno-Hay I, Okeson JP. Does altering the occlusal vertical dimension produce temporomandibular disorders? A literature review. J Oral Rehabil. 2015;42(11):875-82.

82. Laluque JF, Brocard D, d'Incau E. Understanding bruxism: Current knowledge and practice. France: Quintessence publishing; 2017. 159 p.

83. Tosato Jde P, Caria PH, Gomes CA, et al. Correlation of stress and muscle activity of patients with different degrees of temporomandibular disorder. J Phys Ther Sci. 2015;27(4):1227–1231.

84. de Lucena IM, Rodrigues LL, Teixeira ML, Pozza DH, Guimaraes AS. Prospective study of a group of pre-university students evaluating anxiety and depression relationships with temporomandibular disorders. J Clin Exp Dent. 2012;4(2):e102–e106.

85. Korszun A, Papadopoulos E, Demitrack M, Engleberg C, Crofford L.The relationship between temporomandibular disorders and stress-associated syndromes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86(4):416-20.

86. Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans. 2007;35(Pt 5):1147-50.

87. Valavanidis A1, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf. 2006;64(2):178-89.

88. Aschbacher K, O'Donovan A, Wolkowitz OM, Dahabhar FS, Su Z, Epel E. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. Psychoneuroendocrinology 2013; 38:1698–1708.

89. Alajbeg IZ, Lapic I, Rogic D, Vuletic L, Andabak Rogulj A, Illes D, et al. Within-subject reliability and between-subject variability of oxidative stress markers in saliva of healthy subjects: a longitudinal pilot study. Dis. Markers 2017;2697464.

90. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J. Oral Pathol. Med. 2012;41:736–740.

91. Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Kebriaei R. Evaluation of salivary and serum antioxidant and oxidative stress status in patients with chronic periodontitis: a case-control study. Front. Physiol. 2017;8:189.

92. Babaee N, Hosseinkazemi H, Pouramir M, Baboli OK, Salehi M, Khadir F, et al. Salivary oxidant/antioxidant status and hematological parameters in patients with recurrent aphthosus stomatitis. Caspian J. Intern. Med. 2016;7:13–18.

93. Harman D. Free radical theory of ageing. Mutat Res/DNAging. 1992;275(3-6):257-266.

94. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004;266:37–56.

95. Kesarwala AH, Krishna MC, Mitchell JB. Oxidative stress in oral diseases. Oral Dis. 2014;22(1):9-18.

96. Cooke MS, Loft S, Olinski R. Measurement and meaning of oxidatively modified DNA lesions in urine. CEBP. 2008;17:3–14.

97. Pryor WA, Godber SS. Noninvasive measures of oxidative stress status in humans.Free Radic Biol Med. 1991;10(3-4):177-84.

98. World Health Organization & International Programme on Chemical Safety. (2001). Biomarkers in risk assessment: validity and validation. World Health Organization. https://apps.who.int/iris/handle/10665/42363

99. Devasagayam TP1, Boloor KK, Ramasarma T. Methods for estimating lipid peroxidation: an analysis of merits and demerits. Indian J Biochem Biophys. 2003;40(5):300-8.

100. Kamodyová N, Tóthová L, Celec P. Salivary markers of oxidative stress and antioxidant status: influence of external factors. Dis Markers. 2013;34(5):313–321.

101. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009;27(2):120-39.

102. Sova H, Jukkola-Vuorinen A, Puistola U, Kauppila S, Karihtala P. 8-Hydroxydeoxyguanosine: a new potential independent prognostic factor in breast cancer. Br J Cancer. 2010;102(6):1018–1023.

103. Cooke MS, Olinski R, Loft S. Measurement and meaning of oxidatively modified DNA lesions in urine. Cancer Epidemiol Biomarkers Prev. 2008;17(1):3-14.

104. Kurgan Ş, Önder C, Altıngöz SM, Bağış N, Uyanık M, Serdar MA, Kantarcı A. High sensitivity detection of salivary 8-hydroxy deoxyguanosine levels in patients with chronic periodontitis. J Periodontal Res. 2015;50(6):766-74.

105. Marnett LJ. Lipid peroxidation—DNA damage by malondialdehyde. Mutat research/Fundamental and Molecular Mechanisms of Mutagenesis. 1999;424(1-2):83-95.

106. Khoubnasabjafari M, Ansarin K, Jouyban A. Salivary malondialdehyde as an oxidative stress biomarker in oral and systemic diseases. J Dent Res Dent Clin Dent Prospects. 2016;10(2):71–74.

107. Hajhashemi V, Vaseghi G, Pourfarzam M, Abdollahi A. Are antioxidants helpful for disease prevention? Res Pharm Sci. 2010;5(1):1–8.

108. Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. BMC Vet Res. 2016;12(1), 166.

109. Jeeva JS, Sunitha J, Ananthalakshmi R, Rajkumari S, Ramesh M, Krishnan R. Enzymatic antioxidants and its role in oral diseases. J Pharm Bioallied Sci. 2015;7(Suppl 2):S331–S333.

110. Sautin YY, Johnson RJ. Uric Acid: The Oxidant-Antioxidant Paradox. Nucleosides Nucleotides Nucleic Acids. 2008;27(6-7): 608-619.

111. Halliwell B. Antioxidants: the basics—what they are and how to evaluate them. Adv Pharmacol. 1996;38:3–20.

112. Gaté L, Paul J, Ba GN, Tew KD, Tapiero H. Oxidative stress induced in pathologies: the role of antioxidants. Biomed Pharmacother. 1999;53(4):169-80.

113. Liguori I, Russo G, Curcio F, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757–772.

114. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11–26.

115. Al-Gubory KH. Environmental pollutants and lifestyle factors induce oxidative stress and poor prenatal development. Reprod Biomed Online. 2014;29(1):17-31.

116. Rodríguez de Sotillo D, Velly AM, Hadley M, Fricton JR. Evidence of oxidative stress in temporomandibular disorders: a pilot study. J. Oral Rehabil. 2011;38:722–728.

117. De Almeida C, Amenábar JM. Changes in the salivary oxidative status in individuals with temporomandibular disorders and pain. J. Oral Biol. Craniofac. Res. 2016; 6:S1–S4.

118. Sies H. Strategies of antioxidant defense. Eur. J. Biochem. 1993;15:213–219.

119. Vrbanović E, Alajbeg IZ, Vuletić L, Lapić I, Rogić D, Andabak Rogulj A, Illeš D, Knezović Zlatarić D, Badel T and Alajbeg I. Salivary Oxidant/Antioxidant Status in Chronic Temporomandibular Disorders Is Dependent on Source and Intensity of Pain – A Pilot Study. Front. Physiol. 2018; 9:1405.

120. Takahashi A, Mikami M, Yang J. Hydrogen peroxide increases GABAergic mIPSC through presynaptic release of calcium from IP3 receptor-sensitive stores in spinal cord substantia gelatinosa neurons. Eur J Neurosci 2007;25: 705-16.

121. Nishio N, Taniguchi W, Sugimura YK, Takiguchi N, Yamanaka M, Kiyoyuki Y, et al. Reactive oxygen species enhance excitatory synaptic transmission in rat spinal dorsal horn neurons by activating TRPA1 and TRPV1 channels. Neuroscience 2013;247:201-12.

122. Cai HX, Luo JM, Long X, et al: Free-radical and superoxide dismutase activity in synovial fluid of patients with temporomandibular disorders. J Orofac Pain 20:53, 2006

123. Guven O, Tekin US, Durak I, Keller EE, Hatipoglu M. Superoxide dismutase activity in synovial fluids in patients with temporomandibular joint internal derangement. J Oral Maxillofac Surg. 2007;65(10):1940–1943.

124. Nitzan DW, Goldfarb A, Gati I, Kohen R. Changes in the reducing power of synovial fluid from temporomandibular joints with anchored disc. J Oral Maxillofac Surg. 2002;60(7):735–740.

125. Richards RS, McGregor NR, Roberts TK. Association between oxidative damage markers and self-reported temporomandibular dysfunction symptoms in patients with chronic fatigue syndrome. J Chronic Fatigue Syndr. 2004;12(3):45–61.

126. Miller N, Rice –Evans C, Davies MJ. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci. 1993;84:407-412.

127. Motoyama T, Okamoto K, Kukita I, Hamaguchi M, Kinoshita Y, Ogawa H. Possible role of increased oxidant stress in multiple. Care Med. 2003;31:1048-1052.

128. Basi DL, Velly AM, Schiffman EL, Lenton PA, Besspiata DA, Rankin AM, et al. Human temporomandibular joint and myofascial pain biochemical profiles: a case-control study. J. Oral Rehabil. 2012;39:326–337.

129. Etöz OA, Akçay H, Neşelioğlu S, Erel Ö, and Alkan A. Total antioxidant capacity and total oxidant status of synovial fluids in patients with temporomandibular joint pain and dysfunction. Clin. Oral Investig. 2012;16:1557–1561.

130. Vrbanović E, Lapić I, Rogić D, Alajbeg IZ. Changes in salivary oxidative status, salivary cortisol, and clinical symptoms in female patients with temporomandibular disorders during occlusal splint therapy: a 3-month follow up. BMC Oral Health. 2019;19(1):100.

131. Nunes LA, Mussavira S, Bindhu OS. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systematic review. Biochem Med (Zagreb). 2015;25(2):177–192.

132. Lee JM, Garon E, Wong DT. Salivary diagnostics. Orthod Craniofac Res. 2009;12(3):206–211.

133. Malon RS, Sadir S, Balakrishnan M, Córcoles EP. Saliva-based biosensors: noninvasive monitoring tool for clinical diagnostics. Biomed Res Int. 2014;2014:962903.

134. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics - Current views and directions. Exp Biol Med (Maywood). 2017;242(5):459–472.

135. Katsiougiannis S, Wong DT. The Proteomics of Saliva in Sjögren's Syndrome. Rheum Dis Clin North Am. 2016;42(3):449–456.

136. Emshoff R, Emshoff I, Bertram S. Estimation of clinically important change for visual analog scales measuring chronic temporomandibular disorder pain. J Orofac Pain. 2010;24(3):262-9.

137. Dijkstra PU, de Bont LG, Stegenga B, Boering G. J Oral Rehabil. Angle of mouth opening measurement: reliability of a technique for temporomandibular joint mobility assessment. J Oral Rehabil. 1995;22(4):263-8.

138. Hudek-Knežević J, Kardum I, Lesić R. Efekti percipiranog stresa i stilova suočavanja na tjelesne simptome. Društvena istraživanja. 1999; 8,543-561.

139. Rener-Sitar K, Petričević N, Čelebić A, Marion L. Psychometric properties of Croatian and Slovenian short form of oral health impact profile questionnaires. Croat Med J. 2008;49(4):536-44.

140. Barros V de M, Seraidarian PI, Côrtes MI, de Paula LV. The impact of orofacial pain on the quality of life of patients with temporomandibular disorder. J Orofac Pain. 2009;23(1):28-37.

141. Von Korff M, Ormel J, Keefe FJ, Dworkin SF. Grading the severity of chronic pain. Pain 1992;50:133–49.

142. Ohrbach R, Larsson P, List T. The jaw functional limitation scale: development, reliability, and validity of 8-item and 20-item versions. J Orofac Pain. 2008;22(3):219-30.

143. Ray K, Fahrmann J, Mitchell B, Paul D, King H, Crain C, et al. Oxidation-sensitive nociception involved in endometriosis-associated pain. Pain. 2015;156,528–539.

144. Medow MS, Aggarwal A, Baugham I, Messer Z, Stewart JM. Modulation of the axon-reflex response to local heat by reactive oxygen species in subjects with chronic fatigue syndrome. J. Appl. Physiol. 2013;114,45–51.

145. Ishimaru K, Ohba S, Yoshimura H, Matsuda S, Ishimaru J, Sano K. Antioxidant capacity of synovial fluid in the temporomandibular joint correlated with radiological morphology of temporomandibular disorders. Br J Oral Maxillofac Surg. 2015;53(2):114-20.

146. Tomida M, Ishimaru JI, Murayama K, Kajimoto T, Kurachi M, Era S, Shibata T. Intra-articular oxidative state correlated with the pathogenesis of disorders of the temporomandibular joint. Br J Oral Maxillofac Surg. 2004;42(5):405-9.

147. Lee JY, Chung JW, Kim YK, Chung SC, Kho HS. Comparison of the composition of oral mucosal residual saliva with whole saliva. Oral Dis. 2007;13(6):550-4.

148. Goll RD, Mookerjee BK. Correlation of biochemical parameters in serum and saliva in chronic azotemic patients and patients on chronic hemodialysis. J Dial. 1978;2:344–99

149. Nunes LA, Brenzikofer R, Macedo DV. Reference intervals for saliva analytes collected by a standardized method in a physically active population. Clin Biochem. 2011;44:1440–4.

150. Pedlar J, Frame JW. Oral and maxillofacial surgery: an objective-based textbook. Second edition. Elsevier Limited, China, 2007. p. 236

151. Davis MC, Zautra AJ, Reich JW. Vulnerability to stress among women in chronic pain from fibromyalgia and osteoarthritis. Ann Behav Med. 2001;23(3):215–226.

152. Jafri MS. Mechanisms of myofascial pain. Int Sch Res Notices. 2014;2014:523924.

153. Spiers JG, Chen HJ, Sernia C, Lavidis NA. Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. Front Neurosci. 2015;8:456.

154. Gray RJ, Davies SJ, Quayle AA. A clinical approach to temporomandibular disorders: a clinical approach to treatment. Br Dent J. 1994;10;177(5):171-178

155. Kirk WS Jr. Magnetic resonance imaging and tomographic evaluation of occlusal appliance treatment for advanced internal derangement of the temporomandibular joint. J Oral Maxillofac Surg. 1991;49(1):9–12.

156. Augusto VG, Perina KCB, Penha DSG, Dos Santos DCA, Oliveira VAS. Temporomandibular dysfunction, stress and common mental disorder in university students. Acta Ortop Bras. 2016;24(6):330–333.

157. Ekberg E, Nilner M. Treatment outcome of appliance therapy in temporomandibular disorder patients with myofascial pain after 6 and 12 months. Acta Odontol Scand. 2004;62(6):343-349.

158. Alajbeg IZ, Boric Brakus R, Brakus I. Comparison of amitriptyline with stabilization splint and placebo in chronic TMD patients: a pilot study. Acta Stomatol Croat. 2018;52(2):114-122.

159. Wolman BB. Dictionary of behavioural science. Academic Press, 1989.

160. Fricton J, Look JO, Wright E, Alencar FGP, Chen H, Lang M, Ouyang W, Velly AM, et al. Systematic review and meta-analysis of randomized controlled trials evaluating intraoral orthopedic appliances for temporomandibular disorders. J Orofac Pain. 2010;24(3):237-254.

161. Truelove E, Huggins KH, Mancl L, Dworkin SF. The efficacy of traditional, low-cost and nonsplint therapies for temporomandibular disorder: a randomized controlled trial. J Am Dent Assoc. 2006;137(8):1099-107.

162. Alencar F Jr, Becker A. Evaluation of different occlusal splints and counselling in the management of myofascial pain dysfunction. J Oral Rehabil. 2009;36(2):79-85.

163. Linton SJ, Shaw WS. Impact of Psychological Factors in the Experience of Pain. Physical Therapy. 2011;91(5), 700–711.

164. Baş B, Aksoy A, Atmaca E, Öz AA, Kaya Ö, Kazan D, Yılmaz E, Kütük N. Effect of occlusal splint on interleukin 6, malondialdehyde and 8-hydroxydeoxyguanosine levels in synovial fluid of patients with temporomandibular disorders. Int J Oral Maxillofac Surg. 2019;pii: S0901-5027(19)31114-2.

7. AUTHORS' BIOGRAPHY

Ema Vrbanović graduated from the School of Dental Medicine, University of Zagreb, in August 2016. Immediately after graduation, she enrolled in a postgraduate doctoral course and began her work on a project of the Croatian Science Foundation "The role of oxidative stress and opiorfin in temporomandibular disorders", led by mentor Professor Iva Alajbeg. In July 2018, she started working full time as a research and teaching assistant within the Young Researchers' Career Development Programme at the Department of Removable Prosthodontics, School of Dental Medicine in Zagreb. During her doctoral studies, she actively presented the project's results at various international congresses, winning second place in the competition for the best oral poster presentation. In June 2019, she spent a week at the Dental Clinic, Medical University of Graz. Since 2018, she has been actively involved in teaching on both Croatian and English graduate studies.

List of publications:

Vrbanović E, Lapić I, Rogić D, Alajbeg IZ. Changes in salivary oxidative status, salivary cortisol, and clinical symptoms in female patients with temporomandibular disorders during occlusal splint therapy: a 3-month follow up. *BMC Oral Health*. 2019;19(1):100. (**IF: 2.048**, Q2 (WoS), Q1 (Scopus), 2018)

Vrbanović E, Alajbeg IZ. Long-term Effectiveness of Occlusal Splint Therapy Compared to Placebo in Patients with Chronic Temporomandibular Disorders. Acta Stomatol Croat. 2019;53(3):195-206. (Q3 (Scopus), 2018)

Vrbanović E, Alajbeg IZ, Vuletić L, Lapić I, Rogić D, Andabak Rogulj A, Illeš D, Knezović Zlatarić D, Badel T and Alajbeg I. Salivary Oxidant/Antioxidant Status in Chronic Temporomandibular Disorders Is Dependent on Source and Intensity of Pain – A Pilot Study. *Front. Physiol*. 2018; 9:1405. (**IF: 3.201**, Q2 (WoS); Q2 (Scopus))

Alajbeg IZ, Lapic I, Rogic D, Vuletic L, Andabak Rogulj A, Illes D, Knezović Zlatarić D, Badel T, **Vrbanović E**, Alajbeg I. Within-subject reliability and between-subject variability of oxidative stress markers in saliva of healthy subjects: a longitudinal pilot study. *Dis. Markers* 2017;2697464. (**IF: 2.949**, Q2 (WoS) Q1(Scopus))

Vrbanović E, Alajbeg IZ. A Young Patient with Temporomandibular Joint Osteoarthritis: Case Report. *Acta Stomatol Croat*. 2017;51(3):232–239. (Q4 (Scopus))

Alajbeg I, **Vrbanović E**. Temporomandibularni poremećaji. MEDIX. 2019; God. 25 Br. 138: 169-173. (Review Article)

Conference proceedings:

Vrbanović E, Alajbeg, IZ. Effectiveness of Stabilization Splint Compared to Placebo in Temporomandibular Disorders // CED-IADR/NOF, Madrid, 2019; p. 70-70.

Alajbeg IZ; **Vrbanović E**, Brkljačić L, Alajbeg I. Salivary Opiorphin is Dependent of Pain Intensity in Chronic TMD Patients // CED-IADR/NOF Abstract book Madrid, 2019; p. 67-67.

Vrbanović E, Alajbeg IZ. Influence of occlusal treatment on salivary oxidative status in chronic TMD patients. // Acta Stomatol Croat. 2019;53(3):280-292. / Tarle, Zrinka; Klarić, Eva (editors), 2019; p. 285-285.

Alajbeg I, **Vrbanović E**, Lapić I, Rogić D; Alajbeg IZ. Salivary Concentrations Of Antioxidants And Cortisol In Patients With Chronic Temporomandibular Disorders. // 14th Biennial congress of EAOM in conjunction with the world workshop on oral medicine VII. Gothenburg, Švedska, 2018; p. 1-1.

Alajbeg I, **Vrbanović E**, Lapić I, Rogić D, Vuletić L, Andabak Rogulj A, Alajbeg I. Salivary antioxidants levels in chronic temporomandibular disorders patients // Program book of 96th general session of the IADR. London, Engleska, 2018; p. 200-200.

Alajbeg I, **Vrbanović E**, Lapić I, Knezović Zlatarić D, Illeš D, Badel T, Zadravec D, Alajbeg I. Salivary antioxidant profile during temporomandibular disorders treatment // Journal of Dental Research / Giannobile, William V (editor.). Michigan: International Association for Dental Research, 2018; p. 2075-2075.

Vrbanović E, Lapić I, Rogić D, Alajbeg I. Salivary cortisol and antioxidan levels in patients with chronic temporomanidbular disorders // Acta stomatol Croat. 2018; 52(2):162-171. / Tarle, Zrinka; Klarić, Eva (editors). Zagreb, 2018; p. 163-163.

-2nd prize on the 4th International Congress of the School of Dental Medicine

Alajbeg I, **Vrbanović E**, Rogić D, Lapić I, Vuletić L, Andabak Rogulj A. Salivary malondialdehyde – a biomarker of oxidative stress // Acta stomatol Croat. 2017; 51(3):249-264 / Tarle, Zrinka (editor). Zagreb, 2017; p. 251-252