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CGRP plasma level changes in patients with temporomandibular disorders treated with occlusal splints — a randomised clinical trial

Zmiana stężenia CGRP w osoczu u pacjentów z dysfunkcją układu ruchowego narządu żucia leczonych szynami okluzyjnymi — badanie randomizowane

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Abstract

Introduction: Occlusal splint therapy is a well-known method for the treatment of TMD. Muscle stretching and pain relief are effects of occlusal appliance. The aim of this study was to evaluate the plasma level of CGRP in patients with myofascial pain (RDC/TMD Ia) and myofascial pain with limited opening (RDC/TMD Ib) before and after muscle stretching with occlusal splint therapy.

Material and methods: A randomised trial was performed including 35 subjects (males = 10, females = 25) in the experimental group and 30 subjects (males = 9, females = 21) in the control group. Blood samples were taken from the external jugular vein before and after 30 days of occlusal splint therapy. Plasma levels of CGRP were measured with a Radio Immunoassay Kit (Phoenix Pharmaceuticals Inc.) and Cobra Series Auto-Gamma Counting System.

Results: The results of the study demonstrated that CGRP concentrations were significantly higher after occlusal splint than before splint therapy: CGRP2 = 17.02 pg/mL (SD = 5.85), CGRP1 = 13.78 pg/mL (SD = 5.12), in the experimental group ($p < 0.05$).

In the control group, there were no statistically significant changes in CGRP levels: CGRP1 = 14.5 pg/mL (SD = 4.87) to CGRP2 = 13.5 pg/mL (SD = 4.63). In the experimental group, there was a statistically significant reduction in pain intensity, VAS1 = 5 (SD = 2.5) to VAS2 = 1 (SD = 1.04) after splint therapy ($p < 0.05$). In the control group, there were no statistically significant changes in pain intensity: VAS1 = 5 (SD = 2.3) to VAS2 = 4 (SD = 2.6), ($p < 0.05$).

Conclusions: CGRP plays an important role in muscle blood flow, which is altered by changes in muscle length. Further investigation is needed to clarify the mechanism of muscle blood flow and the muscle healing process in patients with TMD. **(Endokrynol Pol 2014; 65 (3): 217–222)**

Key words: *CGRP (calcitonin gene related peptide); TMD (temporomandibular disorder); occlusal splint*

Streszczenie

Wstęp: Szynoterapia jest popularną formą leczenia dysfunkcji układu ruchowego narządu żucia (DURNŻ). Głównymi jej efektami są zmiany napięcia mięśni oraz działanie przeciwbólowe. Celem pracy była ocena stężenia CGRP u pacjentów cierpiących z powodu bólu mięśniowo-powięziowego mięśni żucia (RDC/TMD Ia) oraz bólu mięśniowo-powięziowego z ograniczonym odwodzeniem żuchwy (RDC/TMD Ib) przed i po szynoterapii.

Materiał i metody: Przeprowadzono randomizowane badanie kliniczne obejmujące 35 pacjentów z DURNŻ w grupie badanej: 10 mężczyzn oraz 25 kobiet oraz 30 pacjentów z DURNŻ w grupie kontrolnej: 9 mężczyzn i 21 kobiet. Materiał do badania pobierano z żyły szyjnej zewnętrznej przed oraz po 30 dniach szynoterapii. Do oceny stężenia CGRP zastosowano zestaw Radio Immunoassay Kit (firmy Phoenix Pharmaceuticals Inc.) oraz system Cobra Series Auto-Gamma Counting System.

Wyniki: Uzyskane wyniki wskazują, że stężenia CGRP w badanej grupie był znacząco wyższy po niż przed szynoterapią: CGRP2 = 17,02 pg/ml (SD = 5,85), CGRP1 = 13,78 pg/ml (SD = 5,12), ($p < 0,05$). W grupie kontrolnej nie odnotowano istotnych statystycznie zmian: przed i po szynoterapi: CGRP1 = 14.5 pg/ml (SD = 4,87), CGRP2 = 13,5 pg/ml (SD = 4,63). W grupie badanej zauważono statystycznie istotną redukcję natężenia bólu, VAS1 = 5 (SD = 2.5), VAS2 = 1 (SD = 1,04) ($p < 0,05$). W grupie kontrolnej nie wystąpiły istotne statystycznie zmiany w natężeniu bólu: VAS1 = 5 (SD = 2,3), VAS2 = 4 (SD = 2,6), ($p < 0,05$).

Wnioski: Podsumowując, CGRP odgrywa istotną rolę w regulacji przepływu krwi przez mięśnie żucia. Perfuzja krwi przez tkankę mięśniową zmienia się wraz z długością mięśnia podczas szynoterapii. Niezbędne są dalsze badania, które wyjaśnią mechanizm regulacji przepływu krwi przez mięśnie żucia oraz gojenia tkanki mięśniowej, u pacjentów z DURNŻ. **(Endokrynol Pol 2014; 65 (3): 217–222)**

Słowa kluczowe: *CGRP (peptyd związany z genem kalcytoniny); dysfunkcja układu ruchowego narządu żucia; szyny okluzyjne*

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Introduction

Temporomandibular disorders (TMD) affect 70% of the population, but only 3–7% of these patients are aware of their disorder and seek treatment [1]. Occlusal splint therapy is a well-known and popular method for the treatment of TMD [8]. Many electromyography studies have revealed that occlusal splints reduce muscle tone and muscle pain [2–5, 7]. Only a few authors have studied the biochemical changes in the craniomandibular tissues in TMD patients [6]. Recent data supports an important role for calcitonin gene-related peptide (CGRP) in deep tissue nociceptive processing and tissue blood flow. In addition, peripheral and central sensitisation has been implicated in the pathology of TMD. Bruxism increases muscle tone in central nervous system excitation. The motor nucleus of the trigeminal nerve in the brainstem is influenced by emotional stress, and masticatory muscles are stimulated in an unconscious manner by thalamic and hypothalamic impulses. Shevel et al. analysed CGRP plasma changes in patients with migraines and reported that CGRP plays an important role in extracranial blood flow and migraine pathogenesis [9–11]. The continuum theory describes many similarities between migraines, tension-type headaches and TMD (Wolff's theory). At one end of the continuum theory is a pure vascular-pain migraine, and at the other end is muscle pain that is characteristic of TMD [12, 13]. Electrical stimulation of trigeminal ganglion also elevated CGRP plasma levels in the external jugular vein in cats [14] and in patients with neuralgia during thermocoagulation of the trigeminal ganglion [15]. Similar trigeminal ganglion activation may occur as a result of myofascial pain. Durham et al. demonstrated that botulinum neurotoxin A is effective in inhibiting CGRP release [11]. This neurotoxin is also very successful in treating TMD patients [26]. Goadsby has described elevated levels of CGRP in patients with cluster headaches, paroxysmal hemicranias and autonomic cephalalgias [17, 18]. We have observed CGRP concentration changes in patients treated with occlusal splints, in whom muscle stretching and muscle relaxation was performed. Muscle stretching was the effect of the occlusal appliance, which is one of many different occlusal splints used in TMD therapy [19]. The hypothesis was that an association exists between CGRP plasma level changes and splint treatment efficacy (pain reduction). CGRP sensory fibres are preferentially located in the arterial walls, and nerve fibres containing CGRP accompany small blood vessels in human cranial muscles. It has also been shown that the density of CGRP fibres around arteries is increased in persistently inflamed muscles (chronic myofascial pain Ia and Ib RDC/TMD- Research Diagnostic Criteria/Temporomandibular Disorder) [17].

These findings indicate that ongoing activity in sensory neurons in the cranial muscles may be reflected in changes of the plasma levels of neuropeptides in patients with myofascial pain. CGRP causes vasodilation and a decrease in systemic vascular resistance, resulting in hypotension [19]. CGRP can be released antidromically, eliciting neurogenic inflammation [21]. CGRP also regulates bronchial smooth muscle tone in the human airway [21].

The aim of this study was to evaluate the plasma level of calcitonin gene-related peptide (CGRP) in patients with myofascial pain (RDC/TMD Ia) and myofascial pain with limited opening (RDC/TMD Ib) before and after muscle stretching with occlusal splint therapy. The results were compared to a control group. The study was designed to evaluate whether changes in CGRP levels were associated with pain reduction and muscle relaxation during occlusal splint treatment.

Material and methods

A parallel group, randomised trial was performed including 120 patients. Of these, 48 patients were excluded because 40 of them did not meet inclusion criteria, three patients declined to participate, and five patients were treated with anticoagulants. Seventy-two patients were allocated into one of two groups (by picking a colour card from an envelope). One patient did not accept the occlusal appliance, and six patients did not return for control visits. The experimental group consisted of 35 subjects (males = 10, females = 25), and the control group consisted of 30 subjects (males = 9, females = 21) aged 44–70 years (average = 47 years). The patients were examined using the RDC/TMD clinical and physical examination form. One person enrolled participants in the study, and another dental practitioner assigned them to the interventions. The main eligibility criteria for the participants were a temporomandibular disorder-positive RDC/TMD examination for group Ia and Ib and patient agreement to participate in the experimental study. Exclusion criteria were primary headaches, ophthalmological, neurological, cardiovascular diseases, head trauma in the past six months, secondary headaches, trigeminal neuralgia, anticoagulation treatment and platelet or coagulation disorders. Myofascial pain was an indication for preparing occlusal appliances in both groups. Control subjects were given occlusal appliances 30 days after the second CGRP2 sample collection. We analysed plasma levels of neuropeptide CGRP with a Radio Immunoassay Kit (Phoenix Pharmaceuticals Inc.) using a Cobra Series Auto-Gamma Counting System. Samples

were collected before occlusal splint treatment (CGRP1) and after 30 days of splint therapy (CGRP2) in the experimental group. Blood samples in the control group were collected at the same time without any splint therapy. Blood samples were taken from the external jugular vein on the painful side during the clinic visit. If the myofascial pain was equal on both sides, we preferred the right jugular vein. Patients were in a supine position on a dental chair in the treatment room when the physician performed the venipuncture. The results were analysed with Statistica 7.0 for each group, and an ANOVA-derived pattern analysis was also performed. The study was approved by the Bioethical Committee of Silesian Medical University, document number KNW/0022/KB1/33/I/12 on 22.05.2012r.

Results

The results of the study demonstrated that plasma CGRP concentrations were significantly higher after occlusal splint therapy, CGRP2 = 17.02 pg/mL (SD = 5.85), than before splint therapy, CGRP1 = 13.78 pg/mL (SD = 5.12), in the experimental group ($p < 0.05$) (Fig. 1). In the control group, there were no statistically significant changes in CGRP plasma levels, with the average level changing from CGRP1 = 14.5 pg/mL (SD = 4.87) to CGRP2 = 13.5 pg/mL (SD = 4.63) (Fig. 2). The pain intensity was evaluated according to the 0-10 point VAS scale. In the experimental group, there was a statistically significant reduction in pain intensity, with the average pain intensity changing from VAS 1 = 5 (SD = 2.5) to VAS2 = 1 (SD = 1.04) after splint therapy ($p < 0.05$) (Fig. 3). In the control group, there were no statistically significant changes in pain intensity based on the VAS scale, with the average pain intensity changing from VAS1 = 5 (SD = 2.3) to VAS2 = 4 (SD = 2.6), ($p < 0.05$) (Fig. 4). The trial ended after one month, as planned. There were no complications or any unintended effects in either group. Patients were treated with relaxing splints for three months after the trial was finished. If any additional prosthodontic or orthodontic treatment was needed, the patients were treated in our department.

Discussion

In 1995, Appelgren et al. reported the important role of CGRP in TMD and other debilitating orofacial diseases [22]. Vause and Durham proposed that CGRP was released from neurons of the trigeminovascular system and could promote and maintain trigeminal ganglion inflammation, which mediates peripheral sensitisation and temporomandibular disorders [25]. We found elevated CGRP plasma levels that were accompanied by

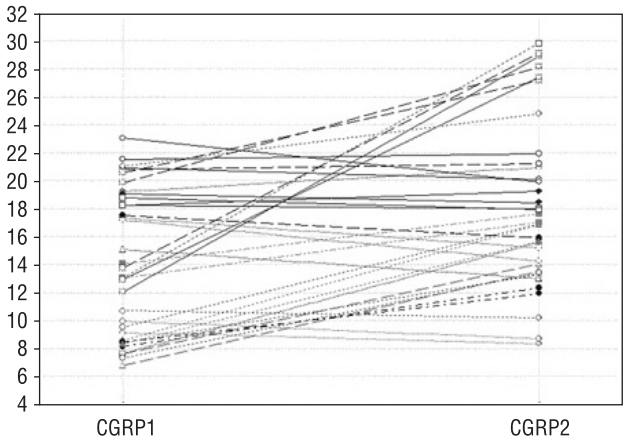

Figure 1. *CGRP level changes before (CGRP1) and after 30 days of treatment (CGRP2) in patients treated with an occlusal appliance*

Rycina 1. *Zmiany stężenia CGRP przed (CGRP1) oraz po 30 dniach szynoterapii(CGRP2)*

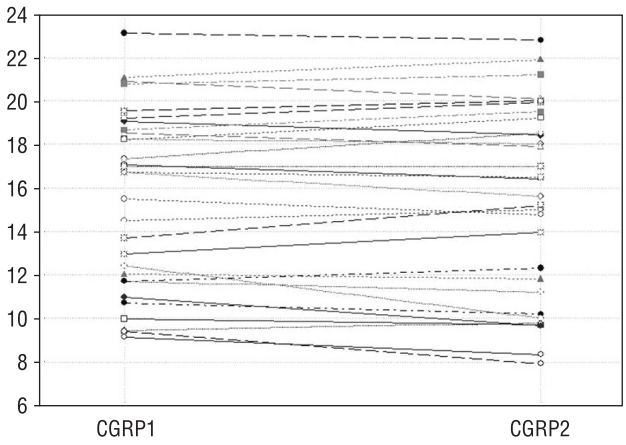

Figure 2. *CGRP level changes in control group patients before (CGRP1) and after 30 days of no treatment (CGRP2)*

Rycina 2. *Zmiany stężenia CGRP u pacjentów w grupie kontrolnej przed (CGRP1) i po 30 dniach bez leczenia (CGRP2)*

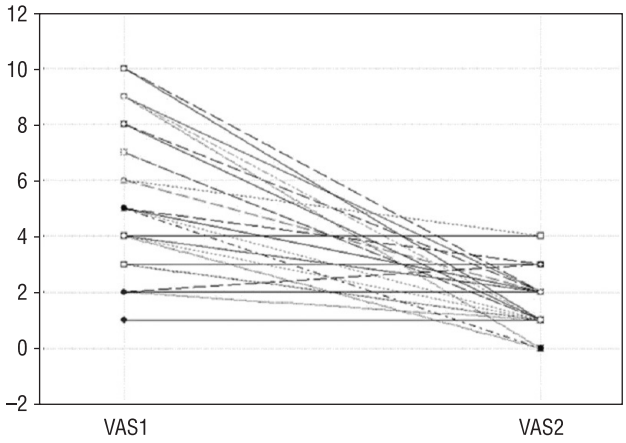

Figure 3. *Pain VAS changes before (CGRP1) and after 30 days of treatment (CGRP2) in patients treated with occlusal appliance*

Rycina 3. *Natężenie bólu przed (CGRP1) i po 30 dniach szynoterapii (CGRP2) u pacjentów leczonych szyną okluzyjną*

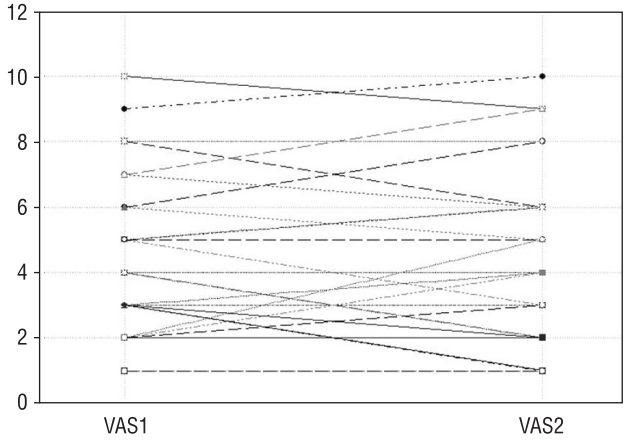

Figure 4. *Pain VAS changes in control group patients before (CGRP1) and after 30 days of no treatment (CGRP2)*

Rycina 4. *Natężenie bólu przed (CGRP1) i po 30 dniach bez leczenia (CGRP2)*

pain relief in patients treated with occlusal splints after 30 days of muscle relaxation. There were no statistically significant changes in the CGRP plasma level or VAS level in patients in the control group. After 30 days of occlusal splint therapy, there was a statistically significant reduction in pain intensity based on the VAS scale in patients treated with occlusal splints, and patients' health improved as well.

There are studies comparing CGRP levels in the external jugular vein and the radial or cubital vein [24, 25]. Blood samples collected from the radial vein would most likely be less useful than those from the external jugular vein because of the lower peripheral CGRP levels. In 2000, Ashina and Bendtsen measured elevated CGRP levels in blood collected from the cubital vein [25]. According to a later study (2005) by Tvedskov and Ashina, there is no difference between CGRP levels in the cubital fossa and the external jugular vein in patients with migraines [26]. New and more sensitive methods of analysis are necessary to clarify whether the CGRP concentration in the radial vein is high enough to be detectable in TMD patients [25, 26]. Venipuncture was one of our trial limitations because the procedure was stressful for the patients and not every patient decided to participate in the trial. Another limitation was the short period of time between the collection of the first and second blood samples. The samples used for measurements can only be frozen at –70°C for one month. In 1999, Parlapiano evaluated CGRP plasma levels and ET-1 plasma levels in normal subjects and reported that the mean plasma CGRP level was 42.8 pg/mL. The plasma concentration was measured using a competitive radioimmunoassay kit (Peninsula Labs), but the authors did not mention which blood vessels the blood samples were collected from [27]. Elevated levels of CGRP were also analysed by Joyce et al., who found that in healthy patients, the level was 2.0 ± 0.3 pg/mL and that in patients with sepsis, the level was 14.9 ± 3.2 pg/mL [19]. These findings contribute to a decreased vascular resistance in dilatory states and increased cardiac output in septic states. The same vascular dilatation may occur in masticatory muscles of patients with TMD. Most studies have analysed the nociceptive CGRP mechanism of action; however, few have studied the vasodilatory properties. Muscle pain causes release of neuropeptides, which initiate and maintain neurogenic inflammation. Peripheral and central sensitisation leads to hyperalgesia and allodynia. Cady et al. injected CGRP into the TMJ capsule of rats and observed the inflammation process and sensitisation [1]. Sato et al. studied CGRP-positive cells in synovial connective tissues around blood vessels in TMJ and found the number to be significantly higher in the experimental group than in healthy volunteers. They concluded that CGRP may play an important role in the pain mechanism and that CGRP elevated levels may be correlated with joint pain. Wu et al. analysed CGRP expression in rats using an image analysis system and concluded that CGRP nerve fibres may be found in pathological TMJ tissues after emotional stress. Bick demonstrated that CGRP causes calcium mobilisation in skeletal muscle cells. High levels of CGRP caused continuous tetanus, which could be the reason for muscle-tension headaches. Bick conducted different studies with various aspects of muscle soreness, exercise and repair. Homonko observed an increased number of CGRP+ motoneurons in muscles after physiological neuromuscular activity [28, 29]. The exercise regimen may result in damage to the muscle tissue, thereby initiating repair and regenerative mechanisms [29]. This repair process may be the reason for the elevated concentration of CGRP in our experimental group of patients with TMD. Jonhagen detected increased CGRP levels after eccentric exercise in healthy subjects, which may reflect tissue regeneration [30]. Eccentric contraction can occur after muscles are stretched during contraction [31]. CGRP muscle levels were estimated *in vivo* by microdialysis followed by radioimmunoassay and were found to be increased a few days after eccentric exercise, and this may also be the reason for the elevated CGRP plasma levels in our experimental group of patients. The eccentric exercise performed in the Jonhagen study may be similar to the masseter and temporalis muscle exercises with occlusal splints in our experimental group [30]. According to Dessem et al., the masseter muscle has a limited ability to repair itself after injury and a tendency to undergo apoptosis [32]. Dessem also presented evidence that masticatory muscles do not adapt to repeated injury (bruxism), such as that occurring in hindlimb muscles;

therefore, masticatory muscles are more susceptible to injuries and chronic muscle pain.

McDaniel noticed that blood flow in muscles is altered by changes in muscle length, i.e. the longer and more relaxed the muscle is, the higher the blood flow [33]. Occlusal splints change the vertical dimension and stretch masseter and temporal muscles. The altered muscle length decreases muscle tone and produces masticatory muscle relaxation, which is probably the reason for increased muscular blood flow. These findings suggest that CGRP may participate in muscle contraction and stretching. Cerebral blood vessels are innervated by sensory nerves that store several neurotransmitters. In primary headaches, there is a clear association between head pain and the release of the neuropeptide CGRP [10, 20, 34, 35]. Durham and Cady reported that decreased amounts of CGRP were released from rat trigeminal ganglion cells after the administration of botulinum toxin type A [BTX-A] [11]. Muscle spasm may also be treated successfully with botulinum toxin type A by local muscle injection, as reported by Guarda-Nardini in an investigation of its efficacy in reducing myofascial pain symptoms in bruxers [36]. Muscle stretching with occlusal splints increases CGRP release from the trigeminovascular system, but BTX-A produces muscle paralysis and decreases the CGRP concentration. An interesting observation in our study is that during the time of elevated CGRP plasma concentration, there was a negative correlation between the VAS scale and the CGRP plasma level concentration. A similar situation was observed by Ashina, who indicated that ongoing activity in sensory neurons in cranial muscles may result in plasma CGRP level changes in patients with chronic headache. Eight patients had higher CGRP plasma levels in the delayed headache-free period. However, a small number of patients were enrolled in this study, and the CGRP plasma levels in the cranial and peripheral circulation may be undetectable [6]. Eccentric muscle contraction and rapid stretching produces scattered myofibres and intramuscular plasma extravasation [31]. CGRP vasodilates blood vessels in muscle tissue and mediates neurogenic inflammation. In his study, Donnerer noted an important regulatory function of neuropeptides such as CGRP and substance P in healing processes [37].

Conclusions

CGRP neuropeptide is most likely also implicated in muscle healing and may serve multiple functions including muscle repair. This study shows that CGRP is involved in muscle contractility and the myofibre repair process in patients with TMD. Further investigation is necessary in this field.

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