Venopunction of the cubital vein as an alternative approach for CGRP plasma level evaluation in tmd patients

Venopunkcja żyły odłokciowej jako alternatywne podejście dla oceny stężenia CGRP w osoczu krwi u pacjentów z dysfunkcją układu ruchowego narządu żucia

Alessandra Nitecka-Buchta¹, Bogdan Marek², Jolanta Batko-Kapustecka¹, Stefan Baron¹

¹Department of Temporomandibular Disorders, Unit SMDZ in Zabrze, SUM in Katowice, Zabrze, Poland
²Department of Pathophysiology and Endocrinology, Medical University of Silesia Katowice, Zabrze, Poland

Abstract

Introduction: Calcitonin gene-related peptide is an important vasodilator. It plays an important role in the metabolism of chewing muscles. The aim of the 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 occlusal splint therapy (Michigan splint).

Material and methods: A randomised trial was performed including 39 patients (males = 3, females = 36). Blood samples were taken from the external jugular vein (JUG) and cubital vein (CUB) before and after 30 days of occlusal splint therapy. Plasma levels of CGRP were measured with ELISA KIT for Human Calcitonin Gene Related Peptide (CGRP) 96T (USCNK Business Co. Ltd.).

Results: The results of the study show that the plasma CGRP level was higher in the external jugular vein (JUG1 = 5.07pg/mL [SD = 1.99]) than in cubital vein (CUB1 = 4.3 pg/mL [SD = 1.6]). After 30 days of the occlusal splint therapy the levels in both veins increased: JUG2 = 6.07 pg/mL (SD = 2.19), and CUB2 = 4.9 pg/mL (SD = 1.4). The CGRP plasma level increase was statistically significant only in the external jugular vein (JUG) (p < 0.05). Statistically significant pain intensity reduction was observed: VAS1 = 5.4 (SD = 2.08) decreased to VAS2 = 1.7 (SD = 2.07) after splint therapy (p < 0.05).

Conclusions: Venepuncture of an external jugular vein is more precise, than venepuncture of a cubital vein in evaluating CGRP plasma level changes in patients with TMD. (Endokrynol Pol 2017; 68 (3): 326–331)

Key words: occlusal splint; external jugular vein; cubital vein; venepuncture; TMD; CGRP

The source of funding was the Silesian Medical University in Katowice, Poland, for the development of the Department of TMD and Orthodontics (KNW-2-017/N/4/K).

Introduction

Calcitonin gene-related peptide is a neuroinflammatory molecule promoting nociceptive and neuroimmune responses [1]. It was recently implicated in TMD (Temporomandibular Disorders) pathophysiology, with chronic muscle pain [2]. CGRP (Calcitonin gene-related peptide) was first discovered by Amara in 1982 in the
thyroid tissue of aging rats [3]. A human form of CGRP was first isolated from medullary thyroid carcinomas. Two isoforms of CGRP have been discovered (α CGRP, β CGRP), but the β CGRP is poorly understood [3]. Human α CGRP is primarily located in unmyelinated, small-diameter sensory C fibres, commonly found in close contact with the vasculature, especially the arterial side. Vasodilator activity of CGRP was first described by Brain in 1985, who characterised it as the most potent microvascular vasodilator [4]. An intradermal injection of CGRP was sufficient to induce erythema and increase blood flow in that area [3]. Small doses injected into human skin produce an erythema that lasts for 5–6 hours [4]. The microvasculature responds strongly to the CGRP: its potency is 10-fold greater than the prostaglandins and 100–1000 times greater than other classic vasodilators. Intravenous CGRP administration causes facial flushing. Higher doses of CGRP cause skin redness, lasting for several hours, and even hypotension. CGRP delivered intravenously causes ionotropic and chronotropic heart effects. The CGRP is released from activated trigeminal sensory nerves, dilates intracranial and extracranial blood vessels, and centrally modulates vascular nociception [5].

Endogenous CGRP promotes tumour-associated angiogenesis and tumour growth. Another recent study supports the cardioprotective role of CGRP against ischaemia/reperfusion injury [6]. Circulating levels of CGRP are reduced in patients with hypertension [7]. After CGRP receptors activation nitric oxide (NO) is synthesised and vasodilation takes place. It was also observed that the effect of CGRP on microcirculation of specific tissues (specific sensitivity) is much more intensive that in the entire circulation. That was the reason for stating the hypothesis in our study: to find out if the CGRP plasma level is the same in the external jugular vein and the cubital vein. The level of this neuropeptide decreases with age. Mice treated with CGRP receptor antagonist presented a decrease in pain duration and pain intensity [2].

This study is a continuation of previous research: “CGRP plasma level changes in patients with temporomandibular disorders, treated with occlusal splints: a randomised clinical trial”, performed in 2014. The aim of this study was to evaluate the plasma level changes of CGRP (in patients with TMD/RDC Ia and Ib- Research Diagnostic Criteria for Temporomandibular Disorders, after an occlusal splint therapy) and to compare CGRP plasma levels between the external jugular vein and cubital vein. Cubital vein venepuncture is much easier to perform and less stressful for the patient. If CGRP plasma levels were comparable in both samples, it would be easier and much more comfortable for a patient to participate in an experimental study.

### Material and methods

A randomised clinical trial was performed including 105 patients. Of these, 66 patients were excluded for the following reasons: 30 of them did not match inclusion criteria, such as RDC/TMD Ia or Ib diagnosis, bruxism symptoms, or agreement to use a splint [8] — a Michigan splint was produced for the upper arch in each patient; in 26 patients venepuncture of the cubital vein (CUB) or external jugular vein (JUG) was impossible to perform twice (before or after the occlusal splint therapy); and 10 patients did not return for follow-up visits. Thirty-nine patients were enrolled to the study (women = 36 and men = 3), aged 19–70 years (average = 46 years). Patients were examined using the RDC/TMD clinical and physical examination form [8]. One physician enrolled participants in the study and another one assigned them to the interventions. The main inclusion criteria were: positive RDC/TMD Ia or Ib, patient’s agreement to participate in the study and to perform two venepunctures. Blood samples were taken from the external jugular vein and cubital vein during the first visit and after 30 days of splint therapy. If the myofascial pain was equal on both sides, we preferred the right jugular vein. Patients were in a supine position on a dental unit chair in the treatment room when the physician performed the venepuncture. The study was approved by the Bioethical Committee of the Silesian Medical University (document number KNW/0022/KBI/104/1/14.)

Exclusion criteria were: anticoagulation treatment, platelet or coagulation disorders, primary headaches, ophthalmological, neurological, or cardiovascular diseases (with hypertension), head traumas in the past six months, secondary headaches, and trigeminal neuralgias.

Myalgia (RDC/TMD Ia) and reduction of mouth opening (RDC/TMD Ib) were indications for preparing occlusal appliances, which patients were given to use during sleep for one month (30 days). Blood samples were collected twice (on the first visit: JUG1, CUB1, and after 30 days of splint therapy: JUG2, CUB2) in each patient, from the external jugular vein (JUG) and cubital vein (CUB).

CGRP plasma levels were measured with ELISA KIT for Human Calcitonin Gene-Related Peptide (CGRP) 96T (USCNK Business Co. Ltd.). Data collected during biochemical analysis were noted in Excel files. The results were analysed with Statistica 7.0 for each group, and Wilcoxon Test analysis was performed (p = 0.05).

### Results

The results of the study show that the plasma CGRP level was higher in the external jugular vein JUG1 =
5.07 pg/mL than in the cubital vein CUB1 = 4.3 pg/mL, but an increase in CGRP level was observed in each group. After 30 days of the occlusal splint therapy, levels in both veins changed and were elevated: JUG2 = 6.07 pg/mL and CUB2 = 4.9 pg/mL. In the external jugular vein (JUG) an increase of CGRP level was statistically relevant, but in the cubital vein (CUB) it was not statistically relevant. In the present study we provide data suggesting that the CGRP plasma level is comparable in the cubital vein and in the external jugular vein, but higher CGRP levels are marked in the external jugular vein. The venepuncture of an external jugular vein provides blood samples of higher CGRP concentration.

Comparison of CGRP plasma level changes in cubital vein before and after a splint therapy
The CGRP plasma level changes in a cubital vein (Fig. 1) before and after a splint therapy in the experimental group were statistically irrelevant, p = 0.054.

Comparison of the CGRP plasma level changes in external jugular vein before and after splint therapy
The CGRP plasma level changes in an external jugular vein (Fig. 2) before and after a splint therapy in experimental group were statistically relevant, p < 0.05 (p = 0.00067).

Comparison of the pain intensity changes in VAS (Visual analogue scale) before and after a splint therapy
The pain intensity reduction after splint therapy in the experimental group (Fig. 3) was statistically relevant, p < 0.05 (p = 0.0).

In both the JUG (external jugular vein) and CUB (cubital vein) an increase in CGRP concentration was observed; it was statistically relevant for the external jugular vein (JUG1 = 5.07 and JUG2 = 6.07 pg/mL). For the cubital vein (CUB1 = 4.3 pg/mL and CUB2 = 4.9 pg/mL) CGRP plasma level changes were statistically insignificant (p > 0.05). The pain intensity reduction after splint therapy in the experimental group was statistically significant: VAS1 = 5.4 (SD = 2.08) decreased to VAS2 = 1.7 (SD = 2.07), (p < 0.05). The results of our previous study with CGRP are similar. The same tendencies in plasma CGRP levels are observed in both trials.

DISCUSSION
CGRP is released after stimulation of TRPV1 (Transient Receptor Potential Ion Channels) receptors and TRPA (Transient Receptor Potential Ion Channels) receptors, by capsaicin, noxious heat, and most importantly in our

Figure 1. CGRP plasma level changes in cubital vein before and after splint therapy
Rycina 1. Zmiany stężenia osoczowego CGRP, w żyle odłokciowej, przed i po terapii szyną okluzyjną

Figure 2. CGRP plasma level changes in external jugular vein before and after splint therapy
Rycina 2. Zmiany stężenia osoczowego CGRP, w żyle szyjnej zewnętrznej, przed i po terapii szyną okluzyjną

Figure 3. Pain intensity before and after splint therapy
Rycina 3. Nasilenie bólu przed i po terapii szyną okluzyjną
found no detectable changes. All migraineurs had an overall elevated mean CGRP value compared to control values [17]. The ELISA KIT used in our study was sensitive enough to detect CGRP in the jugular vein and in the cubital vein in TMD patients. Elevated CGRP plasma levels, in external jugular vein and in cubital vein, were found after 30 days with occlusal splints, accompanied by pain relief. A statistically significant reduction in pain intensity based on the VAS scale was observed and patients’ health improved as well. Venepuncture 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 radioimmunoassay kit (Peninsula Labs), but the authors did not mention which blood vessels the blood samples were collected from [18]. Elevated levels of CGRP were also analysed by Joyce, 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]. CGRP plasma levels in this study are similar to our results. 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.

Muscle pain causes release of neuropeptides, which initiate and maintain neurogenic inflammation. We know that 5-HT (5-hydroxytryptamine, serotonin) and PGE2 (prostaglandin 2 receptor) are involved in the development of pain and hyperalgesia or allodynia of the masseter muscle in patients with fibromyalgia, whereas myofascial pain seems to be modulated by other, as yet unknown, mediators. Interaction between the peripheral nervous system, the immune system, and local cells is probably of great importance for the modulation of pain and inflammation in the TMJ (temporomandibular joint) and orofacial musculature [14]. Peripheral and central sensitisation leads to hyperalgesia and allodynia. Cady injected CGRP into the TMJ capsule of rats and observed the inflammation process and sensitisation [20]. Sato concluded that CGRP may play an important role in the pain mechanism and that CGRP elevated levels may be correlated with joint pain [21]. 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 sore-
The CGRP plasma level is increased in the external jugular vein and in the cubital vein after 30 days of splint therapy in patients with TMD. The mechanism of CGRP accumulation is not well known. In our study the plasma level of CGRP changed in both sample collections (JUG and CUB), but it was statistically relevant only in samples collected from the external jugular vein (JUG). The concentration of neuropeptide CGRP is detectable in the cubital vein, but the increase that we observed was not statistically important. For more precise results, it is better to collect samples from the external jugular vein rather than from the cubital vein. This neuropeptide is probably 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.

Acknowledgements

We would like to acknowledge Piotr Buchta, Krzysztof Nitecki, and Teresa Ogonowska for their technical support. The authors have no conflict of interest regarding this commentary.