VIA MEDICA

ORIGINAL ARTICLE

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Changes of substance P, nerve growth factor and calcitonin gene-related peptide salivary levels among patients undergoing physical therapy

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ABSTRACT

Introduction: Pain is one of the most prevalent health conditions in the world. It is widely accepted that chronic pain persists beyond its biological usefulness and compromises the quality of life. Chronic pain is not the only continuum of acute pain, but the effect of functional and structural reorganization in the central nervous system (CNS) and changes in perception and behaviour.

Materials and methods: The aim of our study was to investigate salivary levels of NGF, CGRP and substance P and assess changes in pain intensity among patients suffering from cervical spine pain or CSD-related headaches before and after physical therapy. The study group consisted of 86 patients. 44 were treated using the McKenzie method and 42 of them underwent suboccipital relaxation therapy. To determine the salivary concentration of Substance P, CGRP, NGF the commercially available enzyme-linked immunosorbent assay kit was used. The intensity of pain was assessed using a VAS score.

Results: In both groups, a significant decrease in VAS score and Substance P concentration after treatment was observed. In the McKenzie group, there was a significant increase in NGF level after therapy; the changes in CGRP level were not significant. In a group undergoing suboccipital relaxation, a significant reduction of the concentration of all investigated molecules was found: Substance P, CGRP and NGF.

Conclusions: Both methods influence the conditions of patients through a decrease in VAS score and changes in salivary levels of Substance P, CGRP and NGF. Further research is needed to completely elucidate the influence of the McKenzie method and suboccipital relaxation on pain markers levels.

Key words: substance P, CGRP, NGF, McKenzie therapy, suboccipital relaxation

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Introduction

Pain is one of the most prevalent health conditions in the world. It is widely accepted that only acute pain has an important protective role and warns the organism of imminent danger, whereas chronic pain usually persists beyond its biological usefulness and compromises the quality of life [1]. Chronic pain is not the only continuum of acute pain, but the effect of functional and structural reorganization in the central nervous system (CNS) and changes in perception and behaviour [2]. Cervical spine pain or CSD-related headaches are conditions for which the occurrence of chronic pain is characteristic. Due to lifestyle changes, low levels of physical activity 10% to 24% of the whole population are affected by these disorders [3].

Substance P belongs to the tachykinin family and is widely distributed among tissues and body fluids [4]. It is considered a potential pain biomarker [5]. Substance P is released from the dorsal horn of the spinal cord and is associated with the transmission and modulation of pain. Its increased secretion is associated with physical pain and intense stress [6].

Nerve growth factor (NGF) is a member of the neurotrophin (NT) family of growth factors (GF) which also include BDNF (brain-derived neurotrophic factor), neurotrophin-3 and 4/5 (NT-3 and NT-4/5) [7, 8]. NGF signals via two receptors, TrkA and p75 neurotrophin

receptor. It plays important role in persistent pain development. NGF has a role in the development of peripheral sensitivity [9]. Binding of NGF to receptors on peripheral nociceptors induces sensitisation of the nociceptive response. NGF is also involved in upregulating expression of many pain-related genes such as substance P and calcitonin gene-related peptide (CGRP) [7, 9]. Anti NGF therapy is a potential method of pain treatment [10].

Calcitonin gene-related peptide is a widely expressed neuropeptide that has a major role in sensory neurotransmission. It is also known to be long-lasting vasodilator in the cardiovascular system. CGRP plays important role in the pathophysiology of migraine pain [11]. There is also evidence that CGRP plays role in central sensitization, which is an enchantment of neurons and circuits in nociceptive pathways that contributes to chronic pain pathogenesis [12] CGRP concentration is correlated with pain intensity and decreases after pain treatment among patients with osteoarthrosis [13].

In this study, the authors investigate salivary levels of NGF, CGRP and substance P among patients suffering from cervical spine pain or CSD-related headaches before and after physical therapy.

Materials and methods

The study group consisted of 86 patients. 44 were treated using the McKenzie method and 42 of them underwent suboccipital relaxation therapy.

There was no significant difference in sex ratio between the two groups. The average age in the group that underwent McKenzie Therapy was 50.12 [standard deviation (SD) = 10.40, range 26–64] and 48.07 (SD = 10.80, range 26–64) in the group undergoing suboccipital relaxation. Again, there was no statistically significant difference in age between the two groups ($p > 0.05$). Inclusion criteria included: diagnosed cervical spine pain or CSD-related headaches, patient age > 18 years and written consent for participation. Patients with cervical spine or head trauma, constant pain-relieving pharmacotherapy, drug abuse, neoplastic diseases, myasthenia gravis, steroid therapy, Arnold-Chiari syndrome and other congenital defects of the head and cervical area and syringomyelia were excluded. The study was approved by the Committee on Research Ethics of the Silesian Chamber of Physicians (No. 46/2015).

Each patient was subjected to 3 treatment sessions, one every 3 weeks. Pain assessment was performed using the VAS scale: before the first session and at the end of observation.

For biochemical determinations, saliva was collected using "salivette" type test tubes. Patients had to abstain from eating, cigarette smoking, brushing teeth, chewing gum and drinking for 20 minutes before sample collection, which occurred before every session and 20 minutes after. Informed consent was obtained from each patient. These samples were then centrifuged and frozen at –85°C for storage. The laboratory examination was performed in the Chair and Department of Medical and Molecular Biology of the School of Medical Sciences in Zabrze, the Medical University of Silesia in Katowice.

Visual Analogue Scale

The Visual Analogue Scale (VAS) is a reliable tool for pain intensity assessment. Repeated VAS evaluations allow, for example, the monitoring of pain-relieving treatment effectiveness. The scale is represented by a 10cm ruler, scaled from 0 to 10. The ends are defining the extreme limits, with 0 representing no pain, 10 — the strongest paint imaginable. The patient indicates the pain level using his finger.

NGF, CGRP, Substance P levels assessment

To evaluate salivary levels of examined molecules, commercial ELISA tests were used. Substance P and NGF were analysed using test provided by USCN Life Science (Wuhan, China). CGRP concentration was determined using the ELISA kit by Abnova (China). The analytic procedures were in correspondence to the manufacturer's instructions. Absorbance assessment was performed with a μQuant reader (manufactured by BioTek USA), data analysis using KCJunior software (BioTek USA).

Statistical analysis

Statistical analysis of the data was performed using the Statistica 13 PL software. The patient age was expressed as an average with a ± SD. To compare the age in both groups, a t-Student test was used. Shapiro-Wilk test was used to determine variables distributions. Non-normal variables were presented as median with interquartile range. To compare non-parametric variables U-Mann Whitney test was performed for groups comparison and Wilcoxon test to assess differences between initial and final values of analysed variables. To determine relations between examined variables Spearman's rank coefficient was used. P values <0,05 were considered significant.

Table 1. VAS score and concentrations of NGF, CGRP, Substance P among examined groups before and after therapy. A-group treated with using McKenzie method, B-group treated with using suboccipital relaxation therapy

Median		Before Treatment			After treatment			p
		Q1	Q3	Median	Q1	Q3		
A	VAS	4.00	3.00	5.00	1.00	0.00	2.00	0.00
	NGF	1189.65	1124.80	1372.80	1234.50	1102.40	1298.70	0.01
	CGRP	396.80	365.80	405.10	398.50	365.80	400.50	0.65
	Substance P	320.95	289.70	399.60	314.10	279.55	397.55	0.00
B	VAS	4.00	3.00	5.00	1.00	0.00	3.00	0.00
	NGF	1273.70	1123.60	1362.50	1244.40	1108.50	1324.50	0.01
	CGRP	365.20	285.80	409.20	328.00	274.30	387.50	0.00
	Substance P	393.70	318.55	432.00	380.00	302.30	405.30	0.00

VAS — Visual Analogue Scale; NGF — nerve growth factor; CGRP — calcitonin gene-related peptide

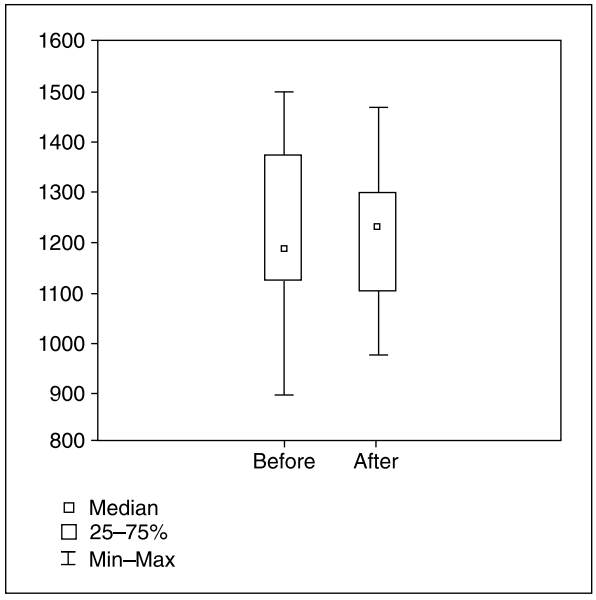

Figure 1. Nerve growth factor levels in patients treated with using the McKenzie method before and after treatment. Data presented as median with interquartile range

Results

A significant drop of VAS score was found among patients in both groups (Tab. 1). A slight but significant drop of CGRP concentration was found after treatment among the group undergoing suboccipital relaxation but not in the McKenzie group (Tab. 1, Fig. 3–4). Significant decrease of salivary substance P levels was observed after treatment in both groups (Tab. 1, Fig. 5–6). NGF concentration decrease was also observed among patients in both groups (Tab. 1, Fig. 1–2).

A significant negative correlation between NGF concentration and VAS score was observed among overall patients after treatment ($R = -0.26$, $p < 0.05$) (Tab. 2). P substance was also negatively correlated with VAS scores among overall patients after treatment. No significant correlations were found inside groups.

No significant correlations were found between examined molecules in each group.

Discussion

Significant changes in examined molecules concentrations were found during the study. Both examined methods of manual therapy lead to a VAS score decrease. Reduction of pain sensations was associated with changes in some examined molecules concentrations.

McKenzie method has been reported to decrease VAS score in patients with lumbar spine discopathy [14]. and low back pain [15, 16] whereas suboccipital relaxation was found to reduce VAS score in individuals with cervicogenic headache [17]. The authors have also shown a decrease in VAS score in patients treated with the McKenzie method and using suboccipital relaxation in their previous study [18].

Significant decrease of P substance concentration after treatment was observed among patients in both groups; however, P substance level correlates negatively with VAS score among overall patients after treatment. Substance P (SP) is an undecapeptide belonging to the tachykinin small-peptide family [19]. SP is generated in primary nociceptive sensory neurons (nociceptors) and is released with noxious stimulation [20]. The release from cutaneous peripheral terminals induces neurogenic inflammation and release from central terminals enhances the glutamate-dependent excitatory postsynaptic potential, thus leading to central sensitization. Increased level of substance P in serum and tissues was confirmed in may disorders such as

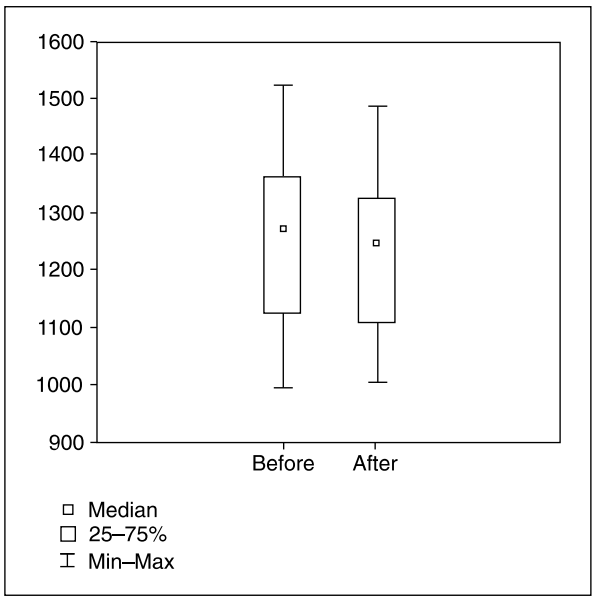

Figure 2. Nerve growth factor levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range

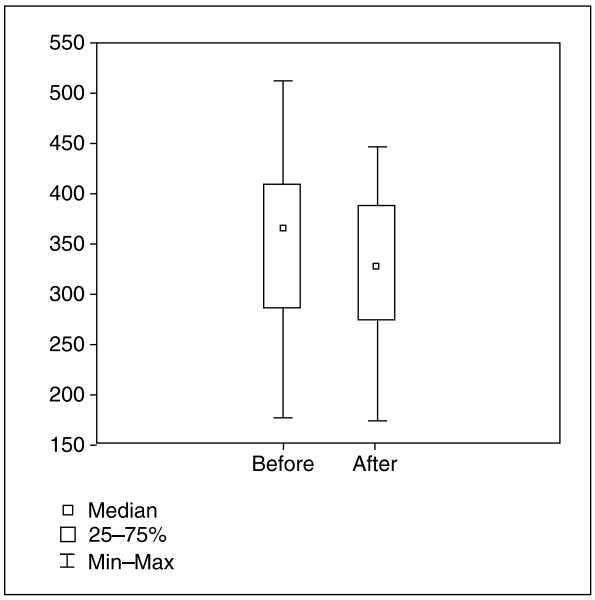

Figure 4. Calcitonin gene-related peptide levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range

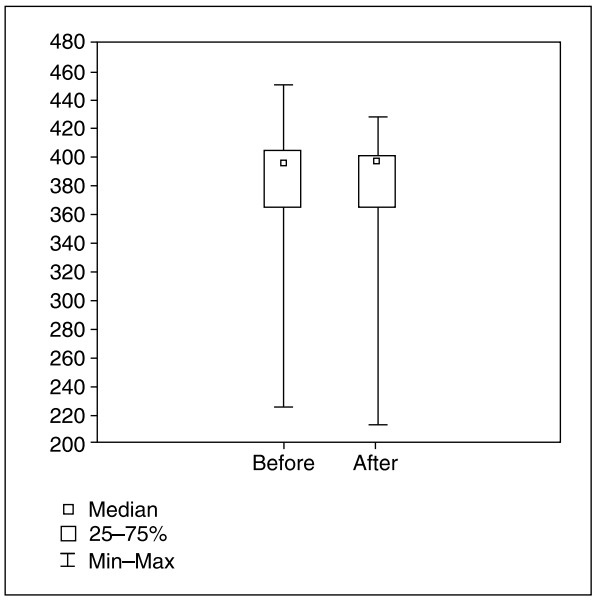

Figure 3. Calcitonin gene-related peptide levels in patients treated with using the McKenzie method before and after treatment. Data presented as median with interquartile range

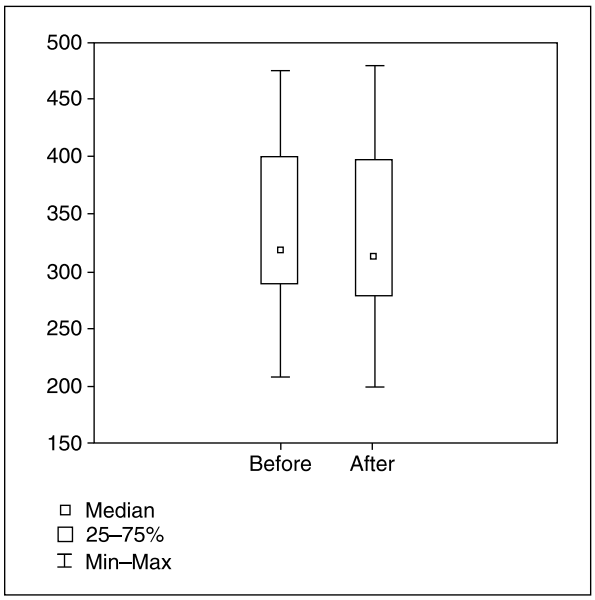

Figure 5. Substance P levels in patients treated with using the McKenzie method before and after treatment. Data presented as median with interquartile range

inflammatory bowel diseases, fibromyalgia, asthma, rheumatological diseases, depressive disorders [21]. Especially fibromyalgia is a disorder in which the changes in serum and salivary level of substance P and pain intensity were thoroughly investigated. This is a condition with characteristic symptoms such as chronic widespread pain, allodynia, myalgia, arthritis, tiredness and sleep disorders which decrease the quality of life [22]. In a randomized controlled clinical trial, Saliha Karatay et al reported a significant drop of serum

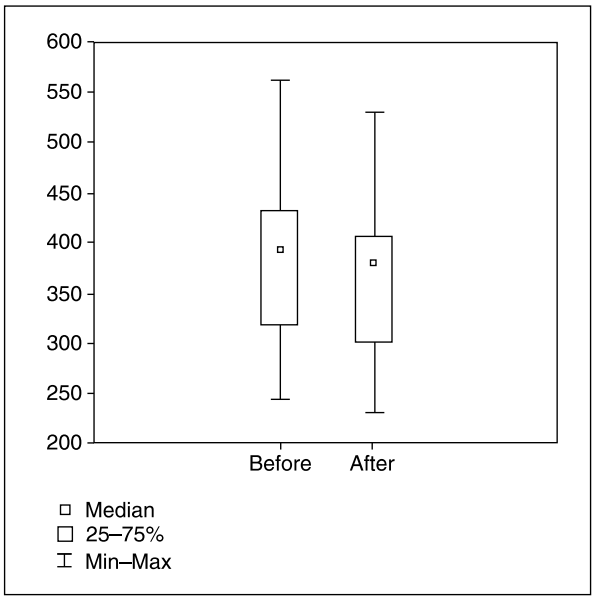

Figure 6. Substance P levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range

Table 2. Significant correlations between the VAS score and investigated proteins **[brak odnośnika w tekście]**

Pair of variables	R	p
VAS score after treatment & NGF level in both groups of patients after treatment	–0.26	0.049
VAS score after treatment & substance P in both groups of patients before treatment	–0.37	0.003
VAS score after treatment & substance P in both groups of patients after treatment	–0.36	0.005

R—Spearman's correlation coefficient; VAS — Visual Analogue Scale; NGF — nerve growth factor

substance P level in patients with fibromyalgia after acupuncture therapy [23]. Also, massage therapy has been shown to decrease pain intensity and the salivary level of substance P in another study [24].

In the presented work, a negative correlation between substance P level and VAS score among overall patients after treatment was found. Some authors suggest that substance P can play an anti-nociceptive effect in tissues [25]. This effect is associated with opioid receptors activation [26]. There are also evidences that SP influences decrease of pain sensations in muscles by alterations in ion channels activity [27]. The findings of the presented study may suggest that increase of SP concentration contributes to pain sensations decrease, however further studies are necessary to verify this effect.

There was a significant drop in CGRP level in both groups after treatment. It has been reported that the increase in the concentration of CGRP is associated with migraine and the decrease in CGRP level was observed in patients after treatment. Nowadays CGRP antagonists are considered as promising new class of anti-migraine drugs and are extensively investigated in clinical trials [28]. It is worth noting that the elevated level of CGRP in plasma, in plasma, synovial and cerebrospinal fluid was confirmed in patients with musculoskeletal pain [13] What is more, the higher concertation of CGRP in serum was reported in patients to complex regional pain syndrome (CRPS) and its decrease after treatment [13]. The is no study which found the correlation between the CGRP level and neuropathic pain intensity. In the presented work, the authors have also not demonstrated this association.

We have found that after treatment NGF level significantly decreased in the group which underwent suboccipital relaxation therapy and significantly increase in the McKenzie group. The ability of NGFT to mediate neuropathic pain, upregulate substance P, cause hyperalgesia was confirmed in animal models of pain [29]. Currently, NGF and NGF receptors are promising targets in therapy of both chronic and acute pain. Monoclonal antibodies able to block NGF or NGF receptor such as Tenazumab are investigated in clinical trials [30]. Interestingly, a negative correlation between VAS scores and NGF level in patients after treatment was observed. This observation such as the increased concentration of NGF in the McKenzie group after treatment requires further studies. One should keep in mind that the role of NGF in pain pathophysiology may have dual nature- NGF can also influence spinal cord and induce analgesia in animal models of neuropathic pain [29] so one cannot expect that the level of NGF reflects a reduction of pain intensity directly and proportionally.

Conclusions

In presented work, both methods influence the conditions of patients through a decrease in VAS score and changes in salivary levels of Substance P, CGRP and NGF. A significant decrease in the level of Substance P both in McKenzie and suboccipital relaxation group were observed. In McKenzie group was found a significant increase in NGF level after treatment; the changes in CGRP levels were no statistically significant. In the suboccipital relaxation group, there was a significant drop both in CGRP and NGF levels after treatment. To the best of the authors' knowledge, no study has compared the salivary levels of Substance P, CGRP,

NGF in patients undergoing McKenzie method with these observed in individuals treated with suboccipital relaxation. Because of a small study group and too short observation period, there were no possibilities to indicate which method is better, but this study indicates that both of them may provide a better quality of life and decrease in pain intensity in patients. Further studies are necessary to completely assess the usefulness of the McKenzie method and suboccipital relaxation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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