Changes of substance P, NGF and CGRP salivary levels among patients undergoing physical therapy.

Authors: Witold Miecznikowski, Sylwia Mielcarska, Paweł Kiczmer, Karolina Cygan, Elżbieta Świętochowska

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Abstract

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 only a continuum of acute pain, but effect of functional and structural reorganization in central nervous system (CNS) and changes in perception and behaviour. 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. Study group consisted of 86 patients. 44 were treated using 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 were used. The intensity of pain was assessed using VAS score. In both groups we observed significant decrease in VAS score and Substance P concentration after treatment. In McKenzie group there was a significant increase in NGF level after therapy; the changes in CGRP level were not significant. In group undergoing suboccipital relaxation we found significant reduction of concentration of all investigated molecules: Substance P, CGRP and NGF. Both methods influence the conditions of patients through 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 McKenzie method and suboccipital relaxation on pain markers levels.

Key words: Substance P, CGRP, NGF; McKenzie therapy; suboccipital relaxation

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

Substance P belongs to tachykinin family and is widely distributed among tissues and body fluids [4]. It is considered as 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 neurotrophin 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 CGRP [7,9]. Anti NGF therapy is a potential method of pain treatment [10].
CGRP (Calcitonin gene related peptide) is a widely expressed neuropeptide that has a major role in sensory neurotransmission. It is also known to be a long lasting vasodilator in cardiovascular system. CGRP plays important role in patophysiology of migraine pain [11]. There is also evidence that CGRP plays role in central sensitization, which is 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 our study we 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**

Study group consisted of 86 patients. 44 were treated using 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 group that underwent McKenzie Therapy was 50.12 (SD= 10.40, range 26-64) and 48.07 (SD=10.80, range 26-64) in 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 a written consent for participation. We excluded 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. 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 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, Medical University of Silesia in Katowice.

**VAS scale**

The Visual Analogue Scale 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 pain imaginable. The patient indicates the pain level using his finger.

**NGF, CGRP, Substance P levels assessment**
In order to evaluate salivary levels of examined molecules, commercial ELISA tests were used. Substance P and NGF were analyzed using test provided by USCN Life Science (Wuhan, China). CGRP concentration was determined using 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 +/- standard deviation (SD). To compare the age in both groups, t-Student test was used. Shappiro-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 Spearaman’s rank coefficient was used. P values <0,05 were considered significant.

**Results**

|        | Before Treatment | After treatment | p     |
|--------|-----------------|----------------|-------|
|        | Median | Q1    | Q3    | Median | Q1    | Q3    |       |
| 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  |
| A Substancja P | 320,95 | 289,70 | 399,60 | 314,10  | 279,55 | 397,55 | 0,00  |
| 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 | 308,50  | 302,20 | 405,30 | 0,00  |
| B Substancja P | 393,70 | 318,55 | 432,00 | 380,00  | 302,30 | 405,30 | 0,00  |

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.
Fig. 1: NGF levels in patients treated with using McKenzie method before and after treatment. Data presented as median with interquartile range.

Fig. 2: NGF levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range.
Fig. 3: CGRP levels in patients treated with using McKenzie method before and after treatment. Data presented as median with interquartile range.

Figure 4: CGRP levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range.
Fig. 5: Substance P levels in patients treated with using McKenzie method before and after treatment. Data presented as median with interquartile range.

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

Table 2: Significant correlations between the VAS score and investigated proteins. R—Spearman’s correlation coefficient

Significant drop of VAS score was found among patients in both groups. Slight but significant drop of CGRP concentration was found after treatment among group undergoing suboccipital relaxation but not in McKenzie group. Significant decrease of salivary substance P levels were observed after treatment in both groups. NGF concentration decrease was also observed among patients in both groups.

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

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

Discussion

Significant changes in examined molecules concentrations were found during study. Both examined methods of manual therapy lead to 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]. We have also showed the decrease in VAS score in patients treated with McKenzie method and with using suboccipital relaxation in our 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 many disorders such as inflammatory bowel diseases, fibromyalgia, asthma, rheumatological diseases, depressive disorders [21]. Especially fibromalgia 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 randomized controlled clinical trial Saliha Karatay et al reported significant drop of serum 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 presented work we have found negative correlation between substance P level and VAS score among overall patients after treatment. Some authors suggest that substance P can play 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]. Our findings 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 increase in 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 concentration of CGRP in serum was reported in patients complex regional pain syndrome (CRPS) and its decrease after treatment [13]. There is no study which found the correlation between the CGRP level and neuropathic pain intensity. In presented work we have also not demonstrated this association.

We have found that after treatment NGF level significantly decreased in group which underwent suboccipital relaxation therapy and significantly increase in 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, we observed negative correlation between VAS scores and NGF level in patients after treatment. This observation such as increased concentration of NGF in McKenzie group after treatment requires further studies. We 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 we can not expect that the level of NGF reflects reduction of pain intensity in a direct and proportional manner.

Conclusions

In presented work, both methods influence the conditions of patients through decrease in VAS score and changes in salivary levels of Substance P, CGRP and NGF. We have observed significant decrease in level of Substance P both in McKenzie and suboccipital relaxation group. In McKenzie group we have found significant increase in NGF level after treatment; the changes in CGRP levels were no statistically significant. In suboccipital relaxation group there was significant drop both in CGRP and NGF levels after treatment. To the best of our 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 small study group and too short observation period we have no possibilities to indicate which method is better, but our 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 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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Figure legends

Fig. 1: NGF levels in patients treated with using McKenzie method before and after treatment. Data presented as median with interquartile range.

Fig. 2: NGF levels in patients treated with using suboccipital relaxation therapy before and after treatment. Data presented as median with interquartile range.

Fig. 3: CGRP levels in patients treated with using McKenzie method before and after treatment. Data presented as median with interquartile range.

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Tables

| Pair of variables                                                                 | R   | p   |
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| Substant | VAS | NGF | CGRP | Substance P | VAS | NGF | CGRP | Substance P | p |
|----------|-----|-----|------|-------------|-----|-----|------|-------------|---|
| Before Treatment | Median | Q1 | Q3 | Median | Q1 | Q3 | Median | Q1 | Q3 | Median | Q1 | Q3 | Median | Q1 | Q3 |
| 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 |
| 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 |

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