Plasma rich in growth factors injections effectiveness for myofascial pain treatment in masticatory muscles. Randomised controlled trial

Running title: Growth factors injections for myofascial pain.

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Background:

Myofascial pain in masticatory muscles is one of the most common temporomandibular disorder. Nowadays the most usable treatment methods are based on the muscle taut band cells membranes disruption, which releases the taut band.

Platelets rich plasma, made with PRGF Endoret ® method, gives an opportunity to use platelet-derived growth factors in treatment processes. It has been proven that platelet derived growth factors can relief pain and activate muscle regeneration.

Objective:

To test a hypothesis that PRGF injections can be effective for treating myofascial pain in masticatory muscles.

Methods:

50 adult patients participated in the study. Participants were randomly divided into two groups. The first group received 1ml lidocaine injections to trigger point in their masseter muscle. The second group of patients received 1ml PRGF injections. The patients’ pain were measured by using visual analogue scale (VAS).

Results:

Statistically significant difference in pain levels before the procedure and 4 weeks after it, was found in both groups.

There were no statistically significant difference between groups in pain levels before the procedure (p=0,063) and 2 weeks after it (p=0,123), however, statistically significant difference was noticed 4 weeks after the procedure (p<0,001). 4 weeks after the procedure patients’ average pain in lidocaine group was 3.4 on VAS, and it was 0.9 in PRGF group.
Conclusions:

PRGF injections in masseter muscle affected by myofascial pain syndrome is an effective treatment method.

PRGF injections more effectively relief myofascial pain in masseter muscle than lidocaine injections.

Key words: myofascial pain, trigger point, masseter muscle, PRP, plasma rich in growth factors.

Background:

Myofascial pain is a chronic pain syndrome localized in skeletal muscles. One of the main etiological factors of myofascial pain formation is muscle overload. Epidemiological studies have shown that myofascial pain syndrome (MPS) of the masticatory muscles is the most common form of Temporomandibular disorders (TMD), accounting for almost a half of the patient cases. It is also known that MPS is found in 9.7% of the general population.

A clinical hallmark of MPS is the trigger point, which is defined as a localized deep tenderness in a taut band of the skeletal muscle that is responsible for pain in the zone of reference. Currently, there are several treatment approaches regarding the release of trigger points that allow pain relief: manual therapy, dry needling, local anesthetics injections, Botulin toxin injections, splint therapy, and low-intensity laser therapies are being used for myofascial pain treatment in masticatory muscles. The effects of manual therapy, dry needling and local anesthetics injections on myofascial trigger point (MTP) release are all based on a single principle. These treatment methods seek to disrupt contracted muscle fiber membranes in order to release
However, the injected substance (during local anesthetic injections) does not affect the pathophysiologic mechanism, it only makes the postoperative period less painful.

Plasma rich in growth factors (PRGF) – is a platelet rich plasma (PRP) preparation protocol created by Eduardo Anitua. The protocol is unique because it does not include leukocytes in its composition, moreover PRGF can be used in different clinical forms. It is known that the proteins derived from platelets such as platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF), also plasma containing certain natural growth factors in the name of insulin-like growth factor (IGF), fibroblast growth factor (b-FGF) and nerve growth factor (NGF) are able to promote angiogenesis and enhance muscle regeneration. Moreover, it has also been proven that platelets rich plasma can reduce chronic pain. The general knowledge about the clinical effectiveness of PRP and proteins derived from platelets shows that PRGF injections could be an effective treatment method for MPS.

Since many of the modern MPS treatment methods are solely based on contracted muscle fiber membrane disruption, not the effect of an injectable substance, furthermore, PRGF is a totally autologous substance with no reported side effects, we decided to set up a randomized controlled clinical trial.

The aim of this study was to find out whether PRGF injections can be an effective treatment method for myofascial pain in the masseter muscle by reducing pain and treatment time.

**Methods:**

This was a prospective, single centre, unstratified (with balanced randomisation [1:1]), controlled, parallel-group study conducted in Lithuania. The study was conducted in accordance with International Standards of Quality for Clinical Trials and the Declaration of Helsinki in its revised version (Seoul, Korea, 2008). Approval for the research was granted by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-40). Study protocol was prospectively registered in ClinicalTrials.gov (Identifier: NCT04040309)

Adult patients diagnosed with myofascial pain syndrome in one side of a masseter muscle were included in the study. The diagnoses were confirmed by one researcher based on Simons et all. diagnostic criteria: 1) pain recognition, 2) palpable taut band, 3) spot tenderness in a
taut band. Only patients who had not had previous injections in the masseter muscle were included in the study. The rejection criteria are as follows: myofascial pain syndrome in other masticatory muscles, myofascial pain syndrome in the masseter muscles of both sides, pain-inducing inflammations of the head and neck, pain-inducing TMJ pathology, neuralgia of the trigeminal nerve, oncological diseases of the head and neck. The study was conducted at the Hospital of Lithuanian University of Health Sciences Kauno klinikos, the Department of Maxillofacial Surgery, Kaunas, 2019. There is no regional data available on the prevalence of myofascial pain syndrome in the masticatory muscles. All patients included in the study signed the clinical trial informed consent form.

The 50 patients selected were randomized into two groups. Patients in the first group received a second fraction injection of 1 ml of PRGF into the trigger point of the masseter muscle. Plasma rich in growth factors was prepared according to the protocol described by E. Anitua. A sample of 9 ml of venous blood was taken from each subject and distributed into test tubes using 3.8% sodium citrate as an anticoagulant. The blood was centrifuged (PRGF Centrifuge System, BTI, Vitoria, Spain) at room temperature for 8 minutes at 1800rpm. During centrifugation, the blood in the tubes breaks down into fractions: plasma fraction 1 (containing physiological blood platelet concentration), plasma fraction 2 (containing 2 to 3 times the platelet concentration found in healthy human blood. This fraction is used for injection), buffy coat and sedimented red blood cells. The fractions are distributed in the order listed from the top of the tube to the bottom. The second plasma fraction was separated via pipet (2 ml second fraction plasma is obtained from a single tube). 1 ml of the second fraction plasma was drawn into a syringe, the plasma was activated with 10% calcium chloride and immediately injected into the trigger point of the masseter muscle. The second group of patients received 1 ml injections of 2% lidocaine at the trigger point of the masseter muscle. All injections were made by the same researcher.

Patient pain was assessed using a visual analogue scale of 0-10. The pain was assessed before the procedure, as well as 2 weeks and 4 weeks afterwards.

The selected patients were randomized into groups. The randomisation sequence was created using Sealed Envelope randomisation tool and was unstratified with a 1:1 allocation ratio using random block sizes of 2, 4 and 6. The patients knew which group they belonged to and what kind of intervention would be performed on them. The first researcher performed the randomization, categorization, pain assessment and statistical analysis of patients. The second
researcher diagnosed the patients and performed the injections. The second researcher had no knowledge of the patient pain assessment results, and had no contact with the patients prior to the procedure, and was not aware of which group the patient belonged to, until the moment of a procedure.

Sample size of this study was calculated by using MedCalc software. A sample size calculation was performed with a desirable Type I error of 0.05 and Type II error of 0.2. The hypothesized area under the Receiver operating characteristic (ROC) curve was expected to be found in this study at 0.75 with a null hypothesis value of 0.5 and the ratio of sample sizes in negative/positive groups to be 1. It was calculated that the total sample size for this study should be 38 patients, however, assuming possible dropouts we decided to include 50 patients in this study.

Our primary endpoint was change in the VAS score 2 and 4 weeks after the procedure. The Kolmogorov-Smirnov test was used to assess the normal distributions. We used the paired samples t-test to assess pain score change in the group. To compare pain scores between different groups we used the parametric Student t test for two independent samples as well as the non-parametric Mann-Whitney test for two independent samples. We also used the non-parametric Mann-Whitney test for two independent samples to compare relapse rates between groups. We used the parametric Student t test for two independent samples and the non-parametric Mann-Whitney test for two independent samples to determine homogeneity of samples by age, sex and disease duration. The significance level was selected at $p < 0.05$. The statistical analysis was performed using IBM SPSS Statistics 20 software.

Results:

A total of 94 patients were examined for inclusion into the study. Of these, 42 patients that did not meet the inclusion criteria were excluded, and 2 patients refused to participate in the study. A total of 50 patients were included and divided into groups of 25. The procedures were performed on all the selected patients. No subjects were lost during the 4 week study period, all 50 patients were included in the statistical analysis (Figure 1).
The mean age of the patients included was 47.8 years. The majority of the participants were women (82%). The average duration of disease before the treatment was 25.8 months. The study groups were homogeneous in terms of age, sex and duration of illness (Table 1).

Pre-treatment, the mean patient pain score was 5.7 on the VAS scale. There was no statistically significant difference in the mean pain scores between the groups before treatment ($p = 0.063$) (Table 2). Mean pain scores before treatment and after 4 weeks showed a statistically significant difference in both groups ($p < 0.001$ in the Lidocaine group and $p < 0.001$ in the PRGF group), indicating that both treatment methods were effective. After 4 weeks, the Lidocaine group had a pain mean reduction score of 45.3%, and the PRGF group a mean reduction of 82.4%. Two weeks after the procedure, there was no significant difference in mean pain scores between the groups, but a statistically significant difference was observed at 4 weeks ($p < 0.001$) (Figure 2). During the 4 weeks after the procedure, 5 cases of pain relapse were observed, when the assessment showed increased pain, even regressing to the initial stage. Four such cases were observed in the Lidocaine group and 1 case in the PRGF group. No statistically significant difference was observed in the incidence of relapses between groups ($p = 0.346$). The statistical power of the study was 0.93 when evaluating the patients mean pain score percentage changes.

During the study, no side effects of the procedures or complications were observed.

**Discussion:**

Myofascial pain is polyetiological secondary muscle damage characterized by acute, repetitive, dilatory and prolonged pain. In the face and neck area, myofascial pain can be caused by irregular posture, unbalanced occlusion, stress, bad habits leading to muscle overload. It is important to note that the causes of trigger point formation and the onset of pain cannot always be accurately identified and promptly resolved, therefore pain management plays an important role in the treatment of the disorder. Masticatory muscle overload is considered to be the leading cause of the syndrome. The mandibular joint is one of the most actively used joints in the human body, which explains the frequent overloads that occur in the masticatory muscles. Myofascial pain relief is based on the release of trigger points. Understanding the trigger point formation mechanisms is essential to effective treatment application and refinement.
Myofascial trigger points in masticatory muscles result from an energy crisis in muscle cells. This lack of energy is caused by two mechanisms: 1) Muscle overload causes a large amount of acetylcholine to be released into the neuromuscular junction, as the nerve impulse extends, the myocyte membrane depolarizes; the depolarization extends to the sarcoplasmic reticulum, which starts to release calcium ions, and the sarcomere contracts. All of these processes lead to increased energy demand in the cell. 2) As the sarcomeres contract, the lumen of muscle fibers increases, resulting in compressed capillaries, which can significantly disrupt blood circulation. Because of the impaired blood flow, myocytes receive less of the necessary substances required for cell energy maintenance\(^1,12\). The energy crisis mechanism operates according to the closed-loop principle: the sarcomere contracts, leading to local ischemia, the increased cellular energy demand leads to energy deficiency, then cell lacks energy to return calcium ions to the sarcoplasmic reticulum, causing sarcomeres to contract and be unable to release\(^1,12\).

Many of the methods used nowadays to treat myofascial pain are based on the same principle. Manual therapy, acupuncture, local anesthetic, and steroidal anti-inflammatory medicine injections are used to to break down the membranes of tight muscle fibers. By breaking down the strained muscle fiber membranes, calcium ions are released from the cell, reducing their concentration in the cell and allowing the sarcomere to release\(^1\). During these injections, the trigger point is released by the needle prick, and the injected substance only makes the procedure less painful and facilitates the postoperative period\(^3\). By researching the potential effect of platelet-derived growth factors on myofascial pain syndrome in masseter muscle, we aimed to test the hypothesis that PRGF injections may be effective in treating myofascial pain syndrome.

Several different protocols for preparing platelet concentrates are used in modern medicine. The major difference between PRGF and other preparation protocols is that PRGF contains a minimal amount of leukocytes\(^13\). Studies have shown that leucocyte-rich platelet concentrates significantly increase IL-6 expression\(^13\)-\(^15\), which is associated with the development of chronic pain\(^16\). Leukocyte-rich platelet concentrates are also known to increase concentrations of IL-1β, TNF-α, cyclooxygenase-2 (COX-2), nitric oxide synthase (iNOS)\(^17\). For these reasons, we decided to use a platelet concentrate containing minimal amounts of leukocytes for our study.

In our study, we found that injections of PRGF into the trigeminal points of the masseter muscle significantly reduced the mean VAS score 4 weeks after the procedure. Also,
within the limits of this study, 4 weeks after the procedure PRGF injections were more effective than Lidocaine injections in pain relief. We believe this may have been influenced by several different platelet-derived growth factors.

Our hypothesized physiological mechanism how injection of PRGF should be effective in treating myofascial pain syndrome, was based on two separate factors: 1) During the injection needle should disrupt the membranes of contracted muscle fibers and by doing that, break the closed-loop intracellular energy crisis mechanism and release the trigger point 1,3. 2) Injected growth factors should reduce pain by inhibiting inflammatory cytokines and also enhance muscle regeneration and vascularisation processes 6-9.

Platelet-derived VEGF plays an important role in angiogenesis 18. Given the trigger point formation mechanism resulting from local ischemia 1, we anticipated that PRGF injections at the trigger point could decrease the occurrence of myofascial pain relapses. Unfortunately however, the results did not show a statistically significant difference in the occurrence of pain relapse in the different groups. Moreover, results would be more reliable if we would evaluate relapses within a longer follow up period.

The biggest limitation of our study is the lack of blindness. Comparing the results between the different test groups would be much more reliable if we had drawn the venous blood from all the patients without telling them which group they were in, and if we had the researcher give the injection via a special syringe that would make impossible for the patient to discern the substance being injected. Patients who had PRGF injections showed a keen interest in the new technology, therefore blindness procedures would have prevented the potential placebo effect of PRGF injections and would have allowed comparisons between the groups to be more reliable. In this study, to confirm myofascial pain in masseter muscle we used diagnostic criteria described by Travel and Simons 1, however, these criteria aren’t so well established and confirmed internationally. To make our differential diagnosis as accurate as possible it would have been better to use more established diagnostic criteria (like Diagnostical Criteria for Temporomandibular Disorders (DC/TMD) described in 2015 19). It also would have allowed to easier compare our study with others in future systematic reviews and meta-analyses, since the diagnostic criteria are matching in more studies. Seeing as how we consider VAS pain assessment as an only partially objective criterion, we believe that finding an objective method for assessing
myofascial pain syndrome in masticatory muscles could provide a more reliable interpretation of the results. In conclusion, multicentre, larger, double-blind randomized trials with a longer follow-up period are needed to obtain more reliable results. It also would be useful to research the effectiveness of multiple PRGF injections within a longer follow up period.

The take home message is for the odontologist to keep in mind that injectable treatment methods for myofascial pain syndrome are only for treating symptoms, not the cause. These methods alone do not prevent potential relapses of pain. It is very important to do a detailed examination of the patient: identification and/or assessment of the patient's posture, possible bruxism, the patient's bad habits (pen biting, opening bottles with teeth, etc.), unbalanced occlusion, irregular bite, TMJ pathology, and collection of anamnestic stress history data. Only after the aforementioned etymological factors have been assessed and eliminated will it be possible to cure myofascial pain syndrome. Injection treatment methods should only be considered as first aid for a patient suffering from myofascial pain.

Conclusions:

Within the limitations of this study, plasma rich in growth factors injections into the masseter muscle trigger points is an effective treatment method for myofascial pain. Moreover, PRGF injections are more effective than Lidocaine injections for myofascial pain relief in masseter muscle.

Author contributions:

G.J. was the author of the idea of this trial. All authors contributed equally to the study protocol creation process. G.J and R. K. supervised the whole randomized controlled trial. JP. R evaluated participants and confirmed diagnosis and included them in the trial. D.S. performed randomization and allocation, also measured participants' VAS scores during the study. JP. R performed injections, D.S. made statistical analysis. All authors discussed results and equally contributed to writing a manuscript.

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Tables:

<table>
<thead>
<tr>
<th></th>
<th>Lidocaine group (n = 25)</th>
<th>PRGF group (n = 25)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean (SD))</td>
<td>46.9 (15.9)</td>
<td>48.8 (17.2)</td>
<td>p=0.696</td>
</tr>
</tbody>
</table>
Male/Female (%)

3(12.0)/22(88.0) 6(24.0)/19(76.0) *p=0.269

Duration of illness (months, mean (SD))

28.7(55.6) 22.9(42.9) **p=0.546


Table 1. Demographic characteristics comparison of the two study groups at baseline

<table>
<thead>
<tr>
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<th>Lidocaine group (n = 25)</th>
<th>PRGF group (n = 25)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (VAS mean (SD))</td>
<td>6.4(2.5)</td>
<td>6.1(2.3)</td>
<td>p=0.063</td>
</tr>
<tr>
<td>2nd week (VAS mean (SD))</td>
<td>3.4(2.6)</td>
<td>2.2(2.6)</td>
<td>p=0.123</td>
</tr>
<tr>
<td>4th week (VAS mean (SD))</td>
<td>3.4(2.7)</td>
<td>0.9(1.7)</td>
<td>*p&lt;0.001</td>
</tr>
</tbody>
</table>

SD – Standard deviation, p - parametric Student t test, *p - nonparametric Mann-Whitney test.

Table 2. Pain evaluation results in VAS from 1 to 10 for Lidocaine and PRGF groups

Figures:

Figure 1. Flow chart of the study, following the CONSORT guidelines.

Figure 2. Comparison of pain (VAS score) during the follow-up period. *p<0.001 - Mann-Whitney test, CI – Confidence Interval.