EFFECT OF PLATELET-RICH PLASMA ON CHRONIC ODONTOGENIC MAXILLARY SINUSITIS: A PILOT STUDY

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ABSTRACT **DOI: https://doi.org/10.25241/stomaeduj.2019.6(2).art.4**

Introduction: Chronic odontogenic maxillary sinusitis (CMOS) is a frequent inflammatory process pertaining to the oro-maxilo-facial pathology. Platelet-rich plasma (PRP) is a natural source of growth factors, which have the potential to stimulate and accelerate the wound healing process.
The aim of this study is to observe the effects of the PRP growth factors in patients with CMOS.
Methodology: Inflammatory oral mucosa was collected from five patients diagnosed with CMOS, and it was incubated with 2 mL PRP for 7 days. PRP was obtained from venous blood collection from each patient. The control samples were represented by inflammatory sinus mucosa without adding PRP. The following biomarkers were measured using cell lysate: insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R), glycogen synthase kinase 3 beta (GSK3β), glycogen synthase kinase 3 alfa (GSK3α) performed by Multiplex technology.
Results: The results revealed statistically increased levels for all four parameters in patients with CMOS *versus* controls ($p < 0.05$). Growth factors from PRP bind to receptors with tyrosine kinase activity, cellular event being correlated with cell proliferation. The radiological control of patients 10 months after PRP administration revealed a decrease in the thickness of the sinus lining up to 3 times.
Conclusions: The growth factors released from platelets should be regarded as a positive effect source in the case of patients diagnosed with CMOS. These growth factors should activate the oligopotente stem cells which will finally lead to sinus mucosa regeneration. Future studies are needed to understand the molecular mechanisms that occur at the sinus level.
Keywords: Inflammation; Platelet-rich plasma (PRP); Sinusitis; Chronic odontogenic maxillary sinusitis (CMOS).

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1. Introduction

CMOS is an inflammation of the maxillary sinus caused by dental infection. Patients with chronic periodontitis have an increased risk of developing maxillary sinusitis caused by intra-antral foreign bodies or by oroantral fistulas after tooth extraction [1,2]. According to the literature in the field, the incidence of this disease in the adult population is 10-12%, but according to the latest studies, the incidence increased to 41% [3-5]. So far, there is no gold standard in choosing the most suitable treatment method for this inflammatory process. Until now, specialized literature suggests 3 main clinical methods to treat CMOS: the Caldwell-Luc operation, functional endoscopic sinus surgery and finally dental extraction or dental treatment. Using these surgical methods presents several disadvantages: bleeding, infraorbital nerve damage, facial swelling, pain, and significant hemorrhage may appear. These treatment methods require patient hospitalization and general anesthesia with possible complications [6,7]. Platelet-rich-plasma (PRP) is a natural source of growth factors being considered an endogenous therapeutic technology used in many medical fields. PRP has the potential to stimulate and accelerate the healing process [8,9,14]. The main aim of this pilot study was to observe the effects of PRP-related growth factors in patients diagnosed with CMOS.

2. Materials and Methods

The pilot study included five patients (4 men and 1 woman aged 40-64) diagnosed with CMOS following a dental exam and computer tomography (CT) (Fig.1). PRP was obtained by venous blood collection in tubes containing separating gel with an inert polymer necessary to ensure the separation between erythrocytes and platelers. Sodium citrate was used as anticoagulant to prevent the initiation of the coagulation cascade. The samples were centrifuged at 4000 rpm for 5 minutes. Using the trepanation technique, inflammatory sinus mucosa was collected from the maxillary sinus in sterile tubes and was incubated with PRP for 7 days. PRP was injected in the inflammatory maxillary sinus PRP was injected in all patients based on a clinical chart.

Along with the clinical study, lab study was conducted in order to underline the PRP procedure on the mucus, to prove the effect of growth factors implied in the local healing process. The control samples were represented by the inflammatory mucosa that were not treated with PRP and were collected in sterile tubes containing phosphate-buffered aline solution (pH 7.2). The samples were sonicated on ice (10 min, Sonoplus HD 2070, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) and the lysate was stored at -80°C. All patients signed an informed consent and agreed to participate in the study. Insulin receptor (IR), insulin-like growth factor 1 receptor (IGF-1R) glycogen synthase kinase 3 beta (GSK3β) and glycogen synthase kinase 3 alfa (GSK3α) were detected using Luminex technology and Magnetic Bead-Based Multiplex assays (Akt/mTOR, Total protein 11-plex Magnetic Bead kit, 96 well plate, 48-612 MAG). This method can detect all the mentioned biomarkers from the same sample.

2.1. Statistical analysis

The statistical analysis was performed using student t-test and p-value less then 0.05 was considered statistically significant.

3. Results

The results of our study reveled statistically increased levels of IR, IGF-1, GSK3β and GSK3α for mucosa samples treated with PRP compared with mucosa

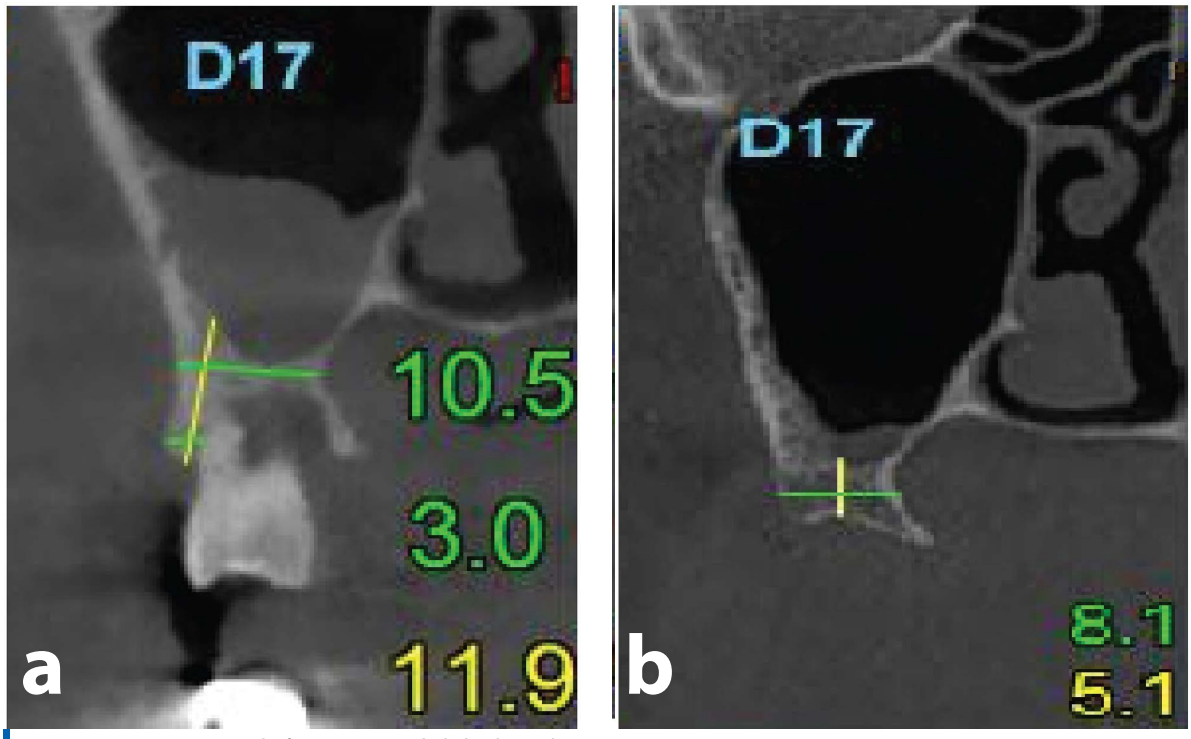

Figure 1. Computer tomography from a patient included in the study:
a. Coronal CT dental scan showing. Opacified right maxillary sinus - before PRP treatment;
b. Opacity of right maxillary sinus reduced after PRP intrasinusal infiltration *(Used with permission of pacient)* - after PRP treatment

Table 1. Mean values for all four parameters detected.

Parameters (MFI)	Patients	Controls	p
IGF-1R	142.33 ± 16.7	63.37 ± 16.7	< 0.05
IR	204.5 ± 8.23	78.97 ± 8.23	< 0.05
GSK3β	70.625 ± 20.44	30 ± 20.44	< 0.05
GSK3α	111.75 ± 43.12	40.375 ± 43.12	< 0.05

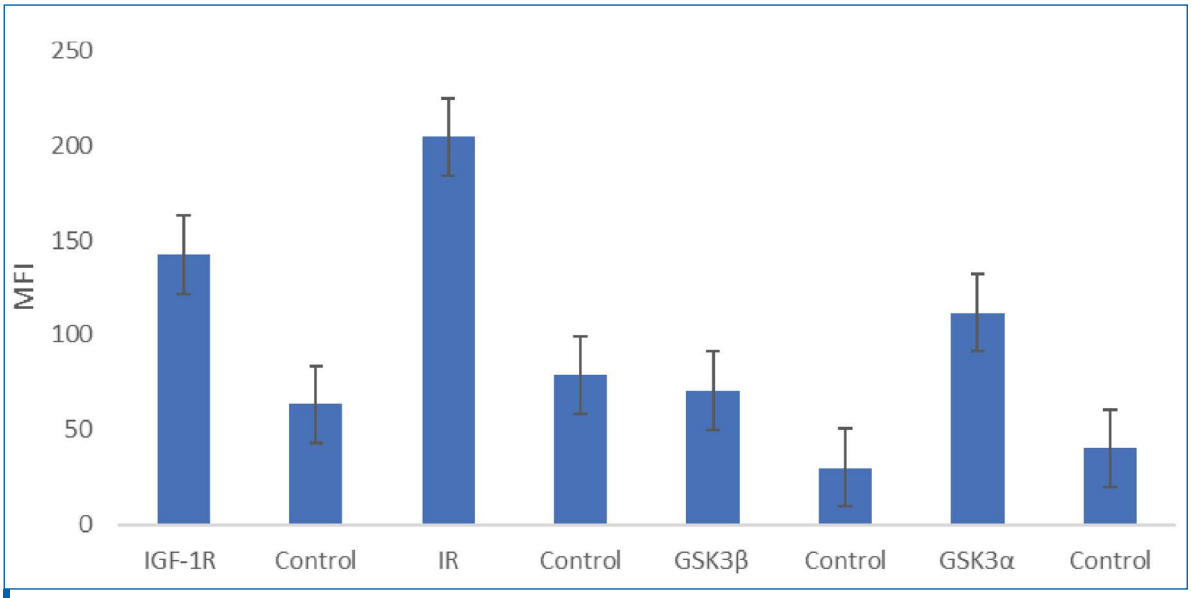

Figure 2. Graphic represenation of all 4 studied parameters versus their controls

samples that did not received PRP. Our results are presented in Table 1 and Fig. 2.

4. Discussions

PRP is currently used in many medical fields such as orthopedic, pediatric, maxillofacial and plastic surgery, sport medicine, and cosmetic and dental implant surgery [9-13]. FDA (Food and Drug Administration) has classified PRP as a minimally manipulated tissue and an autologous blood product with multiple benefits for patients [14]. Growth factors play key roles in the complex process of tissue healing and regeneration, being signaling proteins that influence the metabolism of other cells [15,16]. Transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) are present in PRP [10-16]. PDGF is secreted by platelets, macrophages, keratinocytes, endothelial and muscle cells. PDGF is one of the growth factors that are secreted when an injury occurs, stimulating many metabolic processes such as protein and collagen synthesis. It also promotes the proliferation and migration of endothelial cells, exerting angiogenic effects, stimulates the production of TGF-β that initiates collagen synthesis and stimulates the production of IGF-1. The positive effects of PDGF were seen in the treatment of the ulcerous foot in diabetic patients [17,18]. Platelets, macrophages, fibroblasts and mesenchimal stem cells secrete EGF, which is released at a high concentration at the beginning of the healing process. EGF plays important roles in the proliferation, differentiation, growth and migration of keratinocytes and epithelial cells [14,15].

IGF is secreted by fibroblasts and is involved mainly in the inflammatory and proliferative phase II, 58, 72. Decreased IGF concentrations were observed in diabetic patients with chronic wounds. Clinical trials conducted on laboratory animals such as diabetic and healthy rats, or rabbits showed that the exogenous IGF application accelerates the healing process [16-18]. In combination with other growth factors such as PDGF and EGF, IGF exhibits much more powerful biological effects promoting keratinocyte migration and enhancing tissue repair [19-21]. VEGF is secreted by plateles, keratinocytes, macrophages and fibroblasts and manifests strong paracrine effects on endothelial cells, promotes and supports the process of wound angiogenesis. It is the main growth factor that initiates the angiogenesis process in granulation tissue [21,22]. VEGF growth factors are implicated in the physiological and pathological processes of vasculogenesis, angiogenesis, vascular

permeability and lymhangiogenesis [22,23].
Clinical and experimental studies conducted on diabetic mice and dogs, as well as diabetic patients reported very good results by applying VEGF, which accelerate healing by increasing epithelialization, angiogenesis and granulation tissue formation [24-27]. Many cell types secrete TGFβ such as platelets, T lymphocytes, macrophages, keratinocytes, fibroblasts, muscle and endothelial cells. TGFβ induces synthesis of collagen and fibronectin due to chemotactic effects on macrophages and inhibits the activity of metalloproteases [25]. IGF-1R, IR, GSK3α, GSK 3β, detected in our pilot study are part of the signaling cascade called Akt/mTOR or protein kinase B. This signaling pathway plays a central role in numerous cellular processes such as cell proliferation regulation, survival and glucose metabolism. Akt is activated by insulin, which presents the receptor with tyrosine kinase activity [28]. By binding the insulin to the extracellular domain of the receptor there occurs the autophosphorylation of the receptor that is recognized by the IRS (insulin receptor substrate), and further there is a series of cascade phosphorylates with Akt activation that will lead to cell growth and proliferation. Akt may be activated by many growth factors such as those found in PRP or cytokines [28]. The results of the current study reflect statistically significant increased levels for IGF-1R, IR, GSK3α, GSK3β in the case of PRP-treated oral mucosa versus oral mucosa without PRP treatment. Growth factors from PRP bind to receptors with tyrosine kinase activity, such as the insulin receptor, actives Akt signaling pathway, promotes increase in protein translation and cell growth. The results of the biochemical evaluations are correlated with the findings of CT performed at 10 months after the PRP injection, according to Fig. 1.

5. Conclusions

In conclusion, the growth factors released from platelets should be regarded as a positive effect source in the case of patients diagnosed with CMOS. These growth factors should activate the oligopotent stem cells which will finally lead to sinus mucosa regeneration. Future studies are needed to understand the molecular mechanisms that occur at the sinus level.

Author Contributions

All authors equally contributed to the present manuscript. DM: participated in sample analysis and manuscript writing; AT: participated in sample analysis and manuscript writing, IRR: participated in study design, data collection; VM: participated in the study design and sample collection, being the OMF surgeon to produce the clinical samples; MM: participated in sample analysis, CS: participated in manuscript writing; IIS: manuscript writing; RR: manuscript writing; CT: participated in manuscript writing; GCR: translation of the text, proposing and managing the whole work; MG: participated in critical review of the manuscript.

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Her research field includes salivary studies on oxidative stress in various oral pathologies, the effects of obesity on laboratory animals, the effects of PLGA nanoparticles both in vivo and in vitro, the effects of plasma-rich plasma in patients diagnosed with chronic maxillary sinusitis, and the study of Akt (protein kinase B) signaling pathways in health and disease .

Questions

1. PRP is:

❑a. Natural source of growth factors
❑b. Natural source of proinflammatory agents
❑c. Natural source of antiinflammatory agents
❑d. Natural source of stem cells

2. PRP may be used in:

❑a. Ortopedic surgery
❑b. Dental implant surgery
❑c. Plastic surgery
❑d. Cosmetic

3. IGF:

❑a. Is secreted by fibroblasts
❑b. Is involved in proliferative phase
❑c. Promotes keratinocytes migration
❑d. Promotes tissue repair

4. Akt (protein kinase B) is implicated in:

❑a. Cell proliferation
❑b. Glucose metabolism
❑c. Celular survival
❑d. Necrosis