Concentrated growth factors gel activated with ozone for facial aesthetics purpose after granuloma removal: a case report

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The aim of this case report was to present the management of the aesthetic consequences of the treatment for granuloma removal in the zygomatic region, with concentrated growth factors (CGF) activated by medical ozone. A 54-year-old woman presented with bilateral lesion in the zygomatic region, caused by treatment with hyaluronidase and laser for removal of a granuloma, developed after infiltration with hyaluronate. The lesion was treated by local application of platelet-rich plasma obtained with CGF centrifuge, and containing CD34+ cells, mixed and activated by ozone in a 1:1 proportion, at a concentration of 40 mg/ml for 30 sec, in a syringe. Five consecutive bilateral infiltrations were made at 3-week intervals. Lesion volume was measured, and patient's quality of life was assessed with PGWBI (Psychological General Well Being Index) questionnaire. After the third infiltration of CGF-ozone, a consistent reduction of the lesion was observed, until disappearance at the end of the treatment. The result was maintained after 4-year follow-up. Considerable improvement of patient's well-being was reported. This case report showed that CGF-ozone combined therapy may promote dermal regeneration, achieving excellent facial esthetics outcomes. This result needs to be confirmed by further studies with a larger sample size.

Ozone is an allotropic form of oxygen discovered by Christian Friedrich Schonbein (1799-1868) in 1832. Over the years, several beneficial properties have been demonstrated, such as bactericidal activity to prevent infections during surgical procedures and for the topical treatment of ulcers. Ozone therapy is used for therapeutic purposes in a large number of pathologies (i.e. vasculopathies, acute and chronic infectious diseases, neurodegenerative, dermatological and gastrointestinal diseases), being effective, if correctly carried out and applied at the appropriate doses, and safe, since it does not cause allergic reactions and is free from side effects (1).

Regarding the possible mechanisms of action, ozone is a powerful pro-oxidant capable of inducing the production of highly reactive free radicals that promote oxidative pre-conditioning capable in turn of reducing oxidative stress markers and increasing the reserve of the tissue antioxidant defence system (2-4).

Ozone, therefore, directly or through its metabolites, improves the release of oxygen to the tissues, the perfusion of the microcirculation and induces the removal of toxic catabolites and the formation of small blood vessels. In the human body, ozone is naturally produced by activated neutrophils for increasing their neutralizing activity against microorganisms (5).

In pathological and stress-induced conditions,

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and during the aging, an excess of free radicals (superoxide radicals) production occurs. This can damage membrane phospholipids and alter cell membrane and mitochondrial activity, resulting in insufficient detoxification of reactive compounds, and facilitating the development and progression of degenerative diseases (6, 7).

Ozone has an important effect as an activator of the release of platelet growth factors (8, 9). such as the fibroblast growth factor, (10) which is interesting for applications in the aesthetic medicine field (11). Hyaluronic acid filler-derived granuloma is a frequent issue in aesthetic medicine. The management of this type of granuloma involves the use of hyaluronidase and LASER, but both treatments leave trauma to the tissues.

The aim of this report is to present the management of a bilateral zygomatic lesion developed after treatment of hyaluronic acid-derived granuloma with hyaluronidase and laser therapy. The tissues were successfully regenerated using autologous concentrated growth factors (CGF) activated with ozone.

MATERIALS AND METHODS

This clinical case was performed in accordance with the principles embodied in the WMA Declaration of Helsinki, following standard methodologies currently applied in clinical practice. For this reason, no ethical approval was required, and the patient signed an informed consent form before being treated. The treatment was accomplished by medical doctors trained to the use of the therapy with CGF and oxygen-ozone therapy, assisted by professional nurses.

A female patient, aged 54 years, presented to the private clinics Golden Salus, San Marino, for aesthetic issues to the face. The haematological and biochemical profile fell within the normal ranges. The clinical examination showed a bilateral lesion on the zygomatic area, the result of the removal of a granuloma by infiltration with hyaluronic acid. The granuloma had been removed with LASER by the introduction of a cannula inside the tissue, and subsequently treated with hyaluronidase. Such treatment resulted in bilateral lesions in the zygomatic region.

Ozone generator

The ozone was produced using an EC-approved medical ozone generator (Ozonette, Sedecal, Spain), capable of generating concentrations between 1 and 100-μg / mL according to pharmaceutical guidelines. The device was equipped with an optoelectronic system controlled by a microprocessor connected to the source generator of gas, to monitor and keep constant the generation of ozone.

Cell separator

For the preparation of the CGF 36 ml of venous blood was drawn in four 9-ml sterile tubes containing sodium heparin (15-30 IU). The tubes were placed in the separator Medifuge MF200 (Silfradent srl, Santa Sofia, Italy), that works at programmed centrifugation speed: 30 s of acceleration, 2 min at 2700 rpm, 4 min at 2400 rpm, 4 min at 2700 rpm, 3 min at 3000 rpm, and 36 s for deceleration and stop. For the preparation of the platelet gel, the Activated Plasma Albumin Gel (APAG, Silfradent srl, Santa Sofia, Italy) was used at 75° C for 10 min.

Preparation of the filler

After blood centrifugation, three main fractions resulted: i) the upper fraction, corresponding to the platelet-poor plasma (PPP); ii) the middle fraction, which corresponded to platelet-rich plasma (PRP); iii) the lower layer of red blood cells (RBCs). Between the RBCs and the PRP there is a fourth thin layer, the buffy coat, very rich of white cells. At the border between the buffy coat layer and the PRP a small fraction has been identified, in which are located some CD34+ cells; 0.2ml of this fraction were extracted using a 75mm x 18G needle, without removing the tube cap. Such fraction did not undergo activation nor ozonization. From each tube was then separated the PRP (2.5ml) and the PPP (3ml). PRP was mixed and activated with an equivalent volume of ozone at a concentration of 40 mg/ml for 30 sec, in a syringe. At the end of this phase, ozone reacts with albumin and platelet, and is transformed in O2. Then, 0.05ml CaCl 10% per ml of PRP-ozone are added, to complete the activation phase and stimulating growth factor release from platelets. This phase is completed 10 min before application. PPP is then introduced in the APAG and incubated at 75°C for 10 min, in order to prepare the platelet gel. The filler was prepared by a Luer male-male and 2 Luer Lock syringes, that allowed to combine PPP gel, activated PRP, and CD34+ fraction in the following proportion: 1.0:0.8:0.2.

Application of the filler

The area to be treated was cleaned with skin disinfectant. EMLA local anesthetic cream (2.5% lidocaine + 2.5% prilocaine) was applied topically for 45 min. The EMLA was then removed and the filler was injected intradermally through a 25G x 20 mm cannula on the whole injured area. The treatment was repeated once every 3 weeks, for a total of 5 applications, during the study period, from July 1st, 2014 to January 31st, 2015. Study timing was: T0 (enrolment, soon before starting the therapy with the filler), T1 (21 days after the first application, soon before the 2nd one), T2 (21 days after the 2nd application, soon before the 3rd one), T3 (21 days after the 3rd application, soon before the 4th one), T4 (21 days after the 4th application, soon before the 5th one), T5 (21 days after the 5th application).

The patient returned for scheduled control visits, consisting in clinical observation of the treated region, and collection of information on the postoperative side effects and complications, at 1 month, 6 months, 1 year, 2, 3, and 4 years after the last application.

Statistical analysis

The primary variables were collected at: 1) the size of the lesion. Surface area and perimeter were measured, and a 3D reconstruction was performed using Adobe Photoshop CS© (Adobe Systems Inc., San Josè, CA, USA). The software ImageJ (NIH, Wisconsin, USA) was used to measure the lesion volume; and 2) patient's quality of life, evaluated through a validated questionnaire for measuring the discomfort (PGWBI: Psychological General Well Being Index). The questionnaire was administered before treatment, and soon before the 4th application. Being a case report, only descriptive statistics of the variables collected at the various times was performed.

RESULTS

Lesion volume

The pattern of lesion volume over time, is reported separately for each lesion in Table I. The lesion volume progressively decreased until disappearance, in both sides. The decrease was appreciable since 3 weeks after the first application of the PRP-ozone filler. The most relevant change occurred after the third application (at T3) in both cases. No relevant side effects have been reported, except for slight and transient local pain during application, and small bruises in the region involved.

Quality of life

The results of the PGWBI questionnaire reported in Figure 1, indicate a remarkable improvement of the psychological-emotional wealth status. The higher the score, the better. Values lower than 72 indicate moderate distress; values lower than 60 indicate overload due to severe stress. A relevant increase of all the variables assessed was found, indicating improved pattern. Self- control did not improve much, but it was the only non-critical parameter at

Table I. *Volume lesion in the two zygomatic regions.*

Timing	Right side (mm^3)	Left side (mm^3)
T0	903.6	835.9
T1	710.1	739.3
T2	578.6	436.8
T3	57.0	68.1
T4	37.1	49.6
T5	9.9	8.7

baseline. Figures 2a-c show the successful evolution of the lesion located on the right zygomatic region of the patient.

DISCUSSION

PRP-based technology opens new perspectives in the field of tissue regeneration and is an invaluable tool for treating a wide range of tissue injuries in orthopedics, sports medicine, aesthetic medicine, vascular, dentistry, implantology, maxillofacial surgery, ophthalmology and other fields. The activation of the PRP with ozone has proved very effective from the clinical point of view, even though the positive results herein observed need to be confirmed.

Concentrated Growth Factors (CGF) is an autologous product that collects a large number of growth factors in a small volume of plasma, in addition to circulating CD34+ stem cells (12, 13). The preparation of the CGF is done using the technology of gradient centrifugation, specifically developed and optimized for platelet concentrates products by an Italian company (Silfradent, Santa Sofia, Italy). Blood is an optimal source of cells and bioactive molecules in most tissue engineering techniques for healing of large injuries. The CGF (a second-generation type of PRP) is an autologous, biocompatible, safe and effective biomaterial, and works as a tissue adhesive fibrin with haemostatic properties, being able to enhance tissue sealing (12).

CGF accelerates endothelial, epithelial and epidermal regeneration, stimulates angiogenesis, increases collagen synthesis, promotes tissue healing of soft and hard tissues, reduces skin scarring, improves haemostatic response to a wound (14). The high concentration of leukocytes present in the CGF also adds an antimicrobial effect, which is important for postoperative infection control (15). Furthermore, the lipoxin A4 released by the platelets confers an anti-inflammatory effect (16).

CGF exerts its beneficial effects through the degranulation of platelets alpha granules, which contain growth factors thought to be important in the initial healing phase of a wound. When biphasic platelets present in the CGF activated by thrombin aggregate, they release growth factors, along with others substances that serve to accelerate the healing process, thus increasing the cell proliferation, matrix formation, osteoid production, healing of connective

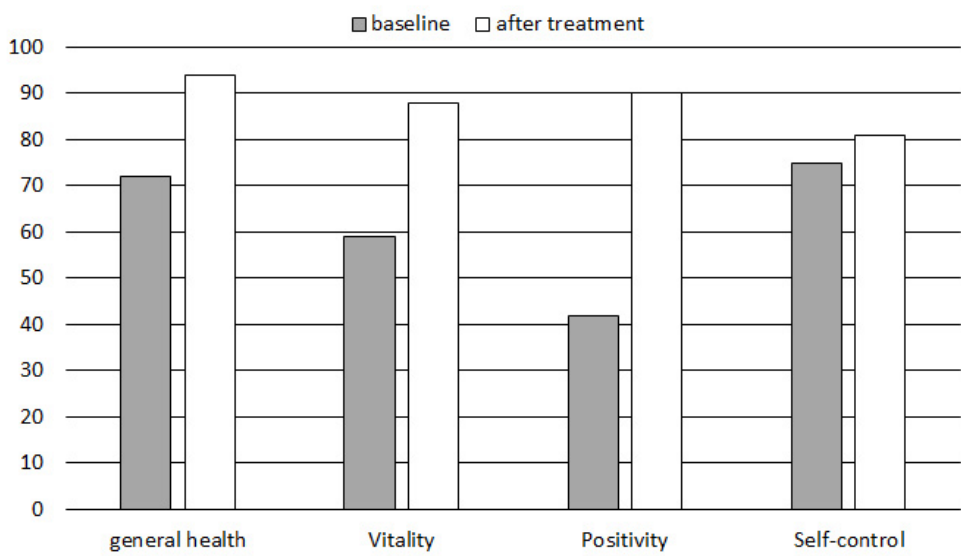

Fig. 1. *Pattern of the psychological-emotional distress scores (PGWBI) before and following treatment with PRP-ozone. Values lower than 72 are considered unsatisfying.*

Fig. 2. ***a)*** *Evidence of granuloma after infiltration with hyaluronic acid;* ***b)*** *Situation after removal of the granuloma with hyaluronidase and laser treatment. A skin depression is visible;* ***c)*** *Picture of the treated lesion, taken 21 days after the 5th application of PRP-ozone.*

tissue, angiogenesis and collagen synthesis (14). The first step, i.e., the active secretion of these growth factors, occurs within a few minutes from the start of the coagulation sequence, and more than 90% of the growth factors are secreted during the first hour. After this initial shot, in a second phase the platelets secrete additional growth factors for the remaining 7 days of their vitality. The CGF also contains: TGF-b1, VEGF and CD34 + cells (12, 13). The use of CFG leads to excellent healing in the case of critical size bone lesions in vivo (17), in the case of hair loss (18), in the impairment of peripheral cutaneous lesions like ulcers (14) and in case of myocardial ischaemia (19).

Human studies have shown that CGF can be beneficial and easy to apply in surgery and medical therapy. In the present case, it was noted that from the first session (21 days later) a reduction in tissue damage begins, manifested by volume reduction of injuries. After three sessions there is a noticeable improvement and finally after 5 sessions the patient totally recovers the normal volume of both areas zygomatic. The evolution of the lesions is accompanied by an improvement significant ($p < 0.05$) of the patient's psychological condition. The application of PRP-Ozone involves a step forward in tissue regeneration and is free of side effects.

This case report suggests that the CGF activated with ozone and mixed with the PPP gel can act as a stimulant in the regeneration of fibroblasts. These results provide new insights into the use of this therapy in facial aesthetics.

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