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Regenerative surgery performed with Platelet-Rich Plasma used in sinus lift elevation before dental implant surgery: an useful aid in healing and regeneration of bone tissue

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Abstract. – OBJECTIVES: Aim of this work is to show the effectiveness of a protocol involving the use of platelet-rich plasma (PRP) as a grafting material in bone regeneration before dental implant rehabilitation.

MATERIALS AND METHODS: 127 patients, requiring maxillary sinus lift, were enrolled in a follow-up study plan, which established clinical and radiological examinations on the day after surgery and six months later. PRP, in combination with autogenous bone, anorganic bone material and organic bone substitutes, was used before implant-prosthetic rehabilitation.

RESULTS AND CONCLUSIONS: After implant placement, 63 patients, previously treated with PRP, reached a statistically significant improvement in implant-prosthetic rehabilitation, established by primary stability and radiographic integration criteria, in comparison with the other 64 patients receiving implant-prosthetic rehabilitation without PRP treatment.

Key Words:
Platelet-rich plasma, Sinus lift, Tissue regeneration, Growth factors.

Introduction

In dentistry, tissue regeneration is a therapeutic aspect which cannot be disregarded for the achievement of a complete and harmonious restoration of appearance and function. During the past few years, several techniques have been suggested and many types of materials have been assessed, all critically evaluated and revised in clinical studies by means of histological investigations.

The "Oral and Maxillofacial Surgery Community" focused its attention on a set of scientific researches discussing the possibility that a platelet derivative could be valid not only for haemostasis, but also for the emerging field of "bone grafting"[1,2].

In 1997 Whitman et al[2] determined the effectiveness of the "Platelet Gel" in speeding up the wound healing process. In 1998 Marx et al[3] paved the way for a new era in "bone grafting", stating that the use of PRP (Platelet Rich Plasma), together with autologous bone, could allow a significantly enhanced result, in comparison with the graft performed without its use. The PRP, of which Marx et al were talking about, consists of a plasma concentrate rich in platelets; it can be obtained by centrifugation of the venous blood of the patient who will then receive it as a bone grafting material. This technique had been already used in dermatology and orthopedics[4-6], with the well known growth factors capacity to involve a wide spectrum of cellular events such as tissue healing, chemotaxis, mitotic induction and cell differentiation, increase in collagen production and angiogenesis. In particular, PRP is rich in the following growth factors: Transforming Growth Factor-beta (TGF-β), Platelet-Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF).

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Their role in tissue recovery and regeneration, as well as in bone regeneration, is confirmed by several studies in this regard. Up to date, seven types of TGF-β are known: they are generated by proteolysis of a higher molecular weight precursor, whereas their activation can occur in a non-enzymatic way or by means of plasmin or cathepsin B[7-10]. The above-mentioned growth factors are released by platelets through the degranulation process, which occurs few hours after contact with the receiving surface: *in vitro* experiments demonstrated that this process exerts its effect even on cells metabolism actively involved in the early stage of tissue regeneration (mesenchymal stromal cells, fibroblasts, osteoblasts and osteoclasts). In particular, the cellular events induced by PRP three days after grafting in the receiving site, are:

1. Capillary regeneration (neoangiogenesis)
2. Increase in osteoblast proliferation
3. Increase in fibroblast proliferation
4. Induction of osteoblast differentiation process, with the consequent stimulation of mineralization into the newly-formed bone matrix.

In this regard, it needs to underline that these events properly occur if the platelet concentration increases from 2 to 3.38 times the basal platelet count[11]. Indeed, the assumption that tissue regeneration results better in case of a higher platelet concentration results widely disproven by a set of *in vitro* examinations[12].

Firstly reported by international literature, PRP has been widely used in odontostomatology to accelerate and improve the wound healing process: it was generally used after radical oncologic surgery, extractive and periodontal surgery whereas, in recent times, the PRP is widely used in pre-implant GBR surgeries for the rehabilitation of toothless areas, requiring an increase in bone volume[1,13-21].

Data reported in a large number of scientific reports are often not in accordance, also because the use of PRP was made both alone or in combination with autogenous bone, anorganic bone material and organic bone substitutes[4,22].

Conflicting opinions also derive from the analysis of studies aimed to evaluate, in terms of neo-apposition and osteointegration, the improvement which can be achieved in case of association between PRP and anorganic bone material (Bio-Oss, Hydroxyapatite), compared with the use of bone material alone.

In a research carried out in 2001, Marx et al[5] stated that the percentage of bone formation was significantly higher with autogenous bone than either Bio-Oss or Bio-Oss with PRP. The effects of PRP alone have been assessed during its use in periodontal and post-extraction surgery and in the management of bone defects before implant. Also in this case, the reported data did not allow to outline a correct and unambiguous conclusion. In fact, whereas some Authors reported an increase in the initial osteointegration[23,24], it results not statistically significant if using PRP or not. The conflicting opinions reported in literature could be partly due to the lack of agreement about the used animal models. Furthermore, a negative role also played the absence of a validated model to obtain the concentrate mix, together with a standardized procedure protocol, universally recognized and assumed. Although the used techniques share the main phases procedure, there are several substances suggested to work within each phase and numerous versions are proposed to optimize a way to obtain PRP[25-28].

The aim of this work is to show the effectiveness of a protocol involving the use of PRP, as a grafting material in bone regeneration, before dental implant rehabilitation.

Patients and Methods

The study protocol includes a cohort of 127 patients (72 women and 55 men aged between 32 and 45) requiring maxillary sinus lift, before implant-prosthetic rehabilitation, by using PRP in combination with autogenous bone, anorganic bone material and organic bone substitutes in 63 patients (test group) and with the only autogenous bone, anorganic bone material and organic bone substitutes in the remaining 64 patients (control group). The typology of grafting material was chosen according to the clinical conditions of each patient.

The bone loss was assessed by means of radiological examinations and computerized tomography (CT): the choice of implants was case-specific, on the basis of the typology of rehabilitation necessity.

The protocol used to obtain PRP from each patient enrolled in our study was developed in compliance with the indications reported in literature[25-28]: PRP can be obtained by centrifugation of the venous blood of the patient who will then receive it as a bone grafting material.

- *First phase:* venous blood was taken (16-24 ml, according to the defect to treat);
- *Second phase:* coagulation was promptly inhibited by using sodium citrate, already present in the 3.5 ml vacuum venojet tubes which were used in the present study. The test tubes are characterized by a smooth glass surface;
- *Third phase:* in all 127 cases, a single centrifugation was performed (speed = 180 rpm = 1000 rpm for 20 minutes), in order to obtain a PRP with optimum physical and biological properties, to use in the surgical site;
- *Fourth phase:* the activation of the preparation was achieved by using calcium chloride, in a ratio of 1 g/10 ml of PRP. This phase is fundamental to guarantee the correct functioning of the biochemical processes resulting from the haemostatic, regenerative and osteoinductive action of PRP. However, before beginning this last phase, the Authors considered it opportune to use liquid PRP: it was prepared for endosinusal injection, and used for the imbibition of fibrin sponges and implant threads. Then, 1 g of calcium chloride/10 ml of PRP was mixed with the platelet concentrate and with the grafting materials in order to fill the bone gaps.

All patients accepted in this protocol were non-smokers and practicing a good home oral hygiene. By selection criteria, subjects with coagulation and calcium metabolism defects were excluded, together with subjects with hepatopathies and immunological deficits.

Before each surgical treatment in both the groups, a professional oral hygiene was performed by supragingival and subgingival scaling; moreover, 2 g of amoxicillin were administered 1 hour before surgery.

The treated patients of both the groups were included in a follow-up plan, which established clinical and radiological examinations on the day after surgery and six months later. Therefore, the clinical progress of the healing process, according with the literature[13,16,18], was supported by radiological investigations. After a dental panoramic X-Ray, a computerized tomographic examination was made, with the purpose to assess the variation in bone density around the implant. From a clinical point of view, the Authors took in account the peri-implant tissue healing together with eventual recessive phenomena of the soft tissues.

Statistical Analysis

At peri-implant surfaces, changes in bone level (mm) between baseline and 6 months after surgery, were recorded and analyzed. Statistical analysis compared data listed into Group 1 (treated patients) with data listed into Group 2 (non treated patients = controls) by means of a two tailed paired *t* test.

Results show an high significance:

$$p < 0.001 \text{ with } t = 4.28 \text{ and df} = 62$$

Mean of differences resulted 0.724 (95%) with a confidence interval from 0.385 to 1.06. R squared = 0.228

These data show that the differences between the two groups result statistically significant indicating that, in the group treated with the PRP, the quality of the response in bone tissue growth is higher than the response quality found in "control group" patients. The achieved results show the ability of the PRP to promote neo-angiogenesis during the period of bone healing. In addition, the PRP acts as a scaffold able to guide the tissue replacement in a functional manner.

Other clinical and radiological criteria of Albrektsson et al[29] also confirmed the successful implant reconstitution.

Results

In all 127 cases included in the protocol, the Authors observed a successful implant-prosthetic rehabilitation, according with the Albrektsson et al criteria[29]. In fact, there was an optimum implant stability in the monitored patients; besides, no signs of tissue pain affecting the peri-implant soft tissues were noticed. The analysis of the volume ratio of regenerated bone was performed using a personal computer and a software called "Master 3D", which allowed a three-dimensional reconstruction of the treated area. The different tomographic sections were then scanned and analyzed on a personal computer by a software of image management, comparing the videos corresponding to both pre- and post-procedure conditions. The radiographic evaluations, made six months after the implant insertions, showed, in the Test group treated with PRP, the presence of a newly-formed bone tissue, well amalgamated with the residual bone, also revealing an average increase in the peri-implant bone quality, according with Lekholm and Zarb classification[30].

Data obtained in the compared groups resulted statistically significant and the radiographic integration, at the moment of the implant placement, reached the 100% of all treated cases.

Table I. Test group (n = 63): changes in bone level at peri-implant surfaces between baseline and 6 months later.

Change in bone level (mm) at peri-implant surfaces	Number of patients
3.1 to 4.0	1
2.1 to 3.0	2
1.1 to 2.0	10
0.1 to 1.0	23
0.0 to -0.9	21
-1.0 to -1.9	5
-2.0 to -2.9	1
-3.0 to -3.9	0
	Total = 63

Discussion

Data reported in literature result sometimes in contrast, also because these studies often suggested the use of PRP both alone or in combination with autogenous bone, anorganic bone material and organic bone substitutes[11,12,14-18].

The conflicting opinions reported in literature are certainly caused by a lack of agreement into study models and especially into an absence of one unique protocol universally approved and applied in order to obtain the platelet concentrate: the repeatability and predictability of results could only be achieved by strictly applying the standardized protocol[31,32].

Once the degranulation process ends, platelets are no more the source of growth factor release. Macrophages, derived from circulating monocytes, intervene in their place. They are drawn back in large quantities into the graft, due to tissue hypoxia (pO_2 between 0.3 and 5 mm/Hg instead pO_2 between 35 and 40 mm/Hg in venous blood).

Table II. Control group (n = 64): changes in bone level (mm) at peri-implant surfaces between baseline and 6 months later.

Change in bone level at peri-implant surfaces	Number of patients
3.1 to 4.0	0
2.1 to 3.0	0
1.1 to 2.0	4
0.1 to 1.0	18
0.0 to -0.9	32
-1.0 to -1.9	8
-2.0 to -2.9	1
-3.0 to -3.9	1
	Total = 64

Thanks to their fast replication, macrophages are remarkably efficient in growth factors production, being actively involved in the second stage of tissue regeneration. As the induced revascularization increases, a decrease in the oxygen pressures values is found and, consequently, with a significant reduction in macrophage action. Complete revascularization occurs on the 14th day, when bone matrix deposition by stem cells (endosteal osteoblasts) is fully detected. Up to now, the exact concentration able to optimize the aforesaid processes has not yet been detected; medical literature is only in agreement with the above-mentioned range[12]. Higher platelet concentrations, in fact, are not able to sustain an increase in osteoblast and fibroblast proliferation.

Conclusions

Even though it is widely stressed the necessity to continue this study on a larger cohort of patients, the Authors verified and documented the positive clinical-radiological examinations achieved by using the described protocol in all treated cases. Therefore, this paper tested, with a standardized methodology, the possibility to satisfactorily use PRP in the clinical practice with a great predictability about its success.

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