

Evaluation of the effect of platelet-rich plasma on intrabony defect repair in glucocorticoids-induced osteoporosis in rabbits (Histological and biochemical study)

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ABSTRACT

Background Osteoporosis has an impact on bone healing process, platelets rich plasma (PRP) ameliorated the deleterious effect of osteoporosis on bone healing process. Autologous (PRP) could be used in many clinical fields of oral and maxillofacial bone, implant reconstructive surgery and periodontology. This study was carried out to evaluate histologically the regeneration capacity of autologous (PRP) on defect in the mandible bone of osteoporotic rabbits.

Materials and Methods Forty-eight female rabbits were used in this study, divided into four groups (12 rabbits for each group), each rabbit was received intrabony defect in the mandible. Two groups were save as control groups one of them left for normal healing (group A), while other group were receiving PRP treatment (group B). The remaining two groups were given 10 mg/B.W hydrocortisone i.m daily for 8 weeks to induce OP-like condition which save as experimental groups. One of them left for normal healing (group C), while other were receiving PRP treatment (group D). After 2, 4, and 6 weeks postoperatively (4 rabbits from each groups), blood sample was taken from each animal for serum alkaline phosphatase, calcium, and phosphorous analysis. Then the animals were sacrificed and the decalcified sections of the bone were studied histomorphologically. These histometric analyses including counting of bone cells: osteoblast, osteocyte, and osteoclast. Bone trabecular, separation, width, and number; cortical width, blood vessels number, and bone marrow space and volume assed.

Results Histological examinations showed that with the use of autologous platelets rich plasma in an osteoporotic and normal rabbit, an obvious enhancement of new bone formation and neovascularization significantly more than that of groups without (PRP) application. The results of osteoporotic group treated with (PRP) nearly reached the levels of normal group without PRP in all the three periods postoperatively. Biochemical serum analysis revealed an increase in serum alkaline phosphatase and calcium concentrations in osteoporotic animals than control one, while serum phosphorous level increased in control animals than osteoporotic ones.

Conclusions This study illustrated that the (PRP) has an osteopromotive activity that accelerated bone-healing process in madibular bone defect in an osteoporotic rabbits as well as in normal rabbits.

Key words: Platelet-rich plasma, intrabony defect. (J Bagh Coll Dentistry 2012;24(3):71-77).

INTRODUCTION

Repair of bone tissue is a complex process involving a number of cellular functions and mineralization of the defect followed by an eventual remodeling of the defect site to attain the original structure⁽¹⁾.

Systemic disease such as diabetic mellitus and osteoporosis (OP) has been suggested as potential conditions that delay bone healing.

OP-like conditions often manifested themselves in the geriatric female population, for which bone fracture has become common⁽²⁾.

OP has received attention in the dental field, as it is characterized by reduction of bone mass, structure, and function. OP is thought to be a result of altered bone remodeling capacity, i.e., bone formation decrease while restorative capacity remains relatively constant⁽³⁾.

Several studies have shown that bone regenerative procedures in normal defect or osteoporotic defect may be enhanced by the addition of specific growth factors⁽⁴⁾.

Growth factor is a naturally occurring substance capable of stimulating cellular growth, proliferation and cellular differentiation. Usually it is a protein or a steroid hormone⁽⁵⁾.

Platelet-rich plasma (PRP) is blood plasma that has been enriched with platelets. As a concentrated source of autologous platelets, PRP contains several different growth factors and other cytokines that stimulate healing of bone and soft tissue. Based on this principle PRP are introduced to stimulate a supra-physiologic release of growth factors in an attempt to jump start healing in chronic injuries. All of the known clinical applications of PRP highlight an accelerated tissue cicatrization due to the development of effective neovascularization, accelerated bone healing with fast tissue remodeling, and nearly total absence of infectious events⁽⁶⁾.

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MATERIALS AND METHODS

Forty-eight female rabbits were divided into 4 groups (12 rabbits for each), Intra bony defect were made for each rabbits in the diastima near the first premolar in the mandible (fig 2-4). These groups are:

Group A: (12 rabbits) control group with

Group B: (12 rabbits) control group received platelet rich plasma.

Group C: (12 rabbits) experimental group - osteoporotic group- with normal healing.

Group D:(12 rabbits) experimental group - osteoporotic group- received platelet rich plasma Four rabbits from each group were sacrificed at 2, 4 and 6 weeks intervals according to this table.

Healing period Study group	2 week postoperatively	4 weeks postoperatively	6 weeks postoperatively	Total
Group A	4	4	4	12
Group B	4	4	4	12
Group C	4	4	4	12
Group D	4	4	4	12
Total	16	16	16	48

Induction of osteoporotic-like condition: Twenty four rabbits were received (10 mg/kg body weight) of hydrocortisone daily by i.m injection for 8 weeks to induced osteoporotic – like condition ^{(7) (8)}

Preparation of Platelet Rich Plasma (PRP):

This processes involve the collection of whole blood that is anticoagulant with citrate dextrose before undergoing two stages of centrifugation designed to separate the PRP aliquot from platelet poor plasma and red blood cell. The platelets poor plasma was separated from platelets rich plasma along with puffy coat ⁽⁹⁾, the P.R.P. was activated by combination with equal volume of sterile solution of 10% CaCl₂

Surgical procedure

After anesthetizing the rabbit by general anesthesia, the operative field was properly draped by sterilized towels. An incision of (1.5-2.5 cm) was made along the alveolar crest in the naturally edentulous space between the incisors and premolar teeth in the mandibular arch (lower left diastema).

Bone penetration was performed by dental engine of low speed hand piece of (2000rpm), round bur (no.012) cooled by a continuous stream of sterile normal saline, used only to perform the orifice in the bone , then with fissure bur (No. 010)the cavity deepened to hold the implanted material

The hole was made on the mandible at the diastema between central incisors and, premlar of rabbit, its size approximately 2mm in diameter and (2-3mm) in depth ⁽¹⁰⁾. Then the hole filled with small piece of gelita tampon immersed in platelet rich plasma in the rabbits of (group B and groupD) While, the hole left unfilled in the rabbits of (group A and group C) for normal healing.

RESULTS

Histological analysis:

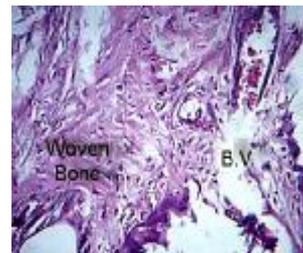


Figure 1: View of group A at the end of 2nd week showing woven bone (WB) and blood vessels (BV) in the area of bony defect. (H&E stain X100)

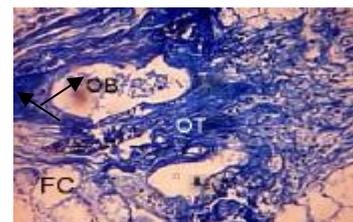


Figure 2: View of group A at the end of 2nd week showing newly osteoid tissue (OT) formation surrounded by osteoblast(OB),fat cells (FC) and haemopoietic cells. (Gomori Blue Trichrome stain X100)



Figure 3: Newly bone trabecula(BT)lined by osteoblast(OB),osteocyte(OC),osteoclast(OCL), numerous blood vessels(BV) and collagen fiber.(H&E stain X200)

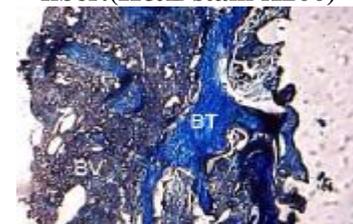


Figure 4: View of group B at the end of 2nd week showing bone trabeculae(BT) and blood vessels. (Gomori Blue Trichrome stain

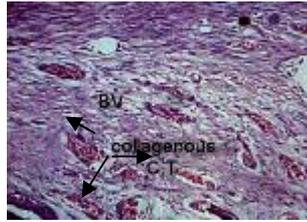


Figure (5) View of group C at the end of 2nd week showing collagenous connective tissue and blood vessels (BV). (H&E stain X100)

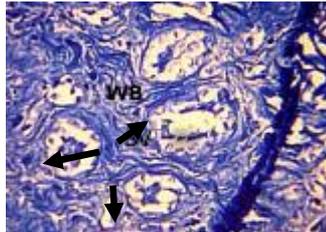


Figure 6: View for group C at the end of 2nd weeks showing woven bone (WB) and blood vessels (BV). (Gomori Blue Trichrome stains X400)

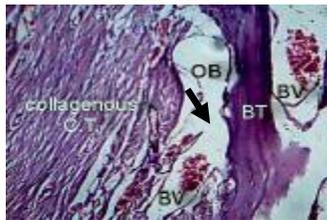


Figure 7: View of group D at the end of 2nd week showing bone trabeculae (BT) lined by osteoblast (OB), collagenous connective tissue (CT) and numerous blood vessels (BV). (H&E stain X100)

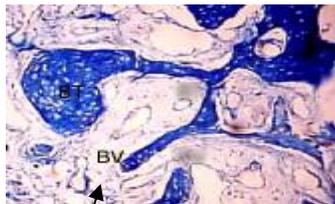


Figure 8: View for group D at the end of 2nd weeks showing speculae of bone trabeculae, surrounded by blood vessels (BV) (Gomori Blue Trichrome stain X100)

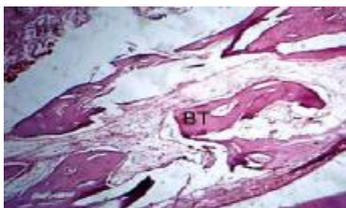


Figure 9: View of group A (control without PRP) at the end of 4th week showing that the defect filled with bone trabeculae (BT) (H&E stain X100)

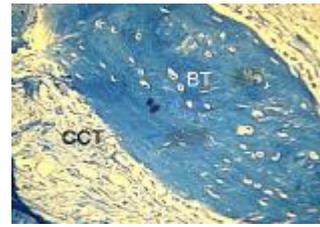


Figure 10: View of group A at the end of 4th week showing (BT) surrounded by collagenous connective tissue (CCT). (Gomori Blue Trichrome stain X100)

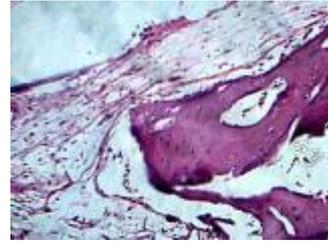


Figure 11: View of group B at 4th weeks duration showing new bone formation (H&E stain X100)

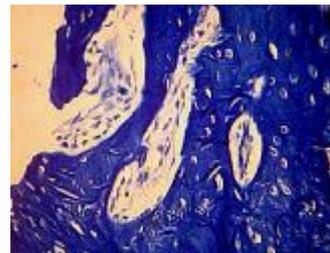


Figure 12: View of group B at the end of 4th week showing new bone formation (Gomori Blue Trichrome stain X200)



Figure 13: View of group C at 4th weeks duration showing cartilage tissue surrounded by new bone (H&E stain X100)

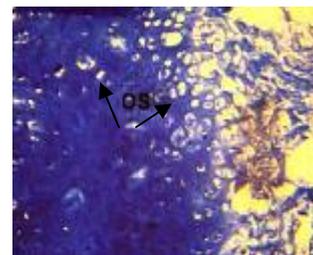


Figure 14: View of group C at the end of 4th weeks showing new bone formation with irregularly arranged osteocytes (OS) (Gomori Blue Trichrome stain X400)

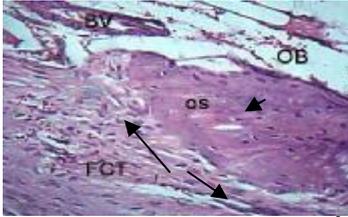


Figure 15: View of group D at 4th weeks duration showing fibrous connective tissue (FCT) surrounded new bone (H&E stain X100)

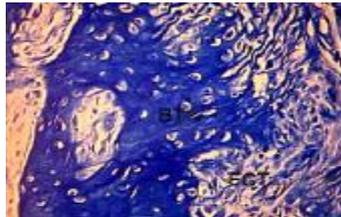


Figure 16: View of group D at the end of 4th weeks showing fibrous connective tissue(FCT) and bone trabeculae (BT) (Gomori Blue Trichrome stain X200)

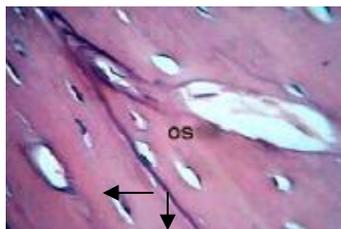


Figure 17: View of group A at the end of 6th week showing irregular arrangement of osteocytes (OS).(H&E stain X400)

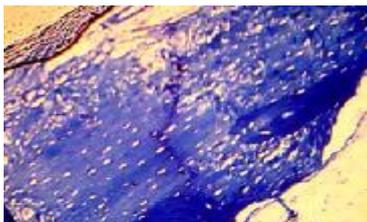


Figure 18: View of group A at the end of 6th weeks showing new bone formation filled the defect area (Gomori Blue Trichrome stain X100)

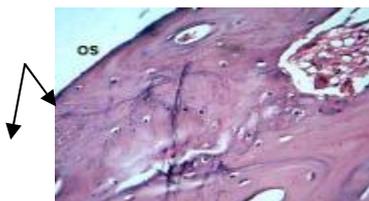


Figure 19: View of group B at the end of 6th week showing mature (lamellated) bone and small size lacunae of osteocytes (OS) (H&E stain X200)

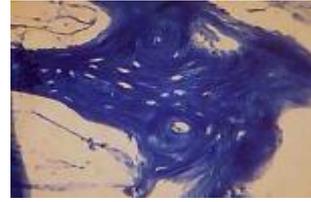


Figure 20: View of group B at the end of 6th weeks showing new bone formation (Gomori Blue Trichrome stain X200)



Figure 21: Higher magnification of previous slide showing preosteocyte(POS), osteocyte(OS), and osteoclast(OCL) (H&E stain X200)

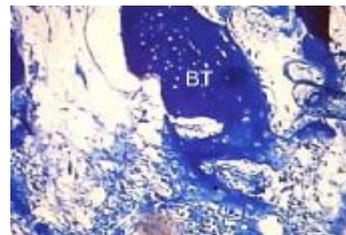


Figure 22: View of group C at the end of 6th weeks showing trabecular bone (BT) formation (Gomori Blue Trichrome stain X100)

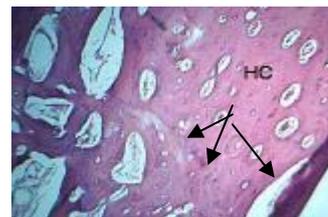


Figure 23: View of group D at the end of 6th week showing new bone formation with increase Haversian canals (HC) no (H&E stain X100)

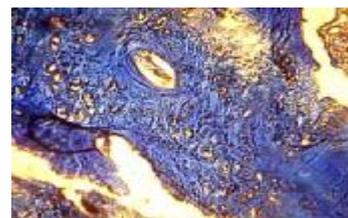


Figure 24: View of group D at the end of 6th weeks showing mature bone formation (Gomori Blue Trichrome stain X100)

Histomorphometric analysis for bone microarchitecture:

Cortical width:

The cortical width values increased significantly in group B (control with PRP) than other three groups in all healing periods. ($P \leq 0.01$). However, there was significant increase in cort.wid in all groups with time lapse in three healing periods $P \leq 0.01$ (Figure 25).

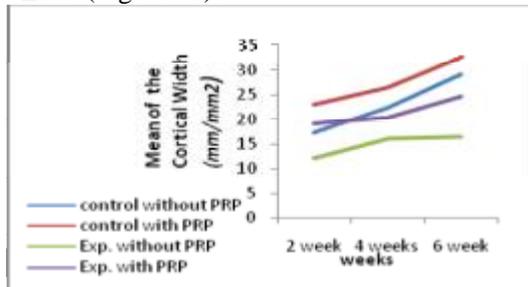


Figure 25: Linear chart showing the mean of cortical width

Trabecular width :

The results of trabecular width revealed that at 2nd week duration showed least significant increase in Tb.wid. Also the results showed that group B (control with PRP) had higher mean value in all healing periods. (figure 26).

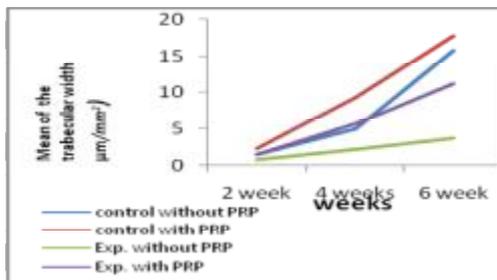


Figure 26: Linear chart showing the mean of the trabecular width

Volume star bone marrow space (V*m):-

The results showed that there were highly significant reduction in V*m with time progression. The highest mean vales of V*m was seen in group C in all healing periods, while the least mean in all healing periods was seen in group B. & Figure 28.

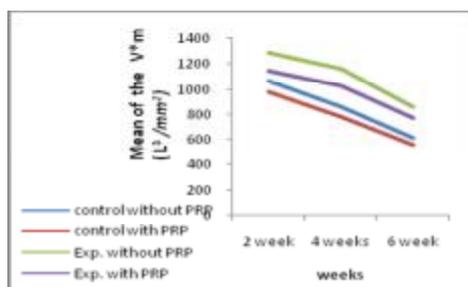


Figure 28: Linear chart showing the mean of the Volume star bone marrow space (V*m)

Osteocyte number:

The highest mean values of osteocyte number were seen in group B (control group with PRP) & the least mean values were seen in group C (exp without PRP) (figure 29)

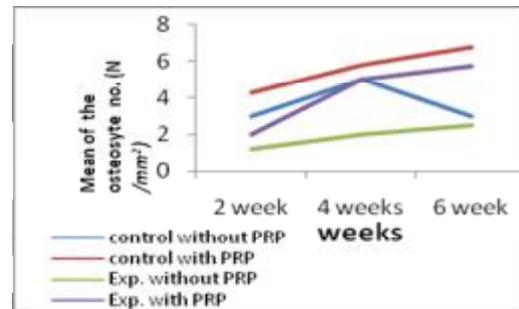


Figure 29: Linear chart showing the mean of the osteocyte number

Blood vessel number:

The results denoted that there was significant reduction in the number of B.V. in almost all groups, except in group A, which showed non-significant differences in B.V. number. On the other hand the highest blood vessels mean number was seen in group B than other groups in all healing period (Figure 30)

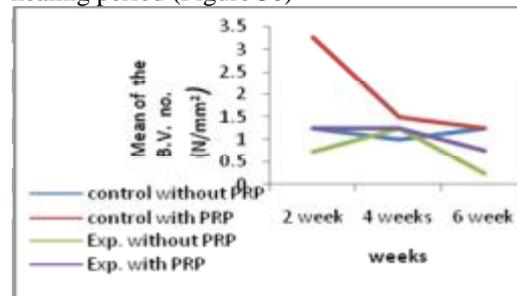


Figure 30: Linear chart showing the mean of the blood vessels number

Alkaline phosphatase level:

The highest mean levels of alkaline phosphatase were showed in groups C and D (osteoporotic groups) than group A and B (control groups). In addition, there were highly significant increased in alkaline phosphatase with time in groups A and B. (figure 31)

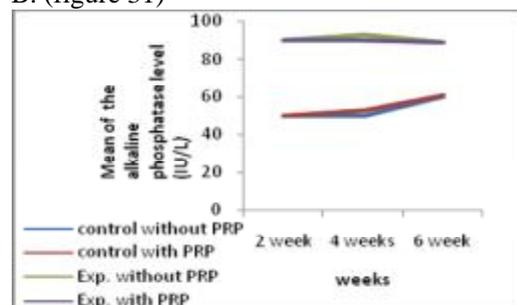


Figure 31: Linear chart showing the mean of the alkaline phosphatase level

Histological and Histochemical analysis:

At the end of 2nd weeks postoperatively: Group A (control group without PRP) showed primitive bone formation while group C osteoporotic group without PRP) the defect still filled with collagen fiber and beginning of osteoid matrix formation, this mean that there was delay in bone healing related to GC administration which lead to reduce bone formation and increase bone resorption⁽¹¹⁾.

Histological findings in control bony defect treated with PRP (group B) illustrated formation of bone trabeculae with active osteoblast and active blood vessels, the findings were not observed in control group without PRP (group A) which showed only primitive bone formation, this is in agreement with study done by⁽¹²⁾, who found platelets can enhance the plasminogen activation capacity of mesenchymal progenitors which responsible for bone formative cell.

At the end of 4th weeks postoperatively: - at this healing period, the histological findings illustrate bone trabeculae formation in all groups but they were thinner, spare and randomly oriented in osteoporotic rabbits (group C) than other three groups.

Moreover, fibrous connective tissue may be still filled the cortical bone in some area of bony defect in the osteoporotic group without PRP, this delay was attributed to improper cell function related to GC administration. This will lead to diminished bone formation, lower mineral density in newly formed bone, and delay bone healing⁽¹³⁾.

Furthermore, the delay of bone formation was very obvious in the defects of experimental group without PRP by the presence of cartilagenous tissue with its chondrocyte in some area, while in defects treated with PRP showed new bone formation with large number of osteocyte This histological finding was in agree with⁽¹⁴⁾, who found that PRP has an osteopromotive activity since it contains a concentrated growth factors that increasing cellular proliferation.

At the end of 6th weeks postoperatively : The histological features of control groups (with and without PRP) revealed the maturity of bone formation, in which, the osteocytes arranged in circular manner around Haversian canal. In addition, there were fewer amounts of spaces between cortical bones in the control groups (group A and B) than that in the experimental groups (group C and D). This might be attributed to the effect of hydrocortisone in delay bone formation⁽¹³⁾.

On the other hand, the application of PRP to the bony defect of group B and D had positive effect on the healing process of these defect. These results could be observed by the presence large

numbers of haversian canals, which mean that there was increased in blood supply. These findings are indicated that the platelets within PRP release growth factors and proteins like osteonectin, fibronectin, and osteocalcin, all of them influence bone healing in different ways.⁽¹⁵⁾

Histomorphometrical analysis:

In general, osteoporotic animals showed reduction in mean value of cortical width, trabecular width, trabecular number and osteocyte number when compared with control animals in three healing period⁽¹⁶⁾.

On the other hand, group C (experimental without PRP) showed the less value of previous objects and highest mean value of trabecular separation and volume star bone marrow space when compared with other groups. Although in this study the results nearly showed no significant differences in the bone architecture analysis between control group without PRP and experimental group with PRP. These findings coincide with that of⁽¹⁷⁾ who concluded that PRP in combination with an osteoconductive synthetic alloplastic substitute has an effect on bone regeneration more significantly in ovariectomized osteoporotic rats than in normal rats.

Results of histomorphometric analysis for bone architecture parameters showed that using of autologous PRP in the normal bone defect or osteoporotic one has benefits for organizing the formative cell (specially osteoblast), formation of neovascularization and more rapid and faster apposition of bone matrix with its mineralization process. The more supplement of blood to healing area in animals treated with PRP, accelerate and potentiate two processes⁽¹⁸⁾. *First process include local hemostasis at sites of vascular injury *Second process include providence of nourishment for undifferentiated cells to be differentiate and provide significant effects for their migration to the healing area and activate its biological role.

The result of the present study reported that there are a significant increment in trabecular number, trabecular width, cortical width and osteocyte number in the defect area as period progress and that is true on the fact that osteoid formation progress to bone trabeculae formation and their opposition and maturation and their establishment to ideal thickness needs for time⁽¹⁹⁾

Biochemical serum analysis:-

Alkaline phosphatase may assist in the diagnoses of OP, including high turnover of osteoporotic bone⁽²⁰⁾.

Results of alkaline phosphatase level increased significantly in group C&D (experimental groups) than group A&B (control groups) in all healing

period. These were in agreement with ⁽¹⁶⁾, which recorded an increase in the level of alkaline phosphatase in ovariectomized rat to induce OP, when it compared with their controls.

These changes in serum total alkaline phosphatase may indicate suppression of bone formation in osteoporotic rabbits. The result suggest that GC inhibits bone growth mainly by decreasing bone formation ⁽²¹⁾.

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