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Effects of single versus double application of human platelet - rich plasma on bone formation of rabbit sagittal suture after rapid expansion

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Abstract :The purpose of this study was to evaluate the effect of single versus double local application of human platelet rich plasma on bone formation of orthopaedically expanded sagittal suture of rabbits. For this purpose thirty apparently healthy male Albino rabbits, aged 2-3 months and weighed 0.75-1kg were used. They were divided into three groups of 10 animals (5 for histological and radiological study of the sagittal suture and the other 5 animals to study the relapse ratio of the expanded suture). GroupI received single human platelet rich plasma (hPRP) application over the expanded suture, group II received double application of hPRP one week apart, and group III was a control group with sagittal suture expansion only. Sutural expansion was carried out for 7 days in all animals using an expansion spring made of 0.7 mm diameter stainless steel wire with two helices. The expansion appliance was calibrated to exert an initial expansion force of 80 g. The suture regeneration was evaluated radiographically and histologically after two weeks of hPRP application. Results showed that the application of hPRP has a positive influence on bone regeneration of orthopaedically expanded sagittal suture in comparison to control group, although no significant difference was noted radiographically. However, the delivery of a second dose of hPRP one week after the first application showed a more stimulatory effect on bone formation, both radiographically and histologically. The relapse ratio was significantly less in single and double application of hPRP than in the control group. Although, the relapse was less in double application group than single application group, it failed to reach a significant difference. It was concluded that the double application of hPRP is more effective than single application in enhancing bone regeneration of orthopaedically expanded rabbit sagittal suture.

Keywords: Bone Formation, Expansion, Human, Platelet Rich Plasma, Rabbits, Suture.

Introduction

Transverse maxillary deficiency is a common problem in orthodontic and rapid maxillary expansion is the treatment approach to solve this problem [1]. The purpose of this technique is to increase the maxillary dental arch width by distracting the midpalatal suture, allowing for new bone formation at the expanded suture[2].Rapid maxillary expansion can exert a significant force on the mid-palatal suture, which results in stretching of collagen fibers, accompanied by new bone formation with associated mitotic figures[3]. Tension -induced osteogenesis in the expanded suture involve initial traumatic response followed by a period of connective tissue repair and wound healing. The expanded suture contains large blood vessels and collagen fibers oriented transversely. Finally, bone is deposited at the edges of the suture in tension areas [4].

The concept of midpalatal suture opening was first described by Angell in 1860 [5].This technique offers solutions for the treatment of several abnormalities such as transverse maxillary deficiency, pseudo-class III malocclusion, rhinologic and respiratory ailments, and cleft palate[6].

Relapse is one of the most challenging issue involving rapid maxillary expansion procedure.[7]. Although the actual causes of relapse are not yet fully understood, regulation of bone metabolism and retention period duration are some of the suggested causes[8]. Circummaxillary resistance, tension produced in the palatal mucosa and supracrestal fibers, and patient's age may also influence post-expansion relapse [9].

Relapse of rapidly expanded suture is regarded as cumbersome and currently mechanical retention is used to prevent relapse and maintain long-term stability of treatment outcomes[10]. Up to six months of mechanical retention have been advocated to prevent relapse. A study by Petrick et al [11] using computerized tomography showed that bone mineralization in the expanded midpalatal suture is not completed even after a conventional 6-months retention period. Inadequate bone formation in the suture could lead to early relapse because the suture cannot withstand the tension and pressure of facial bone structures[11].It would be potentially beneficial; therefore, to enhance bone generation and mineralization in the expanded mid-palatal suture to prevent relapse and to shorten the retention period[12].A variety of agents have been tested locally to stimulate bone formation in the sagittal and midpalatal suture after rapid expansion; such as bone morphogenic protein [13], GSK-3β inhibitor [14],Vitamin C [15],Vitamin E [16],Vitamin D[17], Zoledronic acid [18], periostin -like factor[19] electrical stimulation [20], and Laser[21].

Platelet rich plasma is rich in cytokines and growth factors such as transforming growth factor-β, vascular endothelial growth factor, and platelet derived growth factor, which would accelerate bone formation [22].Up to our knowledge no study have investigated the effect of human platelet rich plasma, in clinical or experimental study, on bone formation of rapidly expanded sutures.

Materials and Methods

Experimental animals

Thirty apparently healthy male Albino rabbits, aged 2-3 months and weighed 0.75-1.0 Kg. were used in the study. They were housed in metallic cages in the animal house of the College of Dentistry, University of Mosul. They were kept at a constant temperature of 24^0C under alternate 12-hour periods of light and dark conditions. They were fed with green vegetables and given tap water ad libitum.
The animals were divided into three groups of 10 animals each (5 for histological and radiological study of the sagittal suture and the other 5 animals were used to study the relapse ratio of the expanded suture). Group I received single human platelet rich plasma (hPRP) application over the expanded suture, group II received double application of hPRP one week apart, and group III was control group with suture expansion only.

Preparation of hPRP

Human PRP was prepared and supplied by Blood Bank in Mosul Medical City. About 500 ml o negative blood from a healthy donor was collected in 70 ml of anticoagulants (citrate–phosphate–dextrose [CPD]) and cooled to about 22^oC. Within 24 h of extraction, the blood was separated through centrifugation into erythrocytes, buffy coat (leukocytes and platelets) and plasma. From the buffy coat the leukocytes were removed through filtration, and the isolated fraction of platelets was human PRP. To obtain information on the increase in platelet concentration and the final concentration of platelets in the PRP, platelet count was performed in the Haematology Department of Ibn Sina Hospital in Mosul, using slide and microscope. The number of platelets in the prepared hPRP was $2400x10^9$/L.

Expansion of sagittal suture

Sutural expansion was carried out for 7 days in all animals using an expansion spring made of 0.7 mm diameter stainless steel wire (Dentaurum, Pforzheim, Germany) with two helices (Fig. 1). The rabbits were anaesthetized via an intramuscular injection of a combination of 0.5 mL of 50 mg/ml ketamine hydrochloride (Gracure Pharmaceutical Ltd., India) and 0.25 ml of 2% xylazine (Alfasan, Holland). The hair over the calvarium was removed with hair romving lotion and the skin disinfected with chlorohexidine; then, the animal was draped to allow aseptic access in the operation field.

A 1.5–2 cm midsagittal incision was made antero-posteriorly through the scalp to expose the sagittal suture. Subsequently, two holes were symmetrically made in the parietal bone with small round bur on either side of the suture with the distance between the two holes on opposite sides of the suture was 3 mm. The expansion appliance was calibrated to exert an initial expansion force of 80 g and was then placed into the holes. The skin was closed with silk suture.
After 7 days of expansion the appliance was removed and the expanded suture was maintained with a wire retainer for 14 days.

Injection of hPRP

hPRP was activated in a 10:1 volume ratio with a mixture of 1000 U/ml bovine thrombin and 10% calcium chloride which activated platelet aggregation to form a platelet gel. The resulting hPRP gel was then loaded into a 1ml insulin syringe. Immediately after placement of wire retainer group I and II, received an 0.5 ml of hPRP gel injected subcutaneously slowly over the expanded suture. GroupIII received no injection and served as control. One week later, group II received a another injection of 0.5ml hPRP subcutaneously over the suture.

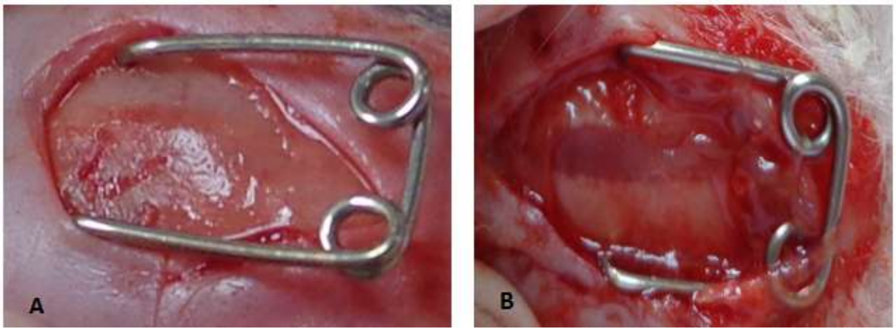

Figure (1): Expansion appliance, (A) before and (B) after expansion

The relapse ratio of the expanded suture

In the relapse ratio group, the wire retainer was removed after the 2 weeks of retention and the relapse ratio was observed one week later. The distance between the gutta percha markers was measured by electronic verneir immediately after appliance placement (A), after retention (B), and at end of 7 days post-retainer removal (C). The relapse ratio was calculated according to the following equation[23]:

(B-C)/ (B-A) x100

Histological and radiological study of the sagittal suture

The group of animals (15 rabbits) intended for histological and radiographic study of bone formation at the sagittal suture was sacrificed after two weeks of retention period. The parietal bone including the expanded suture was dissected from soft tissue and removed with hand saw with at least 5 mm of bone on either side of the expanded suture margins. The specimens were then immediately fixed in 10% neutral formalin for two days.

After fixation the wire retainer was removed and the specimens were subjected to radiographic examination to assess the amount of bone formation and density of the suture with digital dental radiographic system (Planmeca, Finland) using periapical sensor. Radiographic density of the expanded suture was measured, in pixels, using Image J software program (Sun Microsystems, USA).

After radiography, the specimen was then decalcified with 5% nitric acid solution for 5 days. The specimens were then dehydrated with ethanol and embedded in paraffin. The paraffin block was then cut coronally with microtome into 5µm thick sections. For histological examination, the sections were stained with haematoxylin and eosin before examination under light microscope.

Statistical analysis

Statistical analysis was conducted using a statistical software package (SPSS 18.0 for Windows).Mean values and standard deviation were calculated and Duncun multiple range test was used to determine statistical differences among the groups. Statistical significance was set at $P = 0.05$.

Results

All the animals survived during the study period, gain weight, with no mortality and no signs of infection were noted at the site of injection over the suture.

Histological observations

The sections of the expanded suture in the control group showed a large quantity of fibrous tissue bridging the two edges of expanded gap. The fibrous tissue consisted of dense irregularly arranged collagen fibers and fibroblasts with numerous newly formed blood capillaries in various stages of maturation. At both edges of the suture, there was large number of osteocytes and inactive osteoblasts with absence of osteoclasts cells (Fig. 2A).In the group that received single dose of hPRP the two edges of expanded suture were joined by large quantity of dense fibrous tissue that was vertically oriented to the expanded vector. In addition, there was some new bone trabeculae as finger-like projections with various lengths extended from both edges of suture gap (Fig. 2B).In the double dose of hPRP-treated group the suture showed a mature fibrous tissue consisting of various numbers of fibrocytes with huge number of large congested blood vessels filling the expanded gap. In addition, there were some bone islands made of osteoid and osteoblastic cells present among the fibrous tissue. There were some longitudinal new bone trabeculae extended from the edges of both suture margins (Fig. 2C).

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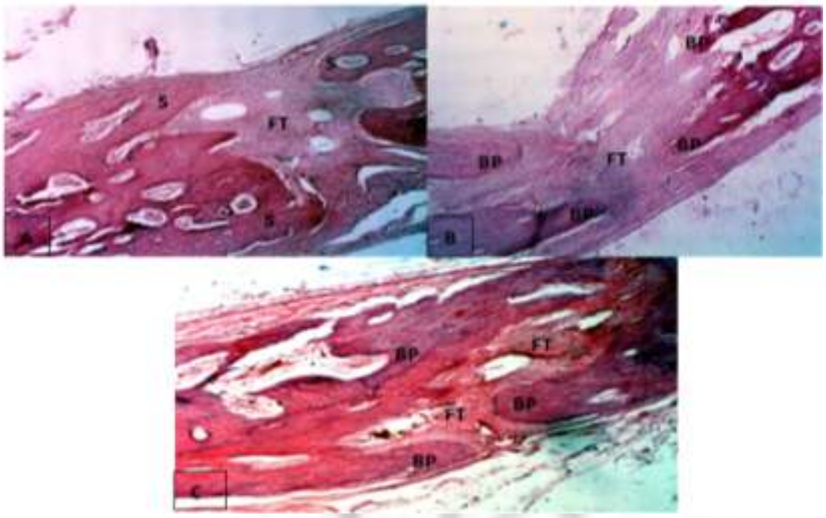

Figure (2): Histological sections of expanded sagittal suture. (A) Control group, dense fibrous tissue (FT) could be visualized joining the tow edges of the suture (S), (B) Single hPRP- treated group, finger-like bony projections (BP) extending from the bones on either side of the suture and dense fibrous tissue (FT) joining the suture edges (S). (C) Double hPRP-treated group, extensive finger-like bony projections (BP) interdigitated with dense fibrous tissue (FT) could be seen in the suture ,HE 40X.

Radiographic observations

The radiograph density of the expanded sagittal suture showed no significant difference between the control group and single hPRP study group. The density of the suture in double hPRP study group was significantly higher than control group and single hPRP study group

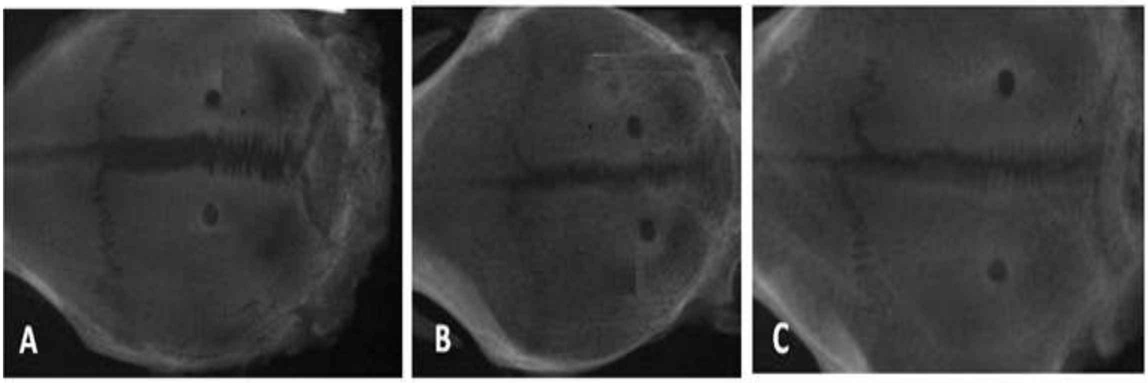

Figure (3): expanded sagittal suture two weeks after retention. (A) control group. (B) single hPRP group. (C) double hPRP group.

Table (1): Radiographic density of sagittal suture in pixels for single and double hPRP groups and control group.

		Radiographic Density in Pixels				
Groups	No.	Minimum	Maximum	Mean	St.D.	St.Error
Control	5	34.16	42.67	38.16[a]	3.76	1.67
Single hPRP	5	39.89	44.81	41.45[a]	1.99	0.89
Double hPRP	5	44.51	47.58	46.47[b]	1.31	0.85

Means carrying different letters are significantly different (Duncun test).

Relapse ratio

Changes in the relapse ratio are shown in Table (2). The relapse ratio was significantly less in single and double application of hPRP than in the control group (24%). Although, the relapse was less in double application group (7%) than single application group (10%),failed to reach a significant difference.

Table (2): Relapse ratio of expanded sagittal suture in single and double hPRP groups and the control group

		Relapse ratio				
Groups	No.	Minimum	Maximum	Mean	St.D.	St.Error
Control	5	18.0	35.5	23.66^{a}	7.27	3.25
Single hPRP	5	7.5	14.6	10.12^{b}	2.77	1.23
Double hPRP	5	4.5	12.0	6.88^{b}	2.99	1.33

Means carrying different letters are significantly different (Duncun test).

Discussion

Palatal expansion is a common orthopaedic procedure in orthodontic practice to increase the palatal width in cases of collapsed upper arch with posterior cross bite. Long time of retention is required to allow consolidation of the expanded suture to prevent relapse.

In this study we used rabbit sagittal suture as a model for human midpalatal suture. The rabbit bone has haversian systems that are similar to that of human, which is an important advantage in terms of extrapolation of results gained from rabbit study for human bone application [24] .The disadvantage of using rabbit models is the more rapid healing in rabbits than humans, so direct transfer of information to the human clinical situation is questionable [25].

Platelet rich plasma contains several growth factors including transforming growth factors (TGF),vascular endothelial growth factors (VEGF),platelet derived growth factors(PDGF) and insulin like growth factors (IGF).Many investigators have concluded that PRP has early role in bone healing. The mechanism of initiation of bone regeneration may start with the release of PDGF and TGF-β from the platelets. PDGF has been shown to stimulate migration of the mesenchymal progenitor cells [26] and also mitosis of osteoblasts [27].TGF- β activates fibroblasts and preosteoblasts to mitosis and promote their differentiation toward mature functioning osteoblasts[28]. Continued TGF-β secretion also causes the osteoblasts to lay down bone matrix and the fibroblasts to lay down collagen [29].

The result of present study showed that the application of hPRP demonstrated a positive influence on bone generation of orthopaedically expanded suture in comparison to control group, although no significant difference was noted radiographically. However, the delivery of a second dose of hPRP one week after the first application showed a more stimulatory effect on bone formation, both histologically and radiographically. The histology of sutures in double hPRP showed islands of bone formation at the central area of the suture with finger like projections of newly- formed bone invading the suture. The density of the suture was significantly higher in the double application group than single application and control groups.

In this study, the relapse ratio was significantly less in the hPRP groups than control group and also less in double than single application, but failed to reach a significant difference. This result demonstrated that the degree of relapse in sagittal suture after rapid expansion is reduced significantly by the injection of hPRP and the effects are dependent on repeating the application.

The role of PRP in enhancing bone formation has shown great variability in both human and animal studies. Some studies demonstrated a positive effect on bone regeneration[30-32] .On the contrary, other investigators have demonstrated that PRP has no benefit or even a negative effect on bone regeneration[33, 34].
In evaluating the benefits of double application of PRP on bone healing in rabbits, Özdemir et al [35] concluded that although new bone formation was histomorphologically remarkable at double-application PRP groups, statistical analysis revealed no significant difference. Our observations are supported by many other studies that evaluate the beneficial effects of multiple application of PRP for healing of cutaneous chronic wounds [36], diabetic foot ulcers [37]

and degenerative cartilage lesion [38].In sagittal suture distraction osteogensis in rabbit model, Xu et al [39]found that autologus PRP gel induces suture bone formation without suture fusion and accelerates the suture wound healing.

Aghaloo et al [40] evaluated PRP delivery to cranial defects in rabbits and found that PRP didn't show significant advantages over empty control defects in both histological and radiographic examinations. Miloro et al [41] also found that the application of PRP alone demonstrated no statistically significant benefit to healing in an osteotomized defect in mandibular defects of rabbits.

The conflictive results of the different studies about the benefits of PRP may be due to different defect models, different evaluation methods and periods. In our study the sagittal suture expansion model is used which is completely different from other models that use surgically created defects or osteotomies. In addition, the present study depends on using human PRP rather than using autologous PRP of rabbits. This is because we intended to study the effects of human PRP in animal model as a base for further clinical studies.

Conclusions

The effects of local application of human platelet rich plasma on bone formation of orthopaedically expanded sagittal suture of rabbits were examined histologically, radiographically and clinically. A relapse ratio of the expanded suture was 24%, 10%, and 7% in the control, single hPRP, and double hPRP application groups respectively The difference was highly significant between the control and double hPRP groups, but not between single and double hPRP groups.

The radiographic density of the expanded suture was significantly higher in the double application of hPRP group than the single hPRP and control groups. Histologically, the double application group showed islands of osteoid tissue in the central part of the expanded suture; in addition to finger like projection of bone invading from the suture margins toward the central part of the suture. The single hPRP group also showed finger like bony projections, but the islands of osteoid tissue were seen in the double group.

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Biography

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