

**Evaluation of Alveolar Bone Healing With the Use of Autologous Platelet Rich Plasma in Extraction Sockets: A Cone-Beam Computed Tomographic Study**

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**Citation of this Article:** Dr Namrata Adhauiliya, Dr I M Ali, Dr Anupama N K, Dr Rajeshwari G. Annigeri, Dr K.S. Srivatsan, Dr Karan Punn, “Evaluation of Alveolar Bone Healing With the Use of Autologous Platelet Rich Plasma in Extraction Sockets: A Cone-Beam Computed Tomographic Study”, IJDSIR- November - 2020, Vol. – 3, Issue - 6, P. No. 01 – 15.

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**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

**Abstract**

**Introduction:** Extraction-socket is a wound which undergoes a process of healing and regeneration. Several techniques aiming at enhancing regeneration process in extraction-socket have been adopted. Platelet-rich plasma (PRP), an autologous concentrate of platelets in a small volume of plasma, promotes soft and hard tissue healing. Literature provides contradictory effects of PRP on bone regeneration. This study explores whether and to what extent application of autologous-PRP to an extraction-socket may influence bone regeneration, as assessed by

Cone-Beam Computed Tomography(CBCT), a modality of radiographic diagnosis considered superior to 2-D imaging and a better alternative to existing 3-D modalities.

**Methods:** Premolar sites undergoing orthodontic extraction were considered. Immediately after extraction, sockets on one side received PRP (Group I), whereas the other side(Group II) was left to spontaneous healing. Patients underwent 3-months of radiographic follow-up. Bone regeneration in terms of regenerated bone volume and density variation was assessed on CBCT images.

**Results:** Increase in bone density over 3-months was statistically highly significant, p-value(0.001) for group I in both jaws. Statistically significant p-values 0.005(maxilla) and 0.002(mandible) were obtained in group II. Regenerated bone volume values were statistically highly significant with p-value(0.001) for both groups in both jaws. On intergroup comparison, there was no significant difference with respect to the parameters in either jaw over respective time-intervals.

**Conclusion:** Autologous-PRP plays a role in enhancing bone regeneration of extraction-sockets although its superiority to natural healing in atraumatic extractions could not be established. CBCT was found to be a valuable diagnostic tool in evaluation of socket regeneration.

**Keywords:** CBCT, PRP, Regenerated Bone Volume, Socket Healing, Wound Healing

### **Introduction**

Oprah Winfrey once remarked, “Turn your wounds into wisdom.” The idea can be remolded by pouring some scientific wisdom into the wounds and appraising the outcomes. Wounds are common in oral cavity. Wound healing in oral cavity starts with the blood clotting, which initially seals the wound. Platelet activation during the primary hemostasis releases a number of cytokines that start the healing process via chemotactic signals to inflammatory and resident cells.[1] One of the most common dental procedures is tooth extraction. The extraction of a tooth initiates a series of reparative processes involving both hard tissue (i.e. alveolar bone) and soft tissues (periodontal ligament, gingiva).[2] The healing of an extraction wound is modified by the peculiar anatomic situation, which exists after the removal of a tooth.[3]

Immediately following extraction, a healing process begins that affects the eventual alveolar bone volume and

ridge architecture. Timely healing is essential to obtain ideal functional reconstruction and tooth support. Patients and clinicians can benefit if a simple, cost-effective technique is developed that decreases bone-healing time. Autologous platelet rich plasma (PRP) has been widely considered as an effective method for improving bone formation.[4] PRP contains a cocktail of growth factors including transforming growth factor- $\beta$  (TGF- $\beta$ 1 and TGF- $\beta$ 2), vascular endothelial growth factor, platelet-derived growth factor and epithelial growth factor.[5] These are believed to accelerate chemotaxis, mitogenesis, angiogenesis, synthesis of collagen matrix and favour tissue repair when applied on bone wounds and reduce postoperative pain after extraction.[6,7] Several studies have shown contradictory results on the effect of PRP on bone healing but most of the studies found favorable results in terms of acceleration potential of PRP on bone healing and new bone formation.

The efficacy of PRP in alveolar socket healing has been assessed in literature using various methods including:[8] Clinical assessment by either self-evaluation or by observer evaluation; histological analysis and radiological analysis.

Radiographic evaluation of the dental and periodontal tissues is a critical segment of the comprehensive oral examination and diagnosis. A variety of radiographic tools have been used to evaluate socket healing, which include the following- Periapical radiographs, Panoramic radiographs, Digital Subtraction Radiography, Cone beam Computed Tomography (CBCT), Computed Tomography (CT), Micro-CT analysis and Static phase scintigraphic evaluation. The limitations of the two-dimensional imaging modalities have been surmounted with the advent of three-dimensional CBCT technique for structures in the craniofacial complex. This novel technique has initiated a new era in the field of dento-maxillofacial radiology,

owing to the acquisition of large data volume in a short scan time and at low radiation dose.[9] The effective dose of CBCT ranges from 25-1025 $\mu$ Sv (ICRP 2007). CBCT offers higher spatial resolution and image sharpness. Reduction in the slice thickness to tens of micrometers, results in accurate demonstration of even the fine structures of the dento- maxillofacial complex.[10] CBCT appears to offer an effective, non-invasive and relatively low radiation technique for assessment of dimensional, volumetric and densitometric changes in the alveolar ridge and extraction socket.[11]

The present study was designed to monitor the radiographic alveolar socket healing, using CBCT in tooth extraction sites packed with autologous platelet rich plasma as compared to the sites undergoing physiologic socket healing. Thus, this study aims to evaluate the role of autologous PRP in bone regeneration of extraction sockets using CBCT as a tool.

### **Materials And Methods**

The present prospective clinical study was conducted on patients undergoing orthodontic extraction of premolars in all four quadrants referred from Department of Orthodontics and Dentofacial Orthopedics provided they met the inclusion and exclusion criteria. A minimum of 30 extraction-sockets were determined as the statistically significant sample. The study encompassed 40 extraction-sockets allowing for drop-outs. Extraction-sockets were randomly divided into two groups in each patient namely the experiment/PRP group and the control group, in a split-mouth design.

### **Inclusion Criteria**

1. Subjects who were physically healthy and well oriented in time, place and as a person
2. Healthy individuals in the age group 15-25 years planned for orthodontic extraction of premolars in all four quadrants

3. Unremarkable medical history
4. Willingness to comply fully with protocol time-line and procedural requirements
5. Able to cognitively understand the proposed study therapy

### **Exclusion Criteria**

1. Patients with uncontrolled diabetes, on anticoagulant therapy, immunosuppressive therapy, suffering from, platelet disorders and other systemic diseases
2. Smoker
3. Patients with oro-sinusal communication
4. Endodontic periapical lesions with diameter > 5 mm (radiographically determined)
5. Periodontally involved teeth
6. Pregnant women
7. Subjects with previous history of any bony pathoses
8. Patients on any medication that may adversely affect bone healing

All participants were explained the need and design of the study, the benefits and the possible adverse effects. Out of the 40 sockets, 8 sockets could not be evaluated for all the follow ups and hence **32 sockets** were finally considered.

### **Methodology**

The subjects undergoing orthodontic extractions bilaterally in either maxillary or mandibular arch or in all four quadrants were selected. Patient's data was collected in relation to age, gender, smoking habits, tooth location, and indication for extraction. Patients were also examined for endodontic and periodontal health. A written informed consent was obtained after explaining the procedure. The first visit consisted of collecting the demographic data, general history, patient's past dental history, clinical and radiographic examinations. Extraction sites were randomly distributed into group I (PRP group) and group II (non-PRP group).

### **Preparation of PRP**

A venous puncture was done in the ante-cubital fossa using a 21-gauge latex-free needle. Whole blood was drawn using a 10 mL syringe. 4.5 mL of blood was then transferred into 2 test tubes each containing 0.45 mL of the anticoagulant tri-sodium citrate (9: 1). The test tubes were centrifuged for 10 minutes at 3000 rpm. After centrifugation the red blood cell/plasma interface was allowed to set for 3 minutes. Premade labels with a central dotted line was positioned at the blood cell/plasma interface, a solid line 3 mm above, and a second solid line 2 mm below. The upper plasma layer (platelet poor plasma [PPP]) was aspirated to the 3 mm mark (top solid line) using a sterile bevelled 20-gauge needle attached to a 5mL latex-free syringe. The PRP was collected from between the upper 3 mm mark and the lower 2 mm mark using a separate bevelled 20-gauge needle.

### **Extraction technique and PRP placement**

The extraction procedure was carried out by administering local anaesthesia obtained using lignocaine hydrochloride with epinephrine (1:80,000). Tooth extraction was performed using extraction forceps by closed extraction technique. The extraction socket was then inspected for the presence of bony septum. The resulting socket was carefully curetted to remove any granulation tissue and also examined for deficiencies of the socket bony walls. In the subjects, the extraction sockets of same side were filled with gelfoam soaked in PRP clot and left without suturing (Group I). No attempt was undertaken to approximate the gingival margins of the socket. Extractions were performed for group II teeth using the same protocol. In the group II, the wound was equally examined and left as such allowing the creation of a naturally forming blood clot after controlling any possible haemorrhage with homeostatic gauzes. The patients underwent a baseline CBCT scan using standardized

positioning and irradiation protocol. All the patients were prescribed acetaminophen only on the day of extraction.

### **Acquisition of image by CBCT**

CBCT scans were obtained with Planmeca Promax 3-D machine. Sterile wet cotton rolls were placed in the region of interest in cases where the patients had orthodontic brackets and wires bonded to the adjacent teeth, to reduce the metallic artifacts. Field of view (FOV) was selected to medium and adjusted according to the patient selection. The patient was made to sit upright with chin resting on chin rest and Frankfort horizontal plane parallel to the floor. Standardized positioning and irradiation protocols were followed. Once a scout image of the region of interest was obtained, patient was subjected for final exposure. After the basis projection frames were obtained, data was processed to volumetric data. Several hundreds of individual projection frames were obtained comprising of millions of pixels with 12 to 16 bits of data designated for each pixel. The raw images from CBCT detectors were corrected for pixel defects, image normalization and removal of inherent electronic detector artifacts. After the images were corrected, they were related to each other and assembled using filtered back projection algorithm for acquiring volumetric data. Once all the slices were reconstructed, they were combined into a single volume for visualization. Finally, the volumetric data was displayed as secondary reconstructed images in three orthogonal planes, i.e. axial, sagittal and coronal on a 1024 x 1280 resolution LED monitor. The image analysis was done under dim light.

### **Follow up**

The steps for CBCT imaging were repeated at the follow up visits (end of first- and third-months following extraction) using the same protocol and were carried out by the same examiner at all the visits for all the patients.

Regenerated bone volume (RBV):

CBCT scans taken at baseline, after 1month and after 3months of extraction were imported into Romexis software. The selected axial section was the one in which there was visual inclusion of the whole boundaries of the socket. The image was sectioned into 2mm slice thickness and viewed in the corresponding coronal plane. The measurements were carried out at the crestal level. The longest distance from the mid-point of the most coronal width to the deepest apex of the socket was considered (Photograph 1). In order to calculate the defect volume, extraction socket was considered as a cone and the following equation was applied:  $V = \frac{1}{3} \pi r^2 h$ . Where V is the volume of the extraction socket, r is half of the most coronal width of the socket, and h is the apico-coronal distance. When an inter-radicular septum was present, the total volume of the extraction socket was calculated as the sum of the volumes of the buccal and palatal root. The volumes for the three scans were termed V1, V2, V3 for baseline, end of first month and end of third month respectively. The new “non-regenerated” socket volume was calculated on the CBCT scan obtained 1month and 3months after surgery as described above. The regenerated bone volume was then calculated by the following equation:  $RBV1 = \frac{(V1-V2)}{V1} \times 100$ . Where RBV1 is regenerated bone volume at end of first month following extraction, V1 is socket volume at baseline, and V2 is “non-regenerated” volume at end of 1month. Similar formulae were applied to calculate the RBV at end of 3 months after extraction as compared to baseline and as compared to end of 1month respectively.

Bone density (BD):

The Hounsfield value of the regenerated socket was determined using axial section in the CBCT scan obtained at baseline, 1 month and 3 months after extraction (Photograph 2). The measurement was performed using a

rectangle of 2.25mm x 1.35mm while avoiding the positioning of the rectangles at the boundaries of the socket. The upper border of the rectangle was positioned at 2mm below the crest and at minimum distance of 2mm from the most apical extension of the socket. When an inter-radicular septum was present, bone density was measured in the socket of the palatal root.

### Statistical analysis

Statistical analysis was carried out using the SPSS package (version 21). Results were expressed as median (first quartile to third quartile). Friedman test and Wilcoxon sign rank test were used for intragroup comparisons followed by Mann-Whitney U test for inter-group comparisons. For all the tests, a p-value of 0.05 or less was considered as statistically significant.

### Results

#### Age, Gender and Site Wise Distribution

The patients enrolled in the study were in the age range of 15-23years. 6 patients were females and 2 were males. The sockets (either right or left) were divided randomly into group I and group II in both maxillary and mandibular arches. Thus, out of the 32 sites evaluated 16 were maxillary and 16 mandibular sites, with 8 extraction sockets under each group.

#### Intragroup Comparison (Group I Maxilla) of variation in the parameters over different time intervals (Table 1)

The median score of bone density at baseline for group I was -17.92. During the follow up period, the median scores at end of first month, and end of third month were 116.38 and 466.23 respectively. During this period, chi-square distribution value for all the follow-ups was 14.25 with a p-value of 0.001, which was statistically highly significant. Similarly, the median score of regenerated bone volume at baseline was 17.77. While the median scores at end of first and third month follow up were

100.00 and 100.00 respectively. During this period, chi-square distribution value for all the follow-ups was 15.08 with a p-value of 0.001, which was statistically highly significant.

A Friedman test was used to compare the variation of parameters (BD & RBV) over time. The difference in the parameters over Day 0, one-month and three-month intervals was found to be significant.

#### **Intragroup Comparison (Group II Maxilla) of variation in the parameters over different time intervals (Table 2)**

The median score of BD at baseline was 94.80. The median scores at end of first month, and end of third month were 218.99 and 458.82 respectively. Chi-square distribution value for all the follow-ups was 10.75 with a p-value of 0.005, which was statistically highly significant. The median score of RBV at baseline was 37.09 and at end of first month, and end of third month were 100.00 and 100.00 respectively. Chi-square distribution value for all the follow-ups was 14.77 with a p-value of 0.001, which was statistically highly significant.

A Friedman test was used to compare the variation of parameters over time, a significant difference was obtained in the parameters over Day 0, one-month and three-month intervals.

#### **Intragroup Comparison (Group I Mandible) of variation in the parameters over different time intervals (Table 3)**

The median score of BD at baseline for group I was 67.61. The median scores at end of first month, and end of third month were 176.79 and 727.73 respectively. Chi-square distribution value came out to be 14.25 with a p-value of 0.001, which was considered statistically highly significant. The median score of RBV at baseline was 28.26 and at end of first month, and end of third month

were 100.00 and 100.00 respectively. The chi-square distribution value for all the follow-ups was 15.08 with a p-value of 0.001, which was statistically highly significant.

According to Friedman test there was a significant difference in all the study parameters over Day 0, 1-month and 3-month intervals.

#### **Intragroup Comparison (Group II Mandible) of variation in the parameters over different time intervals (Table 4)**

The median score of BD at baseline for group I was 148.58 while those at the end of first month and third month were 182.39 and 559.00 respectively. During this period, chi-square distribution value for all the follow-ups was 12.25 with a p-value of 0.002, which was statistically highly significant. The median score of RBV on the day 1 was 27.94 and at end of first and third month were 100.00 and 100.00 respectively. The chi-square distribution value for all the follow-ups was found to be 15.08 with a p-value of 0.001, which was statistically highly significant.

According to Friedman test there was a significant difference in all the study parameters over Day 0, 1-month and 3-month intervals.

#### **Comparison of BD between the groups in maxilla (Table 5, Graphs 1-3)**

The results are presented in terms of the Median values and represented as Median (Q1-Q3) where Q1 is first and Q3 is the third quartile. A Mann Whitney U test was used to determine the differences in BD of the study and control groups in maxilla. The median BD score or increase in bone density was not statistically significant between the study and control group at either Day 0, 1 month or 3month time intervals.

#### **Comparison of BD between the groups in mandible (Table 6, Graphs 1-3)**

The median BD score according to Mann Whitney U test, was not statistically significant between the study and control group at either Day 0, 1 month or 3month time intervals.

**Comparison of RBV between the groups in maxilla (Table 7, Graphs 4-6)**

According to Mann Whitney U test, the median RBV was not statistically significant between the study and control group at either Day 0, 1 month or 3month time intervals.

**Comparison of RBV between the groups in mandible (Table 8, Graphs 4-6)**

According to Mann Whitney U test, the median RBV was not statistically significant between the study and control group at either Day 0, 1 month or 3month time intervals.

**Pairwise comparison between time-intervals in maxilla and mandible for groups (Table 9)**

Wilcoxon sign rank test was used to make non-parametric comparisons between the various time intervals in each of

the study parameters. Statistically significant differences were obtained for gain in BD in maxilla between 1 month-Day 0 (p= 0.01) and 3 months-Day 0 (p= 0.01) for the study group. Statistically significant results were also obtained for the control group, p= 0.02 & p= 0.01 between 1 month-Day 0 and 3 months-Day 0 respectively. Statistically significant differences were also obtained for BD in mandible between 1 month-Day 0 (p= 0.03) and 3 months-Day 0 (p= 0.01) for the study group. A non-significant difference was obtained in control group between 1 month-Day 0 (p= 0.48). Statistically significant result (p= 0.01) was obtained between 3 months-Day 0.

Statistically significant differences were obtained for RBV in maxilla between 1 month-Day 0 and 3 months-Day 0 (p = 0.01) in both study and control groups. Similar results were obtained in mandible between 1 month-Day 0 and 3 months-Day 0 (p = 0.01) for both study and control groups.

Table 1: Comparison of variation in the parameters over different time intervals in study group in maxilla

Group – 1		N	Median (Q1-Q3)	Friedman test	
				Chi square value	p-value
BD	Day 0	8	-17.92	14.25	0.001*
	1 month	8	116.38		
	3 months	8	466.23		
RBV	Day 0	8	17.77	15.08	0.001*
	1 month	8	100.00		
	3 months	8	100.00		
VAS	Day 1	8	1.50	9.29	0.01*
	Day 3	8	0.00		
	Day 7	8	0.00		

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 2: Comparison of variation in the parameters over different time intervals in control group in maxilla

Group – 1		N	Median (Q1-Q3)	Friedman test	
				Chi square value	p-value
BD	Day 0	8	94.80	10.75	0.005*
	1 month	8	218.99		
	3 months	8	458.82		
RBV	Day 0	8	37.09	14.77	0.001*
	1 month	8	100.00		
	3 months	8	100.00		
VAS	Day 1	8	2.00	14.86	0.001*
	Day 3	8	0.50		
	Day 7	8	0.00		

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 3: Comparison of variation in the parameters over different time intervals in study group in mandible

Group - 2		N	Median (Q1-Q3)	Friedman test	
				Chi square value	p-value
BD	Day 0	8	67.61	14.25	0.001*
	1 month	8	176.79		
	3 months	8	727.73		
RBV	Day 0	8	28.26	15.08	0.001*
	1 month	8	100.00		
	3 months	8	100.00		
VAS	Day 1	8	0.50	7.54	0.02*
	Day 3	8	0.00		
	Day 7	8	0.00		

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 4: Comparison of variation in the parameters over different time intervals in control group in mandible

Group - 2		N	Median (Q1-Q3)	Friedman test	
				Chi square value	p-value
BD	Day 0	8	148.58	12.25	0.002*
	1 month	8	182.39		
	3 months	8	559.00		
RBV	Day 0	8	27.94	15.08	0.001*
	1 month	8	100.00		
	3 months	8	100.00		
VAS	Day 1	8	1.00	11.20	0.004*
	Day 3	8	0.00		
	Day 7	8	0.00		

\*p<0.05 statistically significant

p>0.05 Non significant, NS



Table 5: Comparison of BD between the groups in maxilla

Teeth extracted		N	Percentiles			U Statistic	p-value
			Q1	Median	Q3		
BD - Day 0	Study	8	-84.19	-17.92	85.68	19.00	0.17(NS)
	Control	8	-12.06	94.80	106.39		
BD 1 month	Study	8	-5.50	116.38	377.74	25.00	0.46(NS)
	Control	8	152.70	218.99	313.38		
BD 3 months	Study	8	326.25	466.23	511.46	30.00	0.83(NS)
	Control	8	186.41	458.82	685.90		

Mann Whitney U test

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 6: Comparison of BD between the groups in mandible

Teeth extracted		N	Percentiles			U statistic	p-value
			Q1	Median	Q3		
BD - Day 0	Study	8	-212.09	67.61	222.71	22.00	0.29(NS)
	Control	8	16.19	148.58	344.09		
BD 1 month	Study	8	59.93	176.79	303.90	32.00	1.00(NS)
	Control	8	28.17	182.39	348.72		
BD 3 months	Study	8	376.21	586.18	727.73	30.00	0.83(NS)
	Control	8	289.35	559.00	713.04		

Mann Whitney U test

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 7: Comparison of RBV between the groups in maxilla

Teeth extracted		N	Percentiles			U Statistic	p-value
			Q1	Median	Q3		
RBV Day 0 (RBV1)	Study	8	6.99	17.77	25.91	16.00	0.09(NS)
	Control	8	17.19	37.09	54.78		
RBV 1 month (RBV2)	Study	8	91.73	100.00	100.00	32.00	1.00(NS)
	Control	8	95.67	100.00	100.00		
RBV 3 month (RBV3)	Study	8	89.83	100.00	100.00	31.00	0.89(NS)
	Control	8	89.53	100.00	100.00		

Mann Whitney U test

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 8: Comparison of RBV between the groups in mandible

Teeth extracted		N	Percentiles			U statistic	p-value
			Q1	Median	Q3		
RBV Day 0 (RBV1)	Study	8	2.97	28.26	47.89	31.00	0.92(NS)
	Control	8	13.89	27.94	43.24		
RBV 1 month (RBV2)	Study	8	98.47	100.00	100.00	32.00	1.00(NS)
	Control	8	98.61	100.00	100.00		
RBV 3 month (RBV3)	Study	8	94.99	100.00	100.00	32.00	1.00(NS)
	Control	8	97.78	100.00	100.00		

Mann Whitney U test

\*p<0.05 statistically significant

p>0.05 Non significant, NS

Table 9: Pairwise Comparison between time intervals

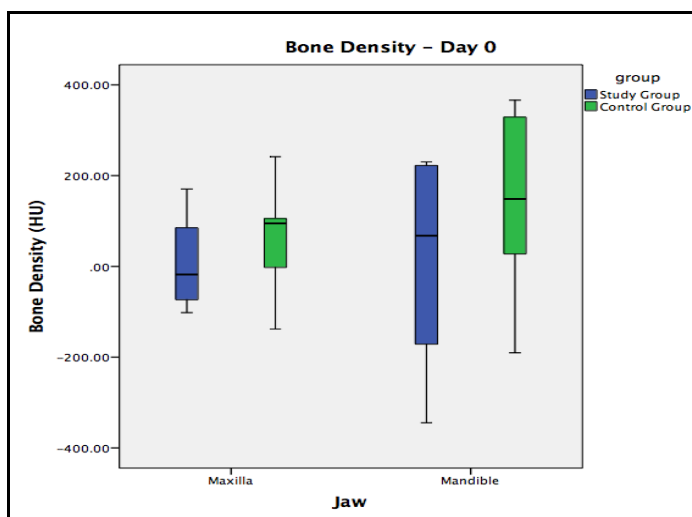
	Group	Maxilla				Mandible			
		Study group		Control group		Study group		Control group	
		Z	p-value	Z	p-value	Z	p-value	Z	p-value
BD	1 month - Day 0	-2.524	0.01*	-2.383	0.02*	-2.243	0.03*	-.701	0.48(NS)
	3 months - Day 0	-2.524	0.01*	-2.524	0.01*	-2.524	0.01*	-2.524	0.01*
RBV	RBV2 - RBV1	-2.521	0.01*	-2.521	0.01*	-2.521	0.01*	-2.521	0.01*
	RBV3 - RBV1	-2.521	0.01*	-2.521	0.01*	-2.521	0.01*	-2.521	0.01*

Wilcoxon sign rank test

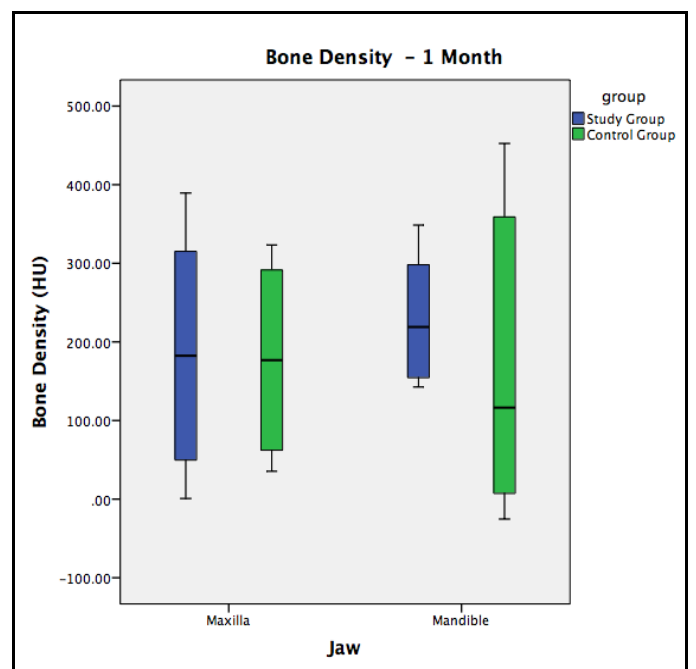
\*p<0.05 statistically significant

p>0.05 Non significant, NS

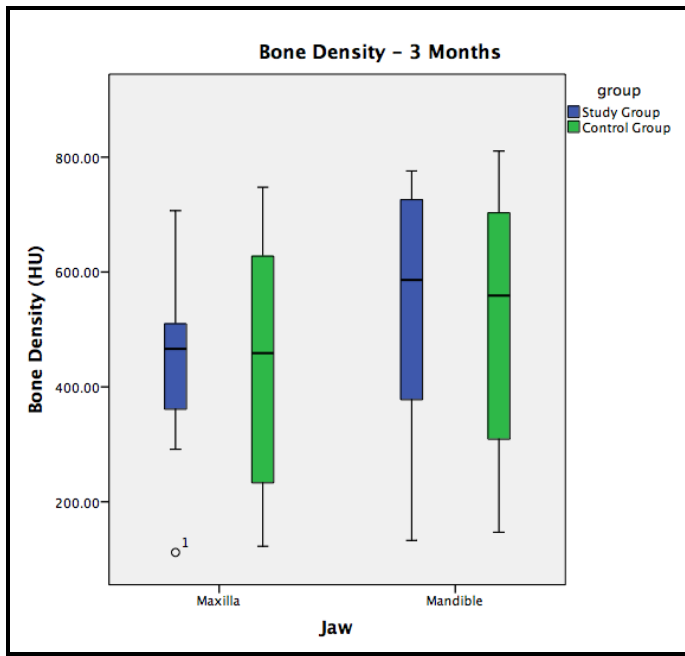
Graph 1-3: Box-and-Whisker plots for Comparison of Bone density between the groups in maxilla and mandible at Day 0, 1 Month, 3 Months.



Graph 1

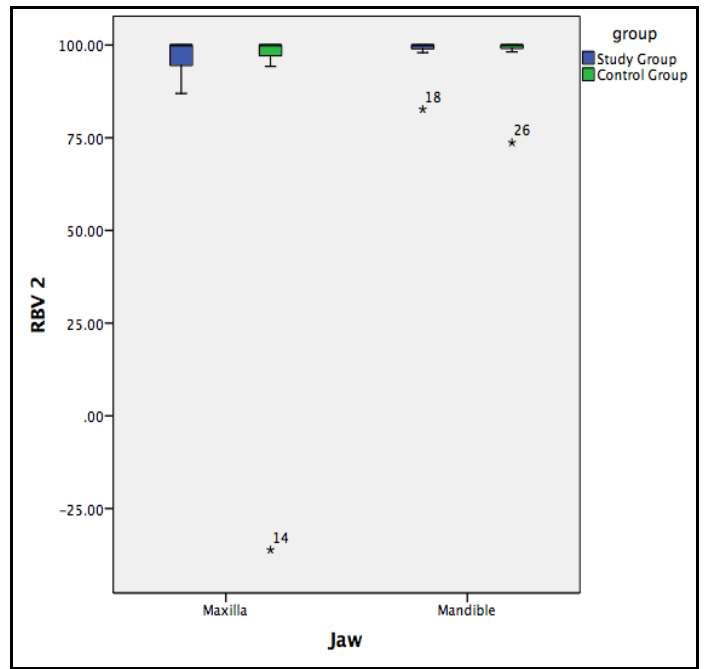


Graph 2

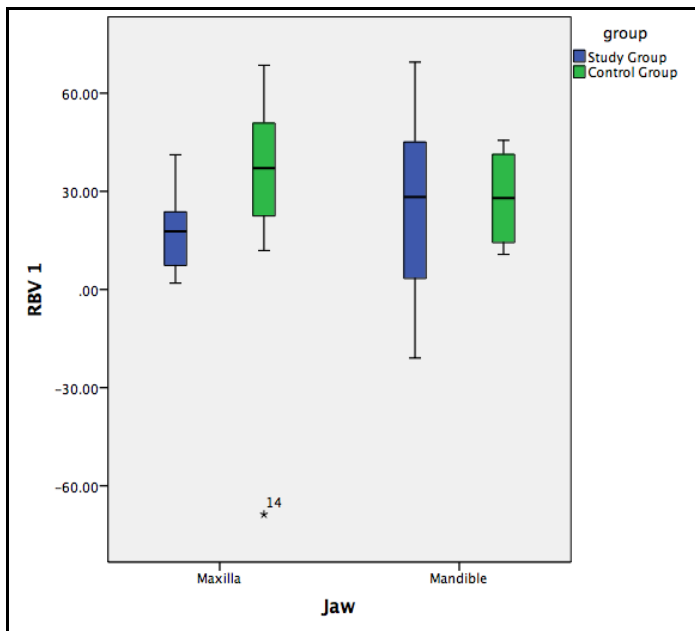


Graph 3

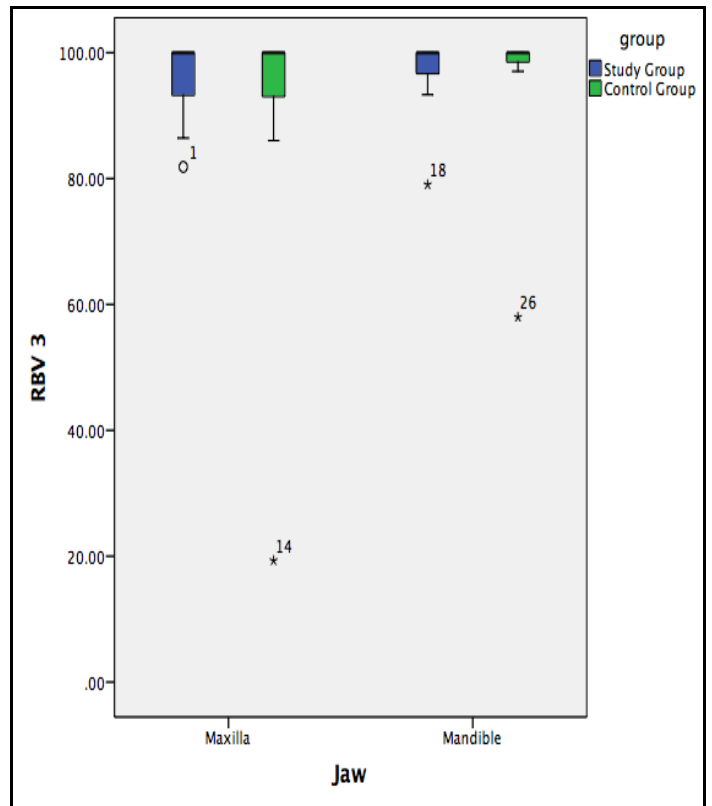
Graph 4 – 6: Box-and-Whisker plots for Comparison of Regenerated Bone Volume between the groups in maxilla and mandible at Day 0, 1 Month, 3 Months



Graph 5



Graph 4



Graph 6



Figure 1: Coronal Section of Mandibular Socket for Radius and Height Measurements

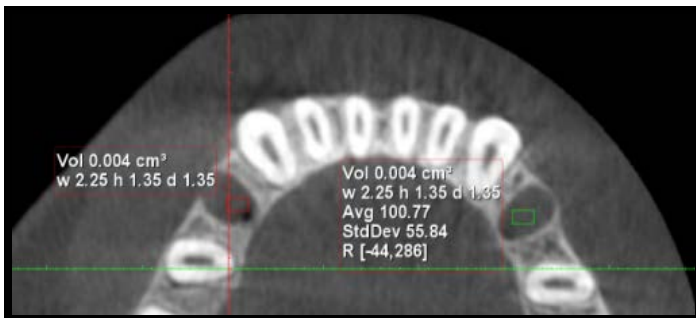


Figure 2: Axial Section Image of Mandibular Sockets for Density Measurements

## Discussion

Platelet-rich plasma has been used in a variety of clinical situations, ranging from filling post-extraction sockets to bone regeneration or sinus lift surgeries. It is claimed to alleviate pain and inflammation, promote the epithelialization of soft tissues and bone regeneration. [12,13] Although there are many authors who extol the virtues of PRP use, there are few randomized clinical trials and the results are controversial.[14]

Several methods have been employed to study the bone regeneration in the extraction socket, but radiology provides a non-invasive, easy and reliable alternative. Amongst the various radiographic techniques, the use of CBCT has taken precedent in the recent years, as it is a 3-

D technique with comparatively less radiation exposure while providing good image details in terms of good spatial resolution, gray density range, contrast, and a good pixel/noise ratio. The dimensional accuracy is also comparable with CT, but the gray density values of the CBCT images (voxel value [VV]) are not absolute.[15] Bone density in Hounsfield units using CBCT can be measured with a high degree of accuracy and reproducibility.[16]

## Evaluation of individual parameters

Role of PRP in bone regeneration:

In the present study, the study group (both in maxilla and in mandible) successfully achieved the goal of fast healing in terms of both BD measurements and RBV by producing statistically highly significant scores at the end of first and third months. Similarly, the control group also showed significant gain in BD & RBV at the end of first and third months but on intergroup comparison, there was no statistically significant difference between the groups with regards to the above-mentioned parameters.

The results are in agreement with a study which concluded that the application of PRP alone into soft tissue impacted mandibular third molar extraction sockets failed to increase the osteoblastic activity in postsurgical weeks 1 and 4 in comparison to non-PRP-treated sockets.[17] Another study did not find any statistically significant differences in bone volume or the amount and density of mineral tissue between the test and control groups at 8 weeks.[4] Another author mentioned that PRP enhanced the osteogenic response in initial bone healing at 1 month duration but there was no added benefit in late bone healing at 4 months period compared in both intervention and control groups.[18] On the other hand, a recent study showed that autologous PRP is biocompatible and significantly improved bone regeneration and bone density in extraction sockets.[19]

To the best of our knowledge, the effect of PRP on maxillary extraction socket healing has previously been evaluated only in one study by means of periapical radiographs. Also, most studies have used extraction sockets of mandibular third molars following surgical removal whereas we have considered premolar extraction sockets which underwent atraumatic extraction using forceps technique. In agreement with several previous studies there was statistically non-significant difference seen in healing rate or density of bone formed in the extraction sockets with the application of autologous PRP as compared to the sockets which were left to heal naturally, in either jaw.

The values of BD in maxilla showed statistically greater significance when compared to those in mandible, in both the groups (more significant in the study group in both jaws). The results of mandibular control socket were non-significant between baseline and end of 1month. This is in agreement with a study where the authors detected greater differences in rate of healing in the maxilla as compared to mandible.[20] But it is found to be in contrast to the authors of another study who have found rate of regeneration of the mandible after three weeks was higher than that of the maxilla and they have speculated that mandible is subjected to higher mechanical forces and consequently has a higher rate of healing than the maxilla.[21] This is in dissimilar to our study and the reason is hypothesized to be due to the application of orthodontic forces which might have hastened the healing in maxilla. Also, maxilla may allow faster healing due to less compact structural organization of the bone and richer blood supply as compared to that of the mandible.[20]

Role of CBCT in evaluation of socket healing/bone density:

CBCT was used to evaluate the healing of the extraction sockets by using two parameters on i.e., BD

measurements and the regenerated socket/bone volume. We found CBCT to be an easy, time-saving, reliable and reproducible technique for making the above-mentioned measurements and thus, for determining the socket volumes and alveolar bone density. The reliability of volumetric measurements obtained in our study is in agreement with other studies carried out using CBCT. A study was carried out to determine the accuracy of volumetric analysis of extraction sockets using direct measurement and CBCT imaging. No significant differences were seen between both volume measurements.[9] Another study, demonstrated the clinical usefulness of CBCT for evaluation of extraction sockets healing by volumetric fill up of extraction sockets.[22] In another study, CBCT values were in overall agreement with data reported by, other studies which had used stone models to study dimensional alterations of the ridge during socket healing.[23]

According to our study, the bone density evaluation can be carried out on CBCT with reliability and reproducibility. A study examined differences in bone density in periapical lesions in Hounsfield units (HUs) by using CBCT. The results supported the use of CBCT to measure bone density.[24] Another study concluded bone density assessment using CBCT is an efficient method.[25] Also as previously stated present day CBCT machines deliver radiation doses in equivalent or only slightly higher range compared to those of panoramic machines hence it is a better diagnostic modality in every aspect.

#### **Side effects**

None of the patients in our study reported any serious side effects at any stage of the study.

#### **Limitations**

The present study is a randomized clinical study. In the study single blinding was done as the patients were not aware on which side PRP was placed. Double blinding

was not feasible as the principal investigator and radiological examiner was the same person. Another major limiting factor was the sample size, which was small in the present study. The results were widely skewed because of this reason, hence we had to rely on the median rather than the mean values.

The lack of a standardized technique for preparation of autologous PRP can be considered as another limiting factor. There are several methods being used for the same and hence the results vary according to the platelet concentration produced. Also, there is no consensus on whether the platelets should be artificially activated or not before PRP is placed in the extraction-sockets.

#### **Future prospects**

Further studies are recommended on a larger sample of patients, possibly using a double blinded design and taking into consideration inter-observer variability. Also, in order to minimize the effects of confounding factors more standardized new generation platelet rich fibrin can be employed instead of platelet rich plasma.

#### **Conclusion**

In conclusion, the results from our study emphasize the need to standardize the technique by which PRP is prepared so that this invaluable resource can be harnessed to its full potential in regards to socket healing and bone regeneration. The results are highly encouraging in terms of the role played by CBCT as a diagnostic modality in evaluation of densitometric and volumetric changes in maxillary and mandibular sockets.

#### **References**

1. Farina R, Trombelli L. Wound healing of extraction sockets. In: Larjava H. (ed.) *Oral Wound Healing: Cell Biology and Clinical Management*. Chichester, West Sussex: John Wiley & Sons; 2012. Pg.195-228.
2. Farina R, Trombelli L. Wound healing of extraction sockets. *Endod Topics*. 2011;25(1):16-43.
3. Sivapathasundharam B. Healing of Oral Wounds. In: Rajendran and Sivapathasundharam (eds.) *Shafer's textbook of oral pathology*. 7<sup>th</sup> ed, New Delhi: Reed Elsevier; 2012. Pg.591-608.
4. Rutkowski JL, Johnson DA, Radio NM, Fennell JW. Platelet rich plasma to facilitate wound healing following tooth extraction. *J Oral Implantol*. 2010;36:11-23.
5. Marx RE. Platelet-rich plasma: Evidence to support its use. *J Oral Maxillofac Surg*. 2004;62:489-496.
6. Oyama T, Nishimoto S, Tsugawa T, et al. Efficacy of platelet rich plasma in alveolar bone grafting. *J Oral Maxillofac Surg*. 2004;62:555-558.
7. Olufemi K. O, Vincent I. Uand , Folusho J. Can Autologous Platelet-Rich Plasma Gel Enhance healing after surgical extraction of mandibular Third Molars?. *J Oral Maxillofac Surg*. 2011;69:2305-2310.
8. Del Fabbro M, Bortolin M, Taschieri S, Weinstein R. Is platelet concentrate advantageous for the surgical treatment of periodontal diseases? A systematic review and meta-analysis. *J. Periodontol*. 2011;82(8):1100-11.
9. Agbaje JO, Jacobs R, Maes F, Michiels K, van Steenberghe D. Volumetric analysis of extraction sockets using cone beam computed tomography: a pilot study on ex vivo jaw bone. *J Clin Periodontol* 2007; 34: 985–990. doi: 10.1111/j.1600-051X.2007.01134.x
10. Lubele M., Guerrero M.E., Jacobs R., et al. A comparison of jaw dimensional and quality assessments of bone characteristics with cone beam CT, spiral tomography and multi slice spiral CT. *Int. J. Oral Maxillofac. Implants*. 2007;22(3):446-454.
11. Arnheiter C, Scarfe WC, Farman AG. Trends in maxillofacial cone-beam computed tomography usage. *Oral Radiol* 2006;22:80–5.

12. Anitua E, Troya M, Orive G. Plasma Rich in Growth Factors Promote Gingival Tissue Regeneration by Stimulating Fibroblast Proliferation and Migration and by Blocking Transforming Growth Factor- $\beta$ 1-Induced Myodifferentiation. *J Periodontol.* 2012;83:1028-37.
13. Anitua E, Tejero R, Zalduendo MM, Orive G. Plasma rich in growth factors promotes bone tissue regeneration by stimulating proliferation, migration, and autocrine secretion in primary human osteoblasts. *J Periodontol.* 2013;84:1180-90.
14. Barona-Dorado C, González-Regueiro I, Martín-Ares M, Arias-Irimia O, Martínez-González JM. Efficacy of platelet-rich plasma applied to postextraction retained lower third molar alveoli. A systematic review. *Med Oral Patol Oral Cir Bucal.* 2014;19 (2):e142-8.
15. Arisan V, Karabuda ZC, Avsever H, Özdemir T. Conventional multi-slice computed tomography (CT) and conebeam CT (CBCT) for computer-assisted implant placement. Part I: relationship of radiographic gray density and implant stability. *Clin Implant Dent Relat Res.* 2013;15(6):907-17. doi: 10.1111/j.1708-8208.2011.00436.x.
16. Daif ET. Effect of autologous platelet-rich plasma on bone regeneration in mandibular fractures. *Dent Traumatol.* 2013;29:399-403; doi: 10.1111/edt.12021.
17. Gurbuzer B, Pikkoken L, Urhan M, Süer BT, Narin Y. Scintigraphic evaluation of early osteoblastic activity in extraction sockets treated with platelet-rich plasma. *J Oral Maxil Surg.* 2008;66(12):2454-60.
18. Gawai KT, Sobhana CR. Clinical evaluation of use of platelet rich plasma in bone healing. *J. Oral Maxillofac. Surg.* 2015;14(1):67-80.
19. Vivek GK, Sripathi Rao BH. Potential for osseous regeneration of platelet rich plasma: a comparative study in mandibular third molar sockets. *J Maxillofac Oral Surg* 2009;8:308-11.
20. Antonello G, Torres do Couto R, Giongo C, Corrêa M, Chagas Júnior O, Lemes C. Evaluation of the effects of the use of platelet-rich plasma (PRP) on alveolar bone repair following extraction of impacted third molars: Prospective study. *J Craniomaxillofac Surg.* 2013;41(4):e70-e75.
21. Kotze MJ, Bütow KW, Olorunju SA, Kotze HF. A comparison of mandibular and maxillary alveolar osteogenesis over six weeks: a radiological examination. *Head Face Med.* 2014;10(1):1.
22. Agbaje JO et al. Bone healing after dental extractions in irradiated patients: a pilot study on a novel technique for volume assessment of healing tooth sockets. *Clin Oral Invest.* 2009; 13:257-61. DOI: 10.1007/s00784-008-0231-7.
23. Misawa M, Lindhe J, Araujo MG. The alveolar process following single-tooth extraction: a study of maxillary incisor and premolar sites in man. *Clin. Oral Impl. Res.* 2016;27:884-89. doi: 10.1111/clr.12710.
24. Kaya S et al. Measuring Bone Density in Healing Periapical Lesions by Using Cone Beam Computed Tomography: A Clinical Investigation. *J Endod* 2012;38(1):28-3
25. Salimov F et al. Evaluation of relationship between preoperative bone density values derived from cone beam computed tomography and implant stability parameters: a clinical study. *Clin. Oral Impl. Res.* 2014;25(9):1016-21. doi: 10.1111/clr.12219.