Effect of Platelet Rich Plasma on Osseo Integration of Dental Implants by Texture Based Evaluation of Patient's Dental Panoramic Radiographs - An In Vivo Study

Dr. Guljot Singh, [1] Dr. Sabiya Sheikh, [2] Dr. Shivani Jain, [3] Dr. Kalyani P Goswami, [4] Dr. Jyoti Beniwal [5]

1. **Dr. Guljot Singh**
   Principal and Head, Department of Periodontology & Implantology, Daswani Dental College & Research Centre, Kota, Rajasthan, India.
2. **Dr. Sabiya Sheikh**
   PG Student, Department of Periodontics & Implantology, Daswani Dental College & Research Centre, Kota, Rajasthan, India.
3. **Dr. Shivani Jain**
   Senior Lecturer, Department of Periodontics & Implantology, Daswani Dental College & Research Centre, Kota, Rajasthan, India.
4. **Dr. Kalyani P Goswami**
   PG Student, Department of Periodontics & Implantology, Daswani Dental College & Research Centre, Kota, Rajasthan, India.
5. **Dr. Jyoti Beniwal**
   Research Assistant, Dr. Harvansh Singh Judge Institute of Dental Sciences Hospital, Sector 25, South Campus, Panjab University, Chandigarh

Abstract

Aim: To analyse the effect of application of autologous Platelet-Rich-Plasma (PRP) on the osseointegration of implants using texture -based analysis of the patient's sequential dental panoramic radiographs.

Methods: Twenty Partially edentulous patients without any localized or generalized pathology in the implant region were selected for this randomised control clinical trial. Patients were equally alloted in two treatment groups: Group A [test group] included 10 patients, who received endosseous implants exposed to autologously extracted platelet rich plasma and GROUP B [control group] included 10 patients, in whom endosseous implants NOT exposed to autologously extracted platelet rich plasma were placed. Effect of PRP application on the peri-implant bone regeneration and osseointegration of the implant was analysed by texture-based analysis of orthopantomograph at baseline, 3months and 6 months postoperatively. Following recording of sequential radiographs, Spatial Gray Level Dependance Method was used to analyse the radiographic difference in the osseointegration of implants belonging to the test and control groups.

Results: The study revealed that no significant difference was demonstrated between the osseointegration of an implant placed conventionally in a freshly created osteotomy site and treatment of the implant surface with platelet rich plasma extracted autologously from the patient's own venous blood sample.

Conclusion: Exposure of an implant surface to autologously generated PRP extract does not have a significant effect on the osseointegration of implants.

Keywords: Dental Implants, Osseointegration, Platelet Rich Plasma, Panoramic Radiographs

INTRODUCTION

Implant treatment mainly aims at functional restoration of stomatognathic system which provides comfort and health for the patient. Osseointegration forms basis of implant success. Platelet plays a fundamental role in the early stages of wound healing and bone regenaration by releasing growth factors and other molecules. Platelet rich plasma is one such autologous source, rich in various growth factors whose release continues for 7 days.

This study evaluates the use of PRP in osseointegrated implant sites & the study effect was analysed using the Spatial Gray Level Dependence Method (SGLDM) involving texture-based analysis of the patient's sequential dental panoramic radiograph.

MATERIAL AND METHODOLOGY

The present study was conducted on 20 patients who reported the OP department (OPD) of the Department of Periodontics & Implantology, Daswani Dental College & Research Centre, Kota for replacement of missing teeth. All the patients included in the study fulfilled the eligibility criteria

INCLUSION CRITERIA - Inclusion criteria was relatively open ended so as case selection could be possible within the limits of standardization. Age limit was 18 years to 60 years to encompass all common causes of tooth loss requiring implant therapy for replacement such as gross decay, trauma, bone loss as well as age related tooth loss. To prevent any compromise in the prosthetic superstructure fabrication, the mesiodistal diameter of the edentulous span was defined to an 8 to 10mm range to cover both anterior and posterior jaw regions. Good to fair oral hygiene was considered (OHI-S: 0.0 to 2.0), as described by Green et al (1964). Bone height for implant placement was kept in the range of 8mm to 15mm to include all/most possible implant sizes

EXCLUSION CRITERIA - Patients with adverse occlusal habits such as bruxism were excluded to prevent possible future implant overload. Patients with higher values of OHI-S along with periodontally, Medically, psychiatrically and physically compromised patients as well as pregnant subjects were excluded.

Formal consent was taken for all patients participating in the study with due explanation of all underlying procedures within the study such as implant surgery, radiography, implant prosthetic superstructure clinical procedures, etc. The study utilised orthopantomography (OPG) as a comparative sample for the analysis of efficacy of osseointegration for the two patient groups at baseline, 3 months and 6 months postoperatively. Sequentially recorded radiographs of each subject were evaluated for differences in peri-implant bone regeneration in the test and control groups via Spatial Gray Level Dependance Method (SGLDM) involving texture based analysis of the radiographs . This involved a vertical pixel to pixel comparative analysis of the gray level segregation of the radiographic image through comparison of the spatial distribution of gray focus levels in the regions of interest i.e. the immediate 500-1000µm radius around the placed implant length. The ROI values were then tabulated for statistical analysis.

RANDOMISATION - patients were randomly assigned in one of the 2 groups

Group A [TEST GROUP]- 10 partially edentulous patient were subjected for endosseous implant exposed to autologous platelet rich plasma.

Group B [CONTROL GROUP]- 10 partially edentulous patient were subjected for endosseous implant NOT exposed to platelet rich plasma.

PROCEDURE

GROUP A - subjects were required to provide 5ml sample of venous blood prior to implant placement surgery for harvesting the autologous PRP.

Steps in PRP Preparation:

1. With sterile gauze and injection 5 ml Venous blood was drawn into a test tube containing an anticoagulant to avoid platelet activation and degranulation.
2. The test tube carrying the processed blood sample was then placed in the centrifuge with another test tube placed contralaterally with the same metric quantity of saline for centrifugal balance.
3. The first cycle of centrifugation was then performed, called the "soft spin[1]" at 3000rpm for 10 mins.

4. Centrifuged blood sample was Separated in three layers, bottom-most RBC layer (55% of total volume), top most acellular plasma layer called PPP (platelet poor plasma) (40% of total volume), and an intermediate PRP layer (5% of total volume) called the "buffy coat" occurred at this stage.
5. Using a sterile syringe, the PPP, PRP and some RBCs were then transferred into another tube without an anticoagulant
6. This test tube was now subjected to a second centrifugation, which was longer and faster than the first, called a "hard spin". This allowed the platelets (PRP) to settle at the bottom of the tube with a very few RBCs, which explains the red tinge of the final PRP preparation. The acellular plasma, PPP (80% of the volume), was found at the top.
7. Most of the PPP was removed with a syringe and discarded, and the remaining PRP was shaken well for homogenisation.
8. This extracted PRP was then mixed with bovine thrombin and calcium chloride at the time of application (This results in gelling of the platelet concentrate. Calcium chloride nullifies the effect of the citrate anticoagulant used, and thrombin helps in activating the fibrinogen, which is converted to fibrin and cross-linked[2])
9. The extracted PRP was then loaded into a syringe and manually coated onto the implant being placed into the freshly prepared osteotomy site for group A patients.

SURGICAL TECHNIQUE

The surgical procedure was same for both the groups and was divided into two stages:

a) **First Stage Surgery:** After assessing the pre-treatment records, and fabricating a surgical stent, crestal incision was given[fig1.A] &[fig 2 A]and full thickness flap was raised at the proposed implant placement site[fig1.B] &[fig.2.B] Implant site was then prepared by sequential drilling to prepare the osteotomy site to receive the implant [fig. 2.C]. The implant fixture (untreated for Group B and treated with autologous PRP for Group A) [fig 1.C] was then placed into the osteotomy site, following which a cover screw was placed atop it and the surgical flap was sutured back into place with the implant submerged under the soft tissue [fig 1.D] & [fig 2.D]. A protective surgical pack was then placed over the implant site to prevent trauma or food lodgement and contamination to the site and the sutures.

b) **Second Stage Surgery:** After the healing period, incision was placed over the implant site and soft tissue reflected sufficiently to allow removal of cover screw. Healing abutments were placed and gingival tissue was sutured around it. Later, the healing abutments were removed and final abutments were placed on which implant prosthesis was fabricated following prosthetic superstructure fabrication steps.

c) **Radiographic Examination**: Parameters were recorded with IOPAR (Intra-Oral Peri-Apical Radiograph) and OPG (Orthopantomograph immediately following the implant procedure at baseline, at 90 days & at 180 days post-operatively by. Following recording of sequential radiographs, the Spatial Gray Level Dependence Method (SGLDM) was used to analyse the radiographic difference in the osseointegration of implants belonging to the test and control groups. Difference in the radiographic gray level saturation of the control and test group were then analysed for notable differences.

RESULTS

The effect of application of autologous Platelet-Rich Plasma (PRP) on the osseointegration of the implant using texture based analysis of the patient's dental panoramic radiographs recorded on the day of implant placement [fig.3A, 3.B], and at 180 days (6 month) [fig. 3.C, 3.D] postoperatively was analysed. The null hypothesis for the present study stated that there is no significant relation between the osseointegration of an implant placed conventionally in a freshly created osteotomy site and treatment of the implant surface with platelet rich plasma(PRP) extracted autologously from the patient's own venous blood sample. Based on the values obtained and the results hence calculated, statistical analysis using means and Paired 't' Test

reveals that the 't' value for all test and control group evaluations approached 0.0000 along with a statistically insignificant p value (p>0.05).

Statistical Analysis: - the clinical data was analysed statistically by paired t test.

STATISTICAL RESULTS BASED ON THE ANALYSIS OF AVAILABLE DATA (PAIRED 'T' TEST):

GROUP A: [0 days] P value and statistical significance: The P value equals 1.0000. By conventional criteria, this difference is considered to be not statistically significant.

GROUP B: [90 days] P value and statistical significance: The P value equals 1.0023 by conventional criteria, this difference is considered to be not statistically significant.

GROUP C: [180 days] P value and statistical significance: The P value equals 1.0008 By conventional criteria, this difference is considered to be not statistically significant.

Group	Test (0)	Control (0)
Mean	77.6	76.8
SD	1.03	0.63
SEM	0.33	0.20
N	10	10

Group	Test (0)	Control (0)
Mean	83.6	83.2
SD	1.08	0.59
SEM	0.41	0.28
N	10	10

Group	Test (0)	Control (0)
Mean	85.7	86.2
SD	1.02	0.88
SEM	0.36	0.22
N	10	10

Fig. 1: Evaluation of Mean

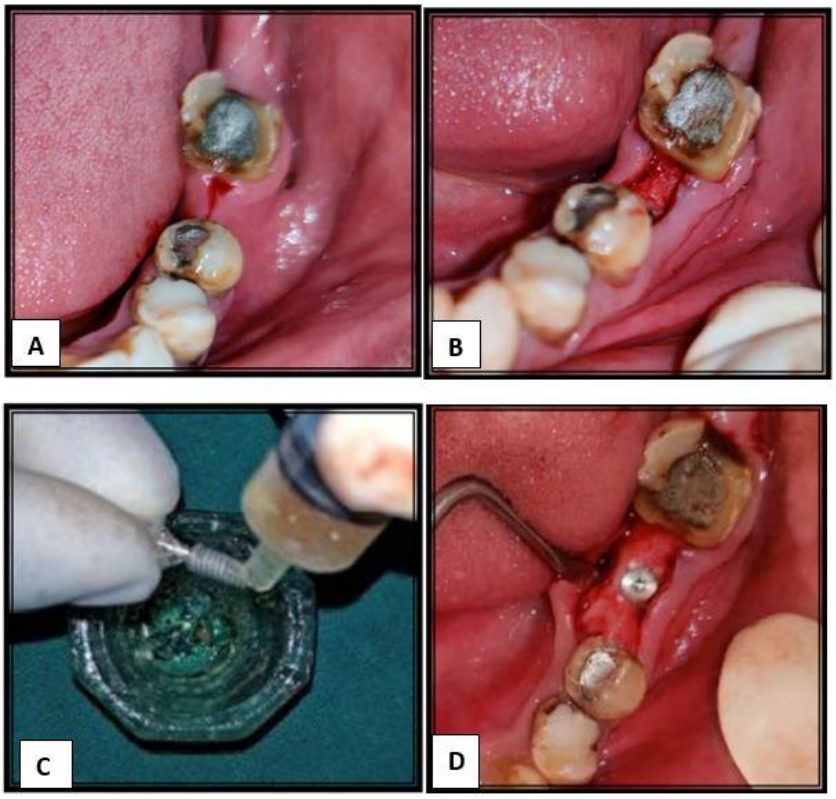


Fig.1 [**A**]Crestal Incision for Mucoperiosteal Flap (Test Group-A). **[B**] Full Thickness Flap reflected. [**C**]Application of Autologously Extracted PRP onto Implant Fixture Surface. [**D**]Implant Fixture placed in Freshly Prepared Osteotomy site.

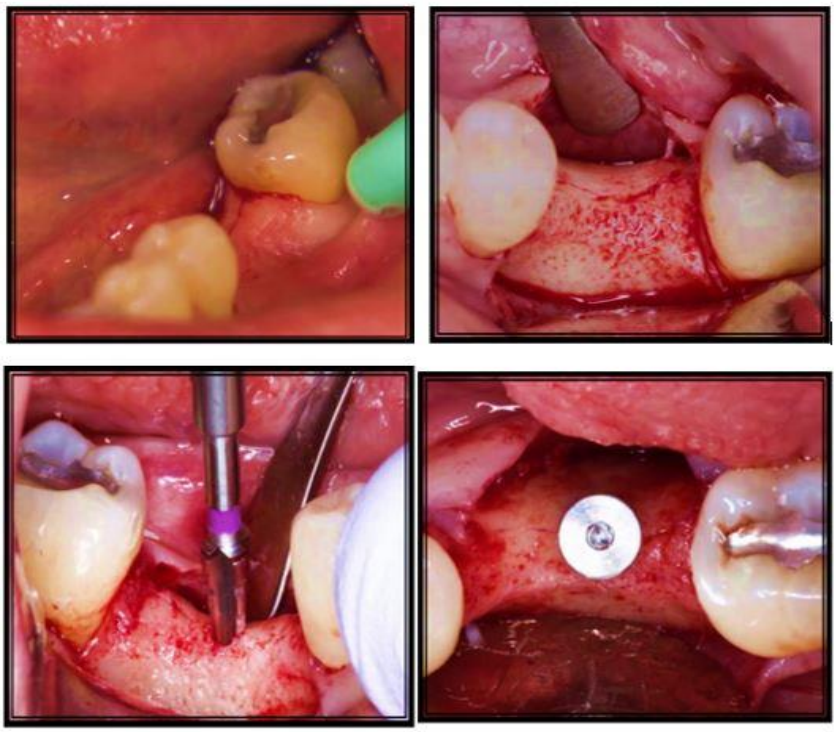


Fig 2[**A**] Crestal Incision for Mucoperiosteal Flap(Control Group-B)[**B**] Reflection of Full Thickness Flap.[**C**]: Sequential Drilling for Endosseous Implant Placement.[**D**] Implant Hexed into Osteotomy Site with Cover Screw

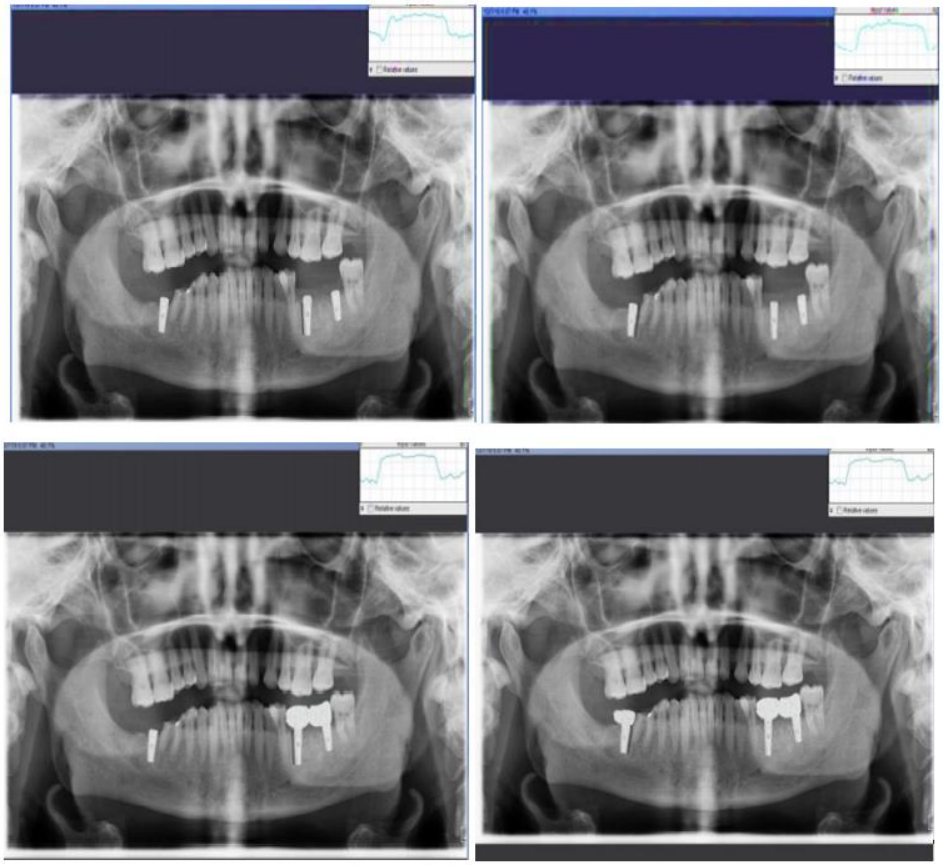
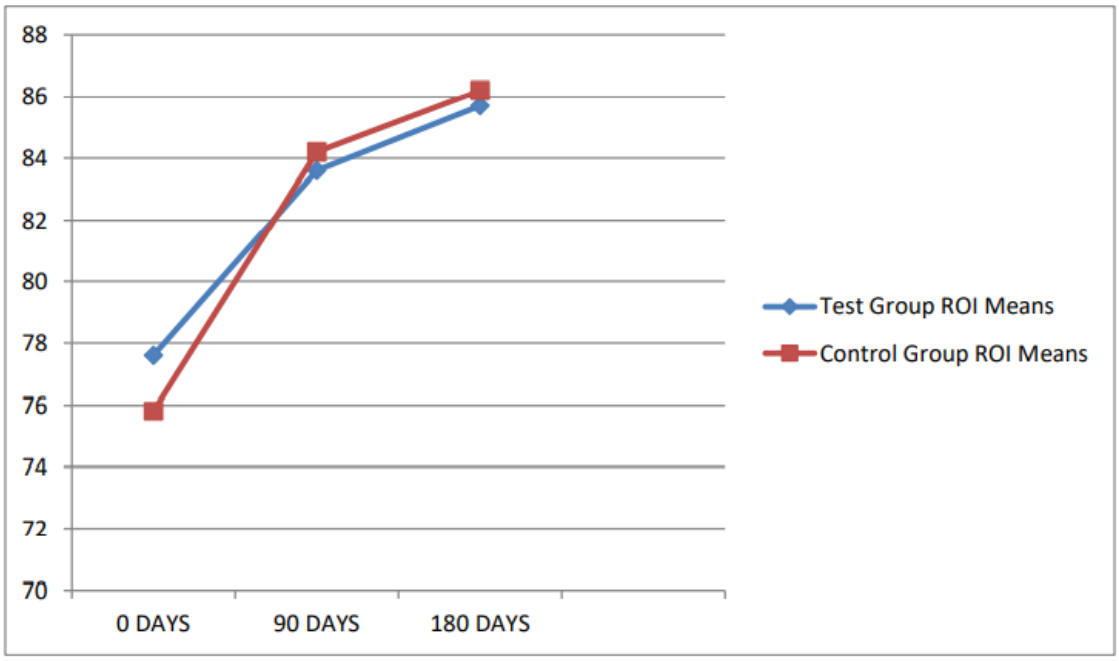


Fig 3[**A**] ROI Analysis of Test Sample at 0 Days**[B]** ROI Analysis of Control Sample at 0 Days **[C]** : ROI Analysis of Test Sample at 180 Days.[**D**] ROI Analysis of Control



Graph 1: Representation of Evaluation of Means of Test and Control Group ROI Values

DISCUSSION

The restoration of missing teeth is an important aspect of modern dentistry. As teeth are lost to decay or periodontal disease, there is a definitive demand for replacement of aesthetics or function.The concept of replacement of missing teeth with dental implants allows the clinician to rehabilitate the patient's edentulism in a near physiologic manner as a biologic union is expected to occur between the dental implant and the patient's alveolar bone . This biologic union is referred as osseointegration, a term coined by Dr.Per-Ingvar Branemark, the father of modern implantology. As the process of osseointegration is primarily a result of a positive tissue response to a biocompatible bone friendly substance, it is also affected by a variety of natural and physiological factors. One very rich source of these growth factors that are known to positively enhance bone formation and growth, is platelet rich plasma (PRP), which contains platelet derived growth factor (PDGF), transforming growth factor (TGF) and many other such bone growth inducing plasma derivatives[3].

The present study was conducted to study the effect of the application of this platelet rich plasma onto the surface of an implant at the time of implant placement in a freshly prepared osteotomy site on the subsequent osseointegration of the implant as compared to that of an implant placed without treatment with platelet rich plasma.

The study design used the orthopantomography (OPG) as a comparative sample for the analysis of efficacy of osseointegration for the two patient groups as it provides a standardized method of radiographic imaging based on well-defined skeletal landmarks, at the same time minimizing the patient's radiologic exposure[4] . A relatively low voltage of 66 kV & current of 9 mA, with a minimalistic exposure time of 16s restricts the patient's radiologic exposure to a maximum of 0.010mSv. The method of comparison used to analyse the effect of the application of platelet rich plasma on the implant surface on the osseointegration of the implant was chosen to be the Spatial Gray Level Dependence Method (SGLDM) as described by Lee et al (1992). It is a radiographic analytic technique based on evaluation of 2-dimensional distribution of gray level matrices on panoramic radiographs in specific parts of the radiographic image denoted as the study areas and known as regions of interest(ROIs), Based on the number of occurrences of gray matrices belonging to various levels of saturation on a scale of 0.0 to 1.0, density of the ROIs signifies increased radiopacity at a microscopic level comparing the subsequent bone formation as a result of osseointegration . Haralick

et al (2014) have compared SGLDM with other radiographic analytic techniques like Pixel Based Texture Analysis (PBTA), Computational Algorithm for Analysis of Image Saturation (CAIS), etc and concluded that SGLDM provides a more defined interpretation and gives a definitive result when a comparison of near equal values of radiographic saturation is required.

The desired physiologic effects of the growth factors present in PRP were expected to enhance the osteo-inductive and conductive environment and appositional bone growth occurring upon and around the implant. But, comparison of the level of osseointegration of the subsequent implant versus an implant placed without PRP treatment, analysed using SGLDM involving texture- based analysis of the patient's sequential dental panoramic radiographs, did not reveal a statistically significant difference in a grayscale- based measurement of predetermined pixel locations of radiographic regions of interest (ROIs) around the implant at all three defined durations, viz. 0 days, 90 days and 180 days. A comparative evaluation of the grayscale saturation levels thus recorded revealed that the test values for both the sample groups were more or less similar.

The region of interest (ROI) values for implants placed in the test group patients demonstrated a mean ROI value of 82.3 units, whereas that of implants placed in the patients in the control group was demonstrated to be 82.6 units. To prevent the possibility that a sample ROI might include some pixels from the dental implant, leading to an additive effect on the grayscale saturation values, the grayscale saturation of bone regions inconsequent to the implant site were also recorded, to define a credible range of bone surface grayscale saturation.

As the study is entirely based on a comparison of these ROIs to arrive at a rejection or acceptance of the null hypothesis, it is imperative to consider the effect of the errors in demarcation on the resultant sample values. Although the evidence presented above describes various lines of reasoning as to why the effect of PRP on the osseointegration of an implant may not always be evident, direct studies supporting the result of this study are also in abundance. Attia32 et al (2019) concluded that no correlation could be found between submicroscopic apposition of bone in the osseointegration of implants treated with PRP. Ultramicroscopic evaluation and comparison of osteoblastic activity revealed a present but statistically insignificant improvement in osseointegration of the samples belonging to the test group [5]. Ullas33 et al (2008) postulated that early bone apposition following implant placement may be encouraged by exposure to PRP but the longterm bone response was similar in both the test and control groups, rendering the study result inconclusive[6]. Ketabi et al (2015) used Periotest to compare the effect of local appli-cation of platelet-rich plasma (PRP) on osseointegration of implants placed in the mandible of edentulous patients. They concluded that no additional effect on implant stability was observed in the test group patients, although beneficial effects in improving soft and hard tissue healing were definitely observed [7]. Several other such studies have arrived at similar lines of resultant research. However, there is definitely a much wider scope of research in the direction of acceptance of a positive effect of PRP on the osseointegration of dental implants. Georgekapoulos et al (2014) demonstrated a significantly positive effect on bone formation in implants treated with PRP using a radiographic analysis that utilizes couccurance of gray level matrices around healing implants[8]. Kundu24 et al (2014) demonstrated that a synergistic effect of PRP was observed on improved implant stability and bone levels. However, they also concluded that this effect was not as markedly observed on implants placed in areas with perpendicular buttress osteocyte activity such as the posterior maxilla. This allowed for reflection upon the effect of buttress arrangement on implant osseointegration.[9] In Study by Monov5 et al (2005), post placement implant stability measurements were made by means of resonance frequency analysis at different time intervals, in implants placed with and without exposure to PRP. study concluded that improved resonance frequencies were noted in implants exposed to PRP[10]. scientific evidence exists both in favor of and against the result of the present study, it is only tactical to conclude that further

research in the field of submicroscopic, histologic and radiographic interpretation of osseous changes or regeneration that occur following the creation of an osteotomy site for the placement of an implant that may better allow a detailed analysis of the effect of osteoinductive and conductive components of blood and its products may allow more light to be shed on a clearer correlation between the said criteria.

CONCLUSION

Study reveals that no correlation was demonstrated between the osseointegration of an implant placed conventionally in a freshly created osteotomy site and treatment of the implant surface with platelet rich plasma (PRP) extracted autologously from the patient's own venous blood sample.

Conflicts of Interest: None.

REFERENCES

1. Froum S, Wallace S, Tarnow D, Cho S. "Effect of Platelet-Rich Plasma on Bone Growth and Osseointegration in Human Maxillary Sinus Grafts: Three Bilateral Case Reports". Int Journal of Periodont & Rest Dent 2002;22(1):45-53.
2. Sebastian F, Olmeido D, Lineras J, Guglimote M. "Effect of Platelet-Rich Plasma on the Periimplant Bone Response: An Experimental Study". Imp Dent 2004;13(1):73-78.
3. G Weibrich, Hansen T, Kleis W, Buch R, Hitzler W. "Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration". Bone 2004;34(4):665-71.
4. 4.Ito K, Yamada Y, Naiki T, Ueda M. "Simultaneous implant placement and bone regeneration around dental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and platelet-rich plasma". Clin Oral Imp Res 2006 oct;17(5):579–86.
5. Attia S, Narberhaus C, Schaaf H, Streckbein P. "Long-Term Influence of Platelet-Rich Plasma (PRP) on Dental Implants after Maxillary Augmentation:Implant Survival and Success Rates". Journal Clin Med 2019;9:382-391.
6. SatyaS, Dhayanand V , Sangeetha S, Prakash PSG. "Plasma Rich Plasma In Periodontal Therapy". J. Pharm. Sci. & Res. Vol. 9(6), 2017, 965-971.
7. Ketabi M, Fahami, Amini S. "Effect of Platelet-rich Plasma on Implant Stability in the Mandible". Journal Periodont Imp Dent 2015;7(2):50-54.
8. Georgakopoulos I, Tsantis S, Korfiatis P, Fanti E"The impact of platelet-rich-plasma(prp) on osseointegration of oral implants in dental panoramic radiography: A texture based evaluation". Clin Min Met 2014 Jan;11(1):59-66.
9. Kundu R, Rathee M. "Effect of Platelet-Rich-Plasma (PRP) and Implant Surface Topography on Implant Stability and Bone". Journal Clin Diag Res 2014Jun;8(6):ZC26-ZC30.
10. Monov G, Fuerst G, Tepper G, Watzak G. "The effect of platelet-rich plasma upon implant stability measured by resonance frequency analysis in the lower anterior mandibles". Clin Oral Imp. Res 2005;16(4):461–5.