

A COMPARATIVE EVALUATION OF SECONDARY IMPLANT STABILITY USING ADVANCED PLATELET RICH FIBRIN ALONE AND IN COMBINATION WITH ADVANCED PLATELET RICH FIBRIN AND LOW LEVEL LASER THERAPY USING RESONANCE FREQUENCY ANALYSIS – A 12 WEEKS RANDOMIZED CONTROLLED SINGLE BLIND CLINICAL STUDY

Periodontology

Samistha Sinha* Consultant Periodontist *Corresponding Author

Vikram Blaggana Professor and HOD

Preeti Upadhyay Professor

Neeru Verma Consultant Periodontist

ABSTRACT

Addition of biologically active molecules during implant placement creates an osteoconductive effect, increases osteoblastic differentiation and enhances healing of peri-implant bone regeneration leading to faster osseointegration. Advanced platelet rich fibrin, Low-level laser therapy (LLLT) has gained greater awareness, reduces postoperative pain, promotes the osseointegration of implants, particularly, improving stability and enhances new bone formation. A total of 22 dental implants were placed in posterior mandibular region and divided into two equal groups (n=11). Group A implant placed with advanced platelet rich fibrin (A-PRF) and Group B implant placed with advanced platelet rich fibrin and low level laser therapy (A-PRF+LLLT). The implant stability quotient (ISQ) values were recorded. The data was statistically evaluated using SPSS version 16.0 Software. The mean ISQ values at the baseline was 75.45 ± 4.204 for Group A, at 2 weeks 73.09 ± 5.873 and at 4 weeks 71.55 ± 3.934 , 75.45 ± 3.328 at 6 weeks, 77.91 ± 2.879 at 8 weeks and 79.73 ± 1.794 at 12 weeks, respectively. For Group B, the mean ISQ values at baseline was 70.73 ± 5.331 , at 2 weeks 68.00 ± 9.143 , 67.55 ± 9.158 at 4 weeks after which the value increased to 70.27 ± 6.724 at 6 weeks, 72.55 ± 6.788 at 8 weeks and finally 74.73 ± 6.214 at the end of 12 weeks. Addition of A-PRF provided positive results

KEYWORDS

Implant Stability Quotient, Resonance Frequency Analysis, Advanced Platelet-Rich Fibrin, Low Level Laser therapy

INTRODUCTION

In the past 50 years, implant dentistry has evolved from an experimental treatment to a highly predictable option to replace missing teeth.¹ Although a number of restorative options for the treatment of missing teeth still exist, none have proven to be as functionally effective and robust as implants.^{1,2}

Implant stability plays a critical role in successful osseointegration, which is the direct structural and functional connection between bone and the surface of a load carrying dental implant. Achieving and maintaining this implant stability is a prerequisite for a successful clinical outcome.⁴

The technique of resonance frequency analysis (RFA) has extensively been examined in clinical studies. RFA values are recorded as Implant Stability Quotient (ISQ) on a scale from 1 to 100. Higher ISQ values are reportedly associated with greater implant stability and osseointegration.⁷

In recent years, the use of platelet-rich concentrates has again gained momentum in the dental field as an autologous source of growth factors. Advanced platelet rich fibrin (A-PRF) was first described in 2014 as a new concept of cell-based tissue engineering, by decreasing the rpm and increasing the centrifugation time as compared to standard platelet rich fibrin (PRF). A-PRF release significantly higher total quantities of growth factors when compared to traditional PRF. The effect of low level laser therapy (LLLT) on bone regeneration has become a focus of recent research. With respect to bone, LLLT has been shown to modulate inflammation, accelerate cell proliferation and enhance healing.⁸

The present article compares secondary implant stability using advanced platelet rich fibrin (A-PRF) alone and in combination with low level laser therapy (A-PRF+LLLT) using resonance frequency analysis (RFA).

MATERIAL AND METHODS

Twenty two sites were randomly selected having at least one edentulous site in mandibular posterior region. Both male/female patients aged between 18 to 55 years with no relevant medical history were included.

They were assigned to either of the two groups:

- I. Group A (11 sites) – Implant placement with Advanced Platelet Rich Fibrin (A-PRF).
- II. Group B (11 sites) – Implant placement with combination of Advanced Platelet Rich Fibrin (A-PRF) and low level laser therapy (LLLT).

The areas were anesthetized using appropriate local anesthetic agent (2% lignocaine hydrochloride with 1 in 80,000 adrenaline bitartrate). After crestal incision on a healed residual ridge a full thickness mucoperiosteal flap was raised. Then series of drills were used sequentially, to prepare the osteotomy site.

Preparation Of Advanced Platelet Rich Fibrin (a-prf):

For both groups, 24-gauge needle was used for venipuncturing of patient's antecubital vein for blood collection. At the time of surgery 10 ml of whole blood was drawn from the patient in sterile silica coated glass-based vacuum tubes i.e. A-PRF vacuum tube.

These tubes were placed into Remi centrifuge machine. Centrifugation was carried out at 1500 rpm for 14 minutes. After centrifugation, tube contains 3 layers:

1. The most upper layer was platelet poor plasma (PRP)
2. The middle layer was fibrin buffy coat containing polymerized fibrin block with concentrated growth factors, and
3. The bottom layer was the red blood cells (RBC)

Middle buffy coat layer was retrieved after removing the upper and lower layers and this layer constitutes advanced platelet rich fibrin (A-PRF). The A-PRF was transferred to a PRF box and compressed to obtain PRF membrane.

The membrane was then placed into the osteotomy site before implant placement in both the groups.

Laser Irradiation Protocol:

Only For Group B, after the implants were placed, low level laser therapy (LLLT) was applied using diode laser 940 nm. (A-PRF + LLLT). Laser was irradiated by tissue probe in non contact continuous mode into the buccal and lingual mucosa for 40 s (14.18 J/cm^2 energy density) from each side.

Energy was 4 J at each side, totaling 8 J per session. Laser irradiation was repeated at 2nd day, 4th day, 7th day, 10th day, and 14th day postoperatively and the final dose was 48 J, accumulated energy of all six sessions.

Healing abutments were placed in both the groups. RFA was used to measure the ISQ (Implant Stability Quotient) with a non-contacting technique. ISQ values were evaluated for both Groups at baseline then at 2, 4, 6, 8, and 12 weeks.

Flaps were sutured using 3-0 non resorbable braided black silk suture. Post surgical medications and instructions were given to the patients. Suture removal was done after 10 days of surgery



Fig 1: Pre operative view



Fig 2: Adequate ridge for implant placement



Fig 3: Mid crestal incision given

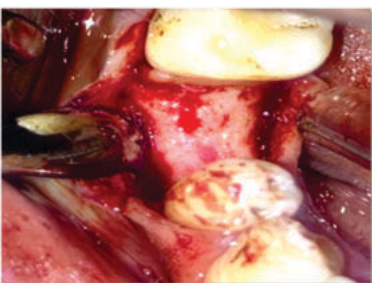


Fig 4 : Full thickness mucoperiosteal flap reflected



Fig 5: Preparation of osteotomy site

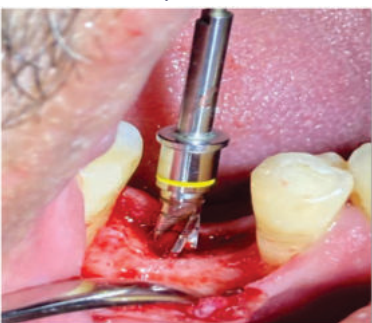


Fig 6: Osteotomy site of desired length completed



Fig 7: Phlebotomy is done to withdraw blood (Group A & B)



Fig 8: Centrifugation of blood to make A-PRF (Group A & B)



Fig 9: PRF clot obtained

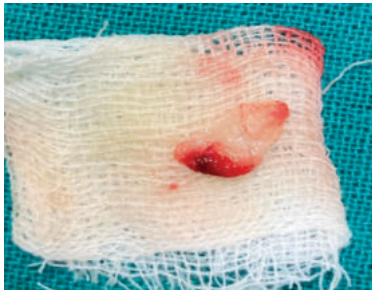


Fig 10: A-PRF membrane obtained



Fig 11: Membrane being inserted into osteotomy site



Fig 12: Implant Paced



Fig 13: Low level laser therapy applied (For Group B)



Fig 14: Osstem Hi Ossen IS3TM RFA Device



Fig 15: ISQ Value being taken



Fig 16: ISQ value recorded and displayed



Fig 17: Healing abutment placed and sutures given



Fig 18: Immediate post operative RVG

Statistical Analysis

All the data collected and entered in MS excel and analyzed using SPSS 16.0 for windows

RESULTS

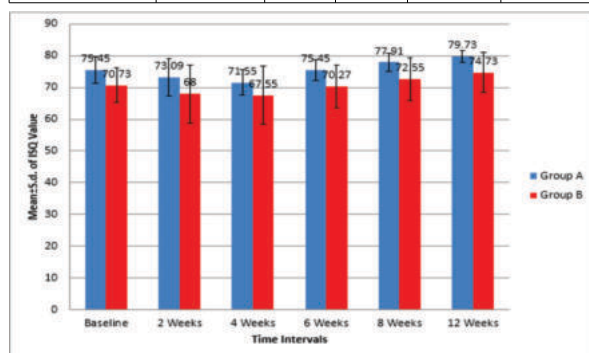
The mean score of ISQ value ascertained clinically at baseline, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks for Group A and Group B were tabulated and graphically represented in Table 1 and Graph 2

The mean Score ($\pm$ standard deviation) of the ISQ value in Group A at baseline, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks were 75.45 ± 4.204 , 73.09 ± 5.873 , 71.55 ± 3.934 , 75.45 ± 3.328 , 77.91 ± 2.879 , 79.73 ± 1.794 respectively. The mean Score ($\pm$ standard deviation) of the ISQ value in Group B at baseline, 2 weeks, 4 weeks, 6 weeks, 8 weeks and 12 weeks were 70.73 ± 5.331 , 68.00 ± 9.143 , 67.55 ± 9.158 , 70.27 ± 6.724 , 72.55 ± 6.788 , 74.73 ± 6.214 respectively. On intergroup comparison, a significant difference was observed at baseline, 6 weeks, 8 weeks and 12 weeks ($p < 0.05$), while no significant difference was observed at 2 weeks and 4 weeks ($p > 0.05$) Table 2

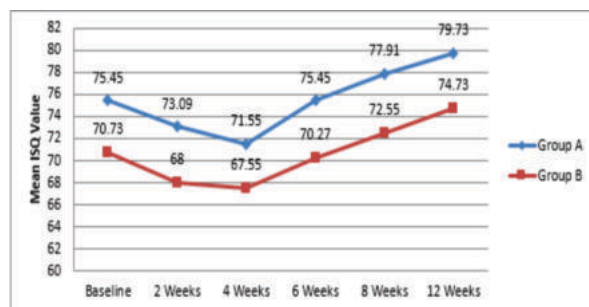
The mean score of change in ISQ value between different time intervals ascertained clinically for Group A and Group B were tabulated and graphically represented in Table 3

Table 1: Distribution of mean and S.D. of ISQ values among two groups at different time intervals

	GROUP	N	Mean	Std. Deviation	Std. Error Mean
BASELINE	Group A	11	75.45	4.204	1.268
	Group B	11	70.73	5.331	1.607
2 WEEKS	Group A	11	73.09	5.873	1.771
	Group B	11	68.00	9.143	2.757
4 WEEKS	Group A	11	71.55	3.934	1.186
	Group B	11	67.55	9.158	2.761
6 WEEKS	Group A	11	75.45	3.328	1.003
	Group B	11	70.27	6.724	2.027
8 WEEKS	Group A	11	77.91	2.879	.868
	Group B	11	72.55	6.788	2.047
12 WEEKS	Group A	11	79.73	1.794	.541
	Group B	11	74.73	6.214	1.874



Graph 1: Distribution of mean $\pm$ S.d. of ISQ values among two groups at different time intervals



Graph 2: Comparison of mean of ISQ values among two groups at different time intervals

Table 2: Intergroup comparison of means of ISQ values among two groups at different time intervals by independent t-test

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	P value	Mean Difference $\pm$ Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	.167	.687	2.309	20	0.032*	4.727 $\pm$ 2.047	.457	8.997
2 Weeks	.917	.350	1.554	20	0.136NS	5.091 $\pm$ 3.277	-1.744	11.926
4 Weeks	13.497	.002	1.331	20	0.198NS	4.000 $\pm$ 3.005	-2.269	10.269
6 Weeks	8.275	.009	2.291	20	0.033*	5.182 $\pm$ 2.262	.463	9.901
8 Weeks	6.877	.016	2.413	20	0.026*	5.364 $\pm$ 2.223	.726	10.001
12 Weeks	13.425	.002	2.564	20	0.019*	5.000 $\pm$ 1.950	.932	9.068

Table 3: Distribution of mean $\pm$ S.d. of change in ISQ values in both Groups at different time intervals

	GROUP	N	Mean $\pm$ Std. Deviation	Std. Error Mean
Change Baseline to 2 Weeks	Group A	11	2.3636 $\pm$ 3.82813	1.15422
	Group B	11	2.7273 $\pm$ 6.43570	1.94044
Change Baseline to 4 Weeks	Group A	11	3.9091 $\pm$ 5.83874	1.76045
	Group B	11	3.1818 $\pm$ 7.04014	2.12268
Change Baseline to 6 Weeks	Group A	11	.0000 $\pm$ 6.58787	1.98632
	Group B	11	.4545 $\pm$ 5.71601	1.72344
Change Baseline to 8 Weeks	Group A	11	-2.4545 $\pm$ 6.17031	1.86042
	Group B	11	-1.8182 $\pm$ 5.98027	1.80312
Change Baseline to 12 Weeks	Group A	11	-4.2727 $\pm$ 5.19790	1.56723
	Group B	11	-4.0000 $\pm$ 5.76194	1.73729
Change 2 Weeks to 4 Weeks	Group A	11	1.5455 $\pm$ 5.59220	1.68611
	Group B	11	.4545 $\pm$ 4.78254	1.44199
Change 4 Weeks to 6 Weeks	Group A	11	-3.9091 $\pm$ 3.08073	.92888
	Group B	11	-2.7273 $\pm$ 3.40855	1.02772
Change 6 Weeks to 8 Weeks	Group A	11	-2.4545 $\pm$ 1.50756	.45455
	Group B	11	-2.2727 $\pm$ 3.82337	1.15279
Change 8 Weeks to 12 Weeks	Group A	11	-1.8182 $\pm$ 2.18258	.65807
	Group B	11	-2.1818 $\pm$ 2.75021	.82922

DISCUSSION

For a successful osseointegration of an endosseous dental implant placed in mandible at least 3 months without loading is important. (Adell et al 1981; Branemark et al 1985).³ As the patients desire a shortening of this period of waiting for the final prosthesis, various researches have been done which would further accelerate osseointegration without compromising the mechanical outcomes and tissue integration.²

This has been accomplished by inducing biologically active molecules for osteoconductivity, increase osteoblastic differentiation and enhance healing of peri-implant bone.⁶

In this case series, a better version of PRF i.e. Advanced platelet-rich fibrin was used, hypothesizing that A-PRF releases a significantly higher amount of growth factors TGF- β , PDGF, VEGF and would further improve early healing and faster osseous integration of implants compared to the original PRF.⁴ A study conducted by Eizaburo Kobayashi reported that A-PRF released the highest bone

morphogenic protein (BMP) when compared to either PRF or PRP.¹⁹ The low level laser therapy (LLLT) is one such adjunctive therapy that has been investigated in recent years to improve the potential for bone regeneration. A systematic review conducted by Prados Frutos et al⁷ demonstrated satisfactory integration of the implant with the bone by low power laser irradiations.

In the present study, all the implants achieved good to excellent primary stability, which was documented by high ISQ value and standard deviation at the baseline.

The mean ISQ values and the standard deviation of ISQ values at the baseline was 75.45 $\pm$ 4.204 for Group A, which started to reduce at 2-weeks and continued to dip till 4th week after which a reversal in trend of the values was seen. The mean ISQ values with standard deviation, increased at 6, 8 and at 12-weeks (79.73 $\pm$ 1.794) respectively. This was in accordance with the study done by Amjed Fouad et al⁸

For Group B, the mean ISQ values and the standard deviation of ISQ values at baseline was 70.73 $\pm$ 5.331 after which an appreciable dip in value was seen at the end of 2-weeks. It further kept on dipping at 4 weeks which was the lowest value, after which the value increased to at 6, 8 and at 12-weeks (74.73 $\pm$ 6.214). Both the groups displayed the same trend of stability change.

The 12 week ISQ value (79.73 $\pm$ 1.794) for Group A was higher than the baseline ISQ value (75.45 $\pm$ 4.204) which was found to be a statistically significant improvement. Same trend was noticed in Group B. On intergroup comparison, we observed non-significant changes in means of change in ISQ values between both Group A and Group B at all baseline and at different time intervals. This implies that both the groups were following similar trend in implant stability. According to this comparison we may infer that adding A-PRF with or without LLLT has provided a similar trend in achieving secondary implant stability over the period of study.

If we observe the mean change in ISQ values (mean $\pm$ standard deviation) from baseline Group A This also indicates that after 4 weeks a significant increase in change in means of ISQ value from baseline to 6 weeks, baseline to 8 weeks and baseline to 12 weeks respectively. Here also, Group B significant change in means of ISQ value from baseline to 6 weeks, baseline to 8 weeks and baseline to 12 weeks was observed. So on overall comparing the means of change in ISQ values obtained from baseline and at different time points in both the Groups, a higher reduction was seen in Group A than Group B in the initial 4 weeks followed by a statistically significant increased ISQ value in the following period up to 12 weeks.

The possible reasoning attributed for the positive results of Group A is the lower centrifugation speed & the ability to release growth factors from 1 to 4 weeks guarantees longer period of healing stimulation explained by Starzynska et al⁹. Another hypothesis explained by Kumar et al¹¹ PRF displayed an inhibitory role in the formation and differentiation of osteoclast cells after 4 weeks, and its molecular mechanism of action was related to the apoptosis induction through intrinsic mitochondrial pathway by activating caspase-9, -3 and -7 which are the most prevalent caspases and they are responsible for the majority of apoptotic effect. The addition of LLLT to the A-PRF protocol of implant placement did have a positive effect on implant stability but to a lesser extent when compared with the A-PRF group alone, measured by RFA. According to Karu et al¹⁰ osteoclasts are multinuclear cells with mitochondria of high activity; they are more readily affected by low-level laser radiation than osteoblast, which might have negatively affected the role of A-PRF, giving an insignificant results. This might indicate that LLLT can have an inhibitory role in the PRF effect in the earlier days of healing. Hentunen et al¹² reported that the bone matrix liberates a protein that stimulates osteoclast formation, which is light-dose dependent, explaining the higher resorption levels in the irradiated animals. However, further investigations to study the effects of PRF and low level laser therapy on implants osseointegration is needed with a larger sample size to evaluate the long term benefits.

CONCLUSION

The knowledge of achieving faster osseointegration and increased bone density can be utilized for earlier loading of implant prosthesis. Addition of A-PRF induced faster rate of healing with increased bone density and enhanced osseointegration as compared to implants placed with A-PRF and LLLT

REFERENCES

1. Branemark P.I, Zarb C, Albrektsson T. Tissue-integrated prostheses: Osseointegration in clinical dentistry. Chicago: Int Dent J 1985;35:259-65
2. Branemark, P.I. Osseointegration and its experimental background. J Prosthet Dent 1983; 50:399-410.
3. Herrero-Climent M, Albertini M, Rios-Santos JV et al. Resonance frequency analysis-reliability in third generation instruments: Osstell mentor. Med Oral Patol Oral Cir Bucal 2012;17:801-6
4. Karu et al. Advanced Platelet-Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells J of Oral Implantology. 2014;24: 6
5. Kobayashi E, Fluckiger L, Fujioka-Kobayashi M & Sawada K. Comparative release of growth factors from PRP, PRF, and advanced-PRF Clin Oral Investig. 2016; 20:2353-2360.
6. Mandić B, Lazic Z, Markovic A, Mandic M. Influence of postoperative low-level laser therapy on the osseointegration of self-tapping implants in the posterior maxilla: A 6-week split-mouth clinical study. Clinic of Oral Surger. 2015
7. J. C. Prados-Frutos, J. Rodriguez-Molinero, M. Prados-Privado, J. H. Torres, R. Rojo. Lack of clinical evidence on low-level laser therapy (LLLT) on dental titanium implant: a systematic review. Lasers Med Sci 2016
8. Hussien AF, Hussien A, Al-Hussaini. Effect of platelet-rich fibrin on implant stability. J Bagh Coll Dentistry 2017;29:58-64
9. Starzynska A, Wieremczuk MK, Lopez MA. The Growth Factors in Advanced Platelet-Rich Fibrin (A-PRF) Reduce Postoperative Complications after Mandibular Third Molar Odontectomy. Int. J. Environ. Res. Public Health 2021
10. Karu et al. Advanced Platelet-Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells J of Oral Implantology. 2014;24: 6
11. Kumar A, Mahendra J, Samuel S, et al. Platelet-rich fibrin/biphasic calcium phosphate impairs osteoclasts differentiation and promotes apoptosis by the intrinsic mitochondrial pathway in chronic periodontitis. J Periodontol. 2018;1-1
12. Hentumen Y, Al-Hijazi, A. Evaluation of the effect of autologous platelet rich fibrin matrix on osseointegration of the titanium implants immunohistochemical evaluation for PDGF-I & IGF-A. J of Baghdad college of dentistry 2013; 21:605-11