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Clinical Evaluation of Keratinized Tissue Thickness around Dental Implants with L-PRF: A Split-Mouth Randomized Clinical Trial

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ABSTRACT

Aim: The aim of this study was to objectively evaluate the effect of leukocyte-platelet-rich fibrin (L-PRF) on increasing the soft tissue thickness around dental implants placed in conventional way. **Materials and Methods**: This split mouth randomized clinical trial included seven patients (4 females and 3 males) received 24 dental implants inserted in conventional (delayed) protocol. Each patient has received at least two implants, one with PRF placement (to be included in study group), and the other without PRF (to be included in control group). The thickness of the soft tissue was measured at buccal side by transgingival measurement using endodontic reamer with a stopper, then the distance from the tip of the reamer to the stopper was measured by a digital vernier. These measurements were taken at baseline, one month and six months after surgery. **Results**: The mean of soft tissue thickness value at baseline was (2.98) and (2.84) for study and control groups respectively. After one month, the value for study group was (3.65) which was higher than control group (3.65), with a mean difference of (0.67) and (0.40) for study and control groups respectively. After six months, the mean value for the study group was (2.67) and for the control group was (2.59), with a mean difference of (-0.31) for the study group and (-0.25) for control group. **Conclusion**: In this study, the use of L-PRF has not been shown to be useful in increasing the soft thickness around dental implants.

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Keywords: Dental implants (DIs), soft tissue thickness (STT) and leukocyte-platelet-rich fibrin (L-PRF).

INTRODUCTION

Dental implants (also known as oral or endosseous implants) have been used to replace missing teeth for more than half a century. Since the goal of modern dentistry is to restore the

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patient to normal contour, function, comfort, esthetics, speech, and health by removing a disease process from a tooth or replacing teeth with a prosthesis, dental implants are considered to be an important contribution to dentistry as they have revolutionized the way by which missing teeth are replaced with a high success rate **(Warreth *et al*., 2017, Resnik, 2020)**.

The clues solidify that an adequate peri-implant width of keratinized mucosa and soft tissue thickness seem to have a positive impact on the long-term stability of peri-implant tissues **(Bassetti *et al*., 2016)**. Recent publications suggest that inadequate keratinized tissue width and soft tissue thickness around dental implants may lead to undesirable outcomes **(Bassetti *et al*., 2017)**.

Nowadays, most implantologists have shifted their focus from obtaining osseointegration to achieving a pleasing esthetic appearance. Hence, soft tissue augmentations around dental implants have slowly become an area of interest **(Lin *et al*., 2018)**. To gain keratinized tissue or soft tissue thickness around dental implants, different surgical techniques and augmentation materials have successfully been used **(Bassetti *et al*., 2016)**. These include the use of free gingival graft, subepithelial connective tissue graft, xenogeneic graft material, or allogenic graft material **(Lin *et al*., 2018)**.

Another trial to influence the peri-implant soft tissue is the use of platelet-rich fibrin (PRF). This second-generation platelet concentrate described by Choukroun., *et al.* in 2001 is a fibrin matrix enriched with cytokines, circulating progenitor cells, and growth factors that can be used in surgery. Several studies show a constant release of growth factors such as PDGF (platelet-derived growth factor) or TGF-b (transforming growth factor) for at least 1 week up to 28 days and proved its accelerating effect on the healing process. The application of PRF has been tested in various disciplines of dentistry so far **(Ayoub and Belal, 2016)**. So, our null hypothesis was that PRF has no effect on thickness of keratinized tissues around dental implants.

MATERIALS AND METHODS

This study was a split mouth randomized clinical trial which was conducted in dental implant unit, department of periodontology, College of Dentistry, University of Baghdad. Randomization was performed by lottery method. A total of 7 patients, 4 females and 3 males, with age ranged from 35-65 years were enrolled in this study between December 2019 to August

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2020 and provided with a written informed consent. This study was approved by the Ethical Committee of the College of Dentistry, University of Baghdad (Ref.132619, in 2/12/2019) and registered in http://ClinicalTrials.gov (Identifier: NCT04864197).

Sample size and primary outcome:

To calculate sample size, one of clinical parameters (the thickness of buccal mucosa) was used as primary outcome of the study. The buccal mucosa thickness, (1.85 mm ± 0.41 SD at baseline and 2.15 mm ± 0.78 SD at 3-month follow-up) **(Hehn *et al*., 2016)**, was utilized to calculate the sample size using software program (g-power3.1) at 95% confidence interval, 5% error margin. The resulted acceptable sample size was consisting of 42 implants divided into two groups, the first group consist of 21 implants with PRF, while the second group consist of 21 implants without PRF. Because of quarantine due to pandemic COVID-19, nineteen patients were examined and only seven of them were indicated to participate.

Inclusion criteria: The patients were selected without any systemic or local contraindications such as local acute and chronic infections, periodontal disease and parafunctional habits that interfere with the surgery.

Exclusion criteria:

- Patients with a history of severe periodontitis.
- Smokers.
- Pregnant or lactating women.
- Patients with bad oral hygiene.
- Patients who were unwilling to participate in the study.

The patients have received 24 DIs (Dentium Co., Korea), 12 implants with PRF (as the study group) and 12 implants without PRF (as the control group). The implants in the study group have been placed with PRF to augment the soft tissue, while in control group, no PRF has been placed.

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Treatment planning

Clinical examination for the evaluation of oral hygiene status and periodontal condition. Space analysis for the proposed implant site was performed to determine the suitability for dental implant placement which included measuring of inter-coronal distance and the inter-ridge distance. A preoperative radiographical examination by cone-beam computed tomography (CBCT) was done to evaluate the available bone height for implant placement, presence of any pathology and proximity to the vital structures.

Clinical measurement of soft tissue thickness

- The planned implant sites (both PRF and control sites) were marked on the stent (vacuum-formed retainer) which was fabricated specifically for each patient to make sure that the measurements will be standardized by keeping them at the same position during the follow up visits (one month and six months after surgery). These marks were placed on the stent according to measurements (intercoronal distances) for the implants determined by CBCT. The marks were placed on the buccal side of each implant site.

- After administration of local anesthesia by infiltration technique with (lidocaine hydrochloride 2% with adrenaline 1:80,000 (Septodent, France)), the stent was placed inside patient's mouth to mark the points on the soft tissue at each implant site with a dental probe (figure 1A).

- After locating the points, the stent was removed and the soft tissue thickness was measured using endodontic reamer size 50 **(Hehn *et al*., 2016)** as in figure 1B and these measurements were confirmed by using a vernier (figure 1C).

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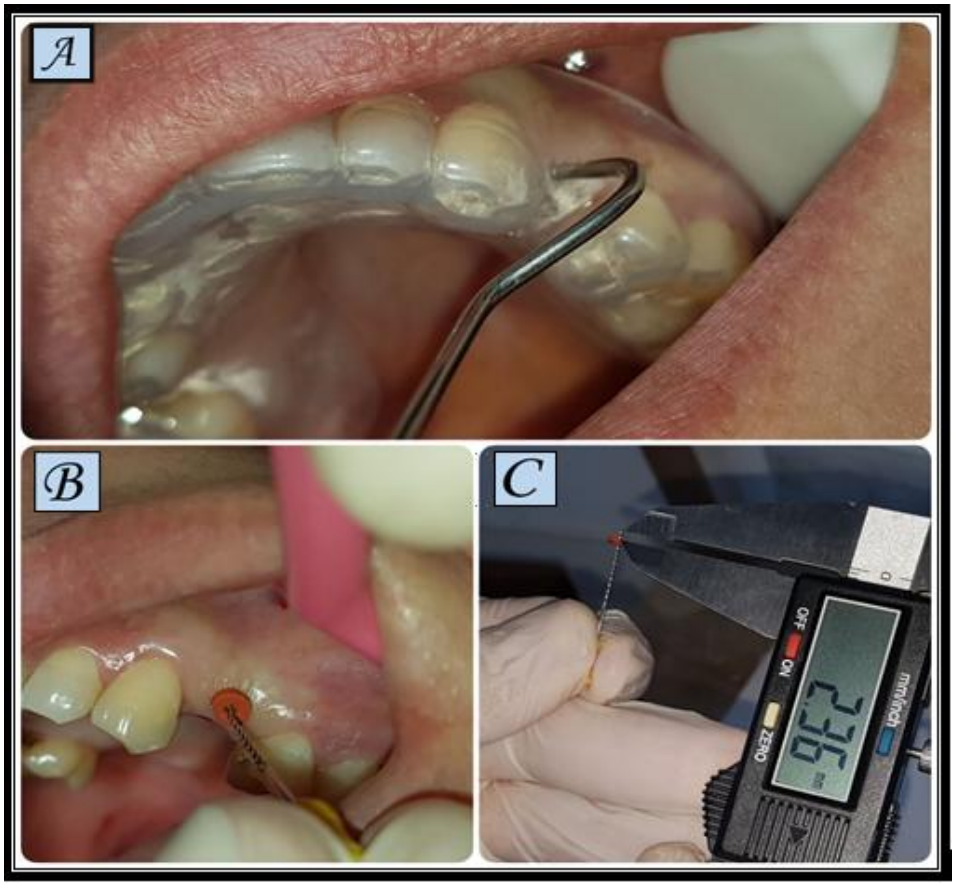

Figure 1: (A): marking the point at which soft tissue thickness will be measured. **(B):** measuring the thickness by endodontic reamer. **(C):** determining the accurate measurement by digital vernier.

Surgical procedure

The surgery is performed under local anesthesia by infiltration or block technique with (lidocaine hydrochloride 2% with adrenaline 1:80,000 (Septodent, France). The conventional implant insertion started with reflection of a 3-sided mucoperiosteal flap that consist of crestal incision in addition to two vertical releasing incisions were made using scalpel and blade number 15, then reflected to expose crestal, labial/buccal and palatal/lingual alveolar bone using mucoperiosteal elevator.

The preparation of implant osteotomy sites was carried out with drills of sequential diameters, starting with 1st drill and increase in size until reaching the required diameter, with the dentium dental implant engine set at a speed of 800 rpm and torque of 35 N/Cm.

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During PRF preparation, the implant fixtures were introduced into the prepared implant sites either by rotary method using the dental implant engine at a speed of 20 rpm with torque of 35 N/Cm or manually by using the ratchet from dentium surgical kit fixed to the adapter, and sometimes combination of two methods were used (figure 2A). The implant fixtures were placed at a level with or just below the crestal bone level, and after that, the cover screws were inserted.

PRF preparation started with minimally invasive venipuncture technique using disposable syringe (10 ml). After tourniquet was applied on the arm, skin was rubbed with alcohol and blood was collected from one of the superficial veins in cubital fossa (cephalic, basilic, median cubital, and median ante brachial veins). Once suitable vein has been identified, the needle was inserted into the vein. 10 ml of blood was collected from the patient and used as a standardized amount into plain glass tube (10 ml of blood for each implant in PRF side). In this case, a tube filled with the same amount of saline should be considered to balance the centrifugation. Then, the centrifuge was loaded with both tubes being placed one opposite to the other and immediately centrifuged at 3000 rpm for 10-12 minutes at the room temperature according to **(Dohan *et al*., 2006)** for preparation of L-PRF protocol.

After completion of centrifugation, PRF clot obtained was removed from the test tube (figure 2B) and separated from the blood clot by milking action with tweezers and the attached red blood cells scraped off and discarded. Then PRF clot was placed on the DI fixture at the study (PRF) site (figure 2C), and the flap was sutured over the PRF clot using a non-resorbable (4/0) silk suture (figure 2D) (we used clot as it contains a great amount of exudate, which is rich in growth factors) **(Newman *et al*., 2018)**. On the control site (the site without PRF), the flap was sutured directly over the fixture without adding any material.

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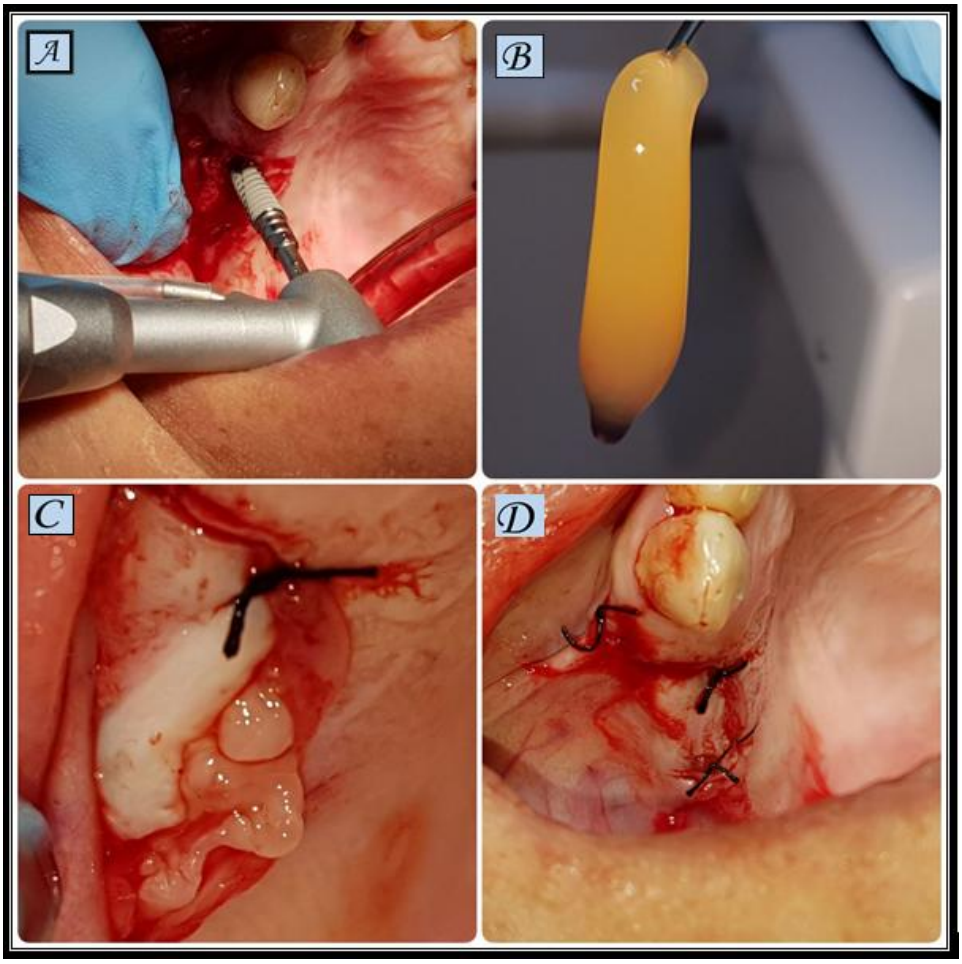

Figure 2: (A): dental implant placement. **(B):** L-PRF clot after removal from the tube. **(C):** placing L-PRF over the implant site. **(D):** suturing the flap over thr L-PRF.

Statistical analysis

Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version 21) Statistical analyses can be classified into two categories:

1-Descriptive Analysis:

A. Frequencies and percentage for nominal variables, minimum, maximum, mean, standard deviation (SD) for quantitative variable.
B. Graphs: Simple and Cluster chart bars.

2- Inferential analysis:

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Repeated Measure One Way ANOVA: use to detect the differences between K related means of the continuous quantitative variable with Bonferroni post hoc test, the Partial eta square effect size range from small (0.01-0.059), medium (0.06-0.139), large >=0.14 [1]

- Level of significance as: Not significant P>0.05, Significant P<0.05.

[1] Cohen, J. (1988) *Statistical power analysis for the behavioral sciences (2nd ed.)* (Hillsdale, NJ: Erlbaum)

- Detection of P value between mean differences was done by paired t-test.

Follow up and data collection

The patients were informed to return back (1) month and again (6) months after surgery to repeat the soft tissue measurements exactly as mentioned previously and evaluate the difference in these measurements between study and control sites.

RESULTS

Seven patients were enrolled in this study whom aged from 35-65 years with an average of **(47.71)** year and a standard deviation (SD) of **± 11.79**. The highest percentage **(71.43 %)** was reported ≤ 45 as shown in Figure 3.

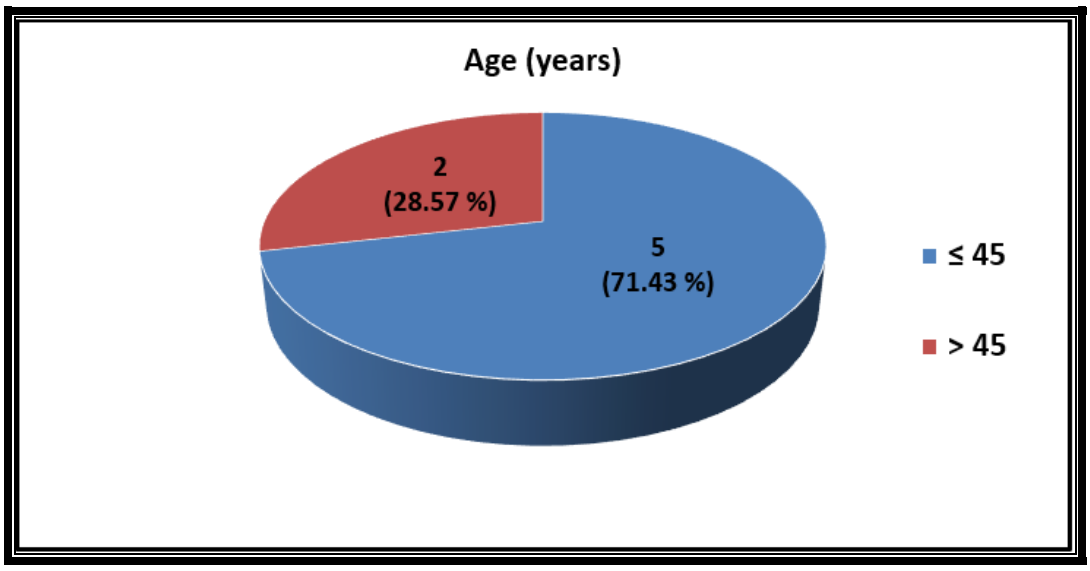

Figure 3: Pie chart demonstrating the age distribution.

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According to the gender, this study included 4 females **(57.14 %)** and 3 males **(42.86%)** as clarified in Figure 4.

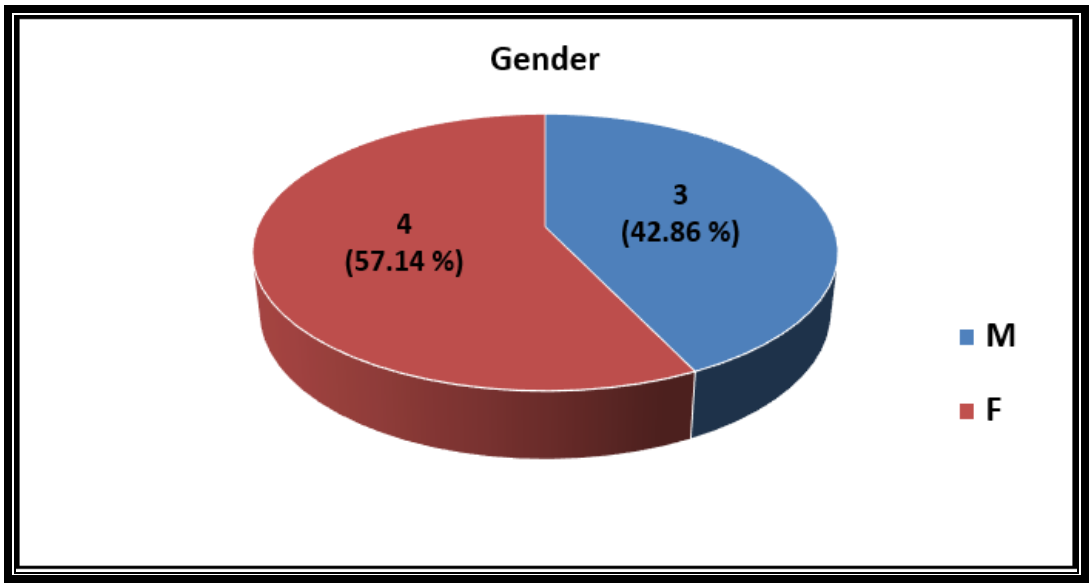

Figure 4: Pie chart demonstrating the gender distribution.

Regarding STT, there was a significant increase in both groups after one month, while after six months, a non-significant decrease has occurred in both groups. More decrease has occurred in PRF group as illustrated by the mean difference between second visit and baseline.

Table 1: Descriptive and statistical test of Soft tissue thickness

Groups		Buccal					F	P
		Base line	1st visit	MD (baseline-1st visit)	2nd visit	MD (baseline-2nd visit)		
PRF	Min.	1.93	2.78	**0.67**	2.00	**-0.31**	36.46 ES=0.77	**0.000 S**
	Max.	3.92	4.70		3.40			
	Mean	**2.98**	**3.65**		**2.67**			
	±SD	0.55	0.54		0.45			
No PRF	Min.	1.84	2.56	**0.40**	1.95	**-0.25**	15.08 ES=0.59	**0.000 S**
	Max.	3.52	3.80		3.04			
	Mean	**2.84**	**3.25**		**2.59**			
	±SD	0.51	0.41		0.38			

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F/T	0.41	0.52	6.61	0.22	1.47		
P	0.52	0.05	0.99	0.64	0.99		
	NS	ES =0.160	NS	NS	NS		

***s: significant at p-value 0.001, NS: non-significant, SD: standard deviation, ES: effect size, MD: mean difference, PRF: platelet rich fibrin.**

Table 2: intra group comparison regarding the buccal side

Variable	Groups	comparisons	P value	
Buccal	PRF	Base line xFirst visit	**0.000**	**S**
		Base line xSecond visit	0.117	NS
		First visit xSecond visit	**0.000**	**S**
	No PRF	Base line xFirst visit	**0.004**	**S**
		Base line x Second visit	0.196	NS
		First visit x Second visit	**0.000**	**S**

PRF: platelet rich fibrin.

Discussion

In this study, there was a significant increase of STT at first visit (after one month) in both control and study groups. The increase in PRF group may be explained by the biology of PRF as it is a second-generation platelet concentrate that contains all the components in the blood sample that are beneficial for healing. It consists of polymerized fibrin and platelets, white blood cells, cytokines and circulating stem cells **(Wang and Ma, 2020)**. The high‐density fibers provide an additional stability of the wounds and promote angiogenesis. Moreover, the concentrated platelet‐derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF), which are the main growth factors in PRF, enhance soft tissue healing by angiogenesis and matrix biosynthesis. These three main platelet cytokines play a fundamental role in initial healing mechanisms owing to their capacity to stimulate cell migration and proliferation, they attract stem cells to the site of release and stimulate cell proliferation, (particularly by platelet‐derived growth factors [PDGFs]) and induce fibrin matrix remodeling as well as secretion of a cicatricial collagen matrix (particularly by transforming growth factor-beta [TGFb]). With these fundamental considerations, PRF can be considered as a

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natural fibrin‑based biomaterial favorable to the development of a microvascularization **(Mufti *et al*., 2017, Ibraheem, 2018)**. Also, PRF appears to stay long enough during healing, it seems to have a sustained release of growth factors in a period between 1 and 4 weeks (one month) **(Borie *et al*., 2015)**.

This interpretation is supported by **(Bozkurt Doğan *et al*., 2015)** who revealed in their study that the higher increase in STT in the PRF group may be explained by biology of PRF, which contains much larger, denser and richer in growth factors fibrin matrix. Also **(Simonpieri *et al*., 2009)** illustrated in their study that PRF allows to obtain thick gingiva, and its action on the periosteum is significant, because of the growth factors and the fibrin itself, and **(Miron and Choukroun, 2017)** declared that direct contact of PRF with periosteum substantially improves the blood supply to the soft tissue favoring its thickness. Furthermore, this opinion is further supported by **(Thamaraiselvan *et al*., 2015)** and **(Dixit *et al*., 2018)** who mentioned that the increase in STT may be attributed to proliferation of gingival fibroblasts under influence of growth factors from PRF or to a spacing effect of the PRF membrane.

These results are in accordance with **(Dixit *et al*., 2018)** as there was an increase in thickness after one month in PRF group, and also with **(Elbattawy *et al*., 2020)** who showed an increase in STT in PRF group in their study at first re-entry after surgery.

On the other hand, a reduction in STT has occurred at second visit (after six months from baseline) which was non-significant. This is also may be attributed to the use of a single PRF clot and thus creating less spacing effect. Also, a small sample size (seven patients due to covid-19 and lockdown) may play a role in this result.

This is in the same line with **(Hehn *et al*., 2016)** where they mentioned a decrease in STT in PRF group. The author attributed the cause of reduction in thickness to the different flap design as a split thickness flap was used, and this resulted in a nutrition problem as the flaps would be too thin and additional nutrition from the periosteum and the bone is necessary to maintain a livid flap. On the opposite side, these data are in disagreement with **(Gupta *et al*., 2015)** as they declared an increase in STT in PRF group after six months.

CONCLUSION

In this study, the use of L-PRF has not been shown to be useful in increasing the soft thickness around dental implants.

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Limitation: a lockdown that has occurred in 2020 due to covid-19 has played a major role in limiting the sample size, larger sample size is required to confirm that whether L-PRF has a positive effect on keratinized tissue thickness around dental implants.

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