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RESEARCH ARTICLE

EVALUATION OF WOUND HEALING AND EPITHELIALIZATION USING TOLUIDINE BLUE DYE INDICATOR IN DIODE LASER FRENECTOMY WITH AND WITHOUT SURGICAL MICROSCOPE

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Abstract

Objective: The study aimed to evaluate the wound healing and epithelialization in diode laser frenectomy using toluidine blue dye as an indicator, comparing outcomes between procedures performed with and without the use of a surgical microscope at 1- and 2-weeks post-operatively.

Materials and Methods: This controlled clinical trial involved patients undergoing diode laser frenectomy, divided into two groups: one group underwent the procedure with the aid of a surgical microscope, while the other group did not use a microscope. Wound healing and epithelialization were assessed using toluidine blue dye at 1 and 2 weeks following the procedure.

Results: The group that received diode laser frenectomy with the use of a surgical microscope demonstrated significantly faster and more complete wound healing and epithelialization at both 1 and 2 weeks compared to the group that did not use the surgical microscope. The toluidine blue stain uptake showed similar results.

Conclusion: The use of a surgical microscope in diode laser frenectomy appears to enhance wound healing and epithelialization, as evidenced by the results at both 1 and 2 weeks. This suggests that incorporating a surgical microscope into the procedure may improve clinical outcomes and reduce recovery time. Further research is needed to confirm these findings across larger and more diverse patient populations.

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Introduction:-

Frenum is a fold of mucous membrane, that provides stability to the upper & lower lip and tongue. It is enclosed by muscle fibres and attaches the mucous membrane of the cheek and lip to the alveolar mucosa and/or the periosteum.¹

When the frenum attaches at an abnormal position on the gingiva, it causes inflammatory changes by interfering with plaque removal². It may also increase the risk of a gingival recession. It may even cause esthetic problems like midline diastema due to widening of interdental space. It also affects stability of removable partial and complete denture³.

In some cases, it is corrected physiologically as the frenal attachment repositions with the growth of alveolar process due to eruption of permanent incisors. However, when this does not happen, a frenectomy /frenotomy is indicated.

Many surgical procedures have been devised to treat an aberrant frenum attachment like Miller's technique, V-Y plasty and Z-plasty⁴.

Although effective, the surgical procedures are invasive and have increased intra-operative bleeding, post-operative discomfort, pain and morbidity⁵. Hence, laser excision and electrocautery provide an added advantage of reduced bleeding and post operative pain. However, electrocautery is accompanied by certain complications, like burns, risk of bone necrosis, interference with pacemakers, the production of surgical smoke and explosion (in rare cases). These complications are overcome by lasers, especially diode and CO₂ lasers⁶.

These surgeries are currently being performed as a chair side procedure with no magnification or macroscopically. Subjects treated under magnification supplied by the microscope is termed microscopic. Microscopic surgeries has several advantages over conventional surgeries, such as, faster wound healing, minimal trauma and patient morbidity.

Thus, the aim of the current study is to evaluate and compare the post operative healing and wound epithelialization after the treatment of aberrant frenum attachment employing a diode laser with or without surgical microscope.

Materials and Methods:-

Study design:

This study was conducted in the Department of Periodontology and Oral Implantology, ITS Centre for Dental Studies and Research, Muradnagar, Ghaziabad. This study was a randomized control clinical trial which included a total of 18 sites with high frenal attachment. The sites were randomized into group I (Laser excision of frenum without magnification) and group II (Laser excision of frenum using surgical microscope) using the envelope method.

Inclusion Criteria:

1. Systemically healthy subjects between 12-55 years of age
2. Subjects with presence of papillary or papillary penetrating type of frenal attachment (Placek et al, 1974)²⁷.
3. Subjects with no active periodontal infections.
4. Subjects who were willing to comply with all study related procedures and willing to sign an informed consent form

Exclusion Criteria:

1. Patients with habits of tobacco chewing, smoking, alcohol consumption or any other deleterious habits
2. Pregnant or lactating mothers
3. Patients with inability to provide informed consent
4. Patients taking medications that affect wound healing of periodontium

Standardized Clinical Parameters:

1. The following clinical parameters were recorded for each site at baseline i.e., pre-operatively and post-operatively on the 7th and 14th day.
2. Wound healing index (Landry, Turnbull and Howley, 1988)¹⁵
3. Wound evaluation using Toluidine blue as indicator for epithelialization.^{16,28}

Pre – Surgical Phase

1. Prior to surgery, all the patients received thorough supra gingival and sub gingival scaling.
2. Oral hygiene instructions were given.
3. Patients were reviewed after a period of 1 week for oral hygiene maintenance.
4. Patients complying with the oral hygiene instructions and maintain it were included for the study.

Surgical Procedure:

Group I:

1. All procedures were performed under aseptic conditions on an outpatient basis.
2. The surgical procedure for Group I was performed as follows
3. The surgical area was anaesthetized using Local anesthesia (2% Lidocaine with adrenaline 1:800000)

4. Using a diode laser, (Settings: Power: 1W, Pulse interval: 0.1 s, Peak power: 5W and Wavelength: 980nm), the muscle fibers were excised with short, slow horizontal strokes.
5. All muscle fibers of the frenulum were severed until the lip no longer gave a positive tension test.
6. The wound was surgically closed using interrupted direct loop sutures (4-0 silk). (Figure 1-4)



Surgical procedure Group I: (from top left) Figure 1. Pre-operative view of patient, 2. Immediate post operative, 3. 1-week follow up with toluidine blue dye indicator 4. 2-week follow up with toluidine blue dye indicator.

Group II:

The surgical procedure was performed in a similar way in Group I except it was performed under surgical microscope at 6x magnification and for closure of wounds 5-0 silk sutures were employed.



Surgical procedure Group II:(from top left) Figure 5. Pre-operative view, 6. Immediate post-operative view, 7. Frenectomy under 6x magnification, 8. Suturing done under magnification, 9. 1 week follow up with toluidine blue indicator dye & 10. 2 week follow up with toluidine blue indicator dye.

Follow-Up Care:

The following routine post-surgical instructions was instructed to the patients as follows:

1. Avoid eating any hot/spicy food for 24 hours after surgery.
2. Not to touch the surgical area or disturb the wound area with tongue.

3. To apply icepack for 24 hours post surgery.
4. Not to brush the teeth in the surgical area for a period of at least 1 week to avoid trauma.
5. To rinse the mouth thoroughly after eating and drinking.
6. In case of any problem regarding the surgery undertaken, contact the doctor as soon as possible.
7. Take 1 tablet Diclofenac sodium 50mg SOS for the 1st 7 days.

All the clinical parameters were recorded at 1 week & 2 weeks, and statistical analysis was performed. Comparison of all variables (Wound Healing Index and Toluidine blue staining) intra and inter-group comparisons were performed. The data was entered into Office 360 Excel sheet and subjected to analysis using SPSS software (version 16). For inter-group comparison Unpaired t-test was used. To evaluate significance of differences at different time intervals Chi-square test and paired t-test were employed. The p-value was taken significant when less than 0.05 (confidence interval of 95% was taken).

The mean between the groups was compared by using Student t- test and the level of significance was taken at 5% (p<0.05).

Results:-

The present clinical study included 18 patients who underwent diode laser frenectomy without surgical microscope (Group I) and under surgical microscope (Group II). All patients enrolled in this study completed a follow up of 14 days post-operatively for evaluation of healing and re-epithelialization.

The results of wound healing scores at week 1 and week 2 (Landry, Turnbull and Howley, 1988)¹⁵ are depicted in Table 1 Graph 1. Intra group comparison revealed an increase in distribution of wound healing index score from week 1 to week 2 in both Group I (p <0.05) and Group II (p<0.05). Median wound healing index score showed statistically significant improvement in both the groups from week 1 to week 2. The median WHI scores at week 1 in Group I was 2, while that at week 2 was 4, which was statistically significant. The median WHI score at week 1 in Group II was 3 while at week 2 was 5. There was a statistically significant increase in the mean and median WHI from week 1 to week 2 in both the groups. (Table 1 Graph 1)

Intergroup comparison, revealed a statistically significant difference between Group I and II at week 1 (p value < 0.05), with 44.5% patients showing a wound healing index score of 3 in Group II whereas 66.7% of patients of Group I showed a score of 2. (Table 2 Graph 1). There was a statistically significant difference in wound healing scores between the groups at week 2 as well, in the favour of Group II (p value < 0.05) with 77.8% of the patients showing a wound healing score 5 while only 33.3% of patients in Group I showed the same score.

Table 1:- Intra-group comparisons of early wound healing index.

Group		Median	Mean	SD	P value
Group I	Week 1	2	2.11	0.60	0.005 S
	Week 2	4	4.33	0.50	
Group II	Week 1	3	3.33	0.71	0.010 S
	Week 2	5	4.78	0.44	

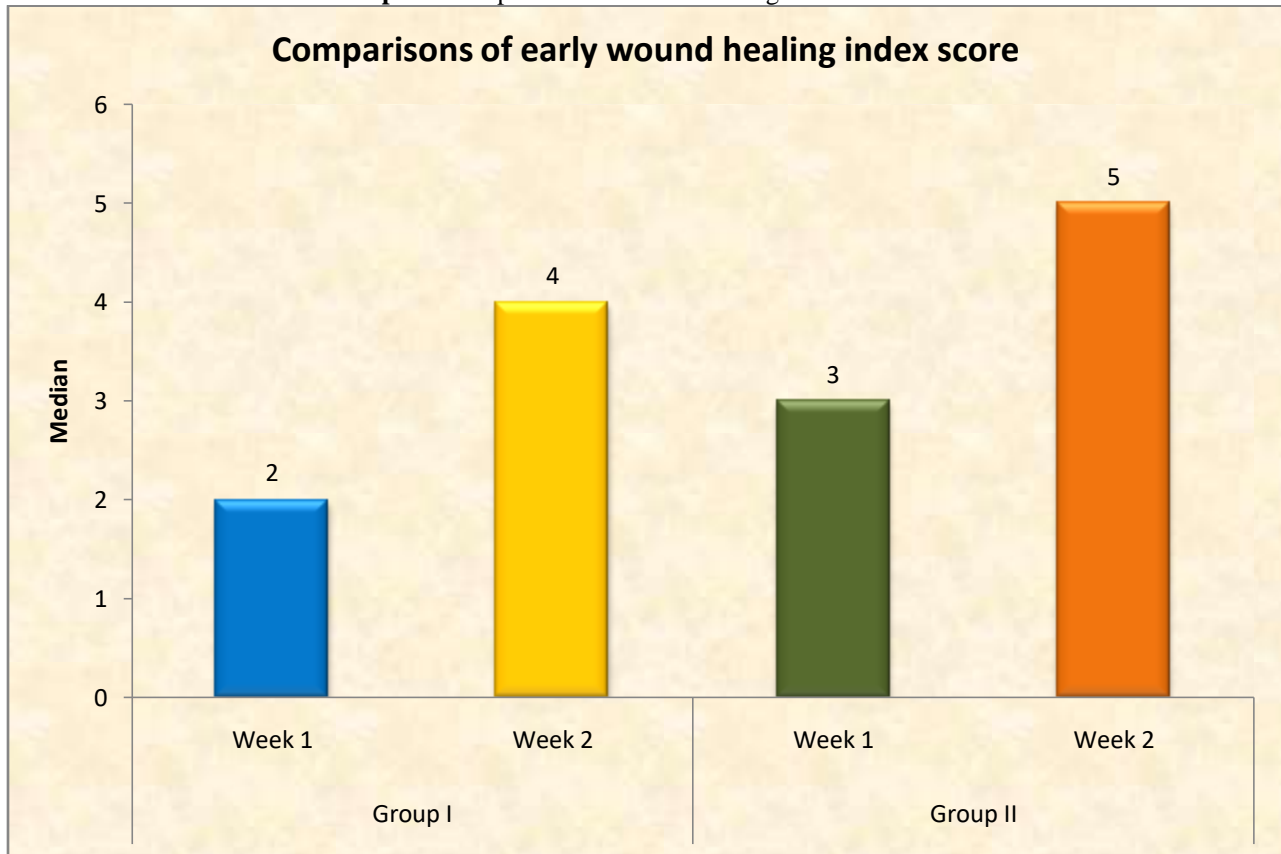
(Wilcoxon signed rank test)

Table 2:- Inter-group comparisons of early wound healing index.

Group	Group	Median	Mean	SD	Percentage	P value
Week 1	Group I	2	2.11	0.60	66.7%	0.003 S
	Group II	3	3.33	0.71	44.5%	
Week 2	Group I	4	4.33	0.50	66.7%	0.065 NS
	Group II	5	4.78	0.44	77.8%	

(Mann Whitney U test)

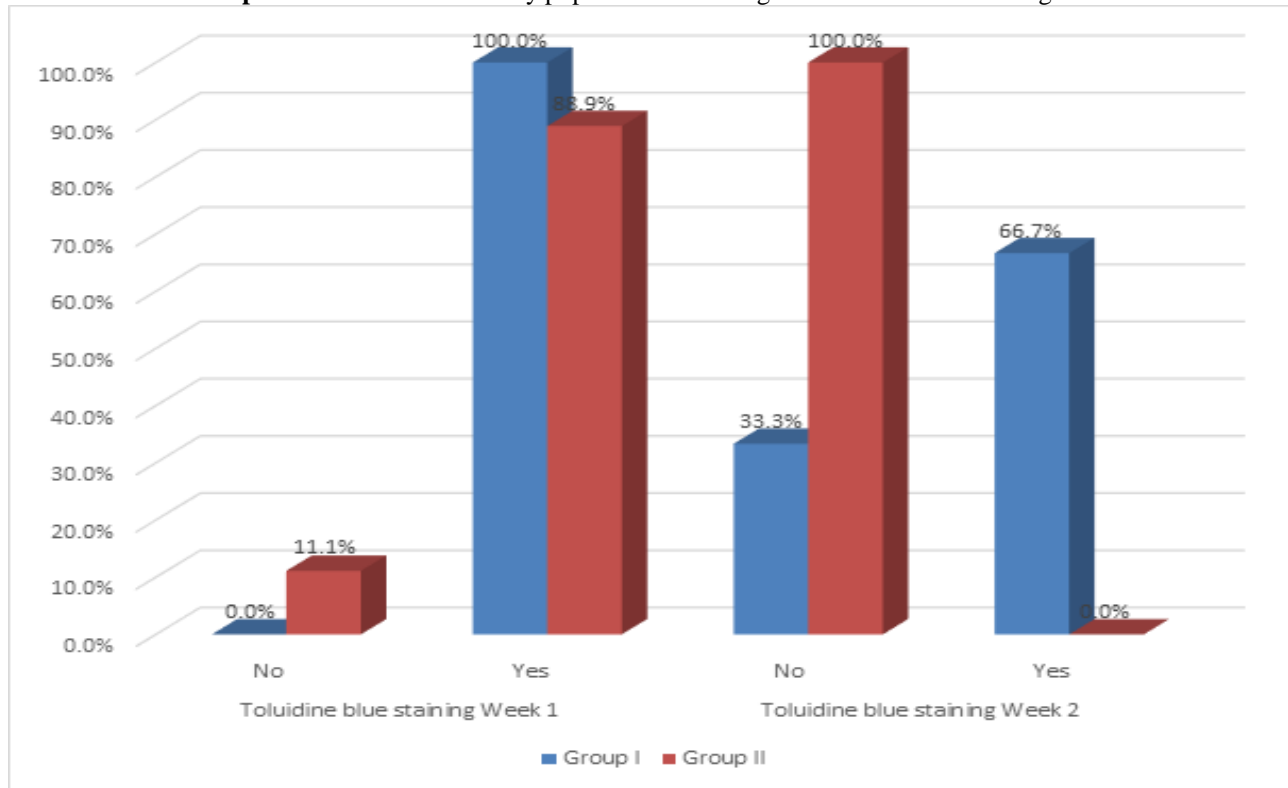
Graph 1: Comparisons of wound healing index scores



Toluidine blue stain has been used in this study as an indicator for epithelialization at week 1 and week 2 post-operatively. Results of Toluidine blue staining have been depicted in Table 3. At week 1 there was a higher number of sites picking up the stain in Group I (9 patients - 100%) as compared to Group II (8 patients - 88.9%) but the difference was not statistically significant ($p > 0.05$). At week 2, none of the surgical sites picked up the stain (100%) in Group II, as compared to 3 patients in Group I. There were a greater number of sites (6 patients) picking up the stain in Group I as compared to Group II (0 patients) which was statistically significant ($p < 0.05$). On intra group comparison, both groups showed statistically significant improvement in epithelialization from week 1 to week 2, with more percentage of sites not getting stained with toluidine blue stain ($p < 0.05$). (Table 3 Graph 2)

Table 3:- Distribution of study population according to Toluidine blue staining.

Toluidine blue staining		Groups			Total	p-value
		Group I	Group II			
Week 1	No	0	1	1	0.325	
		0.0%	11.1%	5.6%		
Yes		9	8	17		
		100.0%	88.9%	94.4%		
Week 2	No	3	9	12	0.003*	
			33.3%	100.0%		66.7%
	Yes	6	0	6		
			66.7%	0.0%		33.3%
p-value		0.048*	0.001*			

Graph 2:- Distribution of study population according to Toluidine blue staining.**Discussion:-**

The present clinical study was conducted in the Department of Periodontology and Oral Implantology, ITS-CDSR, Murad Nagar, Ghaziabad. The aim of this randomized control clinical trial was to compare and evaluate clinically the healing after diode laser frenectomy with or without surgical microscope in the treatment of aberrant frenum attachment.

In this study a total of 18 patients with aberrant frenum attachment were treated with frenectomy procedure using diode laser with or without surgical microscope. The subjects were divided into 2 groups: Group I (Laser frenectomy without surgical microscope) and Group II (Laser frenectomy under surgical microscope). All the patients were subjected to assessment of clinical parameters i.e. Wound healing Index (WHI) (Landry, Turnbull and Howley, 1988) and Clinical assessment of re-epithelialization using Toluidine blue as an indicator.

Laser surgery offers several significant benefits due to its high absorption by chromophores like hemoglobin, melanin, and collagen. This technology not only provides precise cutting but also facilitates coagulation and hemostasis. As reported in recent scientific papers^{9,31}. Lasers also show decreased patient morbidity and faster healing, hence have proven to be an ideal alternative to conventional methods. The laser wound was distinct from a traditional wound and exhibited quicker healing. This is due to the laser causing minimal damage to surrounding tissue, forming a surface coagulum of denatured protein initially. The inflammatory response is reduced, there are fewer myofibroblasts, and minimal wound contraction occurs³².

With the use of surgical microscope, we are anticipating a smaller wound with better approximation of wound edges. This may be attributed to the use of a laser and optical magnification provided by the microscope⁵. Procedures performed with laser under surgical microscope aid in precise surgery and less trauma to adjacent tissues.

Our study is the first to compare clinical wound healing employing surgical microscope and diode laser.

Assessment of healing has been done in our study using Wound healing index at week and week 2 (by Landry, Turnbull and Howley, 1988). By evaluating wound healing at these specific time points, researchers can provide insights into the initial stages of wound closure and monitor the early response to treatment. It is also deemed to be optimal evaluation period as many wounds exhibit significant changes in appearance and size during the first two weeks, allowing researchers to observe and quantify the progress made during this crucial period. Similar healing protocol was evaluated by **Lingameneni et al, 2018**³³, in their study where they evaluated wound healing using Landry's Wound healing index at 3rd, 7th and 14th day post-operatively using LLLT after gingivectomy and concluded that LLLT enhanced healing; can lead to complete wound epithelialization by 14th day.

In the present study, **distribution of study population based on Wound healing index score**, on intra group comparison revealed an increase in wound healing index score from week 1 to week 2 in both Group I ($p < 0.05$) and Group II ($p < 0.05$). On intergroup comparison, there was a statistically significant difference between Group I and II at week 1 ($p < 0.05$), with 44.5% patients showing a wound healing index score of 3 in Group II whereas 66.7% of patients of Group I showed a score of 2. (Table 2 Graph 1). There was a statistically significant difference in wound healing scores between Group I and II at week 2 as well, in the favour of Group II ($p < 0.05$) with 77.8% of the patients showing a wound healing score 5 (Excellent) while only 33.3% of patients in Group I showed the same score (Table 1 Graph 1). Similar results were obtained by **Kumar et al, 2015**³⁴, in which he compared WHI score after correction of mucogingival anomalies by scalpel and by laser and at week 1 and week 2 diode laser showed higher percentage population with greater WHI in diode laser group.

Median wound healing score showed statistically significant improvement in both the groups from week 1 to week 2. The median WHI scores at week 1 in Group I was 2, while that at week 2 was 4 ($p < 0.05$). The median WHI score at week 1 in Group II was 3, while at week 2 was 5. There was a statistically significant increase in the median WHI from week 1 to week 2 in both the groups. Inter group analysis at week 1 and week 2 showed a statistically significant increase in the WHI obtained in Group II ($p < 0.05$) as compared to Group I ($p < 0.05$). (**Table 2 Graph 2**). Similar results were obtained by **Zaaba et al, 2021**³⁵ in their study comparing healing in laser versus scalpel frenectomy using WHI 2-week post operatively and concluded that the laser technique showed better WHI scores than the scalpel technique.

Evaluation of wound using Toluidine blue as indicator of re-epithelialization, At week 1 there was a higher number of sites picking up the stain in Group I (9 patients - 100%) as compared to Group II (8 patients - 88.9%) but the difference was not statistically significant ($p = 0.325$). At week 2 Toluidine blue stain was not picked up by 9 patients (100%) in Group II as compared to 3 patients in Group I. There were a greater number of sites (6 patients) picking up the stain in Group I as compared to Group II (0 patients) which was statistically significant ($p = 0.003$). On intra group comparison, both groups showed statistically significant improvement in epithelialization from week 1 to week 2, with more percentage of sites not getting stained with toluidine blue stain ($p = 0.0048$ and 0.001 , Group 1 and Group 2 respectively). Similar results were observed by **Dahiya et al, 2019**³⁶, where epithelialization was evaluated using toluidine blue post gingivectomy by using either PRF membrane or non-eugenol pack as dressings, concluding that wounds covered with PRF membrane as dressing picked up less stain at 2 week time period. These results are also in accordance with study performed by **Lingameneni et al, 2019**³³ in which they utilized 2 tone dye as an indicator for epithelialization post LLLT therapy in gingivectomy patients and observed decreased stain being picked up at week 2 time period.

The results of the current study suggest, frenectomy when performed under surgical microscope shows better wound healing and complete epithelialization of wound 14 days post-operatively.

The limitations of the study were that the photographs taken for the sites could've been standardized even further to quantify the amount of toluidine blue stain uptake. Some software could've been employed to assess the toluidine blue stain. Also, the use of surgical microscope advocated use of microsurgical armamentarium, (5-0 sutures) which may have caused a bias in this study

Conclusion:-

Both Group 1 & Group 2 resulted in improvement in clinical parameters with the considerable promise for diode laser frenectomy, however WHI was in favor of Group II with surgical microscope. Further, studies with large sample size & long-term follow-up should be conducted to substantiate the result of our study. The results of the present study showed that diode laser with surgical microscope is reliable treatment

modality with more predictable healing and decreased post operative healing period for patients.

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