Soft Tissue Healing and IL-6 Cytokine Levels in Microsurgical and Conventional Open Flap Debridement in Patients with Chronic Periodontitis - A Randomized Clinico-Biochemical Trial

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ABSTRACT

BACKGROUND

The present study was conducted to compare clinical outcomes and gingival crevicular fluid (GCF) interleukin (IL)-6 cytokine levels in microsurgical and conventional open flap debridement procedure.

METHODS

Thirty sites in chronic periodontitis patients were randomly assigned into Group I (microsurgical) and Group II (conventional) open flap debridement in a split-mouth design. Gingival bleeding index (GBI), probing pocket depth (PPD), relative attachment level (RAL) were recorded at baseline and 3 months. GCF IL-6 cytokine levels were assessed at baseline and on 3rd day postoperatively. Pain perception using visual analog score (VAS) and soft tissue healing using early healing index (EHI) were assessed after on 7th day post-surgery.

RESULTS

There was a significant reduction in gingival bleeding index, probing pocket depth, relative attachment level within both the groups. Intergroup gingival bleeding index scores were statistically significant at the end of 3 months. The difference in visual analog scores between the two groups was found to be statistically insignificant whereas early healing index scores between the groups was found to be statistically significant. Group I showed lower levels of IL-6 on 3rd day postoperatively. It was also found that there was positive correlation of IL-6 levels with clinical parameters such as PPD and RAL.

CONCLUSIONS

Open flap debridement using microsurgical approach can substantially improve clinical parameters and wound healing compared with conventional macrosurgical approach. IL-6 levels were lower in microsurgical group indicating less invasive surgical approach.

KEY WORDS

Open Flap Debridement, Periodontal Microsurgery, Wound Healing, IL-6, Cytokine, GCF.

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BACKGROUND

The goal of periodontal therapy is to provide a dentition that functions in health and comfort for the life of the patient. This result is generally achieved by elimination of inflammation resulting from the subgingival colonization of pathogenic bacteria. Conventional periodontal treatments such as scaling and root planning, and open flap debridement are highly effective in repairing disease-related defects and in halting the progression of periodontitis.[1]

The treatment modalities in some areas of periodontal education and research have swung away from traditional mechanical and surgical therapy towards advanced treatment options. Development in the therapies and techniques such as endoscopes, magnification loupes, dental operating microscopes offer advantages to the clinician such as illumination, magnification, visual acuity and increased precision in the delivery of operating skills.[2] Magnification loupes provide better magnification, wider depths of the field and longer working distance. They provide a clear, larger image that allows the clinician to work better in the oral field. Microsurgery has been proposed in various fields of dentistry and gained importance in periodontics. It's perceived advantages in periodontics relate to the enhanced visual acuity associated with magnification and better soft tissue manipulation.[3] A variety of factors play an important role in determining the outcome of flap, such as soft tissue manipulation and importantly the ability for primary closure of the flap.[4]

Gingival crevicular fluid is an inflammatory exudate and contains different substances which includes immunoglobulin, microorganisms, toxins, cells, lysosomal enzymes and markers.[5] Cytokines have been detected in GCF and presence of such constituents can be of effective value in evaluating periodontal disease condition or outcomes of periodontal treatment. Interleukin-6 is a cytokine that is found at an increased level in GCF of periodontitis patients and is also reported to be closely related to clinical severity of periodontitis.[6]

To our knowledge till date, no scientific literature has been documented to compare GCF IL-6 cytokine levels in microsurgical and conventional open flap debridement procedure. Hence, the present study was done to compare the clinical outcomes and GCF IL-6 cytokine levels in microsurgical and conventional open flap debridement procedure.

Objectives

- To clinically evaluate the treatment outcomes of microsurgical open flap debridement and to compare the clinical outcomes with conventional open flap debridement procedure in chronic periodontitis patients.
- To evaluate and compare soft tissue healing outcomes in both the procedures in chronic periodontitis patients.
- To evaluate GCF cytokine levels before and after microsurgical and conventional open flap debridement in chronic periodontitis patients.

METHODS

The present single blinded randomized controlled clinico biochemical trial was carried out at a single centre, Kamineni Institute of Dental Sciences, Telangana from February 2019 to May 2020.

Approval for the conduct of the study was obtained from the Institutional Ethical Committee (IEC/2018/34) which was registered in clinical trials registry - India with registration no. CTRI/2019/02/017691. CONSORT guidelines were followed. The nature and purpose of the study was explained to the patients and written informed consent was obtained.

Patient Selection

A total of fifteen untreated chronic generalized periodontitis patients satisfying the inclusion and exclusion criteria enrolled in the study. The subjects for the study were selected from the outpatient's section, Department of Periodontics.

Inclusion Criteria

- Individuals between 25 - 60 years of age
- At least one site with probing pocket depth ≥ 5 mm
- Similar horizontal bone loss in contralateral quadrants

Exclusion Criteria

- Patients with any systemic diseases
- Without periodontal therapy in the past 6 months
- Pregnant or lactating women

Sample Size

- Sample size was calculated using G power 3.1 software. 30 sites would be sufficient to achieve the required significance under 95 % power with alpha error value set at 0.05.

Screening and Examinations

Total eight patients with chronic periodontitis were included in the study (Figure 1). After taking an informed consent from patient, periodontal examination was done using UNC-15 probe.

The clinical parameters gingival bleeding index (GBI),[7] probing pocket depth (PPD) and relative attachment level (RAL) of selected sites were recorded at baseline and 3 months in Group I and Group II. All measurements were performed by one experienced periodontal examiner, allowing an intra-experimental comparison of the values.

Percentage agreement with another examiner within 1 mm was > 97 %. A customized stent was fabricated to standardize the location and angulation of periodontal probe at all the six sites. Measurements were done at selected sites and the reading was recorded to the nearest millimetres.

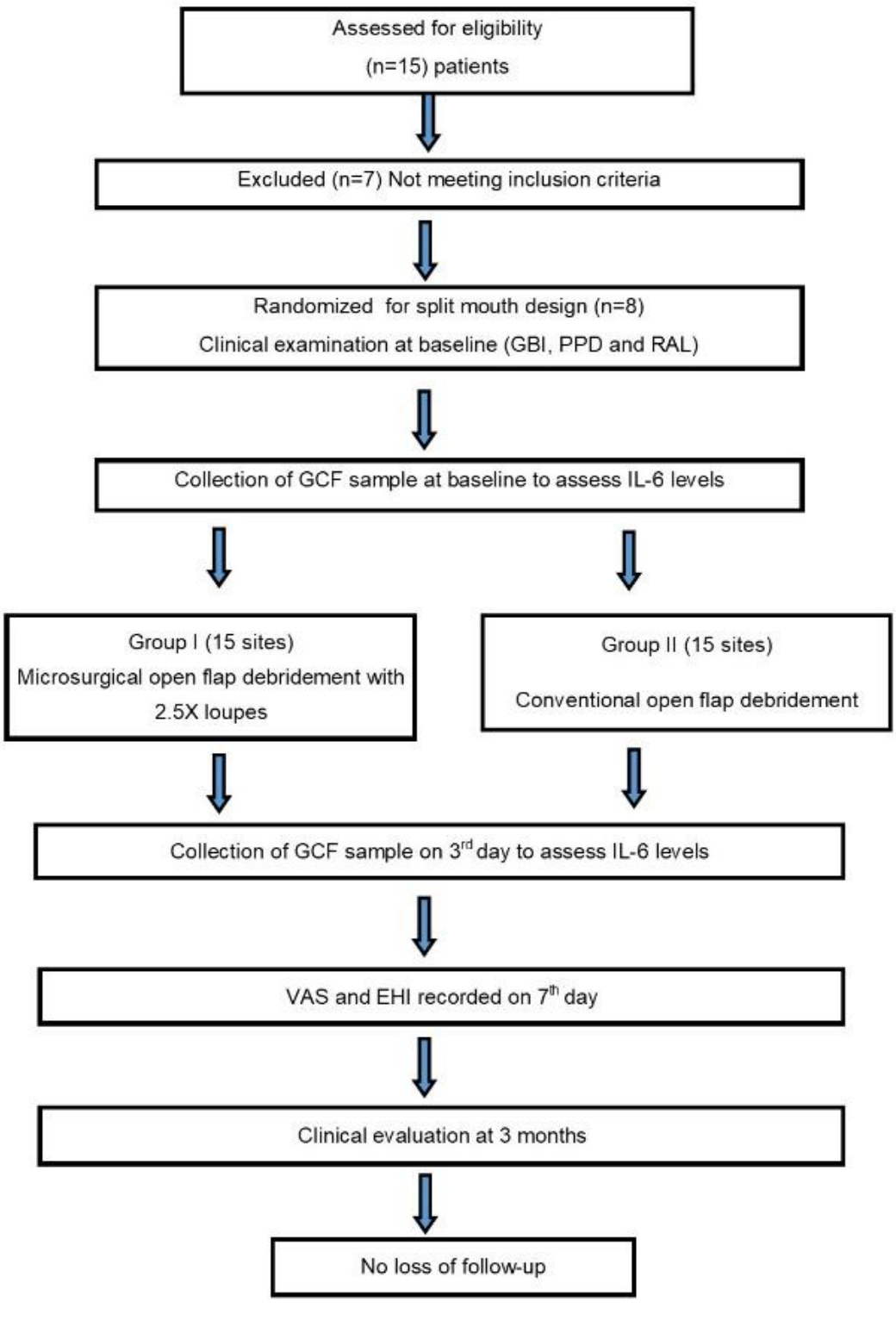

Figure 1. Study Design

Figure 2. Microsurgical Instruments

Randomization

- Total 30 sites were selected according to inclusion and exclusion criteria and the selected sites were randomly divided by flipping the coin into two groups of 15 each.
- Group I (Test): Microsurgical open flap debridement
- Group II (Control): Conventional open flap debridement
- All patients received thorough scaling and root planing.

Collection of Gingival Crevicular Fluid Sample

Pooled GCF samples were collected by micropipettes (Sigma Aldrich Company) at selected site after isolating the area with cotton rolls and air drying in order to prevent any salivary contamination of sample. GCF samples were collected at baseline and on 3rd day post-surgery. Following the isolation and drying of a site, capillary tubes of known internal diameter were inserted into the entrance of the gingival crevice.[8] 2 µl of GCF was collected and transferred to Eppendorf tubes and stored at - 80°c for further analysis of IL-6 by using enzyme linked immunosorbent assay (ELISA) kit.

Surgical Procedure

All periodontal surgical procedures were performed under aseptic conditions and the patient was asked to rinse the mouth before surgery with 10 ml of 0.2 % chlorhexidine digluconate solution for 60s.

After anesthetizing the area with 2 % lignocaine with adrenaline (1: 80000) solution, surgical procedure was performed, the selected sites in Group I microsurgery was carried out with ×2.5 optical magnification loupes (Heine Dental Loupes S - Frame). Sulcular incisions were placed with microsurgical ophthalmic blades (Figure 2). Buccal and lingual full-thickness mucoperiosteal flaps were elevated using periosteal elevators and granulation tissue adherent to the inner surface of flaps was carefully removed with area-specific curettes (Hu - Friedy, USA) to provide full access and visibility to root surfaces. Any remaining plaque and calculus were gently removed using hand instruments. Sutures were placed using 5-0 (non - resorbable) sutures to achieve primary closure. Whereas conventional open flap debridement was carried out at the selected sites in Group II using area-specific curettes (Hu - Friedy, USA) and both the surgical flaps were approximated using a 3-0 (non - resorbable) sutures.

All patients received systemic antibiotic therapy (Cap. Amoxicillin 500 mg thrice daily) for 7 days and analgesics (Ketorol DT 10 mg thrice daily) for 3 days to prevent post-operative pain and oedema. Sutures were removed 7 days post-operative. On 7th day post-surgery pain perception using visual analog scale[9] and soft tissue healing using early healing index (EHI) by Landry et al.[10] were assessed.

Statistical Analysis

The data were analysed using the SPSS (Statistical Package for the Social Sciences) software 20.00 program (SPSS Inc. Chicago, IL, USA). Before analysis, normality was checked by Kolmogorov Smirnov test and found that GBI, PPD, RAL, VAS and EHI were not following normal distribution. Therefore, the non-parametric tests (Mann-Whitney U test) were applied for above mentioned clinical parameters, whereas parametric tests (independent t test and dependent t test were applied) were applied for IL-6 level analysis.

RESULTS

The changes in GBI, PPD, RAL, VAS, EHI and IL-6 between the groups in different visits are shown in Table 1 and 2 respectively. Mean GBI scores at baseline and 3 months in Group I were 88.33 ± 12.91 and 36.67 ± 12.91 whereas in Group II were 93.3 ± 11.44 and 50.00 ± 13.36 respectively. Intra group comparison of GBI was statistically significant (P = 0.0007) between baseline and 3 months in Group I and Group II. Intergroup comparison of GBI was not statistically significant at baseline between Group - I and Group - II (P = 0.3615) but at 3 months it was statistically significant between Group - I and Group - II (P = 0.0327).

Parameter	Time Interval	Group I (Mean ± SD)	Group II (Mean ± SD)	P Value
GBI	Baseline	88.33 ± 12.91	93.33 ± 11.44	0.3615
	3 months	36.67 ± 12.91	50.00 ± 13.36	0.0327*
	P value	0.0007*	0.0007*	
PPD (mm)	Baseline	7.67 ± 1.40	7.13 ± 0.99	0.3297
	3 months	5.00 ± 0.93	4.87 ± 0.52	0.9835
	P value	0.0007*	0.0007*	
RAL (mm)	Baseline	7.67 ± 1.40	7.13 ± 0.99	0.3297
	3 months	5.00 ± 0.93	4.87 ± 0.52	0.9835
	P Value	0.0007*	0.0007*	

Table 1. Comparison of GBI, PPD and RAL at Baseline and Three Months Post–Surgery

*p<0.05 is statistically significant
GBI = Gingival Bleeding Index, PPD = Probing Pocket Depth, RAL = Relative Attachment Level, SD = Standard Deviation

Parameter	Time Interval	Group I (Mean ± SD)	Group II (Mean ± SD)	P Value
VAS	7th Day	3.60 ± 0.51	4.33 ± 1.40	0.0971
EHI	7th Day	4.46 ± 0.49	3.33 ± 0.47	0.0001*
IL - 6 (pg/µl)	Baseline	9.69 ± 1.24	9.27 ± 0.87	0.3018
	3rd Day	20.45 ± 3.74	28.63 ± 4.01	0.0001*
	p value	0.0001*	0.0001*	

Table 2. Comparison of VAS, EHI and IL-6 Levels

*P < 0.05 is statistically significant
VAS = Visual Analog Score, EHI = Early Healing Index, SD = Standard Deviation

In Group I, the mean PPD score at baseline was 7.67 ± 1.40 which was reduced to 5.00 ± 0.93 at 3 months whereas in Group II, the mean PPD score at baseline was 7.13 ± 0.99 which was reduced to 4.87 ± 0.52 at 3 months. Reduction of PPD scores was observed in Group I and Group II from baseline to 3 months which were found to be statistically significant (P = 0.0007). There was no statistically significant difference of PPD scores between Group I and Group II at baseline (P = 0.3297) and at 3 months (P = 0.3297).

In Group I, the mean RAL score at baseline was 7.67 ± 1.40 which was reduced to 5.00 ± 0.93 at 3 months. In Group II, the mean RAL score at baseline was 7.13 ± 0.99 which was reduced to 4.87 ± 0.52 at 3 months. Reduction of RAL scores were observed from baseline to 3 months in Group I and Group II respectively which was found to be statistically significant (P = 0.0007). There was no statistically significant difference of RAL scores between Group I and Group II at baseline (P = 0.3297) and at 3 months (P = 0.3297).

There was no statistically significant difference (P = 0.0971) of VAS in Group I (3.60 ± 0.51) and Group II (4.33 ± 1.40). EHI scores in Group I and Group II were 4.46 ± 0.49 and 3.33 ± 0.47 respectively. Intergroup comparison of EHI scores was found to be statistically significant (P = 0.0001).

In Group I, the mean IL-6 level score at baseline was 9.69 ± 1.24 pg/µl which increased to 20.45 ± 3.74 pg/µl whereas in Group II mean IL-6 level score at baseline was 9.2 ± 0.87 pg/µl which increased to 28.63 ± 4.01 pg/µl. Intragroup comparison showed a gradual increase of IL-6 cytokine level scores in Group I and Group II at baseline to 3 days which were found to be statistically significant (P = 0.0001). On 3rd day post-surgery, IL-6 level scores were found to be statistically significant (P = 0.0001) in between the groups.

DISCUSSION

The current pendulum of clinical opinion in some areas of periodontal education and research has swung far away from traditional mechanical and surgical therapy towards advanced treatment applications. The application of magnification to periodontics has tremendously refined the periodontal surgical care. Microsurgery has been proposed in various fields of dentistry. Its perceived advantages in periodontal surgery relate to the enhanced visual acuity associated with magnification and better soft tissue manipulation.

Dadwal et al.[11] evaluated the amount of remaining calculus after scaling and root planing, with and without magnification loupes and concluded that usage of magnification loupes resulted in less remaining calculus and less damage to tooth surface. According to Wachtel et al.[12] a microsurgical approach may also be of value for regenerative treatment using enamel matrix derivative, as it allows a more precise and less traumatic access to and debridement of the periodontal defect and a well-controlled application of the regenerative technology. In our study, there was statistically significant reduction (P = 0.0007) in GBI scores from baseline to 3 months in Group I and Group II respectively in accordance to a study done by Perumal et al.[13]

In the present study, there was a statistically significant difference of PPD and RAL among both the groups from baseline to 3 months (P = 0.0007) but they were statistically insignificant in between the groups at 3 months post-surgery (P = 0.9835). These results of the present study are in accordance to a study done by Singh et al.[14] where there was no statistically significant difference between the groups (micro-surgical and macro-surgical) in terms of PPD (P = 0.372) and clinical attachment level (P = 0.110).

Early healing was assessed on 7th day after the procedure which showed that Group I was significantly better than Group II. This may be due to delicate handling of the tissues and precise wound closure, which were similar to some of the earlier studies.[15,12]

VAS was assessed on 7th day after the procedure which showed the mean scores in Group I as 3.60 ± 0.51 and in Group II as 4.33 ± 1.40 respectively. In the present study, perceived pain was not statistically significant among groups (P = 0.0971). But however, it can be observed that pain range was wider in the Group II than Group I.

IL-6 is an important cytokine involved in the regulation of host response to tissue injury and infection.[16] In a meta-analysis[17] it was stated that GCF levels of IL-6 were significantly higher in subjects diagnosed with chronic periodontitis than periodontally healthy subjects. The total levels of IL-6 in the GCF of disease sites in chronic periodontitis patients decreased in response to nonsurgical therapy.[18] Therefore, in the present study IL-6 was used as diagnostic and prognostic potential for monitoring disease and therapeutic decisions.

In the present study, there was a statistically significant difference among the groups (P = 0.0001) and between the groups (P = 0.0001) respectively. Group I showed lower levels of IL-6 on 3rd day postoperatively indicating less surgical trauma.

Generally, IL-6 levels were elevated during early phase of wound healing which has been shown to be involved in regulation of leukocyte infiltration and angiogenesis. The results in the present study showed an increase in cytokine levels from baseline to 3 days post-surgery in both Group I and Group II but however, comparatively lower levels were observed in Group I. These are in accordance to a study done by Lin.[19] and Gallucci,[20] where the levels of IL-6 were maintained over the entire observation period of 14 days.

In order to ascertain the possible clinical relevance of these observations, correlation analysis between the clinical parameters and the total cytokine levels in GCF sample sites was performed. Positive correlations were observed between the levels of IL-6 with PPD and RAL. However, it was contradictory to a study, where they found that positive correlations were observed between the levels of IL-1α, IL - 1β, IL-10 and TNF-α but not IL-6 with PPD and CAL.[18] There was no correlation observed between cytokine levels and GBI, VAS, EHI.

The microsurgical approach involves delicate handling of tissues and precise wound closure which accounts for the favourable early wound healing as reported from studies.[21, 22] Comparison between the two groups showed more areas of haemorrhage in the control group than the test group specimens, which is suggestive of more trauma inflicted to the tissues treated by conventional surgery as compared to microsurgery.[3] This further confirms that microsurgical approach may be less traumatic and more favourable.

CONCLUSIONS

Open flap debridement procedures using the microsurgical approach significantly improved the early healing of the wound and decreased post-operative pain when compared to the clinical performance under conventional technique. IL-6 levels were lower in microsurgical group indicating less invasive surgical approach.

Limitations of This Study

The present study had certain limitations which include smaller sample size and shorter follow-up period, longer GCF collection time, difference in clinical parameters could be due to learning curve of surgical procedure with 2.5X magnification loupes and analysis of only IL-6 in GCF. Hence, future longitudinal studies are required to investigate cytokine levels associated with periodontal disease progression and correlate these cytokines with microsurgical procedures along with large sample size which further provides evidence regarding application of microsurgery in periodontal surgical procedures.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jemds.com.

REFERENCES

[1] Deas DE, Moritz AJ, Sagun RS Jr, et al. Scaling and root planing vs. conservative surgery in the treatment of chronic periodontitis. Periodontol (2000) 2016;71(1):128-39.
[2] Shanelec DA, Tibbetts LS. A perspective on the future of periodontal microsurgery. Periodontol (2000) 1996;11(1):58-64.
[3] Shreya S, Thoudam B. Comparative evaluation of microsurgical and conventional open flap surgical procedure outcomes in patients with periodontitis - a histopathological & scanning electron microscopy study. Biomed J Sci & Tech Res 2018;6(5):5503-08.
[4] Tibbetts LS, Shanelec DA. An overview of periodontal microsurgery. Curr Opin Periodontol 1994;2:187-93.
[5] Uitto VJ. Gingival crevice fluid -- an introduction. Periodontol (2000) 2003;31:9-11.
[6] Moreira PR, Lima PMA, Sathler KOB, et al. Interleukin - 6 expression and gene polymorphism are associated with severity of periodontal disease in a sample of Brazilian individuals. Clin Exp Immunol 2007;148(1):119-26.
[7] Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25(4):229-35.
[8] Pradeep AR, Daisy H, Hadge P, et al. Correlation of gingival crevicular fluid interleukin- 18 and monocyte chemoattractant protein-1 levels in periodontal health and disease. J Periodontol 2009;80(9):1454-61.
[9] Hayes MHS, Patterson DG. Experimental development of the graphic rating method. Psychological Bulletin 1921;18:98-99.
[10] Landry RG, Turnbull RS, Howley T. Effectiveness of benzydamyne HC1 in the treatment of periodontal post - surgical patients. Res Clin Forums 1988;10:105-18.
[11] Dadwal A, Kaur R, Jindal V, et al. Comparative evaluation of manual scaling and root planing with or without magnification loupes using scanning electron microscope: a pilot study. J Indian Soc Periodontol 2018;22(4):317-21.
[12] Wachtel H, Schenk G, Bohm S, et al. Microsurgical access flap and enamel matrix derivative for the treatment of periodontal intra-bony defects: a controlled clinical study. J Clin Periodontol 2003;30(6):496-504.
[13] Perumal MPB, Ramegowda AD, Lingaraju AJ, et al. Comparison of microsurgical and conventional open flap debridement: a randomized controlled trial. J Indian Soc Periodontol 2015;19(4):406-10.
[14] Singh SK, Sharma N, Malhotra S, et al. Coverage of localized gingival recession using coronally advanced flap: a comparison between microsurgical and macrosurgical techniques. Indian J Dent Sci 2017;9(2):88-97.
[15] Cortellini P, Tonetti MS. Microsurgical approach to periodontal regeneration. Initial evaluation in a case cohort. J Periodontol 2001;72(4):559-69.
[16] Hirano T, Akira S, Taga T, et al. Biological and clinical aspects of interleukin 6. Immunol Today 1990;11(12):443-9.
[17] Stadler AF, Angst PDM, Arce RM, et al. Gingival crevicular fluid levels of cytokines/chemokines in chronic periodontitis: a meta-analysis. J Clin Periodontol 2016;43(9):727-45.
[18] Reis C, Da Costa AV, Guimaraes JT, et al. Clinical improvement following therapy for periodontitis: Association with a decrease in IL - 1 and IL - 6. Exp Ther Med 2014;8(1):323-7.
[19] Lin ZQ, Kondo T, Ishida Y, et al. Essential involvement of IL - 6 in the skin wound- healing process as evidenced by delayed wound healing in IL - 6 - deficient mice. J Leukoc Biol 2003;73(6):713-21.
[20] Gallucci RM, Simeonova PP, Matheson JM, et al. Impaired cutaneous wound healing in interleukin - 6 - deficient and immunosuppressed mice. FASEB J 2000;14(15):2525-31.
[21] Burkhardt R, Lang NP. Coverage of localized gingival recessions: comparison of micro and macrosurgical techniques. J Clin Periodontol 2005;32(3):287-93.
[22] Fickl S, Thalmair T, Kebschull M, et al. Microsurgical access flap in conjunction with enamel matrix derivative for the treatment of intra-bony defects: a controlled clinical trial. J Clin Periodontol 2009;36(9):784-90.