Effect Of 1% Alendronate Gel on IL-6 Levels in Non-Surgical Management ofChronic Periodontitis - A Randomized Clinical Trial & An Immunological Analysis

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Abstract:

Aim- Alendronate (ALN), an aminobisphosphonate, inhibits osteoclastic activity, thus inhibiting bone resorption. Literature suggests that Alendronate reduces the levels of Interleukin-6 (IL-6) in osteoblastic cell cultures. So, the aim of the study was to evaluate the effect of 1% Alendronate Gel on of IL-6 levels in Gingival Crevicular Fluid (GCF), which was quantified by using ELISA and to compare and evaluate the effect of 1% Alendronate Gel on clinical parameters.

Materials and Methods- 10 patients (5 males and 5 females) suffering from Chronic Periodontitis as per American Academy of Periodontology (AAP) classification of 1999 with PD $\geq$ 5mm and $\leq$ 7mm and radiographic evidence of horizontal bone loss were included in the study. Scaling and Root Planing (SRP) followed by administration of 1% Alendronate Gel as a local host modulating agent into the gingival sulcus was done. GCF collection was done at baseline, 3 and 6 months using a micropippete for estimation of IL-6 and its quantification was

done by ELISA. Statistical Analysis was done by Independent t test, Friedman Test and Wilcoxon Test.

Results- The results showed statistically significant reduction in full mouth Gingival Index (Loe & Silness, 1963) *(P < 0.0001)*, Plaque Index (Silness and Loe, 1964) *(P < 0.0001)*, Pocket Probing Depth *(P < 0.001)*, gain in Relative Attachment Levels *(P < 0.001)* and reduction in IL-6 levels in GCF *(P < 0.0001)* at treated sites from baseline to 6 months.

Conclusion- Scaling and Root Planing along with 1% Alendronate Gel is an effective adjunct in non-surgical management of periodontal pockets.

Clinical Significance- In the present study, 1% Alendronate Gel as a local host modulating agent has shown promising results on clinical parameters i.e. Gingival Index, Plaque Index, Pocket Probing Depth and Relative Attachment Levels; and immunological parameter i.e. IL-6 levels in GCF.

Keywords:

1% Alendronate Gel, Gingival Crevicular Fluid, IL-6, Local Host Modulating Agent, Randomized Clinical Trial

Key Messages:

1. Various studies have substantiated improvement in the clinical parameters such as CAL/RAL and Probing depth using **Alendronate Gel** in different treatment modalities, either alone or with placebo or compared to some other drugs.
2. Till date, literature does not report any study where the effect of **1%Alendronate gel** (as a local drug delivery agent) on IL-6 levels in GCF has been evaluated with the help of ELISA.
3. In the present study, 1% Alendronate Gel has shown promising results on clinical and immunological evaluation.

Introduction:

Deposition of plaque biofilm on the surface of tooth structure leads to an inflammatory response called Periodontitis which causes destruction of periodontal ligament and alveolar bone and can ultimately result in loss of tooth.[1] Although bacteria that colonize the tooth surface and gingival sulcus initiate the periodontal disease process, the host response is accredited to play an indispensable role in the

breakdown of bone and connective tissue, which are fundamental features of the disease process. The host inflammatory response leads to infiltration of leukocytes, creating a barrier against bacterial invasion. Leukocyte recruitment is mediated by bacterial bi-products, chemokines, cytokines, lipid mediators, a combination of adaptive and innate immunity, and complement factors.[2]

Gingival crevicular fluid (GCF) has been examined for cellular immune-response indicators, such as Interleukin- 1 (IL-1), IL-I α, IL-1ß, IL-6, IL-8 and tumor necrosis factor-α by several investigators. Levels of these indicators could serve as possible biomarkers of active periodontal disease.[3]

Cytokines are small glycoproteins belonging to the transforming growth factor - β family produced by a variety of cell types, predominantly leukocytes and T-macrophages that regulate number of physiological and pathological functions including immunity, inflammation and hematopoiesis and can induce chondrogenic and osteogenic differentiation in undifferentiated mesenchymal cells.[4] They have been categorized into **pro or anti-inflammatory cytokines**, depending on their effects on immunocytes.

Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine that stimulates Immunoglobulin secretion by human B-lymphocytes, activates T-cells, stimulates the synthesis and secretion of acute phase proteins by hepatocytes, and triggers the complement system cascade.[5] IL-6 has also been shown to play a major role in the terminal differentiation of B-lymphocytes to plasma cells, which are the predominant inflammatory cells in tissues involved in established and advanced periodontal disease.[6, 7] IL-6 is of great significance because of its ability to induce bone resorption, both by itself and in conjunction with other bone-resorbing agents.[8, 9]

Bisphosphonates (BPs) constitute a class of drugs that modulates mineralization by binding to crystals of hydroxyapatite[6] and it can be divided into nitrogen-containing and non-nitrogen containing. These drugs bind readily to calcified bone matrix and are potential inhibitors of bone resorption. This property has engendered their use in the treatment of various diseases associated with increased bone resorption, such as

Paget's disease, hypercalcemia of malignancy, and osteoporosis.[10, 11, 12, 13]

Alendronate (ALN) is a nitrogen-containing second-generation bisphosphonate, which contains aminobisphosphonates with an amino-terminal group.[10] It is 70-folds more potent as compared to the non-nitrogen containing compound.[11, 12, 13, 14] It coheres to the crystals of hydroxyapatite present in bone and binds preferentially to the bone resorption surface, particularly to those encountering active osteoclastic resorption.[15] Studies have shown that systemic administration of Alendronate in rats reduces the levels of Interleukin-6 (IL-6) in periapical lesions.[16] Yang et al. in 2015 demonstrated that addition of Alendronate to the osteoclast cell culture results in inhibition of proinflammatory mediators such as IL-1β, IL-6, PGE 2 and TNF-α.[17]

Evidences have shown that bone destruction associated with periodontitis is caused by osteoclastic activity (**Reddy & Co-workers).**[18] Therefore any biocompatible inert agent capable of inhibiting osteoclastic activity should be effective in protecting alveolar bone housing. Keeping this tenet in mind, the study aimed to evaluate the efficacy of 1% Alendronate gel from the Bisphosphonate group of drugs in controlling bone resorption by mode of evaluating IL-6 levels in GCF with the objective of finding out the effect of 1% Alendronate gel on levels of IL-6 in non-surgical management of Chronic Periodontitis and comparing and evaluating its effects on clinical parameters.

Materials and Methods:

The present study was conducted in the Department of Periodontics, ITS Dental College, Hospital & Research Centre to evaluate the effect of **1% Alendronate Gel** on the levels of bone resorbing cytokine namely IL-6 in GCF which was quantified by using ELISA method. A study design was formulated based on the inclusion and exclusion criteria **(Flowchart 1)** and Ethical clearance was obtained from institution's review board. The clinical trial was registered under UMIN-CTR Clinical Trial (**Unique ID issued by UMIN - UMIN000032428**). Prior patient consent and ethical protocols were taken from each patient as outlined by Helsinki guidelines [19] after explaining the procedure in patient's language along with the potential risks and benefits involved.

Indigenous Formulation of 1% Alendronate Gel- To get a concentration of 1%, 50mg of Alendronate Sodium and 25 ml of distilled water were mixed and then 50mg of Carbopol 940 was added to it by stirring it gradually. Carbopol 940 was left to soak in the mixture for 2 hours. To this gel 0.12ml of Trietanolamine was added. Separately, 7.5mg of Methyl Paraben and 2.5mg of Propyl Paraben was dissolved in 0.5ml of Ethanol and was then added to the preparation. Then followed the autoclaving of the prepared gel at 121ºC for 30 minutes. The gel was then transferred into syringe and stored in a refrigerator. The shelf life of this gel is 3 years. **(Fig. 01)**

Clinical Procedure- *At baseline:* An acrylic stent was used to record the clinical parameters. 1µL GCF collection was collected from the area under consideration using a microcapillary tube**.** This sample was then transferred into Eppendrof Tubes and 99 µL of Phosphate Buffered Saline was added to it with the help of a micropipette and was stored at -20˚C**.** Ultrasonic Scaling and Root Planing was done. After which 1% Alendronate Gel was administered into the pocket with the help of a syringe and periodontal dressing was placed which was removed after 7 days. **(Fig. 02)**. *After 3 months:* Clinical Parameters were re-recorded. 1 µL GCF collection was done and 99 µL of Phosphate Buffered Saline was added to it with the help of a micropipette and was stored at -20˚C. *After 6 months:* Same procedure was repeated as was done at 3 months. **(Fig.03)**

Evaluation of IL-6 by ELISA- Human IL-6 ELISA test kit was used for the estimation of IL-6 **(Flowcart 2).** The quantitative evaluation of IL-6 was done using Automated Microplate ELISA Reader**.**

Preparation of Standard- Standard samples were prepared by performing serial dilutions of standard IL-6 sample provided with ELISA kit and optical density was measured **(Table 1)** according to which a graph was plotted **(Graph 1).** With the help of this graph an equation between the Optical Density and IL-6 concentration was derived.

Equation: y = 161716700 + (4.36377 - 161716700)/(1 + (x/31593.95)^1.373231)

The data gathered was subjected to statistical analysis by Independent t test, Friedman Test and Wilcoxon Test.

Results:

The results revealed a statistically significant reduction in mean Gingival Index[20] and Plaque Index[20] from baseline to 3 and 6 months *(P < 0.0001).* Also, there was a significant reduction in mean Pocket probing depth and gain in mean Relative Attachment Level both full mouth *(P < 0.0001)* and at treated sites *(P < 0.001)* **(Table 2)**. The results also revealed that there was reduction in optical density and IL-6 levels in collected GCF samples from baseline to 3 and 6 months which was found to be statistically significant *(P < 0.0001)* **(Table 3 & Graph 3)**. Further, the concentrations of IL-6 in the samples were within the range of standard samples prepared from the reagents provided with the ELISA kit.

Discussion:

This study was conducted to evaluate the effect of **1% Alendronate Gel** on IL-6 in GCF which was quantified by using ELISA method. 5 male and 5 female patients were included in the study with the mean age of 30.2 (±8.87130) and 33 (±8.33667) years, respectively. Clinical parameters including **Pocket probing depth (PPD), Relative Attachment Level (RAL), Gingival Index (GI) and Plaque Index (PI)** and **GCF samples** for IL-6 assessment were taken into consideration at **baseline, 3 and 6 months**.

Pathogenesis of periodontitis involves various proinflammatory cytokines such as IL-1, 6, 12, 17, 18, 21, TNF-α and IFN-γ. When healthy controls were compared with Periodontitis patients, an increased expression of IL-6 was found to be present in GCF as well as in gingival tissues of patients suffering from periodontitis.[21, 22] Similarly, nonsurgical periodontal therapy led to a decrease in circulating systemic levels of IL-6 resulting in improvement of the periodontal clinical parameters.[23] Another study was done by **Geivelis M, Turner DW, Pederson ED and Lamberts BL**[3] in which they described whether or not the amounts of IL-6 in GCF are associated with periodontal

clinical measures. Clinical parameters such as Plaque Index (PI), bleeding index (BI), probing depth (PD) were evaluated, IL-6 content in GCF samples were estimated. Significant interrelations were found between BI and IL-6 ($P < 0.005$) and between PD and IL-6 ($P < 0.05$), but not between PI and IL-6.

Various studies[1, 24, 25] have demonstrated the positive effects on various clinical after treatment with **Alendronate Gel**, either alone or with placebo or compared to some other drugs. **Sharma and co-workers**[24] studied local delivery of 1% ALN in patients suffering from Aggressive Periodontitis and concluded that 1% ALN when compared to placebo gel, used as an adjunct to scaling and root planing significantly causes increase in PPD reduction, CAL gain, and improvement in bone fill. **Sharma A and Pradeep AR**[25] studied the efficacy of local drug delivery of 1% ALN gel vs placebo gel as an adjunct to SRP in the treatment of intrabony defects in Chronic Periodontitis patients. The results of the study showed that the mean PPD reduction and CAL gain and mean percentage of bone fill were higher in the ALN group than in the placebo group at 2 and 6 months when compared with baseline values.

The results of the present study are also in accordance with the study done by **Pradeep AR, Kumari M, Rao NS, and Naik SB.**[1] They demonstrated the efficacy of local drug delivery of 1% ALN gel vs placebo gel as an adjunct to SRP for the treatment of Class II furcation defects. Results revealed that mean probing depth (PPD) was reduced, mean relative vertical and horizontal clinical attachment level (RVCAL and RHCAL) was improved and mean bone fill percentage increased in ALN group as compared to placebo group from baseline to 3, 6, and 12 months.

Moreover, there are studies which have shown that Alendronate inhibits the production of IL-6. According to **Giuliani et al.**, Alendronate inhibited the secretion of IL-6 in a dose dependant manner when observed in human osteoblastic osteosarcoma cell culture.[26] A similar study was done by **Olmos et al.**, in which they concluded that Etidronate inhibits IL-6 production by osteoblastic cells in culture stimulated by LPS.[27]

The current study reported that 1% Alendronate Gel reduces the levels of IL-6 in GCF in non-surgical periodontal therapy. Till date, to the best of our knowledge, literature does not report any study where the effect of **1%Alendronate gel** (as a local host modulating agent) on IL-6 levels in GCF has been evaluated with the help of ELISA,

thereby laying a foundation for other studies to compare it with other adjunctive osteoclastic inhibiting agents.

However, the limitation of a small sample size necessitates long-term studies with multicentred and randomized controlled clinical trials to know the clinical, immunological, and radiographic effect on bone regeneration in patients with Chronic Periodontitis. Also, a comparison with placebo could have been used to compare the efficacy of 1% Alendronate Gel.

Conclusion and Clinical Significance:

In the present study, 1% Alendronate Gel as a local host modulating agent has shown promising results on clinical and immunological parameters. The results revealed significant improvement in all clinical parameters and reduction in IL-6 levels in GCF when compared from baseline to 6 months. Further randomized, controlled clinical trials are required with increased sample size to prove the efficacy of 1% Alendronate Gel in the non-surgical management of Chronic Periodontitis. Therefore, in light of the present study, it can be concluded that 1% Alendronate Gel is an effective adjunct in non-surgical management of periodontal pockets.

Manufacturer Name:

1. Human IL-6 ELISA Kit
   Manufactured by: Diaclone SAS,
   6 Rue Docteur Jean-Francois-Xavier Girod,
   BP 1985, 25020 Besancon Cedex, France

2. Erba Lisa Scan EM,
   Automated Microplate Elisa Reader,
   Manufactured by ERBA Diagnostics Mannheim GmbH,
   Mallaustrasse 69-73 68219 Mannheim, Germany.

Compliance with Ethical Standards

Conflict of Interest:

I, Dr. Shradha declare that I have no conflict of interest.

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Ethical Approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. A copy of the Ethical clearance letter obtained from the institution has been enclosed.

Clinical Trial Registration: This clinical trial is registered under UMIN-CTR Clinical Trial (**Unique ID issued by UMIN - UMIN000032428**)

Informed Consent: Informed consent was obtained from all individual participants included in the study.

References:

1. Pradeep AR, Kumari M, Rao NS, Naik SB. 1% Alendronate Gel as Local Drug Delivery in the Treatment of Class II Furcation Defects: A Randomized Controlled Clinical Trial. J Periodontol 2013;84:307-315.

2. Graves D. Cytokines that promote periodontal tissue destruction. J Periodontol 2008;79(8):1585-1591.

3. Geivelis M, Turner DW, Pederson ED Lamberts BL. Measurements of Interleukin-6 in Gingival Crevicular Fluid From Adults With Destructive Periodontal Disease. J Periodontol 1993;64(10):980-983.

4. Reddi AH. BMPs: Actions in flesh and bone. Nat Med 1997; 3: 837-839.

5. Revel M. Host defence against infections and inflammations: Role of the multifunctional IL-6/IFN-beta 2 cytokine. Experientia 1989;45:549-557.

6. Vink A, Coulie PG, Wanters P, Nordan RP, Vansnick J. cell growth and differentiation activity of interleukin 1 and related plasmacytoma growth factors. Synergy with interleukin-1. Eur J Immunol 1988;18:607-612.

7. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease: A summary of current work. Lab Invest 1976;33:235-249.

8. Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, et al. IL-6 is produced by osteoblasts and induces bone résorption. J Immunol 1990;145:3297-3303.

9. Mundy G. Inflammatory mediators and the destruction of bone. J Periodont Res 1991;26:213-217.

10. Tenenbaum HC, Shelemay A, Girard B, Zohar R, Fritz PC. Bisphosphonates and periodontics: Potential applications for regulation of bone mass in the periodontium and other therapeutic/diagnostic uses. J Periodontol 2002;73:813-822.

11. Fleisch HA. Bisphosphonate – Preclinical aspects and use in osteoporosis. Ann Med 1997;29(1):55-62.

12. Weinreb M, Quartuccio H, Seedor JG, Aufdemorte TB, Brunsvold M, Chaves E, et al. Histomorphometrical analysis of the effects of the bisphosphonate alendronate on bone loss caused by experimental periodontitis in monkeys. J Periodontal Res 1994;29:35-40.

13. Hu JH, Ding M, Soballe K. Effects of short-term alendronate treatment on the three-dimensional microstructural, physical, and mechanical properties of dog trabecular bone. Bone 2002;31:591-597.

14. Lin JH. Bisphosphonates: A review of their pharmacokinetic properties. Bone 1996;18:75-85.

15. Tenenbaum HC, Torontali M, Sukhu B. Effects of bisphosphonates and inorganic pyrophosphate on osteogenesis in vitro. Bone 1992;13:249-255.

16. Silva RAB, Sousa AP-Pereira, Lucisano MP, Romualdo PC, Paula FWG-Silva, Consolaro A et al. Alendronate inhibits osteocyte apoptosis and inflammation via IL-6, inhibiting bone resorption in periapical lesions of ovariectomized rats, International Endodontic Journal 2020;53(1):84-96.

17. Yang W, Ko H, Kim H, Kim M. The effect of cathepsin K inhibitor on osteoclastic activity compared to alendronate and enamel matrix protein. Dent Traumatol 2015 Jun;31(3):202-8.

18. Reddy MS, Weatherford TW, Smith AC. Alendronate treatment of naturally occurring periodontitis in beagle dogs. J Periodontol 1995;66:211-217.

19. Krleza-Jerić K, Lemmens T. 7th revision of the Declaration of Helsinki: Good news for the transparency of clinical trials. Croat Med J. 2009;50(2):105–110.

20. Loe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967;38:610.

21. Kurtis B, Develioglu H, Taner IL, Balos K, Tekin IO. IL-6 levels in gingival crevicular fluid (GCF) from patients with noninsulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. J Oral Sci 1999;41:163-167.

22. Prabhu A, Michalowicz BS, Mathur A. Detection of local and systemic cytokines in adult periodontitis. J Periodontol 1996; 67:515-522.

23. Marcaccini AM, Meschiari CA, Sorgi CA, Saraiva MCP, de Souza AM, Faccioli LH, et al. Circulating interleukin-6 and high-sensitivity C-reactive protein decrease after periodontal therapy in otherwise healthy subjects. J

Periodontol 2009; 80:594-602.

24. Sharma A, Pradeep AR. Clinical Efficacy of 1% Alendronate Gel in Adjunct to Mechanotherapy in the Treatment of Aggressive Periodontitis: A Randomized Controlled Clinical Trial. J Periodontol 2012;83:19-26.

25. Sharma A, Pradeep AR. Clinical Efficacy of 1% Alendronate Gel as a Local Drug Delivery System in the Treatment of Chronic Periodontitis: A Randomized, Controlled Clinical Trial. J Periodontol 2012;83(1):11-18.

26. Giuliani N, Pedrazzoni M, Passeri G, Girasole G. Bisphosphonates inhibit IL-6 production by human osteoblast-like cells. Scand J Rheumatol 1998;27(1):38-41.

27. Olmos JM, De Vega T, Perera L, Riancho JA, Amado JA, González-Macías J. Etidronate inhibits the production of IL-6 by osteoblast-like cells. Methods Find Exp Clin Pharmacol. 1999 Oct;21(8):519-22.

28. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1-6.

Figures:

Figure 1: 1% Alendronate Gel after preparation in the laboratory (Image Source: Original).

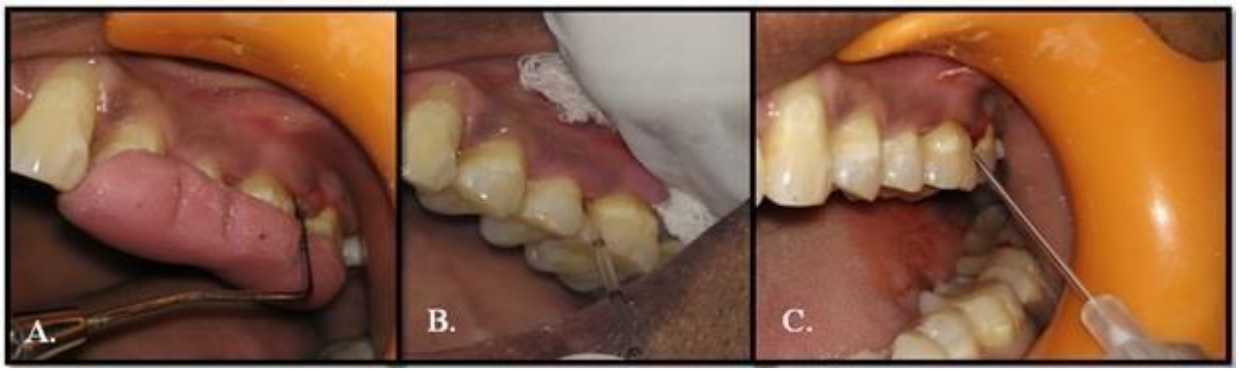


Figure 2: A. Measurement of Pocket Probing Depth and Relative Attachment Level with the help of acrylic stent using UNC-15 Probe, B. Collection of 1µl of GCF Sample using Microcapillary Tubes (Sigma Aldrich Subsidiary of Merck KGaA St. Louis, Missouri, United States), C. Administration of 1% Alendronate Gel into the gingival sulcus through syringe. (Image Source: Original).

Figure 3: Gingival Crevicular Fluid Samples Collected in Eppendrof Tubes (ImageSource: Original).

Tables:

Standard Sample Number	IL- 6 Concentration (in pg)	Optical Density (Dual Wavelength reading; 450 nm & 630 nm)
A	200	1.5459
B	100	0.9332
C	50	0.5526
D	25	0.2480
E	12.5	0.1068
F	6.25	0.0714
G	Blank / Zero	0.0000
H	Positive Control	1.6467

Table 1: IL-6 Concentration versus Optical Density of Standard Samples (IL-6- Interleukin-6; pg- picogram; nm- nanometer).

	Mean GI		**Mean PI**		**Mean PPD (in mm) (Site Specific)**		**Mean RAL (in mm) (Site Specific)**	
	Mean±SD	P value [a]	Mean±SD	P value [a]	Mean±SD	P value [a]	Mean±SD	P value [a]
1.Baseline	1.8970 ±.47067	<0.0001, S	1.4970 ±.25820	<0.0001, S	4.6100 ±.87471	0.001, S	4.1230 ±1.53111	0.001, S
2.3 Months	1.3390 ±.18959		1.2080 ±.07239		2.7350 ±.44250		2.7240 ±2.04402	
3.6 Months	1.1950 ±.19569		1.1000 ±.03464		2.6810 ±1.14179		2.0740 ±.25799	
Post hoc pairwise comparison[b]	1 >2 > 3		1 >2 > 3		1 > 2, 3		1 > 2, 3	

[a]Friedman test, [b]Wilcoxon test

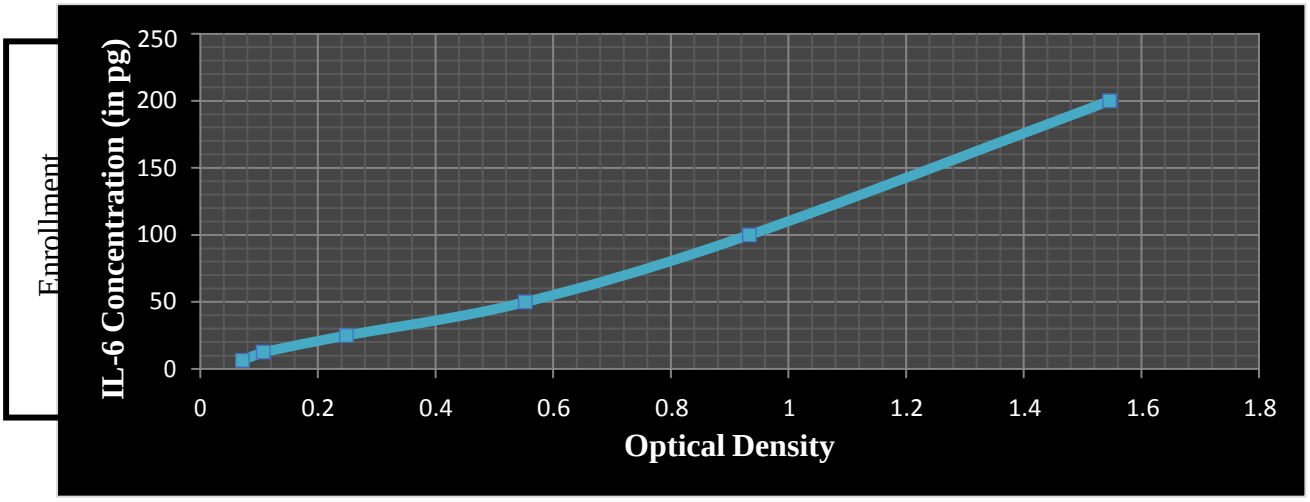
Table 2: Changes in Clinical Parameters from Baseline to 3 Months and 6 Months (GI- Gingival Index[20] ; PI- Plaque Index[20] ; PPD- Pocket Probing Depth, RAL- Relative Attachment Level; mm- micrometer; SD- Standard Deviation; S- Significant).

	Optical Density of GCF Samples (in $L^{-1}cm^{-1}$)				**IL-6 Levels in GCF Samples (mol/L)**			
	1. Baseline	2. 3 Months	3. 6 Months	Post hoc pairwise comparison[b]	1. Baseline	2. 3 Months	3. 6 Months	Post hoc pairwise comparison[b]
Minimum	.10	.00	.00	1 > 2 > 3	9.19	4.38	4.36	1 > 2 > 3
Maximum	.15	.10	.04		12.34	8.63	5.82	
Mean	.1274	.0311	.0136		10.7166	5.4411	4.7221	
Standard Deviation	±.01826	±.02874	±.01421		±1.24356	±1.32457	±.48093	
P value [a]	< 0.0001, S				< 0.0001, S			

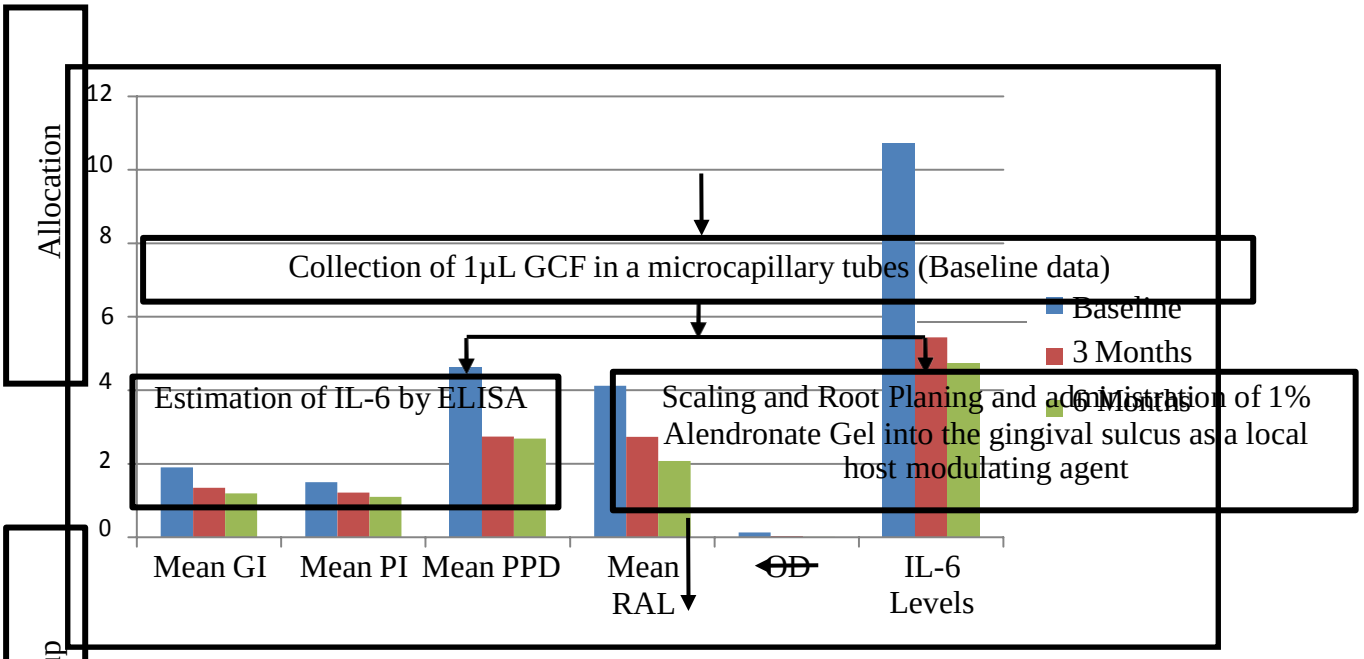
[a]Friedman test, [b]Wilcoxon test

Table 3: Changes in Optical Density and IL-6 Levels from Baseline to 3 Months and 6 Months (GCF- Gingival Crevicular Fluid; $L^{-1}cm^{-1}$ – per litre per centimetre; IL-6- Interleukin-6; mol/L- mole/Litre; S- Significant).

Graphs:

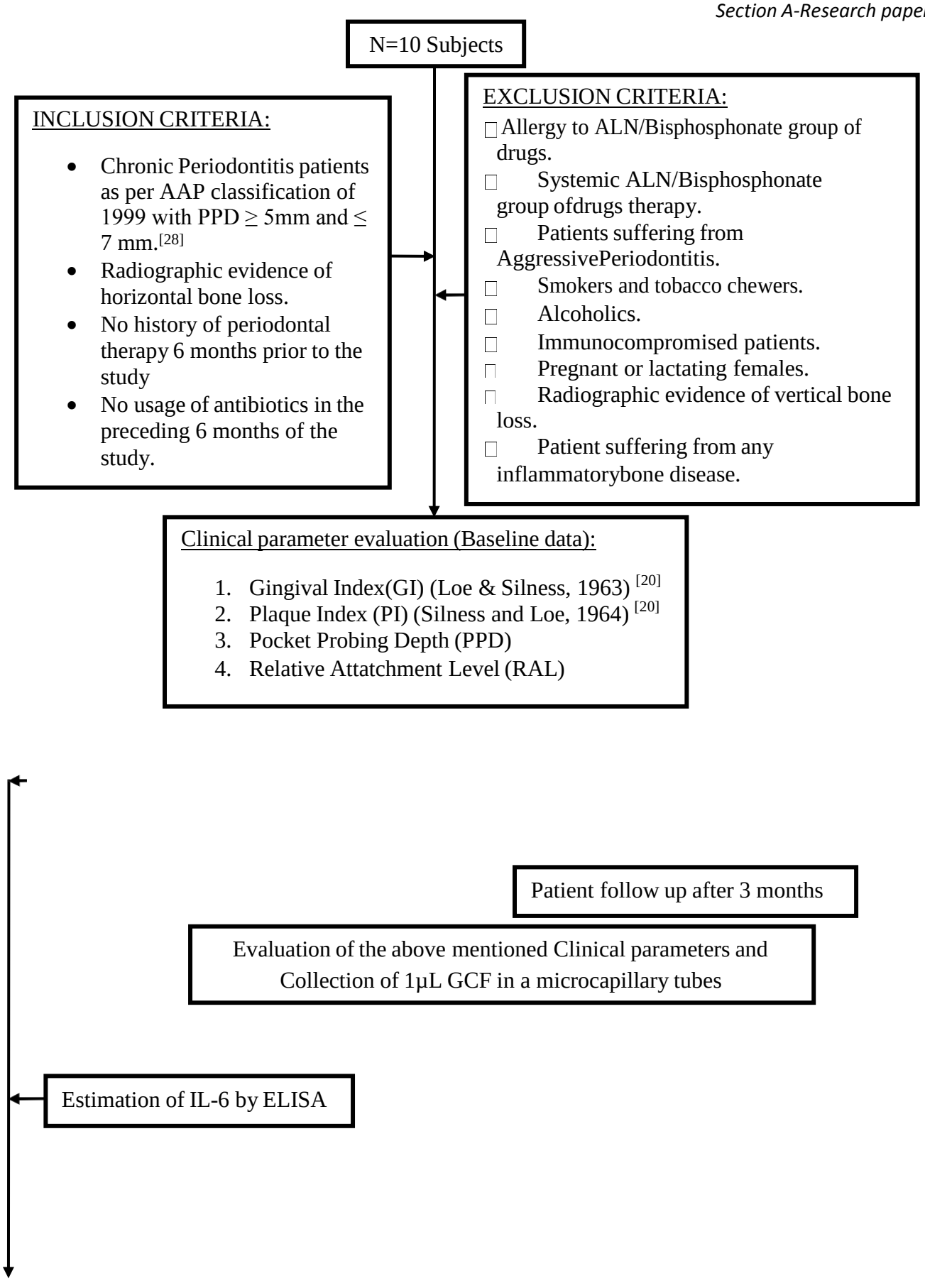


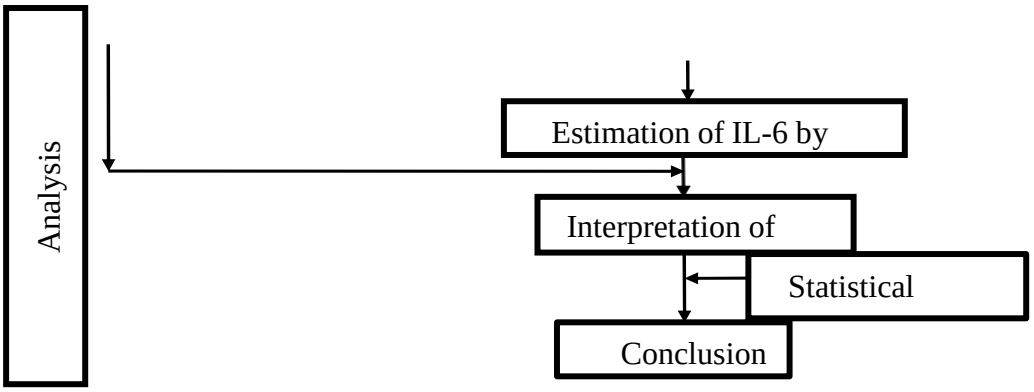
Graph 1: IL-6 Concentration versus Optical Density of Standard Samples (IL-6- Interleukin-6; pg- picogram).



Graph 2: Changes in Clinical Parameters, Optical Density and IL-6 Levels in GCF from Baseline to 3 Months and 6 Months (GI- Gingival Index[20] ; PI- Plaque Index[20] ; PPD- Pocket Probing Depth, RAL- Relative Attachment Level, OD- Optical Density, IL-6- Interleukin-6).

Flowcharts:


N=10 Subjects
INCLUSION CRITERIA:
Chronic Periodontitis patients as per AAP classification of 1999 with PPD ≥ 5mm and ≤ 7 mm.[28]
Radiographic evidence of horizontal bone loss.
No history of periodontal therapy 6 months prior to the study
No usage of antibiotics in the preceding 6 months of the study.
EXCLUSION CRITERIA:
Allergy to ALN/Bisphosphonate group of drugs.
Systemic ALN/Bisphosphonate group ofdrugs therapy.
Patients suffering from AggressivePeriodontitis.
Smokers and tobacco chewers.
Alcoholics.
Immunocompromised patients.
Pregnant or lactating females.
Radiographic evidence of vertical bone loss.
Patient suffering from any inflammatorybone disease.
Clinical parameter evaluation (Baseline data):
1. Gingival Index(GI) (Loe & Silness, 1963) [20]
2. Plaque Index (PI) (Silness and Loe, 1964) [20]
3. Pocket Probing Depth (PPD)
4. Relative Attatchment Level (RAL)
Patient follow up after 3 months
Evaluation of the above mentioned Clinical parameters and Collection of 1µL GCF in a microcapillary tubes
Estimation of IL-6 by ELISA



Flowchart 1: Study Design (**CONSORT** Statement) (AAP- American Academy Of Periodontology; PD- Pocket Probing Depth; ALN- Alendronate; µL- Microlitre; GCF- Gingival Crevicular Fluid; IL-6- Interleukin-6; ELISA- Enzyme Linked Immuno Sorbent Assay).

Total procedure length: 1 hour 45 minutes

Add 100 µl of sample and diluted standard/controls and 50µl Biotinylated anti-IL-6

↓

Incubate 1 hour at room temperature

↓

Wash three times

↓

Add 100 µl of Streptavidin-HRP

↓

Incubate 30 minutes at room temperature

↓

Wash three times

↓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 12-15 minutes.

↓

Add 100 µl H_2SO_4

↓

Read Absorbance at 450 nm

Flowchart 2: Assay Summary (IL- Interleukin; µL- microlitre; HRP- *Horseradish Peroxidase; TMB*- Thermo Scientific Pierce; nm- nanometer).

Figure Legends

- Figure 1: 1% Alendronate Gel after preparation in the laboratory
- Figure 2: A. Measurement of Pocket Probing Depth and Relative Attachment Level using UNC-15 Probe, B. Collection of 1 µl of GCF Sample using Microcapillary Tubes, C. Administration of 1% Alendronate Gel into the gingival sulcus through syringe.
- Figure 3: Samples Collected in Eppendrof Tubes

Table Legends

- Table 1: IL-6 Concentration versus Optical Density of Standard Samples (IL-6- Interleukin-6; pg- picogram; nm- nanometer
- Table 2: Changes in Clinical Parameters from Baseline to 3 Months and 6 Months (GI- Gingival Index [20]; PI- Plaque Index [20]; PPD- Pocket Probing Depth, RAL- Relative Attachment Level; mm- micrometer; SD- Standard Deviation; S- Significant)
- Table 3: Changes in Optical Density and IL-6 Levels from Baseline to 3 Months and 6 Months (GCF- Gingival Crevicular Fluid; $L^{-1}cm^{-1}$ -per litre per centimetre; IL-6- Interleukin-6; mol/L- mole/Litre; S- Significant)

Graph Legends

- Graph 1: IL-6 Concentration versus Optical Density of Standard Samples (IL-6- Interleukin-6; pg- picogram)
- Graph 2: Changes in Clinical Parameters, Optical Density and IL-6 Levels in GCF from Baseline to 3 Months and 6 Months.

  Statistical Analysis: The data gathered was subjected to statistical analysis by Independent t test, Friedman Test and Wilcoxon Test.

Flowchart Legends

- Flowchart 1: Study Design (**CONSORT** Statement)
- Flowchart 2: Assay Summary