ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

EFFECTS OF ALENDRONATE THERAPY ON CYCLOSPORINE INDUCED ALVEOLAR BONE LOSS : A MORPHO-METRIC AND BIO-CHEMICAL STUDY IN RATS.

Dr. Sneha R. Bhat M.D.S[1], Dr. Aravind R. Kudva M.D.S[2], Dr. Dhoom S. Mehta M.D.S.[3].

1. Senior Lecturer, Department of Periodontology, Yenepoya Dental College & Hospital, Mangalore(Karnataka State), India
2. Reader, Department of Conservative Dentistry & Endodontics, Yenepoya Dental College & Hospital, Mangalore(Karnataka State), India
3. Professor & Head, Department of Periodontology & Implantology, Bapuji Dental College & Hospital, Davangere (Karnataka State), India.

Manuscript Info

Manuscript History:

Received: 14 February 2016
Final Accepted: 19 March 2016
Published Online: April 2016

Key words:
Alveolar bone loss; cyclosporine-A; alendronate sodium; calcium; alkaline phosphatase; osteocalcin*;*

Corresponding Author

Dr. Sneha R. Bhat.

Abstract

Background: Various pharmacological agents are known to create an imbalance in the normal physiology of bone remodeling. Cyclosporine-A (Cs-A) is one of the drugs that is widely used in transplantation and has its main side effect as gingival hyperplasia and alveolar bone loss by their action on the inflammatory mediators. Bisphosphonates are a new class of drugs that inhibit bone resorption by decreasing the osteoclast activity and number. The aim of the present study was to evaluate the effect of concomitant administration of alendronate on cyclosporin-A induced alveolar bone loss in a rat model.

Methods: A total of forty male Wistar rats weighing 90-150 grams were randomly divided into four groups, consisting of ten rats in each group. The study was conducted for a period of 60 days. Group I was the control group which received normal saline daily. Group II received subcutaneous injection of Cs-A 10mg/kg body weight daily, Group III received subcutaneous injection of alendronate 0.3mg/kg body weight on a weekly basis. Group IV received subcutaneous injection of both Cs-A 10mg/kg body weight and alendronate 0.3mg/kg body weight. At the end of the study (60 days), blood samples were drawn for the biochemical analysis to evaluate the serum calcium, alkaline phosphatase and osteocalcin levels. Simultaneously, the rats were sacrificed and the mandibles were further processed for the morphometric analysis.

Results: The morphometric analysis exhibited an increased alveolar bone loss in Cs-A treated rats whereas the combination (Cs-A +ALN) group showed marked inhibition of Cs-A induced alveolar bone loss. Also, there was a distinct pattern of increased level of biochemical markers (serum osteocalcin, alkaline phosphatase & calcium levels) in the combination group, though the level was found to decreased in the Cs-A treated rats.

Conclusion: Within the limits of our experimental study, it can be concluded that Cs-A has a distinct resorptive effect on alveolar bone and the adjunctive use of alendronate leads to a reversal of the cyclosporine A-induced bone loss.

Introduction:-

Cyclosporine-A (Cs-A) is a cyclic endecapeptide that was initially isolated from the fungus *Tolypocladium inflatum gams.*[1] The discovery of this immune-suppressive drug has revolutionized organ transplantation, and saving many human lives who are suffering from chronic and life-threatening conditions. However, the prolonged use of Cs-A has been reported to cause significant adverse reactions including metabolic disturbances, nephrotoxicity, hepatic dysfunction, neurological disturbances, hypertension, gingival overgrowth and osteoporosis Though the effect of Cs-A on alveolar bone has received limited attention, there are convincing evidences showing that cyclosporine- A causes increased bone turnover, with higher resorption than formation, resulting in bone loss.[2] However, some of the *in vivo* studies have shown contradictory results i.e. cyclosporine-A decreases bone resorption and increased bone formation in rats.[3]

Bisphosphonates (BP's) are a class of drugs that are chemical analogs of inorganic pyrophosphates and modulate host response by reducing recruitment, attachment and activity of osteoclasts.[4,5] BP's possess two C-P bonds, which are located on the same carbon atom, becoming extraordinarily stable and resistant to enzymatic hydrolysis. These drugs bind avidly to calcified bone matrix and are potent inhibitors of bone resorption. In recent years these compounds are used as therapeutic agents in the treatment of various disorders that are associated with excessive bone resorption, including post-menpausal osteoporosis, Paget's disease, and cancer-related diseases such as hypercalcemia, multiple myeloma, and bone metastases secondary to breast and prostate cancer.[6,7]

Bisphosphonates can be divided into two classes: nitrogen containing and non-nitrogen containing bisphosphonates.[7] Alendronate(ALN), an amino-bisphosphonate is an effective anti-osteolytic agent without having any adverse effects on bone matrix mineralization Alendronate binds to the resorption surfaces and is released during the acidification associated with the osteoclastic activity. This release leads to the local rise in the concentration of alendronate, resulting in the alteration in the ruffled border membrane characteristic of osteoclasts without destroying the cells.[8] Hence, alendronate seems to have a potential to be used as an inhibitor of cyclosporine induced alveolar bone loss.

Serum biomarkers like osteocalcin, alkaline phosphatase and calcium play an important role in the assessment of bone turnover and also in evaluating the effect of therapeutic agents on bone. Osteocalcin (OC) is a calcium binding protein of bone and because of its tissue specific expression, it is considered as a valid marker of bone turnover when resorption and formation are coupled and a specific marker of bone formation when formation and resorption are uncoupled.[9] Serum alkaline phosphatase is an important parameter for monitoring the osteoblastic activity whereas serum serum calcium, being a vital component of bone, is a key biomarker to evaluate bone metabolism. Studies have shown an alteration in serum calcium and alkaline phosphatase levels after the cyclosporine therapy in the rat model.

Nassar et al, [10] reported increased bone remodeling and trabecular bone loss in cyclosporine A administered experimental animals. Similarly, **Fu et al, [11]** observed a distinct pattern of increased osteoclasia and reduced bone formation at periodontal sites after cyclosporine A therapy. **Spolidorio and Co-workers,** [12] recorded an alteration in serum calcium and alkaline phosphatase levels after cyclosporine therapy. Furthermore, in order to investigate the role of bisphosphonates on bone resorption and regional accelerated phenomenon (RAP), **Yaffe et al, [13]** exhibited the inhibitory effect of bisphosphonates on bone resorption, when this drug was administered systemically after the mucoperiosteal flap surgery. Similarly, **Angelo and Co-workers,** [14] found alendronate as an effective drug in the treatment of osteoporosis in renal transplant patients.

Since, there is limited data in the literature relating the role of alendronate in counterbalancing the cyclosporine induced alveolar bone loss, the present study was undertaken with the intend of evaluating the effectiveness of concomitant alendronate therapy on the cyclosporine induced alveolar bone loss in a rat model.

Materials and methods:-

Study Design:-

The experimental protocol was followed in accordance with local guidelines on the welfare of experimental animals and with the approval of the Committee of Ethics in Animal Research of JJM Medical College and Bapuji Dental College & Hospital. Forty adult male Wistar rats (4-8 weeks old), weighing 90-150 gms were housed under similar conditions in cages with access to food and water *ad libitum*.

The rats were randomly distributed into four groups comprising ten animals each. Group I was the control group which received subcutaneous injection of normal saline daily. Group II received subcutaneous injection of Cs-A,[†] 10mg/kg body weight daily, Group III received subcutaneous injection of alendronate,[‡] 0.3mg/kg body weight on a weekly basis. Group IV received subcutaneous injection of both Cs-A 10mg/kg body weight and alendronate 0.3mg/kg body weight. The animals were weighed daily and the drug doses were adjusted accordingly.The total study period was of 60 days. At the end of experimental period i.e. day 60, the animals were anaesthetized using ketamine (0.1mg/100g body weight) anaesthesia 4-5 ml of blood sample was drawn and the rats were sacrificed. The blood samples were centrifuged at 1500 rpm for one minute to separate serum from the cells. The serum samples were immediately stored at -20^0C until subsequent analysis of the biochemical markers. After the blood sample collection, the rats were killed and the mandibles were dissected and fixed in 10% formalin.

Measurement of Alveolar Bone Loss:-
For the morphometric analysis of alveolar bone, images were captured using a 3 chip CCD camera attached to a stereomicroscope with 5x objective. The measurement was done using Image Pro Plus software. The distance from cusp tip to the crest of the alveolar bone was measured using 40x magnification. All measurements were made on the buccal alveolar process in mandibular first molar region.

Laboratory Assays:-
Enzyme-Linked Immunosorbent Assay:-
Levels of osteocalcin in serum samples were analysed by ELISA,[§] carried out according to manufacturer's recommendations, Ninety six well plates coated with appropriate antibodies were used. Samples and standards were added to duplicate wells and incubated for 30±5 minutes at room temperature. The plates were washed and, primary antibody was added, and the plates were incubated for 60±5 minutes at room temperature. After washing, secondary antibody was added and incubated for 60±5 minutes at room temperature. The chromogenic substrate solution was later added and incubated for 30 minutes in darkness. Finally, the stop solution was added and the absorbance was measured compared to the standards. The results for OC were expressed as nanograms per millileter.

Alkaline Phosphatase-SLR Kinetic UV test:-
Total serum alkaline phosphatase activity was measured colorimetrically using para nitrophenyl phosphate as the substrate. The activity was measured by absorbance at 405 nm. The results were expressed as U/dL.

Calcium:-
The serum calcium level was measured using the o-CPC [‖] colorimetric method using o-Cresolphthalein Complexone as the substrate.

Statistical analysis:-
Statistical analyses were performed by one way ANOVA for comparison between the four groups followed by Tukey's post hoc tests for pairwise comparison. P values <0.05 were considered statistically significant.

Results:-
All the animals survived till the end of this experimental study (60 days) and none of them gained body weight irrespective of their therapeutic regimen when compared to their counterparts in the control group.

Alveolar Bone Loss:-
The morphometric analysis revealed alveolar bone loss in the mandibular molar region in both Cs-A and the combination (Cs-A + ALN) groups as compared to the control group. However, the amount of bone loss was significantly higher in the Cs-A group as compared to the combination group. ($P<0.05$) (FIG. 1, 2, 3, 4). Another interesting observation in our study was that the alendronate treated group did not have significant change in the bone levels. ($P>0.05$) (Table 1, Fig 5)

Alkaline Phosphatase Levels in Serum:-
The Cs-A group showed the lowest levels of serum alkaline phosphatase, but no significant differences were found between any of the study groups. ($P>0.05$)(Table 2, Fig 5)

Calcium Levels in Serum:-
The serum calcium levels in the Cs-A group were significantly lower in comparison with the saline control, alendronate and the combination groups. ($P<0.05$). No significant differences were detected in the other group comparisons. ($P>0.05$)(Table 3, Fig 5)

Osteocalcin Levels in Serum:-
The highest serum OC levels were obtained in the combination group and the lowest in the Cs-A group. The combination group exhibited significantly higher serum levels of OC compared to other groups ($P<0.05$).Similarly, the Cs-A group showed significantly lower levels compared to other groups. ($P<0.05$). (Table 4, Fig 5)

Discussion:-

The use of cyclosporin-A to prevent rejection of allograft tissue has stirred the forte of transplantation biology. Cs-A induced gingival alterations have been observed in clinical cases and in animal models. Nonetheless, significance of Cs-A on alveolar bone has been focused upon recently and it's action on long bones and alveolar bone has been explored. In the present study, we evaluated the outcome of alendronate therapy in the prevention of alveolar bone loss associated with Cs-A in a well characterized animal model. The result of the present investigation indicated that concomitant administration of alendronate counteracted the bone resorption induced by Cs-A but it did not have any effect on bone when used alone.

Previous experimental studies have shown that administration of Cs-A in immunosuppressive doses (10mg/kg body weight) for 60 days results in alveolar bone loss around the lower molars[2, 10] In the normal physiological situation, both bone formation and resorption progress in a evenhanded, regulated manner with osteoclastic bone resorption foregoing new bone formation by osteoblasts. It is believed that Cs-A therapy can bring about an imbalance in the dynamic bone remodeling cycle, with excess resorption far exceeding formation, leading to eventual loss of bone.[15] Cs-A exerts its osteopenic effect via the T cell rather than directly on bone,[16] by interfering in the cytokine activity on both osteoclasts and osteoblasts.[17,18] thus influencing bone remodeling.

It is well acknowledged that prostaglandin of the E series can affect both the active mature osteoclasts as well as their precursors and are dominant mediators of osteoclastic bone resorption.[18] Cs-A can also enhance release of arachidonic acid metabolites PG-E_2 which is a cyclooxygenase-2 metabolite and mediator of osteoclastic bone resorption.[19,20] PGE2 formation induced by Cs-A is not due to an enhanced level or activity of the cyclooxygenase enzyme but rather to its action on the production of the metabolites of the lipoxygenase pathway, which can also stimulate osteoclastic bone resorption[21] Cs-A treatment resulted in increased concentrations of IL-1β and inducible nitrous oxide (iNOS) mRNA expression in the absence of inflammation.[22] These cytokines are considered to play a role in bone resorption, probably through the stimulation of osteoclast proliferation and differentiation, leading to bone loss.

Bisphosphonates are a special class of drugs very widely used in the field of medicine. The ability of bisphosphonates to inhibit bone resorption is well described in the literature. This effect has impelled their use in post menopausal osteoporosis, corticosteroid induced osteoporosis, bone metastasis etc. [7, 23] Although their mechanism of action has not been fully elucidated, at the tissue level, bisphosphonates reduce bone turnover, which slows down total bone loss. At the cellular level, the target is inhibition of osteoclast recruitment, adhesion, shortening of lifespan of the osteoclasts, and inhibition of osteoclast activity either through a direct action or by action on cells that modulate osteoclast activity. At the molecular level, the primary mechanism involves the indirect inhibition of prenylation (post-translational lipid modification) of small proteins involved in bone resorption. The result is inhibition of osteoclast activity and apoptosis.[4, 24, 25] Hence, in recent years, bisphosphonates have become powerful agents in combating bone resorption due to their anti-osteoclastic activity. Among these derivatives, the aminobisphosphonates (aminoBPs) exhibit particularly potent activity levels. Alendronate therapy is effective in preventing alveolar bone loss and regional accelerated phenomenon (RAP) following mucoperiosteal flap surgery when administered both topically and parentally.[26,27] Alendronate therapy has been proven to be effective in reducing bone loss around dental implants in estrogen deficiency suggesting the therapeutic benefit of the drug on bone metabolism.[28]

The biochemical assessment of biomarkers of bone turnover correlated well with the other findings in the present study. Biochemical and morphometric analysis data presented in our study suggest that alendronate counterbalanced

the Cs-A induced alveolar bone loss. However, we observed that the administration of alendronate alone did not result in any significant effect on the alveolar bone and also, did not have any side effects as observed by Jeffcoat et al.[29] Osteocalcin, predominantly produced by osteoblasts, is considered as a marker of osteoblast activity, bone formation and bone resorption. Serum OC could be regarded as a specific indicator of the overall activities of cells responsible for bone formation and remodelling.[30, 31, 32] According to our present findings, the combined administration of alendronate and Cs-A but not alendronate alone resulted in significantly higher serum OC levels than all the other groups and Cs-A group resulted in a significantly lower serum OC levels suggesting that Alendronate counteracts the negative effects of Cs-A on alveolar bone.

A significant decrease in serum calcium level in the Cs-A treated group which was statistically significant when compared to the other groups was observed in our study. These findings are in concordance with previous studies[10, 33,34] who suggested that the decreased levels of serum calcium can be attributed to the non specific effect of cyclosporin-A due to increased excretion by kidney and/or increased bone uptake. Cs-A has also shown to induce a change in the calcium flux via cellular membrane permeability.[35] On the contrary, corresponding immunosuppressive doses of cyclosporine cause a severe high turnover osteopenia without changes in ionized calcium, phosphate or PTH levels[36,37] .

The serum alkaline phosphatase may be expressed by both osteoblasts and hepatocytes. We observed a decrease (although non significant) in the serum alkaline phosphatase levels in the Cs-A group compared to the other groups. Similar to our data, recent studies[32,33] have observed a decrease in serum alkaline phosphatase levels following Cs-A administration. This implies that Cs-A could have a probable action on osteoblast maturation and consequently bone resorption suggesting a modest negative effect of Cs-A on bone formation. Conversely, modulation of T cells by Cs-A can increase alkaline phosphatase levels in cyclosporine treated rats as a result of negative feedback mechanism .[2]

As such, and within the limitations of this study, we conclude that alveolar bone loss is a potential side effect of Cs-A in immunosuppressive doses and treatment with alendronate is a beneficial therapeutic option that favours the normalization of cyclosporine A-induced alterations in bone metabolism. Alendronate therapy modulates the host response to the action of Cs-A on the inflammatory cells & cytokines which mediate alveolar bone resorption. Alendronate alone has no negative effects on the alveolar bone in the weekly dosage of 0.3mg/kg body weight when injected subcutaneously. Additional studies should be designed and conducted in order to faciliate understanding of the biological mechanisms for the differential actions of cyclosporine A and alendronate at the molecular levels, which still remain ambiguous.

Figures:-

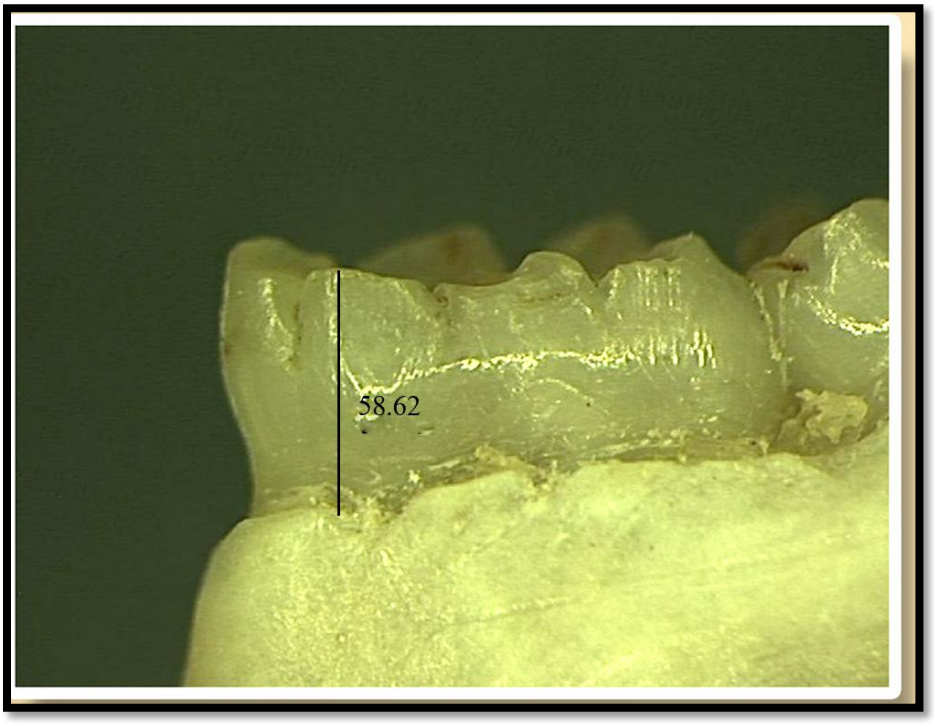


FIG 1: Photomicrograph showing bone levels in group 1, levels measured from cusp tip to the alveolar crest, 40x magnification

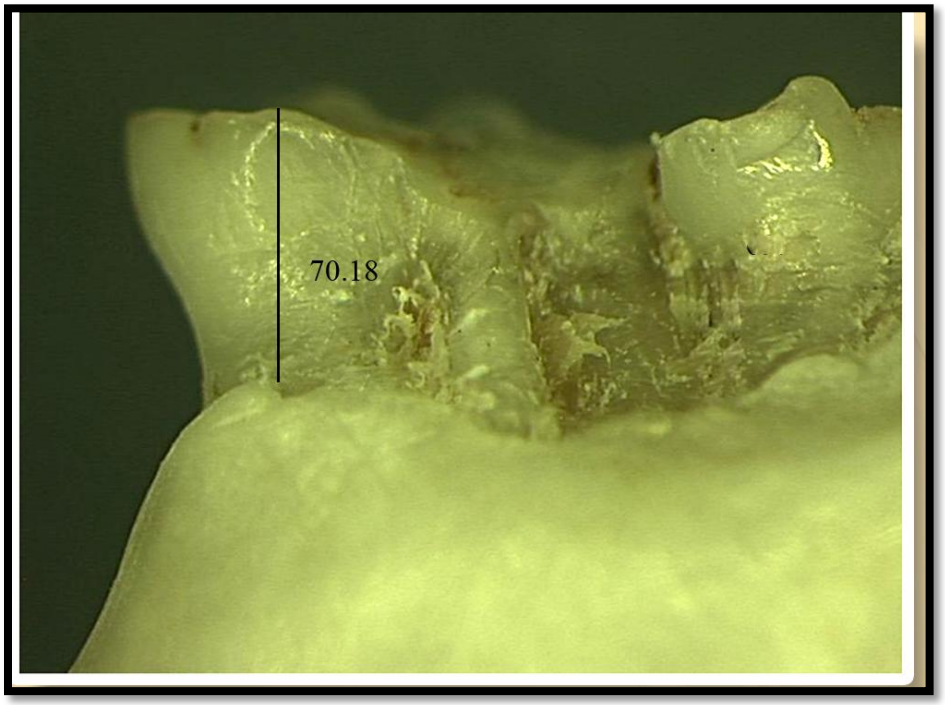


FIG 2: Photomicrograph showing bone levels in group 2, levels measured from cusp tip to the alveolar crest, 40x magnification

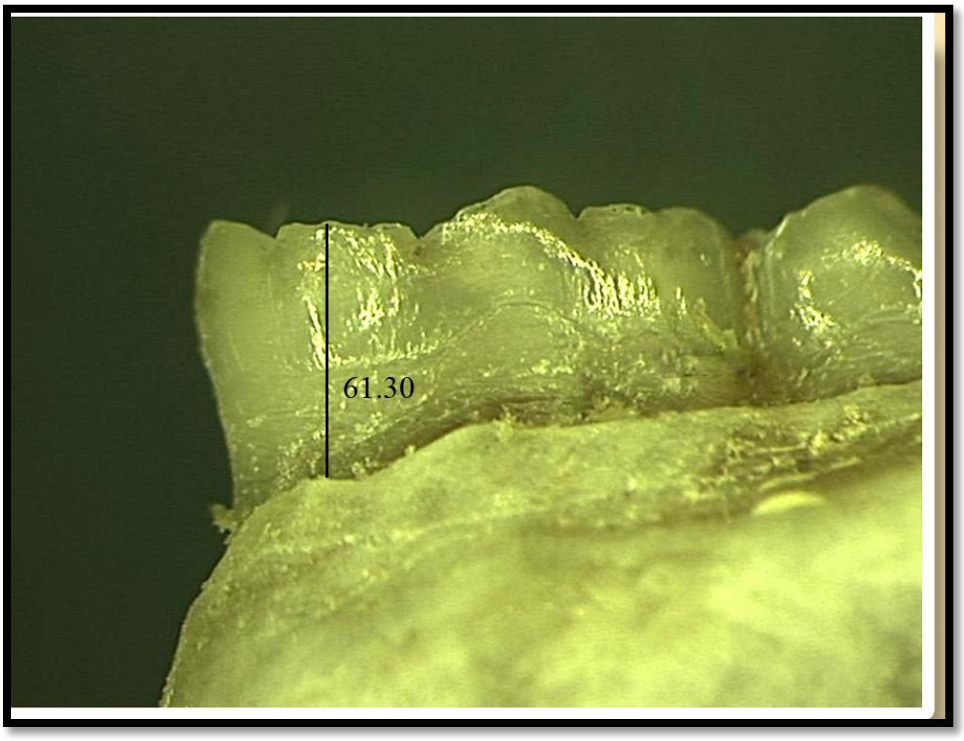
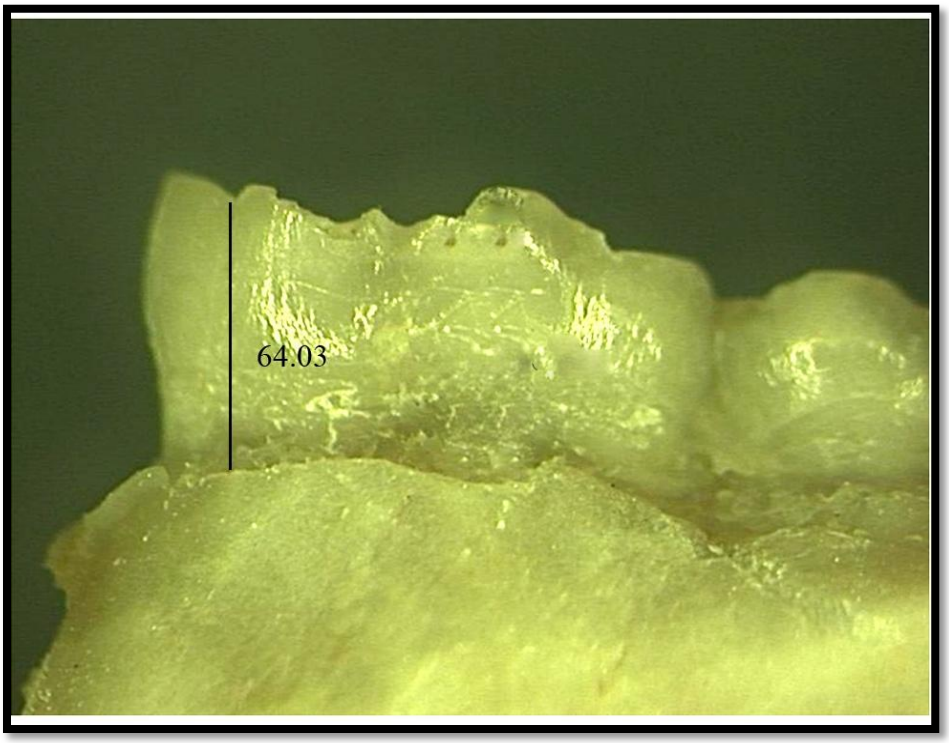


FIG 3: Photomicrograph showing bone levels in group 3, levels measured from cusp tip to the alveolar crest, 40x magnification



Bone loss (µ)				Mean difference, sig**			
Groups	Mean	SD	F* Value, Sig	Group I	Group II	Group III	Group IV
Group I	58.25	4.92	17.7 P=0.001 HS	-	11.56 S	2.7 NS	5.4 S
Group II	67.85	2.24		-	-	8.9 S	6.1 S
Group III	61.30	3.65		-	-	-	2.7NS
Group IV	64.04	3.57		-	-	-	-

FIG 4: Photomicrograph showing bone levels in group 4, levels measured from cusp tip to the alveolar crest, 40x magnification

Tables:-

Table 1: Intergroup comparison of alveolar bone loss

*Oneway ANOVA test ** Tukey's post hoc test p<0.005--Significant
HS- highly significant, NS- not significant, S-significant, SD- Standard Deviation

Table 2: Intergroup comparison of serum alkaline phosphatase levels

AlkalinePhosphate (U/L)				Mean difference, sig**			
Groups	Mean	SD	F* Value, Sig	Group I	Group II	Group III	Group IV
Group I	164.20	26.50	0.39 P>0.05 NS	-	25.8 NS	5.4 NS	14.1 NS
Group II	138.39	52.28		-	-	20.3 NS	11.6 NS
Group III	158.70	56.20		-	-	-	8.6 NS
Group IV	150.08	79.14		-	-	-	-

*Oneway ANOVA test ** Tukey's post hoc test p<0.005--Significant
HS- highly significant, NS- not significant, S-significant,SD- Standard Deviation

Table 3: Intergroup comparison of serum calcium levels

Calcium(mg/dL)				Mean difference, sig**			
Groups	Mean	SD	F* Value, Sig	Group I	Group II	Group III	Group IV
Group I	9.28	0.94	20.9, P<0.001 HS	-	6.08 S	0.15 NS	0.34 NS
Group II	3.19	0.61		-	-	5.9 S	5.7 S
Group III	9.13	1.24		-	-	-	0.19 NS
Group IV	8.94	3.73		-	-	-	-

*Oneway ANOVA test ** Tukey's post hoc test p<0.005--Significant
HS- highly significant, NS- not significant, S-significant, SD- Standard Deviation

Osteocalcin (ng/mL)				Mean difference, sig**			
Groups	Mean	SD	F* Value, Sig	Group I	Group II	Group III	Group IV
Group I	67.68	5.43	30.05, P<0.001 HS	-	9.7 S	1.7 NS	13.5 S
Group II	57.95	5.06		-	-	11.4 S	23.3 S
Group III	69.42	5.57		-	-	-	11.8 S
Group IV	81.26	5.97		-	-	-	-

Table 4: Intergroup comparison of serum osteocalcin levels
*Oneway ANOVA test ** Tukey's post hoc test p<0.005--Significant
HS- highly significant, NS- not significant, S-significant, SD- Standard Deviation

Footnotes
† Sandimmune, Novartis Pharmaceuticals India
‡ Parth Overseas, Mumbai India
§ Prolab Marketing Pvt Ltd, New Delhi, India
II Lab Care Diagnostics Ltd, India

References:-

1. Upton H. Origin of drugs in current use: *Cyclosporin-A story*; 2001.
2. Spolidorio LC, Spolidorio DM, Holzhausen M. Effects of long-term cyclosporin therapy on the periodontium of rats. *J Periodont Res* 2004; 39; 257–262.
3. Orcel P, Josette B, Dominique M, Livia M, Marie CV. Cyclosporin A induces in vivo inhibition of resorption and stimulation of formation in rat bone. *J Bone Miner Res* 1989; 4: 387–391.
4. Fleisch H. Bisphosphonates: mechanisms of action. *Endocr Rev* 1998; 19:80–100.
5. Tenenbaum HC, Shelemay A, Girard B, Zohar H, 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
6. Shinoda H, Takeyama S , Suzuki K, Murakami S, Yamada S. Pharmacological topics of bone metabolism: a novel bisphosphonate for the treatment of periodontitis. *J Pharmacol Sci* 2008; 106:555 – 558
7. Fleish H. Bisphosphonate-Preclinical. In:Fleish H, ed. Bisphosphonates in Bone Disease. *San Diego: Academic Press*;2000: 27-66
8. Sato M, Grasser W, Endo N. Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. *J Clin Invest* 1991; 88:2095-2105.
9. Buduneli E, Buduneli N, Vardar S, Kardesler L, Atilla G, Lappin D, et al. Systemic low dose doxycycline and alendronate administration and serum interleukin-1 Beta, osteocalcin, and C-reactive protein levels in rats. *J Periodontol* 2005;76:1927-1933.
10. Nassar CA, Nassar PO, Abi Rached RS, Holzhausen M, Marcantonio E, Spolidorio LC, et al. Effect of cyclosporin A on alveolar bone homeostasis in a rat periodontitis model *J Periodont Res* 2004; 39; 143–148.
11. Shen EC, Fu E, Hsieh YD, Wikesjo UME, Liu D. Effects of Cyclosporine A on Alveolar Bone: An Experimental study in Rats. *J Periodontol* 1999; 70: 189-194.
12. Spolidorio LC, Spolidorio DM, Nassar PO, Nassar CA, Holzhausen M, Almeida OP. Influence of age on combined effects of cyclosporin and nifedipine on rat alveolar bone. *J Periodontol* 2004; 75:268-272.

13. Binderman I, Adut M, Yaffe A. Effectiveness of local delivery of alendronate in reducing alveolar bone loss following periodontal surgery in rats. J *Periodontol* 2000; 71:1236-1240.
14. Giannini S, Angelo AD, Carraro G. Alendronate prevents further bone loss in renal transplant patients. *J Bone Miner Res* 2001; 16:2111-2117
15. Kawana K, Takahashi M, Kushida K, Hoshino H, Sakata S, Inoue T. The effect of cyclosporine administration on bone metabolism in the rat evaluated by biochemical markers. *J Endocrinol Invest* 1996; 19: 499-504
16. Chowdhury MH, Shen V, Dempster DW. Effects of cyclosporine A on chick osteoclasts in vitro. *Calcif Tissue Int* 1991; 49: 275-279
17. Mc Cauley LK, Rosol TJ, Capen CC. Effects of Cyclosporine A on rat osteoblasts. In vitro. *Calcif Tissue Int* 1992;51: 291-297
18. Schwartz Z, Goultschin J, Dean DD, Boyan BD. Mechanisms of alveolar bone destruction in periodontitis. *Periodontol 2000* 1997;14:158-172
19. Katz IA , Jee WSS, Joffe II et al. Prostaglandin E, Alleviates Cyclosporin A-Induced Bone Loss in the Rat. *J Bone Miner Res* 1992 ;7:1191-1200.
20. Whisler RL, Lindsey JA, Proctor KV, Morisaki N, Cornwell DG. Characteristics of cyclosporine induction of increased prostaglandin levels from human peripheral blood monocytes. *Transplantation* 1984;38:377-388.
21. Gallwitz WE, Mundy GR, Lee CH et al. 5-Lipoxygenase metabolites of arachidonicacidstimulateisolatedosteoclaststo resorb calcified matrices. *J Biol Chem* 1993;268:10087–10094.
22. Nassar PO, Nassar CA, Guimara MR, Aquino SG, Andia DC, Muscara MN, Spolidorio DMP, Ross a J r C , Spalidorio LC. Simvastatin therap y in c yclospor ine A-induced alve olar bone loss in rats. *J Periodont Res* 2009;44:479–488.
23. Wienreb M, Quartuccio H, Seedor JG, 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.
24. Hughes DE, Wright KR, Uy HL. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J Bone Miner Res* 1995;10:1478-1487.
25. Sharpe M, Noble S, Spencer CM. Alendronate: an update on its use in osteoporosis. *Drugs* 2001;61:999-1039.
26. . Binderman I, Adut M, Yaffe A. Effectiveness of local delivery of Alendronate in reducing alveolar bone loss following periodontal surgery in rats. *J Periodontol* 2000; 71: 1236-1240.
27. Kaynak D, Meffert R, Bostanci H, Gunhan O, Ozkaya OG. A histopathological investigation on the effect of systemic administration of the bisphosphonate alendronate on resorptive phase following mucoperiosteal flap surgery in the rat mandible. *J Periodontol* 2003; 74:1348-1354.
28. Giro G, Coelho PG, Pereira RM, Jorgetti V, Marcantonio Jr E, Orrico SR. The effect of oestrogen and alendronate therapies on postmenopausal bone loss around osseointegrated titanium implants. *Clin Oral Implants Res* 2010 Oct 13.
29. Jeffcoat MK. Safety of oral bisphosphonates controlled studies on alveolar bone. Int *J Oral Maxillofac Implants* 2006;21:349-53.
30. Lian JB, Gundberg CM. Osteocalcin: Biochemical considerations and clinical applications. *Clin Orthop Relat Res* 1988; 226:267-291
31. Shibutani T, Inuduka A, Horiki I, Luan Q, Iwayama Y. Bisphosphonates inhibits alveolar bone resorption in experimentally induced peri implantitis in dogs *Clin Oral Implants Res* 2001;12:109-114
32. Tohru T, Mutsumi T, Masaaki F, Takuo F. Can serum osteocalcin levels predict bone turnover in patients with osteoporosis? *J Bone Miner Res* 1988; 6: 12-18.
33. Nassar PO, Nassar CA, Spolidorio DMP et al. Simvastatin therapy in cyclosporine A-induced alveolar bone loss in rats. *J Periodontal Res* 2009; 44: 479–488.
34. Spolidorio DM, Muscara MN, Spolidorio LC, Nassar PO, Nassar CA. Conversion of immunosuppressive monotherapy from Cyclosporine to tacrolimus reverses bone loss in rats. *Calcif Tissue Int* 2007; 81:114-123.
35. Mason J. The pathophysiology of Sandimmune (Cyclosporine) in man and animals. *Pediatric Nephrol* 1990;4:686-704
36. Movsowitz C, Epstein S, Fallon M, Ismail F, Thomas S. Cyclosporin-A in vivo pro- duces severe osteopenia in the rat: effect of dose and duration of administration. *Endocrinology* 1988;123:2571–2577.
37. Rucinski B, Liu CC, Epstein S. Utilization of cyclosporine H to elucidate the possible mechanisms of cyclosporine A-induced osteopenia in the rat. *Metabolism* 1994;43:1114–1118.