COMPARATIVE EVALUATION OF 1% MELATONIN GEL AS AN ADJUNCT TO NON-SURGICAL PERIODONTAL THERAPY IN STAGE III PERIODONTITIS : A CBCT STUDY

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1.	PD	Periodontal disease
2.	CP	Chronic Periodontitis
3.	AAP	American Academy of Periodontology
4.	LDD	Local drug delivery
5.	PPD	Probing pocket depth
6.	CAL	Clinical attachment level
7.	NSPT	Non-surgical periodontal therapy
8.	GCF	Gingival Crevicular Fluid
9.	RANKL	Receptor activator of nuclear factor kappa-B ligand
10.	3D	Three dimensional
11.	2D	Two dimensional
12.	CT	Computed tomography
13.	CBCT	Cone beam computed tomography
14.	IBD	Intrabony defect
15.	PTH	Parathyroid Harmone
16.	BOP	Bleeding on probing
17.	PI	Plaque index
18.	GI	Gingival index
19.	mSBI	Modified sulcus bleeding index
20.	SRP	Scaling and Root Planning
21.	RAL	Relative Attachment Level
22.	PBI	Papillary bleeding index
23.	A. aspera	Achyranthesaspera
24.	P. gingivalis	Porphyromonasgingivalis
25.	GR	Gingival recession
26.	BSAP	Bone specific alkaline phosphatase
27.	NTx	Crosslinked N-telopeptides
28.	MF	Metformin
29.	AZM	Azithromycin
30.	SMV	Simavastatin
31.	CEJ	Cementoenamel junction

32.	AC	Alveolar crest
33.	BD	Base of the defect
34.	CPI	Community Periodontal Index
35.	LPO	Lipid Peroxidation
36.	NOx	Nitrite Plus nitrate
37.	GSH	Glutathione
38.	GSSG	Glutathione disulfide
39.	GPx	Glutathione Peroxide
40.	GRd	Glutathione Reductase
41.	DVT	Digital volume tomography
42.	IOR	Conventional intraoral radiography
43.	RT-PCR	
44.	UNC-15	University of North Carolina-15
45.	Mm	Millimeter
46.	MD	Mesiodistal
47.	BL	Buccolingual
48.	FOV	Field of view
49.	HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
50.	BMP	Bone morphogenic protein
51.		
52.	OPG	Osteoprotegerin
53.	IL	Interleukin
54.	MMPs	Matrix metalloproteinases
55.	RSV	Rosuvastatin
56.	OFD	Open flap debridement
57.	EP	Experimental Periodontitis
58.	DM	Diabetes Mellitus
59.	MEL	Melatonin

INTRODUCTION

Periodontal diseases are multifactorial infections caused by a complex of bacteria interacting with host tissues and cells, which release a wide range of inflammatory cytokines, chemokines, and mediators which lead to obliteration of the periodontal structures. The trigger for the initiation of disease is the presence of complex microbial biofilms that colonize in the sulcus between the tooth surface, and the gingival margin and accumulate due to architectural changes in the sulcus (i.e. attachment loss and pocket formation).[1]

A new classification of periodontal diseases and conditions was introduced in 2018, following the deliberations and the consensus reports of an International Workshop that took place in November 2017.[2] The new classification, introduces the concept of staging, supports a multidimensional view of periodontitis, incorporating severity, tooth loss due to periodontitis, and complexity of management of the

patient's periodontal and overall oral rehabilitation needs.

Stage I periodontitis (mild disease): Individual will have probing pocket depth (PPD) ≤ 4mm, CAL ≤ 1-2mm, with mild horizontal bone loss, requiring only non-surgical therapy. There will be no post-treatment loss of teeth, indicating good prognosis and requires only maintenance.

Stage II periodontitis (moderate disease): Individual will have PPD ≤ 5mm, CAL ≤ 3-4mm, patients will have probing depths ≤5 mm, CAL ≤3-4 mm, with horizontal bone loss, and can be managed by non-surgical or surgical therapy. There will be no post-treatment loss of teeth, indicating good prognosis and requires only maintenance.

Stage III periodontitis (severe disease): Individual will have PPD ≥ 6mm, CAL ≥ 5mm, with presence of either vertical bone loss or class II or III furcation involvement. This would necessarily require periodontal regenerative procedure. There is the possibility loss of 0-4 teeth. Also, the requirement of complex treatment and/or restorative may increase. The need of multi-specialty treatment approach also involve, which makes overall fair prognosis and should be kept on maintenance therapy.

Stage IV periodontitis (very severe disease): Individual will have PPD ≥ 6mm, CAL ≥ 5mm, with presence of either/ or both vertical bone loss and/or class II or III furcation involvement. Less than 20 teeth may be present and potential of loss of 5 or more teeth. May require advanced periodontal regenerative surgery, including hard and soft tissue augmentation therapy to aid in implant therapy. Also, the requirement of very complex treatment and/or restorative therapy may increase. The

need of multi-specialty treatment approach also involves which leads to overall questionable prognosis and should be kept on maintenance therapy.[2]

According to the glossary of terms of the American Academy of Periodontology (AAP), an intrabony defect is defined as a ‘‘periodontal defect within the bone surrounded by one, two or three bony walls or a combination thereof’’.[3] Intrabony defects are usually classified according to the criteria offered by Goldman & Cohen as follows:[4]

1. One-wall intrabony defects: defects limited by one osseous wall and the tooth surface;
2. Two-wall intrabony defects: defects limited by two osseous walls and the tooth surface; and
3. Three-wall intrabony defects: defects limited by three osseous walls and the tooth surface.

The treatment of chronic periodontitis emphases on arresting the destruction of periodontal support by eliminating the bacterial presence in the inflamed pocket.[5] Nonsurgical periodontal therapy (NSPT) is the ‘first line of defense’ in periodontal therapy. It is effective in reducing the gingival inflammation as well as number and depth of periodontal pocket. Majority of patients with periodontitis can be treated by scaling and root planing combined with irrigation of periodontal pocket, systemic antibiotics and proper maintenance of oral hygiene by patients.[6] NSPT eliminates the microbial biofilm from the root surfaces of periodontally diseased teeth and removes diseased root cementum without surgical reflection of site. Moreover it decreases gingival inflammation, periodontal pocket depth and gain in clinical attachment

level.[7]

However, sites with deep periodontal pockets, concavities, grooves, intrabony defects and furcations are difficult to access with periodontal instruments. Thus periodontopathic bacteria may remain at those sites.[8] Moreover, scaled and root-planed pockets can rapidly be recolonized by pathogenic bacteria from residual untreated pockets, or from other intraoral niches.[9] Usually, it is known from research that the pathogenic species like A. actinomycetecomitans and P.gingivalis appear to be resistant to subgingival instrumentation.[9,10] These organisms are capable of invading gingival epithelial tissues, connective tissues and the dentinal tubules.[8]

To overcome the limitations of this conventional treatment, chemical agents such as antibiotics and antiseptics have been used successfully to treat moderate to severe periodontal diseases.[11] Systemic route of antibiotics require the administration of large doses to obtain suitable concentrations at the site of the disease which potentially promote the development of bacterial resistance, drug interactions, and inconsistent patient compliance.[12]

To overcome these shortcomings of systemic administration, local delivery systems containing antibiotic or antiseptic agents were introduced.[13] These systems allow the therapeutic agents to be delivered directly to the diseased site with no appreciable systemic effect.

To achieve best results of NSPT there is need of an agent, which not only acts as an adjuvant to the NSPT but also helps effectively in gaining clinical attachment level, inhibits resorption of the alveolar bone and stimulates new bone formation. Dr. Max Goodson and coworkers introduced concept of local drug delivery (LDD) in

Periodontology. Goodson (1985) has suggested that success of any LDD system designed to target periodontal infections depends upon its ability to:-

- Deliver antimicrobial agents to the base of the pocket.
- Drug should be delivered at microbiologically efficacious concentrations.
- It must also facilitate retention of the medicament long enough and at sufficient concentrations to ensure an efficacious result.[14]

Locally-delivered antimicrobials in periodontal therapy

Antimicrobial agents should be used as an adjunct to scaling or root planing.[9] Thorough subgingival instrumentation is the basic step in the treatment of periodontitis, it eliminates the biofilm which may be impervious to local drug delivery, as well as eliminates the calculus and endotoxin which serve as nidus for plaque accumulation.[15] The periodontal pocket provides a natural reservoir for gingival crevicular fluid (GCF) and with easy access for injecting a LDD. The GCF provides a leaching media for the release of the drug from the solid dosage form and its distribution throughout the pocket. These features together with the fact that the periodontal diseases are localized to the immediate environment of the pocket make the periodontal pocket a natural site for treatment with local delivery systems.

Recent developments in subgingivally-placed delivery technology have made site-specific chemotherapeutic treatment possible. These technologies have provided the profession with a new tool to alter the subgingival microflora, and influence the healing of the marginal apparatus.[16.17]

Goodson's first LDD devices involved hollow fibers of cellulose acetate filled with tetracycline. [14] From then onwards many other antimicrobial agents were introduced in LDD system such as chlorhexidine,[18] doxycycline, minocycline, metronidazole and other class of drugs such as bisphosphonates[19] and statins.[20] Also, ayurvedic and herbal medications are increasingly gaining interest to overcome the drawbacks of the allopathic medication, usage of herbal product has increased because of relatively safe nature of herbal extracts, many herbal products and their component are being used for treating periodontitis in the form of local drug delivery.[21] Various ayurvedic and herbal product used as adjunct to scaling and root planning are Neem, Aloe-vera, Lemon grass, Green tea, Tea tree oil, Curcumin, Oak, Coriander, Babul, Bakul and Pomegranate.[21]

Microbes in dental plaque are the primary cause of periodontal disease. These microorganisms are capable of producing enzymes that can harm host tissue, and in response by activating the innate and adaptive immunity system the host attempts to remove the infection by counteracting the microbial attack, which on the other hand leads to the significant tissue destruction by producing large amounts of inflammatory cytokines, proosteoclastogenic factors, and matrix metalloproteinases.[2] One of the important factor in worsening the damage to the existing periodontium is the generation of free radicals and reactive oxygen species, which could originate from the bacteria and also from neutrophils which are the first line of defence.[22,23] Furthermore, there is an imbalance between the oxidant and antioxidant systems.

Melatonin (N -acety l-5 me thoxytryptamine) whose principal function of which is to regulate the circadian rhythm (day and night cycles), is secreted through

multiple organs such as the pineal gland, retina and bone marrow, the gastrointestinal tract.[24] It plays an anti-inflammatory, anti-oncotic, and immunomodulatory role byacting as a scavenger of free-radicals by interacting with cell membrane and intracellular proteins.[25] Scientists studied the bone healing property of melatonin and demonstrated by histomorphometric, biomechanics, radiology and protein analysis that melatonin prevents the bone fracture. The study show that as the bone resorption is necessary for remodeling during bone healing, melatonin helps in bone healing because of inhibition of bone resorption by RANKL-mediated osteoclast activation.[26] Latter studies have showed which melatonin has an important effect in bone-healing because of its regulation of bone cells, antioxidant properties, and promotion of angiogenesis actions.[27]

It has been documented that melatonin regulates bone growth and bone metabolism (such as IGF-I and also calciotropic, parathyroid, thyroid, adrenocortical and gonadal hormones).[28] In addition melatonin stimulates mineralization of matrix and osteoblast differentiation.[29] Melatonin also regulates the synthesis of collagenic and noncollagenic proteins of bone matrix.[30] Author self-assurance that there is a relationship between serum level of melatonin and bone mass in postmenopausal osteoporosis.[26] There is similar opinion about the effect of melatonin on osteoporosis, bone remodelling, and osseointegration of dental implants.[31] Melatonin, with 3 precept actions, modulates bone remodelling:

1) Direct effects on osteoclast and osteoblast actions[30] including: increase the cell proliferation, collagen expression, stimulation the matrix formation and inhibition osteoclasts differentiation.[32]

2) Indirect regulation of bone metabolism such as estrogen, calcitonin, and PTH.[33,34]

3) Production of antioxidant for neutralization of osteoclasts superoxide anions that is needed for degradative process.

Melatonin has positive effect on bone healing process because of its regulation of bone cells, antioxidant properties, and promotion of angiogenesis actions.[35] In a systematic review performed in 2020 to evaluate the role of melatonin in periodontal disease. From the obtained data it is concluded that the potential use of melatonin in periodontal diseases, showed favourable results. Currently there is paucity of data suggesting the best route of administration and appropriate dosage of this molecule. Therefore, further research should be carried out in this aspect for better clinical reliability. In most of studies, the relationship between melatonin levels in saliva and crevicular fluid decreased with an increase in the severity of periodontal disease, but there are few studies that contemplate the same. There arises a need for randomized controlled clinical trials employing melatonin to understand this paradigm better.[36]

There are various methods of assessing periodontal regeneration such as histological examination, surgical re-entry, probing depth examination, radiographic examinations. Although histological evaluation and surgical re-entry are gold standard but they are invasive methods and also have ethical limitations. Probing depth examination is the easiest way to determine regeneration but all types of periodontal probing have some differences to the measurements due to variations in probing force, probe tip size and shape, angulation and recording errors. Also, the presence of gingival inflammation may allow the probe tip to penetrate the connective tissue

attachment, thereby overestimating attachment loss. A decrease in inflammation after treatment leads to tissue shrinkage and could be misinterpreted as regeneration. Clinical attachment level along a root surface is a one-dimensional linear measurement, whereas the regenerative process is three dimensional (3D) in nature. In the case of bony destruction the visual interpretation of the radiograph often underestimates of the clinical situation. 30-60% of the mineral content of the bone may be lost before a lesion is readily apparent with a conventional transmission radiograph which is 2 dimensional (2D) in nature.[37]

To tackle these issues, computed tomography (CT) has been introduced because it allows cross-sectional and 3D analysis without distortion. Unfortunately, CT is impractical because of machine cost, complexity, high radiation, and relatively low resolution. Another imaging technique i.e. cone beam computed tomography (CBCT) was introduced for head and neck applications. Divergent to CT, it consists of a conical radiographic source and a high-performance digital panel detector. In most CBCT machines, the apparatus is analogous in size to a conventional panoramic machine, the examination takes 30 seconds, and radiation is within range of an intraoral full-mouth series. In addition, CBCT resolution can be as small as 0.2 mm, compared to 0.5 to 1 mm for CT.[37]

The literature search revealed that there are limited literature or researches which have been carried out to check efficacy of Melatonin in treating intrabony defects in stage III Periodontitis along with NSPT. Also, CBCT is one of the latest methods of evaluation and there are very few studies that have used CBCT to assess regeneration.

Considering the above fact, the current study was designed as a randomized clinical trial to evaluate and compare the efficacy of 1% Melatonin gel as an adjunct to Non surgical periodontal therapy (NSPT) and NSPT with placebo gel in treatment of periodontal intrabony defects (IBDs) clinically and radiographically using CBCT.

AIM AND OBJECTIVE

The aim of the present study was to evaluate and compare the efficacy of 1% Melatonin gel as an adjunct to NSPT and NSPT with placebo gel in the treatment of periodontal IBD in Stage III periodontitis.

Objectives:

1. To evaluate the efficacy of NSPT in combination with placebo gel in treatment of periodontal IBDs in Stage III periodontitis.

2. To evaluate the efficacy of NSPT in combination with 1% Melatonin gel in treatment of periodontal IBDs in Stage III periodontitis.

3. To assess and compare the efficacy of NSPT with placebo gel and NSPT with 1% Melatonin gel on the probing pocket depth (PPD) and clinical attachment level (CAL) at 3 and 6 months postoperatively.

4. To assess and compare the efficacy of NSPT with placebo gel and NSPT with 1% Melatonin gel on the radiographic bone fill at 3 and 6 months postoperatively by CBCT.

REVIEW OF LITERATURE

In periodontitis alveolar bone loss is clearly associated with an alteration in the normal balance between bone resorption and formation. NSPT still remains the cornerstone of the treatment of periodontitis. Although after NSPT therapy reduction of the inflammation is obtained; for progressive bone loss in periodontitis adjunctive treatment along with SRP alone is required. It can be hypothesized that a pharmacological agent resulting in suppression of bone resorption or an acceleration of bone formation could protect against alveolar bone loss in periodontitis. This approach might provide a new mechanism by which periodontal disease could be arrested. Since locally delivered melatonin was discovered to be potent stimulators of bone formation, the possibility of using these compounds as practical bone anabolic agents has been postulated.

For the ease of understanding, the review of literature has been segregated into three parts

1. Review of studies on LDD in treatment of periodontitis

2. Review of studies on use of melatonin in periodontics

3. Review of studies on the methods of analysis of regeneration.

Review of studies on LDD in treatment of Periodontitis

Paolantonio M et al (2008)[38] carried out study to evaluate clinical and microbiologic effects of CHX chips when used as an adjunct to SRP. A total of 116 patients with moderate to advanced periodontitis were included in the study. The patients were randomly assigned to either control group were only receive SRP and test group received SRP plus CHX Chip. Clinical parameters like PI and GI were measured at baseline, 15 days, 1, 3, 6 month post-operatively and PPD, RAL, BOP were measured at baseline, prior to treatment, 3 and 6 month post-operatively. The identification of 8 possible periodontopathogens through PCR analysis and total bacterial count from subgingival microbiological were done at at baseline, 15 days,1, 3 and 6 months. Result obtained showed that, the PD and RAL were significantly less at 3 and 6 months compared to the baseline scores ($P < 0.01$) for both treatments. Moreover, PD in the treatment group SRP + CHX was reduced by 3 and 6 months compared to that in the SRP treatment group, whereas RAL for both therapies was similar at 3 months and reduced by 6 months for SRP + CHX therapy. With both treatments, the TBCs decreased substantially. The TBC was significantly lower for the treatment group SRP+CHX than for the SRP group at 15 days and 1 month. ($P < 0.01$ and $P < 0.05$, respectively). In both treatments the percentages 8 suspected periodontopathic bacteria were generally reduced over time, though there were often greater reductions recorded for the treatment group SRP + CHX. From the results it

can be concluded that, compared to the SRP alone, the combined use of the CHX chip leads to significant PD reductions. These results have contributed to an important advantage of SRP + CHX therapy in subgingival microbiota.

Panwar M et al (2009)[39] carried out a case-control study on 30 patients suffering from CP. In each patient two quadrants with PPD of 5 mm were selected which were divided in two groups. Only SRP was carried out in 15 patients of Control Group and experimental Group of 15 patients was treated with SRP and tetracycline fiber. PPD was recorded preoperatively and after 30 and 90 days. The data was subjected to statistical analysis. The PPD after 30 and 90 days was subjected to general linear model. The results showed that decrease in PPD after 30 and 90 days following use of tetracycline as an adjunct to SRP was statistically highly significant. The study concluded that LDD with tetracycline fiber as an adjunct to SRP is highly effective in reducing the PPD.

Deo V et al (2011)[40] in a randomized controlled clinical trial evaluated the efficacy of doxycycline hyclate 10% as an adjunct to SRP in the treatment of CP. Randomized clinical trial including 60 systemically healthy, CP patients was conducted for the 6 months. Test Group was treated by LDD of doxycycline hyclate 10% along with the SRP, while the Control Group was treated by SRP along with placebo. Significantly higher reduction in the mean PPD was demonstrated in the Test Group (3.03 ± 0.92 mm) when compared with the Control Group (2.3 ± 0.65 mm). It was concluded that the use of doxycyline hyclate 10% as an adjunct to SRP provides more favorable and statistically significant reductions in PPD and gain in CAL compared to SRP alone.

Behal et al (2011)[41] compare influence of 2% whole turmeric (gel form) as local drug delivery as a added effect on SRP with the SRP alone, by evaluating their impact on trypsin-like enzyme activity of "red complex" microorganisms (Bacteroides forsythus, Porphvromonas gingivalis and Treponema denticola). A total of 30 subjects selected in a split-mouth study design with chronic periodontitis. SRP alone was done in sites control and SRP plus 2 % whole turmeric gel received in experimental sites. Clinical parameters measured were PI, GI, SBI, PPD, RAL and BAPNA assay was done for microbiological analysis of collected plaque sample for trypsin-like activity of "red complex". The parameters that were recorded at baseline 30, and 45 days. Both groups showed statistically significant reductions in PI, GI, SBI, and PPD, as well as gain in RAL, but greater reduction was observed in the experimental group. Significant higher reduction in the trypsin-like enzyme activity of "red complex" (BAPNA values) was observed in experimental group (2% whole turmeric gel). The findings can lead to the conclusion that the local drug-delivery of 2% whole turmeric gel can be used as an adjunct to scaling and root planning in the treatment of periodontal pockets.

Kudva et al (2011)[42] studied the efficacy of locally delivered catechin green tea as an adjunct to SRP in chronic periodontitis management compared to SRP alone. 14 patients with a bilateral pocket depth of 5−8mm were selected and were divided into control (SRP alone) and test groups (SRP plus catechin green tea). In both the group each site was evaluated for the PI, GI, and PPD at baseline and 21 days, as well as for microbiological analysis at baseline, 1 week, and 21 days. Result obtained shows that intercomparison between PI and GI showed no significant results with $P>0.05$ for test and control teams at 21 days, however significant difference was

observed with P<0.001 in decreasing the probing depth. A significant reduction of Aggregatibacter actinomycetemcomitans, Prevotella Intermedia, Fusobacterium species and Capnocytophaga on microbial analysis in test group. It was concluded form the result that the local drug delivery of green tea catechin along with SRP is more effective in improving all the clinical parametrers than SRP alone.

Rao N et al (2013)[43] investigate efficacy of an indigenously prepared biodegradable 1% Metformin (MF) gel as an adjunct to SRP in smoker with generalized chronic periodontitis. Two treatment groups categorized in SRP plus 1% MF and SRP plus placebo were 50 patients were equally divided. Clinical parameters measured were measured at baseline, 3, 6 months which included PI, mSBI, PPD, and CAL and radiographic assessment included intrabony defect (IBD) fill at baseline and 6 months. At all visits, the mean PD reduction and mean CAL gain were found to be greater in the MF group than in the placebo group. Moreover, the MF group had a significantly higher mean percentage of bone fill (26.17 + 6.66 percent) than the placebo sites (3.75 + 8.06 percent) (p 0.001). The author concluded that the decrease in mSBI and PD was greater and that CAL gain was significantly higher in SRP plus 1% MF group along with significantly increased bone fill in smokers with generalized chronic periodontitis.

AR Pradeep et al (2013)[44] evaluate the adjunctive effects of subgingivally delivered 0.5 % azithromycin (AZM) in the treatment of chronic periodontitis in smokers. A total of 54 patients were randomly assigned to Group 1 (26 subjects) received (SRP) plus placebo gel, and Group 2 (28 subjects) received (SRP) plus 0.5 percent azithromycin. Clinal parameters like PI, mSBI, PPD, CAL were recorded at

baseline3, 6 and 9 months. The result showed considerable improvements in azithromycin group. The AZM applications resulted in a mean gain of 2.44 ± 0.64 mm at nine months compared to a placebo gain of 0.18 ± 0.68 mm after single application. The PPD and PI have also been significantly reduced in test group, but no changes have been noted to the mSBI. From the result obtained it can be concluded that the addition of 0.5 % AZM in the treatment of chronic periodontitis in smokers resulted in a significant improvement in clinical outcome.

Kathariya R et al (2014)[45] evaluated efficacy of subgingivally delivered 0.5% clarithromycin as an adjunct to SRP in the treatment of CP in a short term double blinded randomized controlled clinical trial. Ninety-eight patients were divided into two treatment groups: Test Group i.e. Group I was treated by SRP plus 0.5% clarithromycin and Control Group i.e. Group 2 was treated by SRP plus placebo. Clinical parameters recorded were gingival index (GI), PI, modified sulcus bleeding index (mSBI), PPD, and CAL at the interval of 4, 8 and 12 weeks. Gingival fluid concentration of 0.5% clarithromycin was estimated by reverse-phase high pressure liquid chromatography. Test Group showed enhanced reduction in GI, mSBI, and PPD, and gains in CAL over time, as compared with the Control Group. The mean concentration of clarithromycin was detected in gingival crevicular fluid for up to 7 weeks which proved its controlled-release. Study concluded that use of 0.5% clarithromycin as a controlled drug delivery system as an adjunct to SRP enhanced the clinical outcome.

Gupta D et al (2014)[46] evaluates and compare the efficiency of Ocimum sanctum with gold standard chlorhexidine and normal saline on the dental plaque and

gingival inflammation (placebo). A total of 108 people were randomly assigned to one of three study groups: group 1 received Ocimum sanctum mouthwash (n =36), group 2 received chlorhexidine (active control) (n=36), and group 3 normal saline (negative control) (n= 36). Assessment was carried out according to plaque score and gingival score. Result showed that *Ocimum sanctum* mouthrinse is equally effective in reducing plaque and gingivitis as Chlorhexidine. When compared to the control group, the results showed a significant reduction in gingival bleeding and PI in both groups after 15 and 30 days. Based on the findings, it is possible to conclude that Ocimum sanctum mouthrinse may be an effective mouthwash due to its ability to reduce periodontal indices by reducing plaque accumulation, gingival inflammation, and bleeding. In comparison to chlorhexidine, it has no side effects.

Habeba M et al (2014)[47] to evaluate the microbiological and clinical effects of commercially available Aloe Vera gel in the treatments of chronic periodontitis as an adjunctive SRP therapy. The study included a total of 40 patients with moderate and mild chronic periodontitis. The study design was split-mouth where the patient was thoroughly applied locally with Aloe vera gel to either side of his or her teeth and only SRP was applied on the opposite side of the same teeth. Results showed that P. gingivalis and P. intermedia were lower than SRP alone in sites treated with SRP + Aloe vera gel 100 % concentrate. The Author concluded that in patients with chronic periodontitis, SRP plus aloe vera gel was effective in improving clinical and microbiological parameters. SRP plus aloe vera gel (100 percent concentrate) have a bactericidal effect, which could be used as a complementary therapy for mechanical intervention replacing the periodontitis in chronic aloe vera gel (SRP).

Rathod S et al (2015)[48] carried out study to evaluate the efficacy of turmeric and aloe vera chip in chronic periodontitis. Total 20 patients diagnosed with chronic periodontits were randomly assigned into Group I received SRP plus turmeric chip and Group II received SRP plus Aloe vera chip. The clinical parameters measured PI, GI, PPD and RAL at baseline, 21 and 90 days postoperatively. Result obtained showed that both the group showed improvement in all the clinical parameters at all the followup period. However significant more improvement was noticed in the Group II eith $p < 0.001$. from the result it can be concluded that adjunt use of local drug delivery of Aloe vera chip improved all the periodontal parameters because of its anti-inflammatory and antiseptic nature and can contribute in preventive and therapeutic treatment of periodontal disease.

Babrawala I et al (2016)[49] made use of natural 1% chitosan as LDD in the management of non-surgical periodontal treatment. This was randomized controlled split mouth study including ten patients with PPD $\geq$ 5 mm. They were categorized randomly by an envelope technique into two treatment groups: Test Group which had ten sites and was treated with SRP plus 1% chitosan membrane and Control Group which also had ten sites and was treated with SRP alone. GI, BP, PPD were the clinical parameters recorded at baseline before SRP and after 4 weeks. The Test Group showed significant improvement in all clinical parameters as compared to the Control Group with the mean difference of 1.4 mm between the outcomes of the Test and the Control groups for PPD and zero score for BP for the Test Group at the end of 4 weeks. Study concluded that chitosan membranes as a form of LDD can be used as an adjunct to SRP.

AR Pradeep et al (2016)[50] conducted study to evaluate the clinical efficacy of 1% ALN and 1.2% ATV gel in the treatment of patients with CP. A total of 90 intrabony defects were selected and randomly assigned to either 1% ALN, 1.2 percent ATV, or placebo gel. The clinical parameters like PI, mSBI, PPD, CAL were measured at baseline, 3, 6 and 9 month interval. Standardized radiographs were used to record intrabony defect depth (IBD) and defect depth reduction (DDR%) at 6 and 9 months. At 3,6, and 9 months, the ALN and ATV groups had higher mean PD reduction, CAL gain, and DDR percent than the placebo group. Furthermore, at 6 and 9 months, the ALN group had a significantly higher DDR % than the ATV and placebo groups. Author conclude that both ATV and ALN have been shown to be effective modes of treatment for CP patients; however, ALN was found to be superior to ATV in the treatment of intrabony defects in CP patients.

Vennila K et al (2016)[51] used 10% neem oil chip as LDD to evaluate the efficacy in the periodontal disease management in a clinical and microbiological study. Twenty patients with the bilateral PPD of 5–6 mm were included in the study. After SRP, 10% nonabsorbable neem chip was placed in the periodontal pocket in one side of the arch while other side was treated with SRP only. Clinical parameters were recorded on the baseline, 7th day, and 21st day and plaque samples were obtained for microbiological evaluation on the baseline and 21st day. Quantitative and qualitative polymerase chain reaction assay was used for studying Porphyromonasgingivalis strains. On the neem chip site clinical parameters were statistically improved and presence of Porphyromonasgingivalis (P. gingivalis) strains weresignificantly reduced. Study concluded that 10% neem oil LDD delivers desired effects on P. gingivalis and improves clinical parameters in CP.

Boyapatti R et al (2017)[52] conducted study to evaluate the effectiveness of A.apera gel as LDD in management of chronic periodontitis. In the study, 30 chronic periodontitis patients were considered and divided into 2 equal groups, Group A: A. aspera gel scaling and root planing, Group B: placebo gel SRP. At baseline and 3 months, clinical parameters like gingival index, bleeding on probing, probing pocket depth, and clinical attachment level were recorded. All Clinical parameters were statistically improved between the test and control groups from baseline to 3 months. Whereas it was statistically improved in test group. From the result it was concluded that when A. aspera gel was delivered locally along with SRP, it had a positive effect. A. aspera gel, as a nonsurgical local drug delivery system, was found to be safe and effective in the treatment of periodontitis. In addition to its antioxidant activity, A. aspera gel has potent anti-inflammatory properties.

Gupta A et al (2018)[53] in a randomized controlled clinical trial evaluated the efficacy of LDD of zoledronate gel as an adjunct to SRP for the treatment of human periodontal IBDs clinically and radiographically. Forty intrabony defects in moderate to severely affected forty CP patients with the age range, 30–50 years were randomly divided into two groups and treated either with 0.05% zoledronategel or placebo gel after SRP. Clinical parameters such as PI, GI,PPD and CAL were assessed at baseline and at 3 and 6 months. Radiographic parameters were evaluated at baseline and 6 months. In intragroup comparisons, both groups showed significant PI and GI reduction after treatment at 3 and 6 months using dentascan. In intergroup comparisons, PPD reduction and CAL gain were significant only in zoledronate group at 6 months. Radiographically, significant reduction in defect depth and buccolingual width with volumetric defect gain of 40.24% ± 7.44% in zoledronate gel group

compared to insignificant gain of 1.60% ± 4.06% in placebo gel group was observed at 6 months.

Maske S et al (2019)[54] conducted study to evaluate the efficacy of locally delivered ocimum sanctum gel (OS) and assess 8- hydroxydeoxyguanosine (8-OHdG) levels in gingival crevicular fluid (GCF) of smokers and nonsmokers with chronic periodontitis (CP). Total 50 patients divided into three groups as Group I: 10 periodontally healthy patients, Group II: 20 smokers with CP and Group III: 20 non-smokers with CP. Smokers and non-smokers with CP received the local delivered 10% OS gel as an adjunct to scaling and root planning (SRP) at the test site while SRP alone at control site. GCF samples were obtained from all the participants at baseline and 3 months and clinical periodontal parameters were recorded at baseline and at 1 and 3 months after SRP. 8 hydroxydeoxyguanosine (OHdG) levels were analyzed with enzyme-linked immunosorbent assay. Result obtained showed that the test sites in smokers and non-smokers showed significant reduction in probing pocket depth (PPD), Plaque index(PI), Gingival index (GI) and clinical attachment level(CAL) as compared to control sites. GCF 8-OHdG levels were significantly higher in smokers and non-smokers as compared to controls. So, the study concluded that the application of 10% ocimum sanctum gel showed significant improvement in PPD, CAL and PI and GI and reduction in GCF 8-OHdG levels.

Hasan F et al (2019)[55] conducted study on the efficacy of the treatment of gingivitis and periodontitis using simvastatin gel and mouthwash. The patients had been randomly assigned to three groups: simvastatin gel, simvastatin mouthwash and control groups. Simvastatin gel and mouthwash in 1% preparation showed favourable

results by significantly lowering periodontal parameters and inflammatory biomarkers (p0.001) when compared to traditional treatment. As a result, we strongly recommend using simvastatin via a local drug delivery system as an adjunct treatment for SRP.

Review of studies on Melatonin in Periodontics

Cutando A et al (2003)[56] compared the degree of PD and interleukin-2 (IL-2) levels with melatonin concentrations in plasma and saliva of diabetic patients. A total of 43 diabetic patients (20 with type I and 23 with type II diabetes) and 20 age- and sex-matched controls were studied. The periodontal status was evaluated by the Community Periodontal Index (CPI). Plasma and salivary melatonin levels were determined by specific commercial radioimmunoassays, and plasma IL-2 was measured using a commercial enzyme-linked immunosorbent assay kit. Diabetic patients had plasma and saliva melatonin levels of 8.98 ±7.14 and 2.70±2.04 pg/mL, respectively. These values were significantly lower ($P < 0.001$) than those obtained in plasma and saliva of controls (14.91±4.75 and 4.35±0.98 pg/mL, resp). Plasma and salivary melatonin concentrations show a biphasic response in diabetic patients. Melatonin decreased in patients with a CPI index of 2, and then increased reaching highest levels in patients with a CPI index of 4. The results indicate that, in diabetic patients, the presence of a marked impairment of the oral status, is accompanied by an increase in plasma and salivary melatonin. The increase in salivary melatonin excretion may have a periodontal protective role.

Cutando A et al (2007)[57] determine whether tooth extraction induces changes in plasma oxidative stress levels, and whether melatonin treatment may counteract these changes. A total of 16 adult beagle dogs maxillary and mandibular premolars

and molars teeth were extracted under general anesthesia. Out of 16 dogs, eight dogs were treated with 2mg melatonin placed into the alveolar sockets, whereas the other eight dogs received only vehicle. Lipid peroxidation (LPO) and nitrite plus nitrate (NOx) levels were determined in plasma, whereas glutathione (GSH) and glutathione disulfide (GSSG) levels and glutathione peroxidase (GPx) and reductase (GRd) activities were measured in red blood cells before and 24 hours after tooth extraction. Results obtained shows that removal of the premolars and molars caused a significant rise in plasma LPO and NOx levels and in the erythrocyte GSSG/GSH ratio, whereas melatonin treatment restored the normal values of these parameters. Also, melatonin slightly increased erythrocyte GRd activity without changing GPx activity. The study concluded that during the immediate postoperative period following tooth extraction, there is a significant increase of oxidative stress, which is counteracted by the administration of melatonin into the alveolar sockets.

Srinath A et al (2010)[58] conducted study to detect the presence of melatonin in gingival crevicular fluid (GCF) and to assess the levels of salivary and GCF melatonin in periodontal disease and show the correlation between salivary and GCF melatonin. Methods: Forty-five subjects, based on the gingival and Russell periodontal indexes, were grouped as 15 healthy subjects (group 1), 15 subjects with gingivitis (group 2), and 15 subjects with periodontitis (group 3). Saliva and GCF samples were collected from all subjects. Melatonin levels were assessed using an enzyme-linked immunosorbent assay. Results obtained showed that melatonin was present in GCF within mean range of 1.54 pg/ml with significantly less concentration compared to that of saliva within mean 2.17 pg/ml. Salivary and GCF melatonin levels were reduced to the lowest concentrations in patients with chronic periodontitis

(salivary melatonin: 0.07 pg/ml; GCF melatonin: 0.06 pg/ml; P <0.05), which were inversely proportional to the clinical indices. There was no significant correlation between salivary and GCF melatonin levels (P >0.05). It can be concluded that melatonin was expressed in GCF. The levels of salivary and GCF melatonin were different from those of clinical health (group1) to those of periodontitis (group 3). Both salivary and GCF melatonin levels decreased in periodontitis indicate the protective role of melatonin against periodontal disease.

Shoukheba M et al (2012)[59] conducted study to investigate the resulting histological regeneration after the use of melatonin gel in the treatment of induced periodontal one-wall intrabony defects in Dogs. In total 8 dogs were considered for the study in which one-wall infrabony defects (4x4mm) were surgically created in the mesial aspect of second premolars bilaterally (split mouth study). Each intrabony defect underwent one of 2 treatment modalities were in experimental site (Group I)- melatonin gel/ collagen sponge (experimental site group I) and second is Control Group (Group II) - placebo gel (methyl cellulose)/ collagen sponge. Four animals (8 defects 4 defects from tested side and 4 from control side) were sacrificed with an overdose of anesthesia at one month post-surgically and block sections (8 specimens) of the defects were collected for histological and histometric examinations. At 3 months, the other four animals were sacrificed to obtain another 8 block sections for the same purpose. Result obtained showed that at one month interval the melatonin treated specimens showed moderate amount of newly formed bone, newly formed cementum, poorly organized PL fibers, with no epithelial down growth was observed. On the other hand, the surgical control specimens showed epithelial down growth along the root surface, and minimal amount of bone formation at the apical part of the

defect. At three months, histological results of group I (melatonin treated group) revealed true periodontal regeneration that demonstrated similar features to the native periodontal structures found apical to the notches; no epithelial down growth was observed. Well organized, functionally oriented periodontal ligament fibers were observed with plumps of fibroblast cells after melatonin treatment however at surgical control group similar histologic features that recorded at one month with limited amount of bone and osteoid tissue confined to the apical portion of the defect. From the results obtained the author concludate that, the use melatonin is advantageous in stimulating periodontal regeneration.

Yousuf D et al (2013)[60] evaluate the effect on the healing of periodontal osseous defects in diabetes-induced rabbits from local melatonin gel application. Total 28 rabbits were included in the study, the induction of diabetes was made, and further the acute osseous periodontal defects has been created. Further the selected rabbit were divided in group I were periodontal defects were placebo gel with collaplug placed and group II were periodontal defects filled with 2 mg melatonin gel-saturated collaplug. Real-time RT-PCR was used to examine the expression of RANKL mRNA in gingival biopsies. Both groups had histological and histomorphometric evaluations at 3 and 6 weeks. At 2 weeks after diabetes induction, the levels of RANKL mRNA expression were significantly higher. The level of RANKL expression was significantly reduced ten days after the application of melatonin gel compared to the control group. There was a significant increase in the percentage of new bone formation and the number of osteoblasts, decreased in the number osteoclast decreased significantly in the melatonin-treated group when compared to the control group, when observed histologically and

histomorphometrically. After the local application of 2 mg melatonin gel, a therapeutic approach for efficient bone regeneration and PDL regeneration is promising.

Ortiz F et al (2014)[61] evaluate the molecular pathways involved in oral mucositis and assess if melatonin can prevent this disease. The tongue of male Wistar rats was subjected to irradiation. The rats were divided into four groups at random, 1- control group –nonirradiated, 2- irradiated + vehicle (gel without melatonin), 3- irradiated + local application of 3 % melatonin gel, and 4- irradiated + intraperitoneal injection of melatonin. For 21 days after irradiation, rats were given 45 mg/day melatonin or vehicle, either locally in their mouths (melatonin gel) or subcutaneously. After the experiement the rat were sacrificed, and tongues were harvested and processed for the various analyses. For rabbit primary antibodies, the Vectastain ABC kit was used. True mitochondria, nuclei, and cytosol were isolated from the tongue. LPO, GPx, GRd activities, GSH, and GSSG levels are all measured using different analyzing kit. Result obtained found that a connection between reactive oxygen species, generating mitochondria and the NLRP3 inflammasome, has been reported in mucositis. Here, we show that mitochondrial oxidative stress, bioenergetics impairment and subsequent NLRP3 inflammasome activation are involved in the development of oral mucositis after irradiation and that melatonin synthesized in the rat tongue is depleted after irradiation. The application of melatonin gel restores physiological melatonin levels in the tongue and prevents mucosal disruption and ulcer formation. Our results suggest new molecular pathways involved in radiotherapy-induced mucositis that are inhibited by topical melatonin application, suggesting a potential preventive therapy for mucositis in patients with cancer.

Kose O et al (2016)[62] carried out study were effects of systemic melatonin treatment on serum oxidative stress index and alveolar bone loss in diabetic rats with periodontitis are investigated.. A total of 70 rats were divided into four groups: control (C)- experimental periodontitis (EP), diabetes (DM), EP–melatonin (EP-MEL), DM-MEL, and EP-DM-MEL. Diabetes induced by alloxan and periodontitis induced by ligature was performed for four weeks. A 14 day dose of melatonin (10 mg/bw) for rats in melatonin groups i.e EP-MEL, DM-MEL and EP-DM-MEL groups were given after the removal of a single wire. At the end of the investigation, all rats were euthanised and the biochemical and histological analyzes was done on obtained intracardiac blood samples and mandible tissue. In the serum, total oxidant status / total antioxidant status, as well as the oxidative stress index (OSI), were measured . In addition, neutrophil and osteoclast densities and myeloperoxidase activity, were measured in gingival tissue homogenates, and alveolar bone loss was measured histometrically. Results showed that the fasting plasma glucose level was significantly reduced after melatonin treatment. Also, all the decreased OSI and levels of alveolar bone loss were appreciated in melatonin treated group, with more significant reduction in EP-DM-MEL group. Also the level of increased myeloperoxidase, osteoclast and neutrophil also decreased significantly after melatonin use. Melatonin significantly inhibited hyperglycemia-induced oxidative stress and alveolar bone loss in diabetic rats with periodontitis via antidiabetic and antioxidant effects.

Chitsazi M et al (2017)[63] carried out study to evaluate the effect of NSPT and adjunctive use of melatonin and vitamin C in chronic periodontitis. Total 60 chronic periodontitis were randomly divided into three grouos were in group 1- only NSPT performed (n=20), group 2- received melatonin along with NSPT, and group 3-

received NSPT with combination of vitamin C and Meltonin. Clinical parameter evaluated at baseline, 3 and 6 month interval were GI, PD and CAL. Results showed improvement in all the clinical parmetr after NSPT but there was significant improvement in PD and CAL score at 6 month with p <0.001 in group 3 (melatonin and vitamin C) obtained showed that NSPT improved PD and CAL at 3 and 6 months interval compared to baseline (P<0.05). Therefore adjunctive dose of vitamin C offered an additional effect at this interval. So, it was concluded that combination therapy with melatonin and vitamin C can improve the results of non-surgical periodontal therapy.

Ali E et al (2018)[64] carried out the study, to evaluate the preservation of the alveolar ridge using either alone or combined with melatonin in chronic periodontitis patients in a combination of bioacctive glass and platelet rich fibrin**.** A total of 29 patients (30 extraction sockets) were randomly assigned to the control group, which received only atraumatic extraction, the test 1 group (which received bioactive glass mixed with PRF), and the test 2 group (application of bioactive glass mixed with PRF and melatonin gel). Wound healing index (WHI), socket width, vertical height were measured at 3 months and 6 months post treatment. At baseline and 6 months after surgery, CBCT scans were used to measure the width of the socket. On clinical assessment, both groups II and III showed a significant increase in the length and width of the alveolar ridge, with a significant difference between the two groups throughout the study period. Both Group II and Group III radiografically showed significant increases in all the parameters over time and the control group at the end of the study period showed a significant less improvement. The authors conclude that the combined use of bioglass, PRF, and melatonin gives the best clinical and

radiographic results.

Virto L et al (2018)[65] investigated the effect of melatonin as systemic administration with standard mechanical periodontal therapy in obese rats with experimental periodontitis. A total 42 Wistar rats with an initial body weight of 180 grams, out of them 21 rabbits were kept on high fat diet I order to promote obesity. Further, the periodontitis was induced in both the obese and normal weight rabbits by giving oral gavages with P. gingivalis and F. nucleatum combination. After that, groups were randomly divided into two groups, group I- only mechanical debridement performed. Group II and Group III – along with mechanical debridement adjuctive use of chlorhexidin or melatonin performed. respectively. Clinical parameter measured were GI, PI, BOI, PPD and micro CT were done to measure bone resorption . In plasma and gingival tissue levels of biomarker measured were inflammatory cytokines, insulin, leptin, osteocalcin, osteopontin, plasminogen activator inhibitor-1, intercellular adhesion molecule 1, E-selectin and lipids. It was observed that the adjunctive use of melatonin administration resulted in significant reduction in reduced GI, BOP , PPD and appreciable amount of bone repair (15% reduction in alvrolar bone destruction) was also observed on micro CT in the obese-periodontitis group when compared to normal weight healthy rat group despite of any treatment option. When compared to the other groups, this melatonin-treated obese-periodontitis group had a significant effect on biochemical biomarkers in both gingival and plasma samples, with reductions in pro-inflammatory cytokines. Results showed that an added melatonin therapy significantly reduced alveolar bone loss and had a protective anti-inflammatory effect primarily in the experimental animals affected by periodontitis and with co-morbidity like obesity.

Bazyar H et al (2018)[66] carried out study in Type 2 DM patients with chronic periodontal disease to evaluate the impact of melatonin supplement on periodontal status, serum-melatonin and inflammatory markers .the included paptints were randomly divided into two groups were in experimental group patient received 6 mg and control group received placebo (2 tablets) once daily. In all patients, pre- and postoperative serum levels of melatonin, TNF-α, IL-6, hs-CRP, CAL, PD, BOP and PI were evaluated. The results showed a significant increase in the mean serum levels of melatonin following intervention. The level of IL-6 and hs-CRP was reduced significantly in the intervention group (p=0.008 and p=0.017 respectively). After intervention, PD and CAL were significantly lower in the intervention group (p=0.001). Melatonin levels changes were significantly higher in the intervention group compared to the control group. Based on the findings, the author concluded that melatonin supplementation in combination with non-surgical periodontal therapy could improve inflammatory and periodontal status in T2DM patients with CP.

Sharkawy Het al (2018)[67] studied and assess the additive effect of melatonin supplementation in insomnia individuals with generalized chronic periodontitis (gCP) after SRP. Total of seventy-four primary insomnia patients divided into melatonin group were subjects received 2 month regimen of melatonin capsule once daily along with SRP and control group performed SRP along with placebo capsule. Clinical parameters measured were BOP (in %), CAL gain, PPD. Salivary TNF levels and Anthens insomnia scale (AIS) Score were also measured at 3 and 6 months . Results showed that CAL gain and PD reduction measurements for the melatonin group were considerably greater than the control group at 3 and 6 months ($P < 0.01$). Similarly, salivary TNF-α levels and AIS levels in the melatonin group were significantly lower

than in the placebo group. In both groups, the BOP percent improved considerably without any difference.

Pandey A et al (2019)[68] conducted study to evaluate the effects of melatonin on implant osseointegration. Total of 15 patients with age range between 45-55 years were included in the study. Subjects were divided into three groups. Control group (CG) (n=5) were no treatment was applied, and dental implants were simply inserted, Melatonin Dose 1 (MLT D-1) group (n=5), Melatonin Dose 2 (MLT D-2) group (n=5). Results obtained showed that new bone regeneration reached the top in the melatonin group; however, it reached to about one-third in the control group. Some blood vessels were observed in the newly generated bone in the melatonin groups. The total percentages of areas of new bone were significantly different between the melatonin and control groups at 12 weeks. From the result it can be concluded that melatonin promoted vertical bone regeneration in a secluded space using a plastic cap.

Ahmed E et al (2021)[69] conducted a study to assess the efficacy of topical melatonin gel as an adjunct to nonsurgical periodontal therapy. Total of 24 patient with Grade II periodontitis was included in the study which were equally divided into control group were NSPT was performed with placebo gel application and test group were treated with NSPT plus application of 5% melatonin gel in periodontal pocket. Clinical parameters like PPD and CAL were measured at baseline and 3 months. GCF were collected for biochemical evaluation of Total antioxidative capacity (TAC) and matrix metalloproteinase-9 (MMP-9) were done at baseline and 3 months using ELISA. Result obtained showed that statistically more significant decrease in PD significant CAL gain were observed in melatonin treatment sites compared to control

site. In melatonin-treated sites, there was significant increase in TAC and a significant decrease in MMP-9 levels in GCF in melatonin treated group reflecting its strong potential as an antioxidant. The adjunctive effects of locally delivered melatonin gel have a positive effect on inflammatory and antioxidant parameters in periodontitis patients, adding a new dimension to periodontitis management.

Review of studies on the methods of analysis of regeneration

Misch K et al (2006)[37] compared CBCT measurements of periodontal defects to traditional methods. Artificial osseous defects were created on mandibles of dry skulls. CBCT scanning, periapical radiography, and direct measurements using a periodontal probe were compared to an electronic caliper that was used as a standard reference. Linear measurements for all defects revealed no statistical differences between bone sounding, radiography, and CBCT. There was a significant difference when comparing isolated interproximal measurements using a probe versus the caliper ($P < 0.001$) but no significant difference for CBCT or radiography. All bony defects were identifiable and measurable directly or with CBCT. In comparison, buccal and lingual defects could not be measured with radiographs. Overall, all three modalities are useful for identifying interproximal periodontal defects. Compared to radiographs, the three-dimensional capability of CBCT offers a significant advantage because all defects can be detected and quantified.

Vandenberghe B et al (2008)[70] explored the diagnostic values of digital intraoral radiography and CBCT in the determination of periodontal bone loss, infrabony craters and furcation involvements. Accuracy assessment of the imaging modalities was conducted through bone level measurements, infrabony crater and

furcation involvement classifications. For CBCT, images were obtained at 120 kV and 23.87 mAs, and observations were made on a 5.2 mm panoramic reconstruction view and on 0.4 mm thick cross-sectional slices. Intraoral radiographs of a size 2 charge-coupled device (CCD) sensor were obtained using the paralleling technique, at 60 kV and 0.28 mAs exposure. 71 human cadaver and dry skull bony defects were measured and evaluated by 3 observers. Comparison was made with the gold standard. The mean error (gold standard deviation) of bone level measurements was 0.56 mm for intraoral radiography and 0.47 mm for the CBCT panoramic 5.2 mm reconstruction view. There were no significant differences (P = 0.165) between the two methods. However, on 0.4 mm thick cross-sections, the mean error was 0.29 mm and the Wilcoxon signed-rank test indicated a significant difference when compared with the CCD (P = 0.006). The detection of crater and furcation involvements failed in 29% and 44% for the CCD, respectively, in contrast to 100% detectability for both defects with CBCT. CBCT on the panoramic 5.2 mm reconstruction view allowed comparable measurements of periodontal bone levels and defects as with intraoral radiography. CBCT with 0.4 mm thick cross-sections demonstrated values closer to the gold standard, indicating more accurate assessment of periodontal bone loss. Further research is needed to explore these results in vivo and to determine the use of CBCT in periodontal diagnosis.

Grimard et al (2009)[71] did comparison between the measurements obtained from digital intraoral radiographs and CBCT images to direct surgical measurements for the evaluation of regenerative treatment outcomes. Digital intraoral radiographs

and CBCT images were taken prior to initial bone grafting and at the 6 month reentry surgery for 35 intrabony defects. After carrying out defect debridement, direct bony defect measurements were done with a periodontal probe. These same measurements were made on the radiographs and CBCT images and then compared to the direct surgical values. CBCT correlated strongly with surgical measurements, whereas intraoral radiographs correlated less favorably. Intraoral radiographs measurements were significantly less accurate compared to CBCT for all parameters investigated and underestimated surgical measurements from 0.6 ± 2.3mm to 1.5 ± 2.3mm. No significant difference for the distance from the cementoenamel junction to the alveolar crest, defect fill, or defect resolution was seen between CBCT and surgical measurements. Overall, compared to direct surgical measurements, CBCT was significantly more precise and accurate than intraoral radiographs. Study concluded that CBCT may obviate surgical reentry as a technique for assessing regenerative therapy outcomes.

Vasconcelos K De Faria et al (2012)[72] compared periapical radiographs with CBCT in detecting and localizing alveolar bone loss by comparing linear measurements of the height, depth and width of the defects as well as identifying combined bone defects in tomographic images. The images were selected from a secondary database of patients referred for periodontal evaluation. There were 51 sites in study showing both horizontal and vertical bone loss, assessed by 3 trained examiners. The results showed that there were no statistically significant differences between the imaging methods in terms of recognition of the pattern of bone loss. However, there were differences between the two methods when the distance between the cementoenamel junction and the alveolar crest(CEJ-AC) was measured. The two

methods differ in detecting the height of the alveolar bone crest but present similar views of the depth and width of bone defects. CBCT was the only method that allowed for an analysis of the buccal and lingual/palatal surfaces and improved visualization of the morphology of the defect.

Takeshita et al (2014)[73] evaluated the diagnostic accuracy of different radiographic methods in the assessment of proximal alveolar bone loss (ABL). ABL, the distance between cement‑enamel junction and alveolar bone crest, was measured in 70 mandibular human teeth – directly on the mandibles (control), using conventional periapical radiography with film holders (Rinn XCP and Han-Shin), digital periapical radiography with complementary metal-oxide semiconductor sensor, conventional panoramic, and CBCT. Three programs were used to measure ABL on the images: Image tool 3.0 (University of Texas Health Sciences Center, San Antonio, Texas, USA), Kodak Imaging 6.1 (Kodak Dental Imaging 6.1, Carestream Health®, Rochester, NY, USA), and i-CAT vision 1.6.20. The tomographic images showed the highest means, whereas the lowest were found for periapical with Han-Shin. Controls differed from periapical with Han-Shin ($P < 0.0001$). CBCT differed from panoramic ($P = 0.0130$), periapical with Rinn XCP ($P = 0.0066$), periapical with Han-Shin ($P < 0.0001$), and digital periapical ($P = 0.0027$) Conventional periapicals with film holders differed from each other ($P = 0.0007$). Digital periapical differed from conventional periapical with Han-Shin ($P = 0.0004$). Conventional periapical with Han-Shin film holder was the only method that differed from the controls. CBCT had the closest means to the controls.

Banodkar A et al (2015)[74] evaluated the accuracy of CBCT measurements of

alveolar bone defects caused due to periodontal disease, by comparing it with actual surgical measurements which is the gold standard. Hundred periodontal bone defects in fifteen patients suffering from periodontitis and scheduled for flap surgery were included in the study. On the day of surgery prior to anesthesia, CBCT of the quadrant to be operated was taken. After reflection of the flap, clinical measurements of periodontal defect were made using a reamer and digital vernier caliper. In the case of horizontal defects, the defect depth was measured as distance between the CEJ and the alveolar crest. In the case of vertical defects, the defect depth was measured as distance between the CEJ and the base of the defect. The CBCT measurements followed the same pattern as clinical measurements. The measurements taken during surgery were then compared to the measurements done with CBCT and subjected to statistical analysis using the Pearson's correlation test. Overall there was a very high correlation of 0.988 between the surgical and CBCT measurements. In case of type of defects the correlation was higher in horizontal defects as compared to vertical defects. CBCT is highly accurate in measurement of periodontal defects and proves to be a very useful tool in periodontal diagnosis and treatment assessment.

Chhabra A et al (2016)[75] conducted a prospective cross sectional study to determine the accuracy of CBCT in quantifying intra-osseous periodontal bone defects. 5 patients with intra-bony defects were selected and 10 defects were assessed. A total of 60 measurements were performed. Periapical radiographs and Cone beam CT scan images were obtained. Height and depth of each defects was measured using appropriate software. Direct measurements were done during surgical interventions using a periodontal probe and were considered the standard reference. Measurements made by all three modalities were compared to each other. Linear measurements for

all defects revealed no statistical differences between CBCT and direct intra-surgical measurements with respect to the height as well as the depth of the defect. There was a significant difference when comparing peri-apical radiographs to the other two methods. IOPA measurements were only 74.3% accurate as compared to the standard intra-surgical whereas the CBCT measurements were 86.5%. All three modalities proved to be useful for identifying interproximal periodontal defect but CBCT took the lead with better accuracy in reproducing the clinical measurement of intra-bony periodontal bone defects and better visualization of the extent of the defect.

Guo YJ et al (2016)[76] assessed periodontal bone loss in CBCT images by 6 site method. Total 150 measuring points in 11 molars and 14 premolars from 6 patients i.e. 2 males and 4 females were included. Prior to periodontal surgery CBCT images of the teeth were acquired. Four observers evaluated the distances between CEJ-BD at the mesio–buccal, mid-buccal, disto-buccal, mesio-lingual/palatal, mid-lingual/palatal and disto–lingual/palatal sites in CBCT images. Direct surgical measurements of the six sites were obtained during periodontal surgeries. Difference between the values of distances measured in the CBCT images and direct surgical measurements were checked. Interobserver and intraobserver differences were tested. Results showed that no statistically significant difference was found between the surgical and CBCT measurements. Diagnostic similarity rates of four observers were 86.7%, 87.3%, 88.7% and 88.0%, respectively. The interobserver and intraobserver variances were not statistically significant. Study concluded that the six-site measuring method implicated in study may be a useful 3-dimensional method for evaluation of periodontal defect.

Suphanantachat S et al (2017)[77] compared evaluation of clinical values by CBCT and conventional intraoral radiography (IOR) in IBD assessment. The study included 25 patients suffering from periodontitis and presented at least two IBDs. All patients received clinical periodontal examination, IOR and CBCT. Three periodontists checked periodontal diagnosis and prognosis of each tooth. For teeth with presence of infrabony defects, the number of defect walls was determined. IOR and CBCT assessment was compared. There were total 666 teeth and 123 infrabony defects. The overall value similarity between IOR and CBCT for periodontal diagnosis, prognosis, infrabony defect type and infrabony defect treatment were 79.3%, 69.5%, 44.7% and 64.2%, respectively. Diagnosis, prognosis and the number of infrabony defect walls were underestimated by IOR at 16.4%, 24% and 37.4%, respectively. CBCT showed admirable interexaminer agreement and greater percentage of complete agreement among examiners than IOR for all assessments.IOR underrated the severity and prognosis of periodontal disease. CBCT was finer to IOR for valuation of infrabony defect morphology and treatment. The study concluded that CBCT provides excellent agreement among examiners on IBD assessment and hence is a reliable method to measure them.

Zang W et al (2018)[78] compare and correlate accuracy of molar furcation assessment via three different evaluation methods i.e., clinical detection, intraoral radiography and CBCT images. Total eighty-three patients having chronic periodontitis with an existing CBCT scans were included. Furcation involvement was assessed on maxillary and mandibular first molars. Furcation involvement on buccal and palatal/lingual sites were evaluated by using Periodontal charts (modified Glickman's classification), intraoral (periapical and/or bitewing) radiographs

(recorded as presence or absence) and axial CBCT sections. The correlation of furcation assessment by the three methods was evaluated by Pearson analysis. Significant correlation ($p < 0.05$) was found between clinical detection and intraoral radiography, clinical detection and CBCT, as well as intraoral radiography and CBCT at all the measured sites (r values range between 0.230 to 0.644). While, CBCT exhibited higher correlation with clinical detection relative to intraoral radiography, especially at distal palatal side of maxillary first molar ($p < 0.05$). In addition, CBCT provided more accurate assessment of bone loss measurement up to 2 decimals in millimeters, whereas clinically all 3 classes FI were detected and the intraoral radiographs usually only detected the presence of furcation involvement in Glickman Class 2 and 3. This study thus validated the use of CBCT as a valuable tool in assessing molar furcation defects in addition to clinical detection and intra-oral radiographs.

MATERIAL AND METHOD

The present study was undertaken to evaluate and compare the efficacy of 1% melatonin gel as an adjunct to NSPT and NSPT with placebo gel in treatment of stage III periodontitis. The evaluation was done clinically and radiographically using CBCT.

The study was initiated after the clearance from the Institutional Ethics Committee of our institute. A special proforma was designed so as to have organized a methodological recording of observation and information. This included a detailed case history, clinical examination, radiographic evaluation, periodontal indices and written informed consent of patient.

Sample Size Calculation

Referring to the article by Gupta A. et al. (2018)[53], the authors evaluated the efficacy of local drug delivery system of Zoledronate (ZLN) gel as an adjunct to scaling and root planning (SRP) for the treatment of human periodontal defects clinically and radiographically. The proposed study uses Melatonin gel as an adjunct to SRP in patients with mild to severe periodontitis. The study design in split mouth, hence two treatments will be randomly assigned to each side in the same patient. The data on mean difference of parameters (PI, GI, PPD and CAL) between baseline to 6M for control and ZLN groups, was considered for estimating the effect size (ES). The value of ES ranged between 0.3 – 0.5 for the above parameters.

For the proposed study, an ES estimate of 0.6 was considered, which resulted in a sample of 22. This sample size can provide the desired effect with 95% confidence and 80% power.

The formula used for sample size estimation was:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{ES^2}$$

Where $Z_{1-\alpha/2}$ is the standard normal value at 5% level and $Z_{1-\beta}$ is the standard normal value at 20%, ES is the effect size obtained using

$$ES = \frac{(\mu_1 - \mu_2)}{\sigma}$$

where μ_1 and μ_2 are the mean differences for two treatment groups between baseline and 6M and σ is the pooled standard deviation.

So, Present randomized controlled clinical study was conducted in Department of Periodontology of our institution. The study sample included total of 22 patients (11 males and 11 females) with stage III periodontitis exhibiting bilateral intrabony defects. The patients included were between the age ranges of 20 to 60 years.

Inclusion Criteria

1. Patients with stage III periodontitis as assessed by PPD ≥ 5mm and CAL ≥6mm.
2. Patients with at least one pair of bilateral IBDs in either maxillary or mandibular arch.

Exclusion Criteria

1. Patients with any systemic illness.
2. Pregnant or lactating women.
3. Patient who has undergone periodontal treatment in previous 6 months.

Clinical Parameters Measured:

1. Plaque index (PI)[79]
2. Modified sulcus bleeding index (mSBI)[80]
3. Probing pocket depth (PPD)
4. Clinical attachment level (CAL)

Allotment:

The selected sites were randomly assigned by a coin toss method by the operator who is the second examiner to Group I that is Control group (NSPT + Placebo Gel) and Group II that is Test group (NSPT + 1% Melatonin Gel).

Allocation concealment proposal: To ensure the masked nature of the study, an examination file and a treatment file will be created for each patient after inclusion. The examination file, which will contain case history and the clinical measurements, will be accessible only to the examiner blinded to the study group. The treatment file, which provides data about the randomization modalities, will be accessible only to the operator.

Blinding proposal: The treatment sites will be randomized, assessor and operator will be blinded.

Stent Fabrication

A sterile, perforated stock metal impression tray was selected for each patient. An irreversible hydrocolloid impression material (alginate) was manipulated, carried into the tray and maxillary and mandibular impressions were made. Study casts were prepared for each patient. A customized acrylic occlusal stent was fabricated for each patient to fit over the selected sites. The PPD and CAL were measured by using UNC-15 (University of North Carolina-15) periodontal probe. A groove was made on the stent in relation to each involved tooth to guide the periodontal probe while taking measurements. This technique provided a fixed point and angulation for measurements with the probe at each site.

Probing pocket depth (PPD) and Clinical attachment level (CAL)

The PPD and CAL measurements were obtained using UNC -15 graduated probe with markings to the nearest millimeter (mm). PPD and CAL were measured at four sites around each tooth (mesial, midbuccal, distal and midlingual). PPD was measured from the free gingival margin (GM) to the bottom of the pocket. The distance of gingival margin to cemento-enamel junction (CEJ) was recorded. The distance from the gingival margin to the bottom of the sulcus was measured in mm. The distance of gingival margin to CEJ was subtracted from the above measurements to obtain the CAL.

Plaque Index (PI) (Silness and Loe 1964)

It was examined in the scoring units of teeth: distofacial, facial, mesiofacial and lingual surfaces. A mouth mirror and dental explorer were used to assess PI.

Scoring Criteria

Score	**Criteria**
0	No Plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface

Calculation:

A plaque index per person was obtained by adding all of the plaque scores and dividing by the number of surfaces examined.

$$\text{Plaque Index (PI)} = \frac{\text{Total plaque score}}{\text{No of surfaces examined}}$$

Interpretation	
Excellent	0
Good	0.1-0.9
Fair	1.0-1.9
Poor	2.0-3.0

Modified sulcus bleeding index (mSBI)

The severity of gingival bleeding, a sign of inflammation that is associated with periodontal disease. The tissues surrounding each tooth are divided into four gingival scoring units: disto-facial, facial, mesio-facial and the entire lingual gingival margin. To minimize examiner variability in scoring, the lingual surface was not subdivided because it is mostly likely being viewed indirectly with a mouth mirror. A periodontal probe was used and passed along the gingival margin to provoke bleeding, and the clinical findings were recording to the following scores and criteria.

Calculation:

mSBI per person was obtained by adding all of the teeth scores and dividing by the number of teeth examined.

Modified Sulcus Bleeding Index = $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$

Interpretation	
0	No bleeding when a periodontal probe is passed along the gingival margin.
1	Isolated bleeding spots visible.
2	Blood forms a confluent red line on margin.
3	Heavy or profuse bleeding.

CBCT analysis

CBCT measurements were taken for each Group i.e. the Test and the Control group at baseline and at 6 months. The CBCT analysis included the measurement of bone defect height (CEJ –BD), bone defect depth (AC-BD) and the mesiodistal (MD) bone defect width and buccolingual (BL) width. The total bone fill was measured subtracting the depth of the osseous defect at 6 months from the baseline measurement.

Armamentarium

A. Diagnostic instruments

1. Mouth mirror
2. UNC-15 periodontal probe
3. Tweezers
4. Explorer
5. Kidney tray
6. Gloves, mouth mask & head Cap

B. Instruments for NSPT

1. Hand scalers
2. Ultrasonic scalers
3. Gracey curettes

C. For LDD

1. 1% Melatonin gel
2. Placebo gel
3. Disposable syringe
4. Blunt cannula

Formulation of 1% Melatonin Gel

S. No.	Component	1%
1.	Melatonin raw drug	1gm
2.	Carbopol 934	1 gm
3.	Triethanolamine	0.5ml
4.	Methyl paraben	50 mg
5.	Propyl paraben	25 mg
6.	Propyl glycol and Distilled water	1:4 ratio

Procedure for gel preparation:

- Firstly **Propylene glycol** was added in **distilled water** in 1:4 ratio combination.
- The solution is then stirred nicely.
- In next step the addition of preservative **methylparaben** and

propylparaben was done.

- This solution is then kept for sonication for 30 minutes.
- After sonication 1% **Carbopol 934P** was dispersed in the solution.
- After 24 hours the **1gm of melatonin** raw drug was added in the Solution and stirred well.
- The final gel was prepared by adding **triethanolamine**.

Group I (Control Group)- Stage III periodontitis to be treated with non-surgical periodontal therapy followed by placebo gel placement subgingivally.

Group II (Test Group)- Stage III periodontitis to be treated with non-Surgical periodontal therapy followed by 1% melatonin gel placement subgingivally.

Intraoperative Procedure:

Prior to initiating the study, its purpose and design was explained and informed consent was signed by every patient. All the clinical parameters like plaque index, sulcus bleeding index, Probing pocket depth and clinical attachment level were measured using UNC #15 probe and custom-made acrylic stent made to compare the measurement at each recall visit. Clinical procedure consist of Non-Surgical Periodontal Therapy was performed in both group. Group I patients will be treated by NSPT 0.1 ml placebo gel was injected into the periodontal pockets using a syringe with a blunt cannula subgingivally. Group II patients will be treated by NSPT and 1% melatonin gel were 0.1 ml prepared gel was injected into the periodontal pockets using a syringe with a blunt cannula subgingivally and in both the group periodontal dressing was placed. Patients will be instructed to refrain from chewing hard or sticky

foods, brushing near the treated areas, or using any interdental aids for 1 week. Patients will be recalled at 3 months and 6 months postoperatively.

Post-operative evaluation

Patients were evaluated by CBCT at baseline and 6 months intervals. Measurements of PPD, CAL were taken similar to the pre procedures using UNC-15 periodontal probe at 3 and 6 months.

CBCT Measurements

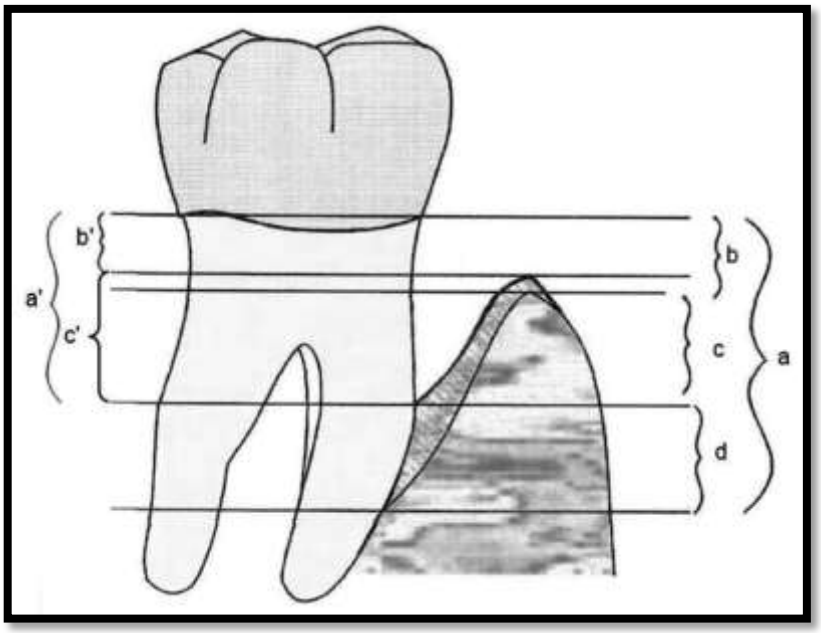
All the sites in both Test and Control groups were subjected to CBCT assessment. The Orthophos® XG 3D manufactured by Sirona Dental Systems GmbH, Germany using 3Diagnosys 4.2 Imaging software was used for the CBCT assessment. Patient was asked to remove all metal objects and wear a lead apron. The patient was asked to bite gently and naturally on the bite block without joining the incisors. The upper incisors centered with the bite block. The patient was adjusted using two positional laser beams-

- The mid-sagittal positioning laser beam
- The 3D field of view (FOV) positioning laser beam

The digital readout was seen on the computer screen.

All the sites in both Test and Control groups were subjected to CBCT assessment at baseline and 6 months postoperatively. The parameters which were measured on CBCT included CEJ-BD, the depth of the defect (AC-BD) and the MD width of the intrabony defect. All parameters above were measured as the same as on the periapical radiographs. Additionally, the BL width of the defect was measured on

the axial plane of the CBCT. When the BL width of the defect was measured, the innermost and the most coronal point for the buccal and lingual alveolar crest was chosen on the axial plane, and the horizontal distance of two points were measured. The linear measurements of CEJ to AC and CEJ to BD for each technique were used to determine IBD depth reduction.



a Distance from CEJ to the base of defect (BD) preoperatively

a^1 Distance from CEJ to the base of the defect(BD) 6 months postoperatively

b Distance from the CEJ to the alveolar crest (AC) preoperatively

b^1 Distance from the CEJ to the alveolar crest (AC) 6 months post-operatively

c Defect depth (pre-operatively) = **a−b**

c^1 Defect depth (6 months post-operatively) = **a1−b1**

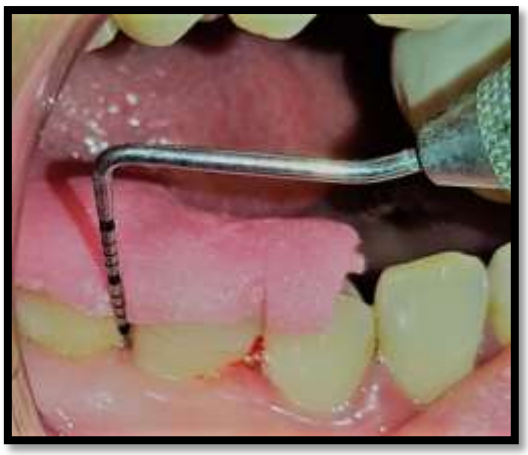
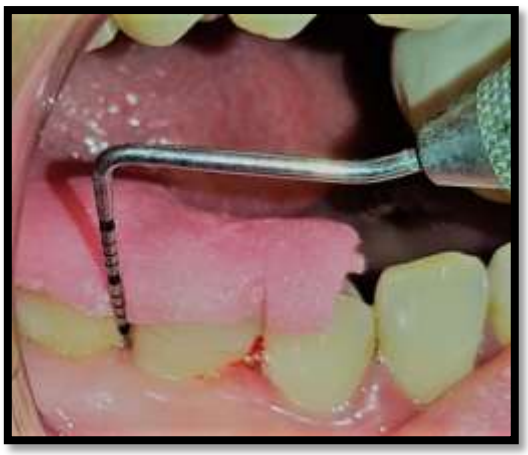
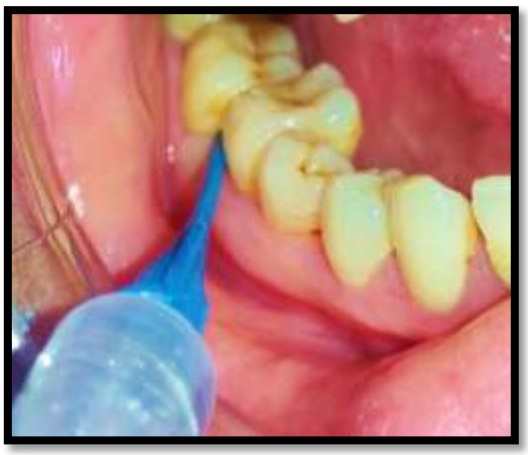
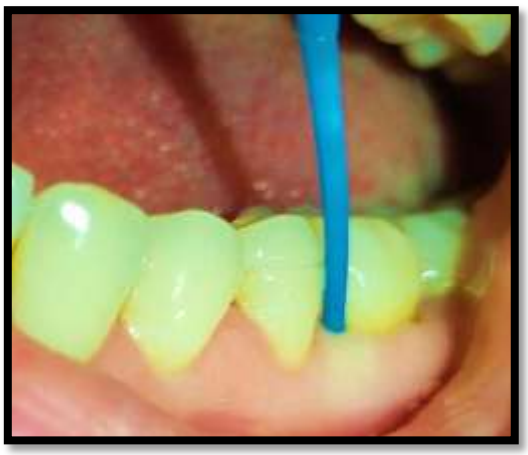
d Amount of defect fill = **c−c1**.

ARMAMENTARIUM FOR NON SURGICAL PERIODONTAL THERAPY

SYRINGE CONTAINING PLACEBO GEL AND 1% MELATONIN GEL

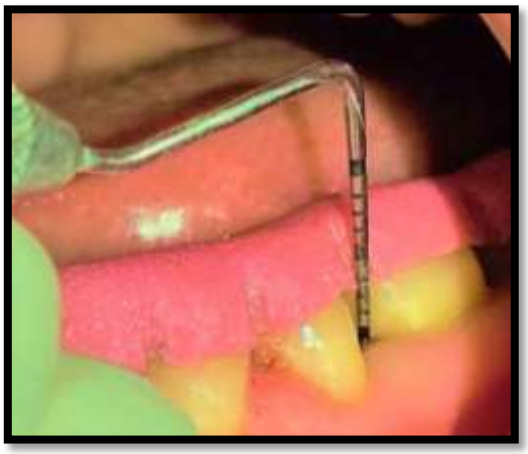
CLINICAL RECORDS

BASELINE

GROUP I	GROUP II
	
	
	

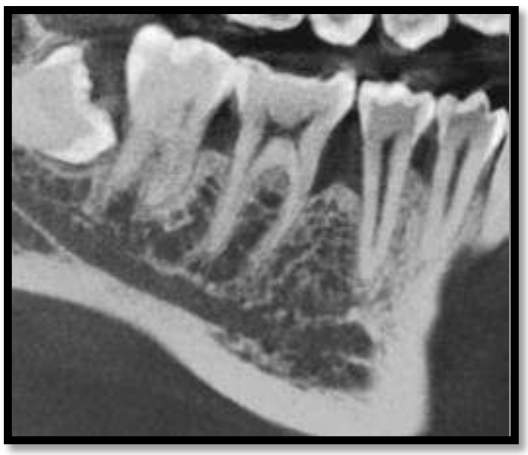
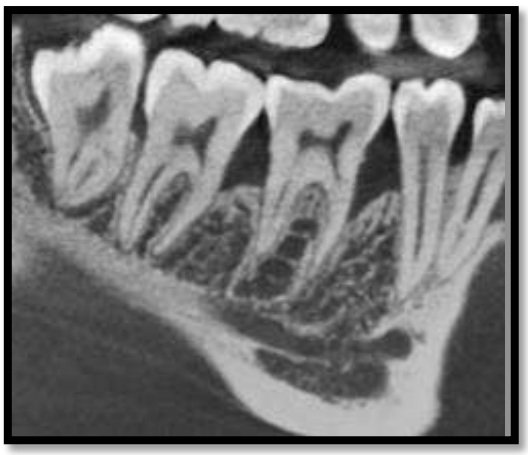
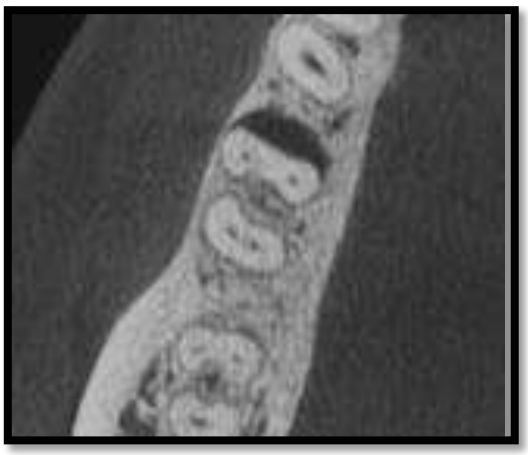
CLINICAL RECORDS

3 MONTHS and 6 MONTHS

GROUP I	GROUP II
	
3 MONTHS	**3 MONTHS**
	
6 MONTHS	**6 MONTHS**

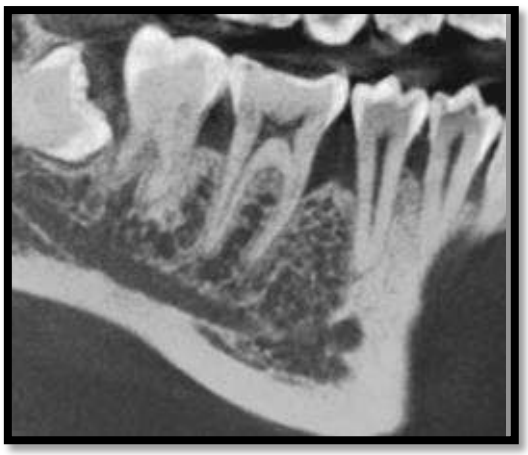
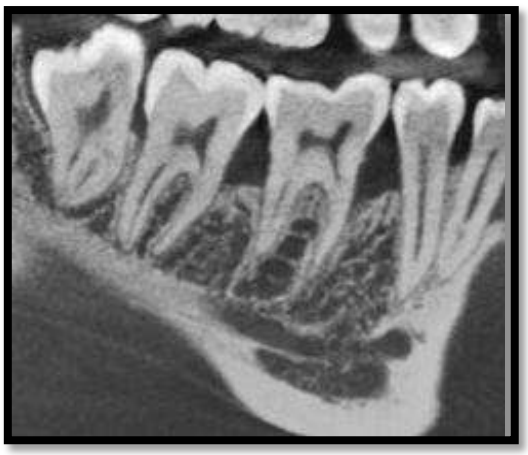
CBCT MEASUREMETNS

BASELINE

GROUP I	GROUP II
	
	

CBCT MEASUREMETNS

6 months

GROUP I	GROUP II
	
	

RESULT

The study has been conducted to evaluate and compare the efficacy of 1% melatonin gel as an adjunct to NSPT and NSPT with placebo gel in the treatment of stage III periodontitis with IBDs, in addition to NSPT and NSPT. The evaluation was done clinically and radiographically using CBCT.

At baseline the clinical parameters assessed were PI, mSBI, PPD, CAL and the intrabony defect parameters such as CEJ- BD, CEJ-AC, AC-BD, BL width, MD width were evaluated by CBCT and RVG for both the groups. PI, mSBI, PPD, CAL were measured at all recall visits of 3 and 6 months. Change in distance from CEJ-BD, CEJ-AC, AC-BD and MD width was measured 6 months recall visit by CBCT. CBCT evaluation at 6 months also included BL width dimension

Statistical analysis:

The clinical parameters like plaque index, gingival index, pocket probing

depth and clinical attachment level were summarized in terms of mean and standard deviation for each time point and for both control and test sites. The comparison of each parameter between sites was performed using paired t-test, while the comparison across time points at each site was carried out using repeated measure analysis of variance (ANOVA). Comparison of CEJ-BD, CEJ-AC, AC-BD, MD, and BL by CBCT between baseline and 6 months was done by performing paired t-test. Further, the CBCT parameters were summarized in terms of mean and standard deviation and compared between baseline to 6. The p value was taken as significant when less than 0.05The analyses were performed using SPSS ver 20.0 (IBM Corp USA) software and the statistical significance was evaluated at 5% level.

The study sample comprised of 22 patients, which included 11 males and 11 females in the age range of 20 to 45 years, with stage III periodontitis exhibiting 22 pairs of bilateral IBDs. The selected sites were randomly divided to receive a combination of NSPT and placebo gel (Group I) or NSPT plus 1% melatonin gel (Group II). The duration of study was 6 months.

In general, patients showed good oral hygiene through the complete duration of the study. Baseline full mouth PI was 1.83 ± 0.24 while at 3 months, it decreased to 1.08 ± 0.23 and at 6 months, the mean PI score was 0.65 ± 0.16. The difference in PI scores when compared with baseline measurements versus 3 months, showed statistically highly significant decrease in PI score ($p<0.0001$). At 6 months post-surgical measurements showed statistically highly significant decrease ($p<0.0001$) when compared to baseline, also, the reduction of PI score from 3 to 6 months was statistically highly significant ($p<0.0001$) **(Table 1 Graph 1).**

The mean mSBI score dropped from 2.69 ± 0.69 at baseline to 1.37 ± 0.19 at 3 months and to 1.05 ± 0.11 at 6 months. mSBI scores when compared from baseline to 3 months showed statistically highly significant decrease ($p<0.001$) and also when compared at 6 months, the difference was statistically highly significant ($p<0.001$). The mean decrease in mSBI score from 3 to 6 months was also statistically highly significant ($p<0.001$) **(Table 1, Graph 2).**

Clinical outcomes

Probing pocket depth (PPD)

In Group I, the mean PPD at baseline was 6.95±0.72 mm and that at **3 months** was 5.45±0.67 mm and in Group II, the mean PPD at baseline was 7.45±0.51 mm and that at 3 months was 5.36±0.49 mm **(Table 2, Graph 3).** At 3 months, the mean PPD reduction was 1.50±0.80 mm for Group I and 2.09±0.43 mm for Group II. There was a statistically highly significant reduction in PPD for Group I as well as Group II at 3 months compared to baseline ($p<0.0001$) **(Table 3, Graph 4).**

In the Group I, the mean PPD at baseline was 6.95±0.72 mm and that at **6 months** was 4. 55± 0.86 mm and in Group II the mean PPD at baseline was 7.45±0.51 mm and that at 6 months was 3.39±0.84 mm **(Table 2, Graph 3**). At 6 months, the mean PPD reduction was 2.40±1.09 mm for Group I and 3.05±0.91 mm for Group II. There was statistically highly significant reduction in PPD for Group I and Group II at 6 months when compared to baseline ($p<0.0001$). **(Table 3, Graph 4).**

Clinical attachment level (CAL)

In Group I, the mean CAL at baseline was 7.41±0.52 mm and that at **3 months** was 5.73±0.70 mm. The mean CAL at baseline in Group II was 7.77±0.69 mm and that at 3 months was 5.68±0.62 mm **(Table 4, Graph 5).** A mean CAL gain of 1.68±0.64 mm was observed in Group I and Group II exhibited a mean CAL gain of 2.29±0.68 mm. Both groups exhibited a statistically highly significant increase in CAL gain at the end of 3 months ($p<0.004$) **(Table 5, Graph 6)**.

In Group I, the mean CAL at baseline was 7.77±0.69 mm and that **at 6 months** was 4.68±0.99 mm. Group II showed a mean baseline CAL of 7.90±0.55 mm and at 6 months, it was 4.36±0.79 mm **(Table 4, Graph 5).** The mean CAL gain at 6 months in Group I was 2.72±1.07 mm and in Group II was 3.79±0.90 mm. There was statistically highly significant CAL gain for Group I and Group II at 6 months when compared to baseline ($p<0.004$). There was a statistically highly significant CAL gain at 3 and 6 months in Group II when compared to Group I ($p<0.004$) **(Table 5, Graph 6)**.

CBCT analysis of IBD parameters

Height of intrabony defect (CEJ-BD)

The mean CEJ-BD at baseline for Group I was 9.33±0.39 mm. At 6 months, the mean CEJ-BD for Group I was 8.83±0.45 mm. The difference in the measurement values of CEJ –BD at baseline and 6 month denotes the bone fill. So bone fill of 0.50± 0.38 mm was noted after 6 months for Group I **(Table 6, Graph 7).**

The mean CEJ-BD at baseline for Group II it was 9.80±0.46 mm and the mean CEJ-BD at 6 months for Group II was 8.34 ± 0.49 mm thus exhibiting a

reduction in CEJ-BD and giving a bone fill of 1.46 ± 0.58 mm. There was a statistically highly significant bone fill in both the groups ($p<0.0001$). When the reduction in CEJ-BD at 6 months was compared between the two groups it was higher in Group II as compared to Group I **(Table 6, Graph 7).**

Level of alveolar crest (CEJ-AC)

The mean CEJ-AC at baseline for Group I was 5.05±0.54 mm. The difference in the measurement values of CEJ –AC at baseline and 6 month denotes the change in level of alveolar crest. At 6 months, the mean CEJ-AC for Group I was 5.00±0.56 mm, showing a mean increase in CEJ-AC of 0.05 ± 0.24 mm **(Table 7, Graph 8).**

The mean CEJ-AC at baseline for Group II was 5.50±0.76 mm . The mean CEJ-AC at 6 months for Group II was 5.21±0.75 mm thus exhibiting mean decrease in CEJ-AC of 0.29 ± 0.21 mm. There was statistically insignificant increase in CEJ-AC distance in Group I ($p=0.341$). There was significant decrease in CEJ-AC distance in Group II ($p<0.0001$).When change in distance CEJ-AC was compared between the two groups was statistically not significant. **(Table 7, Graph 8).**

Intrabony defect depth (AC-BD)

The mean defect depth (AC-BD) at baseline for Group I was 4.28 ± 0.55 mm. The difference in the measurement values of AC- BD at baseline and 6 month denotes the reduction in IBD depth. At 6 months, the mean defect depth (AC-BD) for Group I was 3.82 ± 0.59 mm, showing a mean reduction in defect depth (AC-BD) of 0.45 ± 0.35 mm **(Table 8, Graph 9).**

The mean defect depth (AC-BD) at baseline for Group II it was 4.30 ± 0.56

mm and at 6 months the mean defect depth for Group II was 3.13 ± 0.68 mm thus exhibiting a reduction in defect depth of 1.17±0.59 mm. There was a statistically highly significant defect depth reduction in group II ($p<0.0001$). When the reduction in defect depth at 6 months was compared between the two groups it was higher in Group II as compared to Group I and was statistically highly significant ($p=<0.0001$) **(Table 8, Graph 9).**

Mesiodistal width (MD)

The mean MD dimension at baseline for Group I was 1.86±0.50 mm and at 6 months, the mean MD dimension for Group I was 1.57±0.48 mm, showing a mean reduction in MD dimension of 0.29 ± 0.12 mm **(Table 9, Graph 10)**.

The mean MD dimension at baseline for Group II it was 2.28±0.50 mm and at 6 months for Group II was it is 1.72±0.58 mm thus exhibiting a reduction in MD dimension of 0.56 ± 0.70 mm. There was a highly significant MD dimension reduction in Group II ($p<0.0001$). When the reduction at 6 months was compared between the two groups it was higher in Group II as compared to Group I and was statistically insignificant **(Table 9, Graph 10).**

Buccolingual width (BL)

The mean BL dimension at baseline for Group I was 4.23±0.69 mm. At 6 months, the mean BL dimension for Group I was 3.88±0.74 mm, showing a mean reduction in BL dimension of 0.35 ± 0.21 mm. **(Table 10, Graph 11).**

The mean BL dimension at baseline for Group II it was 4.79±1.32 mm and that at 6 months for Group II was 3.68±1.57 mm thus exhibiting a reduction in BL

dimension of 1.11 ± 0.50 mm. There was a statistically highly significant BL dimension reduction in Group II ($p<0.0001$). When the reduction at 6 months was compared between the two groups it was higher in Group II as compared to Group I and was statistically significant ($p<0.0001$) **(Table 10, Graph 11).**

DISCUSSION

Periodontitis is an infectious disease that destroys the tooth attachment apparatus and, if left untreated, results in progressive attachment loss and early tooth loss.[81] The main periodontal pathogens responsible for periodontal destruction are Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.[82] The overgrowth of several gram negative bacteria in the dental plaque produces several bacterial products which causea an excessive hostimmune response and produce high levels of proinflammatory mediators. These mediators set off a chain of events that in some people, results in irreversible degradation of connective tissue, bone tissues and loss of periodontal attachment. A range of cytokines may have a direct relationship in the biological activity of the periodontal destruction.[83,84] Some proinflammatory mediators such as Il-6, IL-1β and PGE2 are also related to periodontal and alveolar bone resorption. Production of inflammatory mediators like MAPK, AP-1, and NF-B results in IL-1-stimulated synthesis of IL-6, IL-8, PGE2, and MMP-1 in human

periodontal ligament cells via IL-1 signaling cascades.[85,86]

The ultimate goal of periodontal therapy is to regenerate tissues that have been destroyed by periodontal disease. Periodontal regeneration is defined as the complete restoration of lost tissues to their original architecture and function by recreating the critical wound healing events that occur during their development.[87,88] Non-surgical periodontal therapy, is a mainstay and is considered to be a gold standard in terms of the treatment of periodontal diseases. The microbial etiology of human periodontitis also indicates treatment with antimicrobial agents.[89] Systemic agents come into action through their intestinal absorption, leading passage from bloodstream entering into oral tissues. Local administration, on the other hand, requires direct supply of the agent to the subgingival site, reducing adverse reactions at non-oral locations.[90]

Melatonin is primarily responsible for its antioxidant and anti-inflammatory effects and acts as a mediator of bone formation and resorption in physiological as well as pathological processes linked to periodontitis.[91]

Melatonin has the following main advantages:

- Melatonin for periodontal disease treatment: By spreading into the saliva from the bloodstream, melatonin enters the oral cavity. As most of the melatonin remains linked to serum albumin, its saliva level is 1/3rd to that of blood level.[92] It has an effect on cells by interacting with the melatonin 1 and melatonin 2 receptor (MT1 & MT2 respectively).[93,94]
- Melatonin reduces periodontal inflammation by downregulating proinflammatory factors such as C reactive protein (CRP), interleukin 6 and TNF alpha, receptor activators of nuclear factor kappa-B

ligand/osteoprotegrin ratios.[95]

- Melatonin Antioxidant and Free Radical Scavenger: Melatonin is a powerful antioxidant and free radical scavenger.[96,97] Melatonin's metabolites can act as free radical scavengers, which is an important feature that distinguishes it from other free radical scavengers.[96]

- Melatonin as a Bone Formation Promoter**:** Melatonin is known to stimulate osteoblast proliferation and bone formation. It functions as an osteoconductive scaffold. Melatonin promotes the proliferation and differentiation of human osteoblasts in vitro, as well as the production of type I collagen and other bone matrix proteins.[98,99]

- Anti-inflammatory action of melatonin **:** Inflammation is a normal and necessary reaction to any insult. Melatonin directly interacts with COX-2 and also inhibits transcriptional factors that promote the production of pro-inflammatory cytokines.[100]

- Melatonin as a Potential Antimicrobial Agent: Melatonin has antimicrobial properties against a variety of bacteria and viruses. Melatonin and its receptor agonist inhibits the formation of biofilm, capable of reducing Porphyromonas gingivalis viability and showed anti-inflammatory reactions through the acting of lipopolysaccharide.[101,102]

Melatonin, in pharmacological doses, also inhibits receptor activator of nuclear factor-B ligand (RANKL)-mediated osteoclast formation and activation, the differentiation of osteoblasts mediator of angiogenesis, which increases bone formation.[98,103] Taking the above fact into account, the interest in conducting this

randomized controlled clinical trial was encouraged by limited evidence of melatonin use in the treatment of periodontal intrabony defects. The current study was designed as a randomized clinical trial to evaluate and compare the efficacy of 1% Melatonin gel as an adjunct to Non surgical periodontal therapy (NSPT) and NSPT with placebo gel in treatment of periodontal intrabony defects (IBDs) clinically and radiographically using CBCT. The study sample included 22 patients (11 males and 11 females) with the age range of 20-50 years with bilateral IBDs. In Group I IBDs was treated with NSPT with placebo gel placement. In Group II IBDs were treated with NSPT with subgingival delivery of 1% Melatonin gel. PI, mSBI and other clinical parameters such as PPD and CAL were evaluated at baseline, 3 months and 6 months postoperatively. For evaluation of regeneration, CBCT was taken at baseline and 6 months.

The current study has considered the technique of subgingivally delivering Melatonin directly into IBDs in individuals with stage III periodontitis, as the LDD system provides the advantages of high concentrations at the required site with reduced dosage, fewer applications, and high patient acceptability. Compared to a systemic regimen, local delivery may offer important benefits in terms of adverse reactions and patient compliance.

There were no significant differences among Group I and Group II in the study parameters at baseline indicating the precise patient selection devoid of bias. Each patient participating in the study showed good oral hygiene and a healthy clinical gingival condition throughout the duration of study. The PI score was low at the end of six months. This was the result of repeated oral hygiene instructions given

to the patients throughout the study period. Plaque control is essential for the long term stability of clinical outcomes. Bacterial plaque is a major and important factor in the etiology of periodontal destruction and successful therapy depends upon its removal subsequent to treatment. At the end of 6 months, PI and mSBI score were significantly reduced with p<0.0001. Our results are in accordance with the study done by **Ahmed E et al (2021)**[69] in which they have studied the effect of melatonin in patients with grade II periodontitis and clinical parameters were recorded postoperatively up to 3 months. The statistical significant reduction in PI score was observed in melatonin group from baseline to 3 months. Also, same type of reduction in PI and BOP scores was observed by **Sharkawy H et al (2018)**[67] in which melatonin supplementation (10 mg) was given to chronic periodontitis patients at 3 and 6 months.

In Group I, the mean PPD at baseline was 6.95±0.72 mm and in Group II, the mean PPD at baseline was 7.45±0.51 mm. At 6 months, significantly greater reduction in PPD was observed in Group II (3.05±0.91 mm) versus Group I (2.40±1.09 mm) when compared to baseline (p<0.0001).

These results were similar to those reported by **Sharkawy H et al (2018)**[67] who compared melatonin supplementation along with SRP. They found mean reduction in probing depths at the test sites (2 mm) compared to the control sites (1.4 mm), which was greater in the test group as compared to the controls at the end of 6 months. **Bazyar H et al. (2018)**[66] compared the treatment of deep intrabony defects in diabetes with a combination SRP and melatonin supplementation. At baseline, the mean PPD was 4.54±1.01 mm in the control group and 4.45 ±0.96 mm in the test

group (Melatonin group). At 8 week, the mean PPD reduction was 4.36 ± 1.04 mm in the control group and 2.59 ± 1.04 mm in the test group. PPD decreased significantly in both groups compared to the baseline data.

In Group I, the mean CAL at baseline was 7.41±0.52 mm and for Group II the mean CAL was 7.77±0.69 mm. At 6 months, the mean reduction in Group I was 2.72±1.07 mm and in Group II was 3.79±0.90 mm. There was a statistically highly significant CAL gain at 6 months in Group II when compared to Group I ($p<0.004$).

Our results are in accordance with the study done by **Chitsazi M et al (2017)[63]** were they studied the simultenous use melatonin and vitamic C as adjunct to SRP on the clinical parameters in CP and they have found that there was significant improvement in CAL score in test group at 6 month with mean difference of 3.30±0.64 mm. Also, **Cutando A et al (2015)[95]** studied the topical application of melatonin in patient with chronic periodontits with type 1 and 2 diabetes on clinical parameters like PD, CAL and GI. The result showed that significant CAL gain was observed in test group as compared to control group suggesting the added advantage and positive outcome of melatonin in CP with diabetes.[95]

It seems that anti-inflammatory and antioxidant properties of melatonin have positive effect on on the clinical periodontal parameters in periodontitis. This could be explained because the anti-inflammatory characteristics of melatonin are linked with their ability to act as the scavenge agent for the exogenous and endogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS).[104]

Melatonin was found to be an important modulator for calcium metabolism and in some cases prevents osteoporosis and hypocalcemia probably as a result of its

interactions with other regulatory bone conditions, such as parathormone, calcitonin and prostaglandins. It is reported that melatonin lowered expression of pro-inflammatory cytokines, enzymes (NF-KB, iNOS, IL-1β, IFN-γ, TNF-α) and pro-apoptotic genes.[105] Melatonin also promotes osteoblastic differentiation and inhibits osteoclastic activity by down-regulating RANKL ligand. Local melatonin application around dental implants was also found to increase bone-implant contact and trabecular density.[106]

In the present study, the reduction in the CEJ-BD distance signifies bone gain. In our study reduction in CEJ-BD was more in Group II as compared to Group I with mean difference at 6 months in group II is 1.46 mm. This indicates role of melatonin in periodontal regeneration. Change of distance CEJ-AC denotes change in the level of alveolar crest. In our study CEJ-AC distance statistically decreased in the Group II at the end of 6 months with mean difference of 0.29 mm. This indicates that alveolar crest gain was more in Group II were delivery of 1% melatonin gel. The bone fill (AC-BC) at 6 months was compared between the two groups it was found higher in Group II as compared to Group I and was statistically highly significant (p=<0.0001)

This was in accordance with the animal study done by **Vitro L et al (2018)[65]** to evaluate the effect of melatonin in obese rat with periodontitis and found enhanced bone regeneration (reduced CEJ-AC distance) was reported in NW-Perio-Mel and HFD-Perio-Mel (i.e melatonin treatment) groups with a reduction of 11.09(3.19%) vs 57.66(15%) respectively. **Kose O et al (2016)[62]** observed that in diabetic rats the levels of alveolar bone loss with periodontitis were higher than in without diabetes with periodontitis only (p>0.05). Melatonin treatment significantly reduced alveolar

bone loss, and its administration may be associated with its ability to regulate oxidative and inflammatory balances via antioxidant, immunomodulatory, and antidiabetic effects.

In present study the mean MD and BL reduction at 6 months was compared between the two groups it was found higher in Group II (melatonin treated). Similar results was found in the study done by **Ali E et al (2018)[64]** who measured the alveolar ridge preservation with bioactive glass and PRF in combination with melatonin in CP patients and reported that the horizontal ridge width at 1 mm, 3 mm and 5 mm were statistically increased in melatonin treated site (p value = 0.003, 0.004 and 0.004 resp). It can be predicted that melatonin is an important mediator in formation of bone and also stimulate osteoblastic differentiation.[108] In one of the study by **Pandey A et al (2019)[68]** also confirmed in his study that melatonin significantly increased the height of newly generated bone at 12th week with mean height gain of newly generated bone was 63.4±7.2 in test group as compared to control group with mean 25.4±2.6 suggesting that melatonin promote vertical ridge augmentation. For the newly generated bone to reach the top, some scaffolds or growth factor were necessary. Before implanting, melatonin powder and growth hormone (GH) in osteotomy increased new bone growth in the early stage of therapy by the topical application of titanium implant. Melatonin thus worked as a growing factor to improve the formation of new bones.[105,106] **Pandey A et al (2019)[68]** also observed that the number of blood vessels in the melatonin group increased markedly compared to the control group. During repair of bone defects, melatonin increased angiogenesis. As its known that in bone regeneration, angiogenesis plays a key role. Melatonin thus maintained capillary homeostasis.[107] In present study, a significant improvement in all

the clinical and radiographic parameters in combination of local melatonin and NSPT. So, melatonin can be considered to be a novel addition to standard treatment procedures for periodontal treatment to improved treatment outcomes with validated immunomodulatory, anti-inflammatory, and powerful antioxidant functions along with strongly suggesting, melatonin use in periodontal bony defects is promising in terms of promoting bone healing and increasing new bone formation.

CONCLUSION

The current randomized, controlled clinical and CBCT study was designed to assess and compare the efficacy of 1 % Melatonin gel as an adjunct to NSPT and NSPT versus placebo gel in the treatment of periodontal IBDs in stage III Periodontitis. The evaluation was performed clinically as well as radiographically with CBCT. The study included 20 systemically healthy subjects with 20 pairs of bilateral intrabony defects. Clinical parameters such as PI, mSBI, PPD, and CAL were measured at baseline, as were radiographic parameters such as CEJ –BD, AC-BD, MD, and BL width by CBCT. The PI, mSBI, PPD, and CAL were evaluated at 3 and 6 months, respectively, while the CBCT analysis was performed at 6 months.

The reduction in PI and mSBI indicated satisfactory maintenance of oral hygiene by patients throughout the study period. PPD reduction in Group I and Group II was significantly greater at 3 months and 6 months. The mean reduction in bone

defect height, depth and width in Group II was statistically significantly greater than in Group I at 6 months evaluation. Bone fill was greater and statistically significant in Group II as compared to Group I.

From the analysis of results, following conclusions can be drawn:

1. NSPT + 1% Melatonin gel compared to NSPT + placebo gel resulted in statistically significant reduction of PI and mSBI at 3 months and 6 months compared to baseline.

2. NSPT + 1% Melatonin gel compared to NSPT + placebo gel resulted in statistically significant reduction of PPD at 3 months and 6 months compared to baseline.

3. NSPT + 1% Melatonin gel compared to NSPT + placebo gel resulted in statistically significant reduction of CAL at 3 months and 6 months compared to baseline.

4. NSPT + 1% Melatonin gel showed significantly better results in terms of reduction in bone defect height, depth and width at 6 months, compared to NSPT + placebo gel.

It can be concluded within the limits of study that the use of NSPT + 1% Melatonin gel could be more beneficial in achieving better results in terms of periodontal regeneration. Attempting to identify the most accurate method for evaluating hard tissue changes after periodontal therapy is an important task. To date, re-entry procedure appears to be the gold standard and, while no single method can produce similar information consistently. The images obtained by CBCT, combined

with clinical measurements, will definitely increase our ability to determine the treatment outcome without the use of re-entry procedure. It should be noted that the differences in healing patterns, microbial pathogens, study designs, patient population, measurement techniques and human defect variations make it difficult to compare clinical results. Also, different methods like clinical, histological and radiographic evaluations have been used in various studies for assessing the outcomes of treatments. This could be some of the reasons for variations observed amongst clinical trials.

The following were the limitations observed in the present study:

1. A larger sample size would be desirable so as to substantiate the results.

2. Long term analysis is needed to determine the stability of the results and to improve the radiographic assessment of the results.

REFERENCES

1. Stanley C. Holt & Jeffery Ebersole-Porphyromonas gingivalis,Treponema denticola,& Tannerella forsythia: 'the red complex',a prototype polybacterial pathogenic consortium in periodontitis- Perio 2000 vol 38, 2005,72-122.

2. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018 Jun;89 Suppl 1:S159-S172.

3. American Academy of Periodontology:Glossary of periodontal terms, 4th edn. Chicago, American Academy of Periodontology;2001.

4. Goldman HM, Cohen DW. The intrabony pocket: classification and treatment. J Periodontol 1958; 29:272-391.

5. W.A Soskolne, P.A Heasman, A. Stabholtz, J Smart, M. Palmer, M. Flashner

& H.N Newman-Sustained local delivery of chlorhexidine in the treatment of periodontitis: a multicentre study- J Periodontol 1997, vol68:32-38

6. Donos N. The periodontal pocket. Periodontol 2000 2018;76:7-15.

7. Adriaens PA, Adriaens LM. Effects of nonsurgical periodontal therapy on hard and soft tissues. Periodontol 2000 2004; 36:121-145.

8. Makoto Umeda, Yasuo Takeuchi, Kazayugi Noguchi- Effects of nonsurgical periodontal therapy on the microbiota. Perio 2000, vol 30, 2004;98-120

9. Marc Quirynen, Wim Teughels, Marc De Soete & Daniel Van Steenberghe- Topical antiseptics & antibiotics in the initial therapy of chronic periodontitis: microbiological aspects- Perio 2000 vol.28, 2002:72-90

10. Foteini Derdilopoulou, Jorg Nonhoff, Konrad Neumann and Andrej M. Kielbassa- Microbiological findings after periodontal therapy using curettes, Er:Yag laser, sonic, and ultrasonic scalers – J Clin Periodontol 2007:34:588-598

11. Goodson JM. Antimicrobial strategies for treatment of periodontal diseases. Periodontol 2000: 1994; 5: 142-68.

12. Walker CB. The acquisition of antibiotic resistance in the periodontal microflora. Periodontol 2000: 1996; 10:78-88.

13. Goodson JM, Offenbacher S, Farr DH, Hogan PE. Periodontal disease treatment by local drug delivery. J Periodontol. 1985 May;56(5):265-72.

14. Gary Greenstein and Alan Polson. The role of local drug delivery in

management of periodontal disease: A Comprehensive review. Journal of Periodontology 1998; 69:5, 507-520.

15. Daniela C. Grisi, Sergio L. Salvador, Luciene C. Figueredo, Sergio L.S. Souza, Arthur B. Novaes Jr & Marcio F.M Grisi- Effect of controlled- release chlorhexidine chip on clinical & microbiological parameters of periodontal syndrome-J Clin Periodontol 2002 Vol 29;875-881.

16. Kornman KS. Controlled-release local delivery antimicrobials in periodontics: prospects for the future. J Periodontol 1993; 64:782-791.

17. W. Aubrey Soskolne, Howard Proskin & Ayala Stabholz-Probing depth changes following 2 years of periodontal maintenance therapy including adjunctive controlled release of chlorhexidine- J Periodontol 2003 vol74;420-427

18. 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;83:11-18.

19. Elavarasu S, Suthanthiran TK, Naveen D. Statins: A new era in local drug delivery. J Pharm BioallSci 2012;4:248-51.

20. Rangrej U. Dave D, Rai J, Vaghani K. Evidence Based Review on Herbal Local Drug Delivery. Journal of Dental and Medical Sciences. 2017 Aug;16(8):77-85.

21. Gustafson A, Asman B. Increased release of free oxygen radicals from

peripheral neutrophils in adult periodontitis after Fcc-receptor stimulation. J Clin Periodontol; 23: 38–44 (1996).

22. Kimura S, Yonemura T, Kaya H. Increased oxidative product formation by peripheral blood polymorphonuclear leukocytes in human periodontal disease. J Periodontal Res 1993; 28: 197–203.

23. Siu AW, Maldonado M, Sanchez-Hidalgo M, Tan DX, Reiter RJ. Protective effects of melatonin in experimental free radical-related ocular diseases. J Pineal Res 2006;40:101-9.

24. Cutando A, Aneiros-Fernandez J, Lopez-Valverde A, Arias- Santiago S, Aneiros-Cachaza J, Reiter RJ. A new perspective in Oral health: potential importance and actions of melatonin receptors MT1, MT2, MT3, and RZR/ROR in the oral cavity. Arch Oral Biol 2011;56: 944-50.

25. Lau HM, Koyama H, Nakade O, Takada Y, Kuku T. Melatonin increases bone mass by suppressing bone resorption through down-regulation of the RANKI-mediated osteoclatogenesis in mice. J Bone Min Res 2001;16(1): S211

26. Zhang L, Su P, Xu C, Chen C, Liang A, et al. Melatonin inhibits adipogenesis and enhances osteogenesis of human mesenchymal stem cells by suppressing PPARgamma expression and enhancing Runx2 expression. J Pineal Res 2010; 49(4):364-372.

27. Sethi S, Radio NM, Kotlarczyk MP, Chen CT, Wei YH, et al. Determination of the minimal melatonin exposure required to induce osteoblast differentiation from human mesenchymal stem cells and these effects on

downstream signaling pathways. J Pineal Res 2010;49(3): 222-238.

28. Canalis E. Growth factor control of bone mass. J Cell Biochem 2009;108(4):769-777.

29. Picinato MC, Hirata AE, Cipolla-Neto J, Curi R, Carvalho CR, et al. Activation of insulin and IGF-1 signaling pathways by melatonin through MT1 receptor in isolated rat pancreatic islets. J Pineal Res 2008;44(1): 88-94.

30. Radio NM, Doctor JS, Witt-Enderby PA. Melatonin enhances alkaline phosphatase activity in differentiating human adult mesenchymal stem cells grown in osteogenic medium via MT2 melatonin receptors and the MEK/ERK (1/2) signaling cascade. J Pineal Res 2006;40(4): 332-42.

31. Ostrowska Z, Kos-Kudla B, Swietochowska E, Marek B, Kajdaniuk D, et al. Influence of pinealectomy and long-term melatonin administration on GH-IGF-I axis function in male rats. Neuro Endocrinol Lett 2001;22(4): 255-262.

32. Bell NH.Advances in the treatment of osteoporosis. Curr Drug Targets Immune Endocr Metabol Disord 2001:1(1): 93-102.

33. Sahna E, Parlakpinar H, Vardi N, Cigremis Y, Acet. Efficacy of melatonin as protectant against oxidative stress and structural changes in liver tissue in pinealectomized rats. Acta Histochem 2004; 106(5): 331-336.

34. Cardinali DP, Ladizesky MG, Boggio V, Cutrera RA, Mautalen C. Melatonin effects on bone: Experimental facts and clinical perspectives. J Pineal Res 2003;34(2): 81-87.

35. Meenakshi SS, Malaiappan S. Role of melatonin in periodontal disease ‑.A systematic review. Indian J Dent Res 2020;31:593-600.

36. Reddy MS, Jeffcoat MK. Methods of assessing periodontal regeneration. Periodontol 2000 1999;19:87-103.

37. Misch KA, Yi ES, Sarment DP. Accuracy of cone beam computed tomography for periodontal defect measurements. J Periodontol 2006; 77:1261-1266.

38. Paolantonio M, D'Angelo M, Grassi RF, Perinetti G, Piccolomini R, Pizzo G, Annunziata M, D'Archivio D, D'Ercole S, Nardi G, Guida L. Clinical and microbiologic effects of subgingival controlled-release delivery of chlorhexidine chip in the treatment of periodontitis: a multicenter study. J Periodontol. 2008 Feb;79(2):271-82.

39. Panwar M, Gupta SH. Local Drug Delivery with Tetracycline Fiber : An Alternative to Surgical Periodontal Therapy. Med J Armed Forces India. 2009;65(3):244-246.

40. Deo V, Ansari S, Mandia S, Bhongade M. Therapeutic Efficacy of Subgingivally Delivered Doxycycline Hyclate as an Adjunct to Non-surgical Treatment of Chronic Periodontitis. J Oral Maxillofac Res. 2011 Apr 1;2(1):e3.

41. Behal R, Mali AM, Gilda SS, Paradkar AR. Evaluation of local drug-delivery system containing 2% whole turmeric gel used as an adjunct to scaling and root planing in chronic periodontitis: A clinical and microbiological study. J

Indian Soc Periodontol. 2011 Jan;15(1):35-8.

42. Kudva P, Tabasum ST, Shekhawat NK. Effect of green tea catechin, a local drug delivery system as an adjunct to scaling and root planing in chronic periodontitis patients: A clinicomicrobiological study. J Indian Soc Periodontol. 2011;15(1):39-45.

43. Rao NS, Pradeep AR, Kumari M, Naik SB. Locally delivered 1% metformin gel in the treatment of smokers with chronic periodontitis: a randomized controlled clinical trial. J Periodontol. 2013 Aug;84(8):1165-71.

44. Pradeep AR, Bajaj P, Agarwal E, Rao NS, Naik SB, Kalra N, Priyanka N. Local drug delivery of 0.5% azithromycin in the treatment of chronic periodontitis among smokers. Aust Dent J. 2013 Mar;58(1):34-40.

45. Kathariya R, Pradeep AR, Raghavendra NM, Gaikwad R. Evaluation of subgingivally delivered 0.5% clarithromycin as an adjunct to nonsurgical mechanotherapy in the management of chronic periodontitis: a short-term double blinded randomized control trial. Journal of investigative and clinical dentistry. 2014 Feb;5(1):23-31.

46. Gupta D, Bhaskar DJ, Gupta RK, Karim B, Jain A, Singh R, Karim W. A randomized controlled clinical trial of Ocimum sanctum and chlorhexidine mouthwash on dental plaque and gingival inflammation. J Ayurveda Integr Med. 2014 Apr;5(2):109-16.

47. Abdelmonem, H.M., Khashaba, O., Al-Daker, M.A., & Moustafa, M. (2014). Effects of Aloe Vera Gel as an Adjunctive Therapy in the Treatment of

Chronic Periodontitis: A Clinical and Microbiological Study.

48. Rathod S, Mehta P, Trupti S, Raj A. Clinical efficacy of aloe vera chip as an adjunct to nonsurgical therapy in the treatment of chronic periodontitis. Int. J res ayu pharma 2015;6:516-519.

49. Babrawala IS, Mlv P, Bv K, Khanna D. A Novel Approach Using Natural 1% (W/W) Chitosan as a Local Drug Delivery System in the Management of Non-Surgical Periodontal Treatment: A Pilot Study. J Int Acad Periodontol. 2016 Oct 7;18(4):129-133. PMID: 31473701.

50. Pradeep AR, Kanoriy D, Singhal S, Garg V, Balaji M, Chatterjee A. Comparative evaluation of subgingivally delivered 1% alendronate versus 1.2% atorvastatin gel in treatment of chronic periodontitis: a randomized placebo-controlled clinical trial. J Invest Cli Dent; 2016;8.

51. Vennila K, Elanchezhiyan S, Ilavarasu S. Efficacy of 10% whole Azadirachta indica (neem) chip as an adjunct to scaling and root planning in chronic periodontitis: A clinical and microbiological study. Indian J Dent Res. 2016 Jan-Feb;27(1):15-21.

52. Boyapati R, Gojja P, Chintalapani S, Nagubandi K, Ramisetti A, Salavadhi SS. Efficacy of local drug delivery of Achyranthes aspera gel in the management of chronic periodontitis: A clinical study. J Indian Soc Periodontol 2017;21:46-9.

53. Gupta A, Govila V, Pant VA, Gupta R, Verma UP, Ahmad H, et al. A randomized controlled clinical trial evaluating the efficacy of zoledronate gel

as a local drug delivery system in the treatment of chronic periodontitis: A clinical and radiological correlation. Natl J Maxillofac Surg 2018;9:22-32.

54. Maske S. Comparative evaluatuion of. (Grey letrature-Thesis)

55. Hasan F, Ikram R, Simjee SU, Iftakhar K, Asadullah K. Effectiveness of Simvastatin 1% oral gel and mouthwash used as an adjunct treatment of scaling and root planning in the treatment of periodontal diseases. Pak J Pharm Sci. 2019 Nov;32(6):2673-2677..

56. Cutando A, Gómez-Moreno G, Villalba J, Ferrera MJ, Escames G, Acuña-Castroviejo D. Relationship between salivary melatonin levels and periodontal status in diabetic patients. J Pineal Res. 2003 Nov;35(4):239-44.

57. Cutando A, Arana C, Gómez-Moreno G, Escames G, López A, Ferrera MJ, Reiter RJ, Acuña-Castroviejo D. Local application of melatonin into alveolar sockets of beagle dogs reduces tooth removal-induced oxidative stress. J Periodontol. 2007 Mar;78(3):576-83.

58. Srinath R, Acharya AB, Thakur SL. Salivary and gingival crevicular fluid melatonin in periodontal health and disease. J Periodontol. 2010 Feb;81(2):277-83.

59. Malak Yousef Mohamed Shoukheba , Mohamed Hassan Ahmed. Histological Evaluation of the Effect of Melatonin Gel in the Treatment of acute one-wall intrabony defect in dogs. J Am Sci 2012;8(12):929-938.

60. Yousef D, Osama A, Soudany K, Ghoniem S. The effect of local application

of melatonin gel on the healing of periodontal osseous defects in experimentally induced diabetes in rabbits. Dental journal 2013.

61. Ortiz F, Acuña-Castroviejo D, Doerrier C, Dayoub JC, López LC, Venegas C, García JA, López A, Volt H, Luna-Sánchez M, Escames G. Melatonin blunts the mitochondrial/NLRP3 connection and protects against radiation-induced oral mucositis. J Pineal Res. 2015 Jan;58(1):34-49.

62. Kose O, Arabaci T, Kara A, Yemenoglu H, Kermen E, Kizildag A, Gedikli S, Ozkanlar S. Effects of Melatonin on Oxidative Stress Index and Alveolar Bone Loss in Diabetic Rats With Periodontitis. J Periodontol. 2016 May;87(5):e82-90.

63. Chitsazi M, Faramarzie M, Sadighi M, Shirmohammadi A, Hashemzadeh A. Effects of adjective use of melatonin and vitamin C in the treatment of chronic periodontitis: A randomized clinical trial. J Dent Res Dent Clin Dent Prospects. 2017 Fall;11(4):236-240.

64. Ali E, Kilani N, Hazzaa H. Evaluation of Alveolar Ridge Preservation Using a composite of Bioactive Glass and Platelet Rich Fibrin Alone or Combined with Melatonin in Chronic Periodontitis Patient. Al-Azhar Dental Journal for Girls 2018; 5:429-438.

65. Virto L, Cano P, Jiménez-Ortega V, Fernández-Mateos P, González J, Haugen HJ, Esquifino AI, Sanz M. Melatonin as adjunctive therapy in the treatment of periodontitis associated with obesity. J Clin Periodontol. 2018 Nov;45(11):1336-1346.

66. Bazyar H, Gholinezhad H, Moradi L, Salehi P, Abadi F, Ravanbakhsh M, Zare Javid A. The effects of melatonin supplementation in adjunct with non-surgical periodontal therapy on periodontal status, serum melatonin and inflammatory markers in type 2 diabetes mellitus patients with chronic periodontitis: a double-blind, placebo-controlled trial. Inflammopharma cology. 2019 Feb;27(1):67-76.

67. El-Sharkawy H, Elmeadawy S, Elshinnawi U, Anees M. Is dietary melatonin supplementation a viable adjunctive therapy for chronic periodontitis?—A randomized controlled clinical trial. J Periodont Res. 2018;00:1–8.

68. Pandey A, Kaur G, Marwaha J, Biswas A, Kumari S, Signh O. Evaluation of Effects of Melatonin Application on Osseo Integration of Dental Implant. J App Dent Med Sci 2019;5(4):34-38.

69. Ahmed E, Shaker OG, Yussif N, Ghalwash DM. Effect of Locally Delivered Melatonin as an Adjunct to Nonsurgical Therapy on GCF Antioxidant Capacity and MMP-9 in Stage II Periodontitis Patients: A Randomized Controlled Clinical Trial. Int J Dent. 2021 Feb 5;2021:8840167.

70. Vandenberghe B, Jacobs R, Yang J. Detection of periodontal bone loss using digital intraoral and cone beam computed tomography images: an in vitro assessment of bony and/or infrabony defects. Dentomaxillofac Radiol. 2008 Jul;37(5):252-60.

71. Grimard BA, Hoidal MJ, Mills MP, Mellonig JT, Nummikoski PV, Mealey BL. Comparison of clinical, periapical radiograph, and cone-beam volume

tomography measurement techniques for assessing bone level changes following regenerative periodontal therapy. J Periodontol 2009; 80:48-55.

72. de Faria Vasconcelos K, Evangelista KM, Rodrigues CD, Estrela C, de Sousa TO, Silva MA. Detection of periodontal bone loss using cone beam CT and intraoral radiography. Dentomaxillofac Radiol. 2012 Jan;41(1):64-9.

73. Takeshita WM, Vessoni Iwaki LC, Da Silva MC, Tonin RH. Evaluation of diagnostic accuracy of conventional and digital periapical radiography, panoramic radiography, and cone-beam computed tomography in the assessment of alveolar bone loss. Contemp Clin Dent 2014;5:318-23.

74. Banodkar AB, Gaikwad RP, Gunjikar TU, Lobo TA. Evaluation of accuracy of cone beam computed tomography for measurement of periodontal defects: A clinical study. J Indian Soc Periodontol 2015;19:285-9.

75. Chhabra A, Uppoor A, Pralhad S, Singh N, Nayak D. Quantification of intra osseous bone defects by conventional versus 3 dimensional imaging techniques – an in vivo study. Int J Adv Res 2016; 4: 1377-1385.

76. Guo YJ, Ge ZP, Ma RH, Hou JX, Li G. A six-site method for the evaluation ofperiodontal bone loss in cone-beam CT images. DentomaxillofacialRadiol2015;45:20150265.

77. Suphanantachat S, Tantikul K, Tamsailom S, Kosalagood P, Nisapakultorn K, Tavedhikul K. Comparison of clinical values between cone beam computed tomography and conventional intraoral radiography in periodontal and

infrabony defect assessment. DentomaxillofacialRadiol 2017;46:20160461.

78. Zhang W, Foss K, Wang BY. A retrospective study on molar furcation assessment via clinical detection, intraoral radiography and cone beam computed tomography. BMC Oral Health. 2018 May 3;18(1):75.

79. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-135.

80. Newbrun E. Indices to measure gingival bleeding. J Periodontol 1996;67:555-561.

81. Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? Periodontol 2000 2009; 51:208-219.

82. Slots J, Ting M. Actinobacillus and porphyromonas gingivalis in human periodontal disease: occurrence and treatment. Periodontol 2000: 1999; Vol 20: 83-121.

83. Holtfreter B, Kocher T, Hoffmann T et al (2010) Prevalence of periodontal disease and treatment demands based on a German dental survey (DMS IV). J Clin Periodontol 37:211–219.

84. Souza JA, Rossa C Jr, Garlet GP, Nogueira AV, Cirelli JA. Modulation of host cell signaling pathways as a therapeutic approach in periodontal disease. J Appl Oral Sci. 2012;20:128-38.

85. Thunell DH, Tymkiw KD, Johnson GK, Joly S, Burnell KK, Cavanaugh JE, et al. A multiplex immunoassay demonstrates reductions in gingival crevicular fluid cytokines following initial periodontal therapy. J Periodontal

Res.2010;45:148-52.

86. Deschner J, Arnold B, Kage A, Zimmermann B, Kanitz V, Bernimoulin, JP. Suppression of interleukin-10 release from human periodontal ligament cells by interleukin-1beta in vitro. Arch Oral Biol. 2000;45:179-83.

87. Gómez-Moreno G, Cutando-Soriano A, Arana C, Galindo P, Bolaños J, Acuña-Castroviejo D, et al. Melatonin expression in periodontal disease. J Periodont Res. 2007;42:536-40.

88. Polimeni G, Xiropaidis AV, Wikesjo UM. Biology and principles of periodontal wound healing/regeneration. Periodontol 2000 2006; 41:30-47.

89. Needleman IG. Controlled drug release in periodontics: A review of new therapies. Br Dent J 1991;170:405-8.

90. Slots J, Rams TE. Microbiology of periodontal disease. In: Slots J, Taubman MA, ed. Contemporary oral microbiology and immunology. St. Louis: CV Mosby Co., 1992:425-43.

91. Suzuki N, Somei M, Seki A, Reiter RJ, Hattori A. Novel bromomelatonin derivatives as potentially effective drugs to treat bone diseases. J Pineal Res 2008;45:229-34.

92. Cutando A, Galindo P, Gomez-Moreno G, Arana C, Bolanos J Acuna-Castroviejo D, et al. Relationship between salivary melatonin and severity of periodontal disease. J Periodontol; 77: 1533e8 (2006).

93. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: distribution and functions. Mol Cell Endocrinol; 351: 152e66 (2012).

94. Garcia-Maurino S, Gonzalez-Haba MG, Calvo JR, Rafii-El- Idrissi M, Sanchez-Margalet V, Goberna R, et al. Melatonin enhances IL-2, IL-6, and IFN-gamma production by human circulating CD4û cells: a possible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes. J Immunol 159: 574e81 (1997).

95. Cutando A, Montero J, Gomez-de Diego R, Ferrera MJ, Lopez- Valverde A. Effect of topical application of melatonin on serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in patients with type 1 or type 2 diabetes and periodontal disease. J Clin Exp Dent; 7: e628e33 (2015).

96. Arnao MB, Hernandez"Ruiz J. The physiological function of melatonin in plants. Plant Signal Behav; 1(3) : 89–95 (2016).

97. Tan DX, Chen LD, Poeggeler B, Manchester LC, et al. Melatonin : a potent, endogenous hydroxyl radical scavenger. Endocrine J; 1: 57–60 (1993).

98. Roth JA, Kim BG, Song F,. Melatonin promotes osteoblast differentiation and bone formation. J Biol Chem. 1999; 274: 22041–22047

99. Luchetti F, Canonico B, Bartolini D, Arcangeletti M, Ciffolilli S, Murdolo G, et al. Melatonin regulates mesenchymal stem cell differentiation: a review. J Pineal Res; 56: 382e97 (2014).

100. Anwar MJ, Muhammad BY, Bader AA, Abdulghani M, Mahmood D, Haider M. An insight into the scientific background and future perspectives for the potential uses of melatonin. Egyptian Journal of Basic and Applied Sciences.;2: 139-152 (2015).

101. Tekbas OF, Ogur R, Korkmaz A, Kilic A, Reiter RJ. Melatonin as an

antibiotic: new insights into the actions of this ubiquitous molecule. J Pineal Res; 44:222e6 (2008).

102. Boga JA, Coto-Montes A, Rosales-Corral SA, Tan DX, Reiter RJ. Beneficial actions of melatonin in the management of viral infections: a new use for this "molecular handyman"? Rev Med Virol; 22: 323e38 (2012)

103. Koyama H, Nakade O, Takada Y, Kaku T, Lau KH. Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. J Bone Miner Res 2002;17:1219-29.

104. Najeeb S, Khurshid Z, Zohaib S, Zafar MS. Therapeutic potential of melatonin in oral medicine and periodontology. Kaohsiung J Med Sci. 2016;32:391-396.

105. Kireev RA, Cuesta S, Ibarrola C, et al. Age-related differences in hepatic ischemia/reperfusion: gene activation, liver injury, and protective effect of melatonin. J Surg Res 2012;178:922-934.

106. Tresguerres IF, Clemente C, Blanco L, Khraisat A, Tamimi F, Tresguerres JA. Effects of local melatonin application on implant osseointegration. Clin Implant Dent Relat Res 2012;14:395-399.

107. Clafshenkel WP, Rutkowski JL, Palchesko RN, Romeo JD, McGowan KA, Gawalt ES, et al. A novel calcium aluminate-melatonin scaffold enhances bone regeneration within a calvarial defect. J Pineal Res. 2012; 53:206-18.

TABLE

Table 1: Comparison of clinical parameters across time

Clinical Parameter	Mean	SD	P-value*
Plaque Index			
Baseline	1.83	0.24	< **0.0001 (S)**
3 Months	1.08	0.23	
6 Months	0.65	0.16	
Bleeding Index			
Baseline	2.69	0.69	< **0.0001 (S)**
3 Months	1.37	0.19	
6 Months	1.05	0.11	

*Obtained using repeated measures of ANOVA; S: Significant

Table 2: Comparison of PPD across time and between two sites

PPD (mm)	**Group I (n=22)**		**Group II (n=22)**		P-value*
	Mean	SD	Mean	SD	
Baseline	6.95	0.72	7.45	0.51	0.008 (S)
3 months	5.45	0.67	5.36	0.49	0.540 (NS)
6 months	4.55	0.86	3.95	0.84	0.061 (NS)
P-value[Ŧ]	< 0.0001 (S)		< 0.0001 (S)		

[Ŧ]Obtained using repeated measures of ANOVA; *Using paired t-test; S: Significant; NS: Not significant

Table 3: Comparison of reduction of PPD change between two groups at different time intervals

Time points	PPD (mm)				P-value*
	Group I		Group II		
	Mean	SD	Mean	SD	
Baseline vs. 3 months	1.50	0.80	2.09	0.43	**0.004 (S)**
Baseline vs. 6 months	2.40	1.09	3.50	0.91	**0.004 (S)**
3 months vs. 6 months	0.90	1.02	1.40	0.85	0.142 (NS)

*Using paired t-test; S: Significant; NS: Not significant

Table 4: Comparison of CAL across time and between two sites

CAL (mm)	Group I (n=22)		Group II (n=22)		P-value*
	Mean	SD	Mean	SD	
Baseline	7.41	0.59	7.77	0.69	0.017 (S)
3 months	5.73	0.70	5.68	0.57	0.803 (NS)
6 months	4.68	0.99	4.36	0.79	0.329 (NS)
P-value[Ŧ]	< 0.0001 (S)		< 0.0001 (S)		

[Ŧ]Obtained using repeated measures of ANOVA; *Using paired t-test; S: Significant; NS: Not significant

Table 5: Comparison of reduction of CAL change between two groups at different time intervals (in mm)

Time points	CAL (mm)				P-value*
	Group I		Group II		
	Mean	SD	Mean	SD	
Baseline vs. 3 months	1.68	0.64	2.29	0.68	**0.004 (S)**
Baseline vs. 6 months	2.72	1.07	3.79	0.90	**0.004 (S)**
3 months vs. 6 months	1.04	1.13	1.31	0.83	0.418 (NS)

*Using paired t-test; NS: Not significant

Table 6: Comparison of CEJ-BD between two time points in each group and comparison of change in CEJ-BD between two groups for time interval baseline to 6 months by CBCT (in mm)

Time point	Group I		Group II	
	Mean	SD	Mean	SD
Baseline	9.33	0.39	9.80	0.46
6 Months	8.83	0.45	8.34	0.49
P-value*	< **0.0001 (S)**		< **0.0001 (S)**	
Change in CEJ-BD				
Baseline to 6 months	0.50	0.38	1.46	0.58
P-value*	< **0.0001 (S)**			

*Using paired t-test; S: Significant

Table 7: Comparison of CEJ-AC between two time points in each groups and comparison of change in CEJ-AC between two groups for time interval baseline to 6 months by CBCT (in mm).

Time point	Group I		Group II	
	Mean	**SD**	**Mean**	**SD**
Baseline	5.05	0.54	5.50	0.76
6 Months	5.00	0.56	5.21	0.75
P-value*	0.341 (NS)		**< 0.0001 (S)**	
Change in CEJ-AC				
Baseline to 6 months	0.05	0.24	0.29	0.21
P-value*	**0.001 (S)**			

*Using paired t-test; S: Significant; NS: Not Significant

Table 8: Comparison of AC-BD between two time points in each groups and comparison of change in AC-BD between two groups for time interval baseline to 6 months by CBCT (in mm)

Time point	Group I		Group II	
	Mean	**SD**	**Mean**	**SD**
Baseline	4.28	0.55	4.30	0.56
6 Months	3.82	0.59	3.13	0.68
P-value*	**< 0.0001 (S)**		**< 0.0001 (S)**	
Change in AC-BD				
Baseline to 6 months	0.45	0.35	1.17	0.59
P-value*	**< 0.0001 (S)**			

*Using paired t-test; S: Significant

Table 9: Comparison of MD between two time points in each groups and comparison of change in MD between two groups for time interval baseline to 6 months by CBCT (in mm)

Time point	Group I		Group II	
	Mean	**SD**	**Mean**	**SD**
Baseline	1.86	0.50	2.28	0.50
6 Months	1.57	0.48	1.72	0.58
P-value*	**< 0.0001 (S)**		**0.001 (S)**	
Change in MD				
Baseline to 6 months	0.29	0.12	0.56	0.70
P-value*	0.096 (NS)			

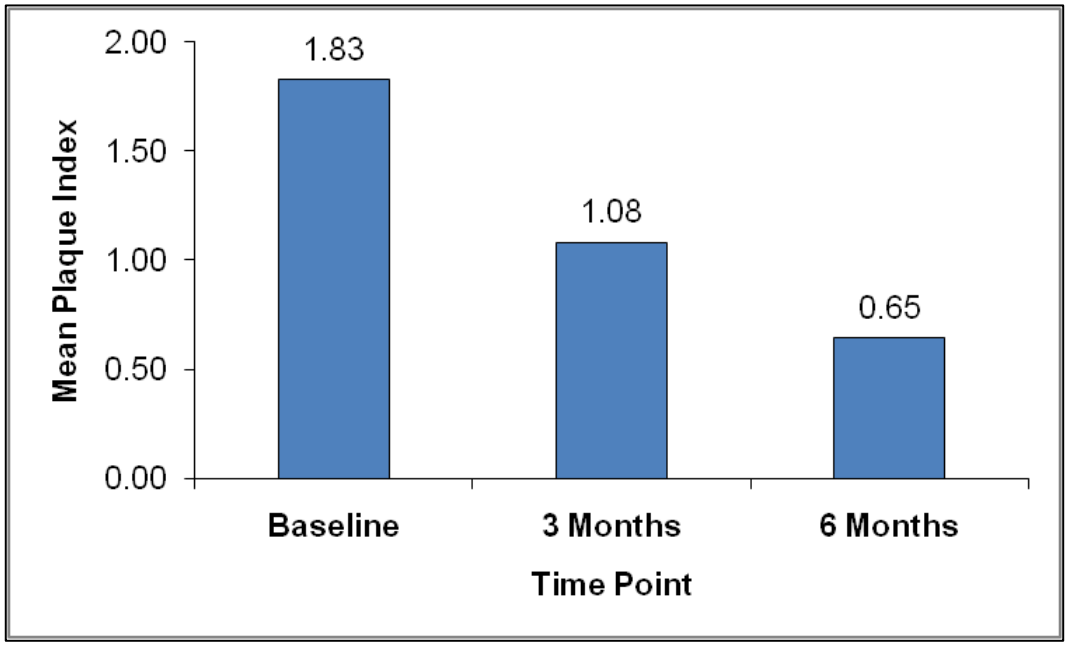
*Using paired t-test; S: Significant; NS: Not Significant

Table 10: Comparison of BL between two time points in each groups and comparison of change in BL between two groups for time interval baseline to 6 months by CBCT (in mm)

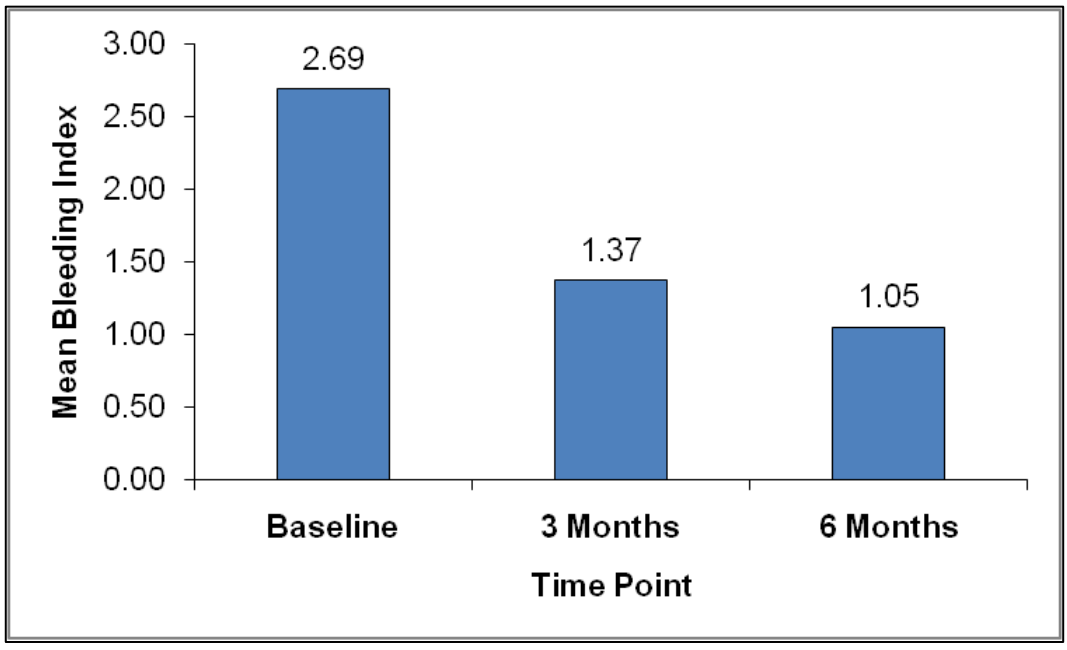
Time point	Group I		Group II	
	Mean	**SD**	**Mean**	**SD**
Baseline	4.23	0.69	4.79	1.32
6 Months	3.88	0.74	3.68	1.57
P-value*	**< 0.0001 (S)**		**< 0.0001 (S)**	
Change in BL				
Baseline to 6 months	0.35	0.21	1.11	0.50
P-value*	**< 0.0001 (S)**			

*Using paired t-test; S: Significant

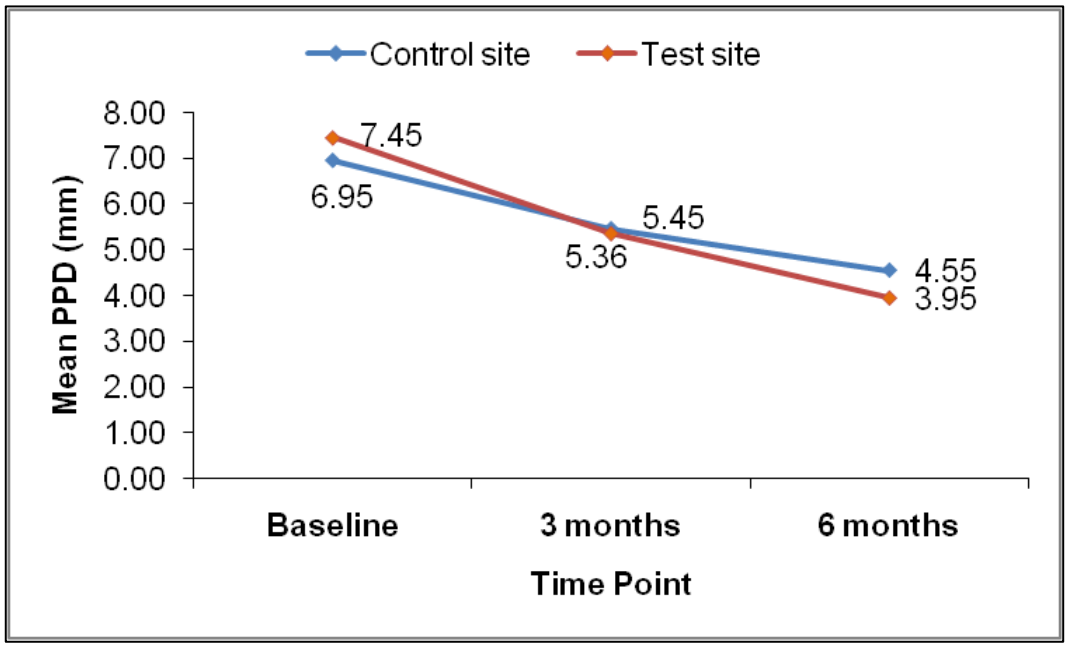
GRAPHS



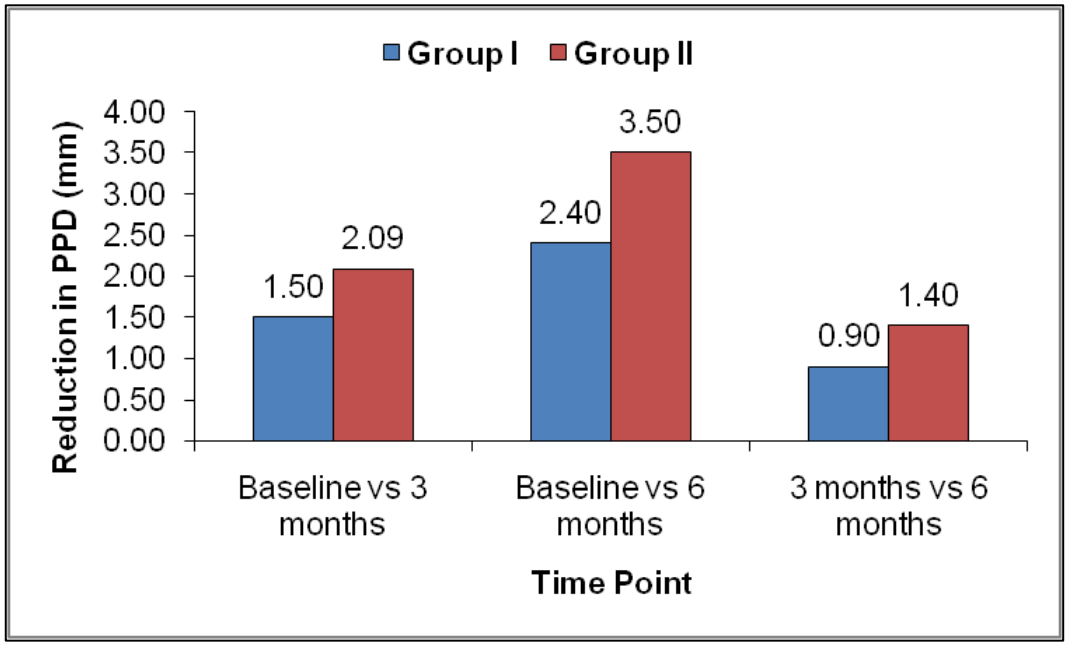
Graph 1: Bar chart showing mean plaque index across time



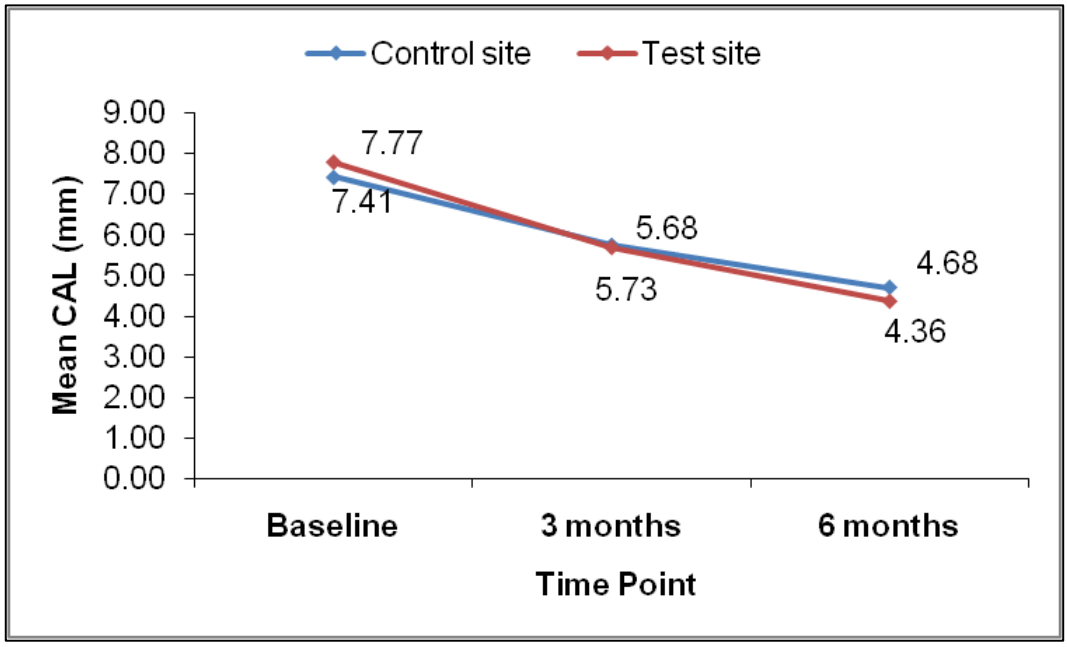
Graph 2: Column chart showing means bleeding index across time



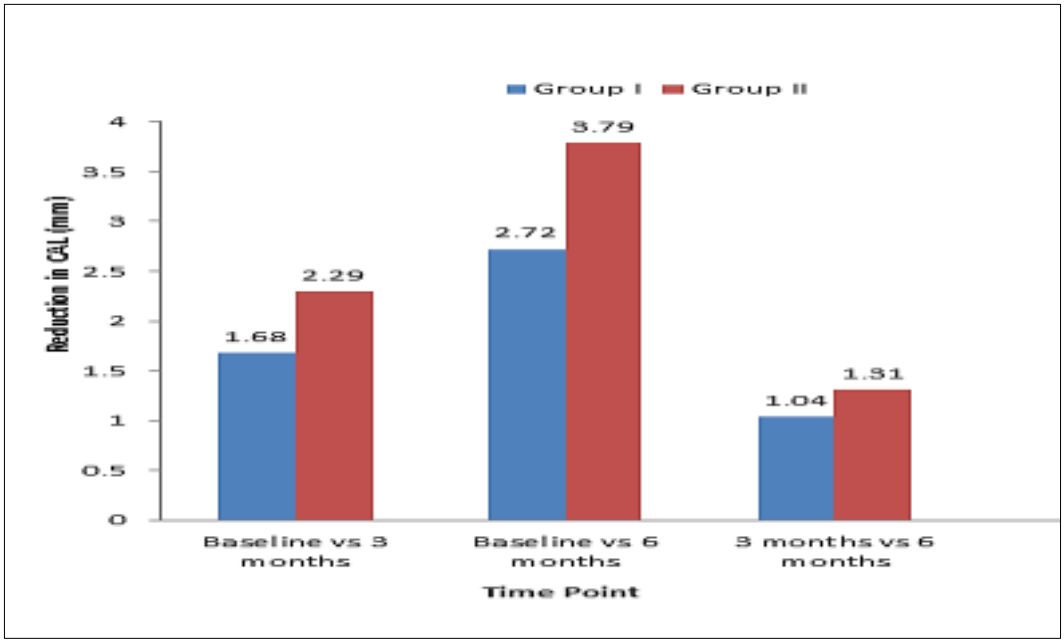
Graph 3: Line chart showing mean PPD between two groups and across time



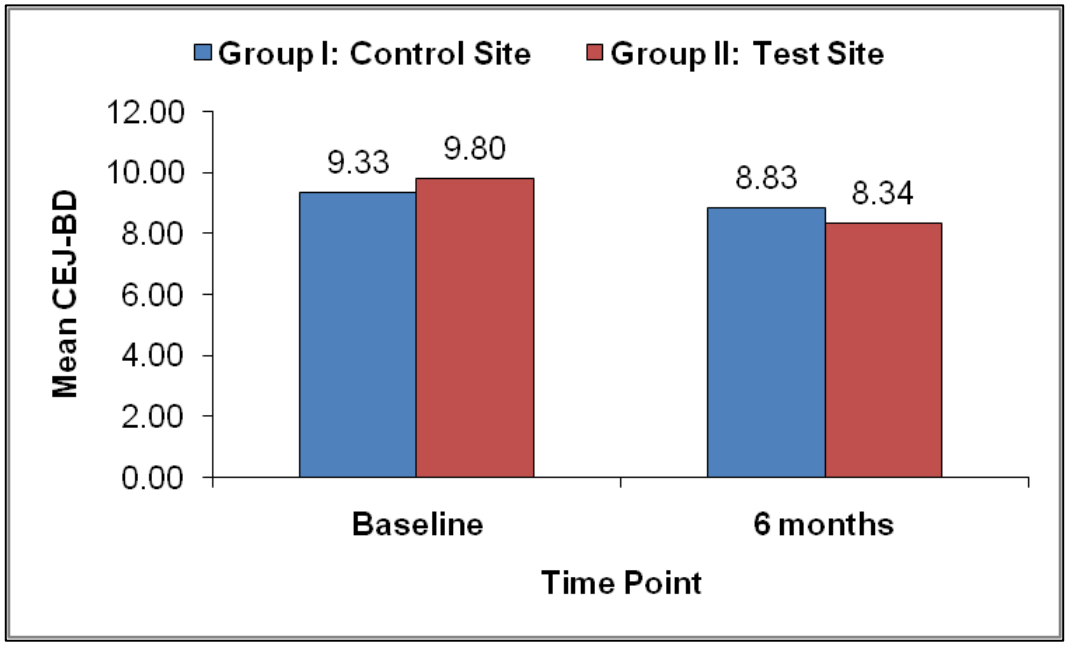
Graph 4: Column chart showing mean of reduction in PPD (mm) between two groups at different time points



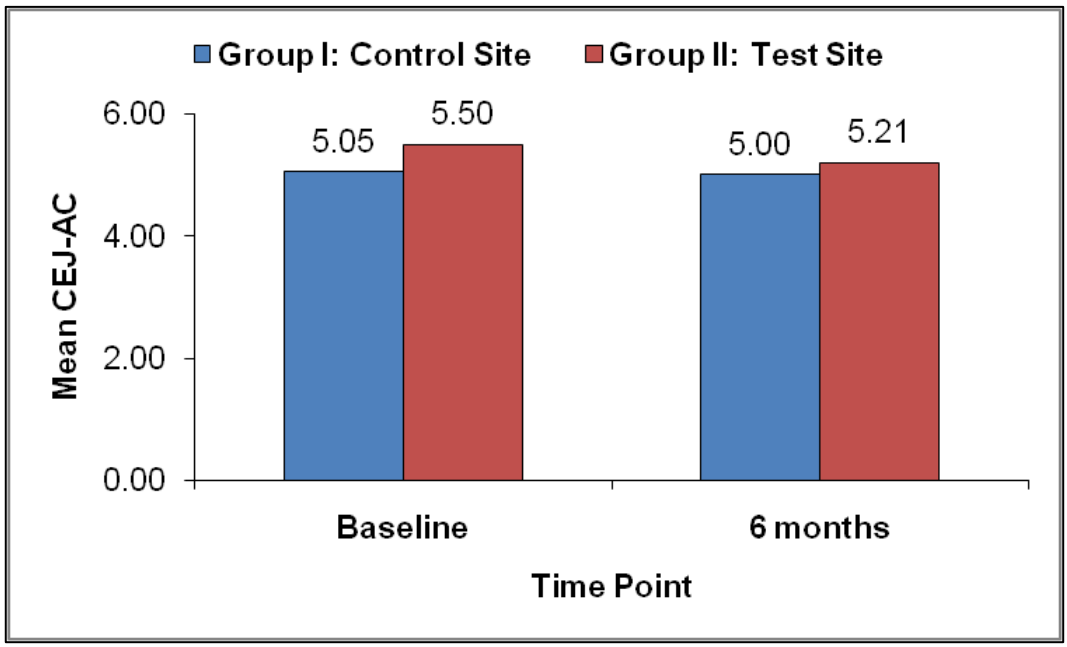
Graph 5: Line chart showing mean CAL between two groups and across time



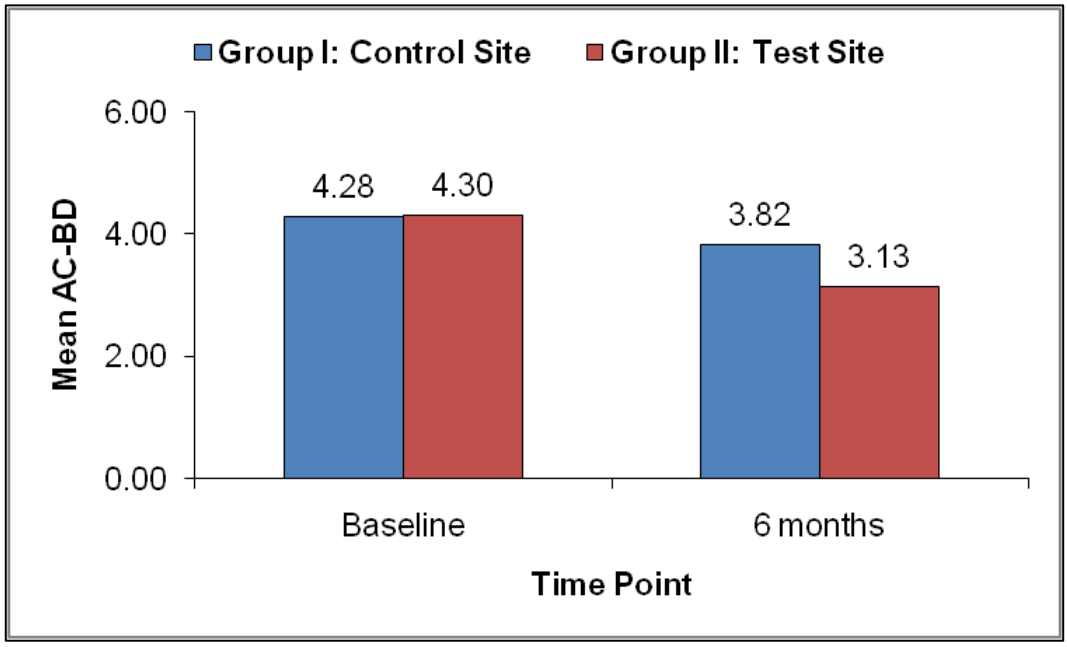
Graph 6: Bar chart showing mean of reduction in CAL (mm) between two groups at different time points



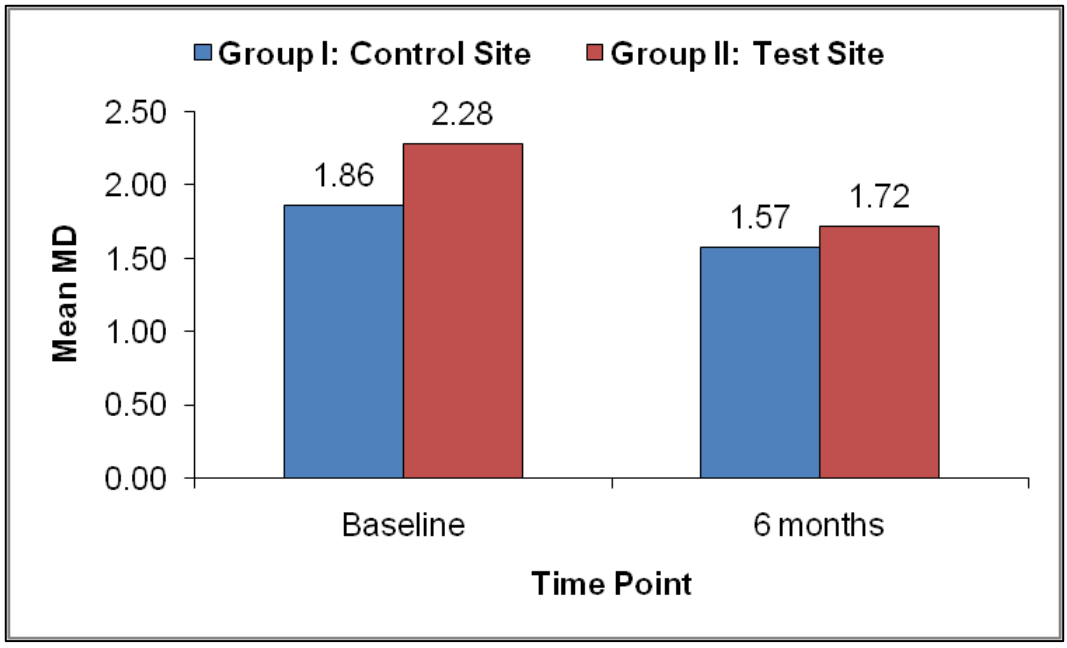
Graph 7: Column chart showing mean CEJ-BD at baseline and 6 months in two groups



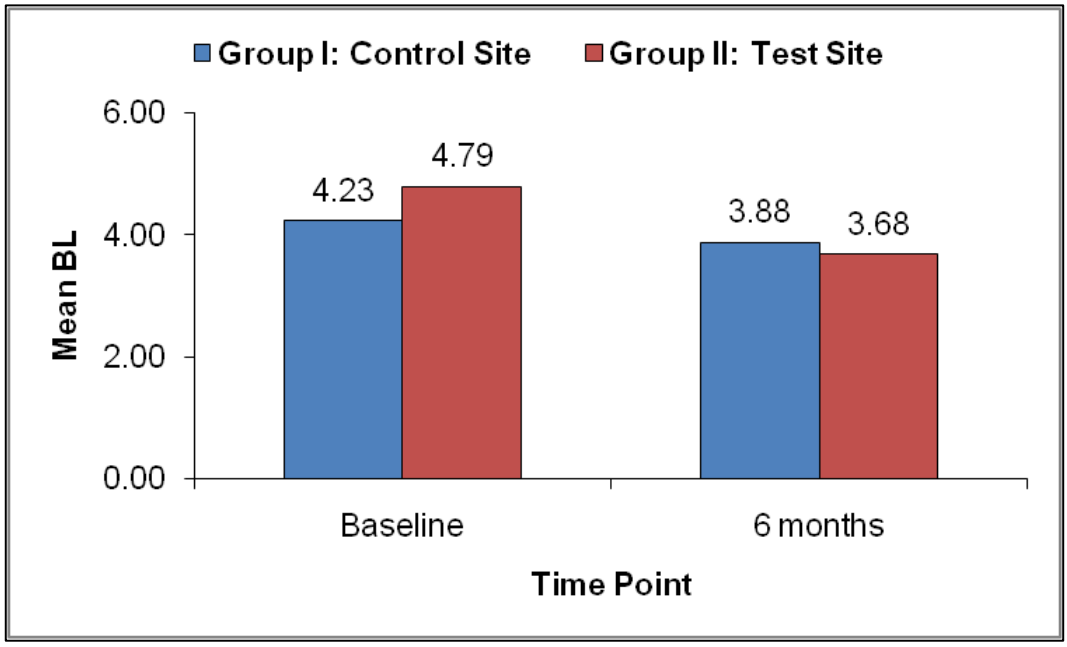
Graph 8: Column chart showing mean CEJ-AC at baseline and 6 months in two groups



Graph 9: Column chart showing mean AC-BD at baseline and 6 months in two groups



Graph 10: Column chart showing mean MD at baseline and 6 months in two groups



Graph 11: Column chart showing mean BL at baseline and 6 months in two groups

MASTER CHART

Sr. No	Plaque Index		
	Baseline (mm)	**3 Months (mm)**	**6 Months (mm)**
1	1.8	0.7	0.4
2	2.1	0.8	0.5
3	1.4	0.9	0.6
4	1.8	0.6	0.4
5	1.9	0.8	0.6
6	1.7	1.2	0.9
7	1.8	1.3	0.8
8	1.7	1.2	0.7
9	2.2	1.3	0.8
10	1.8	1.2	0.6
11	1.7	1.2	0.7
12	2.2	1.3	0.8
13	1.7	1.2	0.7
14	2.2	1.3	0.8
15	1.8	1.2	0.6
16	1.9	0.9	0.4
17	1.9	1.4	0.7
18	1.4	1.2	0.8
19	1.4	1.1	0.7
20	1.8	1.3	0.8
21	1.9	0.9	0.4
22	2.1	0.8	0.5

MODIFIED SULCUS BLEEDING INDEX

Sr. No	Bleeding Index		
	Baseline (mm)	**3 Months (mm)**	**6 Months (mm)**
1.	2.9	1.18	0.9
2.	2.25	1.08	1
3.	3.35	1.42	1.1
4.	2.89	1.32	0.9
5.	1.88	1.5	1.14
6.	3.25	1.66	0.8
7.	1.98	1.33	1.14
8.	3.3	1.88	1.12
9.	3.35	1.42	1.1
10.	1.98	1.33	1.14
11.	1.77	1.16	1.13
12.	2.25	1.35	0.9
13.	2.25	1.08	1.18
14.	3.35	1.42	1.1
15.	1.69	1.41	1.13
16.	2.86	1.52	0.9
17.	2.24	1.6	1.15
18.	3.91	1.2	1
19.	3.4	1.17	1.1
20.	3.24	1.32	0.99
21.	3.35	1.42	1.1
22.	1.69	1.41	1.13

PPD AND CAL MEASUREMENT

CONTROL SITE

Sr. No	PPD Control Site			CAL Control site		
	Baseline (mm)	3 months (mm)	6 months (mm)	Baseline (mm)	3 months (mm)	6 months (mm)
1.	6	5	4	7	5	4
2.	7	6	5	8	7	6
3.	6	6	5	7	6	5
4.	7	6	5	8	6	5
5.	7	5	5	7	5	6
6.	8	7	6	8	7	6
7.	7	6	4	8	5	4
8.	6	5	6	7	6	6
9.	7	6	5	7	6	5
10.	8	5	6	8	5	6
11.	7	6	4	8	7	4
12.	7	5	5	7	6	5
13.	8	6	4	8	6	5
14.	6	5	4	6	5	3
15.	7	5	5	7	5	5
16.	7	6	4	8	6	4
17.	7	6	3	7	6	3
18.	8	5	4	8	6	4
19.	6	5	5	7	5	5
20.	7	5	3	8	6	3
21.	8	5	4	7	5	4
22.	6	4	4	7	5	5

PPD AND CAL MEASUREMENT

TEST SITE

Sr No	PPD Test site			CAL Test site		
	Baseline (mm)	**3 months (mm)**	**6 months (mm)**	**Baseline (mm)**	**3 months (mm)**	**6 months (mm)**
1.	8	6	5	8	6	6
2.	8	5	4	9	5	4
3.	8	6	4	9	6	4
4.	7	6	3	8	5	4
5.	7	5	3	8	6	4
6.	8	6	4	8	6	4
7.	7	5	5	8	6	5
8.	8	5	3	8	6	4
9.	7	5	3	8	5	5
10.	8	6	5	8	6	5
11.	7	5	5	8	6	5
12.	7	5	4	7	5	4
13.	8	6	3	8	6	3
14.	7	5	4	7	5	4
15.	7	5	3	7	5	3
16.	8	6	5	8	6	5
17.	7	5	5	8	6	5
18.	8	5	3	8	7	5
19.	7	5	4	7	5	5
20.	8	6	5	8	6	5
21.	7	5	4	6	5	4
22.	7	5	3	7	6	3

CBCT MEASUREMENTS OF INTRABONY DEFECT AT BASELINE (in mm)

Sr. No	GROUP I - CONTROL GROUP BASELINE					GROUP II - TEST GROUP BASELINE				
	CEJ-BD	CEJ-AC	AC-BD	MD	BL	CEJ-BD	CEJ-AC	AC - BD	MD	BL
1	9.1	5.5	3.6	2.5	2.9	10.1	6.3	3.8	2.7	3.1
2	9.5	4.4	5.1	1.7	4.1	10.3	6.7	3.6	1.5	4.9
3	8.8	4.4	4.4	2.4	3.8	8.7	3.9	4.8	2.6	5.1
4	9.9	5.7	4.2	1.1	3	9.7	5.4	4.3	2.2	3.2
5	9.8	5.6	4.2	3.1	4.9	9.8	4.9	4.9	2.4	6.1
6	9.7	5.4	4.3	1.2	4.8	9.3	5.3	4	2.4	6.2
7	8.8	5.2	3.6	2	3.9	9.8	5.4	4.4	1.9	3.2
8	9.2	4.3	4.9	1.8	4.2	9.7	5.8	3.9	1.3	5.7
9	9.8	5.9	3.9	2.2	4	10.2	7.4	2.8	2.3	6
10	9.2	4.3	4.9	1.8	3	9.6	4.8	4.8	1.7	5.6
11	9.5	4.2	5.3	2.2	5.5	9.6	5.8	3.8	2.1	4.9
12	9.5	5.4	4.1	1.7	3.7	9.3	5	4.3	2.5	4.3
13	8.8	5.3	3.5	2.1	4.9	9.5	4.7	4.8	1.7	5.1
14	9.2	5.3	3.9	2.3	4.4	10.4	5.3	5.1	2.2	3
15	9.2	5.3	3.9	1.8	4.9	10.6	6.1	4.5	2.4	5.2
16	9.5	5.6	3.9	1.5	4.3	10.3	6.3	4	2.9	7.2
17	8.8	5.2	3.6	1.5	4.9	9.6	5.9	3.7	3.3	3.8
18	9.9	4.9	5	2.1	4.8	10.5	5.6	4.9	2.2	3.1
19	9.9	5.4	4.5	2.1	4.2	9.5	5	4.5	2.4	2.7
20	8.9	5.1	3.8	1.4	3.8	9.8	4.9	4.9	3.2	6.8
21	9.1	4.3	4.8	1.3	4.8	9.8	5.3	4.5	2.3	5.4
22	9.2	4.5	4.7	1.1	4.3	9.4	5.2	4.2	1.9	4.8

CBCT MEASUREMENTS OF INTRABONY DEFECT DIMENSION AT 6 MONTHS (in mm)

SR. NO	GROUP I - CONTROL Site 6 MONTHS					GROUP II - TEST Site 6 MONTHS				
	CEJ - BD	CEJ-AC	AC-BD	MD	BL	CEJ-BD	CEJ-AC	AC - BD	MD	BL
1.	8.3	5	3.3	2	2.3	8.5	6.2	2.3	2.3	2.5
2.	8.1	4.1	4	1.5	3.9	8.8	6.3	2.5	2.9	4
3.	8.4	4.2	4.2	1.8	3.5	7.5	3.3	4.2	2.7	4.8
4.	9.2	5.5	3.7	0.9	2.6	8.9	5	3.9	2.6	2
5.	9.3	5.7	3.6	2.9	4.6	9.1	4.8	4.3	1.9	4.8
6.	9.6	5.4	4.2	1	4.5	8.9	5	3.9	2.3	4.5
7.	8.4	5.3	3.1	1.8	2.7	8.8	5.1	3.7	1	1.6
8.	9	4.3	4.7	1.5	4	8.8	5.3	3.5	0.9	4.3
9.	9.6	5.6	4	1.9	3.8	8.7	7	1.7	1.9	5.4
10.	9	4.1	4.9	1.3	2.8	8.3	4.8	3.5	1.3	4.3
11.	9.3	4.3	5	2	5.2	8.6	5.5	3.1	1.4	3.7
12.	9.1	5.6	3.5	1.3	3.5	7.9	5.2	2.7	1.3	3
13.	8.5	5.4	3.1	1.9	4.4	7.6	4.1	3.5	1	4
14.	8.9	5.3	3.6	1.9	4.1	8.1	5	3.1	2.3	1.6
15.	8.8	5.4	3.4	1.4	4.5	8.6	5.8	2.8	1.7	3.1
16.	9.1	5.5	3.6	1.3	4	8.1	5.7	2.4	1.6	6.9
17.	8.3	5.2	3.1	1.2	4.5	7.9	5.4	2.5	1.3	2.5
18.	9.3	5	4.3	1.9	4.5	7.7	5.3	2.4	1.6	2
19.	8.2	5.1	3.1	1.9	4	7.6	4.7	2.9	1.4	0.7
20.	8.5	5.1	3.4	1.2	3.5	8.1	4.8	3.3	1.6	6.1
21.	8.7	4.9	3.8	1	4.3	8.3	5.3	3	1.7	5
22.	8.6	4.1	4.5	1	4.1	8.6	5	3.6	1.1	4.1

CASE HISTORY

COMPARATIVE EVALUATION OF 1% MELATONIN GEL AS AN ADJUNCT TO NON-SURGICAL PERIODONTAL THERAPY IN STAGE III PERIODONTITIS: A CBCT STUDY

NAME: **OPD NO.**

AGE/SEX: **DATE:**

OCCUPATION: **ADDRESS:**

CHIEF COMPLAINT:

PAST DENTAL HISTORY:

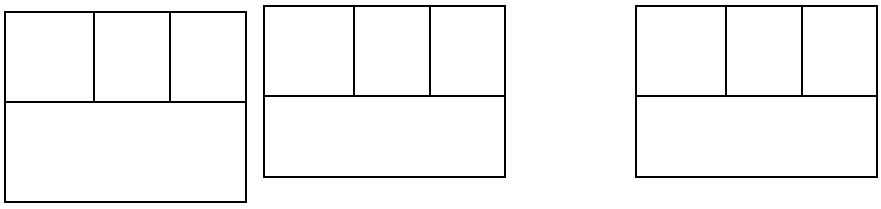
PAST MEDICAL HISTORY:

FAMILY HISTORY:

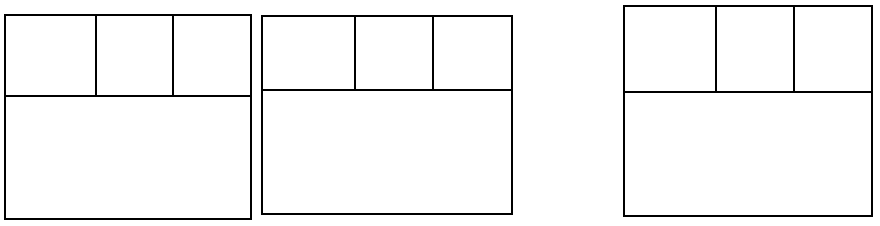
ORAL HYGIENE HABIT:

TEETH PRESENT:

INDICES
PLAQUE INDEX (PI) (Silness & Loe 1964) (Baseline)



16 **12** **24**

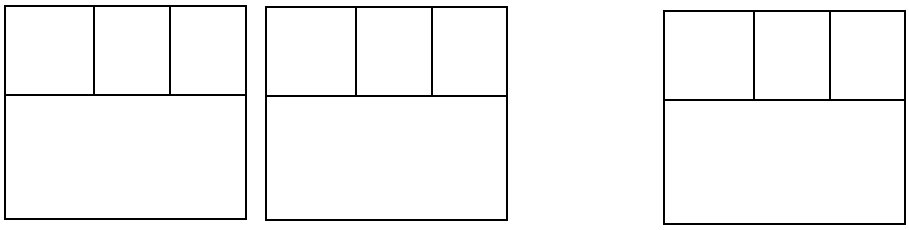


44 **32** **36**

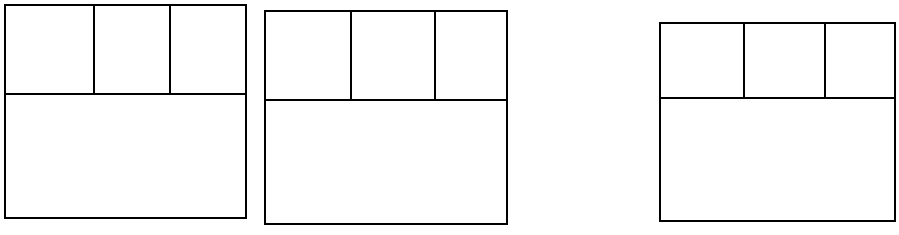
Total scores of all teeth

Total number of teeth examined

PLAQUE INDEX (PI) (Silness&Loe 1964) (3 Months)



16 **12** **24**

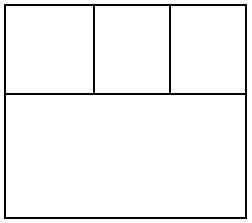


44 **32** **36**

Total scores of all teeth

Total number of teeth examined

PLAQUE INDEX (PI) (Silness& Loe 1964) (6 Months)



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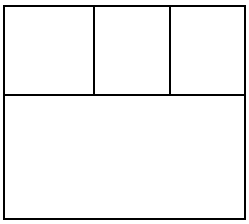

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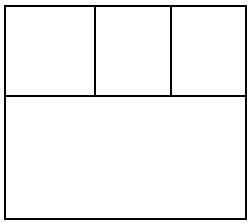

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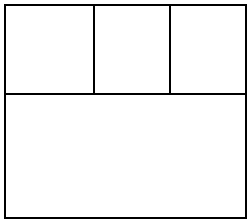

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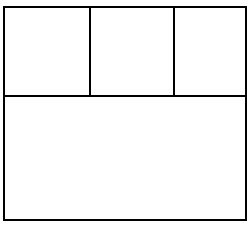

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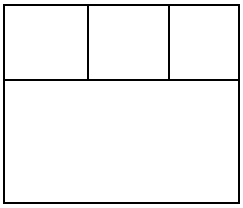


36

Total scores of all teeth

Total number of teeth examined

MODIFIED SULCUS BLEEDING INDEX (mSBI) (Baseline):

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Total scores of all teeth

Total number of teeth examined

MODIFIED SULCUS BLEEDING INDEX (mSBI) (3 Months)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

Total scores of all teeth

Total number of teeth examined

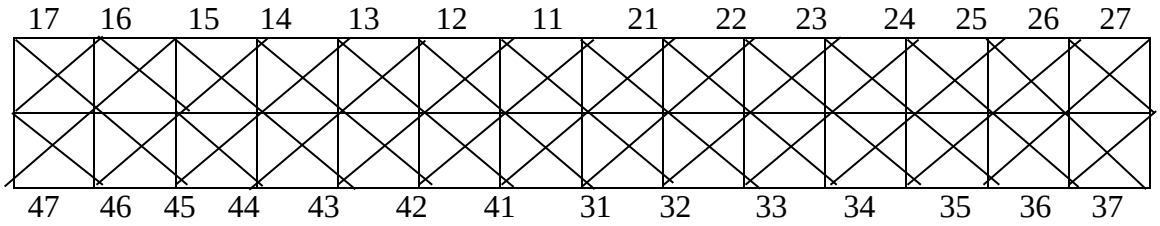
MODIFIED SULCUS BLEEDING INDEX (mSBI) (6 Months)

18	**17**	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
48	**47**	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**

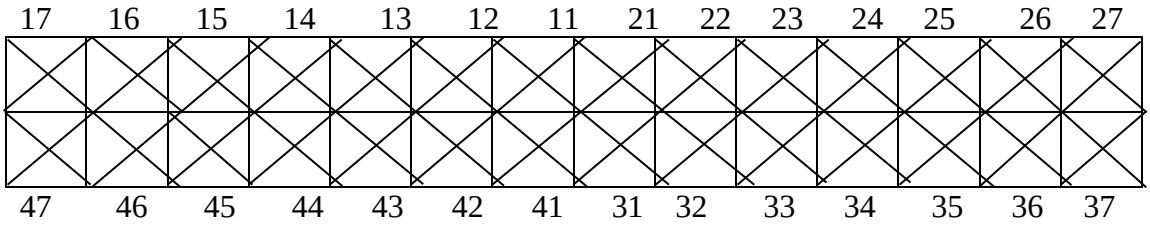
Total scores of all teeth

Total number of teeth examined

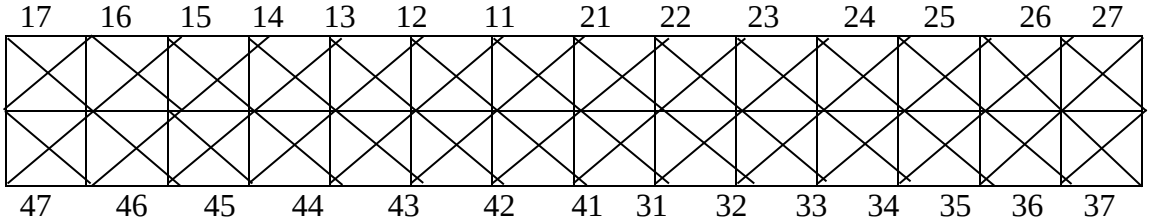
PROBING POCKET DEPTH (mm) (Baseline):



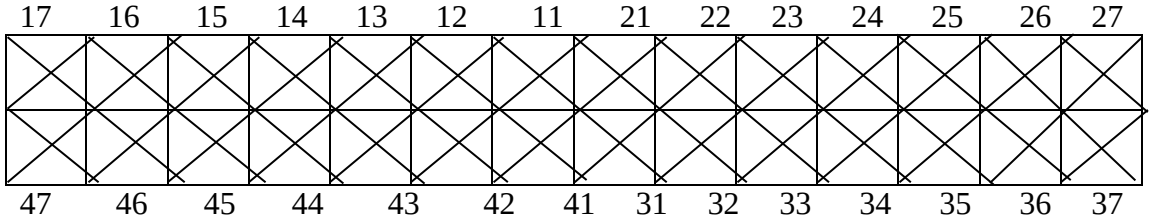
PROBING POCKET DEPTH (mm) (3 Months):



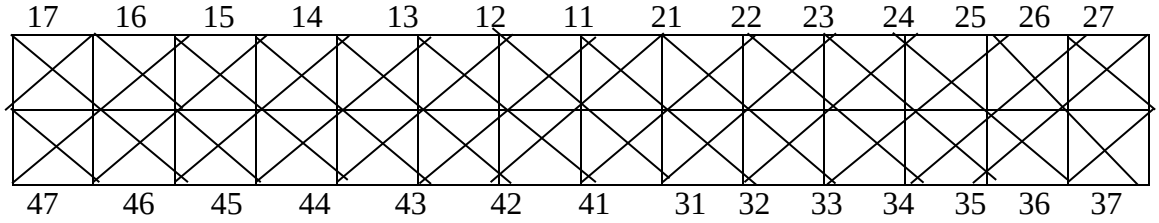
PROBING POCKET DEPTH (mm) (6 Months):



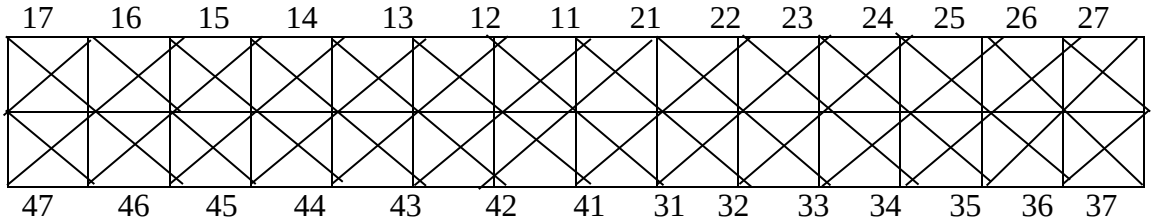
CLINICAL ATTACHMENT LEVEL (mm) (Baseline):



CLINICAL ATTACHMENT LEVEL (mm) (3 Months)



CLINICAL ATTACHMENT LEVEL (mm) (6 Months)



CLINICAL DIAGNOSIS:

RADIOGRAPHIC INVESTIGATIONS:

Intrabony Defect Dimensions (CBCT) at baseline:

Site	Tooth No.	Material Used	CEJ-BD dimension	CEJ-AC dimension	AC-BD dimension (defect depth)	MD width	BL width
Control							
Test							

Intrabony Defect Dimensions (CBCT) at 6 Months:

Site	Tooth No.	Material Used	CEJ-BD dimension	CEJ-AC dimension	AC-BD dimension (defect depth)	MD width	BL width
Control							
Test							

Bone Fill at 6 months

Method of analysis	Test Group	Control Group
CBCT		

(Confidential)
Informed Consent Form

"**Comparative Evaluation of 1% Melatonin gel as an adjunct to non surgical periodontal therapy in stage III periodontitis: A CBCT Study**"

NAME: Mr./Master/Mrs./Miss._____

Resident of: _____aged _____ years, exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted by

Dr. _____.

I acknowledge the receipt of "patient's information sheet", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to undergo this surgical periodontal treatment upon me which includes placing 1% Melatonin gel at one site and Placebo gel at another site in the defects. Potential benefits, risks and complications related to the materials used in surgery have been explained to me. I agree to let my X-rays, photographs, blood investigations, other investigations to be taken as required. I consent to the administration of anesthesia or other medications before, during or after the procedure by qualified personnel. I understand that all anesthetics or sedation medications include the very rare potential of risks or complications, such as damage to vital organs including the brain, heart, lungs, liver and kidneys; paralysis; cardiac arrest; and/or death from both known and unknown causes. I understand that there are potential risks, complications and side effects associated with any dental procedure. Although it is impossible to list every potential risk, complication and side effect, I have been informed of some of the possible risks, complications and side effects of periodontal surgery.

I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time.

I certify that I have read or had read to me the contents of this form.

_____Date _____

Patient /legally authorized representative signature