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RESEARCH ARTICLE

EVALUATION OF EFFICACY OF DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT WITH AND WITHOUT PLATELET-RICH FIBRIN IN THE TREATMENT OF INTRABONY DEFECTS BY CONE BEAM COMPUTED TOMOGRAPHY: A CLINICAL AND RADIOGRAPHIC STUDY

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Abstract

Background: Eventhough the combination of DFDBA (demineralized freeze dried bone allograft) with PRF (platelet-rich fibrin) has been attempted in periodontal practice with significant results, assessment of the intrabony defect and defect bone fill largely done with two-dimensional imaging modalities. Three dimentional analysis of the intrabony defect and defect bone fill has not been attempted with cone beam computer tomography (CBCT). The present study evaluated the efficacy of DFDBA with and without PRF in the treatment ofintrabony defects by CBCT.

Methods: 60 defects in systemically healthy patients ranging from 18 to 50 years of age will be included in the study.30 defects were treated with PRF+DFDBA and 30 defects were treated with DFDBA alone. The study will include the assessment of clinical parameters involving probing depth (PD), relative attachment level(RAL), full mouth bleeding scores(FMBS),plaque index and gingival index from baseline to 3 , 6, 9 months. Hard tissue changes will be assessed radiographically by evaluating defect fill and defect resolution by CBCT at baseline& 9 months.

Results: The results of the present study are statistically significant in both groups in terms of clinical and radiographical parameters ($P < 0.001$). In inter-group comparison, there was a statistically significant greater PD reduction and attachment gain while there was not significant reduction in terms of PI, GI, FMBS, defect fill and defect resolution in DFDBA+ PRF group.

Conclusion: DFDBA along with PRF failed to provide additional value in terms of defect fill and defect resolution over DFDBA alone.

Introduction:-

Periodontal regenerative procedures may restore lost supporting structures of the dentition such as cementum, periodontal ligament (PDL) and bone on a previously diseased root surface. Various bone graft materials have demonstrated regenerative potential and have been successfully used in the treatment of intrabony defects. The bone replacement grafts may aid in providing a scaffold for the host's resident cells or provide factors that aid in stimulating regeneration via osteoinductive or osteoconductive pathways.[1,2]

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For the last three decades, DFDBA has been used alone and in combination with other treatment modalities for periodontal therapy. DFDBA has shown to be both osteoconductive and osteoinductive. The presence of Bone Morphogenic Proteins (BMPs) within DFDBA, aids in mesenchymal cell migration, attachment and osteogenesis when implanted into bony defects.Regeneration of the lost periodontium through the process of tissue engineering is the greatest hallmark of recent studies in the field of periodontics. The 2nd generation platelet concentrate is called PRF or Choukroun's PRF after its inventor. PRF consists of an intimate assembly of platelets, growth factors, cytokines, glycanic chains and structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well-known synergistic effects on healing process.[2-5]

Several techniques have been used to evaluate hard tissue responses to regenerative therapy around periodontally involved teeth. CBCT is used to assess the amount of bone fill following periodontal regenerative procedures. This may be of particular significance because conventional radiographs tend to underestimate the amount of bone fill following such procedures. CBCT may be a valuable tool for periodontal regeneration imaging and assessment, both preoperatively and postoperatively.[6,7]

Even though the combination of DFDBA with PRF has been attempted in periodontal practice with significant results, assessment of the intrabony defect (IBD) and defect bone fill largely done with two-dimensional imaging modalities such as conventional and digital radiography. Three-dimensional analysis of the intrabony defect and defect bone fill has not been attempted much with CBCT. Thus in the present study, the combination of DFDBA and PRF will be studied and three-dimensional analysis of the intra bony defect and defect bone fill was done by CBCT.

Materials and Methods:-

The study sample included 60 intrabony defects in 48 patients with chronic periodontitis visiting Government Dental College & Hospital, Vijayawada were randomly divided in to two groups of 30 each (Group I (DFDBA) or Group II (DFDBA+PRF)) and the study period was 9 months. Ethical clearance was obtained from the Institutional Ethics Committee. The study was designed as a single blinded, randomized, controlled, two arm parallel study. Patients age between 18-50years having intrabony defect depth of $\geq$ 3mm and should not undergone any periodontal therapy prior to six months before initial treatment with good oral hygiene are included in the study. All the patients were explained about the study and written informed consent was obtained before the commencement of the study. (FIG 1)

Following initial examination, at first visit (pre surgical visit) clinical parameters were recorded and impressions were taken to prepare study casts and fabricate occlusal stents for the treatment teeth. Scaling and root planing were performed using hand curettes and an ultrasonic device under local anesthesia. Occlusal adjustment was performed if trauma from occlusion was diagnosed. A periodontal re-evaluation was performed after 4 weeks to confirm the suitability of the sites for this periodontal surgical study. Patients who are suitable for the procedure are advised to take baseline CBCT before surgery.

At the second visit (surgical visit) Periodontal assessment was done by recordingRelative attachment level (distance from apical border of stent to base of pocket),Probing Depth (The distance from the free gingival margin to the base of the pocket). Plaque index(Loe H et al [8]) and gingival index(Loe H et al [8]) FMBS (full mouth bleeding score)(Ainamo and Bay[9])The oral hygiene maintenance of the patient was evaluated and only those patients maintaining optimum oral hygiene (PI $\leq$ 1) proceeded for the surgery. The surgical area was anaesthetized by local anaesthetic techniques using 2% lignocaine with adrenaline 1:200000 dilution. The procedure was done under proper aseptic precautions using continuous aspiration to keep surgical site clean. Buccal and lingual sulcular incisions were made and mucoperiosteal flaps elevated. Care was exercised to preserve as much interproximal soft tissue as possible. Complete debridement of the defects, as well as scaling and root planing to ensure root smoothness, were achieved with the use of an ultrasonic device and hand curettes. In the PRF–DFDBA group, DFDBA granules (Tata Bone Graft) with particle sizes of 500-1040µ were mixed with PRF(prepared by Choukran protocol using a research centrifuge (REMI laboratories) for 15 min at 3000 rpm) that had been minced into pieces about 0.5 mm × 0.5 mm at a proportion of 1:1 (v/v). The PRF+DFDBA mixture was delivered to the defect and packed with amalgam condensers to the level of the surrounding bony walls. Care was taken not to overfill defects. Defects in the DFDBA group were filled with DFDBA granules only.Appropriate post-operative instructions with medication were given to the patients.

After one week following surgery, sutures were removed. Symptoms regarding discomfort, pain, swelling, and fever were asked and recorded. Oral hygiene instructions were reinforced. Clinical parameters including PI, GI, FMBS, PD and RAL were recorded after 3rd, 6th, and 9th months Oral hygiene instructions were reinforced and scaling was done if necessary.A 9 month post- operative CBCT was taken and radiographic parameters defect fill and defect resolution were recorded.

Radiographic Measurement:
Before performing regenerative procedure, paralleling cone technique was used to take peri-apical radiographs with XCP devices for screening the defect. CBCT imaging was taken to check the 3D architecture of the intrabony defect for better treatment planning and evaluate the measurements preoperatively at baseline and postoperatively after 9 months.All the CBCT (NEW CS 9000 System®) scans were taken by a single trained technician at baseline and 9 months. The voltage (70.00KV), Current (10.00mA), Exposure time (10.8 sec) and Detection field were kept constant for each patient at baseline and at 9 months. The reference chosen to standardize the axial and sagittal planes was the bi-spinal line, coinciding with the vertical and horizontal planes,respectively. The reference employed to standardize the coronal plane was the linebetween infra-orbital points, named the infra-orbital line thus concluding the positioning of images over the three spatial planes. The sagittal and coronal sections were reconstructed after 9 months at the same axial slicing to that of the baseline.

The linear measurements of CEJ to AC (Measured from cemento-enamel junction to alveolar crest) and CEJ to BOD(Measured from cemento-enamel junction to base of the defect) were used to determine defect fill and defect resolution. Defect fill was calculated by subtracting CEJ to BOD at baseline from CEJ to BOD at 9 months. Defect resolution was calculated by the formula [(CEJ-BOD) – (CEJ-AC) at baseline] – [(CEJ-BOD) – (CEJ-AC) at 9months].

Statistical analysis
Mean and standard deviation were calculated for all the clinical and radiological parameters. The significance of differences between intervention and control groups in terms of numerical data was evaluated via univariate analysis using the Mann- Whitney U test. The significance of the differences within each group before and after treatment was evaluated using the Friedman test.

Results:-
All patients showed good compliance and the healing was uneventful for both treatment groups, without infection or complications. The mean PI, GI, FMBS decreased from baseline to 9 months with in group I & group11 with statitistical significance. intergroup comparison was not significant (P>0.05) from baseline to 9 months.The mean PD at baseline was 7.27 ± 0.46 mm in group I and 7.8 ± 1.08 mm in group II, the mean PD at 9 months was 4.07 ± 0.26 mm and 3.07 ± 0.26 mm respectively. The mean RAL at baseline was 10.47±0.64 mm in Group I and 11.0 ±1.0 mm in group II, the mean RAL at 9 months was 7.33±0.62 mm and 6.87±0.52 mm respectively. The difference in PD and RAL between group I and group II (intergroup) from baseline to 9 months postoperatively was significant (P<0.05). (Table 1)

The mean CEJ-BOD Level at baseline was 7.23± 1.13 mm in group I and 7.05 ± 1.07 mm in group II, the mean CEJ-BOD Level at 9 months was 5.59 ± 1.24 mm and 5.08 ± 0.98 mm respectively. The mean CEJ-AC Level at baseline was 3.447 ± 0.90 mm in group I and 3.513 ± 0.81 mm in group II, the mean CEJ-AC Level at 9 months was 3.582 ± 0.75 mm and 3.61 ± 0.58 mm respectively. The mean defect fill and defect resolution after 9 months in group I was 1.63 ± 0.51 mm and 1.50 ± 0.52 mm, in group II was 1.97 ± 0.62 mm and 1.75± 0.54 mm respectively. The difference in defect fill and defect resolution between group I and group II (intergroup) after 9 months postoperatively was insignificant (p>0.05). There was a statistically significant difference for group I (P<0.05) and group II (P<0.05) from baseline to nine months.(TABLE 2&3)

Discussion:-
The first evolutionary stage of periodontal regeneration focused on using avariety of bone graft materials. Presently, a lot of research being carried out in evaluating the combination oftherapies that would promote maximum resolution of the defects. Accordingly, in thisstudy we decided to combine PRF containing platelet growth factors to DFDBA, aclinically effective periodontal regenerative therapeutic modality.[10]Radiological parameters like defect fill and defect resolution was assessed by CBCT at baseline and 9 months postoperatively. More recently, CBCT

hasbeen used to assess head and neck structure which has ability to visualize thesestructures in three dimensions while producing images that have high resolution andaccuracy. Grimard BA et al.[6]states that CBCT is an equivalent substitution for direct surgical measurements of bony changesoccurring after bone replacement graft procedures, especially defect fill and defectresolution.

Intragroup comparison of PI and GI and FBS showed statistically significant results (P<0.001) while intergroup comparison was not significant (P>0.05). These results are similar to studies of Agarwal A et al.[11]Piemontese M et al.[12], Markou N et al.[13]The significant mean change of PI, GI, FMBS in both the groups could be due toregular and frequent recall visits in which the patient underwent regular supragingivalscaling and also because of careful patient selection who were able to maintainacceptable oral hygiene. Numerous reports have indicated that good oral hygiene, as reflected by low plaque scores, is associated with better regenerative response[14,15]

PD and RAL from baseline to nine months in intra group and inter group comparison showed statistically significant reduction (p<0.05). PD reduction is not only a desirable outcome of periodontal regenerative procedures but may also be the most important parameter in patient care for the clinician because it directly impacts one's ability to instrument a treated area during maintenance appointments. The present study showed a mean PD reduction of 3.2±0.56 mm in group I and 4.73 ±0.96 mm in group II from baseline to 9 months postoperatively. A mean attachment gain in group II (4.13±0.83) was higher compared to group I (3.13±0.74) in the present study. These are similar to studies done by Parashis A etal.[16], Mellonig JT et al.[17] Bansal C et al.[10,] Demonstration of better results (attachment gain and PD reduction) in DFDBA + PRF group in the present study may be explained by the additional biologic effects of PRF. unlike other platelet concentrate, it is able to progressively release cytokines during fibrin matrix remodeling.[4]PRF organizes as a dense fibrin scaffold with a high number of leukocytes concentrated in one part of the clot. Leukocytes seem to have a strong influence on growth factor release, immune regulation, anti-infectious activity, and matrix remodeling during healing. It is an optimal matrix for migration of endothelial cells and fibroblasts. It permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue. Such a mechanism might explain the clinically observed soft tissue healing properties of PRF.[18,10]It can also be speculated that because BMPs are members of the TGF super family, their effect, if BMPs exist in DFDBA and are active will add to the effects of the growth factors within the platelets, ensuring a synergetic impact on the cell population of the wound.[12]These observations revealed that DFDBA when used along with PRF results in good healing compared to use of DFDBA alone. [19]

There is a statistically significant mean defect fill of 1.63± 0.51 mm in group I from baseline to 9 months and 1.97 ± 0.62 mm in group II respectively. Mean defect resolution also showed significance in group I (1.50 ± 0.52 mm) and group II (1.75 ± 0.54 mm) from baseline to 9 months. Inter group comparison was not significant (p>0.05) for both defect fill and defect resolution after 9 months. Defect fill in group I is due to osteogenic potential of DFDBA that is due to presence of bone morphogenic protein which elicits mesenchymal cell migration, attachment and osteogenesis when implanted in well vascularized bone.[20]When mixed with the graft, PRF fragments serve as a biological connector between bone particles. Moreover, the gradual release of cytokines plays a significant role in the self regulation of inflammatory and infectious phenomena within the grafted material.[10]Even though the results are significant in both the groups, defect fill and defect resolution is less in the present study compared to several similar studies.[10,11,21]Thereason for this may be due to random selection of the intrabony defect with remaining bony walls and also due to less intrabony defect depth.[22] In clinical practice, pure two orthree wall angular defects are uncommon, whereas the majority of the defects are present in combinations. Intrabony Defects treated in this study were two wall, threewall or a combination of two and three wall defects. Regenerative potential of verticaldefects depends on the defect topography[11]and percentage osseous repair is directly proportional to the number of bony walls lining the defect[23,24]

The uniqueness of the present study is using a three dimensional approach (CBCT) for evaluating defect fill and defect resolution inintrabony defects treated with DFDBA and DFDBA+PRF. As per now there are very few studies regarding this context in literature.

Currently, there is sparse evidence to support the adjunctive use of PRF with bone graft in the management of periodontal osseous defects. Studies using larger samples and additional long term documentation with standardization of the study design, surgical technique and other variables are needed to assess the efficacy of adding PRF to bone graft materials and to allow for more valid and meaningful comparisons between studies.

Conclusion:-

Within the limits of the study it can be concluded that the use of DFDBA along with PRF failed to provide additional value in terms of defect fill and defect resolution over DFDBA alone. However further studies with large samples and long follow up are needed to clarify and confirm results.

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Table 1:- Clinical Parametres Of Both Group I (Dfdba)And Groupii(DFDBA+PRF).

	PLAQUE INDEX		GINGIVAL INDEX		FMBS		PD		CAL	
	GROUP I MEAN ±SD	GROUPII MEAN ±SD	GROUP I MEAN ±SD	GROUPII MEAN ±SD	GROUP I MEAN± SD	GROUPII MEAN ±SD	GROUP I MEAN ±SD	GROUPII MEAN ±SD	GROUP I MEAN ±SD	GROUPII MEAN ±SD
baseline	0.73±0.05	0.72±0.05	0.74±0.06	0.75±0.06	19.9±1.39	20.2±1.39	7.27±0.46	7.80±1.08	10.47±0.64	11.00±1.00
3months	0.66±0.06	0.65±0.06	0.65±0.07	0.67±0.07	17.87±1.35	18.2±1.35	4.67±0.49	4.27±0.70	8.40±0.74	8.00±0.93
6months	0.57±0.09	0.58±0.09	0.54±0.09	0.54±0.09	15.93±2.23	16.2±2.23	4.07±0.26	3.00±0.00	7.47±0.64	7.00±0.63
9 months	0.55±0.07	0.53±0.07	0.59±0.08	0.59±0.08	14.5±1.95	14.5±1.95	4.07±0.26	3.07±0.26	7.33±0.62	6.87±0.52
Baseline-3months	0.05±0.03	0.05±0.03	0.05±0.04	0.04±0.04	1.66±1.17	1.66±1.17	2.60±0.51	3.53±0.64	-2.07±0.70	-3.00±0.65
Baseline-6months	0.12±0.04	0.12±0.04	0.13±0.04	0.14±0.04	3.68±1.50	3.68±1.50	3.20±0.56	4.80±1.08	-3.00±0.65	-4.00±0.65
Baseline-	0.19±0.06	0.19±0.06	0.21±0.07	0.22±0.07	5.71±1.93	5.71±1.93	3.20±0.56	4.73±0.96	-3.13±0	-4.13±0

9months									.74	.83
P-VALUE (Intragroup)	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS	P=<0.01 HS
P-VALUE (Intergroup)	P=1.00 NS		P=1.00 NS		P=1.00 NS		P=<0.01 HS		P=<0.01 HS	

Table 2:- Radiological Parametres Of Both Group I (DFDBA)And Groupii(DFDBA+PRF) At Baseline And 9 Months.

	CEJ TO BOD		CEJ TO AC	
	GROUP I MEAN±SD	GROUPII MEAN±SD	GROUP I MEAN±SD	GROUPII MEAN±SD
baseline	7.22±1.13	7.05±1.07	3.44±0.91	3.51±0.75
9months	5.59±1.23	5.08±0.98	3.58±0.80	3.61±0.58
	P=<0.01 HS	P=<0.01 HS	P=<0.05 S	P=<0.01 HS

Table 3:- Radiological Parametres Of Both Group I (DFDBA)And Groupii(DFDBA+PRF) From Baseline - 9 Months.

	Baseline -9months		
	GROUP I MEAN± SD	GROUPII MEAN±SD	p-value
CEJ TO BOD	-1.63±0.51	-1.97±0.62	0.18 NS
CEJ TO AC	0.13±0.39	0.10±0.47	>0.05 NS
Defect fill	1.63±0.51	1.97±0.62	0.18NS
Defect resolution	1.50±0.52	1.75±0.54	0.88 NS

Fig 1:- Flow Chart Of Study Design.

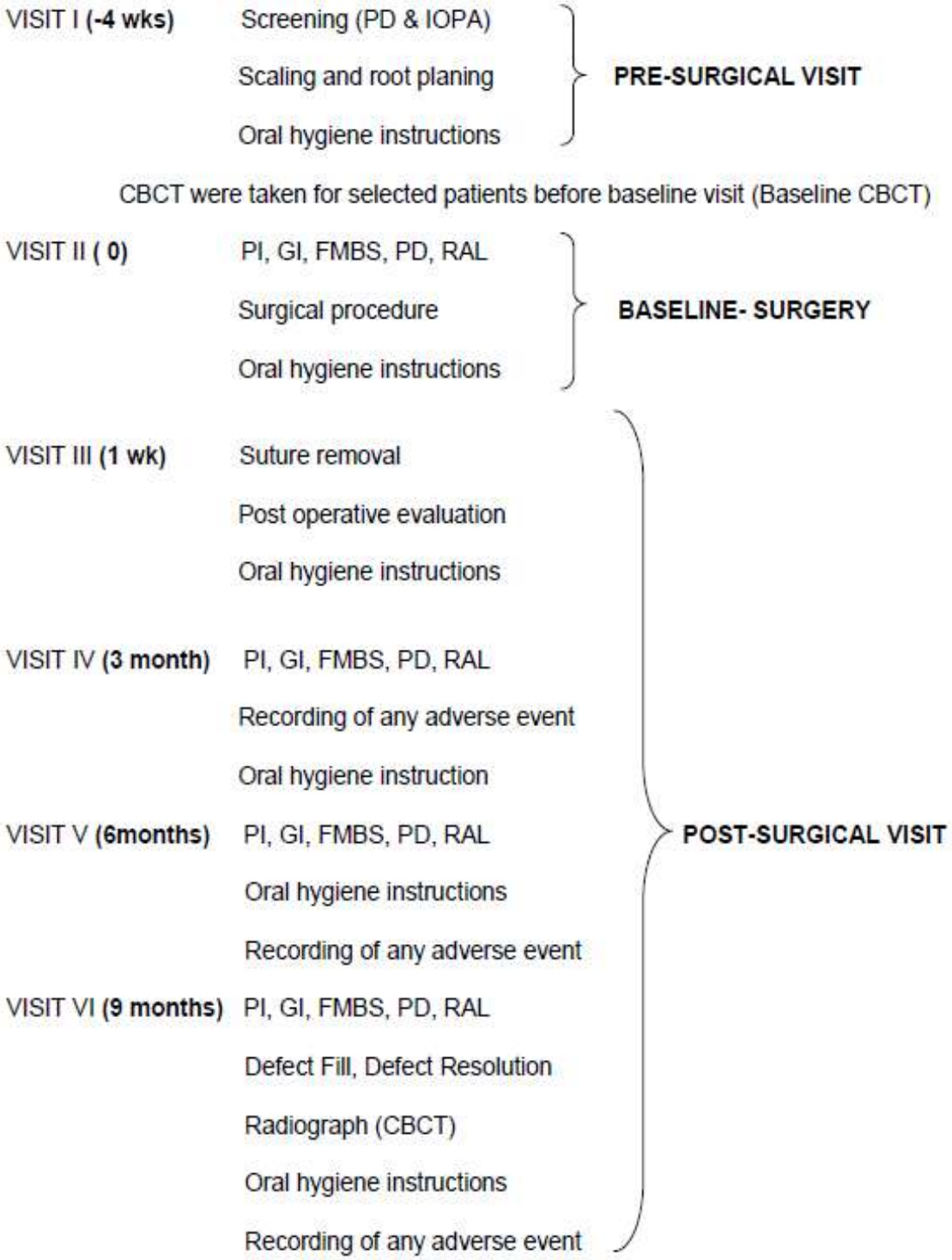

Fig 2:- Radiographic Measurement By Cbct At Baseline.

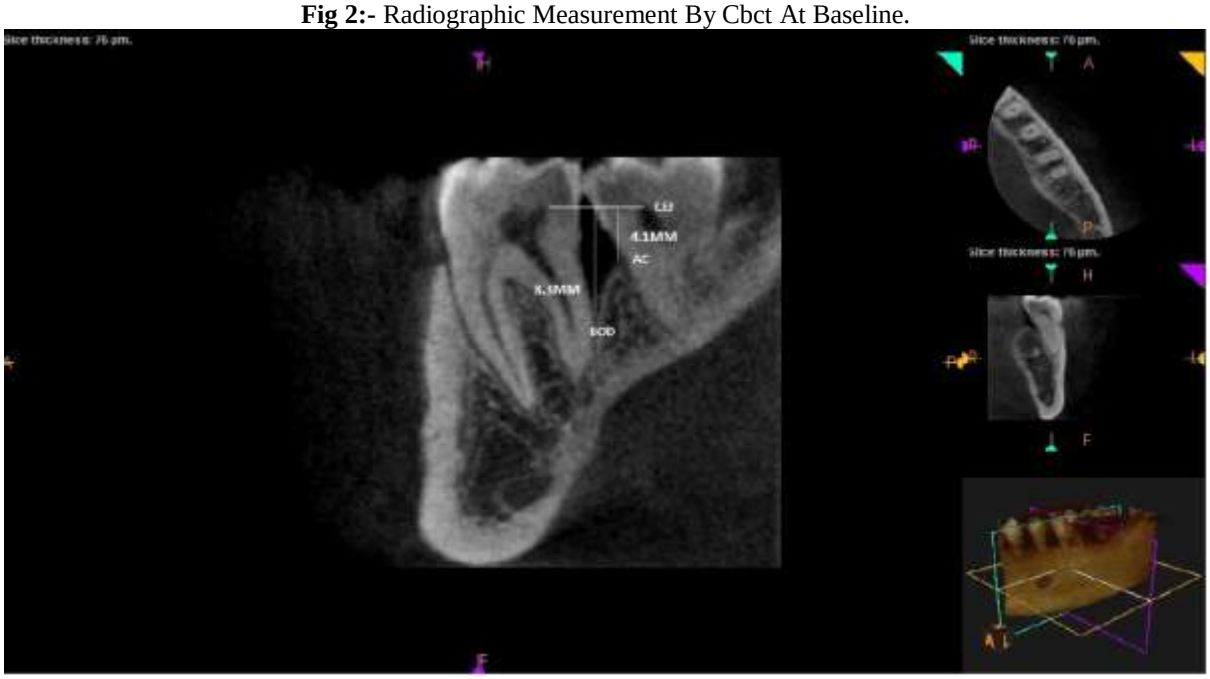

Fig 3:- Radiographic Measurement By Cbct At 9 Months.