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RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF EFFICACY OF DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT WITH AND WITHOUT MELATONIN FOR SOCKET PRESERVATION OF SINGLE ROOTED TEETH: A 6 MONTHS RADIOGRAPHIC & HISTOLOGICAL STUDY

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Abstract

Background: The aim of the present study was to evaluate the efficacy of demineralized freeze-dried bone allograft with and without Melatonin for socket preservation based on clinical, radiological and histological parameters.

Methods: A total of 20 single-rooted teeth indicated for extraction were included in this randomized controlled clinical study of 6 months' duration. The subjects were allocated into two groups for socket preservation viz. test group (DFDBA with Melatonin Powder) and control group (DFDBA only) randomly with the toss of coin.Clinical,radiological and histological parameters were recorded at baseline and 6 months.

Results: Both the groups showed comparable results in clinical, radiographic and histological parameters with considerable promise of regeneration, thus can be used in maintaining the ridge width and height post extraction.

Conclusion: It be concluded that DFDBA with or without melatonin can be used for socket preservation with predictable and comparable results. No significant advantage of mixing melatonin with DFDBA was observed in our study. However, further studies with larger sample size and use of melatonin with other bone grafts should be conducted to ascertain the findings of our study.

Introduction:-

A tooth which cannot be restored or maintained in acceptable conditions for long-term health, function, and/or esthetics is generally indicated for extraction. Loss of tooth has a direct impact on quality of life leading to impairment in the ability to masticate, speak, and, in some instances, socialize.[1]

The main reasons for socket preservation are to prevent future bone loss and ridge resorption, to support the labial plate of bone, support of adjacent teeth and implants; planning for future options such as implants or a fixed bridge and avoidance of additional surgeries for implant site development.[2]Site preservation through socket preservation will help to optimize bony fill within the extraction socket, thereby maintaining vertical bone height and help stabilize the marginal tissues.[3]

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Several types of bone grafts have been studied and widely used in dentistry for the past four decades. Literature has reported alveolar ridge preservation using autogenous, allogeneic (e.g: demineralized freeze-dried bone allografts and freeze-dried bone allografts), xenograft (e.g., bovine bone and coral), and alloplast (e.g., ceramics for biologic use, b-tricalcium phosphate [β-TCP] and hydroxyapatite) graft materials.).[4]

One type of allograft widely used in dentistry is Demineralized Freeze-Dried bone allograft (DFDBA). DFDBA was first used in dentistry and medicine in 1965.[5] A study conducted by Brugnami et al (1996) reported that commercially available DFDBA has the potential to function physically as a nidus for appositional new bone growth in alveolar sockets following tooth extraction.[6] DFDBA provides osteoconductive surface, and it also acts as a source of osteoinductive factors.[7] Through a study done by Urist and Strates (1960), demineralized bone was showed to have osteoinductive potential by stimulating bone formation in extra skeletal sites.[8]These properties of DFDBA makes it one of the most frequently used bone replacement graft.

There are various adjunctive which are frequently used with bone grafts to enhance their osseointegrative properties. One such modality used is Melatonin in the form of powder or gel. Melatonin (N-acetyl-5-methoxytryptamine) is a powerful hormone derived from an essential amino acid tryptophan. It has anti-inflammatory effects and plays an immunomodulatory role.[9,10] Melatonin has theability to directly neutralize a variety of reactive oxygen species, including superoxide anion radical O_2^-, the hydroxyl radical –OH, and hydrogen peroxide H_2O_2.

According to a study by Cutando et al (2008), the dose required for melatonin to enhance osseointegration of dental implants and minimize the marginal bone resorption is reported to be 1.2 mg melatonin powder for each implant.[11] Melatonin acts in increasing bone mass by suppressing resorption through down-regulation of the receptor activator of nuclear factor-kappa B ligand (RANKL) mediated osteoclast formation and activation. All these data point towards an osteogenic effect of melatonin that may be of clinical importance, as it could be used as a therapeutic agent in situations in which bone formation would be advantageous, such as in the treatment of fractures, osteoporosis and hypocalcaemia[12].

Thus, the purpose of this study is to determine the radiographic and histologic efficacy of DFDBAwith Melatonin in preserving extraction sockets. Both DFDBA and Melatonin have osseoinductive properties and are hoped to act in synergy to enhance bone formation after extraction and socket preservation.

Materials and Methods:-

The study was designed as a single-blinded, randomized and controlled trial of 6 months duration. The study protocol was reviewed and the subjects were allocated into two groups viz. test group and control group randomly with the toss of a coin. This study was approved by I.T.S institutional ethics committee (IIEC). Informed and written consent was taken from all the patients. A total of 20 sites ofsingle rooted teeth indicated for extraction (of endodontic or periodontal reasons) were taken up for extraction followed by socket preservation by placement of DFDBA in combination with Melatonin (Test group) and socket preservation by placement of DFDBA (Control Group). Inclusion Criteria were systemically healthy patients within the age of 18-60 years, isolated alveolar sockets (a socket located between two sound teeth) of maxillary and mandibular mono-radicular teeth, indicated for extraction, with atleast 7mm residual alveolar bone height as measured clinically and radiographically from periapical radiographs and with intact socket walls in all dimensions (four walled bony defects) with an occlusion suitable for planned prosthetic and implant treatment, can undergo the treatment under local anaesthesia with or without intravenous sedation and could provide a signed consent. Exclusion criteria included any systemic diseases associated with bone metabolism, uncontrolled diabetes, uncontrolled hypertension, or hepatic and renal disorders, any history of allergy to any of the materials used in this study, antibiotics or antimicrobial therapy for last 6 months before the study, habit of smokeless/smoking tobacco, lactating or pregnant women and subjects unable to provide informed consent.

Acrylic stents made of cold cure acrylic for positioning the probe and measuring the defects from a fixed reference point (FRP) from vertical aspect was fabricated for standardization of clinical measurements using a UNC/15 periodontal probe. Qualified participants were prescribed 0.2% chlorhexidine as the pre-procedural rinse. Selected teeth were extracted with minimal trauma to surrounding tissues, and sockets were thoroughly irrigated. After extraction, the socket was evaluated for hard tissue measurements using a standardized ridge-mapping instrument and the reference stent. Residual sockets were then randomly treated for either of the experimental materials. Lastly,

to retain the grafted material and to close the wound, cross-mattress sutures were given for both the groups to avoid tension.

Postoperative Care
Both groups received the same postoperative treatment and instructions. They were instructed not to clean the surgical sites but to rinse with 0.2% chlorhexidine digluconate twice daily for a 2-week period. Antibiotic coverage with Amoxicillin, 500 mg, 3 times daily was prescribed for 7 days, and analgesic medication (ibuprofen, 500 mg) was prescribed in case of postoperative pain. The sutures were removed 10 days following the surgical procedure. Post-operative evaluation was performed at baseline and 6 months, and all clinical, radiographic and histological parameters were reassessed.

Re-entry Procedure
Sites were re-entered at 6 months during implant placement and ridge dimensions were measured and recorded using the original stents. A 2.7-mm-diameter core biopsy was harvested from the central portion of the previously grafted extraction site. The trephine core biopsy specimens of cases obtained were fixed with 10% neutral buffered formalin, processed and embedded in paraffin wax after mild decalcification procedure in 10% EDTA solution. The tissue specimens were processed following standard tissue processing protocols of dehydration, clearing, infiltration and embedding in paraffin wax. Areas of new bone formation and remnant graft material were measured and compared in the two study groups using Olympus Magnus Pro morphometric software. The sections were also stained with Picrosirius Red stain and observed under Polarising microscope for collagen fiber characterization.

Radiographic Analysis
Radiograph analysis was done utilizing radiographic stents, fabricated on diagnostic casts of each patient covering the site of the tooth to be extracted and extended to the adjacent teeth for stabilization within the dental arch. Radiopaque fiducial markers i.e., Gutta Percha points were impregnated into the stents and served as references on the cone-beam computed tomography (CBCT) images at the central aspect of the site to be treated to allow for standardization of the measurements of the alveolar ridge on pre-and post-operative CBCT images. The voltage, current, exposure time, and field of view were kept constant for each patient at the time of both exposures. For measuring the radiographic parameters same reference points and lines were used as described by Das et al[13] both at baseline and at 6 months.

Statistical Analysis
All the data was collected and analysed. The statistical software SPSS 16.0 is used for analysis of data.

Result:-
The clinical parameters assessed in the present study were buccolingual width, mid-buccal crestal height, mid-palatal/lingual crestal height, and relative socket depth. Standardised CBCT scans were taken to assess the radiological parameters buccal cortical plate height, lingual cortical plate height, distance from the anatomical landmark to the base of the socket and horizontal width at coronal 3rd, middle 3rd and apical 3rd of the socket. Finally, bone cores were extracted from the preserved sites at 6 months post-op with a trephine bur during implant placement and histo-morphometric analysis was done. The following results were obtained.No significant difference ($p>0.05$) in buccolingual width and mid buccal crestal height, mid palatal/lingual crestal height, residual socket height of the socket between control and test group at baseline and 6 months was seen. CBCT measurement of buccal cortical height, palatal cortical height showed statistically non-significant decrease in both control and test group at 6 months from baseline levels. Intergroup comparison of distance from the anatomical landmark to the base of the socket, horizontal width at coronal 3rd, middle 3rd and apical 3rd using CBCT measurements showed horizontal width at coronal 3rdand middle 3rd in favor of test group which was statistically significant ($p<0.05$). New bone volume and residual graft volume and Collagen characterization showed comparable results in test and control groups at 6 months and were found to be statistically non-significant ($p>0.05$).

Discussion:-
In our study, when comparing the buccolingual socket width, statistically non-significant difference ($p>0.05$) in buccolingual width of the socket between control and test group at baseline and 6 months was found. In contrast to our study, **Mayer et al (2016)**[14]reported a statistically non-significant reduction of the buccolingual width at -3mm ($p>0.05$) and -6mm($p>0.05$) from the crest in the biphasic calcium sulphate (BCS) group. While similar to our

study, **Nunes et al (2018)**[15]**,** reported significant reduction in the horizontal width in the HA + β-TCP group at the crest ($p<0.05$).

More decrease in Mid buccal crestal height could be seen from baseline to 6 months in Test group as compared to Control Group. The mean difference was statistically non-significant. Similarly, the mean **mid palatal/lingual crestal height** (in mm) of the socket was found to be statistically non-significant between control and test group at baseline and 6 months. Similarly, **Abdullah et al (2021)**[16] showed statistically non- significant difference in bone height after 6 months when compared with the baseline in both group A (socket preservation using Melatonin with Beta-Tri-Calcium Phosphate) and group B (sockets grafted with β-TCP alone).

In the present study the change in residual socket height before and after the intervention was measured from the standardized acrylic stent till the apical end of the socket. There was statistically no significant difference ($p>0.05$) in residual socket height (in mm) of the socket between control and test group at baseline and 6 months. The results showed comparable effect of DFBDA used alone and DFDBA used with melatonin in increasing socket height after extraction. **Abdullah et al (2021)**[16] showed similar results for residual socket height while comparing Melatonin with Beta-Tri-Calcium Phosphate and Beta-Tri-Calcium Phosphate alone. Whereas, **Mogharehabed et al (2014)**[17]observed contradictory results in the DFDBA + PRGF group as compared to the DFDBA+saline group and the control group.

The difference in the results may be due to the different techniques and bone graft used for socket preservation. In our study, both groups showed increase in socket fill at 6 months, which could be attributed to new bone formation at grafted site as DFDBA has both osteoconductive as well as osteoinductive properties. However, there was no inter group significance suggesting addition of melatonin did not enhance properties of DFDBA.

Radiographic Parameters

In our study, there was statistically no significant difference in buccal cortical height between control group and test group at baseline and 6 months. This result is in line with the results obtained by **Jung et al (2018)**[18]who found non-significant loss of buccal cortical height when using Demineralized dentin matrix with Rh BMP2.

Also,there was statistically no significant difference ($p>0.05$) in palatal/lingual cortical height (in mm) of the socket between control and test group at baseline and 6 months in our study. Similarly, **Brkovic et al (2012)**[19] reported a non-significant reduction in the lingual cortical height when Beta-Tri-Calcium Phosphate-Collagen cones were used for socket preservation. The difference in the results could be because of the difference in the biomaterial used.

In our study, the **distance from the anatomical landmark to the base of the socket** representative of the distance from the tangent drawn at the nearest visible anatomical landmark till base of the socket was done to evaluate bone fill in the socket. In the 6 months follow up evaluation, the base of the socket was considered as the point where the apical border of the graft material was radiographically discernible. The difference in the distance from the anatomical landmark to the base of the socket from 6 months to baseline was representative of the socket fill at 6 months. Intergroup comparison showed increase distance from the anatomical landmark to the base of the socket for both control and test group at 6 months. All these radiographic parameters findings were analogous to the findings of clinical parameters recorded.

On intergroup comparison of both the mean **horizontal width at coronal 3rd**and**horizontal width at middle 3rd,** a statistically significant higher reduction was seen in the test group at 6 months. While, there was statistically no significant difference in horizontal width at apical 3rd between control group and test group at baseline and 6 months.

In our study, mean horizontal width was found to be highest in the apical region and lowest in the coronal portion in both the control and test group. This is in accordance to a study conducted by **Jung et al (2018)**[18] while it's in contrast to the results obtained by **Fathy et al (2019)**[20]

The decrease in assessed radiographic parameters was seen in the present study. Consideration of both horizontal and vertical measurements from this study confirms previous reports that post-extraction healing involves loss of ridge width and height as shown in studies by **Lekovic et al (1997, 1998)**[21,22]

Histological Parameters

The histological sections stained with H and E showed well-formed mature bone with cortical as well as cancellous bone. The bony trabeculae are well formed with adequate medullary spaces. No evidence of inflammatory infiltrates was seen. The results for **new bone formation** (%) and **residual graft volume** (%) were statistically non-significant.While, similarto our study, **Beck et al (2010)**[23]showed that there was no significant increase in the amount of new bone formation or graft clearance from 3 to 6 months using a mineralized allograft material.

The present study results show that there was favourable host response in terms of new bone formation with minimal residual graft volume in both test and control groups.

Varying colours seen on polarizing microscopy after Picrosirius red stain are based on collagen fiber thickness and arrangement. The colour changes seen in our study are similar with the study done by **Velidandla et al (2014).**[24] In the present study, 60% of the samples in test group and 40% of the samples in the control group showed Reddish Orange birefringence whereas 30% of the samples in test group and 60% samples in the control group showed Orange Yellow birefringence. 10% of the samples showed Greenish Yellow birefringence. The above results were statistically non-significant. This shows that both the groups were able to induce production of good amount of thick and tightly packed collagen fibres, and it indirectly shows enhanced density of tissue being formed at the site, which may provide good initial stability during implant placement.

In the present study, more amount of residual graft material was seen in the test group and lesser new formation seen in the test group which suggests that addition of melatonin to DFDBA does not enhance new bone formation as compared to DFDBA alone.

The present study has shown positive results with respect to clinical and radiographical vertical and horizontal bone changes in the form of bucco-lingual width, relative socket depth, mid-buccal and mid-palatal/lingual crestal heights, socket fill in both the groups, exhibiting noticeable preservation of the extraction socket. Use of DFDBA with melatonin and when DFDBA used alone showed comparable results in all aspects evaluated. The results of the present study corroborate the importance of using a graft material to fill the socket after tooth extraction.

Srinath et al (2010)[25] reported that melatonin acts on prostaglandin E2, thereby inhibiting the differentiation of osteoclasts induced by cell-to-cell contact between osteoblasts and osteoclasts. But the results shown in the present study were comparable to the results obtained when DFDBA was used alone. This might be cause DFDBA itself contains bone morphogenic protein (BMP) that causes new bone formation to take place during healing. Thus, true to the results obtained in previous studies, both the DFDBA and Melatonin when used with DFDBA showed comparable positive results.

Table 1:- Intergroup Comparison Of Clinical Parameters At Various Time.

Clinical Parameter (In mm)	Time Interval	Control Group (Mean±SD)	Test Group (Mean±SD)	Mean Difference	p-value
Buccolingual Width	Baseline	7.68±1.89	7.81±1.30	0.13	0.855
	6 Months	6.68±1.15	6.86±1.13	0.18	0.722
Mid-Buccal Crestal Height	Baseline	11.90±1.19	11.20±3.12	-0.70	0.516
	6 Months	13.00±0.70	13.50±1.84	0.50	0.430
Mid Palatal/Lingual Crestal Height	Baseline	11.90±2.72	10.30±1.63	-1.60	0.129
	6 Months	13.20±1.09	13.10±1.72	-0.10	0.877
Residual Socket Height	Baseline	23.30±1.82	22.00±1.24	-1.30	0.080

	6 Months	15.00±0.66	14.20±1.31	-0.80	0.104

Independent t-test; significance at p<0.05*

Table 2:- Intergroup Comparison Of Radiographic Parameters At Various Time Intervals.

Radiographic Parameter (In Mm)	Time Interval	Control Group (Mean±SD)	Test Group (Mean±SD)	Mean Difference	p-value
Buccal Cortical Height	Baseline	20.13±9.10	13.64±4.68	-6.49	0.06
	6 Months	20.00±7.98	14.40±5.27	-5.60	0.08
Palatal Cortical Height	Baseline	21.20±7.53	16.56±5.51	-4.64	0.13
	6 Months	21.03±7.23	15.06±6.25	-5.97	0.06
Distance From AL^ To Base of Socket	Baseline	11.28±6.85	6.89±6.34	-4.39	0.15
	6 Months	13.30±6.80	7.80±5.61	-5.50	0.06
Horizontal Width At Coronal 3rd	Baseline	8.96±2.03	10.03±1.41	1.07	0.19
	6 Months	6.58±1.61	8.11±1.18	1.53	0.02*
Horizontal Width At Middle 3rd	Baseline	9.01±2.18	10.57±1.80	1.56	0.09
	6 Months	7.30±2.00	9.0±1.33	1.70	0.03*
Horizontal Width At Apical 3rd	Baseline	9.78±2.90	11.23±2.23	1.45	0.22
	6 Months	8.25±2.28	9.94±1.34	1.69	0.05

Independent t-test; significance at p<0.05*
AL^- Anatomic Landmark

Table 3:- Intergroup Comparison Of Histological Parameters At 6 Months.

Histological Parameters	Time Interval	Control Group (Mean±SD)	Test Group (Mean±SD)	Mean Difference	p-value
New Bone Volume In %	At 6 Months	31.13±4.77	30.58±10.23	-0.54	0.912
Residual Graft Volume In %	At 6 Months	1.73±1.63	2.50±1.74	0.76	0.428

Independent t-test; significance at p<0.05*

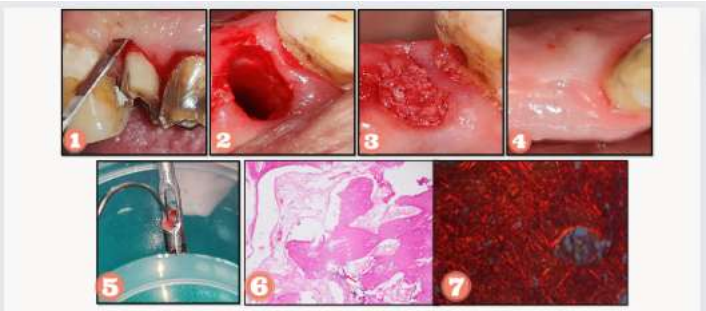
Table 4:- Intergroup Comparison Of Picrosirius Red Stain At 6 Months.

Collagen Characterization	Test Group	Control Group	Total	Chi	p-value

Reddish Orange	6 (60%)	4(40%)	10(50%)	2.400	0.301
Orange Yellow	3(30%)	6(60%)	9(45%)		
Greenish Yelow	1(10%)	0(%)	1(5%)		

Pearson's Chi SquareTest At P<0.05.

Control Group: Fig 1. Atraumatic extraction using Periotome, Fig 2. Extraction socket after atraumatic extraction, Fig 3. Grafting done of extraction site with DFDBA, Fig 4. Post-op 6 months, Fig 5. Bone core extraction with trephine bur at 6 months post-op during implant placement, Fig 6. Photomicrograph showing areas of new bone formation and residual graft (red arrows) in Control Group (H and E stain, 10x magnification), Fig 7. Photomicrograph showing reddish orange collagen fiber group under polarising microscopy in Control Group (Picrosirius Red stain, 10x magnification)



Test Group: Fig 1. Atraumatic extraction using Periotome, Fig 2. Extraction socket after atraumatic extraction, Fig 3. Grafting done of extraction site with DFDBA mixed with Melatonin, Fig 4. Post-op 6 months, Fig 5. Bone core extraction with trephine bur at 6 months post-op during implant placement, Fig 6. Photomicrograph showing areas of new bone formation and residual graft (red arrows) in Test Group (H and E stain, 10x magnification), Fig 7. Photomicrograph showing reddish orange collagen fiber group under polarising microscopy in Test Group (Picrosirius Red stain, 10x magnification)

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