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RESEARCH ARTICLE

EVALUATION OF EGGSHELL DERIVED HYDROXYAPATITE AS A BONE REGENERATIVE MATERIAL IN THE TREATMENT OF INTRABONY OSSEOUS DEFECTS - CLINICAL AND RADIOGRAPHIC ASSESSMENT

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Abstract

Eggshell contains 98.2%calcium carbonate and can be transformed into hydroxyapatite which is an environment friendly process and can be used as bone regenerative grafts which not only reduces the treatment cost but also high in biosafety.

Introduction:-

Periodontitis is a disease of inflammation of periodontal tissues and one of the most prevalent oral diseases in the world is periodontal disease which not only damages the periodontal tissues but also act as a risk factor for systemic diseases like diabetes, preterm low birth weight and cardiovascular diseases.1,2 Chronic nature of periodontitis is normally seen in adults containing variety of inflammatory mediators and cytokines within the inflamed pocket surface area of affected region mainly responsible for systemic diseases like atherosclerosis, etc.2 This upsurges the need for periodontal management for which the patients has to be aware of with their possible risks associated, if left untreated.

Vertical bone defects are more to be considered for periodontal therapy as they are more prone to disease progression than horizontal bone defects and has a better regenerative capacity than horizontal bone loss.4,5
Bone grafts has been in use for reconstructive intraosseous defects and was introduced by Hegedus in 1923 and was later revived by Nabers and O'leary for the treatment of two-wall defects.6Various bone grafting materials are available including -autografts, allografts, alloplasts, xenografts. Because of the ability to keep the progenitor cells vital and inability to evoke any immunological reactions, autogenous bone grafts are considered gold standard for regenerating the osseous defects.

Alloplastic bone grafts are considered alternative to autogenous bone graft materials as their histologic comparison shows no significant differences in amount of newly bone forms in osseous defects.

Hydroxyapatite is apatite calcium phosphate and found in abundance in teeth and bone. In recent years, for bone repair and regeneration of osseous defects, the use of synthetic and processed hydroxyapatite (alloplast) has gained popularity over the traditional grafting methodbecause of factors involved like-

1. Donor site morbidity
2. Time and skill required for second surgery to harvest graft and inadequate quantity available with respect to autografts

Hydroxyapatite can be synthetically prepared or commercially available and both the hydroxyapatites are equally effective in early bone formation.

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AvinashKavarthapu et al in 2019 compared the regenerative effectiveness of osseograft with membrane vs eggshell and its membrane in animals by creating defects of 1.5 ×6 mm at 45th day and found that eggshell powder along with the membrane can be used for bone regeneration.

The eggshell which are poultry waste can be transformed into hydroxyapatite and nanohydroxyapatite which is an environment friendly process and also reduces the treatment cost in bone repair or replacement.13

The composition of eggshell is approximately 98.2%calcium carbonate, 0.9% magnesium and 0.9% phosphorus and also shows low autoimmune and allergic reactions as well as high in biosafety and its composition is similar to bone and teeth. Eggshell calcium is 90% and is the best natural source of calcium and proved to be better than lime stone or coral sources.

Other uses of egg shell/ membranes are as follows-
1. Wound healing and Sinus lifting procedure
2. Eggshell calcium in prevention and treatment of osteoporosis
3. Eggshell membrane possess anti-inflammatory activity thus can be used as local drug in gingivitis.20
4. Eggshell membranes have anti-inflammatory, anti-wrinkle, antimicrobial activity, thus can be used as cosmetic agents to protect skin.
5. As direct pulp capping material.

S Pokhrelin 2018 gave an overview of preparation, properties and biomedical application of eggshell derived hydroxyapatite which is mainly composed of calcium and phosphorus, the main components of bone. The physical and chemical characteristics of hydroxyapatite and eggshell are similar which makes the substance biocompatible for biomedical use. Also, its porous structure, biodegradability, and bioactivity of the bio ceramics, it is in forefront of the discussion.

Chemical bonding with the surrounding bone, characteristic similarity with the bone, scaffold prepared from hydroxyapatite shows good proliferation, differentiation of osteoblasts, porosity and wettability allows ceramic loading with the drug, corporation of silver ions promotes antibacterial property of the graft and osteoinductive property makes the bio ceramic useful for biomedical application.

Sandra Janeth Gutierrez-Prieto et al in 2019 conducted an experiment to assess the compatibility of modified hydroxyapatite to overcome the limitations of hydroxyapatite derived from eggshells like fragility and low mechanical strength. For this, he has added silicon (Si) and poly(lactic-co-glycolic) acid (PLGA) to the original hydroxyapatite graft and found that silicon modified hydroxyapatite is compatible with the osteoblastic cells pertaining high mechanical strength to the original graft.

Hence considering the osteogenic property of eggshell as a hydroxyapatite, we have conducted the first clinical study in india in intrabony defects using eggshell as a bone regenerative material in the management of periodontal intrabonyosseous defect in periodontitis patients and evaluated its osteogenic potential through clinical and Radiographic evaluation.

Subjects and Methods:-

A total of 20 sites of intraosseous defects in periodontitis patients were included in this study in an age group between 30-55 years. They were randomly selected from the Outpatient Department of Periodontics and Implantology at Hitkarini Dental College and Hospital, Jabalpur, M.P, and were assigned into two groups as Experimental Group and Control Group.

Experimental Group was treated with open flap debridement and the placement of Eggshell Derived Hydroxyapatite whereas; Control Group was treated with open flap debridement alone.
Inclusion Criteria

Patients diagnosed as having chronic generalized periodontitis with periodontal pockets of ≥5mm with radiographic evidence of vertical bone loss.
Age group between 30-55 years.

Patients with good general health, without any history of systemic disease or under medication.

Exclusion Criteria
Patients showing unacceptable oral hygiene during pre-surgical (Phase I) period.
2. Smokers, pregnant women and lactating mothers.
Study Design

Informed consent: Written informed consent form explaining the nature of the study and surgical procedure was signed by the patient.

Phase I therapy: Involved oral hygiene instructions, scaling and root planing.
Maintenance phase: Evaluation of initial Phase I therapy.

Baseline recording of clinical parameters
1. Gingival Index (Loe and Silness, 1963)
2. Probing pocket depth (using UNC-15 probe with occlusal stent).
3. Relative attachment level (using UNC-15 probe with occlusal stent).
4. IOPA assessment by paralleling technique.

Pre-surgical Protocol
1. Revaluation of Phase I therapy will be done.
2. The selected sites will be assigned to either Experimental Group or Control Group based on randomization.

Surgical phase
1. Control group – Open flap debridement alone
2. Experimental group – Open flap debridement + Eggshell derived hydroxyapatite as bone regenerative material.

Maintenance phase
1. Removal of periodontal pack and sutures and then irrigation with saline.
2. Reinforcement of oral hygiene instruction.

After six months
1. Recording of clinical parameters (gingival index, probing depth, relative attachment level) using stent.
2. IOPA radiograph
3. Reinforcement of oral hygiene instructions

Pre-Surgical Protocol
After initial examination and treatment planning discussion, all the selected patients were given detailed instructions regarding the surgical procedure and were then subjected to phase I therapy with oral hygiene instructions. Revaluation and Occlusal adjustments were carried out after initial therapy wherever indicated. All the patients were subjected to routine blood examination that included % haemoglobin, bleeding time, clotting time, total leucocyte count and random blood sugar and ethical acceptance were taken. (fig 1 to 13)

Post Surgical Protocol: After one week following surgery, the dressing and sutures were removed, and surgical site was irrigated thoroughly with saline. Symptoms regarding discomfort, pain, and sensitivity were asked from the patient. Patients were instructed to rinse with Chlorhexidine (0.2%) mouthwash twice daily for another week. Recall appointments were made at 3 months, 6 months intervals. At each visit, oral hygiene instructions were reinforced, and the surgical sites were professionally irrigated with normal saline.

At the end of six months post therapy, patients were evaluated clinically and radiographically. Clinical parameters (Gingival Index, Probing Pocket Depth, Relative Attachment Level) and radiographic measurements were repeated for both control and experimental group sites like previous pre-surgical measurements.

Results:-

The Relative Attachment Level and Mean Radiographic area of patients in Group A and Group B doesn't show any significant change after completion of phase I therapy but shows significant improvement after 3 and 6 months. The

Gingival index values and pocket probing depth decreases continuously after completion of phase l , 3 months and 6 months and there were significant differences between all time intervals from baseline to six months.

The different parameters compared between the groups at different time period were given in Table 3. At Baseline there was non-significant difference between the groups. Both the groups were identical with respect to these parameters. After competition of phase one therapy the mean Relative Attachment Level and Mean Radiographic area shows no difference between the groups but Gingival index values and pocket probing depth decreases significantly in Group B in comparison to Group A. All the parameters values at 3 Month and 6 Month show significant improvement in Group B in comparison to Group A. (table 1,2,3 and graph 1)

Discussion:-

Periodontitis results because of imbalance between proinflammatory and anti-inflammatory cytokines like IL-1, IL-6, PGE2, TNF-α, MMPs and IL-4, IL-10, IL-1, TIMPs respectively because of etiologic factors like poor compliance, poor plaque control, subgingival bioburden and risk factors e.g. genetics, smoking, diabetes resulting in underactivity or overactivity of aspects of host response.

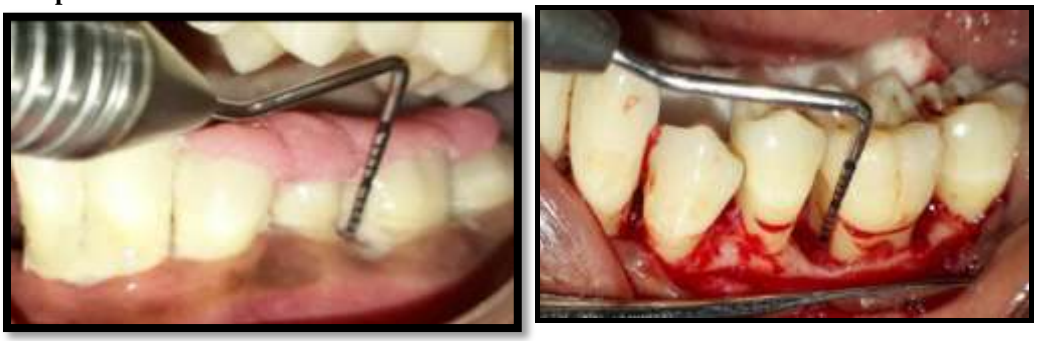
Periodontal flap surgical procedure, which is a conventional method of debridement, have only little potential of restoring the lost periodontium. On contrary, periodontal regenerative procedures not only focuses on formation of new PDL, cementum and bone but also restores the architecture of altered periodontium.20

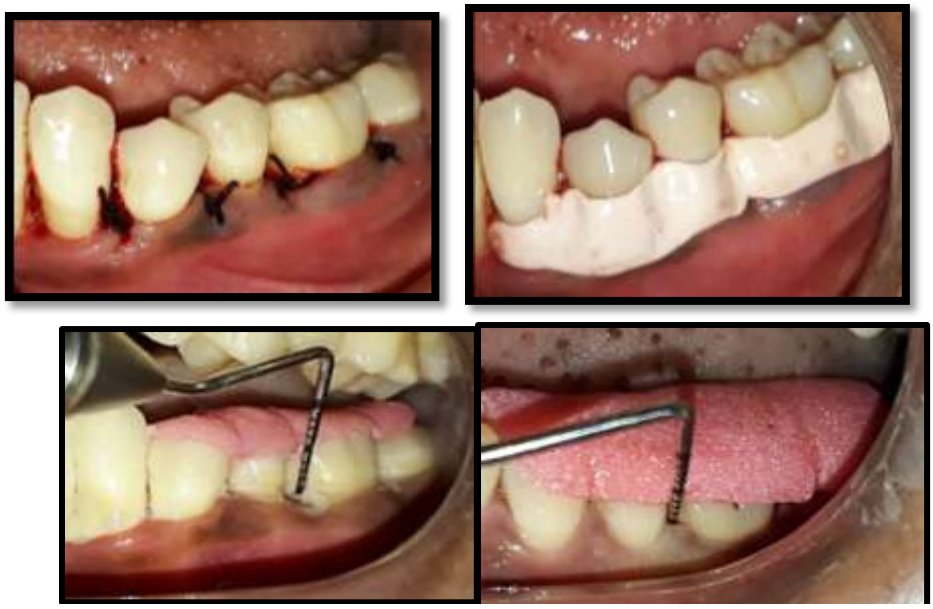
There was a significant reduction in gingival inflammation after the use of Hydroxyapatite extract through its anti-inflammatory property through NF-κB in LPS-triggered human immune cells as proposed by Vuong TT et al, 2017.21

The amount of bone fill was increased after 6 months postoperatively in both groups but significantly higher in the experimental group. Radiographic assessment of the bone fill revealed mean gain of 14.32% and 4.97% in experimental group and control group respectively after 6 months with mean defect reduction from 39.45% to 34.48% in control group and reduction from 41.02% to 26.70% in experiment group after 6 months which shows more significant reduction and bone fill in Group B as compared to Group A. The bone regeneration is contributed to osteoconductive nature of the graft material.

Limitations and future considerations In our study only Radiographic assessment was done for 20 sites which was small sample size and duration of the study was 6 months. For future consideration histological and molecular assessment of the bone fill can be done with a larger sample size. Also, the duration of the study can be extended by 9 months which should be accompanied by CBCT evaluation instead of radiographic assessment.

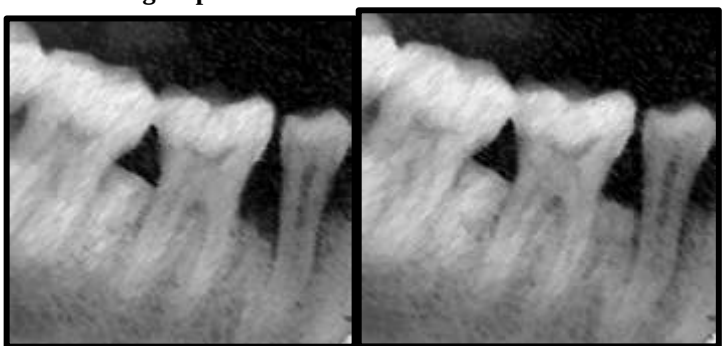
Control Group





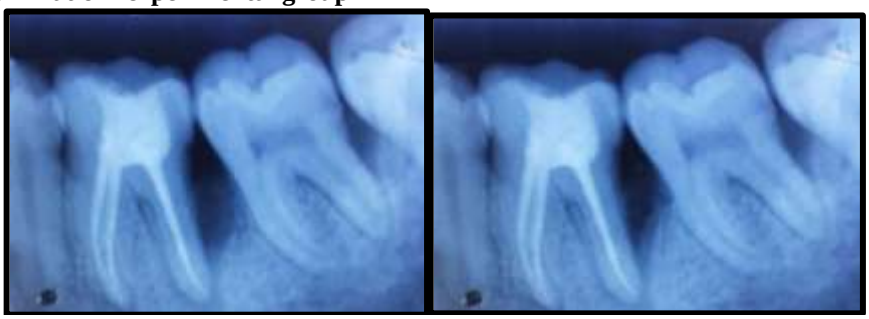
Experimental Group

Radiographic examination -control group



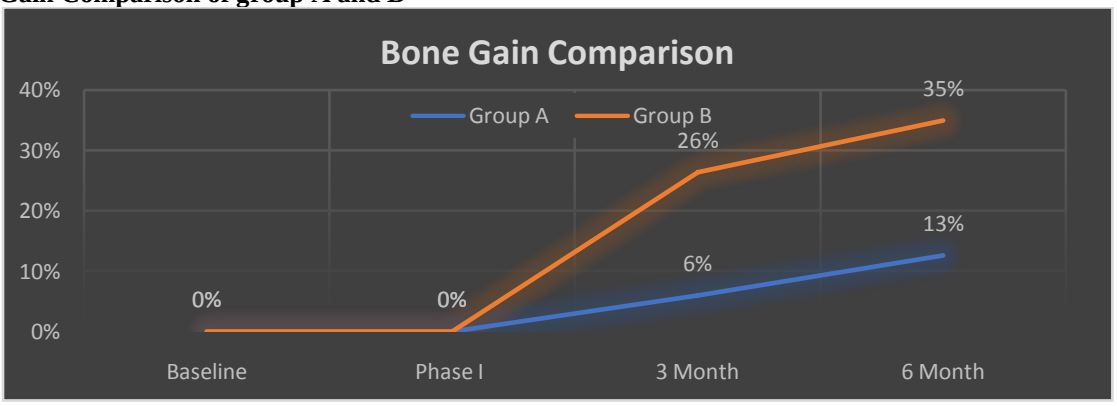
Pre-op radiogarph **post op radiograph**

Radiographic examination -experimental group



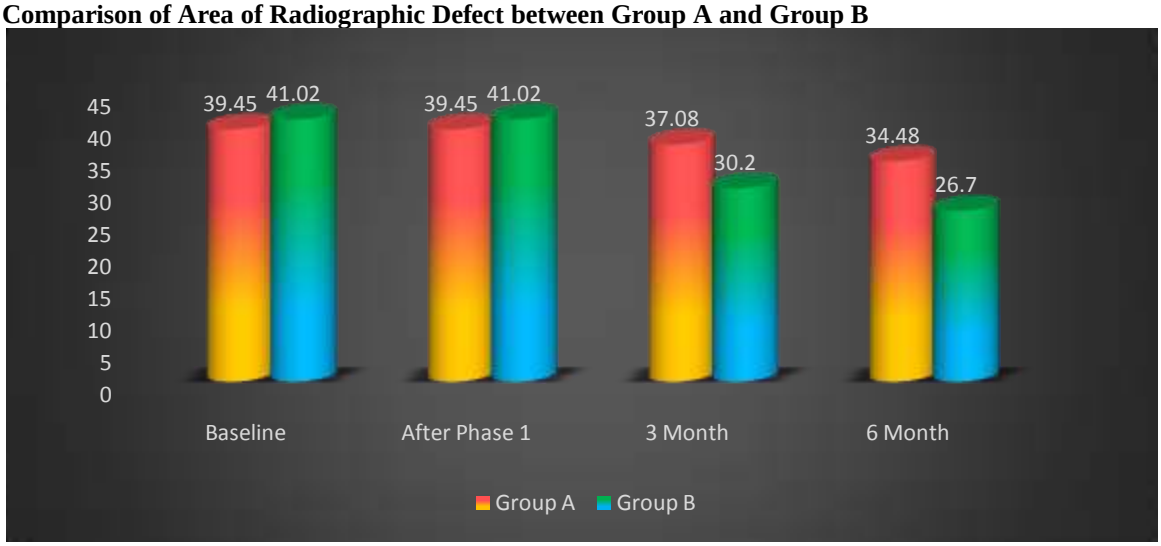
Pre-op radiograph **6 months radiograph**

Bone Gain Comparison of group A and B

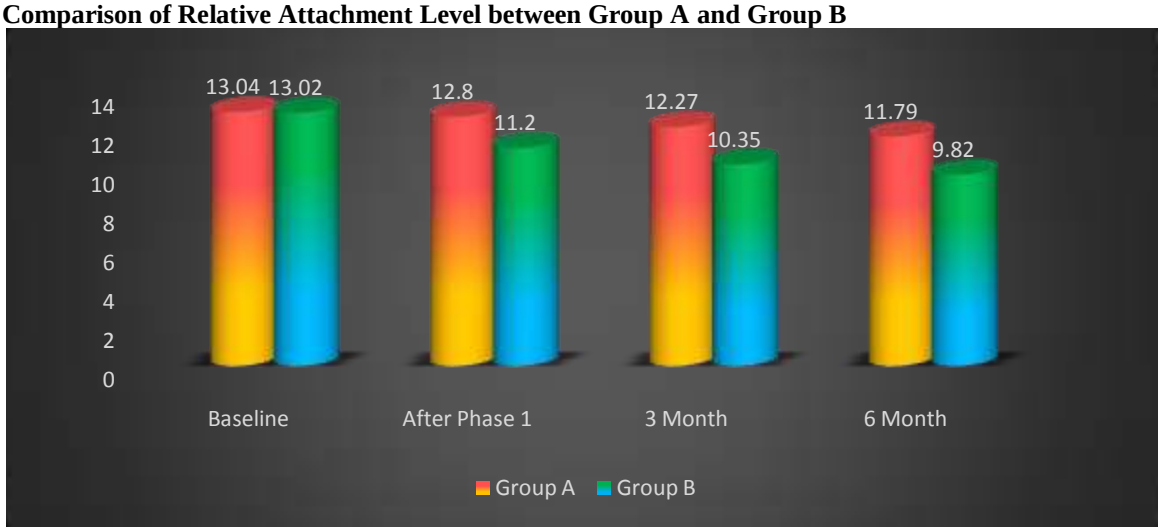


Group A:- Control Group **Group B:-** Experimental Group

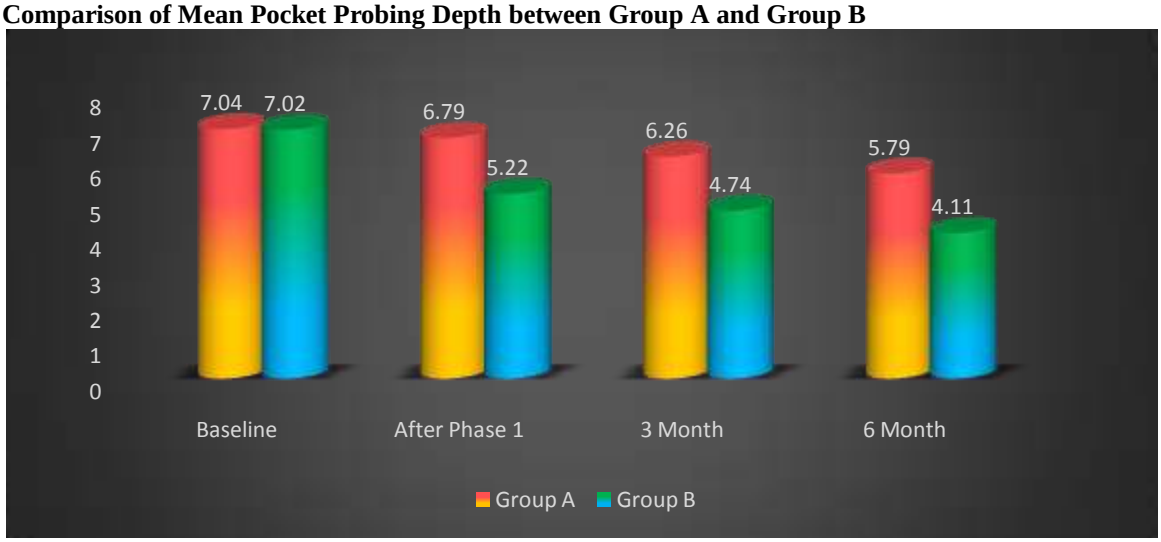
Comparison of Area of Radiographic Defect between Group A and Group B



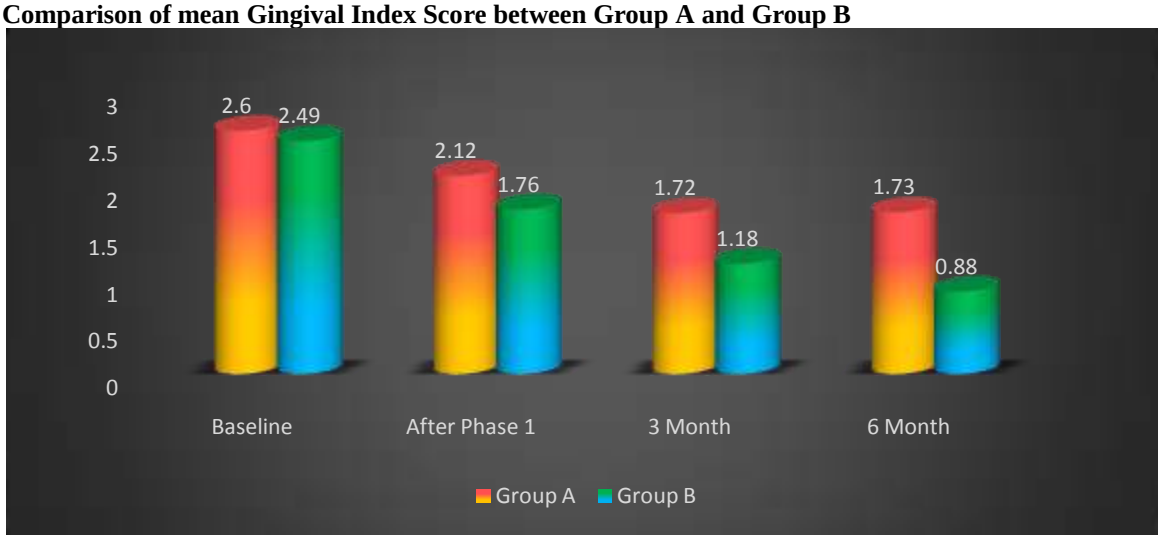
Comparison of Relative Attachment Level between Group A and Group B



Comparison of Mean Pocket Probing Depth between Group A and Group B



Comparison of mean Gingival Index Score between Group A and Group B



Conclusion:-

A radiographic assessment of bone regeneration using Eggshell derived hydroxyapatite was performed in Chronic Generalised periodontitis patients with intrabony defects. There was statistically significant improvement of Gingival Index, Probing Pocket Depth and gain in Relative Attachment Level between baseline and six months in Experimental group and in intergroup comparison of both the groups.

Summary

This study was a clinical and radiographic assessment to evaluate the efficacy of Eggshell Derived Hydroxyapatite as a bone regenerative material in the treatment of intraosseous defects. In this present study, the results showed significant difference in the clinical parameters including the reduction in probing pocket depth and gain in relative attachment level in the Experiment group. This clinical study showed a greater bone fill in the radiographic assessment in the Experimental group when compared to the Control group.

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