COMPARISON OF EFFICACY OF HUMAN AMNIOTIC MEMBRANE VERSUS COLLAGEN MEMBRANE IN GUIDED BONE REGENERATION OF ALVEOLAR BONE

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In

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By

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BATCH: 2018-2021

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LIST OF ABBREVIATIONS

HAM	:	Human Amniotic Membrane
GBR	:	Guided Bone Regeneration
GTR	:	Guided Tissue Regeneration
RVG	:	Radiovisiography
CBCT	:	Cone-beam computed tomography
IOPARs	:	Intraoral periapical radiographs
OPGs	:	Orthopantomograms
VAS	:	Visual Analog Scale
CT	:	Computed tomography
bFGT	:	Basic Fibroblast GrowthFactor
HLA	:	Human Leukocyte Antigen
DFDBA	:	Demineralized Freeze Dried Bone Allograft
PTFE	:	Polytetrafluoroethylene
DBBM	:	Deprotienized bovine bone mineral
IL	:	Intereukin
TGF	:	Tumor Growth Factor

ABSTRACT

ABSTRACT

GBR has emerged as the most preferred technique for alveolar bone grafting since past few years. Resorbable membranes like HAM and collagen membrane, have gained popularity for GBR. Though both membranes have their own unique properties, there is a need to compare and evaluate the efficacy of Amniotic membrane with collagen membrane in GBR of alveolar bone.

A study was conducted which encompassed 8 patients with maxillary or mandibular alveolar bone deficiency. They were randomly divided into two groups, with 4 patients treated with Freeze dried irradiated HAM and 4 patients treated with Collagen membrane. The groups were compared according to the presence of swelling, infection, pain and change in alveolar bone height.

The degree of swelling was lower in the Freeze Dried Irradiated HAM group than the collagen membrane group, although the difference was found to be insignificant. A statistically significant result in favour of Amniotic membrane was found with regard to presence of infection and pain. There was no evidence of infection in the Freeze Dried Irradiated HAM treated group, however, there was a significant development of infection in the collagen membrane group. It was observed that the pain score was much higher in group treated with collagen membrane than in the group treated with freeze dried irradiated HAM. Alveolar bone height increased within both the groups over a period of 180 days. The two groups, however, did not show any significant differences.

When compared to Collagen, Amniotic membrane had good cell occlusivity, anti-inflammatory, antibacterial, osteoinductive and analgesic properties. Its low cost of procurement and storage makes it promising barrier membrane for GBR.

INTRODUCTION

INTRODUCTION

Reconstruction of resorbed alveolar ridge and bone defects poses a great challenge in maxillofacial surgery. The irreversible process of alveolar bone resorption commences as early as 6 months after the loss of tooth or its extraction. In the era where dental implants have gained immense popularity for oral rehabilitation, inadequate volume of bone would jeopardize its long term prognosis[1].

Despite of availability of several methods for bone reconstruction, they all possess specific indications and limitations[2]. Few of them are distraction osteogenesis, onlay and inlay grafting, sinus floor augmentation, bone growth factors. Despite being well documented and highly successful procedures, they still pose risk of postoperative infection, surgical difficulty and graft resorption. This is overcome by GBR technique. GBR is a surgical procedure for bone reconstruction that uses a Barrier Membrane to prevent epithelial migration into the osseous defects, eventually enhancing the regeneration of bone[3]. A successful design of a functional material to be utilized as GBR membrane should own the following characteristics:

1) biocompatibility

2) space maintainance

3) occlusivity

4) easy handling

5) bioactivation property[4]

Although different resorbable and non resorbable membranes have been developed and their uses have been meticulously investigated, research is still going on to develop the "ideal" membrane for clinical application[2].

Non Resorbable membranes like PTFE membranes are good for structural integrity during implantation yet simultaneously require a second surgical procedure for its removal, presenting a potential risk to newly generated tissue and secondary infection. Bioresorabable membranes on the other hand do not need second surgical intervention for removal. They secure the graft as well as can be moulded and pre-fabricated. These are of 2 types, natural membranes, made of collagen and synthetic membranes, made of aliphatic polyesters. They are radiolucent so allow imaging and their bioresorption eliminates potential effects of stress shielding of regenerated bone. Synthetic membranes may present moderate cytotoxic reaction during degradation[2], whereas Collagen Membranes are biocompatible, reduce the risk of infection, show host tissue adherence, biological space making ability, cell occlusion, and better clinical manageability. Conversely, Collagen membranes exhibit variable resorption rates ranging from 4 to 32 weeks and the choice of material depends on the intended use. If it is intended to be used for GBR, a more durable collagen membrane with longer resorption time may be used because of its reported 6 to 9-month resorption period. However, if the intended use is simply to control bleeding, products with the shortest reported resorption time of 10 to 14 days may be preferable[5].

Due to this variability and lack of control over the rate of membrane resorption of collagen membranes[3] , novel membranes like alginate membranes, degradable copolymers, hybrid as well as Amniotic membrane came into picture[2].

HAM is one of the oldest biomaterials used as a scaffold. It has specifically gained importance because of its ability to reduce scarring, inflammation and pain, enhance wound healing, anti-bacterial properties, cell proliferation, differentiation and epithialialisation, good mechanical strength[6], easy adaptability to surgical site, low immunogenicity[7], osteoinduction and osteoconductivity[8], easy availability and cost effectiveness. It is the innermost of fetal membranes, lining the amniotic cavity with a thickness of 0.02 to 0.5mm. It consists of three layers : an epithelial layer, a thick basement membrane and a mesenchymal layer. In the past few years, use of amniotic membrane has emerged out as an effective treatment not only

for soft tissue repair but also for hard tissue reconstruction. It is prepared with cryopreservation, glycerol preservation, Freeze drying (lyophilisation) and gamma irradiation and Peracetic acid/ethanol sterilisation[6] and can be sealed and stored at room temperature, protected from light upto six months.

The promising results shown by both collagen and HAM prompted us to design this study, comparing the efficacy of each for GBR of alveolar bone.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

AIM:

- To compare the efficacy of freeze dried irradiated HAM versus collagen membrane in GBR of alveolar bone.

OBJECTIVES:

- To assess the alveolar bone height in patients treated with freeze dried irradiated HAM and collagen membrane.
- To compare alveolar bone height between patients treated with freeze dried irradiated HAM and collagen membrane.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Gomes MF, dos Anjos MJ, Nogueira TO, Guimaraes SA (2001)[9] performed an investigation aimed at evaluating the osteoinductive property of autogenous demineralized dentin matrix on experimental surgical bone defects in the parietal bone of rabbits using the GBR technique incorporating HAM. They concluded that HAM had no interference in bone repair and was resorbed eventually. Slices of autogenous demineralized dentin matrix induced direct bone formation and were incorporated by the newly formed bone tissue and remodeled. The bone defects healed faster in the autogenous demineralized dentin matrix + HAM group than in the group with HAM only.

Kubo M, Sonoda Y, Muramatsu R, Usui M (2001)[10] studied the immunogenicity of amniotic membrane. They concluded that the immunogenicity of cryopreserved tissues is less than that of fresh tissues and that cryopreserved cells are expected to be nonviable. However, the amniotic membrane is originally fetal tissue, and some proportion of amniotic cells was still viable, even after cryopreservation. Thus it seemed to be immune-privileged tissue and contained immunoregulatory factors, such as HLA-G and Fas ligand.

Pruss A, Perka C, Degenhardt P, Maronna U, *et al* (2002)[11] used Peracetic acid/ethanol for sterilisation of amniotic membrane. Amniotic Membrane is sterilised with 2 % peracetic acid under the addition of 96 % ethanol under a negative pressure of 200 mbar and permanent agitation of the jar for 4 hours.

Wang HL, Boyapati L (2006)[12] described four major biologic principles (i.e., PASS) necessary for predictable bone regeneration: primary wound closure to ensure undisturbed and uninterrupted wound healing, angiogenesis to maintain blood supply and space and maintainance to allow bone ingrowth, and stability of wound for formation of blood clot and uneventful healing. In addition, a novel flap design and clinical cases using this principle were presented.

Von Arx T, Buser D (2006) analyzed the clinical outcome of horizontal ridge augmentation using autogenous block grafts covered with an organic bovine bone mineral and a bioabsorbable collagen membrane. 42 patients with severe horizontal bone atrophy, were augmented with an organic bovine bone mineral and a collagen membrane. The sites were re-entered after a tension-free primary wound closure and a mean healing period of 5.8 months, and the crest width was re evaluated prior before placement of implant. They concluded that the presented technique of ridge augmentation using autogenous block grafts with an organic bovine bone mineral filler and collagen membrane coverage demonstrated successful horizontal ridge augmentation with high predictability. The surgical method was further simplified by using a resorbable membrane. The collagen membrane showed ease of application, and no wound infection.

McAllister BS, Haghighat K (2007)[14] reviewed the techniques for reconstruction of bony defects that included the use of particulate bone grafts and bone graft substitutes, barrier membranes for guided bone regeneration, allogenic and autogenous block grafts, an distraction osteogenesis. The approach largely depended on the extent of the defect and specific procedures to be performed for the implant reconstruction. Best research based approach was used when a treatment plan was developed for augmentation of bone.

Llambés F, Silvestre FJ, Caffesse R (2007)[15] performed Vertical Ridge Augmentation on 11 patients at the time of implant placement. The exposed implant was covered with autogenous bone graft, and a slow resorbing collagen membrane was placed. Primary closure was done with horizontal mattress and interrupted sutures. 4 to 6 months later, a second-stage surgery was performed , and placement of healing abutments was done. At stage 1 and 2 surgeries, the length of the implant out of bone was determined on a periapical x-ray 1 year after loading of implant. At second-stage surgery, histology was obtained from one case. They concluded that there is a potential to promote vertical ridge augmentation with slow resorption collagen membranes when used with autogenous bone at the time of implant loading.

Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, et al (2008)[7] reviewed the properties of amniotic membrane as a scaffold for tissue engineering. The special structure and biological viability of the Amniotic Membrane allows it to be an ideal

candidate for creating scaffolds used in Tissue Engineering. Epithelial cells derived from the Amniotic Membrane have the advantages of stem cells, yet are a more suitable source of cells for Tissue Engineering than stem cells. The components of the basement membrane create provide a framework for Tissue Engineering. They concluded that Amniotic Membrane had biological properties important for Tissue Engineering, including anti-inflammatory, anti-microbial, anti-fibrosis, anti-scarring, as well as reasonable mechanical property and low immunogenicity, which made it suitable for tissue engineering.

Samandari MH *et al* (2011)[16] investigated the effects of amniotic membrane in bone induction and wound healing after vestibuloplasty surgery on animal samples while receptacle proteins such as growth factors facilitate wound healing and bone induction. The authors indicated that the Amniotic Membrane is a suitable cover for different injuries and acellular Amniotic Membrane has the potential for rapid improvement and bone induction. The components of Amniotic Membrane are collagen, and fibronectin, and laminin, that help in bone induction. This substrate promoted bone induction and would contribute to induction of the progenitor cells and/or stem cells in the area where surgery had been undertaken and is also differentiated into bone.

Cordaro L, Torsello F, Morcavallo S, di Torresanto VM (2011)[17] performed a study to amalyse the effect of use of DBBM and collagen barrier membrane in combination with mandibular bone block grafts were evaluated in reducing bone block graft resorption during healing. Twenty-two ridges presenting horizontal alveolar deficiency (crest width <4mm) and at least two adjacent missing teeth were included in the study. In the control group, one or multiple mandibular blocks were used to gain horizontal augmentation of the ridge. In the test group, DBBM granules were added at the periphery and over the graft. The reconstructions were covered by two layers of Collagen Membrane . Implants were placed 4 months after grafting. Direct measurements of crest width were performed before and immediately after bone augmentation, and immediately before implant placement. The results from this study showed that the addition of bovine bone mineral and a Collagen Membrane around and over a mandibular bone block graft could minimize graft resorption during healing. On the other hand, the use of bone substitutes and barrier membranes in

combination with block grafts increased the frequency of complications and the difficulty of their management.

Rodella LF, Favero G, Labanca M (2011)[18] reviewed the available informations on regenerative bone technique using reasorbable membranes and bone grafts. In particular, biocompatibility, immunological response, tissue reaction, reabsorption time and histological features of materials daily use in dentistry and in maxillofacial surgery were emphasized. They concluded that In GBR technique, many graft materials can be chosen and many relative factors had to be considered, such as bone defect site, surgical objective, patient examination and knowledge of graft materials. The graft materials had to induce inflammation responses and they had to be osteoconductive to maintain trophism under the membrane and rapid reabsorption. The results and performances obtained by different biomaterials (membranes and grafts) did not underline clear differences within bone regeneration induced by heterologous materials from animal origin or synthetic materials. There were no significant differences, reported in literature, in the use of animal heterologous grafts or synthetic alloplastic grafts. Nevertheless, a correct choice had been fundamental to minimize the possibility of disease transmission and development; in particular, synthetic biomaterials are better compared to heterologous animal biomaterials, which had a higher risk of inflammatory reactions and disease trasmission.

Saad M, Assaf A, Maghaireh H (2012)[3] firstly defined the GBR concept and identified different used materials. Secondly, and after performing a literature review on the application of GBR in different clinical situations, some hints and tips concurring to attain optimal results were suggested. Finally, this paper tested the level of available evidence when using GBR. Within the limits of this mini-review which aimed to analyze the outcome of the use of GBR for hard tissue reconstruction, it was concluded that GBR was successful treatment modality for dehiscence-and fenestration type defects around dental implants. As for using GBR in a staged approach for horizontal and/or vertical bone augmentation, some of the studies revealed a high percentage of success. However, many of them had a short-term follow-up. Moreover, complications raised with vertical reconstructions, while in the case of horizontal augmentation, studies showed less complications.

Arai N, Tsuno H, Okabe M, Yoshida T, *et al* (2012)[19] proposed a novel technique involving initial hyper-drying instead of freeze drying. After being washed, the amnion was dried under consecutive far-infrared rays and microwaves at temperatures lower than 60 °C by using a hyper-drying device. Final sterilisation was performed with 25 kGy gamma radiation.

Tsuno H, Arai N, Sakai C, Okabe M, *et al* (2014)[20] used Hyperdry amniotic membrane, a novel preservable material derived from the human amnion to treat two cases of intraoral alveolar wounds with bone exposure successfully.

Kesting MR, Wolff KD, Nobis CP, Rohleder NH (2014)[6] reviewed and underlined the versatile properties of HAM and also pointed out the need for more clinical evidence the field of maxillofacial surgery for its indications. They explained Amniotic Membrane to be the innermost of the fetal membranes lining the amniotic cavity. With a thickness of 0.02 to 0.5 mm, the human amnion consists of five layers. A single cell layer, which rests on its basement membrane, is in contact with the amniotic fluid. The underlying connective tissue attaching the basement membrane comprises another three layers, namely a compact layer, the fibroblast layer and the spongy layer, which in turn is connected with the cellular layer of the chorion. Amniotic Membrane reveals to be a transparent, thin, avascular composite membrane composed of three major layers: an epithelial layer, a thick basement membrane, and mesenchyme. It produces TGF-ß and bFGF growth factors that are regarded as being significant for epithelial regrowth. They also explained that the application of Amniotic Membrane to wounds markedly reduces the pain intensity experienced by the treated patients. This resulted from the adherence of the amnion to the lesion and the coverage of exposed nerve endings. In addition, the adherence of Amniotic Membrane stops the contact of lesions with the environment, whereas its porosity allows the evaporation of wound fluid. These mechanisms were proposed to decrease plasma loss and are advocated to prevent infection and sepsis. The antimicrobial effect of amniotic membranes in vitro was attributed to their close adherence to the wound surface preventing further contamination. Their study investigated amniotic tissue and cells that demonstrated the mRNA expression of antimicrobial peptides, such as human-ß-defensin-3, and cytokines, such as IL-10, which was known to be a potent inhibitor of inflammation.

Almazrooa SA, Noonan V, Woo SB (2014)[5] described 6 cases of retained resorbable collagen membranes noted in oral curettage specimens, and described the histopathology of this exogenous material. They found Collagen to be an insoluble fibrous protein that was an essential component of the connective tissue stroma. There were at least 16 types of collagen found in interstitial tissues, matrix of bone, cartilage, epithelial and blood vessel basement membrane, and the vitreous of the eye among others 10. Type I, II and III collagen comprise 80-90% of the body's collagen; commercially available collagen products were composed mainly of Type 1 collagen. Many of the most commonly used collagen membranes were derived from bovine tendon-. Cross-linkage between collagen molecules (such as with formaldehyde or glutaraldehye) during the manufacturing process strengthens the collagen fibrils, increases their stability, prolongs resorption time, and increased its biocompatibility. Resorption of collagen membranes occured through biodegradation by inflammatory cells. In 1985, the Food and Drug Administration approved CollaCote , a rapidly resorbable collagen membrane derived from bovine tendon; subsequently many of the other commercially available membranes were approved in 1990. Resorbable collagen membranes are commercially available as membranes alone or impregnated with other materials such as bone morphogenic protein. Resorbable collagen membranes are frequently used as wound dressings because they act as a scaffold, promote platelet aggregation, stabilize clots, and attract fibroblasts, facilitating wound healing; they are therefore often used for GBR. Other applications included ridge augmentation and grafting of extraction sockets, as well as for sinus lift procedures, the repair of sinus membrane tears, soft tissue recontouring and GBR during apicoectomy, with implants for bone deficient sites. Collagen membranes exhibit variable resorption rates ranging from 4 to 32 weeks and the choice of material depends on the intended use. If the intended use is GBR, a more durable collagen membrane with longer resorption time may be used because of its reported 6 to 9-month resorption period. However, if the intended use is simply to control bleeding, products with the shortest reported resorption time of 10 to 14 days may be preferable. They concluded that although resorbable collagen membrane had been fairly rapidly resorbable, this material sometimes persisted within wound sites without any obvious foreign body reaction.

Liu J, Kerns DG (2014)[21] in an attempt to minimize or prevent post-extraction bone resorption and to preserve ridge integrity, recommended to place a space maintaining

graft in the alveolus at the time of extraction. They reviewd and utilised various ridge preservation techniques and materials and methods of GBR and concluded that it can be achieved with using particulate autogenous bone grafts, allografts, xenografts, or alloplasts grafting materials and resorbable or non-resorbable barrier membranes techniques Their study suggested that most of the commercially available collagen membranes were developed from type I collagen or a combination of type I and type III collagen. The source of collagen comes from tendon, dermis, skin or pericardium of bovine, porcine or human origin. They listed the advantages of the resorbable collagen membranes including-Hemostasis, chemotaxis for periodontal ligament fibroblasts and gingival fibroblasts, weak immunogenicity, easy manipulation and adaption, direct effect on bone formation ability to augment tissue thickness.

Li W, Ma G, Brazile B, Li N, *et al* (2015)[22] performed a study and their results demonstrated that both the collagen membrane and the lyophilized 8-layer Acellular Human Amniotic Membrane acted effectively as a shielding layer to prevent the invasion of the fibrous tissue, and promoted bone-to-implant connection when compared with the Bio-oss only repairing (no membrane coverage). Moreover, the lyophilized acellular HAM barrier membrane further induced the massive bone growth and maturation when compared with the collagen membrane. The authors developed a new barrier membrane produced from lyophilized multilayered acellular HAM. The advantages of the acellular HAM barrier membranes were excellent biomechanical properties, preservation of natural extra cellular matrix structure and composition, easiness in preparation and handling, flexibility in adjusting the thickness and mechanical properties to suit the application, and efficiency in inducing the massive bone growth and avoiding fibrous tissues invasion.

Kumar A, Chandra RV, Reddy AA, Reddy BH, *et al* (2015)[23] evaluated the anti-inflammatory, anti-infective and clinical properties of amniotic membrane when used for GTR in contained interdental defects. From this trial conducted over a period of 24 weeks, Amniotic Membrane demonstrated a marked anti-inflammatory effect and its use resulted in an improvement in periodontal parameters. Amniotic Membrane potentiates to function as a barrier for GTR and the unique properties associated with this material augmented its potential as a matrix for periodontal regeneration.

Sowjanya NP, Rao N, Bhushan NS, Krishnan G (2016)[24] studied about the versitality of the use of Collagen Membrane in Oral Cavity. This study was conducted to evaluate the clinical efficacy of collagen membrane as a biological dressing material for intraoral wounds, to check for haemostasis, pain control, granulation tissue formation, rapid re-epithelialization and minimal contracture. A total of 30 patients 19 male, 11 female were taken for excision of various intraoral lesions like leukoplakia patches, mucocele, epulis growths, irritational fibroma, frenectomy and the surgical defects were closed with collagen membrane. Postoperatively healing was assessed by taking five clinical parameters of Haemostasis, Pain, Granulation tissue, Epithelialization, Contracture. Authors concluded that reconstituted bovine derived collagen membrane used in the study was found to be an effective intraoral wound dressing material for faster uneventful healing of intraorally also.

Hassan M, Prakasam S, Bain C, Ghoneima A, Liu SS (2017)[25] did a comparison of amnion chorion membrane vs. dense polytetrafluoroethylene membrane in ridge preservation procedures. The purpose of this study was to examine if a biologically active commercially available amnion chorion membrane was as effective as the commercially available inert dense PTFE in preserving jaw bone dimensions and whether it provided the added benefit of reducing post-operative discomfort after dental surgery. The authors proved the hypotheses for the study that the use of Amnion chorion membrane in preservation of bone dimensions in extraction socket resulted in greater remaining horizontal and vertical ridge dimension, and reduced postoperative discomfort when compared with dense PTFE in sites where extraction socket were not closed by advancing the gums.

Khojasteh A, Kheiri L, Motamedian SR, Khoshkam V (2017)[1] reviewed to categorize and assess various GBR approaches for the reconstruction of human alveolar bone defects. This review introduced a therapeutically oriented classification system of GBR for treating alveolar bone defects. High heterogeneity among studies hindered drawing definite conclusions in regard to superiority of one to the other GBR technique.

Schnutenhaus S, Doering I, Dreyhaupt J, Rudolph H *et al* (2018)[26] conducted a trial to test the hypothesis that dimensional changes in the alveolar bone after tooth extraction would be reduced by inserting an equine collagen membrane and a collagen

cone to fill and seal the alveolus , in comparison to extraction with untreated alveoli. They concluded that the proposed hypothesis that inserting a combination material comprising a collagen cone and membrane led to a difference in alveolar bone preservation could be accepted for the clinically relevant buccal distance. In this area, implantation of the collagen material led to significantly less alveolar bone resorption.

Al-Askar M, Alsaffar D (2018)[27] proposed a case report demonstrating the feasibility of using allograft bone with a resorbable collagen membrane to correct an alveolar ridge defect and achieve a highly esthetic restoration. A 30-year-old woman with advanced periodontal vertical bone loss and periodontally hopeless upper left right premolar required a fixed restoration. A staged surgical strategy was devised. First, a resorbable collagen membrane and allograft bone grafts were used to guide the bone regeneration in the vertical alveolar defect. After 6 months, complete bone regeneration was achieved and the dental implants were submerged in the bone. Three months later, the implants were exposed and subsequently restored with a crown. The vertical GBR strategy of using allograft bone and a resorbable collagen membrane was found to have the potential to eliminate the need for additional procedures, which were required with non-resorbable membranes, sinus lift procedures, and extensive block graft procedures.

Koushaei S, Samandari MH, Razavi SM, Khoshzaban A, *et al* (2018)[28] evaluated the bone induction effects of an amnion membrane protected graft compared with collagen membrane protected graft in repair of tibial bony defects in dogs. This study was performed using the tibial bone of dogs. Authors found out that osteogenesis in amnion membrane group was better than collagen group but not statistically significant. Using the amniotic membrane appeared to accelerate the bone formation in GBR.

Elangovant R (2019)[29] discussed the properties of amnion derived cells. He concluded that the high tensile strength and the elasticity of Amniotic Membrane makes it an ideal membrane to withstand mechanical intra-uterine stress. The molecular basis was provided by the elastin detected in Amniotic membrane. Amnion-derived cells also have the potential to differentiate into all three germ layers: endoderm, mesoderm and ectoderm. Surface markers associated with embryonic stem cells, such as stage-specific embryonic antigen 3 and 4 and TRA-1-60 and TRA-1-81,

were expressed by amniotic cells. The pluripotent potential to differentiate was underlined to be both epithelial and mesenchymal amniotic cells also express various stem cell markers such as octamer-binding transcription factor 4, hepatocyte nuclear factor 3ß, nanog and nestin. The author also described methods of preparation and preservation of these membranes by Cryopreservation and glycerol preservation, Freeze drying (lyophilisation) and gamma irradiation, and Peracetic acid/ethanol sterilisation. Freezing of the Amniotic membrane was done by passing through liquid nitrogen at -196°F. The Cooling process made the membrane void of microorganisms, immunologically inert material without antigenicity and preserves the membrane for an indefinite time, Cryopreservation with dimethyl sulphoxide at -80°C allowed retention cells in the Amniotic Membrane at approximately 50% for several months. The several angiogenic growth factors and cytokines were removed during cryopreservation of the Amniotic Membrane. The author noted that the storage of the Amniotic Membrane in glycerol at 4°C resulted in immediate cell death. On the other hand for freeze drying, after obtaining the Amniotic membrane from the placenta it was pasteurized at 60 degree celsius, cleansed and treated with 70% ethanol[22], and then was freeze-dried at -60°C under vacuum (atmospheric pressure 102) for 48 hours. Then it was irradiated with 2.5 megarads (25 K Gray) inside a batch type cobalt-60 irradiator. By the method of freeze-drying, sublimation of liquid moisture of membrane to the gaseous state took place without having undergone the intermediate solid stage. By this method, the membrane maintained its original size and shape by minimizing cell rupture. It was sealed and stored at room temperature and protected from light up to 6 months. The freeze-dried membrane can be used immediately after soaking it in normal saline for 1 minute.

Etchebarne M, Fricain JC, Kerdjoudj H, Di Pietro R, *et al* (2021)[30] published a meticulous review on the therapeutic benefits of Amniotic Membrane and amniotic membrane-derived products for bone defect healing. They concluded that the Amniotic Membrane and its derivatives were an attractive source of biological tissue and stromal cells for bone regeneration. Thanks to its low immunogenicity, Amniotic Membrane and its derivatives were used either as a xenograft or as an allograft. The lyophilized or decellularized lyophilized Amniotic Membrane are a promising alternative to the commercial membranes used for GBR procedures and achieved satisfactory outcomes in oral and maxillofacial surgery. Amniotic Membrane was

mainly applied as a single layer and provide better results when used as a membrane covering the defect rather than as a filling material. It was found better to decellularize Amniotic Membrane to enhance its potential to act as a natural scaffold seeded with primary cells before its implantation in bone defects. Amniotic Membrane derived stromal cells also showed their potential to be used successfully in the field of bone regenerative medicine.

MATERIAL AND METHODS

MATERIALS & METHODS

Place of study

The present study was conducted in the Department of Oral and Maxillofacial Surgery, Babu Banarasi Das College of Dental Sciences, B.B.D University, Lucknow after obtaining clearance from Institutional Ethical Committee.

Study Design

A prospective, randomized, single center study was performed among patients with maxillary or mandibular alveolar bone deficiency. Patients were selected as per the inclusion criteria, reporting to the out-patient department of Oral and Maxillofacial Surgery, Babu Banarasi Das College of Dental Sciences, Lucknow.

Sample size

Total (n=8) Patients were divided into two groups-

1. Group A (n-4) -Placement of freeze dried irradiated HAM
2. Group B (n-4) -Placement of Collagen Membrane

Clearance was obtained from the Research Committee & Institutional Ethical committee of Babu Banarasi Das College of Dental Sciences. All patients involved in the study were informed of the procedure and gave a written consent to proceed.

Freeze dried irradiated HAM was procured from TATA memorial hospital, Mumbai. Thickness of the membrane is in the range of 0.02 to 0.5mm.

Eligibility Criteria

Inclusion Criteria

- Patient with maxillary or mandibular alveolar bone deficiency.
- Patient that fall in the age group of 18 to 60 years.
- Patients with a fairly good general health without any contraindication for minor oral surgery and/or local anesthesia, and its components.
- Patient who gave their written informed consent for the surgery and agreed for 6 months follow up.

Exclusion Criteria

- Patient with systemic disease which may have its significance in normal healing.
- Patient unwilling for the surgery.

Material Required

- Mouth mirror and probe
- Metallic scale and divider
- Non elastic measuring thread
- Metzenbaum scissor
- Periosteal elevators -Howarths and Molts
- Tissue holding forceps
- Dappen dish
- Spatula
- Bone graft substitute
- Freeze Dried Irradiated HAM (TATA memorial Hospital, Mumbai)
- Collagen Membrane (Commercially available)
- Suture cutting scissors
- Needle holder
- Bard Parker handle- No. 3

- Blade- No. 15
- Suture materials- 3-0 Mersilk
- Disposable syringes
- Povidone Iodine

Amniotic membrane

Procurement of Freeze dried and irradiated HAM (2× 2.5cm) was done from a commercial tissue bank (Tata Memorial Hospital-Tissue Bank, Mumbai, India). The tissue complies with the International Atomic Energy Agency recommendations and the Asia Pacific Association of Surgical Tissue Banks standards. Storage of the Amniotic Membrane can be done at room temperature because of its stable nature.

Fig 1. Image showing Freeze dried irradiated HAM

Collagen Membrane

Collagen membrane used was commercially obtained- a bioabsorbable GTR membrane derived from bovine source, which is non-friable, resorbable barrier membrane obtained from highly purified Type- l collagen obtained from standardized and authorized animals and is highly purified to prevent any antigenicity. The sizes of the membrane used in the study were -

- 10X15mm
- 15X20mm

Fig 2. Figure showing Collagen Membrane

Bone Graft Material

The graft material used was a commercially available synthetic bone graft substitute, which was prepared by wet chemical processing.

It is biocompatible, Osteoconductive and non- immunogenic, particulate bone grafting material.

METHODOLOGY

Pre surgical records

- Detailed medical history was taken, whether the patient was suffering from any major systemic disease (uncontrolled diabetes, hemophilia, hypertension, myocardial infraction etc.) and any past allergy due to any drug or food. Detailed dental history including previous restorative, periodontal, endodontic treatment reasons for loss of teeth or experience with orthodontic appliance and dental prosthesis was taken.
- General examination, extraoral examination, intraoral examinations was done and diagnostic records (periapical radiograph, facial measurements) were obtained before surgery. From the radiograph, the height of bone was measured and recorded in the case sheet.

Pre-surgical protocol

- Patients were prescribed prophylactic antibiotics, 2 gm. of amoxicillin or 600 mg. of clindamycin one hour prior to the surgery.

- Part preparation of patient was done extraorally with savlon followed by betadine solution and intraorally with betadine solution, then patient was draped with sterile drape.
- Local anesthesia (2% Lignocaine hydrochloride with 1:80,000 adrenaline) was administered to anesthetize the surgical site by suitable nerve block.

Surgical procedure

- Surgical access was achieved by making an incision through the tissue overlaying the defect upto the alveolar bone.
- After incision, hand instruments (Molts and Howarth's periosteal elevator) were used to elevate a full-thickness mucoperiosteal flap, giving direct visual access to the surgical site.
- Thorough degranulation and root planning of the bone defect was accomplished with the help of appropriate site-specific curettes.
- The defect was filled with particulate bone graft mixed with blood and condensed properly with the help of bone scoop and condenser.
- In Group A patients, hydrated freeze dried irradiated HAM, cut according to the size of the defect, was placed over well condensed bone graft.
- Same procedure was followed for placement of collagen membrane in Group B patients.
- Flaps were reapproximated and primary closure was achieved with the help of suturing using 3-0 Mersilk.

Post Operative Guidelines

- Injection dexamethasone 8mg was administered intravenously, post operatively.
- Patients were prescribed Tab-Cefixime 200mg twice daily or Augmentin 625mg twice daily for 5 days and tab-Diclofenac twice daily for 3 days per orally.
- Instructions were given and Chlorhexidine mouthwash was prescribed.
- Sutures were removed on 7^{th} post surgical day.

ASSESSMENT PARAMETERS

1. Radiographic Evaluation

Height of the bone assessed by Intra oral Periapical Radiograph- Pre-operatively, postoperatively (immediate post operative, 7^{th} day, 30^{th} day, 90^{th} day and 180^{th} day).

2. Clinical Evaluation

i. Swelling- Pre-operatively, 1^{st} day, 7^{th} day, 14^{th} day post operatively

ii. Infection - at 7^{th} day, 14^{th} day post operatively

iii. Pain (VAS) – at 1^{st} day, 7^{th} day, 14^{th} day post operatively

HEIGHT OF ALVEOLAR BONE (*Mardas et al)*[31]

- Standardized digital radiographs were obtained with the paralleling/long-cone technique at preset parameters using a commercially available RVG system.
- Height of alveolar bone was evaluated pre-operatively and postoperatively (immediate post-operative, at 7^{th} day, 30^{th} day, 90^{th} day and 180^{th} day) with the help of IOPAR using digital calipers on Illustrator software (Version 23.1.0).
- The linear measurement to evaluate bone height was the line drawn from the cervical margin of the adjacent tooth to the mesial, distal or central most depth of the deficient site. So, any decrease in linear measurement indicated an increase in actual bone height of that area and vice versa.
- The central linear measurement was taken from midpoint of the distance between distal aspect of the cervical line of adjacent proximal tooth and mesial aspect of the cervical line of adjacent distal tooth from the deficient site.
- The Mesial Linear measurement was taken from cervical line of the adjacent mesial tooth to depth of the deficient site.
- The Distal Linear measurement was taken from cervical line of the adjacent Distal tooth to depth of the deficient site.
- The total change in alveolar bone height was then compared to pre operative findings in both groups periodically.

- The periodic mean change in alveolar bone height was then compared between both the groups.

SWELLING

Presence or absence of swelling was checked visually, Pre-operatively, 1st day, 7th day, 14th day post operatively.

In the preoperative period, distances between various groups of guide points in all patients were measured with a flexible non elastic thread. All measurements were evaluated by a single observer. The patients were seated in an upright position and instructed to close their mouth. Measurements were obtained at rest and with no gestures. Men were evaluated with shaved beard and shoulders because these would have affected the evaluation of extraoral edema.

Laskin's[32] method of measuring facial swelling

Laskin's method, used by many researchers, involves recording of three measurements during the evaluation period of 7 days. These measurements were distributed at the following duration: firstly, immediate post operatively, then 24 hours post operatively, and later, 7 days after the removal of sutures. In order to measure the development of the inflammation at the determined points, they were marked with a dermographic pencil and a 00-thickness suture thread fixed with two clamps. Measurements were made between the these points marked previously with the dermographic pencil. Following are the reference points and distances measured in centimetres -

- The distance measured from the lowest edge of the earlobe to the midpoint of the symphysis Hirota, called as horizontal distance to the symphysis.
- The distance measured from the lowest edge of the earlobe to the external corner of the mouth, called as horizontal distance to the corner.
- The distance measured from the palpebral outboard angle to the goniaco angle, called as vertical distance.

The distance between the tragus rim corners and between the outer canthus of the eye and the angle of the mandible, defined by **Amin and Laskin**, were modified for the study for simplification. Instead, the selected guide points were-

Ala, Tragus and corner of the mouth.

A non elastic thread was used to measure-

1. Ala-tragal distance on the affected side.
2. Distance between tragus and corner of the mouth on the affected side.
3. Distance between tragus and chin on affected side only in mandibular alveolar bone defects.

Post operative measurements were substracted from the pre operative measurements to get the net inflammatory edema value.

- Present-scored as 1
- Absent-scored as 0

INFECTION

Infection was checked by presence or absence of inflammation and purulance at the surgical site at 7^{th} day and 14^{th} day post operatively.

Presence of redness, raised localised temperature, purulent discharge, and tenderness was checked.

Infection was recorded as follows-

- Present-scored as 1
- Absent-scored as 0

PAIN

The pain was assessed using ***VAS scale***[33]. The pain VAS is a continuous scale that consists of a horizontal or vertical line that is usually 10 centimetres (100 mm) long and is pegged with two verbal descriptions, one for each symptom extremes. For measurement of the intensity of pain, the scale was pegged with “no pain” (score of 0) and “pain as bad as it could be” or “worst imaginable pain” (score of 100 [100-mm scale]).The patient filled out the pain VAS on their own. The patients were instructed to draw a line perpendicular to the VAS line at the point on the scale that was depicting

their pain intensity most accurately. The score was assessed by with the help of a ruler, measuring the distance (cm) on the 10-cm line between the "no pain" anchor and the patient's mark, providing a range of scores from 0–10. The pain score that was recommended -

no pain = 0 cm

mild pain = 1-3 cm

moderate pain = 4-6 cm

severe pain = 7-10 cm

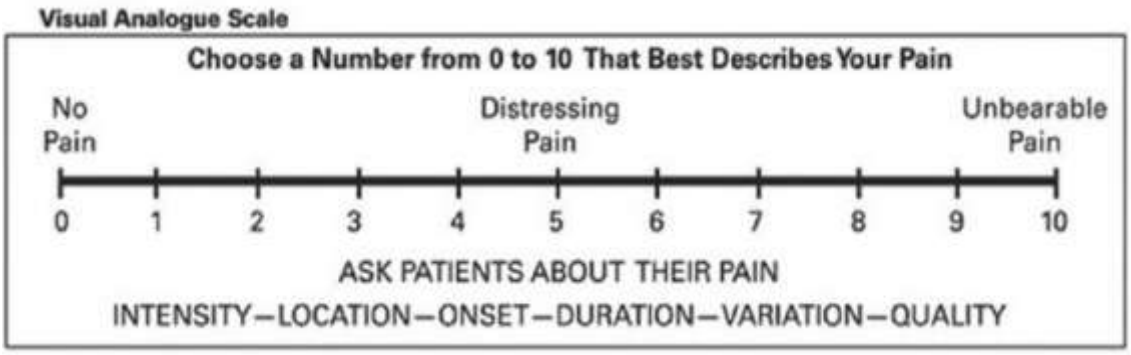


Fig. 3 showing visual analog scale

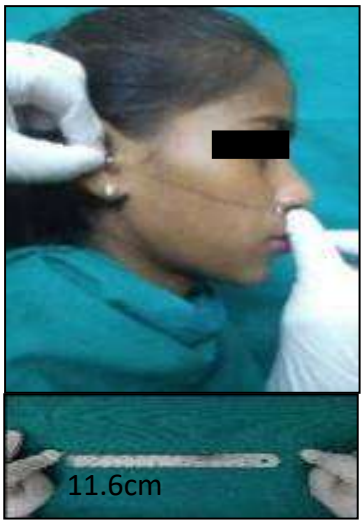
STATISTICAL ANALYSIS

The data was recorded in a preformed case sheet, according to the parameters mentioned and was tabulated and statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 22.0 statistical Analysis Software. Data were summarised as Mean ± SD (standard deviation). The comparison between the groups was done by applying independent Student's t test. Groups were also compared by two factor repeated measure (RM) analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was performed with the help of Tukey's HSD (honestly significant difference) post hoc test after assuring normality using Shapiro-Wilk's test and homogeneity of variance

between groups was tested using Levene's test. Discrete (categorical) data were summarised in number (n) and percentage (%) and compared by chi-square ($\chi 2$) test. A two-tailed ($\alpha=2$) $P < 0.05$ was deduced to be statistically significant.

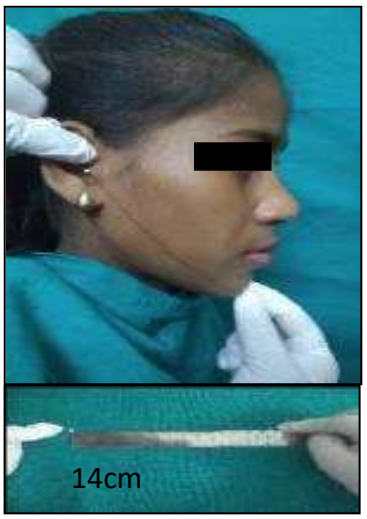
PHOTOGRAPHS

GROUP A



4.Tragus to ala

5.Tragus to corner of mouth



6.Tragus to chin

Fig. 4,5,6 -Showing pre operative swelling measured with different guide points

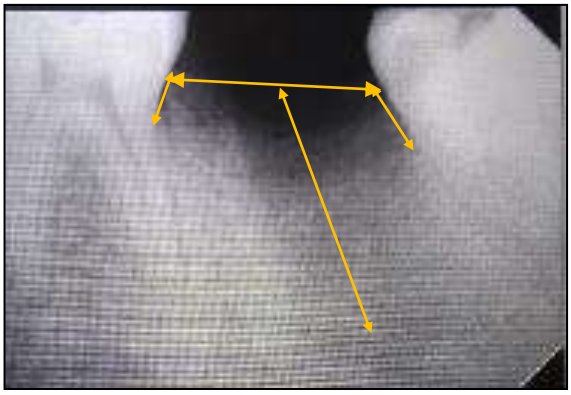
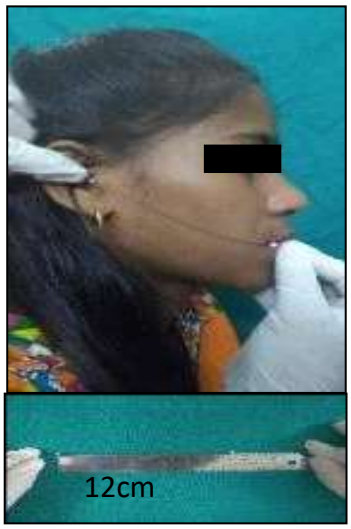


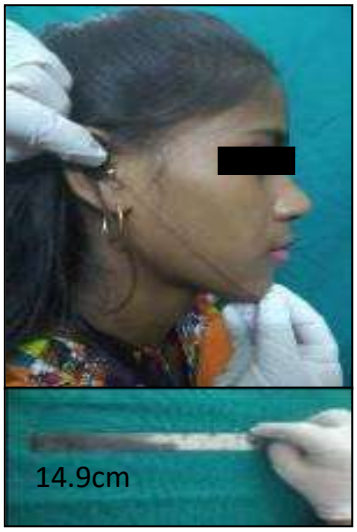
Fig. 7 -Height of bone- Pre operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP A

8. Tragus to ala



9.Tragus to corner of mouth



10. Tragus to chin

Fig. 8,9,10 -Showing 1st day post-operative swelling measured with different guide points

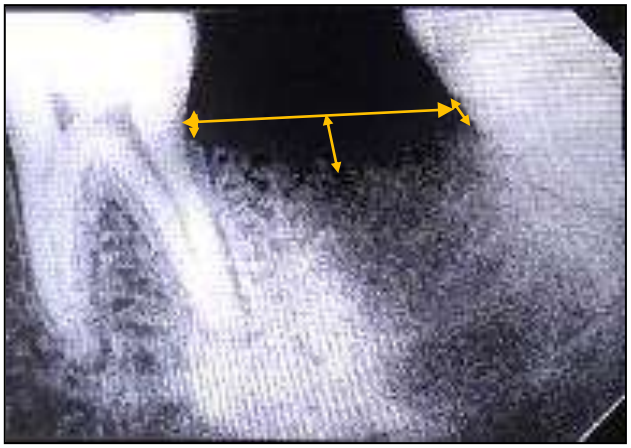


Fig. 11 -Height of bone- Immediate post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP A

12.Tragus to ala

13.Tragus to corner of mouth

14.Tragus to chin

Fig. 12, 13, 14 -Showing 7th day post-operative swelling measured with different guide points

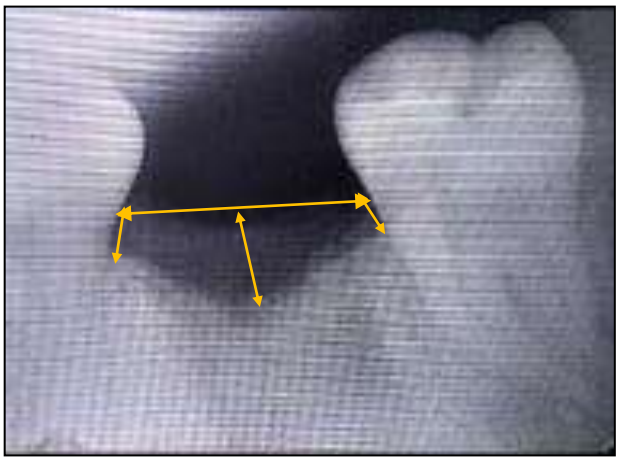
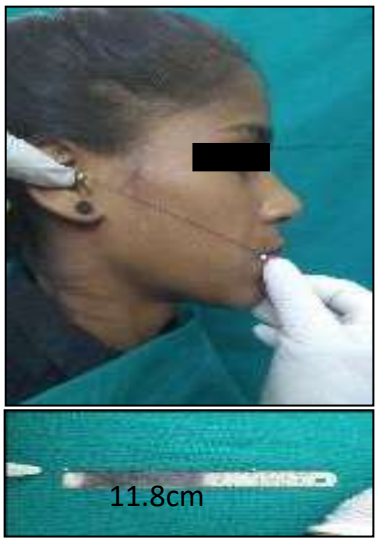


Fig. 15-Height of bone- 7th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP A

16.Tragus to



17.Tragus to corner of mouth

18.Tragus to chin

Fig. 16,17,18 -Showing 14th day post-operative swelling measured with different guide points

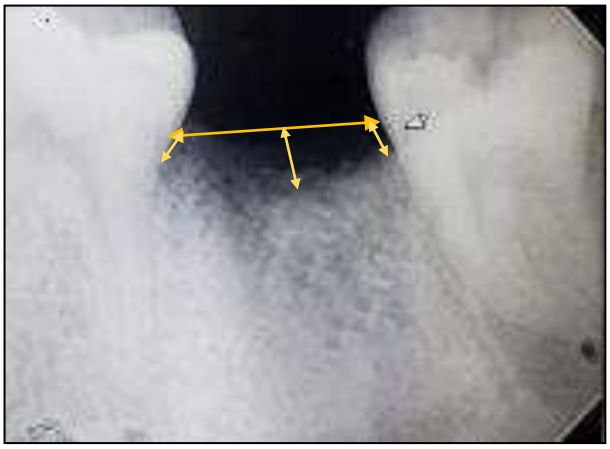


Fig. 19-Height of bone- 30th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP A

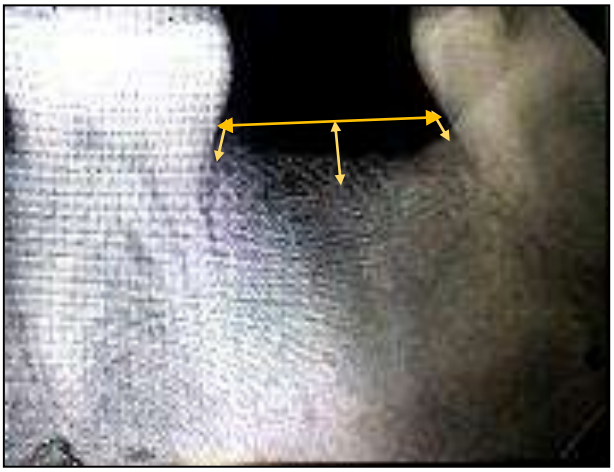


Fig. 20 -Height of bone- 90th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

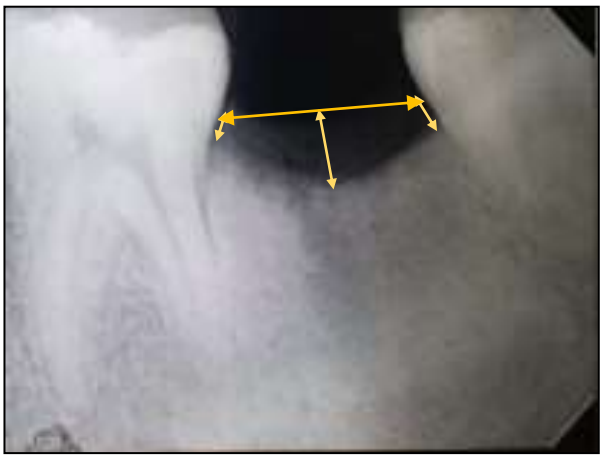
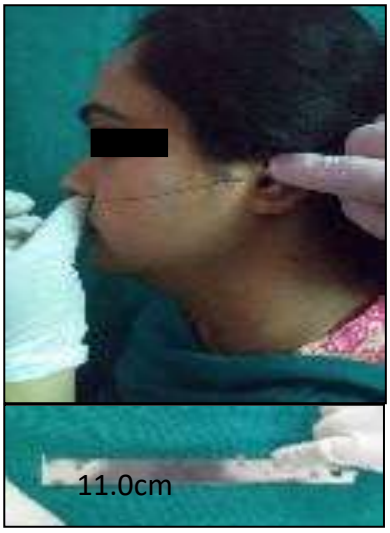
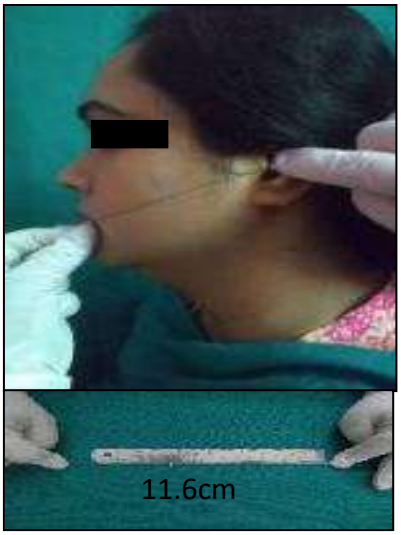


Fig. 21-Height of bone- 180th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP B



22 Tragus to ala



23 Tragus to corner of mouth

Fig. 22,23 -Showing pre operative swelling measured with different guide points

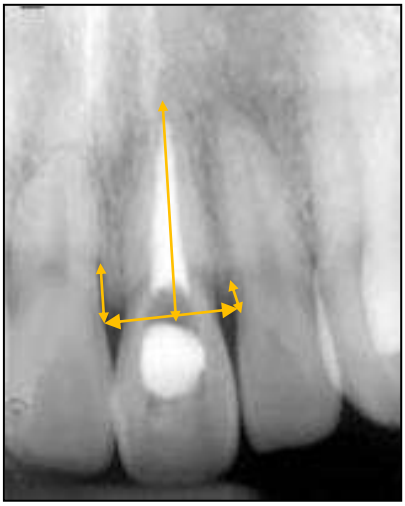
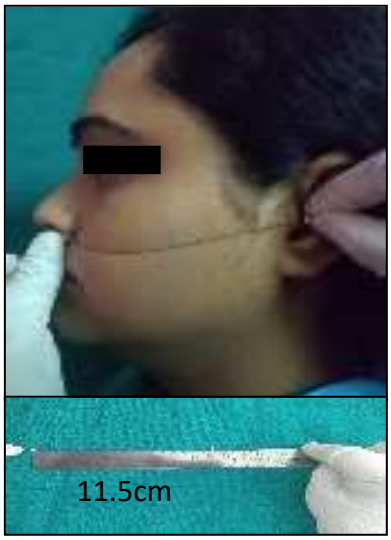
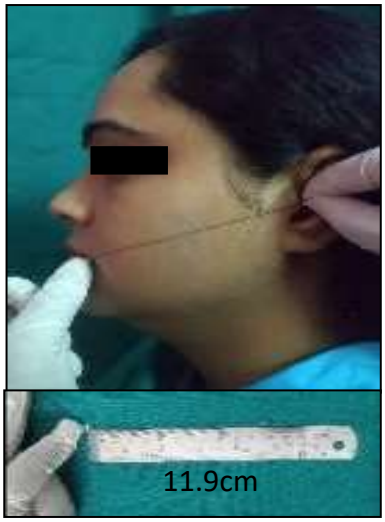


Fig. 24 -Height of bone- Pre operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP B



25 Tragus to ala



26 Tragus to corner of mouth

Fig. 25,26 -Showing 1st day post-operative swelling measured with different guide points

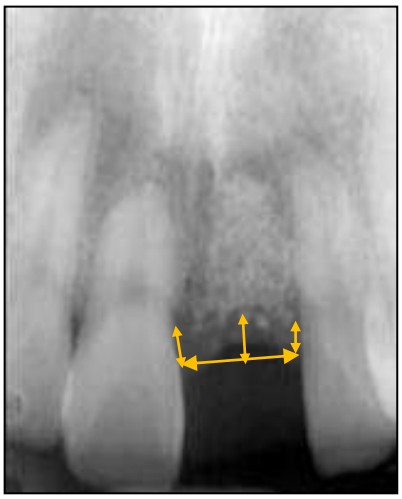
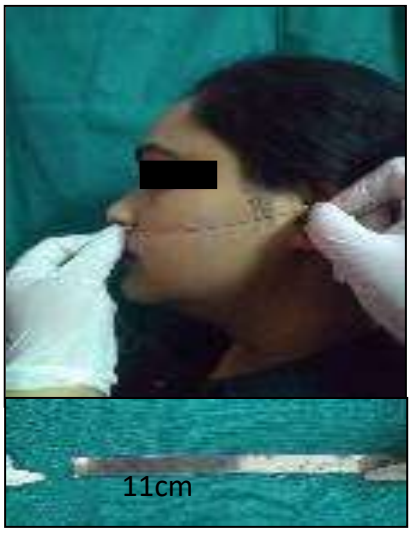
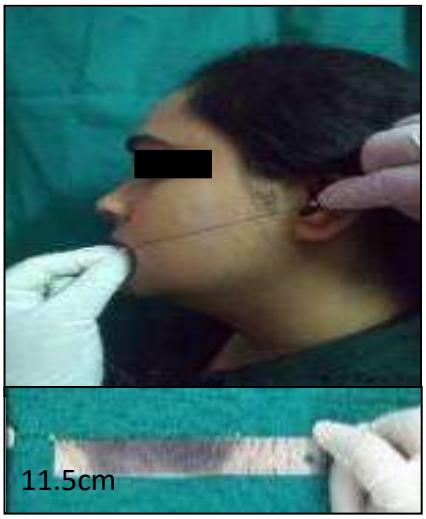


Fig. 27 -Height of bone- immediate post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP B



28 Tragus to ala



29 Tragus to corner of mouth

Fig. 28,29 -Showing 7th day post-operative swelling measured with different guide points

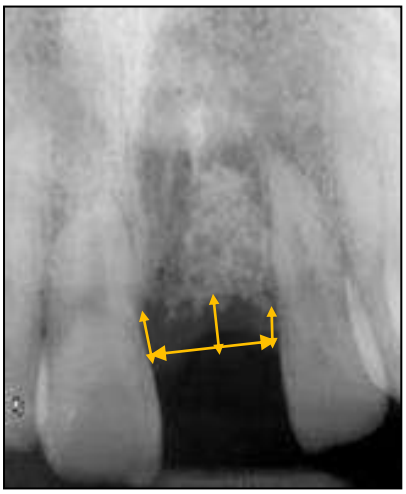
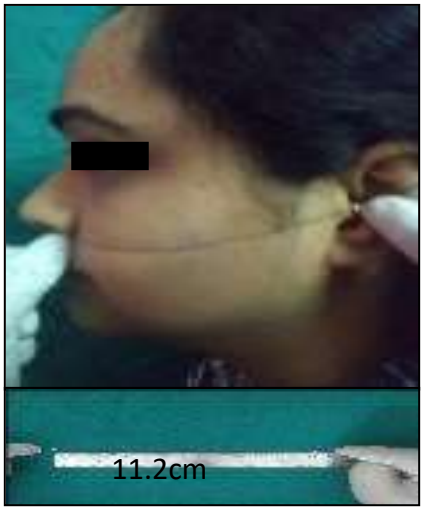


Fig. 30-Height of bone- 7th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP B



31 Tragus to ala

32 Tragus to corner of mouth

Fig. 31,32-Showing 14th day post-operative swelling measured with different guide points

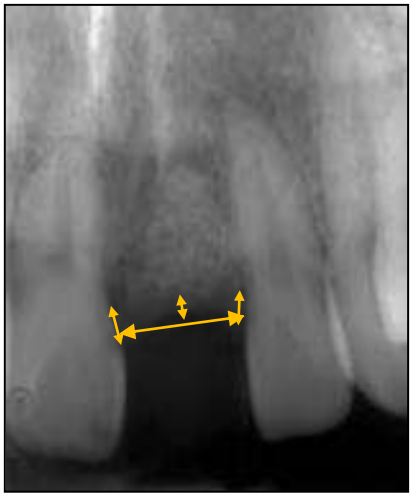


Fig. 33-Height of bone- 30th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

GROUP B

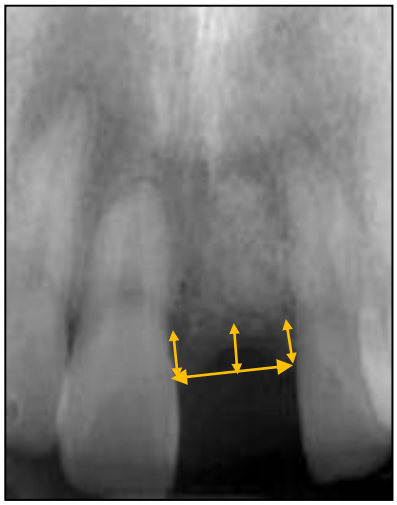


Fig. 34-Height of bone- 90th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

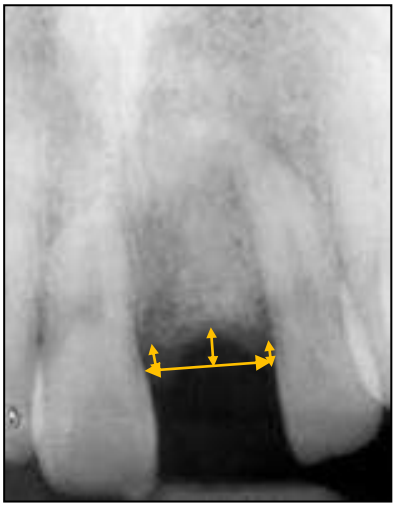


Fig. 35-Height of bone- 180th day post operative radiograph with Arrows showing Central, mesial and distal linear measurements

INTRA OPERATIVE PHOTOGRAPHS

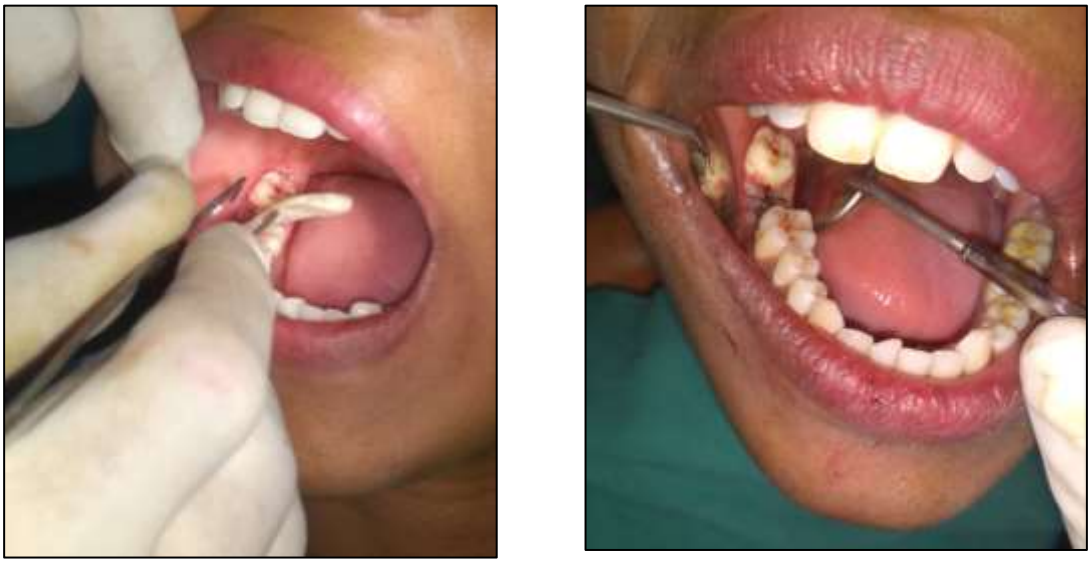


Fig 36. Showing intraoperative photographs of Group A

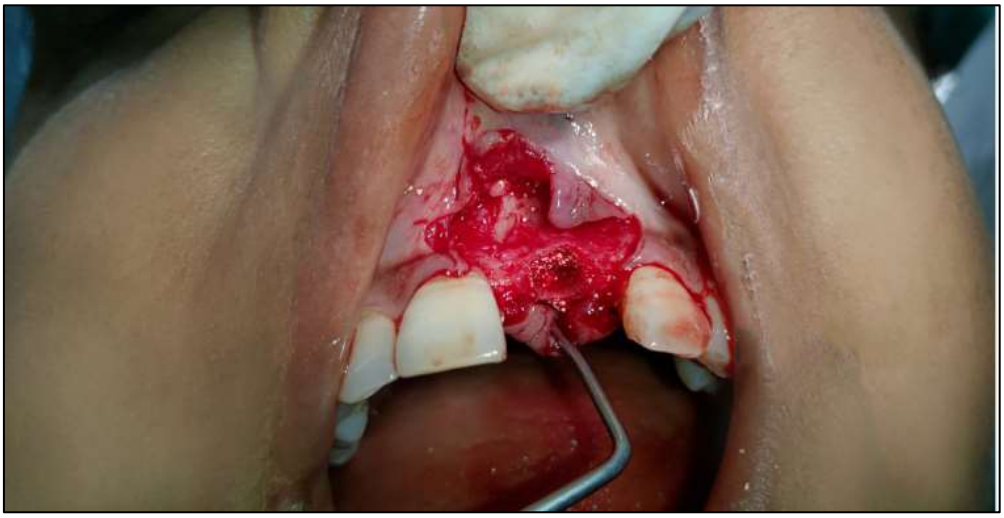


Fig 37. Showing intraoperative photographs of Group B

RESULTS

&

OBSERVATIONS

RESULTS AND OBSERVATIONS

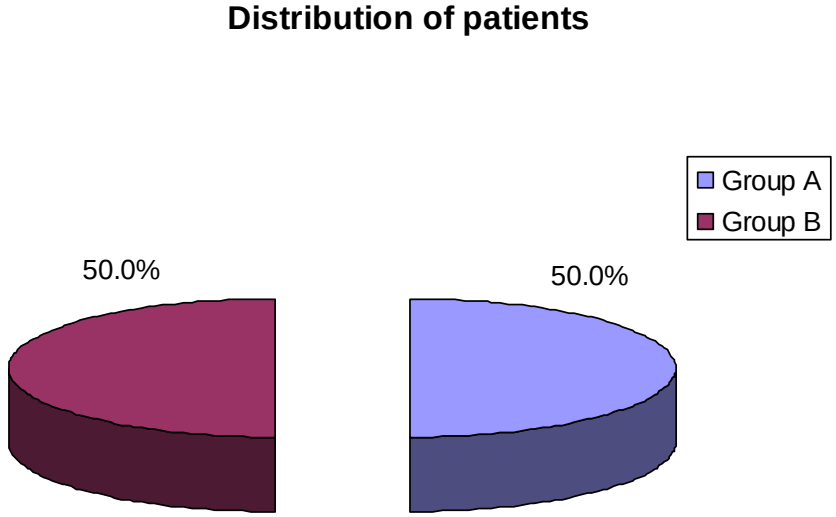
The present study compares the efficacy of HAM versus collagen membrane in GBR of alveolar bone. Total 8 patients of both gender were randomized through simple randomization, equally into two groups and treated either with freeze dried irradiated HAM (*Group A, n=4*) or collagen membrane (*Group B, n=4*) (Table 1 and Graph. 1).

The various parameters used for the study were swelling, infection, changes in mesial linear measurement, distal linear measurement and central linear measurement, and pain. The swelling was assessed at pre treatment (pre or day 0) and post treatment (day 1, 7 and 14). Similarly, changes in mesial, distal and central linear measurement were assessed at pre treatment (pre) and post treatment (immediate post and day 7, 30, 90 and 180). However, both infection (day 7 and day 14) and pain (day 1, 7 and 14) were assessed at post treatment. The swelling and changes in mesial, distal and central linear measurement were measured in millimetre (mm) whereas infection in score (0: absent and 1: present) and pain in visual analogue scale (VAS: 0-10 mm) score.

The objectives of the study were (i) to assess the efficacy of both the treatments on outcome measure within the groups (i.e. intra group or between periods), and (ii) to compare the efficacy of both the treatments on outcome measure between the groups (i.e. inter group or between group).

Table 1: Group allocation and distribution of patients in two groups

Treatment	Group name	Total patients (n=8) (%)
Amniotic membrane	Group A	4 (50.0)
Collagen membrane	Group B	4 (50.0)



Graph. 1. Pie charts showing distribution of patients in two groups.

Demographic characteristics

The demographic characteristics (age and sex) of two groups (Group A and Group B) at presentation (enrolment) is summarised in Table 2 and also depicted in Graph. 2-3, respectively. The age of Group A and Group B ranged from 23-56 and 27-52 yrs respectively with mean (± SD) 37.50 ± 15.72 and 38.25 ± 11.79 yrs, respectively and median 36 and 37 yrs, respectively. The mean age of Group B was slightly higher than Group A. Comparing the mean age of two groups, Student's t showed similar ($P > 0.05$) age between the two groups (t=0.08, $P = 0.942$) i.e. did not differ significantly (Table 2 and Graph. 2).

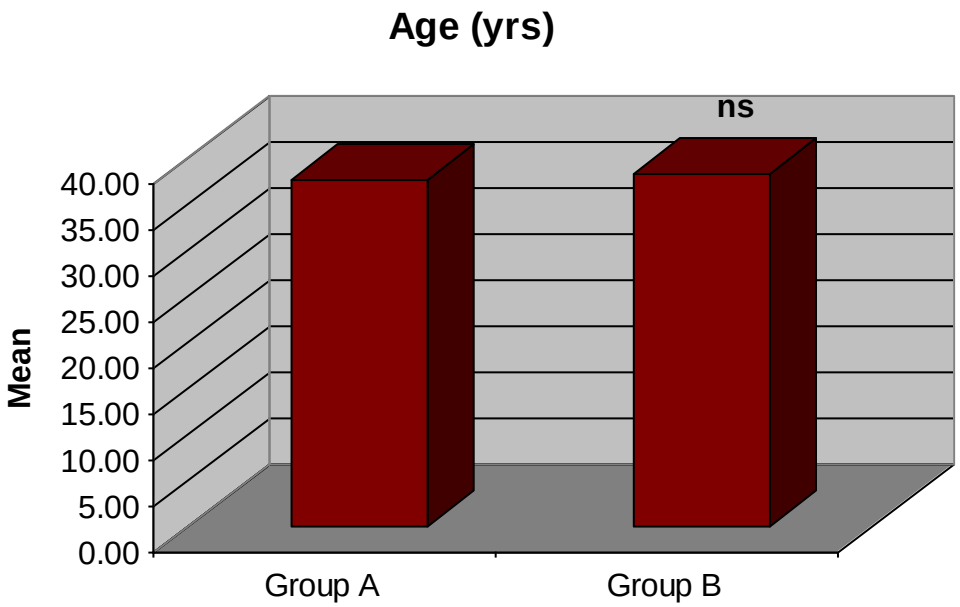
Further, in both groups, there were 2 (50.0%) females and 2 (50.0%) males. Comparing the sex proportion (F/M) of two groups, χ^2 test showed similar ($P > 0.05$) sex proportions between the two groups (χ^2=0.00, P = 1.000) i.e. also not differ significantly (Table 2 and Graph. 3).

The above comparisons concluded that patients of two groups were age and sex matched and thus comparable and hence these may also not influence the study outcome measures (swelling, infection, changes in mesial, distal and central linear measurement, and pain).

Table 2: Demographic characteristics of two groups

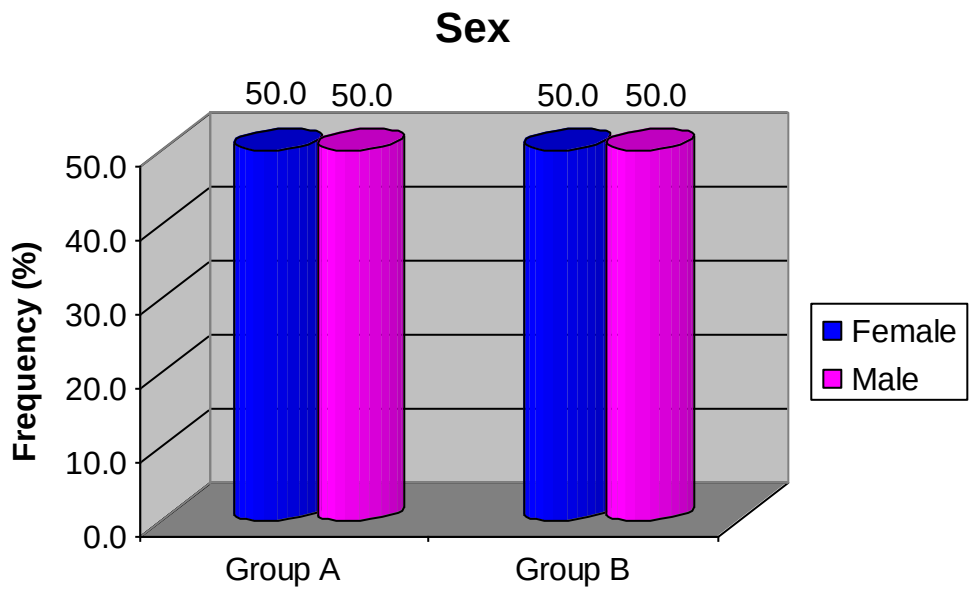
Variable	**Group A (n=4) (%)**	**Group B (n=4) (%)**	**t/χ^2 value**	***P* value**
Age (yrs)	37.50 ± 15.72	38.25 ± 11.79	0.08	0.942
Sex:				
Female	2 (50.0)	2 (50.0)	0.00	1.000
Male	2 (50.0)	2 (50.0)		

The age of two groups were summarised in Mean ± SD and compared by Student's t test (t value) whereas distribution of sex were summarised in number (n) and percentage (%) and compared by χ^2 test (χ^2 value).



$^{ns}P > 0.05$- as compared to Group A

Graph. 2. Bar graphs showing comparison of difference in mean age of two groups.



Graph. 3. Bar graphs showing distribution of sex of two groups.

Outcome measures

I. Swelling

The pre and post treatment (day 1, 7 and 14) swelling of two groups (Group A and Group B) is summarised in Table 3 and also shown in Graph. 4.

For each period, comparing the difference in mean swelling between the two groups (i.e. inter group), Tukey test showed greater (P < 0.05) swelling in Group B as compared to Group A during 1-7days which differs significantly (Table 3 and Graph. 5).

In Group A, the mean swelling remained higher at both post periods (day 1 and day 7) but lower slightly at day 14. In contrast, in Group B, it remained higher at all post periods (day 1, 7 and 14) as compared to pre treatment.

Furthermore, for each group, comparing the difference in mean swelling between the periods (i.e. intra group), Tukey test showed significantly (P < 0.05) different and higher swelling at day 1 as compared to pre treatment in both groups (Group A: mean difference=*0.75±0.9*, P< 0.05; Group B: mean difference=0.71±0.6; P< 0.05) (Table 4

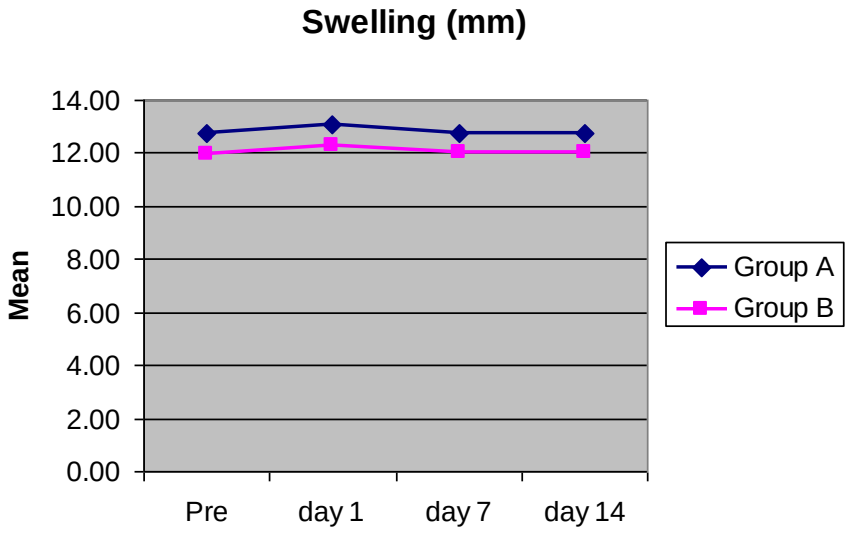
and Graph. 6). Group A showed better and faster reduction in swelling mean difference when compared to that of Group B which was significant at day 1, 7 and 14. ($p<0.05$)

At final evaluation (i.e. mean change from pre to day 14), Group A showed significant decrease in swelling as compared to group B.

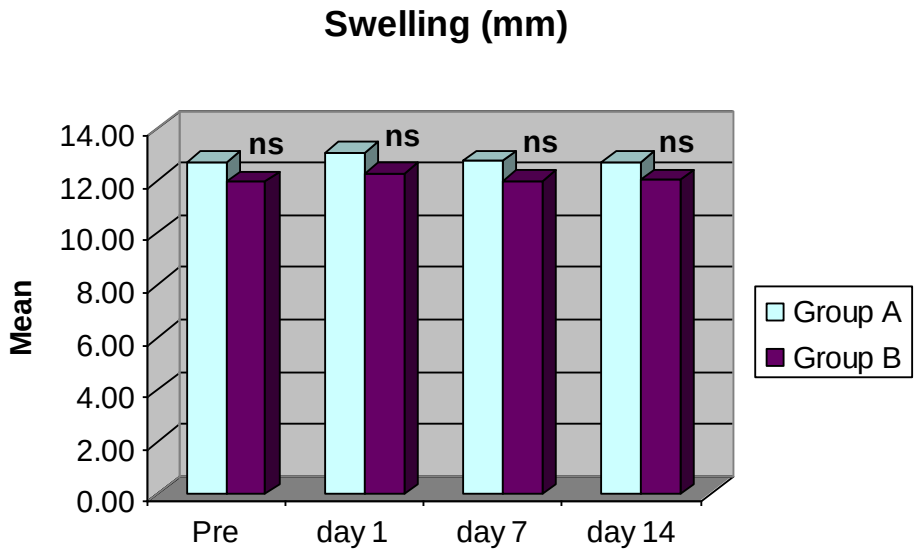
Table 3: Distribution of pre and post treatment swelling (mm) of two groups over the periods

Time period	Group A (n=4)	Group B (n=4)	Mean difference	*P* Value
Pre	12.73 ± 0.69	11.98 ± 0.81	0.75±0.12	0.782
day 1	13.08 ± 0.78	12.69 ± 0.75	0.39±0.03	0.043
day 7	12.76 ± 0.66	12.38 ± 0.72	0.38±0.06	0.032
day 14	12.72 ± 0.68	12.36 ± 0.89	0.36±0.21	0.031

The pre and post swelling of two groups were summarised in Mean ± SD and compared by Tukey test (*P* value).



Graph. 4. Line graphs showing pre and post mean swelling of two groups over the periods.

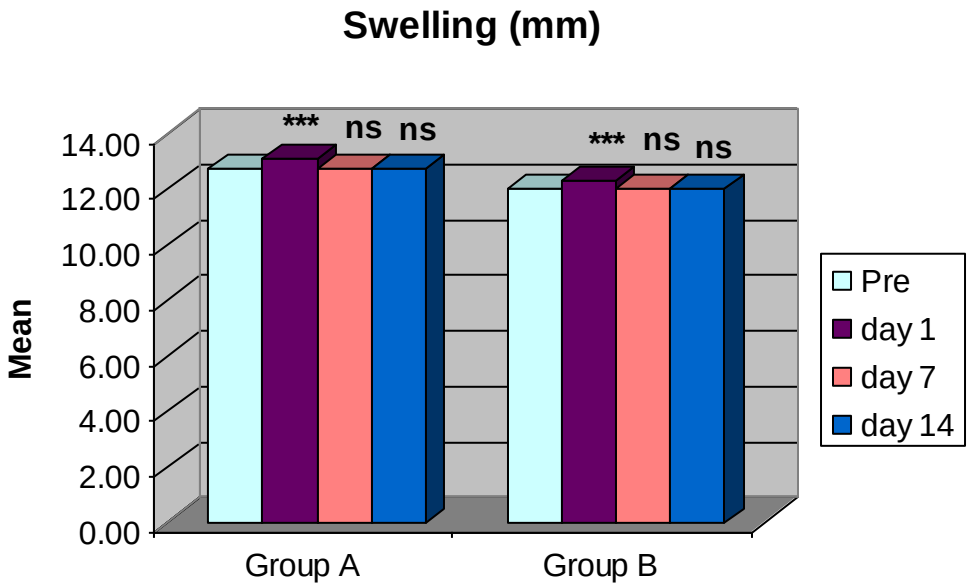


[ns]*P* > 0.05- as compared to Group A

Graph. 5: For each period, bar graphs showing comparison of difference in mean swelling between the groups.

Table 4: For each group, comparison (*P* value) of difference in mean swelling (mm) between the periods by Tukey test

Comparison	Group A		Group B	
	Mean Difference	*P* Value	Mean Difference	*P* value
Pre vs. day 1	0.35±0.9	<0.05	0.71±0.6	<0.05
Pre vs. day 7	0.03±0.03	0.985	0.40±0.09	0.999
Pre vs. day 14	0.01±0.01	1.000	0.38±0.08	0.930
day 1 vs. day 7	0.32±0.12	<0.05	0.36±0.06	<0.05
day 1 vs. day 14	0.36±0.10	<0.05	0.29±0.03	<0.05
day 7 vs. day 14	0.04±0.02	0.972	0.25±0.04	0.999



$^{ns}P > 0.05$ or $^{***}P < 0.05$- as compared to Pre

Graph. 6: For each group, bar graphs showing comparison of difference in mean swelling between the periods.

II. Infection

The post treatment (day 7 and 14) infection of two groups (Group A and Group B) is summarised in Table 5 and also shown in Graph. 7-8, respectively. At day 7, comparing the frequency and percentage of infection (A/P) of two groups, the χ^2 test showed significant difference in (P <0.05) presence of infection between the two groups (χ^2=2.67, P = 0.013) as it was absent in Group A as compared to group B on day 7.

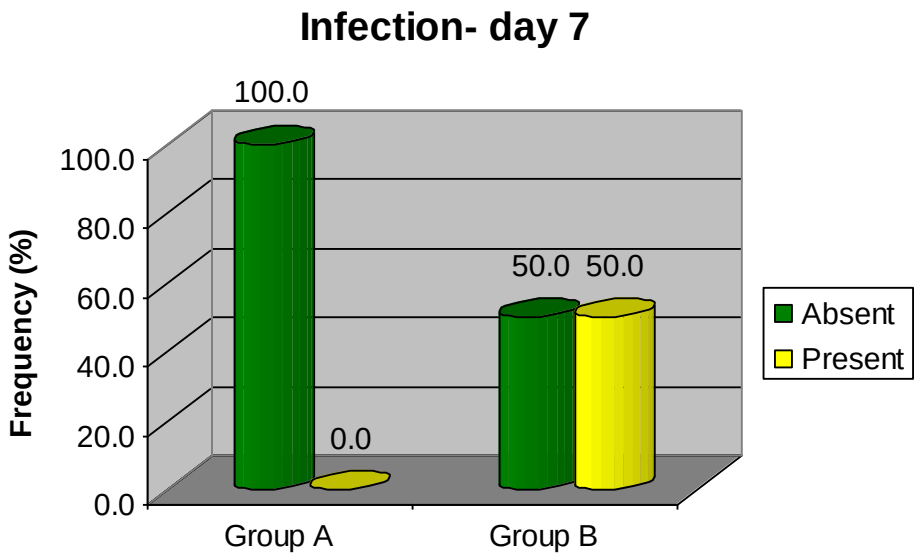
At day 14, group A showed absence of infection, while group B showed 33.33% presence of infection and was found to be significant.

Further, for each group, comparing the frequency (%) of infection (A/P) between two periods (i.e. intra group), a non significant difference was found in group A from Day 7 to day 14 (X^2=0, p=0.09), which can be due to the absence of infection in Group A throughout the period whereas, intra group comparison of Group B shows a significant difference (X2=2.67 ,p=0.034). The percentage of infection decreased from 50% to 33.33% respectively.

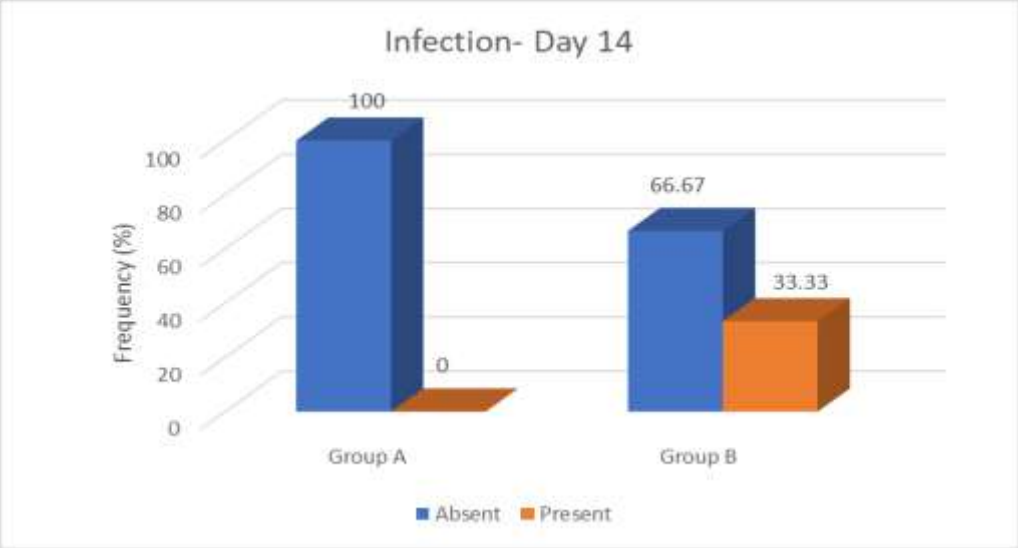
Table 5: Frequency distribution of post treatment infection of two groups over the periods

Time period/ Infection	**Group A (n=4) (%)**	**Group B (n=4) (%)**	**χ^2 value**	***P* Value**
day 7:				
Absent	4(100.0)	2 (50.0)	2.67	0.034
Present	0(00)	2 (50.0)		
day 14:				
Absent	4 (100.0)	3 (66.67)	0.00	0.009
Present	0 (0.0)	1 (33.33)		
χ^2 value, *P* value	0.00, 0.009	2.67, 0.034	-	-

Frequency distribution of post treatment infection of two groups were summarised in number (n) and percentage (%) and compared by χ^2 test (χ^2 value).



Graph. 7. Bar graph showing frequency distribution of post treatment infection of two groups at day 7.



Graph. 8. Bar graph showing frequency distribution of post treatment infection of two groups at day 14.

III. Change in linear measurement

The linear measurement showed the line drawn from the cervical margin of the adjacent tooth to the mesial, distal or central-most depth of the deficient site. So any decrease in linear measurement indicated an increase in actual bone height of that area and vice versa.

(i) Mesial linear measurement

The pre and post treatment (immediate post, day 7, 30, 90 and 180) changes in mesial linear measurement of two groups (Group A and Group B) is summarised in Table 6 and also shown in Graph. 9. In both groups, the mean mesial linear measurement decreased after the treatment and remained lower at all post periods as compared to pre treatment. The results indicate post treatment increase in mean mesial bone height within Group A was more as compared to within Group B.

For each period, comparing the difference in mean mesial linear measurement between the two groups (i.e. inter group), Tukey test showed similar ($P > 0.05$) mesial linear measurement between the two groups at all periods (Pre: mean difference=1.00, $P = 0.997$; immediate post: mean difference=0.60, $P = 1.000$; day 7: mean difference=0.60, $P = 1.000$; day 30: mean difference=0.85, $P = 0.999$; day 90: mean

difference=0.75, P = 1.000; day 180: mean difference=0.60, P = 1.000) i.e. did not differ significantly (Table 6 and Graph. 10).

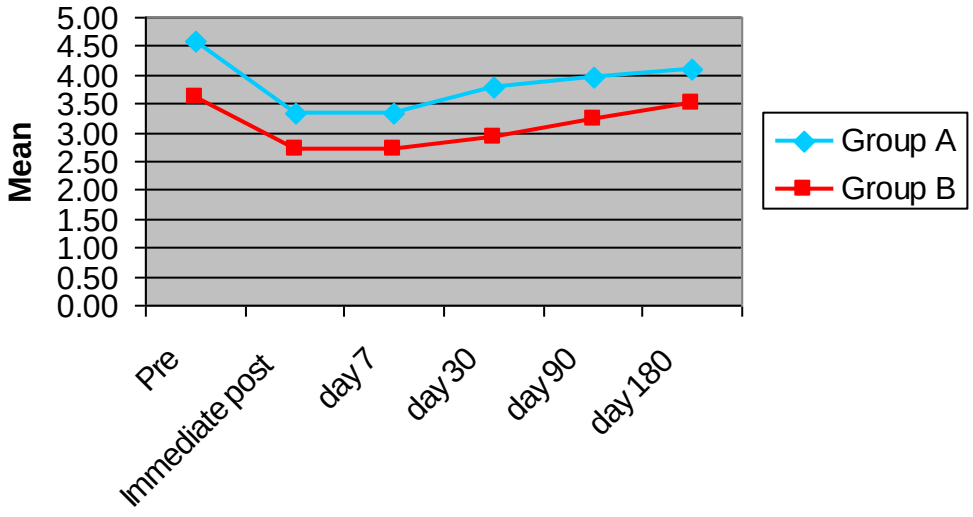
Further, for each group, comparing the difference in mean mesial linear measurement between the periods (i.e. intra group), Tukey test showed significantly ($P < 0.05$ or $P < 0.01$ or $P < 0.05$) different and lower mesial linear measurement at immediate post (mean difference=1.28, $P < 0.05$), day 7 (mean difference=1.28, $P < 0.05$), day 30 (mean difference=0.82, $P < 0.05$), day 90 (mean difference=0.63, $P = 0.05$) and day 180 (mean difference=0.50, $P = 0.017$) as compared to pre treatment in Group A (Table 7 and Graph. 11). Further, in Group A, difference in mean mesial linear measurement between the periods lowered significantly ($P < 0.05$ or $P < 0.01$ or $P < 0.05$) at day 30 (mean difference=0.45, $P = 0.045$), day 90 (mean difference=0.65, $P = 0.05$) and day 180 (mean difference=0.77, $P < 0.05$) as compared to both immediate post and day 7. In contrast in Group B, it lowered significantly ($P < 0.01$ or $P < 0.05$) at immediate post (mean difference=0.88, $P < 0.05$), day 7 (mean difference=0.88, $P < 0.05$) and day 30 (mean difference=0.68, $P = 0.05$) as compared to pre treatment. Further, in Group B, it also lowered significantly ($P < 0.05$ or $P < 0.05$) at both day 90 (mean difference=0.50, $P = 0.017$) and day 180 (mean difference=0.78, $P < 0.05$) as compared to both immediate post and day 7. Furthermore, in Group B, it also lowered significantly ($P < 0.01$) at day 180 (mean difference=0.58, $P = 0.004$) as compared to day 30. However, it did not differ ($P > 0.05$) between other periods in both groups, i.e. found to be statistically the same.

At final evaluation the intra group comparison of difference in mean mesial linear measurement between both the group showed a statistically significant difference between pre and immediate post operative day, indicating increase in mean mesial bone height over the period of immediate post operative to day 180.

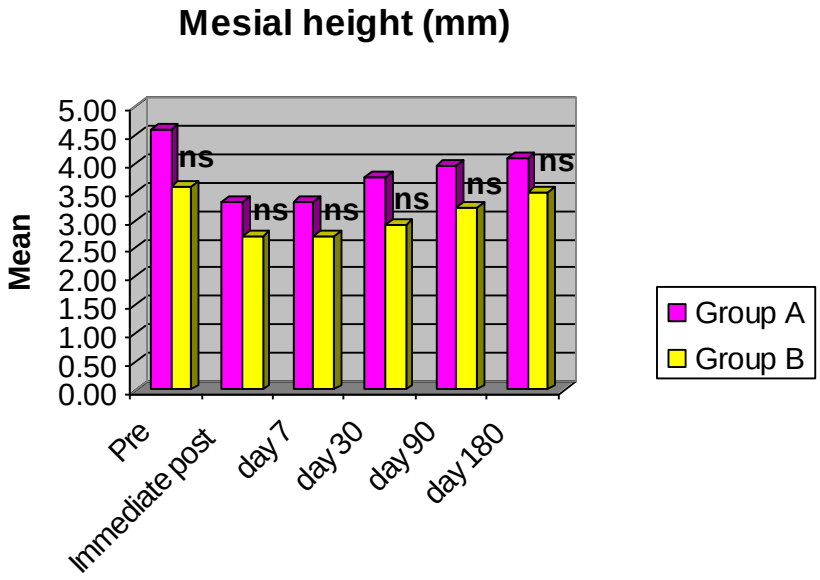
Table 6: Distribution of pre and post treatment mesial linear measurement (mm) of two groups over the periods

Time Period	Group A (n=4)	Group B (n=4)	Mean difference	*P* Value
Pre	4.60 ± 1.94	3.60 ± 0.45	1.00	0.997
Immediate post	3.33 ± 2.32	2.73 ± 0.79	0.60	1.000
day 7	3.33 ± 2.32	2.73 ± 0.79	0.60	1.000
day 30	3.78 ± 2.18	2.93 ± 0.74	0.85	0.999
day 90	3.98 ± 2.15	3.23 ± 0.69	0.75	1.000
day 180	4.10 ± 2.17	3.50 ± 0.70	0.60	1.000

The pre and post mesial linear measurement of two groups were summarised in Mean ± SD and compared by Tukey test (*P* value).



Graph. 9. Line graphs showing pre and post mean mesial linear measurement of two groups over the periods.



$^{ns}P > 0.05$- as compared to Group A

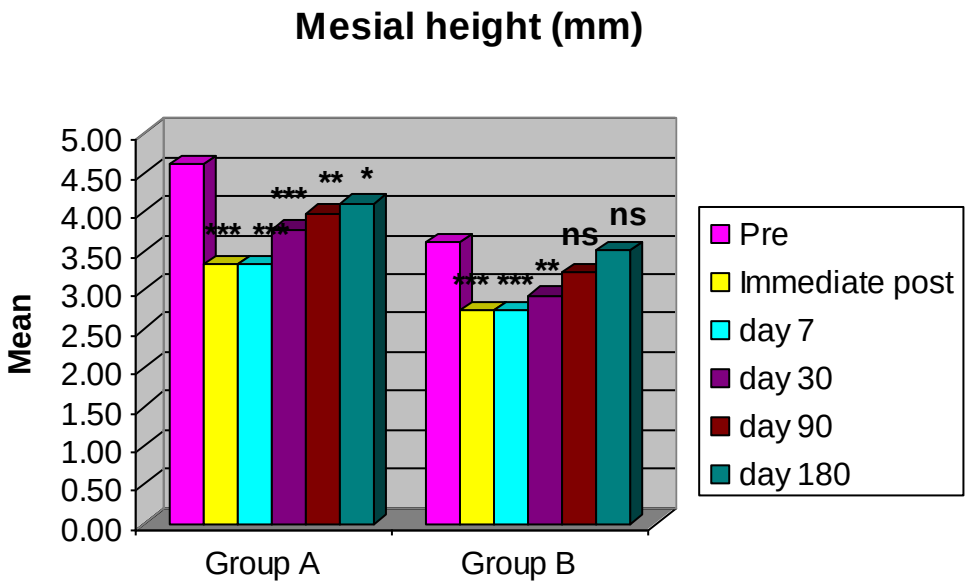
Graph. 10: For each period, bar graphs showing comparison of difference in mean mesial linear measurement between the groups.

Table 7: For each group, comparisons (*P* value) of difference in mean mesial linear measurement (mm) between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
Pre vs. Immediate post	1.28	<0.05	0.88	<0.05
Pre vs. day 7	1.28	<0.05	0.88	<0.05
Pre vs. day 30	0.82	<0.05	0.68	0.561
Pre vs. day 90	0.63	0.05	0.38	0.163
Pre vs. day 180	0.50	0.017	0.10	1.000
Immediate post vs. day 7	0.00	1.000	0.00	1.000
Immediate post vs. day 30	0.45	0.045	0.20	0.899
Immediate post vs. day 90	0.65	0.05	0.50	0.017
Immediate post vs. day 180	0.77	<0.05	0.78	<0.05
day 7 vs. day 30	0.45	0.045	0.20	0.899

day 7 vs. day 90	0.65	0.05	0.50	0.089
day 7 vs. day 180	0.77	<0.05	0.78	<0.05
day 30 vs. day 90	0.20	0.899	0.30	0.443
day 30 vs. day 180	0.32	0.330	0.58	0.764
day 90 vs. day 180	0.13	0.997	0.28	0.569

$^{ns}P > 0.05$ or $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.05$- as compared to Pre



Graph. 11: For each group, bar graphs showing comparison of difference in mean mesial linear measurement between the periods.

(ii) Distal linear measurement

The pre and post treatment (immediate post, day 7, 30, 90 and 180) changes in distal linear measurement of two groups (Group A and Group B) is summarised in Table 8 and also shown in Graph. 12. In both groups, the changes in distal linear measurement showed similar trend as of changes in mesial linear measurement. In both groups, the mean distal linear measurement decreased after the treatment and remained lower at all post periods as compared to pre treatment, indicating increase in distal bone height after bone grafting and membrane placement . Thus, the post treatment increase in mean distal bone height was evidently higher in Group A as compared to Group B, which was found to be statistically non significant (p>0.05).

For each period, comparing the difference in mean linear distal measurement between the two groups (i.e. inter group), Tukey test showed similar ($P > 0.05$) distal linear

measurements between the two groups at all periods (Pre: mean difference=1.00, *P* = 0.997; immediate post: mean difference=0.68, *P* = 1.000; day 7: mean difference=0.68, *P* = 1.000; day 30: mean difference=0.88, *P* = 0.999; day 90: mean difference=0.78, *P* = 1.000; day 180: mean difference=0.75, *P* = 1.000) i.e. did not differ significantly (Table 8 and Graph. 13).

Further, for each group, comparing the difference in mean distal linear measurement between the periods (i.e. intra group), Tukey test showed significantly (*P* < 0.05 or *P* < 0.05) different and lower distal linear measurement at immediate post (mean difference=1.20, *P* < 0.05), day 7 (mean difference=1.20, *P* < 0.05), day 30 (mean difference=0.80, *P* < 0.05) and day 90 (mean difference=0.60, *P* = 0.012) as compared to pre treatment in Group A (Table 9 and Graph. 14). Further, in Group A, it also lowered significantly (*P* < 0.05 or *P* < 0.05) at both day 90 (mean difference=0.60, *P* = 0.012) and day 180 (mean difference=0.80, *P* >0.05) as compared to both immediate post and day 7. In contrast in Group B, it lowered significantly (*P* < 0.01 or *P* < 0.05) at immediate post (mean difference=0.88, *P* < 0.05), day 7 (mean difference=0.88, *P* < 0.05) and day 30 (mean difference=0.68, *P* = 0.003) as compared to pre treatment. Further, in Group B, it also lowered significantly (*P* < 0.01) at day 180 (mean difference=0.73, *P* = 0.05) as compared to both immediate post and day 7. However, there was no significant difference (*P* > 0.05) between other periods in both groups, i.e. found to be statistically the same.

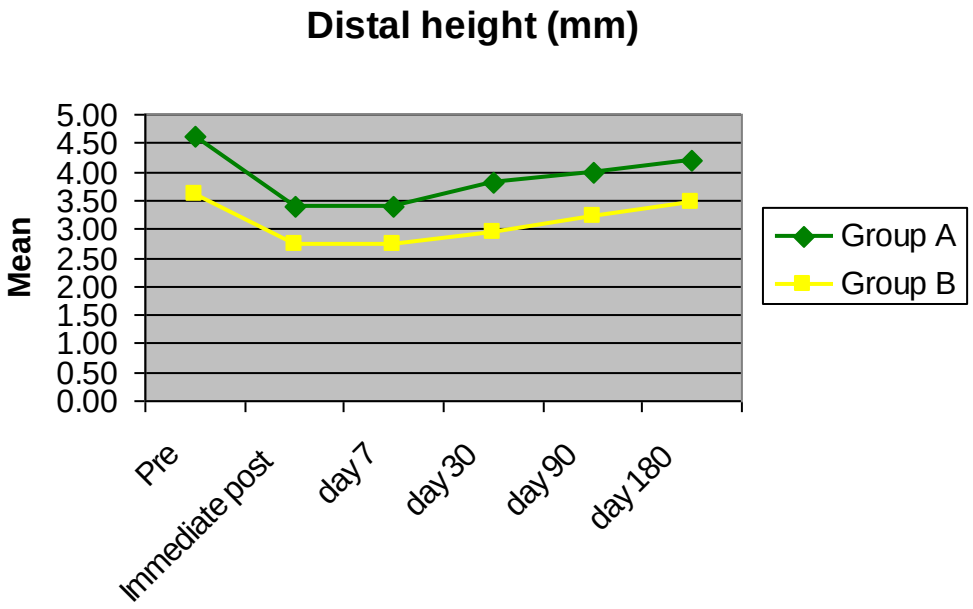
At final evaluation the intra group comparison of difference in mean distal linear measurement between both the group showed a statistically significant difference between pre and immediate post operative day, with an increase in mean distal bone height over the period of immediate post operative to day 180.

Table 8: Distribution of pre and post treatment distal linear measurement (mm) of two groups over the periods.

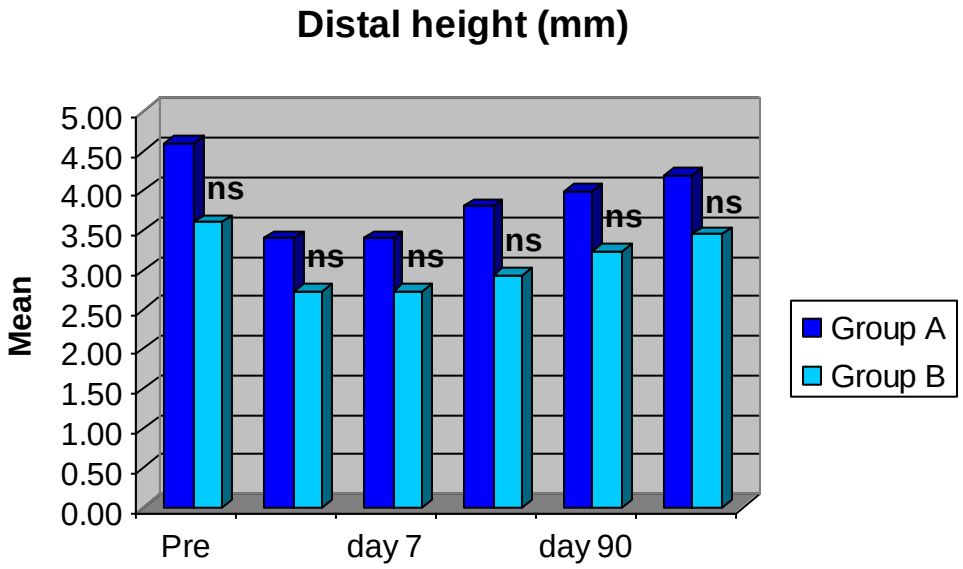
The pre and post distal linear measurement of two groups were summarised in Mean ± SD and compared by Tukey test (*P* value).

Time Period	**Group A (n=4)**	**Group B (n=4)**	**Mean difference**	***P* Value**

Pre	4.60 ± 1.94	3.60 ± 0.45	1.00	0.997
Immediate post	3.40 ± 2.46	2.73 ± 0.74	0.68	1.000
day 7	3.40 ± 2.46	2.73 ± 0.74	0.68	1.000
day 30	3.80 ± 2.23	2.93 ± 0.74	0.88	0.999
day 90	4.00 ± 2.20	3.23 ± 0.69	0.78	1.000
day 180	4.20 ± 2.17	3.45 ± 0.73	0.75	1.000



Graph. 12. Line graphs showing pre and post mean distal linear measurement of two groups over the periods.



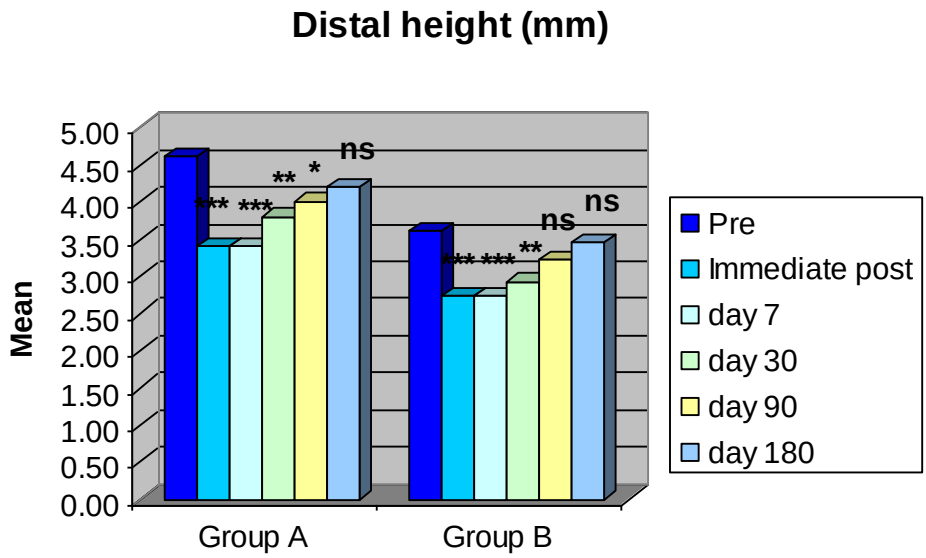
^{ns}P > 0.05- as compared to Group A

Graph. 13: For each period, bar graphs showing comparison of difference in mean distal linear measurement between the groups.

Table 9: For each group, comparisons (*P* value) of difference in mean distal linear measurement (mm) between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
Pre vs. Immediate post	1.20	<0.05	0.88	<0.05
Pre vs. day 7	1.20	<0.05	0.88	<0.05
Pre vs. day 30	0.80	<0.05	0.68	0.003
Pre vs. day 90	0.60	0.012	0.38	0.047
Pre vs. day 180	0.40	0.054	0.15	0.049
Immediate post vs. day 7	0.00	1.000	0.00	1.000
Immediate post vs. day 30	0.40	0.254	0.20	0.960
Immediate post vs. day 90	0.60	0.012	0.50	0.064
Immediate post vs. day 180	0.80	<0.05	0.73	0.05
day 7 vs. day 30	0.40	0.254	0.20	0.046

day 7 vs. day 90	0.60	0.012	0.50	0.054
day 7 vs. day 180	0.80	<0.05	0.73	0.05
day 30 vs. day 90	0.20	0.960	0.30	0.653
day 30 vs. day 180	0.40	0.254	0.53	0.431
day 90 vs. day 180	0.20	0.960	0.23	0.916



$^{ns}P > 0.05$ or $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.05$- as compared to Pre

Graph. 14: For each group, bar graphs showing comparison of difference in mean distal linear measurement between the periods.

(iii) Central linear measurement

The pre and post treatment (immediate post, day 7, 30, 90 and 180) changes in central linear measurement of two groups (Group A and Group B) is summarised in Table 10 and also shown in Graph. 15. In both groups, the mean central linear measurement decreased after the treatment and remained lower at all post periods as compared to pre treatment. However, the post treatment decrease in mean central linear measurement was slightly higher in Group A as compared to Group B.

For each period, comparing the difference in mean central linear measurement between the two groups (i.e. inter group), Tukey test showed similar ($P > 0.05$)

central linear measurement between the two groups at all periods (Pre: mean difference=0.85, $P = 1.000$; immediate post: mean difference=0.85, $P = 1.000$; day 7: mean difference=0.85, $P = 1.000$; day 30: mean difference=0.68, $P = 1.000$; day 90: mean difference=0.65, $P = 1.000$; day 180: mean difference=0.48, $P = 1.000$) i.e. did not differ significantly (Table 10 and Graph. 16).

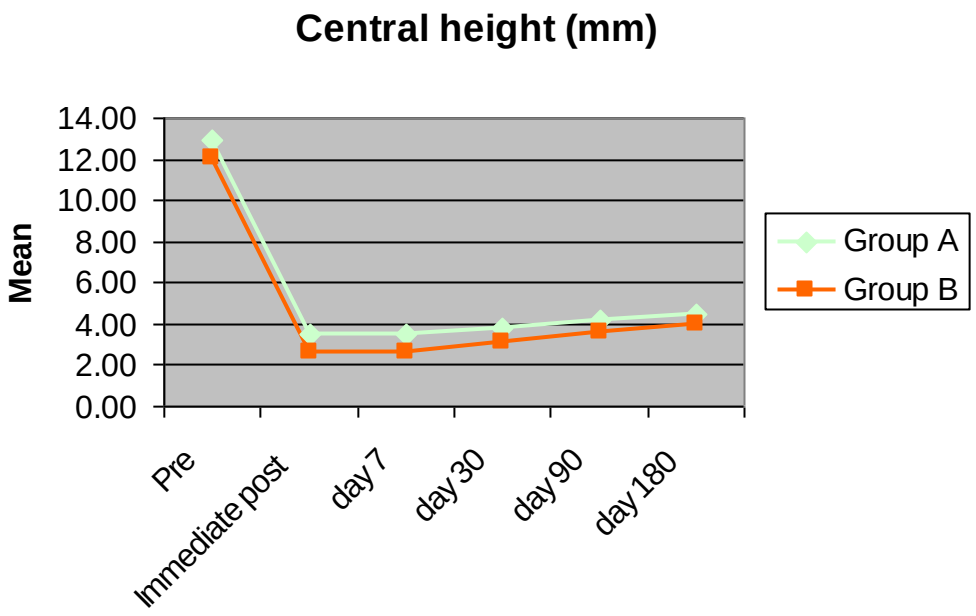
Further, for each group, comparing the difference in mean central linear measurement between the periods (i.e. intra group), Tukey test showed significantly ($P < 0.05$) different and lower central linear measurement at immediate post (mean difference=9.45, $P < 0.05$), day 7 (mean difference=9.45, $P < 0.05$), day 30 (mean difference=9.10, $P < 0.05$), day 90 (mean difference=8.73, $P < 0.05$) and day 180 (mean difference=8.50, $P < 0.05$) as compared to pre treatment in Group A (Table 11 and Graph. 17). Similarly, in Group B, it also lowered significantly ($P < 0.05$) at immediate post (mean difference=9.45, $P < 0.05$), day 7 (mean difference=9.45, $P < 0.05$), day 30 (mean difference=8.93, $P < 0.05$), day 90 (mean difference=8.53, $P < 0.05$) and day 180 (mean difference=8.13, $P < 0.05$) as compared to pre treatment. However, it did not differ ($P > 0.05$) between other periods in both groups, i.e. found to be statistically the same.

At final evaluation the intra group comparison of difference in mean central linear measurement between both the group showed a statistically significant difference between pre and immediate post operative day, with increase in mean central linear measurement over the period of immediate post operative to day 180. Although the intergroup comparison showed a non significant difference between the two groups, however there was less difference in Central Mean Linear Measurement from immediate post operative to day 180 in group A as compared to Group B.

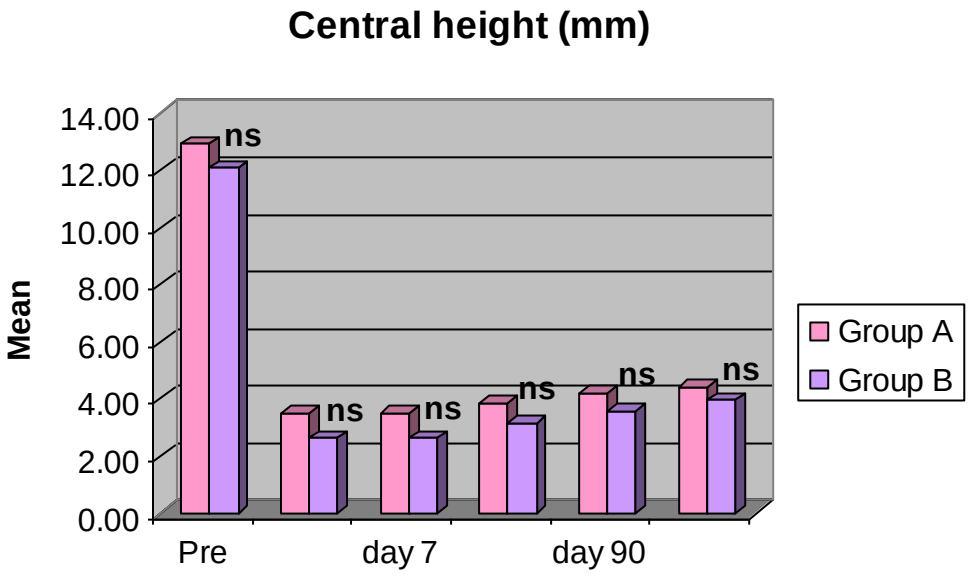
Table 10: Distribution of pre and post treatment central linear measurement (mm) of two groups over the periods

Time Period	Group A (n=4)	Group B (n=4)	Mean difference	*P* Value
Pre	12.93 ± 2.74	12.08 ± 2.70	0.85	1.000
Immediate post	3.48 ± 2.49	2.63 ± 0.75	0.85	1.000
day 7	3.48 ± 2.49	2.63 ± 0.75	0.85	1.000
day 30	3.83 ± 2.39	3.15 ± 0.87	0.68	1.000
day 90	4.20 ± 2.31	3.55 ± 0.83	0.65	1.000
day 180	4.43 ± 2.31	3.95 ± 0.95	0.48	1.000

The pre and post central linear measurement of two groups were summarised in Mean ± SD and compared by Tukey test (*P* value).



Graph. 15. Line graphs showing pre and post mean central linear measurement of two groups over the periods.



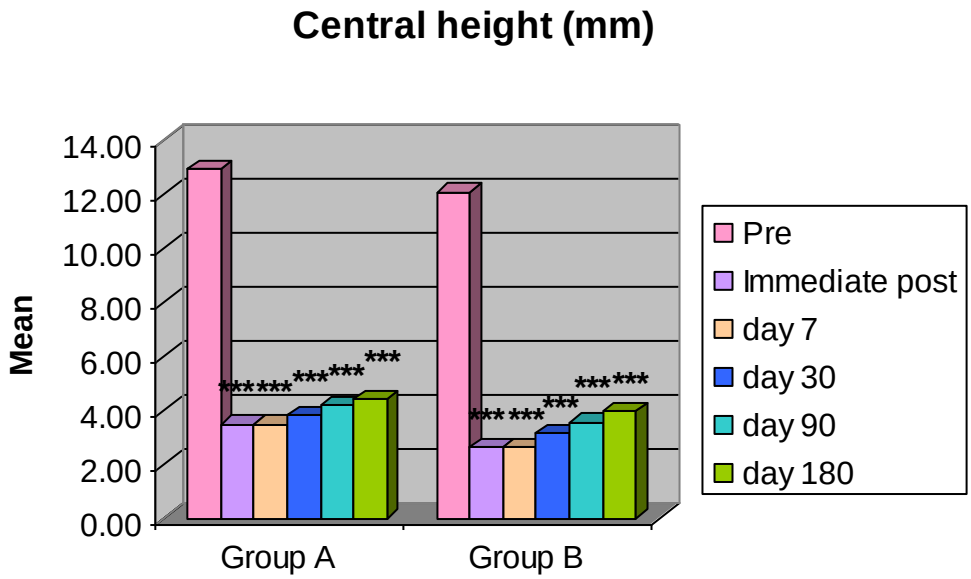
[ns]*P* > 0.05- as compared to Group A

Graph. 16: For each period, bar graphs showing comparison of difference in mean central linear measurement between the groups.

Table 11: For each group, comparisons (*P* value) of difference in mean central linear measurement (mm) between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
Pre vs. Immediate post	9.45	<0.05	9.45	<0.05
Pre vs. day 7	9.45	<0.05	9.45	<0.05
Pre vs. day 30	9.10	<0.05	8.93	<0.05
Pre vs. day 90	8.73	<0.05	8.53	<0.05
Pre vs. day 180	8.50	<0.05	8.13	<0.05
Immediate post vs. day 7	0.00	1.000	0.00	1.000
Immediate post vs. day 30	0.35	1.000	0.53	0.985
Immediate post vs. day 90	0.73	0.872	0.93	0.615
Immediate post vs. day 180	0.95	0.578	1.33	0.148
day 7 vs. day 30	0.35	1.000	0.53	0.985

day 7 vs. day 90	0.73	0.872	0.93	0.615
day 7 vs. day 180	0.95	0.578	1.33	0.148
day 30 vs. day 90	0.38	0.999	0.40	0.998
day 30 vs. day 180	0.60	0.960	0.80	0.789
day 90 vs. day 180	0.23	1.000	0.40	0.998



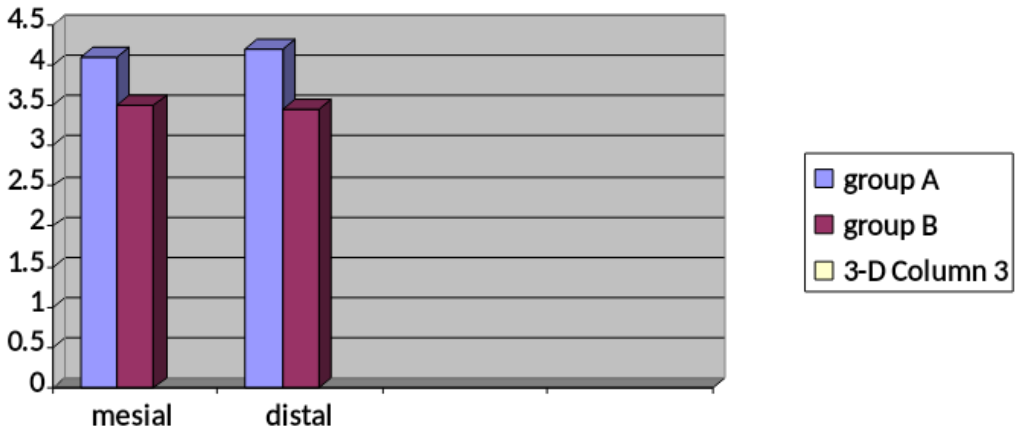
$^{ns}P > 0.05$ or $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.05$- as compared to Pre

Graph. 17: For each group, bar graphs showing comparison of difference in mean central linear measurement between the periods.

Table 12 shows Distribution of mesial and distal linear measurement of two groups over the periods. The result showed that Mean mesial linear measurement showed more reduction as compared to mean distal linear measurement in both the groups, during the study which was found to be non significant.

Table 12: Distribution of mesial and distal linear measurement of two groups over the periods

Group	*Mean mesial linear measurement*	*Mean distal linear measurement*	*P value*
GROUP A	***4.10 ± 2.17***	***4.20 ± 2.17***	***0.751***
GROUP B	***3.50 ± 0.70***	***3.45 ± 0.73***	***0.876***



Graph 18: Distribution of mesial and distal linear measurement of two groups over the periods

IV. Pain

The post treatment (day 1, 7 and 14) pain (VAS score) of two groups (Group A and Group B) is summarised in Table 13 and also shown in Graph. 19. In both groups, the post mean VAS score decreased linearly with time and the decrease was evidently higher in Group A as compared to Group B.

For each period, comparing the difference in post mean VAS score between the two groups (i.e. inter group), Tukey test showed similar ($P > 0.05$) VAS score between the two groups at both day 1 (mean difference=1.75,) and day 14 (mean difference=0.50, $P = 0.935$) (Table 13 and Graph. 19). However, at day 7, it differed and lowered

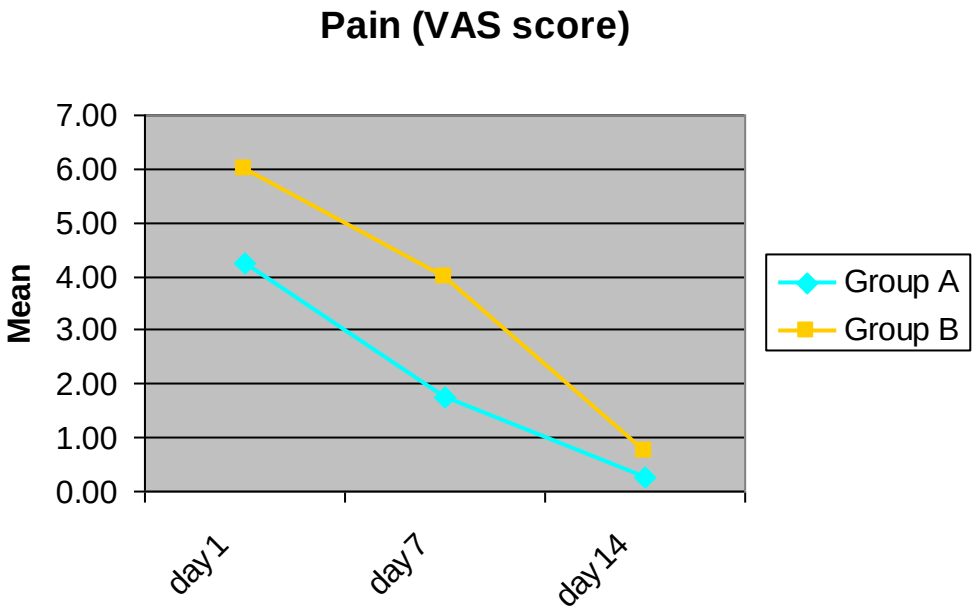
significantly ($P < 0.05$) in Group A as compared to Group B (mean difference=2.25, $P = 0.027$).

Further, for each group, comparing the difference in post mean VAS score between the periods (i.e. intra group), Tukey test showed no significant difference. There was a lower VAS score at both day 7 (mean difference=2.50, $P < 0.05$) and day 14 (mean difference=4.00, $P < 0.05$) as compared to day 1 in Group A (Table 13 and Graph. 20). The result was found to be non significant when compared for both groups.
At final evaluation (i.e. mean change from day 1 to day 14), Group A (94.12% decrease) showed 6.62% higher decrease in pain as compared to Group B (87.50%).

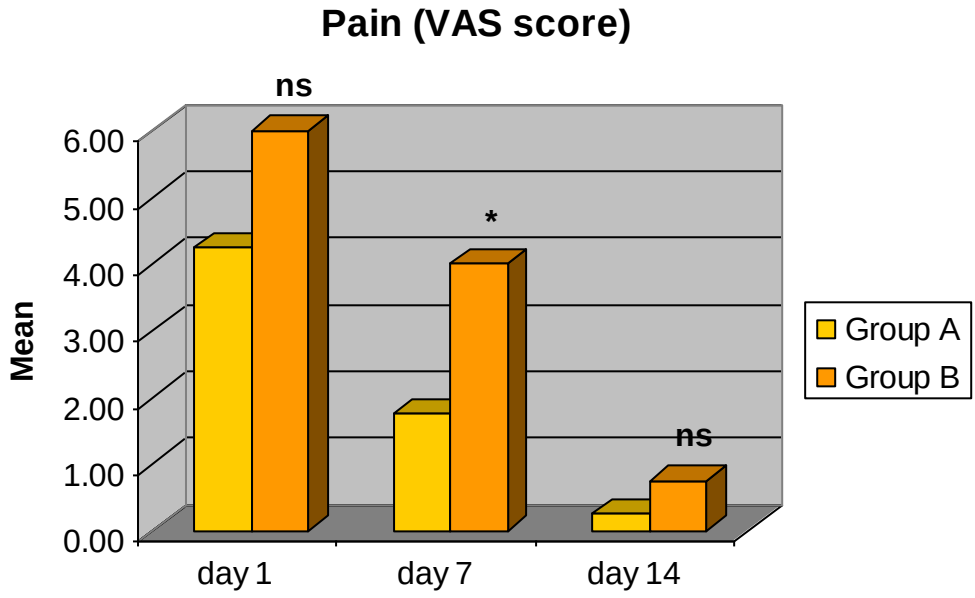
Table 13: Distribution of post treatment VAS score of two groups over the periods

Time period	Group A (n=4)	Group B (n=4)	Mean difference	*P* Value
day 1	4.25 ± 0.96	6.00 ± 0.82	1.75	0.091
day 7	1.75 ± 0.50	4.00 ± 0.82	2.25	0.027
day 30	0.25 ± 0.50	0.75 ± 0.96	0.50	0.935

The post VAS score of two groups were summarised in Mean ± SD and compared by Tukey test (*P* value).



Graph. 19. Line graphs showing post mean VAS score of two groups over the periods.

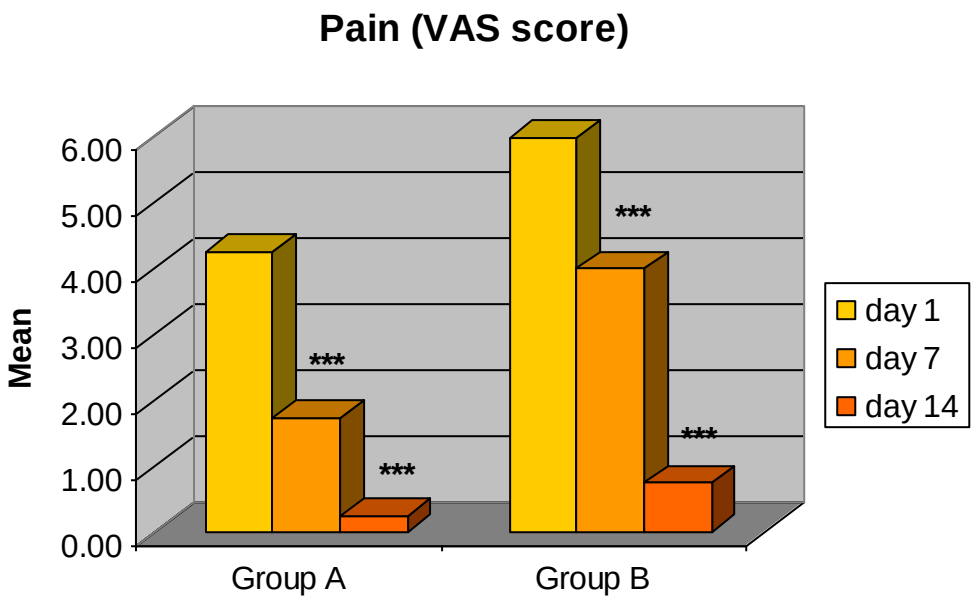


$^{ns}P > 0.05$ or $^{*}P < 0.05$ - as compared to Group A

Graph. 20: For each period, bar graphs showing comparison of difference in post mean VAS score between the groups.

Table 14: For each group, comparisons (*P* value) of difference in post mean VAS score (mm) between the periods by Tukey test

Comparison	Group A		Group B	
	Mean difference	*P* value	Mean difference	*P* Value
day 1 vs. day 7	2.50	0.876	2.00	0.989
day 1 vs. day 14	4.00	0.989	5.25	0.675
day 7 vs. day 14	1.50	0.651	3.25	0.242



Graph. 21: For each group, bar graphs showing comparison of difference in mean VAS score between the periods.

DISCUSSION

DISCUSSION

Reconstruction of bone defects has been posing a major clinical challenge in plastic , orthopedic, oral and maxillofacial surgery[34,35].

Conventionally, autologous block grafting has been advocated as the gold standard for bone augmentation so far. However, there have been advances in biomaterials and clinical techniques that have led to the incorporation of GBR as a potential alternative in case of alveolar bone deficiency[36]. GBR is a surgical procedure for reconstruction of bone that employs a membrane to promote the growth of new bone. This membrane acts as a barrier that fences fibroblast invasion into the bone deficient sites and eventually leads to a better osteogenesis. Selecting the best membrane for a predictable GBR has been controversial, because different materials have different characteristics. Various researches have been done to find the most effective material for GBR and bone induction[3].

Niknejad H et al[7] explicated the following basic properties of an ideal GBR membrane-

1) GBR membrane is known to be biocompatible when its interaction with the host doesn't impair the encompassing tissue, the process of healing, or the patient's safety. Particularly, when the membrane is resorbable, it should either integrate or degrade with the host tissue.

2) The ability of a GBR membrane to preserve space is related to its mechanical stability, which is necessary to prevent the defect from collapsing during the healing process and to safeguard the defect space for new bone formation.

3) The barrier membrane must prevent cells from the mucosa from intruding the defect space while ensuring oxygen and nutrition exchange (i.e., occlusivity).

As a result, occlusivity is tightly linked to porosity; a greater pore size enables banks from the surrounding connective tissue to infiltrate and proliferate into the defect area, impeding bone-forming cell activity. The total size of the pores may have an

impact on cell adhesion. Small pores may impede cell migration and increase collagen deposition, lowering the capacity of cells to migrate.

4) A GBR membrane would have to be easy to handle during surgery without being too rigid, as this could impede tissue integration or cause soft tissue dehiscence.

5) Although the membrane's original purpose was to serve as a passive barrier, this idea may need to be revisited in the context of next-generation membranes. Indeed, a rising number of research are proposing new bone regeneration techniques that incorporate bioactive substances into the membrane, giving a far more active role in the process.

Amniotic Membrane justifies these properties. Being the innermost layer of fetal membranes, Amniotic Membrane consists of a thick basement membrane and avascular stroma[16]. ***Davis***[55] applied the Amniotic Membrane as a surgery material for the first time. It was later introduced as a treatment of scorches and skin burns. It decreased the infection and pain and also promoted the procedure of epithelialization. Amniotic Membrane was reported to be used for chemical eye burns[7].

In the Amniotic membrane, there are two types of cells with different embryological origins-
(1) Human amnion epithelial cells that are derivatives of embryonic ectoderm, and
(2) Amnion mesenchymal cells derivatives of embryonic mesoderm[12].

According to ***Zhang et al***[38], human placental mesenchymal stem cells have the ability to develop into osteogenic, adipogenic, and chonrogenic lineages, as well as restrict T-cell proliferation. These findings were corroborated by ***Yen et*** **al**[39]. They discovered that placenta-derived stem cells share the same surface markers as embryonic stem cells and can differentiate into neurons. The Amniotic Membrane consists of a larger number of mesenchymal stem cells with bipotential osteogenic and adipogenic development, according to ***In't Anker et al***[40].

The majority of commercially available collagen membranes are derived from type I collagen or a combination of types I and III collagen. Collagen is derived from tendon, dermis, skin, and pericardium from bovine, porcine, or human origin. Hemostasis, mild immunogenicity, chemotaxis of fibroblasts, easy manipulation and adaptability, a direct influence on bone production, and the capacity to increase tissue thickness are all benefits of collagen materials for its use as a barrier membrane. Hence, collagen material seems to be a material of choice for a bioresorbable GTR or GBR barrier[21].

They help wound healing by acting as scaffolds for bone deposition in GBR, promoting platelet aggregation, stabilising clots, and attracting fibroblasts. They are biocompatible, simple to handle, and only mildly immunogenic, and are intended to resorb in 2 to 32 weeks. For simplicity of usage, they are offered as membranes, plugs, or a pad[5].

In the present clinical and radiographic study an attempt is made to evaluate efficacy of freeze dried irradiated HAM compared with collagen membrane in guided bone regeneration of alveolar bone. A total of eight patients were randomly selected and equally divided into two groups; Group A(50%) and Group B(50%). The barrier membrane used in group A was Freeze dried irradiated HAM and in group B, collagen membrane(Graph.1) (Table 1).

The primary outcome measures of the study were Swelling, Infection, Pain. The secondary outcome measures of the study were radiographic analysis to assess gain in bone height at follow up evaluations upto 6 months.

Both groups had equal distribution of gender, rendering us with no gender bias eventually (Table 2.)(Graph.3). The mean age of the subjects in group A was 37.50 ranging from 23-56 years and that of Group B was 38.25 ranging from 27-52 years indicating no significant difference among the groups (table 2)(Graph 2.). Therefore, it did not influence the outcome measures of the present study.

SWELLING

The five cardinal signs of manifestation of inflammation are -

- Swelling - Edema development due to increased interstitial fluid.
- Blushing - redness caused by a rise in blood pressure caused by vasodilation.
- Heat - Due to vasodilation and increased local oxygen demand, the temperature in the swollen region elevates.
- Pain - pain is caused by the release of chemicals such as prostaglandins, which activate nociceptors.
- Functionality is lost or reduced (trismus)[32].

The collection of serous fluid in the interstitial space as a result of surgical trauma causes swelling. The degree of swelling depends on the patient, surgical method, degree of invasive surgery, and length of surgical intervention. Swelling early during the healing process can cause severe pain to the patient and may lead to dehiscence of the predicate, resulting in delayed healing. ***Kwoen MJ et al***[41] reported about various drugs and methods that have been used to reduce postoperative swelling, such as low-level laser therapy, cold therapy and steroid and nonsteroidal anti-inflammatory agents. All patients in the present study were prescribed non- steroidal anti-inflammatory drugs post treatment twice daily for three days. The intensity of postoperative inflammatory reactions peaks on the second day after surgery and gradually fades during the next week.

Kocer G et at[42] reported the advantages of assessment of facial swelling in evaluation of the effect of anti-inflammatory drugs and to predict the presence of infection and pain. In the last 60 years, several methods have been employed to assess various forms of facial deformities, including the Face bow method, ultrasound method, and stereophotographic approach[44,43].

In terms of application, reproducibility, and simplicity and ease of procedure, the most frequently used approach for measuring distances between distinct groups of guide points on the face is the contact measurement method. ***Amin*** and ***Laskin***[32]

devised this approach for measuring facial swelling. However, non-contact measuring is rapidly replacing it; the newer methods typically require complicated measurement equipment in order to ensure that the head is oriented accurately during photography and radiography.

Owing to its advantages, the present study utilised the contact method, which is inexpensive, non-invasive, simple and oldest technique and can be performed with rulers and thread. It has a high applicability, accuracy and satisfies the minimal requisites for objective assessments of post-operative facial swelling or other induced changes in face dimensions in clinical studies[42]. Presence or absence of swelling were checked first preoperatively, then at 1st day, 7th day, 14th day post operatively. Within both groups, there was a significant increase in the mean swelling upto 7th day in comparison to pre-treatment, which eventually reduced by 14th day (Table 3,Graph 4,Graph 5). On evaluation of swelling between both groups (inter group) Freeze Dried Irradiated HAM treated group showed better and faster reduction in swelling when compared with collagen membrane group, although not very significant ($p<0.05$)(Table 4,Graph 6). As demonstrated in a previous study by ***Kumar A et al[23]*** **,** this could be due to various reasons, one, being administration of non steroidal anti-inflammatory drugs and dexamethasone post treatment to all patients, secondly and most importantly, Amniotic Membrane demonstrates a direct suppressive effect on IL-1ß and has an upregulatory effect on the expression of hBD-2. The significant reduction of Gingival Crevicular Fluid IL-1ß levels in sites treated with Amniotic Membrane seems to indicate that Amniotic Membrane has a significant anti-inflammatory effect.

INFECTION

Infection was evaluated visually by presence or absence of inflammation and purulence at the surgical site at 7th day and day 14th day post operatively. The presence of redness, raised localised temperature, purulent discharge, and tenderness were checked and recorded to be scored 0 if absent and 1, if present.

Although inflammation is a normal and beneficial step of wound healing, its exacerbation and spread can be aggravating and even result in treatment failure.

In the present study, there was no evidence of infection in the Freeze Dried Irradiated HAM treated patients, however, there was a significant development of infection in the collagen membrane treated patients on 7th day post operatively(Table 5, Graph 7) that lowered to 33.33% over the period of 14 days (Table 5, Graph 8). Similar findings in another study by ***Koushaei S et al***[28] suggested significant difference in inflammation between amnion and collagen groups. This could be explained by the antibacterial activity of HAM, in congruence with the findings of ***Monica Fernande Gomes et al***[9].

PAIN

Traditional techniques of grafting and membrane placement mostly lead to mild-to-moderate postoperative pain, and the intensity of pain could be attributed to various patient factors, and surgical factors. Thus pain is a subjective phenomena.

The interclass correlation coefficients indicate that the VAS for acute pain measurement has a high level of reliability. Ninety percent of pain assessments were repeatable within nine millimetres. The VAS appears to be sufficiently trustworthy to be used to assess acute pain, based on the findings of the studies done by ***Bijur PE et al***[33].

The pain was assessed using VAS scale at 1st day 7th day, 14th day in the present study. In each group, the post mean VAS score decreased linearly with time (Table 13, Graph 20) and it decreased evidently in group treated with amniotic membrane as compared to group treated with collagen membrane (Table 12, Graph 19). In other words, patients treated with amniotic membrane presented with lesser pain when compared to the ones treated with collagen membrane. Moreover, pain decreased with a faster rate in the patients treated with amniotic membrane. This would be due to adherence of the amnion to the lesion and the coverage of exposure of nerve endings concurring with the results of the studies carried out by ***Mermet I et at***[44]***, Subrahmanyam M et al***[41] and ***Ley-Chavez E et al***[46]. In addition, the adherence of The Amniotic Membrane prevents lesions from coming into contact with the environment, while its porosity allows wound fluid to evaporate. These mechanisms

have been advocated by ***Kesting MR et al***[6] to decrease plasma loss and prevent infection and sepsis.

BONE HEIGHT- LINEAR MEASUREMENTS

The solitary study that reported the osteoinduction impact of amniotic membrane was a previous study in vestibuloplasty technique by ***Samandari MH et al***[16]. It was suggested in that study that Amniotic Membrane could give adequate components for bone induction and promote the process of bone formation. According to the observations, Amniotic Membrane alone as a biological dressing has distinctive properties in bone induction that could be effective in the reconstruction of maxillofacial bone defects. Later on, ***Tsuno et al***[20] employed super dry amniotic membrane to cover surgically exposed bone surfaces in the oral cavity. They described two cases in which they used hyper dry amniotic membrane to treat intraoral alveolar lesions with bone exposure. The findings revealed that the hyper dry amniotic membrane is an effective dressing material for soft tissue lesions as well as exposed bone in the oral cavity.

The Amniotic Membrane stroma comprises of natural protease inhibitors, growth factors, anti-inflammatory proteins, and antiangiogenic factors. The basement membrane contains collagen type IV and VII, laminine 1 and 5, fibronectin, and basic fibroblast growth factor. Considering that Amniotic Membrane has different growth factors, it not only contributes to improving and accelerating physiologic wound healings, but also, stimulates bone induction[28]. Later ***Etchebarne M et al***[30] did a meticulous review of 42 previous studies dedicated to the application of amniotic membrane and its derivatives for regeneration of bone. This validates the findings of the current study, indicating that, as compared to collagen or synthetic membranes frequently employed for GBR techniques, Amniotic Membrane has an array of biological features that makes it particularly appealing in this field.

Since the late 1800s, collagen membranes have been studied for their role in regeneration of intrabony defects[43]. Collagen membranes do not have the space-making ability of non-resorbable membranes. The use of bone graft material to preserve space enhanced GBR results. Alveolar bone augmentation is facilitated if

the space under the collagen membrane is created and preserved in an appropriate period while the new bone is being formed. It is therefore advisable to use materials which will provide support as to prevent collapse of the barrier due to pressure of overlay tissue or due to chewing forces. These membranes are frequently employed in conjunction with tenting or supporting materials such as bone grafts or bone fillers to prevent the collapse of space. According to a study conducted by ***Bubalo M et al***[47], when grafting materials are combined with bioresorbable membranes, the results of GBR treatments are generally successful and even comparable to the results obtained with non-resorbable barriers.

In the present study, height of alveolar bone was evaluated pre-operatively and postoperatively (immediately, at 7th day, ^{30th} day, 90th day and 180th day) with the help of Intraoral periapical radiograph using Paralleling cone technique. Height of alveolar bone crest was measured at mesial, distal and central aspects of the bone deficient site. The total change in alveolar bone height was then compared to pre operative findings in both groups periodically.

In another study by ***Gomes MF et al***[9] a follow-up period of 120 days significantly showed mature bone formation and new formed bone was appreciable after 30 days, similar to the outcomes of the present study.

Our results concur with a similar study by ***Koushaei S et*** al[28] comparing the bone induction effects of an amnion membrane protected graft to a collagen membrane protected graft and found that the collagen group had less bone formation than the amnion group after 6 weeks, but this difference was not statistically significant. The presence of several factors, such as basic fibroblast growth factor and laminine 1 and 5, appeared to be beneficial for conversion of woven bone to lamellar bone. The presence of fibronectin and laminine may most likely explain the amniotic membrane's osteoinductive properties[48].

The present investigation indicated that GBR of alveolar bone with either Freeze Dried Irradiated HAM or Collagen membrane resulted in comparatively similar radiographic changes in bone-level. This is consistent with the clinical findings of ***Mardas et al***[31], in which the two biomaterials exhibited comparable ability in

preserving a substantial percentage of the pre-extraction clinical dimensions of the alveolar ridge and supporting bone growth. The distance between the alveolar bone crest at the mesial and distal aspects of the socket and the relative cemento enamel junction or restoration margin of the neighbouring teeth was measured intra surgically at baseline and 8 months after tooth extraction and alveolar ridge preservation in that clinical study. The mean differences between the two groups were not found to be statistically significant.Furthermore, the mean values assessed at baseline were not statistically different from the mean values taken at 8 months within each group, demonstrating that interproximal bone may be entirely preserved after ridge preservation with both biomaterials.

In the present study, the radiographic analysis showed a mild increase in the radiographic bone levels at 180^{th} day following treatment in both groups. In the Freeze Dried Irradiated HAM group, the changes in mesial linear measurements and distal linear measurements, at the mesial and distal site were 4.10 ± 2.17, 4.20 ± 2.17, respectively. For the same period in the group treated with collagen membrane, the Mesial and distal linear measurement showed a mean difference of 3.50 ± 0.70, 3.45 ± 0.73 respectively, indicating bone formation of similar extent in both the groups. The results indicated post treatment increase in mean mesial bone height within group treated with Amniotic membrane was more as compared to within the group treated with collagen membrane but intergroup mean difference was not statistically significant (Table 6, Graph. 9). The changes in distal bone height showed similar trend as of changes in mesial bone height(Table 8, Graph.12). In both groups, the mean central bone height increased after the treatment and remained higher at all post operative periods as compared to pre treatment. However, the post treatment increase in mean central bone height was slightly higher in the group treated with Amniotic membrane as compared to the group treated with collagen membrane, but the results were statistically insignificant to draw any conclusion (Table 10, Graph. 15).

Care was taken in interpreting data of changes in bone levels between different observation periods as the linear measurement showed the line drawn from the cervical margin of the adjacent tooth to the mesial, distal or central most depth of the deficient site. So any decrease in linear measurement indicated an increase in actual

bone height of that area and vice versa. Because of the vast number of statistical comparisons made in this study, it's conceivable that some of the findings were due to statistical chance.

Another intriguing finding of this study was that the pre operative linear measurements at mesial sites were found to be significantly different between the two groups. No evident biological or methodological basis could be found to explain this disparity. The implementation of a strict randomization process, as well as the masking of the examiner who performed the measures, reduced the chance of a systematic error causing such a variance in the measurements. As a result, we had to attribute this disparity to an inadvertent fact.

Intraoral radiographic examination to assess bone levels after tooth extraction, or to detect changes in bone deficient sites or infrabony defects or after regenerative treatment, have been used at a previous clinical study by ***Schropp et al***[49] and ***Zybutz et al***[50]. However, such an approach had distinct limitations as a tool for assessment, beginning with the fact that periapical radiographs only provide two-dimensional images of three-dimensional structures. Furthermore, as the projection geometry changes, the radiographic image of the mesial and distal bone may also alter. Consequently, it is critical that the images be obtained under uniform parameters of film type, exposure time, film processing and with standardised projection geometry. This was also suggested in another study by ***Wenzel & Sewerin***[51].

In the present study standardization of projection geometry has been accomplished by using the paralleling cone technique. Despite the fact that intraoral radiographs were standardised, some degree of magnification is inevitable. This magnification could be caused by tooth movement or occlusal alterations.

Apart from standardization, the identification of anatomical landmarks in X-rays and the measurements of the distances between them poses a significant factor of bias in all studies that use conventional methods of radiography for evaluation of bone level changes. When compared with the gold standard of intra surgical measurements as suggested by ***Shrout et al***[52] both conventional methods (direct measurements on X-rays using magnifying means) and the use of computer assisted digital image analysis systems underestimate the true linear distances between reference

anatomical landmarks such as cemento enamel junction or the alveolar bone crest to a varying degree.

Different factors can affect the precision of radiographic linear measurements.. ***Wolf et al[53]*** tested the reproducibility of the radiographic linear measurements of interproximal loss of bone at infra bony defects (intra and inter examiner) and explained that the radiographic measurements often overestimate the amount of loss of bone as assessed by intra surgical measurements and the reproducibility of the measurements were found to be significantly influenced by the examiner.

In the present study, a single examiner performed all the measurements and was comparable to previous reports.

Bone loss observed between the two groups could be explained by either an increase in resorption of bone in the bone deficient site treated with collagen membrane or, an increase in rate of resorption the collagen membrane or a combination of these both resulting in reduced radiopacity in all the cases. However, the amount of bone resorption or bone formation cannot be estimated with the methodology used in this study.

Besides its multifactorial utility potential, Amniotic Membrane is also found to be immune privileged tissue that contains a few immunoregulatory factors, that include HLA-G (an immunosuppressive factor) and Fas ligand demonstrated in a study by ***Kubo et al[10]***. This property was also advocated by the reduction or absence of expression of HLA class I molecules and the absence of HLA class II molecules deduced in the results of a study by ***Ilancheran et al[54]***, avoiding allograft or xenograft rejection of HAM.

It is easy to handle and to adapt to the surgical site. Amniotic epithelial cells and amniotic stromal cells have showed their ability to develop into multiple cell types, including osteogenic cells, and can be employed safely as a reserve of two pluripotent cell types for tissue engineering. Teratoma development and in vivo tumorigenicity of amniotic epithelial cells and amniotic stromal cells have not been reported, in contrast to embryonic stem cells or induced pluripotent stem cells[30].

LIMITATIONS

1. Firstly, the study showed low level of evidence due to the limited sample size.
2. Although the study measures change in alveolar bone height, yet the amount of bone resorption or bone formation cannot be estimated with the methodology applied in this study.
3. Due to heterogenicity of the site of treatment, it was difficult to make a fair comparison in both inter group and intra group.
4. Use of radiographic method for measurement of bone height allowed only two dimensional measurement of bone level changes. In addition to it, it increased the chances of error.
5. Post surgical administration of non steroidal anti inflammatory drugs and dexamethasone contributed to altered swelling results.

CONCLUSION

CONCLUSION

- Based on the present clinical trial, the differences in swelling were not statistically significant; however, the degree of swelling was lower in the group that received Freeze Dried Irradiated HAM than the group that received collagen membrane treatment.
- Patients treated with amniotic membrane presented with lesser pain when compared to the ones treated with collagen membrane. Moreover, pain decreased with a faster rate in the patients treated with amniotic membrane.
- In the present study, there was no evidence of infection in the patients treated with Freeze Dried Irradiated HAM, however, there was a significant development of infection in the patients treated with collagen membrane, that decreased eventually, rendering results in favour of Freeze Dried Irradiated HAM.
- Alveolar bone height increased within both the groups over a period of 180 days. However, no statistically significant difference was observed between the two groups.
- All properties of the amniotic membrane and collagen membrane, biocompatibility, occlusiveness, analgesic, antimicrobial, anti inflammatory, pleuripotency, osteoinductivity were comparable.
- In addition, Freeze Dried Irradiated HAM can be easily obtained, and it is inexpensive when compared to commercially available collagen membrane.
- Freeze Dried Irradiated HAM is a promising alternative to the commercially available collagen membranes used for guided bone regeneration procedures.
- It is safe to conclude that owing to its cost effectiveness, better handling characteristics, faster healing, less inflammatory response, Freeze Dried Irradiated HAM has the potential to emerge as the treatment of choice not only in soft tissue healing but also in bone regenerative procedures in the field of oral and maxillofacial surgery.
- Previous studies have already compared the properties and effectiveness of collagen membrane and other barrier membranes with amniotic membrane on

soft tissue healing. The present study compared ability of collagen membrane with amniotic membrane for reconstruction of alveolar bone defects.

- Further studies should be performed to include and study the clinical, radiographical and histologic properties simultaneously, to draw conclusive results over osteoinductive potential of these membranes.

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APPENDICES

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparison of Efficacy of Human Amniotic Membrane Versus Collagen Membrane in Guided Bone Regeneration of Alveolar Bone."** submitted by **Dr Rohie Jawarker** Post graduate student from the **Department of Oral & Maxillofacial Surgery** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **26th November 2018** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VII[th] Institutional Ethics Sub-Committee

IEC Code: 36 **BBDCODS/01/2019**

Title of the Project: Comparison of Efficacy of Human Amniotic Membrane Versus Collagen Membrane in Guided Bone Regeneration of Alveolar Bone.

Principal Investigator: Dr. Rohie Jawarker **Department:** Oral & Maxillofacial Surgery

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Rohie Jawarker,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10[th] January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Lakshmi Bala
21/01/19

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

Forwarded by:

(Prof. D. Rajkumar)
PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

Principal
BBDCODS

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Consent Form (English)

Title of the Study ……..

Study Number……..
Subject's Full Name…….
Date of Birth/Age ……
Address of the Subject…….
Phone no. and e-mail address……
Qualification ……
Occupation: Student / Self Employed / Service / Housewife/
Other (Please tick as appropriate)
Annual income of the Subject……
Name and of the nominees(s) and his relation to the subject…… (For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated ……..for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ] No [ ] Not Applicable [ ]**
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:……..
Signatory's Name……. Date …….
Signature of the Investigator…… Date……..
Study Investigator's Name..... Date……..
Signature of the witness…… Date……..
Name of the witness……
Received a signed copy of the PID and duly filled consent form
Signature/thumb impression of the subject or legally Date……..

BBDCODS

Acceptable representative

BBDCODS

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

सहमति पत्र

अध्ययन शीर्षक.....
अध्ययन संख्या.....
प्रतिभागी के पूर्ण नाम.....
जन्म तिथि / आयु.....
प्रतिभागी का पता .....
फोन नं. और ई-मेल पता .....
योग्यता .....
व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी .....
अन्य (उचित रुप मे टिक करें) .....
प्रतिभागी की वार्षिक आय .....
प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले मे मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैने अध्ययन हेतु सुचना पत्र दिनांक ..... को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैंने यहॉ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हुॅ ।

3. मैंने यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हुॅ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूॅ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।
5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हुॅ।

हॉ [ ] नही [ ] अनउपयुक्त [ ]

BBDCODS

6. मै परीक्षण की अनुमति देता हुॅ। मुझे इसके द्वारा यदि कोई परेशानी होती है, इसके बारे में जानकारी दे दी गई है। मैने रोगी जानकारी सूचना पत्र को पढ तथा समझ लिया है।

प्रतिभागी / कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान.....

हस्ताक्षरकर्ता का नाम..... दिनांक .....अन्वेषक के हस्ताक्षर ..... दिनांक .....

अध्ययन अन्वेषक का नाम .....

गवाह के हस्ताक्षर ..... दिनांक .....गवाह के नाम .....

मैनें पीआईडी और विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की.

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर/ अंगूठे का निशान ..... दिनांक.....

BBDCODS

Babu Banarasi Das College of Dental Sciences

(A constituent institution of Babu Banarasi Das University)

BBD City, Faizabad road, Lucknow – 227105 (INDIA)

Patient Information Document (PID)

1.Study title

Comparison of efficacy of Human Amniotic Membrane versus Collagen Membrane in Guided Bone Regeneration of Alveolar Bone.

2.Invitation paragraph

You are being invited to take part in a research study,therefore it is important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your treating physician/family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information. It is up to you to decide whether or not to take part.

3.What is the purpose of the study?

To assess the efficacy of amniotic membrane in comparison to collagen membrane in guided bone regeneration of alveolar bone.

4.Why have you been chosen?

You have been chosen for this study as fulfilling the required criteria for the diseased condition.

5.Why would you take part?

Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

6. What will happen to you if you take part?

My study will last for 3 years and you will be involved in my study for 6 months, Guided Bone Regeneration procedure will be used in which Amniotic Membrane and Collagen Membrane will be placed on the deficient bone with or without bone grafting,

so more bone is available to support a dental implant or other oral rehabilitation procedure.

7. What would you have to do?

You do not have to change your regular lifestyles for the investigation of the study.

8. What is the procedure that is being tested?

To compare the efficacy of amniotic membrane to collagen membrane in guided bone regeneration.

9. What are the interventions for the study?

There are no such interventions, risk and adverse effects related to the study. There is clinical benefit to the volunteer as her/his bone height will increase for placement of implants and other oral rehabilitation procedures.

10. What are the side effects of taking part?

There are no side effects on patients of this study.

11. What are the possible disadvantages and risks of taking part?

Some disadvantages which may happen such as graft rejection, infection etc. but no long term irreversible changes would be seen.

12. What are the possible benefits of taking part?

We wish that you will get benefits after taking part in our study. Your participation in the study may help others, because this participation will help us determine which procedure is efficacious. It will help in your implant replacement or any other oral rehabilitation leading to better chewing ability and esthetics.

13. What if new information becomes available?

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you weather you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

14. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to you.

15. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and IEC. Cost to be born by the patient.

16. Shall I take part in this study be kept confidential?

Yes it will be kept confidential.

17. What will happen to the results of the research study?

The result of the study will be published in the indexed journal. Your identity will be kept confidential in case of any report/publications.

18. Who is organizing the research?

This research study is organized by the candidate and Department of Oral & Maxillofacial Surgery.

19. Will the results of the study be made available after study is over?

Yes, only the data obtained will be published

20. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Department, the IEC and RDC of the institution.

Contact for further information

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Secretary Ethics committee
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Name of principle investigator…….

Signature of principle investigator …….

Date……...

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज
(बाबू बनारसी दास विश्वविद्यालय का एक घटक संस्थान)
बीबीडी सिटी, फैजाबाद रोड, लखनऊ - 227105 (INDIA)

रोगी सूचना दस्तावेज (पीआईडी)

1. अध्ययन शीर्षक

एल्वोलर हड्डी के निर्देशित हड्डी पुनर्जनन में मानव एमनियोटिक झिल्ली बनाम कोलेजन झिल्ली की प्रभावकारिता की तुलना।

2. इनवेशन पैराग्राफ

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जा रहा है, इसलिए आपके लिए यह समझना महत्वपूर्ण है कि अध्ययन क्यों किया जा रहा है और इसमें क्या शामिल होगा। कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने और दोस्तों, रिश्तेदारों और अपने इलाज करने वाले चिकित्सक / परिवार के डॉक्टर से चर्चा करें। हमसे पूछें कि क्या ऐसा कुछ है जो स्पष्ट नहीं है या यदि आप अधिक जानकारी चाहते हैं। यह आपको तय करना है कि आपको हिस्सा लेना है या नहीं।

3. अध्ययन का उद्देश्य क्या है?

वायुकोशीय हड्डी के निर्देशित हड्डी पुनर्जनन में कोलेजन झिल्ली की तुलना में एम्नियोटिक झिल्ली की प्रभावकारिता का आकलन करने के लिए।

4. आप क्यों चुना गया है?

रोगग्रस्त स्थिति के लिए आवश्यक मानदंडों को पूरा करने के लिए आपको इस अध्ययन के लिए चुना गया है।

5. आप क्यों भाग लेंगे?

अनुसंधान में आपकी भागीदारी पूरी तरह से स्वैच्छिक है। यदि आप करते हैं, तो आपको रखने के लिए यह सूचना पत्र दिया जाएगा और सहमति पत्र पर हस्ताक्षर करने के लिए कहा जाएगा। अध्ययन के दौरान आप बिना किसी कारण के किसी भी समय वापस लेने के लिए स्वतंत्र हैं।

6. अगर आप हिस्सा लेंगे तो आपका क्या होगा?

मेरा अध्ययन 3 साल तक चलेगा और आप 6 महीने के लिए मेरे अध्ययन में शामिल होंगे, निर्देशित हड्डी पुनर्जनन प्रक्रिया का उपयोग किया जाएगा, जिसमें एमनियोटिक झिल्ली और कोलेजन मेम्ब्रेन को हड्डी के प्रारूपण के साथ या बिना हड्डी की कमी वाले हड्डी पर रखा जाएगा, इसलिए अधिक हड्डी है एक दंत प्रत्यारोपण या अन्य मौखिक पुनर्वास प्रक्रिया का समर्थन करने के लिए उपलब्ध है।

7. आपको क्या करना होगा?

अध्ययन की जांच के लिए आपको अपनी नियमित जीवन शैली को बदलने की आवश्यकता नहीं है।

8. वह प्रक्रिया क्या है जिसका परीक्षण किया जा रहा है?

निर्देशित हड्डी पुनर्जनन में कोलेजन झिल्ली को एमनियोटिक झिल्ली की प्रभावकारिता की तुलना करने के लिए।

9. अध्ययन के लिए हस्तक्षेप क्या हैं?

अध्ययन से संबंधित ऐसे कोई हस्तक्षेप, जोखिम और प्रतिकूल प्रभाव नहीं हैं। स्वयंसेवक को नैदानिक लाभ है क्योंकि प्रत्यारोपण और अन्य मौखिक पुनर्वास प्रक्रियाओं के लिए उसकी हड्डी की ऊंचाई बढ़ जाएगी।

10. भाग लेने के दुष्प्रभाव क्या हैं?

इस अध्ययन के रोगियों पर कोई दुष्प्रभाव नहीं हैं।

11. भाग लेने के संभावित नुकसान और जोखिम क्या हैं?

कुछ नुकसान जो इस तरह के भ्रष्टाचार अस्वीकृति, संक्रमण आदि के रूप में हो सकते हैं, लेकिन कोई दीर्घकालिक अपरिवर्तनीय परिवर्तन नहीं देखा जाएगा।

12. भाग लेने के संभावित लाभ क्या हैं?

हम चाहते हैं कि हमारे अध्ययन में भाग लेने के बाद आपको लाभ मिलेगा। अध्ययन में आपकी भागीदारी दूसरों की मदद कर सकती है, क्योंकि यह भागीदारी हमें यह निर्धारित करने में मदद करेगी कि कौन सी प्रक्रिया प्रभावोत्पादक है। यह आपके प्रत्यारोपण प्रतिस्थापन या किसी अन्य मौखिक पुनर्वास में बेहतर चबाने की क्षमता और एस्थेटिक्स के लिए अग्रणी होगा।

13. यदि नई जानकारी उपलब्ध हो जाए तो क्या होगा?

यदि अनुसंधान के दौरान अतिरिक्त जानकारी उपलब्ध हो जाती है, तो आपको इन के बारे में बताया जाएगा और आप अपने शोधकर्ता के साथ इस पर चर्चा करने के लिए स्वतंत्र हैं, आपका शोधकर्ता आपको बताएगा कि आप अध्ययन में जारी रहना चाहते हैं। यदि आप वापस लेने का निर्णय लेते हैं, तो आपका शोधकर्ता आपकी वापसी की व्यवस्था करेगा। यदि आप अध्ययन जारी रखने का निर्णय लेते हैं, तो आपको एक अद्यतन सहमति पत्र पर हस्ताक्षर करने के लिए कहा जा सकता है।

14. जब शोध अध्ययन रुक जाता है तो क्या होता है?

यदि निर्धारित समय से पहले अध्ययन रुक जाता है / समाप्त हो जाता है, तो यह आपको समझाया जाएगा।

15. अगर कुछ गलत हो जाए तो क्या होगा?
यदि कोई गंभीर प्रतिकूल घटना होती है, या अध्ययन के दौरान कुछ गलत होता है, तो संस्थान (एस), और आईईसी को रिपोर्ट करके शिकायतों को नियंत्रित किया जाएगा। रोगी द्वारा पैदा की जाने वाली लागत।

16. क्या मुझे इस अध्ययन में भाग लेना गोपनीय रखा जाएगा?
हां इसे गोपनीय रखा जाएगा।
17. शोध अध्ययन के परिणामों का क्या होगा?
अध्ययन का परिणाम अनुक्रमित पत्रिका में प्रकाशित किया जाएगा। किसी भी रिपोर्ट / प्रकाशन के मामले में आपकी पहचान गोपनीय रखी जाएगी।

18. अनुसंधान का आयोजन कौन कर रहा है?
यह शोध अध्ययन उम्मीदवार और ओरल एंड मैक्सिलोफेशियल सर्जरी विभाग द्वारा आयोजित किया गया है।

19. क्या अध्ययन के परिणाम अध्ययन के बाद उपलब्ध कराए जाएंगे?
हां, केवल प्राप्त डेटा प्रकाशित किया जाएगा
।
20. अध्ययन की समीक्षा किसने की?
विभाग के प्रमुख, आईईसी और आरडीसी द्वारा अध्ययन की समीक्षा और अनुमोदन किया गया है।

अधिक जानकारी के लिए संपर्क करें

डॉ। रोही जवारकर डॉ। लक्ष्मी बाला
ओरल और मैक्सिलोफेशियल सर्जरी सचिव आचार समिति के विभाग
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सिद्धांत अन्वेषक का नाम .....।

सिद्धांत अन्वेषक का हस्ताक्षर .....

CASE SHEET

DATE:

OPD NO:

1. NAME :
2. AGE :
3. Gender :
4. Address :

5. Contact no. :
6. Chief Complaint :

7. History Of Present Illness :

8. Past Dental History :

9. Medical history :

10. Personal Habits :

11. Extra Oral Examination:-

- Facial Measurements (affected side):

	Ala to tragus	Tragus to corner of the mouth	Tragus to chin (in mandible)
Measurement in mm			

- Facial Symmetry :

- Lymph Nodes :

- TMJ Movements :

12. Intra Oral Examination :-
    - Hard Tissue Examination :-
        - Teeth Present –
        - Decayed Teeth --
        - Missing Teeth –
        - Filled Teeth –
        - Mobile Teeth –
        - Root Stumps -
    - Soft Tissue Examination :-
        - Gingival –
        - Tongue –
        - Oral Mucosa –
13. Provisional Diagnosis :
14. Investigations :
15. Final Diagnosis :

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16. Pre – Operative Clinical Evaluation:-

a. Periodontal Status :

b. Attached Gingival Status :

c. Any Existing Prosthesis :

d. Maxillomandibular Arch Relations:

17. Radiographic Assessment : -
    a) IOPAR–

18. Evaluation of Bone Height measurements in mm :-
    - Central –
    - Mesial –
    - Distal –

19. Treatment Planning :-

i) Membrane Selection :

- Type- FDIHAM/Collagen membrane
- Graft Placement (if involved) – (in cc)

20. Treatment Done :-

21. Post-Operative Clinical Evaluation

1) Swelling :-
   - Facial Measurements (affected side):

	Ala to tragus	Tragus to corner of the mouth	Tragus to chin (in mandible)
After 1 day			
After 7th day			
After 14th day			

2) Infection:-

   - Present / Absent- (Score 1/0)

	At 7th day	At 14th day
score		

3) Pain (VAS) –

	After 1 day	After 7th day	After 14th day
Score			

4) Radiographic Assessment to Assess Bone height :-

IOPAR (in mm):-

	Immediately after	7th day	14th day	30th day	90th day	180th day
Mesial linear measurement						
Central linear measurement						
Distal Linear measurement						

5) Complication If Any :-

(SIGNATURE OF THE CONSULTANT)

FORMULA USED FOR THE ANALYSIS

Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Standard deviation and standard error

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

and SE (standard error of the mean) is calculated as

$$SE = \frac{SD}{\sqrt{n}}$$

where, n= no. of observations

Minimum and Maximum

Minimum and maximum are the minimum and maximum values respectively in the measure data and range may be dented as below

Range = Min to Max

and also evaluated by subtracting minimum value from maximum value as below

Range = Maximum value-Minimum value

Median

The median is generally defined as the middle measurement in an ordered set of data. That is, there are just as many observations larger than the median as there are smaller. The median (M) of a sample of data may be found by first arranging the measurements in order of magnitude (preferably ascending). For even and odd number of measurements, the median is evaluated as

M= [(n+1)/2]th observation- odd number

M= [n(n+1)/2]th observation – even number

Student's t Test

Student's t-test was used to calculate the differences between the means of two groups

$$t = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

where,

$$SE = \sqrt{S^2 X \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

S^2 is the pooled variance and n1 and n2 are number of observations in group 1 and 2 respectively. The degrees of freedom (DF) is calculated as

DF = n1 + n2 – 2

Chi-square test

The chi-square (χ^2) test is used to compare the categorical data as

$$\chi^2 = \Sigma\Sigma \frac{(Fij - fij)^2}{fij}$$

where, Fij is the observed frequency while fij the expected frequency. The degrees of freedom (DF) is calculated as

$$DF = (r-1)\,(c-1)$$

Analysis of Variance

Analysis of variance (ANOVA) is used when we compare more than two groups simultaneously. The purpose of one-way ANOVA is to find out whether data from several groups have a common mean. That is, to determine whether the groups are actually different in the measured characteristic. One way ANOVA is a simple special case of the linear model. For more than two independent groups, simple parametric ANOVA is used when variables under consideration follows Continuous exercise group distribution and groups variances are homogeneous otherwise non parametric alternative Kruskal-Wallis (H) ANOVA by ranks is used. The one way ANOVA form of the model is

$$Y_{ij} = \alpha_{.j} + \varepsilon_{ij}$$

where;

- Y_{ij} is a matrix of observations in which each column represents a different group.
- $\alpha_{.j}$ is a matrix whose columns are the group means (the "dot j" notation means that α applies to all rows of the j^{th} column i.e. the value α_{ij} is the same for all i).
- ε_{ij} is a matrix of random disturbances.

The model posits that the columns of Y are a constant plus a random disturbance. We want to know if the constants are all the same.

Tukey multiple comparison Test

After performing ANOVA, Tukey HSD (honestly significant difference) post hoc test is generally used to calculate differences between group means as

where,

$$q = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

$$SE = \sqrt{\frac{S^2}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

S^2 is the error mean square from the analysis of variance and n_1 and n_2 are number of data in group 1 and 2 respectively.

Level of significance "*P*" is the probability signifies level of significance. The mentioned *P* in the text indicates the following:

P > 0.05- Not significant (ns)

P < 0.05- Just significant (*)

P < 0.01- Moderately significant (**)

P < 0.05- Highly significant (***)

Urkund Analysis Result

Analysed Document: thesis rohie merged.pdf (D110195941)
Submitted: 7/6/2021 11:20:00 AM
Submitted By: hemantmehra121@bbdu.ac.in
Significance: 6 %

Sources included in the report:

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