"EVALUATION OF WOUND HEALING AND BONE REGENERATION USING TOPICAL COMBINED APPLICATION OF VITAMIN C, DEXAMETHASONE AND BETA-SODIUM GLYCEROPHOSPHATE FOR HEALING OF EXTRACTION SOCKET- A COMPARATIVE STUDY"

By

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Dissertation

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Under the Guidance of

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KAHER's KLE V K INSTITUTE OF DENTAL SCIENCES

BELAGAVI, KARNATAKA

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I dedicate my work to

my family

Dr. Arindam Mandal,

Mrs. Rietu Mandal,

&

Miss Akanksha Mandal

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DR. RISHABH MANDAL

LIST OF ABBREVIATIONS USED

1. ALP	Alkaline phosphatase
2. BMSC	Bone marrow mesenchymal stem cells
3. AA	Ascorbic Acid
4. Beta-Gp	Beta sodium glycerophosphate
5. Dex	Dexamethasone
6. PDLSCs	Periodontal ligament stem cells
7. Vc	Vitamin C
8. hBMMCs	Human bone marrow-derived mesenchymal cells
9. MSC	Mesenchymal stem cells
10. rhBMP2	Recombinant human bone morphogenetic protein-2
11. LD	Lamina dura
12. ACS	Absorbable collagen sponge
13. BMP	Bone morphogenetic protein
14. RVG	RadioVisioGraphy
15. TGF-beta	Transforming growth factor-beta
16. DME	Dulbecco's modified Eagle medium

ABSTRACT

Background and Objectives:

In the first three months after extraction, the bone loss is substantial and can result in both hard and soft tissue defects affecting the potential of the site to regenerate with an adequate amount of bone. This bone loss accounts for the reduced vertical as well as horizontal dimension of alveolar bone decreasing the quality of bone available for rehabilitation.

Nowadays, autogenic bone graft is gold standard and is used commonly among Oral and Maxillofacial surgeons. Numerous researchers and clinicians have developed over time different socket preservation materials, which have their pros and cons like problem of supply, complex surgical procedure, risk of contamination and high cost.

This study aims to evaluate the efficacy of osteogenic inducer (Beta-Sodium Glycerophosphate + Vitamin C + Dexamethasone) when placed in the extraction socket and also to assess the potential use of the material for better wound healing in humans as this combination has successfully been used in rabbits and on human bone marrow cells as an in-vitro study.

Materials and Method:

- Computer generated random allocation of36 patients was done into case group and control group.

- The surgical procedure was performed by a single surgeon under all aseptic conditions.

- As this study involves comparison between osteogenic inducer and usual method of wound healing and bone regeneration. Patients were divided into two groups of 18 each.

    1. Case Group- Osteogenic inducer was placed in the third molar extraction socket and would act as the case group.

    2. Control Group - No material was placed in the third molar extraction socket and would act as the control group.

- Patientswere recalled at intervals of 1day, 7 days, 1 month and 3 months to assess the healing of the socket clinically and radiographically (RVG).

  A. 1^{st} and 7^{th} day for assessment of wound healing.

  B. 1^{st} day, 1^{st} month and 3^{rd} month for assessment of boneregeneration.

Results:

We observed that case group showed significantly better wound healing as compared to control group, in terms of tissue colour, bleeding on palpation, suppuration and granulation tissue at the end of 7th day following extraction.

Bone regeneration was also assessed and case group showed faster bone healing when compared to control group, in terms of presence of lamina dura, overall bone density and trabecular patterns when assessed after 3 months following extraction.

No postoperative complications were seen with the case group patients.

Interpretation and conclusion:

Depending on our research, we can conclude that topical application of osteogenic inducer serves as effective scaffold for wound healing as well as for faster bone regeneration as the material is easily available, cost-effective and does not require any special tools for preparation. It has shown positive results, with the added benefits of no additional donor site requirement, reduced postoperative complications and is not technique sensitive.We recommend more clinical trials with larger sample size, longer follow-up duration and use in normal extraction socket. In order to reaffirm the findings and arrive at a stronger conclusion, histological analysis should also be used.

Keywords: gelatin sponge; osteogenic inducer; tooth extraction socket.

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INTRODUCTION

Tooth extraction leads to reduction in alveolar bone width and length owing to the lack of forces generated during mastication and will lead to distortion of occlusion causing further problems to the patient[1]. As the patient waits functional and aesthetic changes starts to take place which eventually increases the difficulty of rehabilitation. Horizontal loss of bone is approximately three times the vertical loss of bone, as horizontal loss of bone is four to six millimeters (i.e. fifty percent of the preoperative dimensions) over the following six to twelve months, whereas after extraction there is a vertical loss of bone accounting up to one to three millimeters within the first six to twelve months[2]. In addition, the epithelial lining of the cortical bone is thin and the pressure of the lip over the alveolar ridge will also lead to loss of bone[3]. Consequently, additional procedures would be required to increase the alveolar ridge height, resulting in higher expense for the patient as the number of procedures increase as well as the waiting period for rehabilitation[4].

Lot of materials have been designed to modulate the alveolar bone like allogenic bone graft and growth enhancing factors[5]. However, each of these has their demerits like problem of availability, complex surgical procedure, high chances of infection and are not cost effective. Depending on type of bone grafts they can induce various mechanisms of bone healing which can be categorised as osteogenesis, osteoinduction and osteoconduction[6].

Out of the various forms of bone grafts available, the most promising is autogenous bone grafting because it is capable of osteogenesis, osteoinduction and osteoconduction with reasonable healing period, no chances of graft rejection or an

immune response[1]. The shortcoming of autogenous bone graft is that its harvesting is limited and creates a second defect from the donor site which if not taken proper postoperative care can lead to further complications.

Allogenic, xenogeneic and alloplastic graft materials have been used as alternatives even though they have a significant drawback compared to autogenous grafts, like the decreased efficiency, heightened risk of infection, unreliable resorption pattern, extended healing phase and exorbitant cost[7].

A suitable material for bone grafting must secure the blood clot, create a biomechanical matrix for migration of cells, induce proliferation and differentiation, provide essential proteins, such as growth factors and display acceptable resorption and remodelling throughout formation of new bone[8]. Nowadays researchers have directed their attention to develop a bone graft material which shows all the above suitable criteria as mentioned above with minimal to no side effects for patients. In fact, there are no specific standards validated by credible evidence that suggests which kind of biomaterial to be used[9].

Therefore, it is essential to develop a simple, convenient and low-cost procedure to mitigate for these constraints. To reduce or eliminate the need for second surgical procedure to re-establish the proportionate alveolar ridge for rehabilitation one has to plan and intervene at the time of extraction for better treatment outcome and patient satisfaction[2].

In 2015, **J.Chen, et al**[10] conducted a study to determine the efficacy of the combined topical application of Dexamethasone, Vitamin C and Beta-Sodium Glycerophosphate (Osteogenic Inducer) in healing of extraction sockets of rabbits. The

result clearly showed that the osteogenic group had better healing and maintained the alveolar ridge height for future dental rehabilitation. Dexamethasone itself is an effective osteogenic inducer and has the potential to stimulate alkaline phosphatase (ALP) activity in mesenchymal stem cells (BMSCs) of the bone marrow as well as can stimulate calcium deposition and mineralization, but it must be carefully measured as high dexamethasone density by triggering the steroid receptor on the cell surface will prevent the proliferation of BMSCs and their differentiation into osteoblasts[10].

Tao Li et al[11] reported best density range is between $10^8$to 10^{10} mol/l. **Morsczeck, et al**[12] stated that dexamethasone will also contribute to formation of osteoblasts by differentiation of undifferentiated mesenchymal cells. Vitamin C is found to enhance the production of gelatin that leads to cellular proliferation, calcification formation, and stimulation of ALP activity, ultimately triggering bone formation.

Taira et al[13] observed a synergy between vitamin C and other bioactive molecules that could enhance the cell proliferation. Beta-Sodium glycerophosphate is noted to provide osteoblasts with phosphate ions, facilitate deposition and calcification of the calcium salt, thereby accelerating the calcification and maturation of newly formed bone.

The blend of Dexamethasone, Vitamin C, and Beta-Sodium Glycerophosphate was already considered a popular Osteogenic Inducer as this combination has already been used in rabbits and on human bone marrow cells as an in-vitro study but a human in-vivo is not done yet until our study. It has the potential to trigger BMSCs to transform into osteoblasts, enhancing regeneration of bone[10].

The effect of osteogenic inducer on tooth extraction site's healing is unclear. The tooth extraction socket contains several undifferentiated mesenchymal cells, in addition to a more number of BMSCs which infiltrate the socket during the wound healing via the blood supply[10]. The purpose of this study was, therefore, to analyze the influence of this specific osteogenic inducer on the preservation of alveolar ridges after it is introduced into a fresh extraction socket in humans.

AIMS & OBJECTIVES OF THE STUDY

AIM:

- To evaluate the efficacy of combination of Vitamin C, Dexamethasone and Beta-Sodium Glycerophosphate in terms of wound healing and bone regeneration in mandibular third molar extraction sockets.

OBJECTIVES:

- To assess and compare wound healing in both control group and case group.
- To assess and compare bone regeneration in both control group and case group.

NULL HYPOTHESIS:

- There is no difference in wound healing and bone regeneration when osteogenic inducer is used in the extraction socket.

RESEARCH HYPOTHESIS:

- There is significant difference in wound healing and bone regeneration when osteogenic inducer is used in the extraction socket.

REVIEW OF LITERATURE

1. **Douglas Darr, et al**[14] in 1993 studied that ascorbic acid has a beneficial impact on collagen synthesis, the most abundant extracellular protein. The mechanism by which ascorbic acid mediates the elevated synthesis is debated. One recent theory indicates that ascorbic acid causes an increase in lipid peroxidation, and that, this rise in collagen gene expression is somehow up-regulated. The decrease seen in collagen is completely reversed by the treatment of superoxide dismutase and catalase in the cell cultures while the measurement of lipid peroxidation is not impaired by co-incubation with these antioxidant enzymes.

2. **M.J. Coelho, et al**[15]in 2000, a biocompatibility study was conducted to test the outcome of ascorbic acid, beta-glycerophosphate and dexamethasone on differentiation of osteoblasts, using human bone marrow cells which were then cultivated in α-minimum essential medium supplemented with 10 % foetal bovine serum (standard medium) in ascorbic acid (AA, 50 mgml-1), beta-glycerophosphate (beta-GP, 10 mm).and dexamethasone (Dex, 10 nmol)within specified testing environment. Cultures were evaluated in regards to morphology of cell, proliferation of cell, ALP activity and the potential to form deposits of calcium phosphate. The presence of dexamethasone also resulted in a substantial induction of ALP activity. Cultures grown in a medium containing beta-GP displayed differentiated population of osteoblastic cells relative to cultures lacking the process of mineralization.

3. **Dr. SeongHo Choi, et al**[16] in 2002 studied the recombinant human bone morphogenetic protein-2 (rhBMP2) and was evaluated as a candidate therapy for periodontal regeneration in an absorbable collagen sponge (ACS) carrier. The aim of the exercise was to evaluate alveolar bone and cement regeneration, and the associated root resorption and ankylosis. Surgical implantation of rhBMP2 / ACS contributed to rapid bone development in 3-wall intrabony periodontal defects, but no significant enhancement of cementum regeneration was seen.

4. **Randal R Betz, et al**[17]in 2002 studied autogenous cancellous bone which is generally considered to be an ideal form for grafting procedures, offering osteoinductive growth factors, osteogenic cells and a structural scaffold. Low immunogenic rejection and risk of disease transmission are, however, unresolved problems. Such risks are reduced by synthetic grafting materials, but these materials do not move osteoinductive or osteogenic elements to the host site. In the study it was derived that a hybrid graft can be considered to provide the advantages of autograph and allograft. Such a graft may combine a synthetic scaffold with biological elements to promote cell infiltration and the formation of new bone.

5. **M Taira**, **et al**[13]in 2003conducted a study with the aim to investigate the effects of adding 5 cytokines such as vit C, vit D, bone morphogenetic protein (BMP), transforming growth factor-beta (TGF-beta) and dexamethasone (Dex) to Dulbecco's modified Eagle (DME) medium on the propogation of bone marrow stromal cells in Sprague-Dawley (SD) rats. The addition of four cytokines studied (vitamin C, vitamin D, TGF-beta,

and BMP) did not increase cell proliferation significantly. Although Dexamethasone with vitamin C and BMP increased cell proliferation moderately.

6. **Takafumi Yoshikawa**,**et al**[18] in 2004 published an article in which a 3-mL marrow of iliac bone was obtained from 27 patients, proceeded by culture in standardized culture medium. In all 27 cases, porous ceramics were infused with marrow cells and inoculated in an osteogenic culture medium (standard medium supplemented with sodium β-glycerophosphate, vitamin C phosphate, and dexamethasone) to evaluate the in-vivo ability of human bone for osteogenesis. The outcome indicates that human marrow cells' bone-regenerating potential does not rely on age, and that harvested artificial bone may be useful for bone regeneration treatment if sufficient cultured marrow cells can be successfully prepared.

7. **Xiao-jing Liu et al**[19] in 2004 examined the feasibility of generating in vitro mesenchymal stem cells (MSCs) derived from adult human bone marrow differentiate into osteoblasts and possible applicability of the MSCs as the tissue engineering seed cells. The MSCs proliferated rapidly in in-vitro culture and the cells started to produce large amounts of expanded endoplasmic reticulum, Golgi complexes and mitochondria with immature cell nuclei after 2 to 3-weeks of induction. Human bone marrow-derived MSCs can be induced by relatively simple procedures to differentiate into osteoblasts which provide the ideal autogenous source of seed cells for bone tissue engineering.

8. **HidekazuOshina et al**[20]in 2007 used dexamethasone as a differentiation reagent to separate hBMMCs into osteoblasts, adipocytes and chondrocytes. They hypothesized that dexamethasone will improve BMMCs' sensitivity to other differentiation reagents and would not establish the lineage. This research investigated the effect of consistent 100 nM dexamethasone administration on the differentiation of hBMMCs into three distinct lineages. hBMMCs cultivated with sustained dexamethasone therapy (100 nM) reported a higher level of osteogenic marker mRNA expression and better colony-forming positive rates of osteogenesis biomarkers compared to hBMMCs treated with dexamethasone only during differentiation culture. The results demonstrated that dexamethasone preferentially induced apoptosis of some hBMMC populations which were suspected to have poor differentiation potential.

9. **Fulan Wei, et al**[21]in 2011, discovered in his study that vitamin C (Vc) can stimulate periodontal ligament stem cells (PDLSCs) by initiating telomerase activity and can form cell sheet frameworks owing to enhanced synthesis of cell matrix.

10. In the systematic review carried out by **Fabio Vignoletti et al**[22] in 2011 the empirical data on the efficacy of the surgical procedures was evaluated which was designed to maintain the alveolar ridge after tooth extraction, and to determine how these techniques influence the placement of dental implants and the restoration assisted by the final implant. The benefit of socket preservation treatment was being displayed leading to comparability reduced vertical as well as horizontal resorption of the crest bone. The

scientific evidence does not somehow offer specific recommendations for the type of biomaterial or surgical procedure; although a noticeable positive effect has been observed with the flap technique.

11. **Wah Lay Tanet al**[23] conducted a systematic review in 2011 which demonstrated that tooth removal results in modification of the hard and soft tissue dimensions both horizontally and vertically. For decision-making and thorough recovery plan, the severity of these changes is significant, with provisions for potential solutions to the anticipated complications during prosthetic reconstruction.29–63 per cent horizontal bone loss and 11–22 per cent vertical bone loss after 6 months of tooth extraction was seen in these studies. This concluded that rapid reduction is seen in the first 3–6 months, followed by incremental dimensional reduction subsequently.

12. **Robert Horowitz et al**[24] in 2012, he assessed the problem faced by clinicians in determining if the use of bone graft substitutes and/or barrier membranes improves their capacity to ensure the potential positioning of an implant or optimize alveolar bone measurements after tooth removal versus no further treatments. When alveolar ridge preservation procedures were applied and no graft material was inserted into fresh alveolar sockets, some studies observed less ridge resorption. The study showed no grafting material has a definite advantage over any other material.

13. **T Chackartchi et al**[25]in 2013 studied the qualitative and quantitative changes that occur at the alveolar bone level at the extraction site. Varying levels of bone resorption occurs after the tooth removal. Alveolar bone is a tooth-dependent framework and therefore, after the extraction of a tooth, the

dimensional reduction of the bone occurs both horizontally and vertically resulting in changes that can lead to esthetic and functional problems. Therefore, in some cases it is not necessary at the time of tooth extraction to perform ridge preservation and can be postponed by a few weeks (6–8). They discussed the various methods of socket / ridge preservation and the different materials used to fill or avoid collapse of the defective tissues.

14. **O.W. Majid, et al**[26]in2013 used dexamethasone to minimize post-operative sequelae after third molar extraction by administering 4 mg of dexamethasone by five different routes and comparing each route to check for better results. A total of 72 patients were included in the study and were randomly divided into 6 groups. 5 treatment groups received dexamethasone 4 mg as intramuscular injection, intravenous injection, oral tablets, submucosal injection, endoalveolar powder and a control group which received no dexamethasone. All 5 routes showed significant results, however endoalveolar powder group showed better results proving that more extended contact with tissue provides more enhanced effect.

15. **Sebastian J. Padayattyet al**[27]in 2013 showed that Vitamin C must be consumed by humans for survival. Vitamin C is a potent water-soluble antioxidant in humans and act as a donor of electrons. Numerous in vitro studies have demonstrated antioxidant properties of vitamin C. Studies of the dosage concentration of vitamin C in healthy people showed a sigmoidal relationship between concentrations of oral dose, plasma and tissue vitamin C. Hence optimum dosage is crucial to vitamin C-based study designs.

16. **Isaac A. Rodriguez et al**[28] conducted a study in 2013. The aim of this research was to carry out a number of primary in-vitro assessments for use in a bone graft application on a series of modified gelatin gel sponge scaffolds. The gelatin gels were enhanced by adding few components all of which had specific characteristics that are advantageous to bone formation and regeneration. Based on compressive strength, volume loss / decay, protein secretion, and cellular activity, in vitro scaffolds were assessed, with results showing the capacity of gelatin gel sponge scaffolds for use in regeneration of bone.

17. **Antonino Albanese et al**[29] studied the use of PRP in 2013 and saw that surgical practice may have beneficial effects of reducing bleeding, increasing healing of soft tissue and regeneration of the bones. Human studies have shown encouraging results with respect to the implementation of PRP to several dental and oral surgical techniques. The purpose of this narrative analysis was: (a) To identify the various need of PRP in dental (extractions and periodontal surgery) and oral surgery (soft tissue and hard tissue surgery, implants and etc.); and (b) To address its effectiveness and pros /cons ratio. This analysis indicated that PRP when placed extraction socket will enhance healing of soft tissue.

18. **F.O.Castro et al**[30] in 2014 explained the use of mesenchymal stem cells (MSC) for regenerative therapy which will greatly help in rehabilitation of damaged tissues in horses (soft and hard). Vitamin-C and platelet-rich plasma was used to initiate the differentiation of MSC in vitro. The objective of this research was to analyze the influence of vitamin C, platelet-

rich-plasma and their combined effect on the in vitro differentiation of MSC present in adipose tissue of horse. The synergy of vitamin C and plasma-rich platelet has the potential to affect MSC positively to divide in vitro into mesodermal lines during fourteen days of cultivation. When differentiation was attempted for 21 days the result was not significant.

19. **Patrick Aghajanian et al**[31] in 2015 discussed how vitamin C is a critical antioxidant and cofactor that regulates the development, function and maintenance of several forms of cells inside a body. Deficiencies in vitamin C may contribute to conditions such as scurvy, which cause infections, among other things like bone pain, and healing of infected wounds. This study examines the functional value of vitamin C, as it contributes to bone tissue growth and maintenance. There is some ambiguity in human research, the majority point to the assumption that decreased serum vitamin C concentrations or consumption could be correlated with osteoporosis development and increased risk of fracture.

20. **J.Chen, et al**[10]2015 conducted a study to evaluate the efficacy of topical combined application of Dexamethasone, Vitamin C and Beta-Sodium Glycerophosphate (Osteogenic Inducer) in rabbits to assess healing of extraction sockets. A total of 75 male white rabbits were used in this study and were divided into three groups to evaluate three parameters i.e. gelatin sponge with the osteogenic inducer group, the only gelatin sponge group and the control group. The results clearly showed that the osteogenic inducer group had better healing and maintained the alveolar ridge height for future dental rehabilitation.

AN OVERVIEW OF OSTEOGENIC INDUCER

- Sodium glycerophosphate is one of the salts containing glycerophosphate. It is commonly used to treat low phosphate levels. In systemic circulation glycerophosphate is hydrolyzed into inorganic phosphate and glycerol. The magnitude of the reaction depends on the serum alkaline phosphatase activity. Beta Sodium Glycerophosphate provides phosphate ions to osteoblasts, to promote the deposition and calcification in physiological calcium salt and thus accelerate calcification. Similarly, Dexamethasone is known as an approved osteogenic inducer with ability to induce alkaline phosphatase activity in bone marrow mesenchymal stem cells (BMSCs) to enhance calcium production, deposition and mineralization. Vitamin C is proven to increase the formation of gelatin which can lead to cell differentiation, calcification development, and regulation of ALP activity, hence inducing calcification and facilitating bone formation. The combination of Dexamethasone, Vitamin C and Beta-Sodium Glycerophosphate is identified to be a quintessential osteogenic inducer and has the ability to induce the differentiation of BMSCs into osteoblasts and promote bone formation.

MATERIALS & METHODS

STUDY DESIGN:

- Prospective study
- Randomized control trial

SOURCE OF DATA: The present study was undertaken in the Department of Oral and Maxillofacial Surgery, KAHER's KLE V.K. Institute of Dental Sciences. The osteogenic inducer (combination of Vitamin C, Dexamethasone, Beta-Sodium Glycerophosphate) used for the study will be prepared in KLE College of Pharmacy, Belagavi, Karnataka, India, with due permission of the institutional ethical committee. All the patients were explained the procedure and an informed consent were signed by them.

SELECTION OF SUBJECTS

- Computer generated random allocation of 36 patients was done which reported to the Department of Oral and Maxillofacial Surgery, KAHER's KLE V.K. Institute of Dental Sciences, Belagavi, for extraction of impacted mandibular third molar.

INCLUSION CRITERIA:

1. Patients between 18 to 50 years of age.
2. Patients having impacted mandibular third molars.
3. Patients willing to give informed consent for the study.
4. The position of impacted tooth will be assessed using PEDERSON'S DIFFICULTY INDEX and tooth with a score of 3-6 will be included in the study.

EXCLUSION CRITERIA:

1. Medically compromised patients (diabetes, immune diseases or other contraindicating systemic diseases).
2. Radiation therapy/ Chemotherapy in the 12 month period earlier to the proposed therapy.
3. Smoker.
4. Pregnant women.
5. An unwillingness to commit to a long-term post therapy maintenance program.
6. Presence of any acute local infection.

INVESTIGATIONS:

- Random Blood sugar levels
- Haemoglobin levels
- Bleeding time
- Clotting time

METHODOLOGY:

- Computer generated random allocation of patients will be done into case group and control group.

- The surgical procedure will be performed by a single surgeon under all aseptic conditions.

- As this study involves comparison between OsteogenicInducer and usual method of wound healing and bone regeneration patients were divided into two groups of 18 patients each.

1. Case Group- OsteogenicInducer was placed in the third molar extraction socket and would act as the study group.

2. Control Group - No material was placed in the third molar extraction socket and would act as the control group.

- Patient recalled at intervals of 1day, 7 days, 1 month and 3 months to assess the healing of the socket clinically and radiographically (RVG).

  A. 1^{st} and 7^{th} day for assessment of wound healing.

  B. 1^{st} day, 1^{st} month and 3^{rd} month for assessment of bone regeneration.

DETAILS OF THE PROCEDURES TO BE CONDUCTED DURING THE RESEARCH

All patients underwent the same surgical procedure. A standard inferior alveolar, lingual and long buccal nerve block will be given using 1.8ml syringe of 2% lignocaine hydrochloride with epinephrine 1:100,000 followed by surgical extraction of impacted mandibular third molar. Extraction socket inspection is done for any sharp bony margins and granulation tissue removed if present, followed by copious irrigation. A 10mm X 10mm X 10 mm absorbable gelatin sponge is soaked with 0.8 ml of osteogenic inducer (10^{-8}mol/l Dexamethasone, 50mg/l Vitamin C and 10mmol/l Beta-Sodium Glycerophosphate) and placed inside the extraction socket of the case group and control group will not receive any material and will heal in usual manner. Flap is returned to its original position, sutured back by 3 interrupted sutures using 3-0 silk suture. A pressure pack placed on the extraction site and post-operative instructions are given to the patient in the usual manner. In order to prevent

postoperative infection, patients in both the groups were prescribed antibiotics to avoid any discrepancy in the outcome. Patients recalled at intervals of 1 day, 7 days, 1 month and 3 months for follow-up. Clinical evaluation consisted of:

1. Wound healing assessment using the Landry and Turnbull index[32]
2. Bone regeneration of the third molar socket assessment radiographically using standard RVG image. The criteria for bone healing and scoring system are based on a modification of the method used by Kelly et al[33].

   Three radiographic parameters will be used for the assessment of bone regeneration: -

   a) Lamina Dura
   b) Overall Density
   c) Trabecular Pattern

Table 1. Wound Healing index (Landry and Turnbull index)

Score	Clinical signs
Healing index 1: Very poor	Tissue color: ≥50% of gingiva red
	Response to palpation: Bleeding
	Granulation tissue: Present
	Incision margin: Not epithelialized with loss of epithelium beyond incision margin
	Suppuration: Present
Healing index 2: Poor	Tissue color: ≥50% of gingiva red
	Response to palpation: Bleeding
	Granulation tissue: Present
	Incision margin: Not epithelialized with connective tissue exposed
Healing index 3: Good	Tissue color: ≥25% and< 50% of gingiva red
	Response to palpation: No bleeding
	Granulation tissue: None
	Incision margin: No connective tissue exposed
Healing index 4: Very good	Tissue color: <25% of gingiva red
	Response to palpation: No bleeding
	Granulation tissue: None
	Incision margin: No connective tissue exposed
Healing index 5: Excellent	Tissue color: All tissues pink
	Response to palpation: No bleeding
	Granulation tissue: None
	Incision margin: No connective tissue exposed

Table 2. Bone Healing index (Kelly et al)

LD	+2: LD essentially absent, may be present in isolated areas
	+1: LD substantially thinned, missing in some areas
	0: Within normal limits
	−1: Portions of LD thickened, milder degrees
	−2: Entire LD substantially thickened
Overall density	+2: Severe increase in the radiographic density
	+1: Mild to moderate increase in radiographic density
	0: Within normal limits
	−1: Mild to moderate decrease in radiographic density
	−2: Severe decrease in radiographic density
Trabecular pattern	+2: All trabeculae substantially coarser
	+1: Some coarser trabeculae; minor degrees
	0: Within normal limits
	−1: Delicate finely meshed trabeculations
	−2: Granular, nearly homogenous patterns, individual trabeculations essentially absent

Table 3. Pederson Difficulty index

Criterion	Value
Position of the molar	
Mesioangular	1
Horizontal	2
Vertical	3
Distoangular	4
Relative depth	
Class A	1
Class B	2
Class C	3
Relation with ramus and available space	
Class 1	1
Class 2	2
Class 3	3
Difficulty score	Total
Easy	3–4
Moderate	5–6
Difficult	7–10

INSTRUMENTS AND MATERIALS:

Osteogenic Inducer Preparation Material (Image 1):

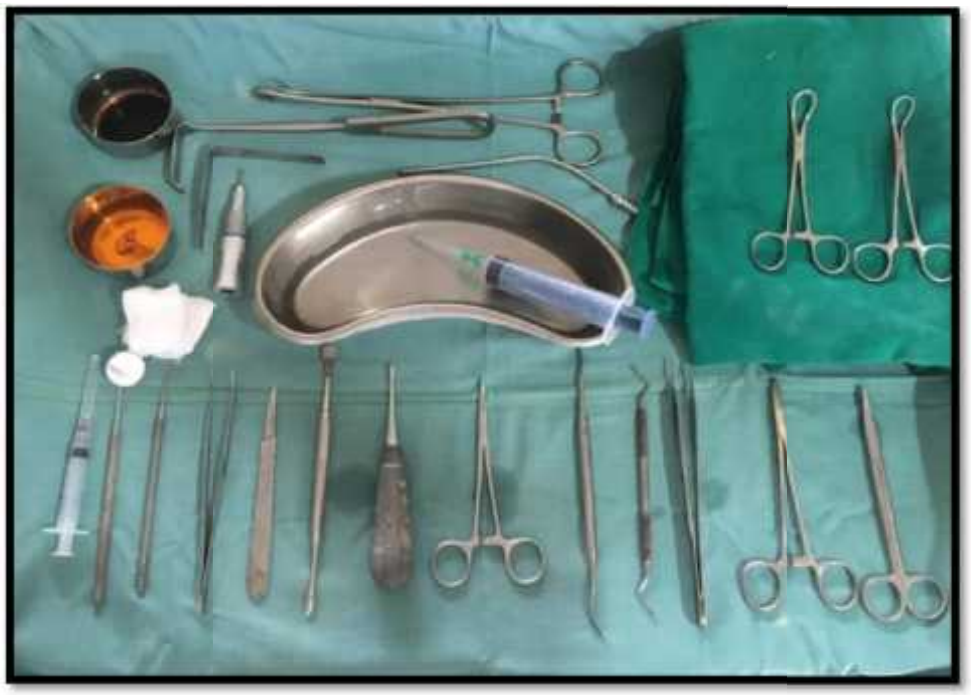
1. Dexamethasone (10^{-8} mol/l)
2. Vitamin C (50mg/l vitamin)
3. Beta SodiumGlycerophosphate (10mmol/l)

Image 1 :Preparation ofOsteogenic Inducer

Surgical Armamentarium (Image 2):

- Surgical gloves
- Mouth mirror
- Dental explorer
- Tweezer
- 2ml Disposable Syringe
- Adson's tissue forceps
- Scissors
- Surgical handpiece and bur
- Kidney tray
- Irrigation syringe 20 ml
- Surgical drape
- Towel clip
- Suction tip
- Langenbeck retractor
- Sponge holder
- Gauze piece
- Surgical scalpel blade no. 15
- Periosteal elevator
- Straight elevator
- Artery forceps
- Curette
- Bone file
- Needle holder

Image 2 :Surgical Armamentarium



SURGICAL REMOVAL OF IMPACTED THIRD MOLAR:

Surgical Technique:

The patient's face was prepared with betadine and patient was draped. Intra-oral irrigation with betadine and normal saline was done.

1. Local anesthesia

Inferior alveolar nerve block, lingual nerve block and long buccal nerve block were administered using 2% lignocaine hydrochloride with 1: 100,000 epinephrine (Image 3a & 4a). Subjective as well as objective signs and symptoms of nerve block were assessed.

2. Incision and flap reflection

In all the cases standard Ward's incision was used. A full thickness mucoperiosteal flap was raised using a periosteal elevator to expose sufficient bone on lateral and distal aspect of the impacted molar.

3. Removal of surrounding bone

Removal of bone was done with stainless steel bur (No.8). Buccal and distal bone was removed and, in some cases, a notch was made in bone near cement-enamel junction of impacted tooth for elevation. Constant irrigation with saline was used while removing bone to prevent thermal necrosis.

4. Extraction

Tooth was luxated with the help of straight elevator and then extracted with molar forceps employing minimal forces (Image 3b & 4b). In some cases, sectioning of tooth was done.

5. Wound Toilet

The surrounding bone was smoothened. The wound was gently irrigated with sterile saline solution and checked for any small detached fragments of bone or tooth pieces.

6. Osteogenic Inducer Placement

In case group 0.8 ml osteogenic inducer is placed in the extraction socket and secured (Image 3c & 3d) while in control group this step was eliminated.

7. Wound closure

Trimming of the irregular margins of the wound was done and primary closure of the wound was done using 3-0 black braided silk suture. Interrupted sutures were placed and pressure pack was given.

Post-operative instructions: –

Regular post extraction instructions were given.

Medication Prescription: -

1. Tab. Amoxcillin 500 mg/TID X 5 days
2. Tab. Flagyl 400 mg/TID X 5 days
3. Tab. Dolonex DT 20 mg/BD X 5 days
4. Chlorhexidine mouthwash gargle QID

Patients were recalled on the 1st, 7th day to assess the soft tissue healing and on 1st and 3rd month to assess the bone healing.

Image 3 :Case Group Photos

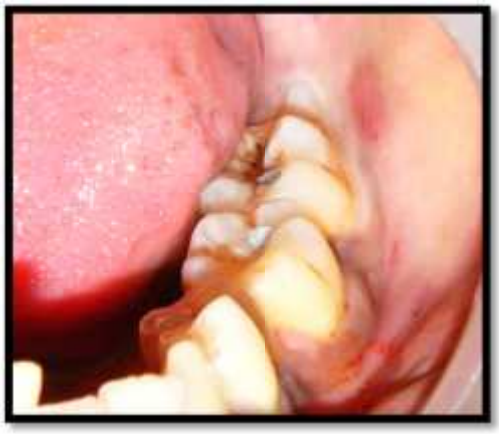


Image 3a :Preoperative 38

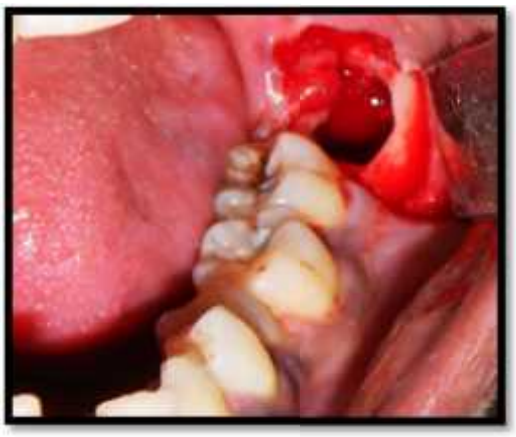


Image 3b :Surgical extraction socket with 38

Image 3c :Osteogenic inducer placement with 38

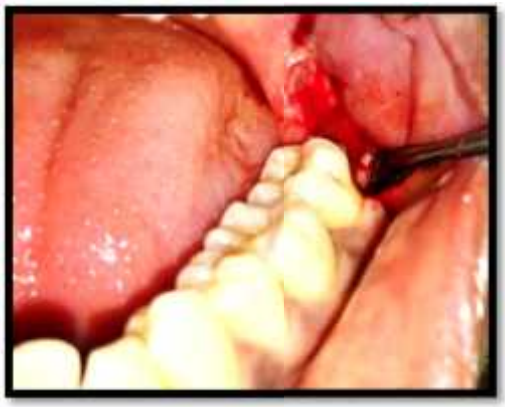


Image 3d:Osteogenic inducer placed with 38 and secured

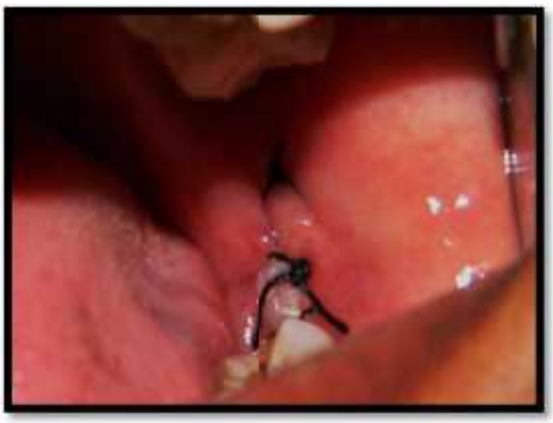


Image 3e:Post-op 1st day (wound healing assessment)

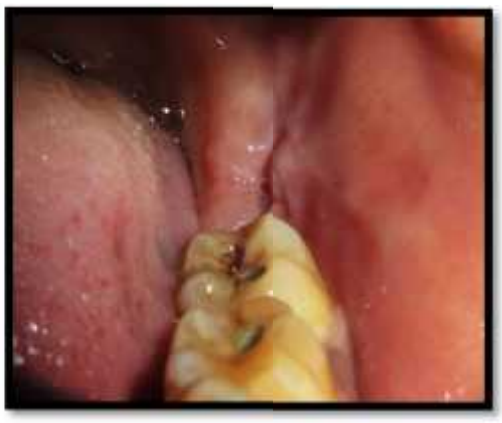


Image 3f :Post-op 7th day (wound healing assessment)

Image 4 :Control Group Photos

Image 4a :Preoperative 48

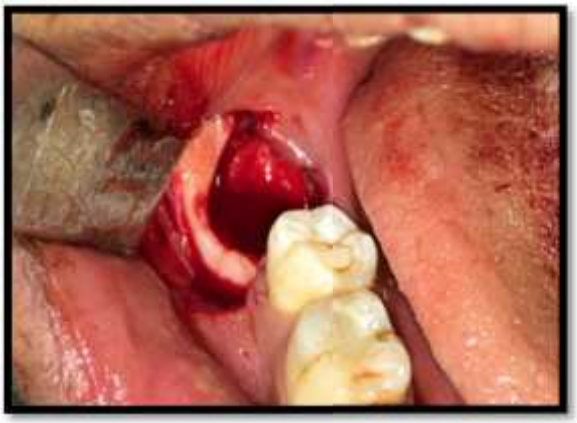


Image 4b :Surgical extraction socket with 48

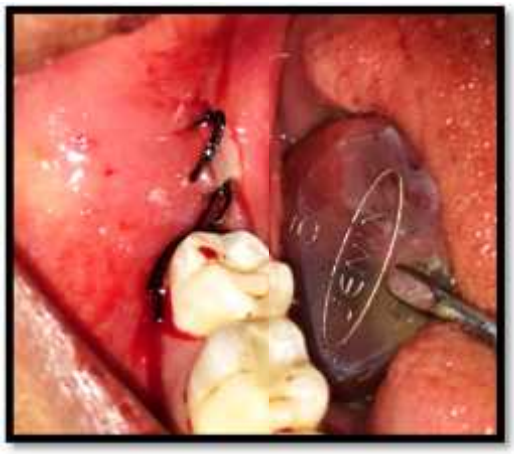


Image 4c:Post-op 1st day (wound healing assessment)

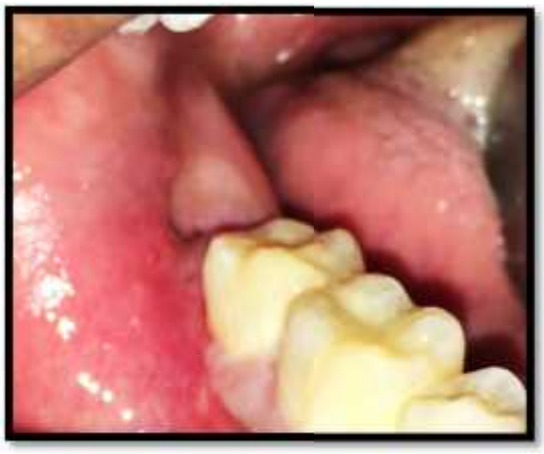


Image 4d :Post-op 7th day (wound healing assessment)

Image 5 :Case Group RVG (Bone regeneration assessment)

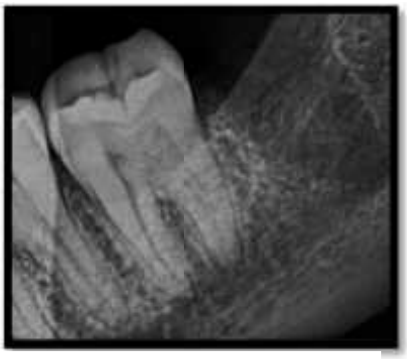


Image 5a :Post-op 1st day

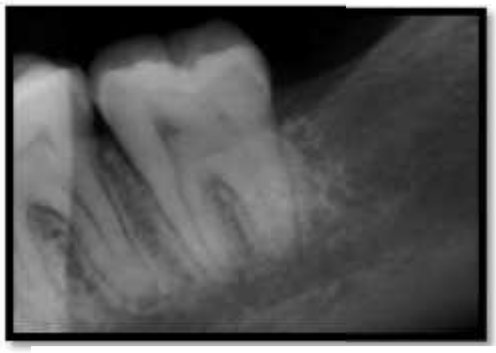


Image 5b :Post-op 1 month

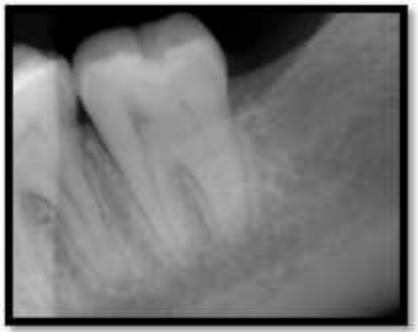


Image 5c :Post-op 3 months

Image 6 :Control Group RVG (Bone regeneration assessment)

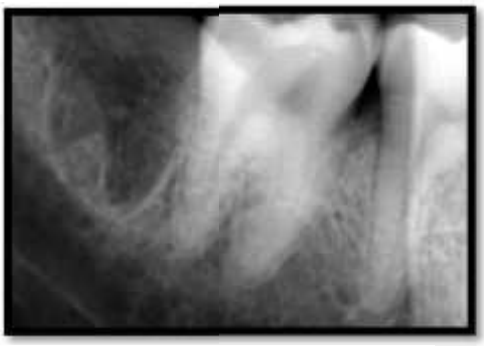


Image 6a :Post-op 1st day

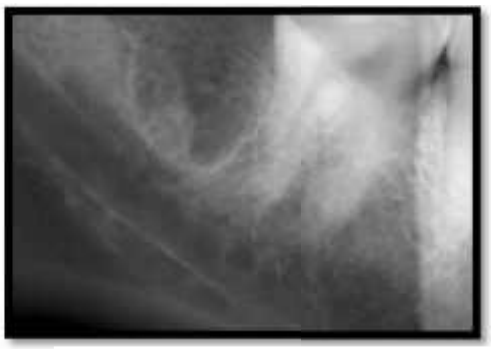


Image 6b :Post-op 1 month

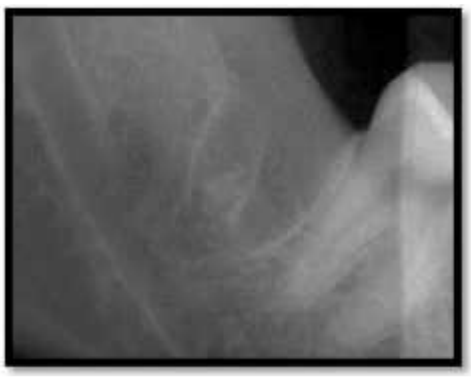


Image 6c :Post-op 3 months

1st Post-operative day:

- Any collection of hematomas or wound dehiscence was investigated for intraoral wound.
- Tissue color, palpation responsiveness, granulation presence, incision margins, suppuration were analysed and scores were calculated accordingly using the Soft Tissue Healing Scale (Image 3e & 4c). (*Landry and Turnbull Index*[32]).
- RVG imaging of the surgical site was done and bone healing assessment was done using the Bone healing Index given by *Kelly et al*[33]. where in three radiographic parameters were assessed i.e. lamina dura, overall density and trabecular pattern and scores were assigned accordingly (Image 5a & 6a).
- Any fresh complaints were noted.
- On days that followed, patient was asked to gargle with mouth wash 3-4 times daily.

7th Post-operative day

- Intra-oral sutures were removed.
- Intra oral wound was examined for any hematoma collection or wound dehiscence.
- Tissue colour, response to palpation, presence of granulation, incision margins, suppuration were assessed and scores were assigned accordingly based on the Soft tissue Healing index (Image 3f & 4d). (*Landry and Turnbull Index*[32]).

- Any fresh complaints were noted

1st Post-operative month:

- RVG imaging of the surgical site was done and bone healing assessment was done using the Bone healing Index given by *Kelly et al*[33]. wherein three radiographic parameters were assessed i.e. lamina dura, overall density and trabecular pattern and scores were assigned accordingly (Image 5b & 6b).

3rd Post-operative month.

- RVG imaging of the surgical site was done and bone healing assessment was done using the Bone healing Index given by *Kelly et al*[33]. wherein three radiographic parameters were assessed i.e. lamina dura, overall density and trabecular pattern and scores were assigned accordingly (Image 5c & 6c).

RESULTS AND OBSERVATION

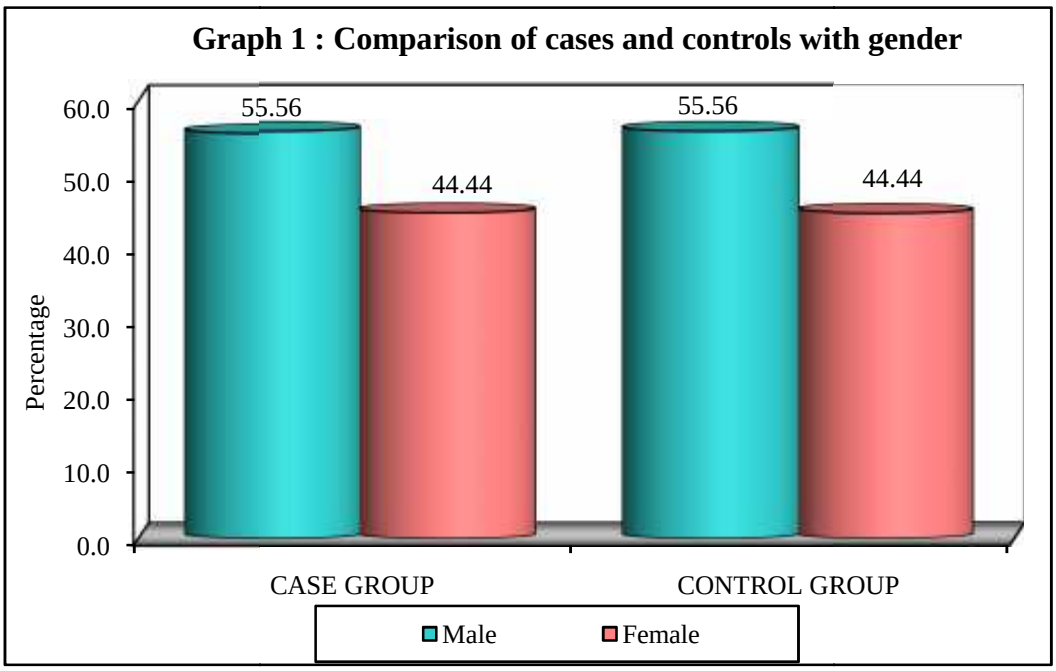
A prospective single blinded randomized control study was conducted including 36 cases undergoing surgical extraction of mandibular third molars. The samples were divided into Case Group and Control Group of 18 patients each.

Gender wise distribution of the participants: -

- As it was a randomised control trial, the case group as well as the control group consisted of 10 males and 8 females.

Table 4: Comparison of cases and controls with gender

Gender	Cases	%	Controls	%	Total	%
Male	10	55.56	10	55.56	20	55.56
Female	8	44.44	8	44.44	16	44.44
Total	18	100.00	18	100.00	36	100.00

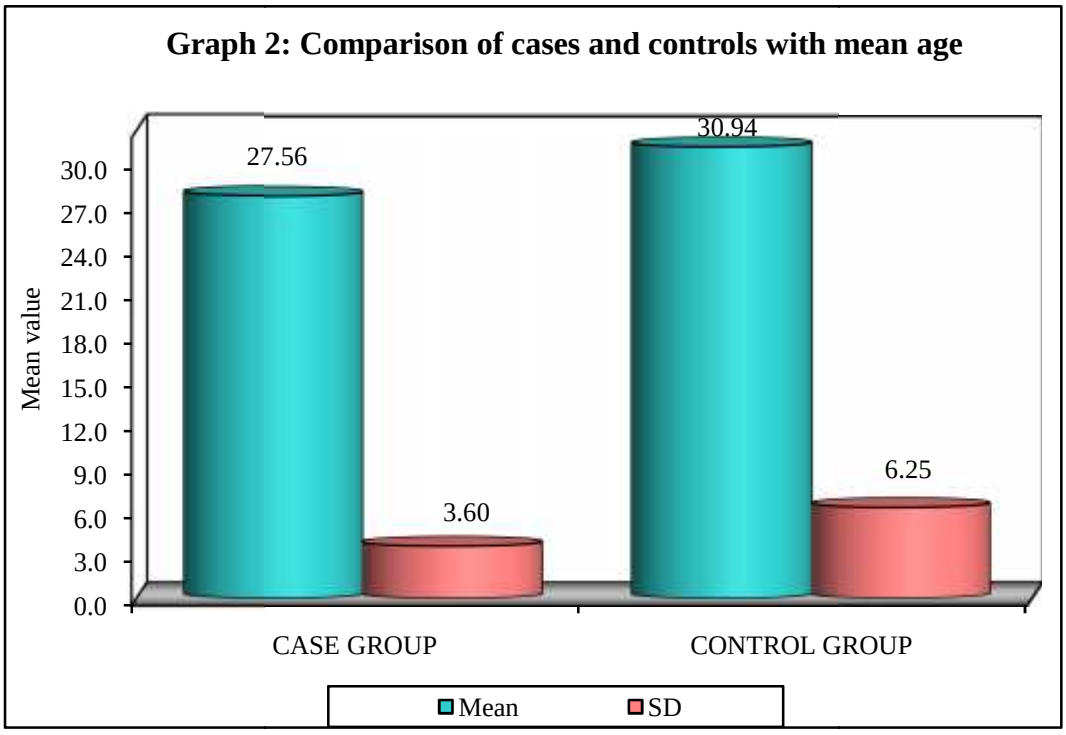


Age wise distribution of the participants:-

- The mean age of participants noted in case group was 27.56 and in control group was 30.94 which was not significant and did not affect the study.

Table 5: Comparison of cases and controls with mean age by t test

Groups	Mean	SD	SE	t-value	P-value
Cases	27.56	3.60	0.85	-1.9923	0.0544
Controls	30.94	6.25	1.47		

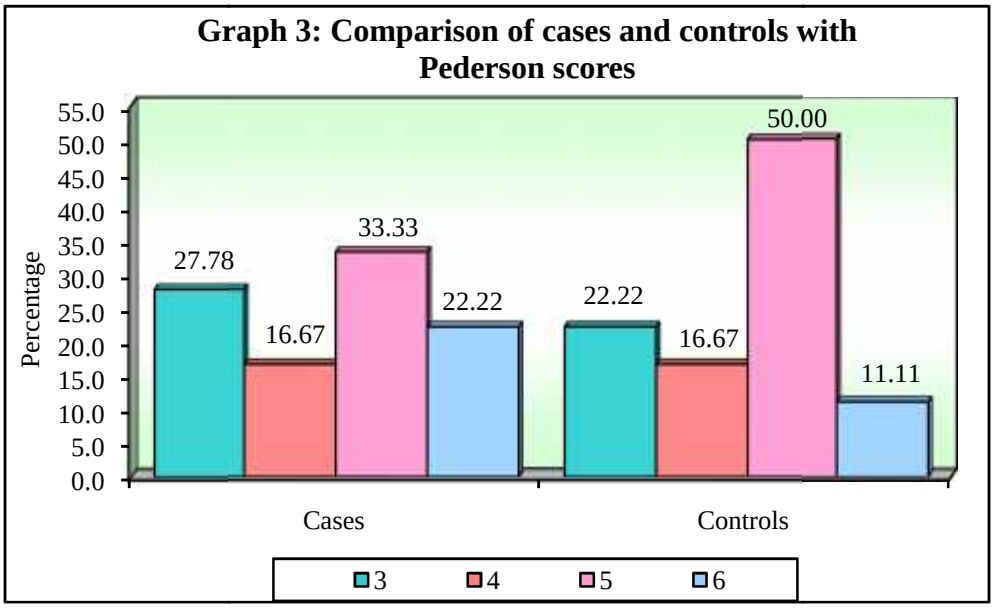


Pederson difficulty index estimation of impacted mandibular 3rd molars

- The Pederson Difficulty Index (PDI) was used in the study to estimate the level of difficulty of impacted mandibular 3rd molars, which confirmed that both groups had a greater number of PDI score 5 cases and required equal amounts of bone removal, thus removing any bias.

Table 6: Comparison of cases and controls with Pederson scores

Pederson score	Cases	%	Controls	%	Total	%
3	5	27.78	4	22.22	9	25.00
4	3	16.67	3	16.67	6	16.67
5	6	33.33	9	50.00	15	41.67
6	4	22.22	2	11.11	6	16.67
Total	18	100.00	18	100.00	31	100.00
Chi-square= 1.3782 P = 0.7111						



Wound healing assessment

- When determining the wound healing ability of the osteogenic inducer we used the Landry and Turnbull Index[32]. The criteria used were the tissue colour, bleeding on palpation, suppuration and granulation tissue.

- The wound healing was assessed on the 1st day and 7th day post-extraction and the healing score was compared using Mann- Whitney U test and Wilcoxon matched pairs test.

- There was no significant difference on day 1 post extraction, as both groups had similar findings with mean of case group was 2.00 and of control group was 1.94. On the 7th day, however, the osteogenic inducer group demonstrated mean score of 4.33 compared to the control group which had the mean score of 3.22. In terms of wound healing the combination of drugs used topically had significant difference on 7th day post extraction.

Table 7.1: Comparison of cases and controls with wound healing scores at different time points by Mann-Whitney U test

Time points	Cases			Controls			U-value	Z-value	p-level
	Mean	SD	Rank Sum	Mean	SD	Rank Sum			
Day 1	2.00	0.49	341.00	1.94	0.54	325.00	154.00	-0.2531	0.8002
Day 7	4.33	0.77	441.00	3.22	0.73	225.00	54.00	-3.4170	0.0006*
Day 1-day 7	2.33	0.59	455.50	1.28	0.57	210.50	39.50	-3.8757	0.0001*

*p<0.05

Graph 4.1: Comparison of cases and controls with wound healing scores at different time points

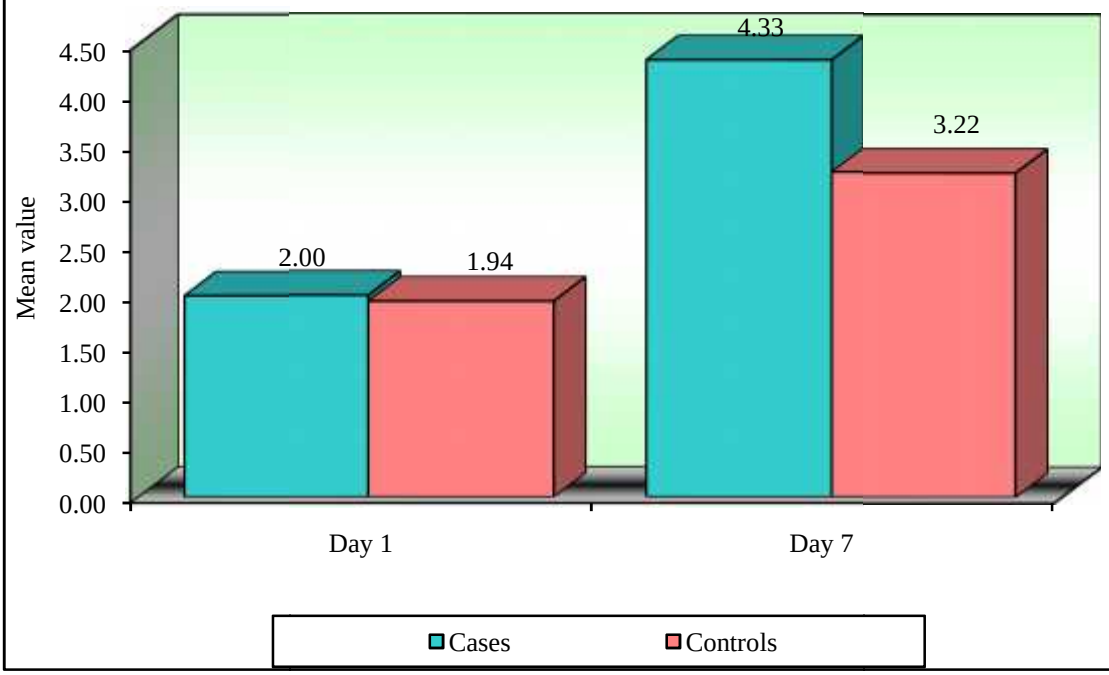
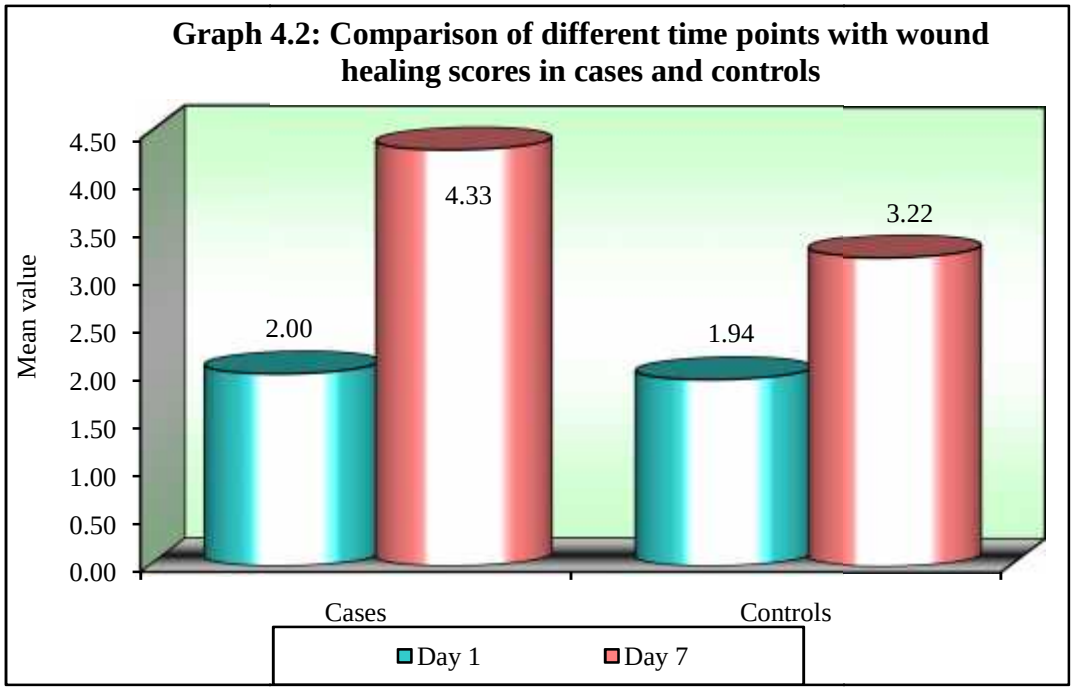


Table 7.2: Comparison of different time points with wound healing scores in cases and controls by Wilcoxon matched pairs test

Groups	Time points	Mean	Std.Dv.	Mean Diff.	SD Diff.	%of change	Z-value	P-value
Cases	Day 1	2.00	0.49					
	Day 7	4.33	0.77	-2.33	0.59	-116.67	3.7279	0.0001*
Controls	Day 1	1.94	0.54					
	Day 7	3.22	0.73	-1.28	0.57	-65.71	3.7236	0.0002*

*p<0.05

Graph 4.2: Comparison of different time points with wound healing scores in cases and controls



Bone regeneration Assessment

- In this study, bone regeneration was evaluated on digital RVG images using Kelly et al's[33] healing Index to assess the lamina dura, overall density and trabecular pattern. On the 1st day, after 1 month and 3 months after extraction, we recalled the patients for follow-ups.

- Pair wise comparison of the two groups with respect to Lamina dura, overall density and trabecular pattern scores at 1st day, after 1 month and 3 months after extraction was done using independent t test and Wilcoxon matched pairs test.

- **LAMINA DURA**: The mean lamina dura score of case group was higher as compared to that of control group and was statistically significant. On the 1st day post extraction, the mean LD score of case group was 0.28 whereas that of control group was -0.28. At the end of 1-month post-operative the mean LD score of case group was 1.17 whereas that of control group was 0.50. At the end of three months post-operative the mean LD score of case group was 1.89 whereas that of control group was 0.83. In terms of duration, there was a significant difference in the LD scores of both the groups at the end of three months post-operative.

Table 8.1: Comparison of Cases and controls with day 1, 1 month and 3 months' time points with Lamina Dura scores by independent t test

Time points	Cases			Controls			U-value	Z-value	p-value
	Mean	SD	Rank Sum	Mean	SD	Rank Sum			
Day 1	0.28	0.57	403.00	-0.28	0.67	263.00	92.00	-2.2147	0.0268*
1 month	1.17	0.51	423.00	0.50	0.51	243.00	72.00	-2.8475	0.0044*
3 months	1.89	0.32	464.00	0.83	0.62	202.00	31.00	-4.1447	0.0001*
Day 1- 1M	0.89	0.58	347.00	0.78	0.43	319.00	148.00	-0.4429	0.6578
Day 1- 3M	1.61	0.61	393.00	1.11	0.76	273.00	102.00	-1.8983	0.0577
1M- 3M	0.72	0.46	396.00	0.33	0.49	270.00	99.00	-1.9932	0.0462*

*p<0.05

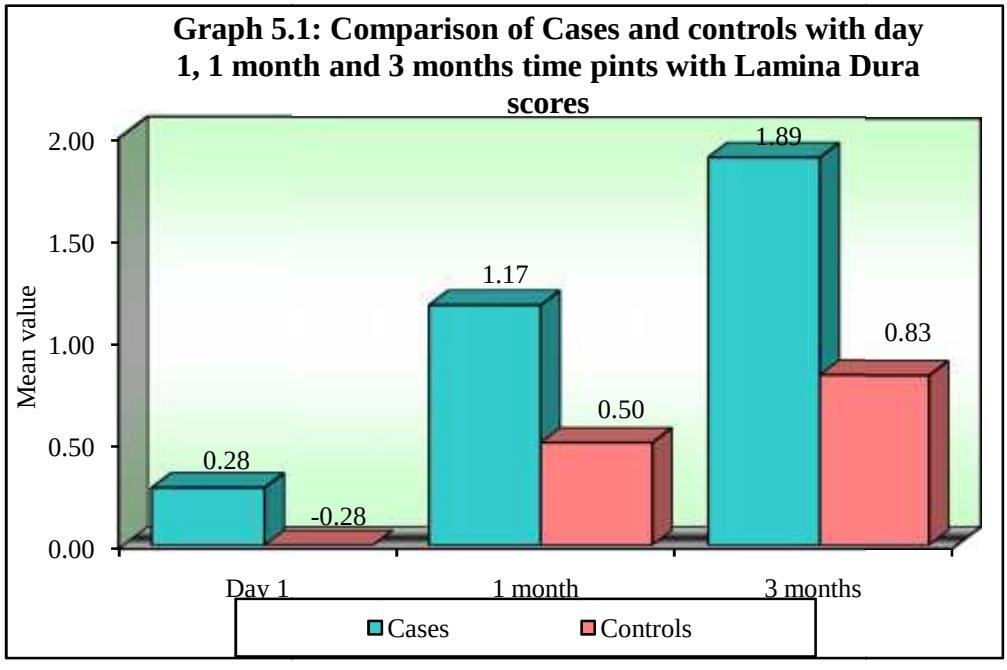
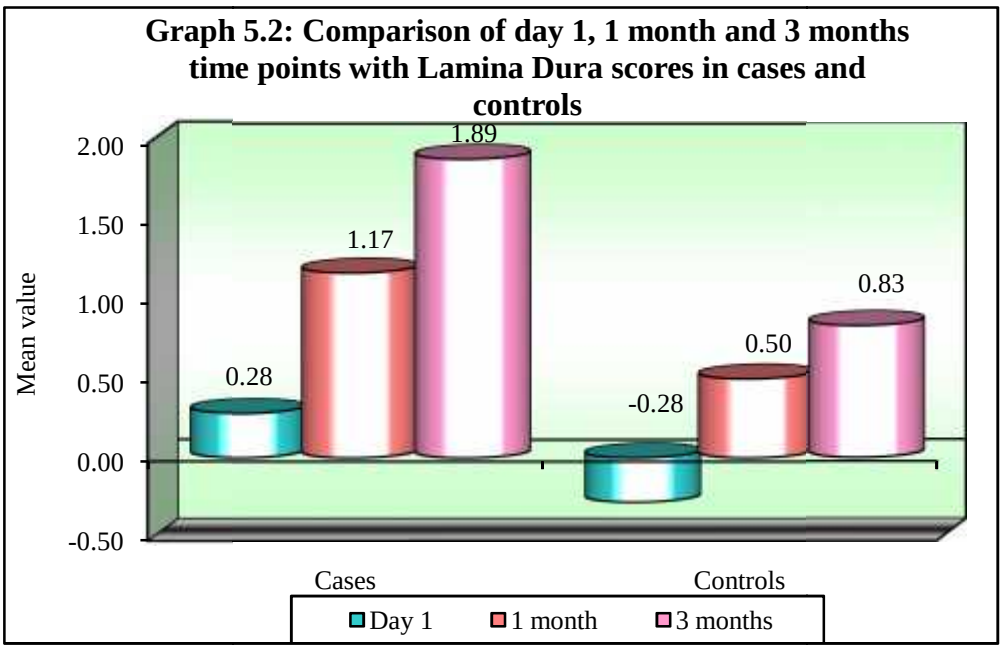


Table 8.2: Comparison of day 1, 1 month and 3 months' time points with Lamina Dura scores in cases and controls by Wilcoxon matched pairs test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	Z-value	p-value
Cases	Day 1	0.28	0.57				
	1 month	1.17	0.51	-0.89	0.58	3.2958	0.0010*
	Day 1	0.28	0.57				
	3 months	1.89	0.32	-1.61	0.61	3.6214	0.0003*
	1 month	1.17	0.51				
	3 months	1.89	0.32	-0.72	0.46	3.1798	0.0015*
Controls	Day 1	-0.28	0.67				
	1 month	0.50	0.51	-0.78	0.43	3.2958	0.0010*
	Day 1	-0.28	0.67				
	3 months	0.83	0.62	-1.11	0.76	3.2958	0.0010*
	1 month	0.50	0.51				
	3 months	0.83	0.62	-0.33	0.49	2.2014	0.0277*

*$p<0.05$



OVERALL DENSITY: The score for overall density was evaluated using radiovisiography (RVG) after 1st day, 1 month and after 3 months. As the osteogenic inducer was infused into a gelatin sponge, the substance density was not the same as that of a grafting material. Hence, radiographically the overall bone density was not significant on the 1st day and after 1 month post extraction. The overall density mean score for case group was -0.44 and that of control group was -0.56. At the end of 1st month postoperative the mean score of case group was 0.28 whereas control group had a mean score of 0.22. At the end of 3 months postoperative the mean score of case group was 1.72 whereas that of control group was 0.94. In terms of duration a significant difference was noted in both the groups at the end of 3rd month postoperative as compared to 1st day and after 1 month proving good bone regeneration capacity of the osteogenic inducer.

Table 9.1: Comparison of Cases and controls with day 1, 1 month and 3 months' time points with Overall Density scores by independent t test

Time points	Cases			Controls			U-value	Z-value	p-value
	Mean	SD	Rank Sum	Mean	SD	Rank Sum			
Day 1	-0.44	0.51	346.00	-0.56	0.62	320.00	149.00	-0.4113	0.6809
1 month	0.28	0.46	342.00	0.22	0.43	324.00	153.00	-0.2847	0.7758
3 months	1.72	0.57	439.00	0.94	0.54	227.00	56.00	-3.3537	0.0008*
Day 1- 1M	0.72	0.46	326.50	0.78	0.55	339.50	155.50	-0.2057	0.8371
Day 1- 3M	2.17	0.62	408.50	1.50	0.99	257.50	86.50	-2.3887	0.0169*
1M- 3M	1.44	0.62	415.50	0.72	0.75	250.50	79.50	-2.6102	0.0091*

*p<0.05

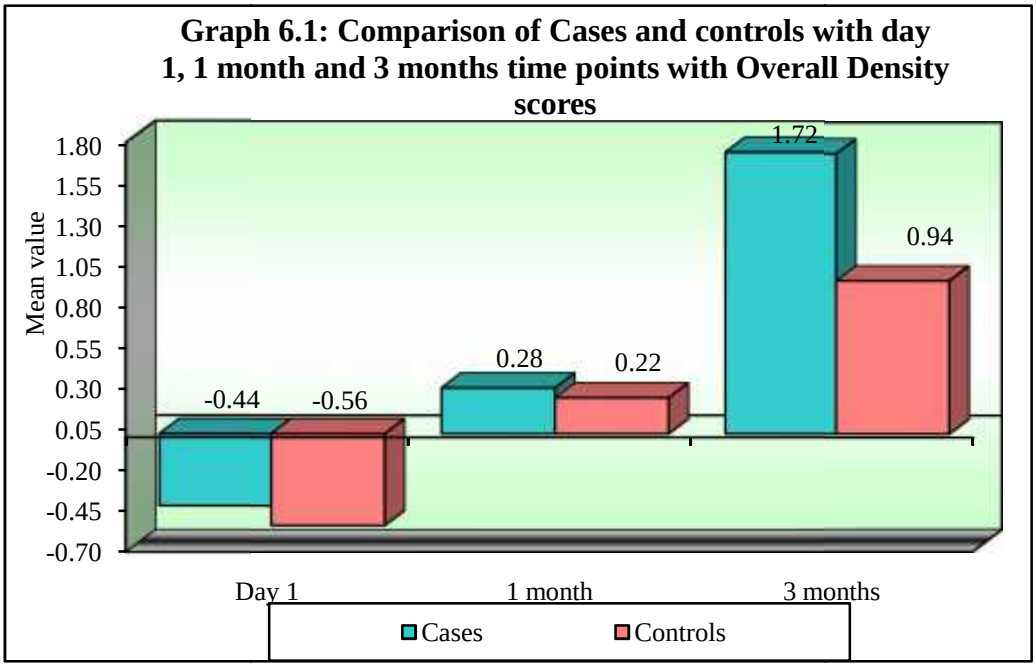
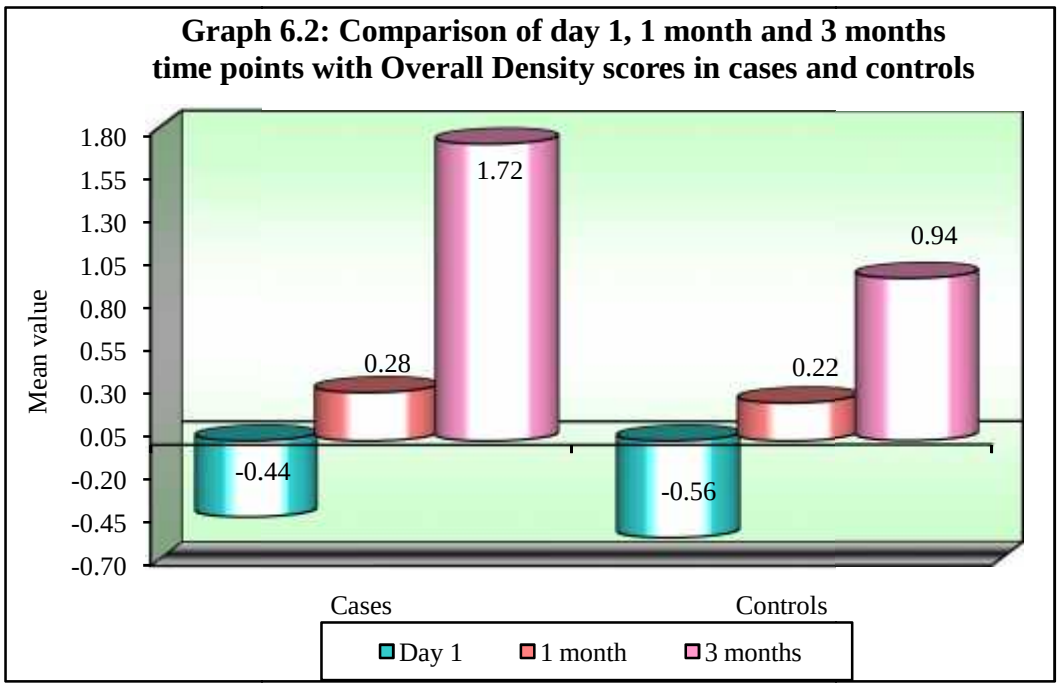


Table 9.2: Comparison of day 1, 1 month and 3 months' time points with Overall Density scores in cases and controls by Wilcoxon matched pairs test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	Z-value	p-value
Cases	Day 1	-0.44	0.51				
	1 month	0.28	0.46	-0.72	0.46	3.1798	0.0015*
	Day 1	-0.44	0.51				
	3 months	1.72	0.57	-2.17	0.62	3.7236	0.0002*
	1 month	0.28	0.46				
	3 months	1.72	0.57	-1.44	0.62	3.6214	0.0003*
Controls	Day 1	-0.56	0.62				
	1 month	0.22	0.43	-0.78	0.55	3.1798	0.0015*
	Day 1	-0.56	0.62				
	3 months	0.94	0.54	-1.50	0.99	3.5162	0.0004*
	1 month	0.22	0.43				
	3 months	0.94	0.54	-0.72	0.75	2.7605	0.0058*

*$p<0.05$



TRABECULAR PATTERN: In terms of trabecular pattern, the mean score was not significant on the 1st day and after 1 month between the case group and the control group. When seen radiographically the mean score on 1st day in the study group was -0.39 whereas in control group was -0.50. After 1 month in the case group mean score was 0.39 whereas in control group was 0.28. Although, after 3 months in case group the mean score was 1.61 whereas in the control group the mean score was 1.22. It was observed that significant difference was present in the case group when results were compared between 1st day – 1 month, 1st day – 3 months and 1 month – 3 months giving positive results that the osteogenic inducer has good bone regeneration potential.

Table 10.1: Comparison of cases and controls with day 1, 1 month and 3 months' time points with Trabecular Pattern scores by independent t test

Time points	Cases			Controls			U-value	Z-value	p-value
	Mean	SD	Rank Sum	Mean	SD	Rank Sum			
Day 1	-0.39	0.50	351.00	-0.50	0.51	315.00	144.00	-0.5695	0.5690
1 month	0.39	0.50	351.00	0.28	0.46	315.00	144.00	-0.5695	0.5690
3 months	1.61	0.50	390.50	1.22	0.55	275.50	104.50	-1.8192	0.0689
Day 1- 1M	0.78	0.43	335.00	0.78	0.55	331.00	160.00	-0.0633	0.9495
Day 1- 3M	2.00	0.34	375.50	1.72	0.57	290.50	119.50	-1.3446	0.1788
1M- 3M	1.22	0.43	376.00	0.94	0.24	290.00	119.00	-1.3605	0.1737

Graph 7.1: Comparison of Cases and controls with day 1, 1 month and 3 months time points with Trabecular Pattern scores

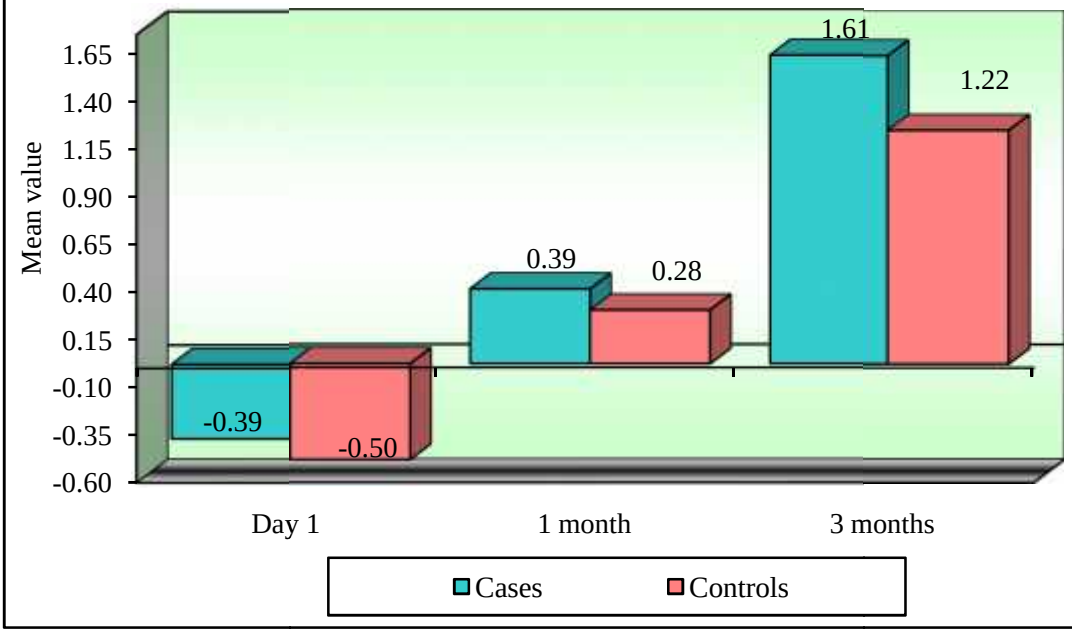
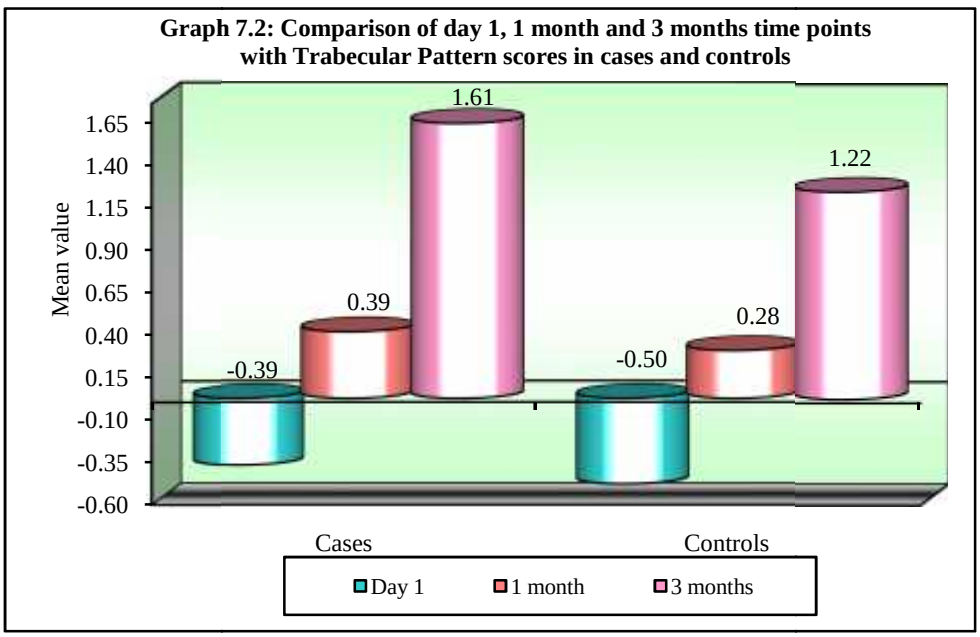


Table 10.2: Comparison of day 1, 1 month and 3 months' time points with Trabecular Pattern scores in cases and controls by Wilcoxon matched pairs test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	Z-value	p-value
Cases	Day 1	-0.39	0.50				
	1 month	0.39	0.50	-0.78	0.43	3.2958	0.0010*
	Day 1	-0.39	0.50				
	3 months	1.61	0.50	-2.00	0.34	3.7236	0.0002*
	1 month	0.39	0.50				
	3 months	1.61	0.50	-1.22	0.43	3.7236	0.0002*
Controls	Day 1	-0.50	0.51				
	1 month	0.28	0.46	-0.78	0.55	3.1798	0.0015*
	Day 1	-0.50	0.51				
	3 months	1.22	0.55	-1.72	0.57	3.7236	0.0002*
	1 month	0.28	0.46				
	3 months	1.22	0.55	-0.94	0.24	3.6214	0.0003*

*p<0.05

Graph 7.2: Comparison of day 1, 1 month and 3 months time points with Trabecular Pattern scores in cases and controls



DISCUSSION

The agony of nature is once destroyed everything takes time to heal. The healing mechanism, following the same concept in human beings, is a complex process of cell induction, conduction, and conversion that eventually leads to regeneration.

In Oral and Maxillofacial surgery, extraction of tooth is the most common procedure performed by surgeons, the procedure being removal of tooth from the bony socket irrespective whether it's a difficult extraction or a simple one, a wound is created in the patient's jaw bone. As studied have shown this wound takes approximately 3-4 months to completely heal on its own with both horizontal and vertical bone loss thus sacrificing the bone structure for rehabilitation[34].

In modern era, with numerous medical advancements, researchers have focused their attention towards grafting of this wound after it is completely healed to compensate for the bone loss which increases patient's rehabilitation time resulting in development of distress and social anxiety among patients. As we progressed and learned about treating a defect, we have taken a step forward in attempt to preventing one from occurring.

In recent time, numerous materials have been developed to regulate the alveolar bone growth, such as allogenic grafts, artificial materials, and growth factors with the intention that a second surgical procedure could be avoided by minimizing amount of bone loss and eventually decreasing patient's waiting period for rehabilitation, leading to increase in quality of health care as well as patient satisfaction. Depending on the type of bone grafts, they can induce different bone

healing mechanisms, which can be classified as osteogenesis, osteoinduction, and osteoconduction[6], although each material has its constraints such as the supply issue, the complex surgical technique, the risk of contamination and the exorbitant prices. Autogenous bone graft is the gold standard grafting material in present times, as it is capable of osteogenesis, osteoinduction and osteoconduction with a fair healing time and no risk of graft rejection or immune response[1]. The drawback of autogenous bone grafts is that its availability is constrained and generates a second defect (the donor site) in the process with associated complication of surgery. A decent bone graft should stabilize the blood clot, establish a biomechanical scaffold for cell migration, proliferation and differentiation, provide essential proteins and peptides and exhibit adequate resorption and remodelling during new bone formation[8].

Lately, researchers and clinicians have shown interest in the discovery and development of combination of drugs which in synergy will increase the rate of bone formation, are easy to prepare, be cost-effective and avoid second surgical procedure.

In this study, we have evaluated wound healing as well as bone regeneration efficacy of topical combined application of Beta-Sodium Glycerophosphate, Vitamin C and Dexamethasone (Osteogenic Inducer) when impregnated into a gelatin sponge and placed inside a fresh extraction socket. This drug combination was first used by **M.J. Coelho, et al**[15]in 2000 on human bone marrow cells that were then grown in a standard medium designed to assess the amount of osteoblastic differentiation. The cultures grown with the drug combination showed significant increase in ALP activity, increased deposition of calcium phosphate as well as displayed differentiated population of osteoblastic cells.

M Taira, **et al**[13] in 2003 assessed proliferation of bone marrow stromal cells in Sprague-Dawley (SD) rats using Dulbecco's modified Eagle (DME) medium and adding five cytokines such as vitamin C, vitamin D, bone morphogenetic protein (BMP), transforming growth factor-beta (TGF-beta) and dexamethasone to it. Later on he derived that the combination of dexamethasone, vitamin C and BMP showed moderately increased growth of cells.

J.Chen, et al[10]in 2015 conducted a study on extraction sockets of rabbits using the same combination of drugs, where they divided the rabbits into different groups to evaluate three parameters i.e. gelatin sponge with the osteogenic inducer group, the only gelatin sponge group and the control group. The results clearly showed that the osteogenic inducer group had better healing and maintained the alveolar ridge height for future dental rehabilitation.

The three drugs used in this study have been previously used many a times separately for assessment of their mechanism of action. **Douglas Darr, et al**[14] in 1993 studied ascorbic acid has impact on collagen synthesis and developed a theory that ascorbic acid increases lipid peroxidation and in turn increases collagen synthesis.

In his 2011, **Fulan Wei, et al**[21] found that vitamin C (Vc) was able to induce telomerase activity in periodontal ligament stem cells (PDLSCs) and can develop cell sheet structures due to increased development of the cell matrix.

In 2007 **HidekazuOshina, et al**[20]used dexamethasone as a differentiation agent to separate hBMMCs into osteoblasts, adipocytes and chondrocytes. However, the study concluded that cells which received higher concentration of dexamethasone

showed a differentiation potential and that dexamethasone induced apoptosis of cells which were suspected to have poor differentiation potential.

Administration of these drugs is another concern which had to be checked to understand which route of administration will provide maximum effect with minimum exposure to the drug. **O.W. Majid, et al**[26]in 2013 studied 5 routes of administration of dexamethasone to reduce post-operative sequelae following third molar extraction. 5 routes which were used were intramuscular injection, intravenous injection, oral tablets, endoalveolar powder and submucosal injection. 4 mg dexamethasone was administered into patients and result showed that the group of patients who received endoalveolar powder presented with highest activity of the drug and minimal post-operative sequelae giving an assurance that topical application or local drug delivery of dexamethasone is more safe and effective option available.

Beta-sodium glycerophophate as reported in the studies by **Xiao-jing Liuet al**[19] provides phosphate ions to osteoblasts to increase the rate of deposition and calcification of calcium salts. In some studies, beta-sodium glycerophosphate is recognized as a skin irritant, but patients did not experience any post-operative allergic reactions in our study and it can be presumed that presence of dexamethasone reduced the irritant effect of the aforesaid drug.

In his research on dogs, **Maxwell D.Finn et al**[35]used gelatin sponge to confirm its potential to promote bone regeneration while acting as a hemostatic agent. The gelatin sponge was seen to act as a scaffold for osteoblastic differentiation in this study.

J.Chen, et al[10] conducted his study on rabbits and observed that gelatin sponge infused with osteogenic inducer group showed better results when compared with plain gelatin sponge group. So, in our study we have used gelatin sponge as a carrier for delivery of osteogenic inducer in the extraction socket. However, future experiments can also be conducted with gelatin sponge independently to validate its ability to function as a scaffold.

Since we randomly selected patients for the study, the findings showed no male or female predilection towards the drug's effectiveness. Similarly, no age-related variation was found but with a rise in age the fact had to be considered that the body's healing ability often steeps down in the curve.

This study was conducted on impacted mandibular 3rd molars, cases in which bone guttering is done and a defect larger than the extraction socket is created and the osteogenic inducer showed excellent results in concern of healing in merely 3 months of time, so it can be considered that if used in normal extraction sockets the healing will be much faster eventually reducing the time of rehabilitation.

Joseph J. Schreiber et al[36] used computed tomography to determine bone mineral density as CT gives the precise idea for bone density assessment but in our study we used RVG to make the evaluation technique user-friendly as RVG provides focused details of the bone architecture which is sufficiently acceptable for our study, is cost-effective and widely accessible across all dental clinics, so that this drug combination can be used for day-to-day care and can be easily monitored for its progress.

When evaluating the osteogenic inducer's wound healing potential we used the Landry and Turnbull Index[32]. On the 1st and 7th post extraction day, wound healing

was evaluated. The criteria used were the color of the tissue, the presence or absence of bleeding on palpation, the presence of suppuration and whether or not granulation tissue was present. On day 1 post extraction there was no significant difference as both the groups had similar results but on the 7th day the osteogenic inducer group showed significant results in comparison to the control group proving that the combination of drugs when applied topically has effect on wound healing as well.

Since our study was primarily concerned with reducing bone loss followed by tooth extraction, the osteogenic inducer was used. Previous benefits of the drug combination were explored and when it was used in our patients, the findings demonstrate substantial improvement as bone regeneration was quicker and bone quality was much better than that of the control group as well.

In this study, bone regeneration was evaluated using Kelly et al's[33] healing Index to assess the lamina dura, overall density and trabecular pattern on digital RVG images. We recalled the patient for follow up on 1st day, after 1 month and after 3 months post extraction.

It was found out that on 1st day, 1 month and after 3 months, the mean Lamina Dura score of the study group was significantly higher than the control group[33].

The score for overall density was evaluated using radiovisiography (RVG) after 1st day, 1 month and after 3 months. As the Osteogenic Inducer was infused into a gelatin sponge, the substance density was not the same as that of a grafting material. Hence, radiographically the overall bone density was not significant on the 1st day and after 1 month post extraction. In the case group significant difference in the overall density of bone was noted radio-graphically after 3 months[33].

In terms of trabecular pattern, the mean score in the case group on the 1st day and after 1 month was not significant when compared to the control group. When seen radiographically fine coarse trabeculae were seen after 1 month in both case group as well as control group with only difference that more fine coarse trabeculae were present in the case group in comparison to the control group. Although, after 3 months dense trabeculae were seen radio-graphically among the case group patients giving positive results that the osteogenic inducer has good bone regeneration potential[33].

Based on the results and observation certain drawback of the study came into light like a longer follow up period could help in assessing whether the regenerated bone is stable or if it resorbs after a certain period of time. Histological analysis should only be used in attempt to reaffirm the results and reach a stronger conclusion[10].

A larger sample size would also provide more definitive data and contrary to our study a split mouth study with one side being the control group and other being the case group, researchers can get a clearer picture of healing potential of the osteogenic inducer.

In order to better predict bone density and to search for suitability for implant placement in the field of rehabilitation, CBCT may also be included in the study.

On the basis of this study we observed that osteogenic inducer has the potential to promote faster wound healing and bone regeneration while eliminating the need for second surgical procedure. On top of that it is easy to formulate and conveniently available at reasonable prices. Thus, we can state that osteogenic inducer is a game changer and can unravel new concepts in the field of bone regeneration.

CONCLUSION & SUMMARY

The goal of this study was to assess the effectiveness of the topical combination application of Vitamin C, Dexamethasone and Beta Sodium Glycerophosphate in wound healing and bone regeneration of the impacted third mandibular molar extraction socket. This study was conducted in the Department of Oral and Maxillofacial Surgery, KAHER KLE V K Institute of Dental Sciences, Belagavi. Osteogenic Inducer was used as a graft material impacted mandibular third molar extraction socket and clinical as well as radiographic features were evaluated.

The study clearly indicates that Osteogenic Inducer used as a graft material showed improved wound as well as bone healing when placed in the third molar extraction sockets. It has shown positive results, with the added benefits of no additional donor site requirement, reduced postoperative complications and is not technique sensitive. We can conclude that topical application of Osteogenic Inducer serves as effective scaffold for wound healing as well as for faster bone regeneration[10].

We recommend more clinical trials with larger sample size, longer follow-up duration and use in normal extraction socket. In order to reaffirm the findings and arrive at a stronger conclusion, histological analysis should also be used.

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ANNEXURE – I

ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
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Sl. No. : 1212

CERTIFICATE

This is to Certify that the synopsis titled

Evaluation of Wound Healing and Bone Regeneration using topical combined application of vitamin C, dexamethasone and beta-sodium glycerophosphate for healing of extraction socket - A comparative study.

Submitted by

Dr. Rishabh Mandal *P. G. Student / Staff, Guided by* Dr. Shridhar D. Baliga *from Department of* Oral and Maxillofacial Surgery *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II – BIOSTATISTICS CLEARANCE CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
☎ 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work of **Dr. Rishabh Mandal** entitled **"EVALUATION OF WOUND HEALING AND BONE REGENERATION USING TOPICAL COMBINED APPLICATION OF VITAMIN C, DEXAMETHASONE AND BETA-SODIUM GLYCEROPHOSPHATE FOR HEALING OF EXTRACTION SOCKET- A COMPARATIVE STUDY"** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 05/09/2020

Name & Signature of Biostatistician
(Dr. S.N. Javali)

ANNEXURE III – PLAGIARISM ACCEPTED LETTER

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences
A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
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Date : 24.9.20 Serial No. : 041

PLAGIARISM CHECK REPORT

Name of the Applicant : Dr. Rishabh Mandal
UG / PG / Ph.D / Staff : Post Graduate
Batch & Year : 2018 - 2021
Department : Oral and Maxillofacial Surgery

The soft copy of Research Work / Manuscript by Rishabh Mandal entitled "Evaluation of wound healing and bone regeneration using topical combined application of vitamin C, dexamethasone and Beta-sodium glycerophosphate for healing of extraction socket - a comparative study."
under the guidance of .....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of 8%, which is **within** / **not within** the acceptable limits of 10% as per the UGC guidelines.

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KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

ANNEXURE - IV

CASE HISTORY PROFORMA

NAME: **AGE/ SEX:**

OCCUPATION: **O.P.NO.:**

ADDRESS: **DATE:**

CHIEF COMPLAINT:

HISTORY OF PRESENTING ILLNESS:

PAST DENTAL HISTORY:

PAST MEDICAL HISTORY:

DRUG ALLERGY:

PERSONAL HISTORY:
Smoking/ Alcohol/ Tobacco chewing

GENERAL PHYSICAL EXAMINATION:

EXTRA-ORAL EXAMINATION:

Facial Symmetry:

TMJ:

Lymph Node:

Mouth Opening:

INTRA-ORAL EXAMINATION:

- Soft Tissue Surrounding The Impacted Tooth: Normal/ Inflamed
- Ulcer: Present/ Absent
- Fibrosed: Yes/ No
- Pericoronitis:
- Swelling:
- Discharge:
- Pain/ Difficulty In Chewing:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:

RVG:

Routine Blood Investigation**:**

RADIOGRAPH AND CLINICAL CORRELATION:

DIAGNOSIS:

TREATMENT PLANNING:

DETAILS OF SURGERY:

DATE:

START TIME (INCISION):

END TIME (CLOSURE):

SURGICAL PROCEDURE:

Local Anesthesia:

Incision:

Flap:

Method of Extraction:

Closure of Site:

MEDICATION:

ANNEXURE – V – CONSENT FORM

KAHER'S K.L.E.'s V.K. Institute of Dental Sciences
Department of Oral and Maxillofacial Surgery, Belagavi
CONSENT TO SURGERY & ANAESTHETICS

Date Time a.m./ p.m.

1. I authorize the performance upon self or Mr./Miss./Mrs._____the following operation (Nature and extent) to be performed under the guidance of Dr. And by Dr._____
2. The procedure of extraction under LA and possible complication has been explained to me in my own language and I understand the nature of the treatment.
3. I consent to the administration of anesthetics as may be considered necessary or advisable by the doctor responsible for this service.
4. I consent to the photographing or television of the operation or procedures to be performed including appropriate portions of my body, for medical, scientific or educational purposes provided my identity is not revealed by the pictures or by the descriptive texts accompanying them. For the purpose of advancing medical education I consent to the admittance of observers to the operating room.
5. I consent to the disposal by Oral & Maxillofacial Surgery Department authorities of any tissues or parts which may be removed.
6. I am participating in this study with my own wish and will and the dentist has explained the nature and the effect of the procedure including extraction of tooth followed by placement of gelatin sponge containing osteogenic inducer (combination of vitamin C, dexamethasone and beta-sodium glycerophosphate) for wound healing and bone regeneration in my vernacular language.
7. The nature and purpose of the operation and the materials being used, possible alternative methods of treatment, the risk involved and the possibility of complications have been fully explained to me in my mother tongue. No guarantee or assurance has been given by anyone as to the results that may be obtained.
8. I have read and understood the above information given by surgeon about the study and Igive my consent for the study and for its publication.

Patients / Witness Signature: Date:
Surgeon's name:
Surgeon's signature:
Doctor's contact:
Hospital contact:

ANNEXURE – VI- MASTER CHART

Case Group- Wound Healing Assessment

Serial no.	1st day	7th day
1	2	5
2	2	4
3	3	5
4	3	5
5	2	5
6	2	5
7	2	5
8	2	4
9	2	4
10	2	3
11	2	5
12	1	3
13	2	4
14	2	5
15	2	5
16	1	3
17	2	4
18	2	4

Control Group- Wound Healing Assessment

Serial no.	1st day	7th day
1	2	3
2	2	4
3	1	2
4	3	4
5	2	3
6	1	3
7	2	3
8	2	3
9	2	3
10	2	3
11	3	4
12	2	5
13	2	3
14	1	2
15	2	3
16	2	4
17	2	3
18	2	3

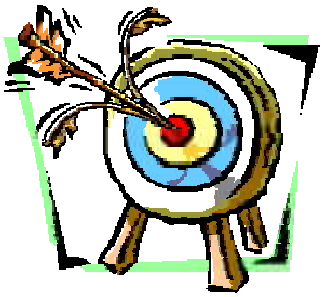
Case Group - Bone Regeneration Assessment

Serial no.	Lamina Dura			Overall Density			Trabecular Pattern		
	1st Day	1 Month	3 Months	1st Day	1 Month	3 Months	1st Day	1 Month	3 Months
1	0	1	2	-1	0	2	0	0	2
2	0	1	2	0	0	2	0	0	2
3	0	1	2	0	1	2	-1	0	2
4	1	2	2	-1	0	2	0	1	2
5	0	2	2	0	1	2	0	0	2
6	0	2	2	-1	0	2	-1	0	1
7	0	1	2	0	0	2	-1	0	1
8	1	1	2	0	1	2	0	0	1
9	1	1	2	0	0	2	-1	0	1
10	0	1	2	-1	0	1	0	1	2
11	1	1	1	0	1	2	0	1	2
12	-1	0	1	-1	0	2	-1	0	1
13	1	1	2	0	1	2	0	1	2
14	0	1	2	-1	0	2	0	1	2
15	0	1	2	-1	0	1	-1	0	1
16	0	1	2	-1	0	0	0	1	2
17	0	1	2	0	0	1	0	1	2
18	1	2	2	0	0	2	-1	0	1

Control Group - Bone Regeneration Assessment

Serial no.	Lamina Dura			Overall Density			Trabecular Pattern		
	1st Day	1 Month	3 Months	1st Day	1 Month	3 Months	1st Day	1 Month	3 Months
1	0	1	1	-1	0	1	-1	0	1
2	0	1	1	0	1	1	-1	0	1
3	1	1	1	-1	0	1	0	0	1
4	-1	0	1	-1	0	0	0	0	1
5	-1	0	1	0	1	1	-1	0	1
6	-1	0	1	-1	0	1	0	1	2
7	-1	0	0	0	0	1	0	0	1
8	-1	0	0	0	0	1	-1	1	2
9	-1	0	0	-1	0	2	0	0	1
10	0	0	0	0	1	1	0	1	2
11	0	1	2	-1	0	1	-1	0	1
12	0	1	2	-1	0	1	-1	0	0
13	0	1	1	0	1	0	0	0	1
14	0	0	0	-1	0	1	0	1	2
15	1	1	1	0	1	1	-1	0	1
16	-1	0	1	-2	0	2	-1	0	1
17	0	1	1	0	0	1	-1	0	1
18	0	1	1	0	0	0	0	1	2

Introduction



Objectives

Review of Literature

Methodology

Results

Discussion

Conclusion & Summary

Bibliography

Annexures