EFFICACY OF PLATELET RICH PLASMA (PRP) IN THIRD MOLAR IMPACTION SURGERY

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH III

ORAL AND MAXILLOFACIAL SURGERY

APRIL 2013

RAJAS DENTAL COLLEGE

RAJA NAGAR, KAVALKINARU - 627 105, TIRUNELVELI DISTRICT.

DCI Recognition No.DE-3 (44) - 93/2246, dated 09/11/1993

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

DEPARTMENT OF ORAL AND MAXILLOFACIAL SURGERY

CERTIFICATE

This is to certify that this dissertation entitled "Efficacy of platelet rich plasma (PRP) in third molar impaction surgery" is a genuine work done by **Dr.Joy R.Das** under my guidance during his post graduate study period 2010-2013.

This Dissertation is submitted to THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY in partial fulfillment for the Degree of **MASTER OF DENTAL SURGERY IN ORAL AND MAXILLOFACIAL SURGERY, BRANCH III.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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Dedicated To

My Papa, Mummy and dear Wife

Acknowledgement

I express my heartfelt thanks to **Prof.Dr.M.Baskaran,MDS,FDSRCS (Eng)**, Professor and Head of the Department,Department of Oral and Maxillofacial Surgery,Rajas Dental College and Hospital,Kavalkinaru,for his constant support,expert critique and encouragement throughout my dissertation.I am indebted to him for his valuable guidance and immense support he has provided me in my entire Post Graduate course.

With deep gratitude and humbleness I wish to express my indebtedness to the college management especially **Dr.Sardar S.A Raja** our beloved Founder Chairman,and **Dr.Jacob Raja, MDS** ,our Chairman for giving me an opportunity to study in this esteemed institution.

I express my gratitude to **Dr.I Packiaraj,M.D.S.,(**Director of Administration and Professor, Department of Oral and Maxillofacial Surgery,for his valuable thoughts, and constant support whenever needed the most, throughout my PG course and for the completion of this dissertation.

I sincerely thank the Institutional Ethical Committee for granting me permission to start this project.

I extend my gratefulness to **Dr.S.Subramonian, MDS,** Reader**, Dr.Retnakumar, MDS,**for their timely help and suggestions throughout my entire course. I specially thank **Dr.J.Pratheep MDS**(Sr.Lecturer) for this untiring guidance in helping me fulfill this dissertation.

I thank **Dr.Jayakrishnan.R MDS** (Sr.Lecturer) for his everlasting support in the department throughout my entire curriculum. I also thank **Dr.Gen Morgan,MDS** (Sr.Lecturer) for his guidance in my studies. I owe my sincere gratitude to **Dr. Suresh Sathiasekar, MDS,** Principal,

Rajas Dental College, for having fulfilled the requirements in my department and the college and a source of inspiration to all.

I thank **Dr.Ramadevi**,Clinical Epidemiologist Trivandrum Medical College for her valuable assistance in completing my dissertation.

My special thanks to **Dr. Meenakshi Chauhan** for helping me with my dissertation work

I would also like to express my gratefulness to my colleagues,**Dr.Varun**.M,**Dr. Dhilip Samji, Dr.Shahin V.R, Dr.Divakar T.K**, and **Dr.Gidean Arularasan** for sharing their views and for extending a helping hand in times of need and encouraging me during my study.

I express my sincere thanks to all my study participants for their co operation and support in this study. My sincere gratitude to Nursing Staff and theatre assistant **Mrs.Mani** for her support towards my departmental activities throughout my course.

Last but not the least I would like to thank my **Parents**, and my **Wife** without whose constant support and encouragement , this study would not have been feasible.

Above all I thank **The Lord Almighty** in guiding me through in all my endeavours.

Efficacy of platelet rich plasma in third molar impaction surgery

Abstract : The use of autologous platelet concentrates mainly platelet rich plasma has been of relevance recently in the field of Oral and Maxillofacial surgery. These concentrates had the ability to improve the hard and soft tissue healing.This ability of the platelet concentrates had been studied in the healing after mandibular third molar impaction surgery. The various parameters like bone density,soft tissue healing, post operative pain,swelling and alveolar osteitis were taken into account. The patients were evaluated on day 1,day 2,day 3,day 7, first month and second month post operatively. The values for each parameters were found out and tabulated.

Materials and methods:12 patients , 7 males and 5 females with bilaterally impacted mandibular third molars were included in the study. Autologous platelets were concentrated.This was applied on one of the sockets which was randomly taken as the test side. The other socket was treated as the control.

Results: Statistical analysis was done using Mann Whitney U test for pain and soft tissue healing, which revealed the values for pain being statistically significant for the test side and no statistical difference was noted for soft tissue healing. Bone density and swelling were analysed with T-test . Bone density values were statistically significant for the test side, while swelling did not show much statistical significance. None of the patients presented with alveolar osteitis in any side.

Summary and conclusion: platelet rich plasma is a surgical additive that can be used to improve bone and soft tissue healing. It contains a wide array of growth factors that are responsible for their healing potential.

Keywords: platelet concentrates, growth factors, bone density, gray value scale, alveolar osteitis,soft tissue healing, pain,swelling**.**

Introduction

One of the greatest challenges in clinical research is the development of surgical additives that can increase the healing potential . The healing of hard and soft tissues is mediated by a wide range of intracellular and extracellular events that are regulated by signalling proteins. Platelets play an important role not only in hemostasis, but also in wound healing.

A lot of surgical additives have been introduced into the field of Oral and Maxillofacial Surgery with the aim of better soft and hard tissue healing, pain control, reduction of dry socket and control of swelling.

Platelet concentrates are blood derived products used for the prevention of haemorrhages due to serious thrombocytopenia of central origin. Oral surgery in cardiac patients under anticoagulant therapy may be facilitated with platelet concentrates[1,2].

The development of platelet concentrates as bioactive surgical additives that are applied locally to promote wound healing stems from use of fibrin adhesives. The evolution of platelet concentrates, thus dates back to 1970 when fibrin glue was introduced. This was prepared by polymerizing fibrinogen with calcium and thrombin. This product was available in blood bank and this carried a risk of disease transmission, like hepatitis and HIV [3]. So the fibrin adhesives were banned in the United States as early as 1977. After this ,studies were concentrated on the preparation of autologous platelet concentrates .

INTRODUCTION

Platelet Rich Plasma was first used in cardiac surgery by Ferrari in 1987 as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion[4]. PRP has also been utilized to treat intra-articular injuries. Examples include arthritis, arthrofibrosis, articular cartilage defects, meniscal injury, and chronic synovitis or joint inflammation[5]. The use of platelet rich plasma in sports medicines were elaborated by various authors. This was introduced into the field of Oral and Maxillofacial Surgery in 1998 , Marx being the pioneer.

Thus the first generation platelet concentrates otherwise called as Platelet Rich Plasma was introduced. The various uses of platelet rich plasma in Oral and maxillofacial surgery includes sinus lift procedures, ridge augmentations, socket preservation, alveolar cleft and palate repair , alveolar bone grafting, oral or nasal fistula closure , intrabony defects , jaw reconstruction , mandibular fractures [6], and soft tissue gingival procedures.

Third molars, the last teeth to erupt into the human dental arch, have been shown to be the most frequently impacted teeth in all human ethnicities. The main factors contributing to impaction are an inadequate dental arch space and erratic eruption paths. The impaction surgery done for the removal of the third molars presents itself with a variety of factors which are uncomfortable to the patient. Pain, swelling, trismus, infection, bone loss, pocket formation distal to the second molar being a few factors .

INTRODUCTION

Immediately following tooth removal, a healing process begins that affects the eventual alveolar bone volume and architecture of the alveolar ridge. Satisfactory and timely healing are essential to obtain ideal functional reconstruction. Traumatic removal of a tooth, or a poor healing response, may lead to excessive bone loss . Patients and clinicians could benefit if a cost-effective, simple technique were available that decreased bone-healing time and increased the predictability of favorable results. The objective of this study was to determine whether the application of platelet rich plasma (PRP) to a tooth extraction site would facilitate bone healing.

Following extraction of a tooth, a blood clot forms in the socket and the healing process begins. The early healing events include the formation of granulation tissue within the socket, which is progressively replaced with newly formed bone.

The healing process is considered complete after 4–6 months, once bone remodelling takes place. In order to reduce alveolar bone dimensional changes, several techniques aiming at enhancing the regeneration process in the extraction socket , better soft tissue healing, reduction in post operative pain and swelling were introduced .Recently, the use of platelet concentrates has been proposed as an aid for enhancing regeneration of osseous and epithelial tissues in oral surgery.

Several in vitro studies, animal experiments and clinical trials suggested that platelet concentrates may effectively trigger stimulation of osseous and

soft tissue regeneration, and reduce inflammation, pain and unwanted side effects. Platelets are known to perform multiple functions during injury and tissue repair. Platelets initiate the body's response to a normal sequence of events that provide clotting and healing of the damaged tissue.

There are 4 phases of wound healing: the hemostatic phase (clot formation), the inflammatory phase (clean-up and recruitment),the proliferative phase (regeneration), and the tissue remodeling phase. Although these phases appear independent from one another, they overlap significantly during the healing process. Naturally occurring materials present in the body provide the signals and structures for repair, regeneration, and healing. These may be considered as "autologous biomaterials" distinguishing them from recombinant or synthetic "biomaterials."

Postsurgically, blood clots initiate the healing and regeneration of hard and soft tissues. Using platelet-rich plasma, is a method to accelerate and enhance the body's natural wound-healing mechanisms. Platelets primarily are involved in wound healing through clot formation and the release of growth factors that initiate and support wound healing.

Increasing the concentration of autologous biomaterials to a wound site enhances healing. Two important autologous biomaterials, platelets and fibrin, play a central role in the formation of the clot. A number of derived growth factors are released from the platelets. They are critical for any wound to heal and are involved in

every phase of wound healing. They are contained in the α granules of platelets as well as other cells such as macrophages and endothelium. Platelet growth factors are responsible for the early migration of cells to the injury site and the triggering of mitosis of these cells .

After tissue injury, platelets become exposed to damaged blood vessels, which places them in direct contact with collagen, the basement membranes of capillaries, and sub endothelial microfibrils. This interaction causes the platelets to aggregate at the site and change from a rounded shape to one that includes large, sticky protuberances, or pseudopodia. This process is called activation. During activation, the alpha granules fuse with the platelet plasma membrane and release their protein contents to the surroundings.

Specific platelet-isolated growth factors include platelet-derived growth factor (PDGF), transforming growth factors-beta (TGF- β),vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF)[7]. Growth factors released from platelets and contained within the clot send out signals to trigger cell division. The growth factors initiate connective tissue healing, bone regeneration and repair, increase mitogenesis of fibroblasts, stimulates angiogenesis in the wound bed and activates macrophages. The newly created blood vessels and blood flow brings necessary nutrients and oxygen for optimal healing. The growth factors act on stem cells , osteoblast precursors and fibroblasts.

INTRODUCTION

Platelet-rich plasma (PRP) is a new biomaterial that consists of a high concentration of human autologous platelets in a small amount of plasma .Platelet-rich plasma can be defined such that ,it is a volume of autologous plasma that has a platelet concentration above baseline.

Because the scientific proof of bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/μL, it is this concentration of platelets in a 5-ml volume of plasma which is the working definition of PRP today[8]. Lesser concentrations cannot be relied upon to enhance wound healing, and greater concentrations have not yet been shown to further enhance wound healing .

Because PRP is developed from autologous blood, it is inherently safe and is free from transmissible diseases such as HIV and hepatitis. Within PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area.

Thus PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration such as is seen in osteoconduction, wound epithelialization, and osseointegration.

Platelets are attracted to a wound or injury site stimulating the clotting and healing cascades. Growth factors signal undifferentiated stem cells to the site, promote cell mitosis, and stimulate osteogenesis and angiogenesis. Cytokines,which attract neutrophils,

are also released from platelet granules. Concentrating platelets 4-5 times the baseline level accelerates the healing process. When platelet rich plasma is mixed with an activator, a platelet gel will form. Clinical applications of platelet gel are numerous. Some benefits include a marked decrease in post-surgical swelling,reduction in surgical site pain, and acceleration of bone growth and soft tissue healing.

The clinical implications of the plasma gel are many in the field of reconstructive surgery. In neurosurgery it is used as a biologic sealant to aid in watertight dural closure.In facial and reconstructive plastic surgery the gel is used as a hemostatic agent in the surgical bed. During radical neck dissections this is used as a hemostatic agent as well as a lymphatic sealant. This gel has also been found to be important in treating chronic wounds and burns.

The current study deals with the efficacy of autologous platelet concentrates in third molar impaction surgery. The hard and soft tissue healing initiated by the platelet concentrates are analysed.

AIM

Comparison of the efficiency of platelet rich plasma (PRP) in third molar impaction surgery in patients with bilaterally impacted mandibular third molars where one side was treated as test (PRP side) and the other side treated as control (non PRP) side.

OBJECTIVES

To reduce the post operative complications like pain, swelling,dry socket, improvement of soft tissue healing and bone density with the use of platelet rich plasma on the basis of a comparitive study on the same patient with bilaterally impacted third molars.

Review of literature

Sidney Shaw[9] (1970) did the estimation of platelet adhesiveness on whole blood and platelet rich plasma. Compared the variation in platelet adhesiveness using whole blood and citrated plasma.

Robert E Marx[7] (1998) stated that platelet rich plasma contains a concentration of platelets and growth factors . The study evidenced that the growth factor additions to bone grafts produced a quantifiably enhanced result in comparison with grafts performed without its use. The fact that PRP is an autologous preparation eliminates concerns about disease transmission and immunogenic reactions.

Regina Landesberg[10] (1998) evaluated the risks of using platelet rich plasma. And she has given the various other thrombin preparations like recombinant human thrombin and autologous thrombin to replace the bovine thrombin

Regina Landesberg et al[11] (2000) emphasized on quantification of growth factor levels using a simplified method of platelet rich plasma gel preparation. The study compared two methods of preparing platelet rich plasma and the levels of PDGF and TGF beta in each preparation. Use of gel preparation is equivalent to using calcium chloride and bovine thrombin. The levels of DPGF and TGF β were same regardless of which method was used for clot formation.

Dugrillon [12] (2001) showed the effects of autologous concentrated platelet rich plasma for local application in bone regeneration. The study shows a blood bank procedure to generate PRP with defined quality control. A comparison was evaluated between the concentrated PRP (c PRP) and PRP. The concentrated PRP showed a 12- 17 times increase in the growth factor content.

Su-Gwan Kim et al [13] (2002)compares study of osseointegration of avana implants in a demineralized freeze dried bone alone or with platelet rich plasma. The results showed that bone defects around titanium implants can be treated successfully with demineralized freeze dried bone powder and that PRP increases bone formation. The study showed that the freeze dried bone used as a mixture with PRP increased the osseointegration.

Tara L Aghaloo[14] (2002) did a pilot study in rabbit cranial defects with platelet rich plasma. The study showed that no significant difference in bone formation was seen when PRP was used alone . There was a significant increase in bone formation and bone density when the PRP was used along with bone.

Gernot Weibrich[15] (2002) compared the growth factor levels in platelet rich plasma in relation to age , sex and platelet count. PRP contains many growth factors. Three of those PDGF αβ, TGF β 1, and IGF 1 were found in high concentrations and PDGF ββ and TGF β 2 were found in low quantities. Major age and gender specific differences were not noted.

Arlene Rodriguez[16] (2003) indicated the use of platelet rich plasma with deproteinated bovine bone in maxillary sinus augmentation. The article concluded that the combination of these two materials is effective in maxillary sinus augmentation in severely resorbed posterior maxillae. They produced new bone with higher density. The endothelial cells, adipocytes and fibroblasts maybe transformed into osteoblasts on a variety of biochemical signals via cytokines provided in the PRP.

Sanchez et al[17] (2003) in their review has elaborated certain potential risks of using PRP. This was with the use of bovine thrombin,which may lead to development of antibodies to factor V , leading to life threatening coagulopathies.Bovine thrombin is known to contain factor V which could stimulate the immune system.

Hiroshi Kitoh et al [18](2004) showed the clinical results of distraction osteogenesis with transplantation of marrow derived mesenchymal cells and platelet rich plasma were reviewed. PRP contained several growth factors , coagulate immediately by a minute introduction of thrombin or calcium. They concluded that PRP reduce the treatment period by acceleration of bone regeneration during distraction osteogenesis.

M.A.W Merks et al [19] (2004) in his article mentions the reconstruction of the mandible with preshaped 2.3 mm titanium plates , autogenous particulate cortico cancellous bone grafts and platelet rich plasma. Describes all the growth factors present in PRP, which have positive effects on vascular ingrowth and bone healing. Because of this PRP in combination with non vascularised bone grafts is advantageous in skeletal reconstruction. Since the reconstruction was mainly done after ablative surgery the article also proves that there are no factors in PRP that causes new tumour formation.

J. Camilo Roldan et al [20] (2003)did a comparative study on bone formation in the presence of platelet rich plasma and bone morphogenic protein 7. This article explains the preparation of PRP. Showed that the TGF β present in the PRP has the osteoinductive property.

Tomoki Oyama et al [21] (2004)showed the efficacy of platelet rich plasma in alveolar bone grafting. They found that PRP was a safe and cost effective source for growth factors and was easy to extract. It could enhance the osteogenesis of alveolar bone grafting in cleft lip and palate patients. Here platelet rich plasma was added to the particles of cancellous bone .Human fibrin glue was added to consolidate the bone chips and PRP. It was revealed that PRP could enhance osteogenesis in a remodeling phase within the first 4 months.

Candan Efeoglu et al [22] (2004) showed the preparation of low cost autogenous platelet rich plasma for use in minor bone grafting procedures. Their methods proved that PRP could be prepared within 30 minutes. Platelet counts in PRP were increased with respect to venous platelet counts. Most of the studies on PRP preparation was focused on machines used in its preparation, presence of growth factors and the anticoagulants used. EDTA was the preferred anticoagulant used. Studies using flow cytometric techniques and automated blood analyzers reveal that various anticoagulants including citrates activate platelets but the activation done by EDTA and heparin are much stronger.

Robert E Marx[23] (2004) showed in this article the evidence to support the use of PRP in maxillofacial surgery. Shows how PRP works , the amount of platelets enough, what all clinical conditions that benefit from PRP, the safety of PRP, and whether it promotes infection. Today PRP remains the sole growth factor available to maxillofacial surgeons. PRP works via degranulation of alpha granules in platelets. A PRP count of 1 million/µL as measured in the standard 6 mL aliquot is the benchmark for therapeutic PRP. PRP enhances osteoprogenitor cells in the host bone and in bone grafts. Shows the use of PRP in continuity defects, sinus lift augmentations , horizontal and vertical ridge augmentations and periodontal and peri implant defects. Since it is an autogebous preparation, it is safe to use.PRP has a pH of 6.5 – 6.7. No evidence to show it promotes infection.

Earl G Freymiller et al[24] (2004) Showed the use of platelet rich plasma with autogenous bone,with anorganic bone material, with organic bone matrix and when used alone. When PRP was used alone in extraction sockets, improved epithelialization and bone density was noted. There is a lower rate of alveolar osteitis, less pain and more dense radiographic bone healing. Explains the growth factors present in PRP and how they are released (degranulation).

JJ Thorn et al [25](2004) Compared the autologous fibrin glue with the platelet rich plasma. Also showed how the fibrin glue can be used as the transplant material for PRP. The platelet gel is a modification of the fibrin adhesive. The gel contains a high concentration of platelets, nearly 300-400% of blood. The concentration of fibrinogen was 12 times less in PRP. Also the PDGF also was higher in fibrin glue. The clinical application of fibrin glue also appeared to be easier than PRP.

BH Choi et al [26](2004) Evaluated the effect of platelet rich plasma on bone regeneration in an autogenous bone graft. Variations in the concentration of platelet derived growth factors are known to influence bone healing. Platelets and growth factors are known to likely act more during the early stage of bone graft healing.

Gilberto sammartino et al [27] (2005) Showed that PRP is effective in inducing and accelerating bone regeneration for the treatment of periodontal defects at the distal root of mandibular second molarafter surgical extraction of a deeply impacted third molar. There is a notable reduction in probing depth and an improvement in the probing attachment level in those cases treated with PRP. The mechanism by which PRP can influence the periodontal regeneration was due to the presence of PDGF and TGF -β. Due to the presence of the fibrin clot the PRP gel permits stabilized coagulation of blood , thereby favouring regeneration of the osseous defect, mostly in the early stages. TGF -β could favour the differentiation of osteoblasts and cementoblasts and the production of fibronectin.

Regina Landesberg et al [28](2005) Showed the activation of platelet rich plasma using thrombin receptor activator peptide. Gives an alternate preparation of PRP. Shows the use of TRAP to activate clot formation in the preparation of PRP. This is used as an alternative to bovine thrombin. Bovine thrombin is associated with the formation of antibodies to factor V and XI and thrombin that may result in coagulopathies. TRAP minimizes the amount of clot retraction.

Rick C Tsay et al[29] (2005) PRP preparation with thrombin results in a large immediate release of growth factors that could be lost into the interstitium in vivo. Thrombin Receptor Activator Peptide -6 (TRAP) proves more effective than thrombin in sustaining growth factor levels critical for the cascade of events leading to bone formation. Growth factor retention was a function of both the substrate used and the specific growth was examined.

BH Choi et al (2005)[30] The result of their study showed that the viability and proliferation of alveolar bone cells were suppressed by high PRP concentrations while they were stimulated by low concentration of PRP 0.5 -5 % PRP stimulates osteoblastic proliferation. A dilution of PRP concentrations to a level suitable for cell proliferation may be necessary for use in bone grafts in order to enhance bone cell growth within PRP treated bone graft

GRJ Swennen et al [31](2005) Showed the effect of platelet rich plasma in the cranial distraction osteogenesis in an animal model. All the growth factors in PRP were given. The preliminary result of the study showed that PRP only had an effect on bone regeneration if active distraction was started immediately after application of PRP in the distraction gap.

Yoichiro Ogino et al[32] (2006) Showed the contribution of platelet derived growth factors transforming growth factor beta 1, and insulin growth factor in platelet rich plasma to the proliferation of osteoblast like cells.PRP could stimulate the mitogenic activity of osteoblast cells. The amount of growth factors present in PRP were measured by ELISA. PDGF was the most dominant factor that could stimulate the proliferation of osteoblast like cells.

Barry L Eppley et al[33] (2006) Gives the technical concepts and evaluation of platelet rich fibrin a second generation platelet concentrate. Compares the biomechanical properties of fibrin adhesives, concentrated PRP and platelet rich fibrin. Emphasis is given more on the technical aspects in the production of PRP and PRF. Describes the earlier use of PRP in thrombopenias. Also describes the functions of platelets, haemostatic mechanisms and clotting cascades.

PA Everts et al [34](2006) healing of hard and soft tissue is mediated by a complex array of intracellular and extracellular events regulated by signaling proteins. Detailed emphasis of all the various growth factors present in platelet rich plasma is given.

MZ Casati et al[35] (2006) Studied the efficacy of platelet rich plasma in bone regeneration around the peri implant bone defects. It was found that platelet rich plasma did not improve bone regeneration around peri implant defects in dogs

Faleh M Tamimi et al[36] (2007) Did a comparative study of 2 methods for obtaining platelet rich plasma. Double and single centrifugation are the most commonly used technique . Double centrifugation method yielded 336% while the single centrifugation yielded 227 %. The PRP obtained is calcium activated before being applied over the surgical site. Platelet concentration was calculated using the given equation in the article. Both these methods were easy to use , can concentrate platelets without damaging them despite the centrigugation process the platelets suffer. The studies used immunohistochemistry to analyse the growth factors present in PRP.

Mozzati M et al[37] (2007) Studied on the clinical application of PRP in the extraction of impacted mandibular third molar. A control side and a PRP side were selected. Various parameters were set including probing depth in relation to adjacent molar, pain, swelling and bone density measured by digital OPG. Soft tissue healing criteria was also set. Digital OPG was taken at the end of first week, second week and one month post operatively. The results of the study showed a remarkable improvement in bone healing and regeneration, pain control and soft tissue healing. The edema did not have any change. The study also showed that the side where PRP was applied had a remarkable soft tissue healing. This was evaluated in the first 2 weeks.

Helen H Lu et al [38](2007) Studied on the controlled delivery of platelet rich plasma derived growth factors for bone formation. The bioactivity of released factors were maintained in vitro and they promoted cell proliferation and alkaline phosphatase activity. Described an alginate based delivery system for regulating the release of PRP derived growth factors.

James L Rutkowski et al[39] (2007) Showed in his retrospective study about the benefits of PRP in prevention of alveolar osteitis. The results showed that the alveolar osteitis occurring following tooth extraction was 2.81 times greater in patients not receiving PRP. The growth factors present in PRP are responsible for this. Mesenchymal stem cells differentiate into osteoblasts when stimulated by the growth factors.

RECM Mooren et al [40] (2007) Animal study was conducted in goats in similar sized cranial defects which were created. This study found that PRP did not have an effect in either the early or late bone healing.

Jan Slapnicka et al [41](2008) Study purpose was to evaluate the effect of progressively increasing concentrations of activated and non activated platelet rich plasma on proliferation of human osteoblasts. Activated PRP resulted in higher proliferation of osteoblasts compared with non activated PRP (0.38 times to 0.95 times). Here autologous human thrombin was used for platelet activation.

XL Griffin et al [42](2008) Studied the clinical use of platelet rich plasma in the promotion of bone healing. Augmentation of PRP with autologous bone graft alone is not as effective as in combination with bone marrow or stromal cells. Concentration of platelets determine the efficacy. The lack of consistency in platelet concentration makes comparative studies less reliable.

Bahadir Gurbuzer et al [43] (2008) Healing process with PRP after the use in impacted third molar extraction sockets were evaluated. Scintigraphic study was conducted. The activity was noted in post operative weeks 1 and 4. Bone regeneration process starts with release of PDGF and TGF beta. Since the lifespan of the platelets are less than 7 days , the growth factors present in PRP exerts their effect in early stages of wound healing (post operative weeks 1 and 2). PRP in conjunction with other graft material yielded better results.

Elisabeth Semple et al [44](2008) Comparison was done between the patients own thrombin and bovine thrombin as an activator for platelets. Thrombin from the patient had a lower growth factor content than the bovine thrombin. The amount of growth factor released depends on the rate of activation and the number of platelets.

HC Kroese –Deutman et al [45] (2008) The effect of platelet rich plasma was evaluated in a rabbit segmental bone defect. Histomorphical studies revealed that bone formation was higher in implants with PRP. It was concluded that PRP had a stimulatory effect on bone formation in titanium fiber mesh filled with autologous bone graft in bone defects. The titanium fiber mesh is shown to be an excellent scaffold material for application for autologous bone grafts. There are no adverse effects with the use of PRP since it is autologous and non carcinogenic because the proliferative growths are induced by differentiation factors only, not mutagens.

Miguel Gustavo Setubal Andrade et al [46](2008) Discusses a protocol for obtaining PRP and evaluate which factors modify PRP cytometry and coagulation time. Whole blood parameters can predict PRP features. Whole blood hematocrit is an important variable for PRP preparation and PRP cytometry characterization. PRP platelet count is dependent on whole blood platelet count. Platelet growth factors are able to intensify matrix formation and stimulate osteoblastic differentiation. Best biological response occurs when 4 to 5 fold increase over the baseline platelet number is achieved.

Agata Cieslik- Bielecka et al[47] (2008) Other applications of PRP in maxillofacial surgery were studied . Basically by concentrating platelets , healing process is stimulated. After platelet degranulation there is massive relaease of growth factors. In this study PRP gel was used for mandibular odontogenic cysts. The oral mucosa healed faster in patients treated with this. Radiographic features showed considerable enhancement of bone regeneration beginning from 4th week onwards. This was the first report that showed the use of PRP in cysts.

Foster TE et al[48] (2009) Autologous PRP is increasingly used in treatment in a variety of soft and bony tissue defects. Accelerates bone formation and management of chronic non healing wounds.Topical gel was used topically in soft tissue wounds. There was better healing rate and adequate tissue regeneration in order to undergo plastic reconstruction. Indicates the use of PRP in sports medicine and in the management of tendonitis, tennis elbow etc.

Gilberto Sammartino et al[49] (2009) combined autologous platelet rich plasma and resorbable membrane for prevention of periodontal defects after deeply impacted mandibular third molar extraction. The study with PRP alone versus PRP and bio resorbable membrane showed similar results in bone density, but the latter showed a high degree of bone maturation but not regeneration when the studies were carried out histologically.

Emilio Lopez- Vidreiro et al [50](2009) Studied the use of platelet rich plasma in arthroscopy and sports medicine. Gives the properties of platelet rich plasma. PRP can be used in sports related injuries like chronic tendinosis, tendinopathy, osteoarthritis, muscle injury, hamstring tears and osteochondritis.

Zhen Ming Hu et al[51] (2009) The molecular level of platelet rich plasma was evaluated. Showed that PRP induces mRNA expression of VEGF and PDGF in bone marrow stromal cell differentiation. This study indicates a potential contribution of PRP as the starting process of angiogenesis, recruiting the endothelial cells which line blood vessels and beginning the initiation of bone regeneration. It jump starts the beginning of a cascade of events that continue to form a mature graft. The addition of PRP to a graft reduces the time period for the consolidation of the graft.

Michael P Hall et al[52] (2009) Demonstrated the current uses of PRP in the field of sports medicine. Explained the various growth factors and the specific function for each of the growth factor. Compared the various PRP therapy preparation systems and evaluated the final concentration of platelets derived from each method. Explains the tendon graft healing induced by PRP and the effect of PRP in gycosylaminoglycans and chondroitin sulphate.

Volker LW et al [53](2009) The objective of this study was to compare the growth factor relaease from PRP and PRF in vitro.The study showed that the PRP application in cell cutures leads to higher levels of growth factors than from PRF applications. In osteoblasts the cytokine concentrations were significantly higher for PRP than PRF. Same results were obtained for fibroblast culture too.

Sunitha J et al[54] (2010) The study was done using a combination of PRP and hydroxyapatite bone graft in the treatment of infra bony defects. Explains the ability of PRP to consolidate the bone grafts . Increased bone ingrowths into a porous hydroxyapatite crystal was demonstrated when combined with PRP.

Shengyun Huang et al [55] (2010) Studied the influence of platelet rich plasma on proliferation and osteogenic differentiation of skeletal muscle satellite cells. The result of the study suggested that PRP containing osteoinductive growth factors stimulate cell proliferation and osteogenic differentiation of skeletal muscle satellite cells. The Alkaline phosphatase activity was kept as a marker for osteoblastic differentiation.

James L Rutkowski et al[56] (2010) Explained how PRP facilitated wound healing following tooth extraxction. Following extraction bone formation normally takes 12 -15 weeks . Stated that it took nearly 6 weeks for control extraction sites to reach the bone density achieved at PRP sites in 1 week. Also the control sites required 16 weeks to reach the bone density levels as reached by the test sites in 8 weeks. Bone densities were measured using Digital subtraction radiohrapghy and CT . Also used Image J software for dental radiograph analysis.

Norbert Pallua et al[57] (2010) Investigated the application of PRP in burns. Showed theoretically the effect of PRP in burn wounds. Currently in burns PRP could stimulate dermal regeneration , increase te take rate after skin graft and speed up re-epithelialisation. PRP can be mixed with fat and this has showed a promising result in scar reconstruction.

Bahadir Gurbuzer et al [58](2010) Showed the effect of platelet rich fibrin on early bone healing process with scintigraphy based on technetium-99m methylene diphoshonate uptake in third molar extraction socket. Their study showed that PRF might not lead to enhanced bone healing in soft tissue impacted mandibular third molar extraction socket 4 weeks after surgery.

Shobha Prakash et al [3](2011) Compared the various platelet concentrates right from their history beginning with fibrin adhesives. Compares the efficiency , method of preparation , growth factor contents , advantages and disadvantages of platelet rich plasma and platelet rich fibrin. Also evaluates both their uses in maxillofacial surgery.

Olufemi K Ogundipe et al[59] (2011) This study assess the effect of PRP gel on postoperative pain, swelling, trismus and bone regeneration on mandibular third molar extraction sockets. The mean postoperative pain score (visual analog scale) was lower for PRP group . The scores for lamina dura, trabecular pattern and bone density were better in PRP groups. Explains how they measured the various post operative sequale like trismus and swelling. Radiographically thy proved the scores for bone density, lamina dura and trabecular pattern was better in PRP group. The beneficial effects of PRP are more noticeable during the early phase of healing.

Javier Montero et al[60] (2011) Evaluates the change in the periodontal status of mandibular second molars following the extraction of adjacent impacted third molars. Molar depth seemed to be the main factor modulating the baseline probing depth and change in probing depth during follow up. Reported that the use of PRP in bone regeneration , afforded a satisfactory reduction in pocket depth and a gain in attachment as well as formation of new bone in the bone defect.

Akira Matsuo et al [61] (2011) Evaluated the bone quality of mandibles reconstructed with particulate cellular bone marrow (PCBM) and platelet rich plasma. Deals on the evaluation of bone density using computer software. The study indicated both PCBM and PRP yielded same bone density.

Kedarnath et al[62] (2011) Estimated the role of PRP in healing after impacted third molar surgery. Elaborately deals with the various growth factors present in PRP and the role of each of the factors. Photo stimulating phosphor was used to determine the changes in the IOPA. The authors concluded that the PRP contributed to the better healing of soft tissues and bone and is a means of growth factor delivery.

Eriko Marakawa et al [63](2011) Studied on the reduction of bone resorption by the application of platelet rich plasma in bone grafting of alveolar cleft. Satisfactory bone bridging formation was seen in PRP treated patients than the control group. The added PRP reduced the resorption of regenerated bone post operatively. The PRP preserves the width and height of the graft without resorption. The addition of PRP to autogenous cancellous bone grafts appears to significantly reduce post operative bone resorption after one year.

M Del Fabro et al[64] (2011) Assess the healing potential of PRP in the healing of extraction sockets. Favourable effects on hard and soft tissue healing was noted and post operative discomfort reduction was seen. Reduces post operative pain and inflammation. A favourable effect on soft tissue healing and post operative discomfort was noted. There has also been a reduction in postoperative pain .

MD Batstone et al[65] (2011) Studied on the effect of PRP inprevention of osteoradionecrosis. PRP failed to show an effect on osteoradionecrosis. There was no effect on mucosal healing and pain reduction.

Olga Garcia-Martinez et al [66] (2012) This study aimed the effect of PRP on the cell cycle, antigenic profile, and proliferation of primary cultured human osteoblasts. The treatment of osteoblasts with PRP modified the expression of CD 54, CD80 , CD86 and HLA-DR antigens. PRP increased cell proliferation in the short term. This study showed that PRP induces neo bone formation without any change in cell cycle, thereby proving there is no malignant potential involved.

Ronaldo Célio-Mariano et al [67](2012) There was significantly faster bone formation in sockets treated with .Significant bone formation was observed in the first month ,second month and thirdmonth were noted for the PRP group than the non PRP group. The radiographic bone density was calculated using the HLIamge ++ software. No statistical differences were observed on the seventh day and sixth month of investigation, yet there were higher means of radiographic bone density in sockets treated withPRP. In the control group, men exhibited significant bone repair compared with women . Autologous PRP was found to accelerate alveolar bone regeneration, and men presented better repair after tooth extraction.

Materials and methods

Materials and methods:

This prospective, randomized double blinded control trial was conducted in Rajas Dental College and Hospitals, Tirunelveli dist, Tamil Nadu , between January and July 2012. This study protocol was approved by the Institutional Review Board and Ethical Committee. Informed consent was obtained from all patients.(Annexure I)

Inclusion criteria

1. Healthy non diabetic individuals between 18 and 34 years of age.
2. Patients having bilaterally impacted mandibular 3rd molars.
3. Absence of pericoronitis .
4. Absence of opposing traumatic occlusion or impinging upper 3rd molars
5. Patients without any systemic diseases.
6. Female patients not on oral contraceptives.

Exclusion criteria

1. Recent antibiotics or steroid use.
2. Systemic immunodeficiency , chemotherapy and radiation therapy.
3. Non localized odontogenic infection.
4. Surrounding tissue pathology or lesion.

5. Smokers/ alcoholics.

6.Associated medical conditions contraindicating dental extraction.

7.Diabetic history > 10 years (RBS > 180 mg/dL).

Materials

After obtaining the complete history (Annexure II), patients were examined clinically and were explained about the procedure, its complications and the follow-up period involved in the study. The patients who were willing were enrolled for the study and preoperative Intra Oral Periapical Radiographs and Orthopantomograms were taken. Informed consent was obtained from all patients.

Sample selection

The study sample consisted of 12 patients, 7 males and 5 females,(chart 1 and 2) who presented with bilaterally impacted mandibular molars and in similar positions. In the 24 impacted teeth, 17 were mesioangular,3 distoangular and 4 vertical , according to Pell and Gregory classification (Chart 3).

Study groups

All patients underwent bilateral surgical removal of impacted 3rd molars .The two operated sides in each patient were randomly divided into two study groups: test and control.

Extraction of the impacted mandibular third molar at one side whose socket was simply sutured was called the control side, while the opposite impacted third molar socket ,which was filled with PRP gel and sutured was called the test side. PRP , prepared prior to start of the procedure was activated to form PRP gel .The sockets where PRP is to be placed were randomly selected. Patients were recalled on first, second day,third day, first week, fourth week and 2 months postoperatively, for follow up study.

Preparation of PRP gel

The PRP was not prepared in this study using the centrifuge. The gel was prepared from the method introduced by Thrombodyne Inc, Salt Lake City , Utah, United States. The method was described as Concentrated Autologous Platelet System (CAPS)[68]. This yielded a solution of concentrated platelets in an isotonic medium without plasma.[FIGURE I A]

The preparation system is available as a single use kit with the following contents. A vacutainer blood collection kit (10 mL) with a Citrate Phosphate Dextrose Anticoagulant (CPDA)[FIGURE I B] ,a mixing syringe kit with a 10 mL mixing syringe and a syringe to syringe coupler, a filter assembly kit with a 3 way stop cock and a Barb Leur connector, a rinsing kit with a rinsing syringe, Calcium Chloride ,Sodium Chloride vial and an amorphous Hydrogel.[FIGURE I C]

Using a tourniquet 10 mL blood is drawn from the antecubital region with the vacutainer kit with the adaptor connected.[FIGURE I D]The contents in the vacutainer are thoroughly mixed and transferred into the mixing syringe using the syringe to syringe coupler. Now the contents are again mixed inside the mixing syring with the metal mixer inside it. Approximately 60 inversions of the syringe must be done. This is done to activate the platelets.[FIGURE I E]

Transfer the entire contents in the mixing syringe to the filtering system. [FIGURE I F] Connect to the 3 way stop cock and the collection kit.Transfer the contents to the collection kit (collection bag in the filter assembly).Now the calcium chloride is mixed with sterile water (10% calcium chloride) and is pushed through the stop cock to the filter assembly. Sodium chloride is also added to make the solution isotonic.[FIGURE I G]Now take the recovery kit and the recovery syringe and connect to the 3 way stop cock. Draw the contents from the filter where the platelets have accumulated. 2 mL of platelet solution is collected.[FIGURE I H]This is mixed with the amorphous hydrogel to make it into a gel form.[FIGURE II]

Surgical technique[FIGURE III- ARMAMENTARIUM]

All the cases were done under conscious sedation with intravenous Midazolam upto 5 mg with monitoring of blood pressure and oxygen saturation using a multiparameter. Inferior alveolar nerve block, lingual nerve block and long buccal nerveblock were administered using 2% lignocaine hydrochloride with 1: 2,00,000 adrenaline, standard Ward's incision was followed in all the cases. Full thickness mucoperiosteal flap was raised to expose sufficient bone on lateral and distal

aspect of the impacted molar. Removal of bone was done with 702 carbide bur.

Constant irrigation was done with normal saline while removing bone to prevent thermal necrosis. Surgical removal of impacted tooth was done. 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. Surgical removal of impacted mandibular 3rd molar was done on the opposite side in the similar way and one of the extraction socket was randomly chosen by the operator for PRP placement.

PRP placement [FIGURE IV]

The pre processed PRP was taken into the sterile stainless steel bowl and 0.5ml of CaCl2 was mixed to obtain the PRP gel, which was placed into the selected extraction socket and primary closure of the wound was done . No graft material was added to PRP in this study. Wound was closed with a 3-0 black braided silk interrupted suture. [FIGURE V,VI.VI A &B AND VII]

Pressure packs were given and the normal post extraction instructions were given. The patients were adviced amoxicillin 500 mg thrice daily and diclofenac 50 mg thrice daily for three days. All the patients were given dexamethasone 2mg intravenously after the procedure. All were adviced chlorhexidine mouthwash four times a day for one week.

First, third and seventh post operative day

Evaluation for pain , soft tissue healing alveolar osteitis.

Post operative IOPA and OPG was taken on the seventh day.[FIGURE VIII A]

Sutures were removed on the seventh day.

4 weeks post operatively

Evaluated for pain and soft tissue healing

IOPA and OPG taken

8 weeks post operatively

Evaluated for pain and soft tissue healing

IOPA and OPG taken.[FIGURE VIII B]

Clinical evaluation

Clinical evaluation included assessment of pain, swelling, soft tissue healing , bone density and alveolar osteitis.

Pain

Describes the evolution of self-reported pain measured in a visual analogue scale (VAS) in the first 7 days after extraction.. A 10-point visual analog scale (VAS) [69] with a score of 0 indicating “no pain” and 10 indicating “very severe pain” was used to assess pain on post operative days 1 , 3 and 7.

Visual analogue scale (VAS) – intraoperative pain intensity (Annexure IV)

The scoring criteria is as described below :

0 = absolutely no pain

1= very mild pain

2-4 = mild pain

5-7= moderate pain

8-9 = severe pain

10= unbearable pain

Swelling [70]

Swelling was assessed using 5 fixed points on surgical side of the face and finding the average. The fixed points used were; the most posterior point at the midline on the tragus ,lateral canthus of the eye, the most lateral point on the corner of the mouth, soft tissue pogonion which is the most prominent point at the midline on the chin and most inferior point on the angle of the mandible. Three lines are drawn joining the points .[A,B andC] The sum of the measures of the 3 lines are found out . A baseline measurement was carried out just before the surgery and similar measurements were carried out on second and seventh day post operatively. The difference between the postoperative and preoperative measurements was calculated. (swelling post op – swelling pre op). This method was described by Yakup Ustun et al. [FIGURE IX.A AND IX. B]

Soft tissue healing[71]

Evaluation of soft tissue healing by Landry , Turnbull and Howley.

Healing index 1 :very poor and has 2 or more of the following

Tissue colour > 50 % of the gingival red

Response to palpation – bleeding

Granulation tissue -present

Incision margin- not epithelialised with loss of epithelium beyond the incision

Margin

Healing index 2 : poor

Tissue colour > 50 % of gingiva red

Response to palpation – bleeding

Granulation tissue present

Incision margin – not epitheliailsed with exposure of connective tissue

Healing index 3 : good

Tissue colour > 25% < 50 % gingival red

Response to palpation –no bleeding

Granulation tissue none

Incision margin – no connective tissue exposed

Healing index 4 : very good

Tissue colour > 25% gingival red

Response to palpation –no bleeding

Granulation tissue –none

Incision margin – no connective tissue exposed

Healing index 5 : excellent

Tissue colour – all tissue pink

Response to palpation –no bleeding

Granulation tissue –none

Incision margin – no connective tissue exposed

Radiographic evaluation for bone density

Orthopantomograms were taken preoperatively,at the first and eighth week respectively to assess and compare radiographic bone densities between PRP and non PRP sites. The radiographic bone densities were evaluated by Adobe Photoshop Software. The bone densities could be measured at the alveolar crest levels, furcation level and the apical region of the surgically removed mandibular third molars. In the present study the apical region of the removed molars were taken to measure the bone densities.

Orthopantomogramic images were digitalized using the Sidexis software. The image was transferred to Adobe Photoshop. The grey scale values were measured. The grey scale values were compared at the PRP and non PRP sides in the same OPG. The values were noted. [FIGURE X A &B]

Assessment for alveolar osteitis

Assessment was done on the third day postoperatively. Patient was asked if they experienced increasingly severe pain in and around the extraction socket. The socket is checked for necrotic odour or grayish discolouration. The pain experienced during alveolar osteitis or dry socket is refractory to the post operative analgesics .

All the values and scores obtained for the parameters described above were recorded in the patient data form (Annexure III). This was done by an investigator who did not know the test and the control side. The values and scores thus obtained were tabulated [pain assessment – table I, soft tissue healing score – table II, gray value scales at the end of first week and second month respectively – table III a and III b , swelling assessment – table IV and assessment of alveolar osteits – table V).

ARMAMENTARIUM

FIGURE I A . Platelet Concentration Kit

FIGURE I B. Blood collection kit (vacutainer)

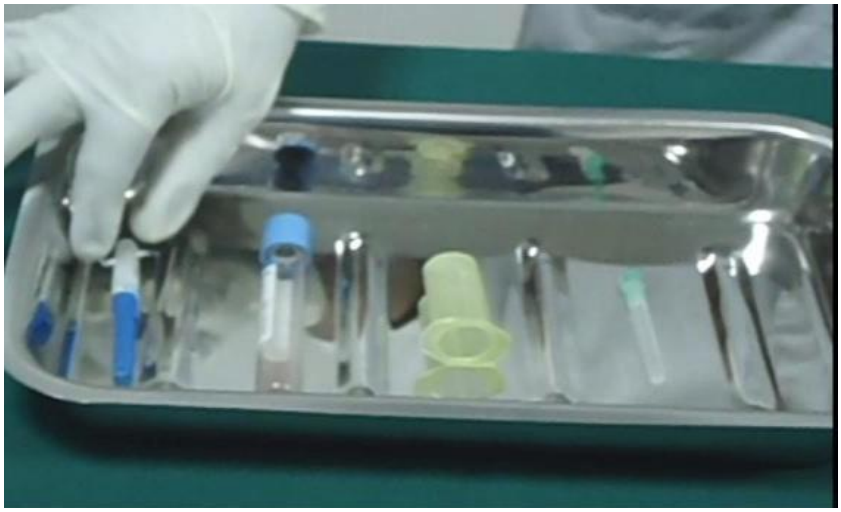


FIGURE I C . Rinsing kit

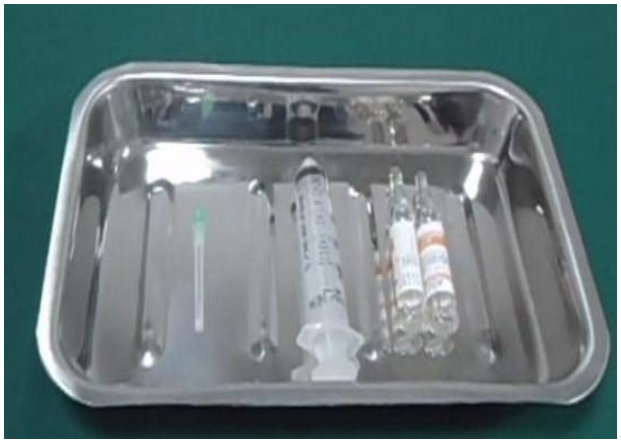


FIGURE I D. blood drawn from patient mixed

FIGURE I E. Blood and Anticoagulant

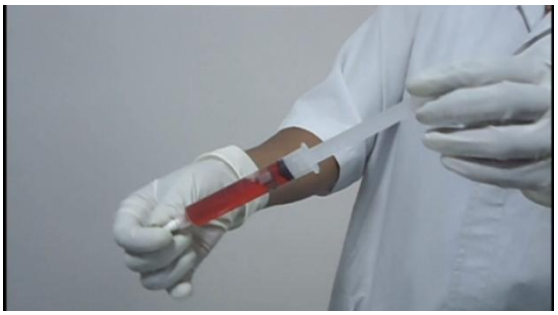


FIGURE I F. Filter assembly

FIGURE I G. addition of sodium chloride

FIGURE I H. Filtering the platelet concentrates

FIGURE II Final platelet concentrates

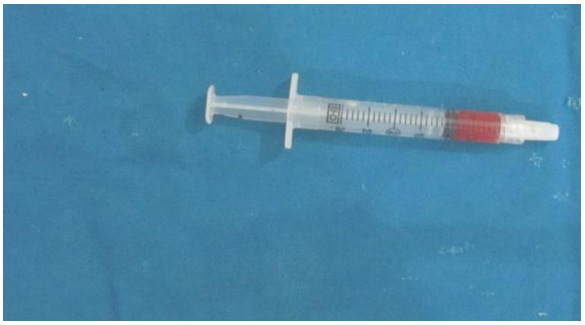


FIGURE III. Armamentarium for impaction surgery

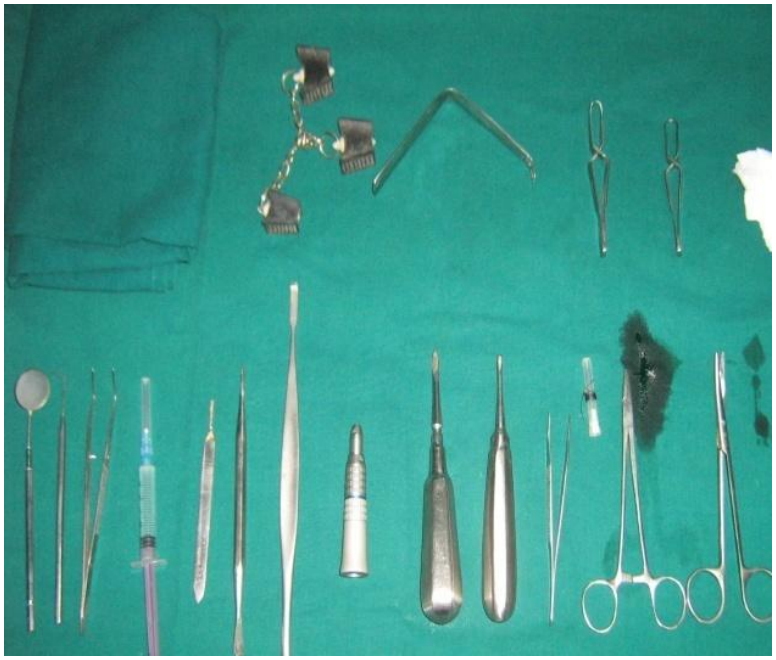


FIGURE IV.Platelet concentrates mixed with hydrogel

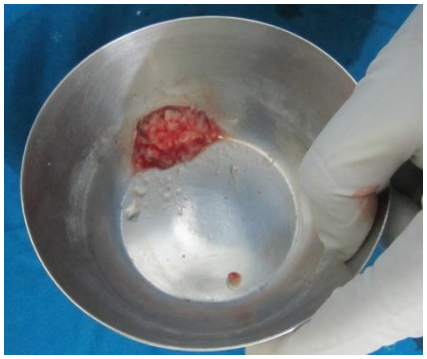
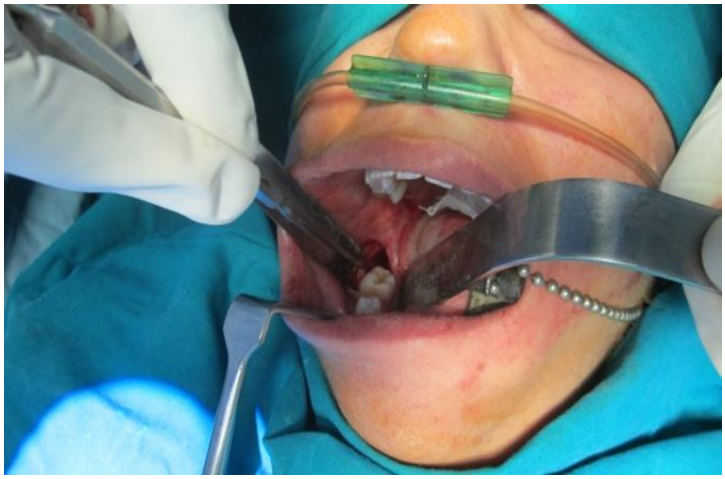
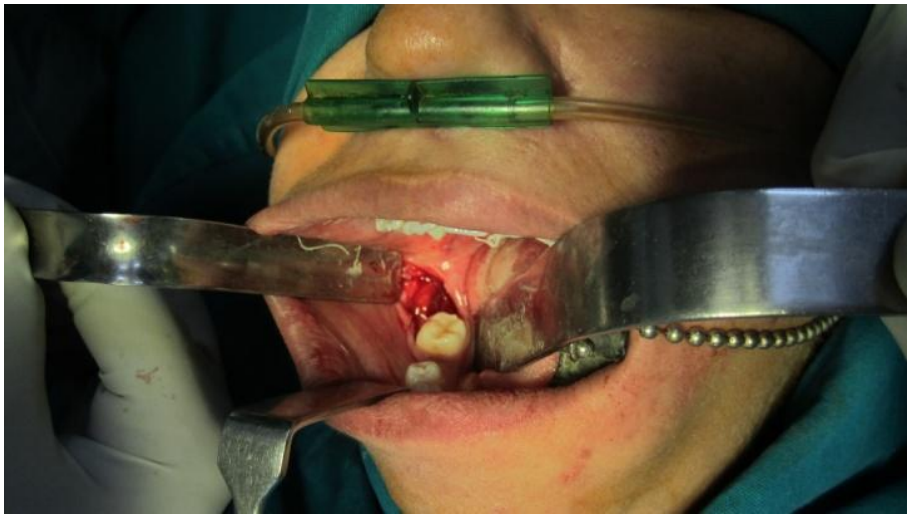


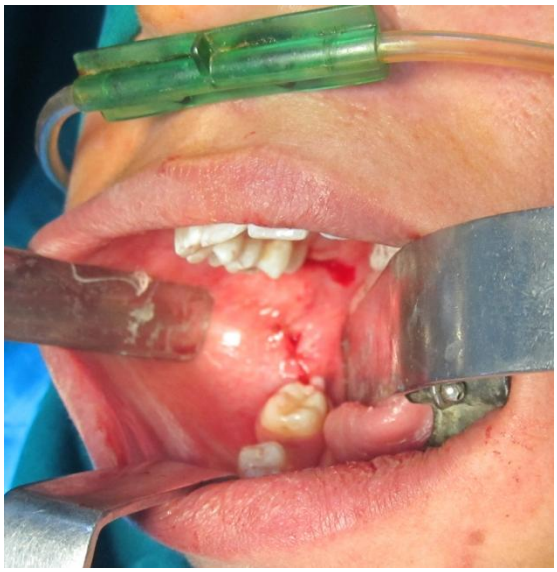
FIGURE V. Extracted socket



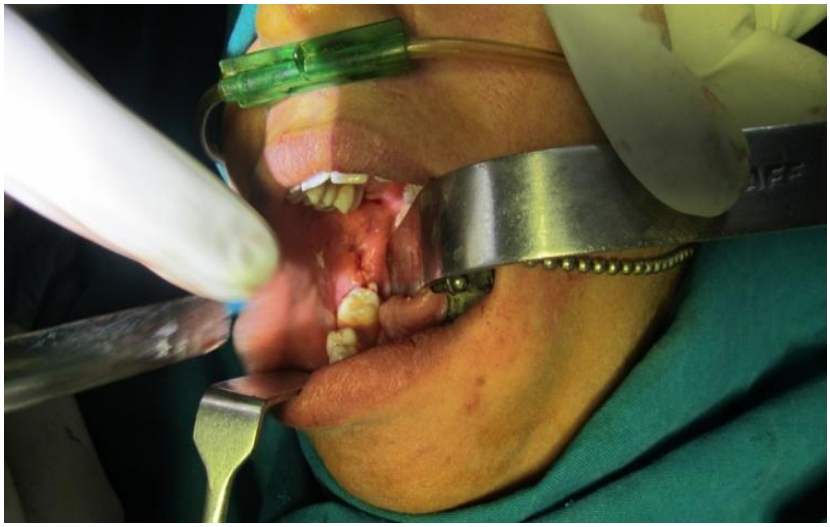
VI.A Placement of the platelet concentrates into the socket



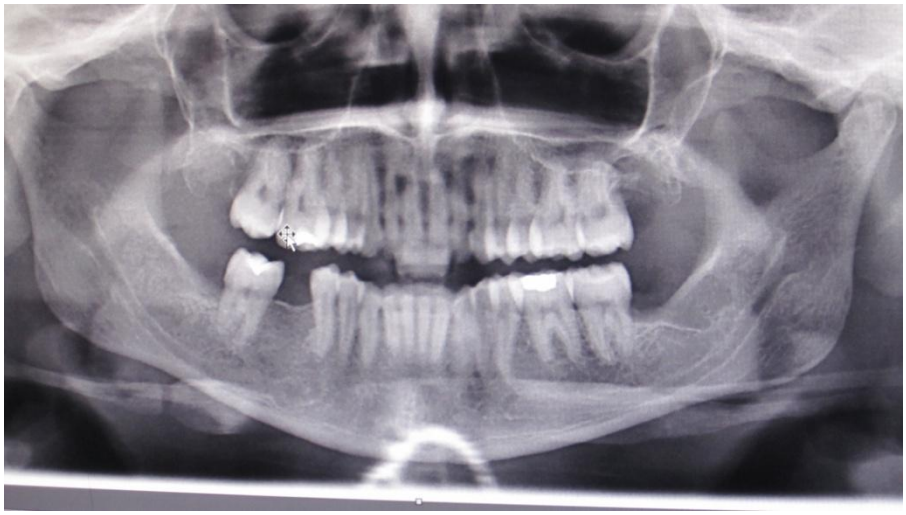
VI B. Socket irrigated



VII. Socket primarily sutured



VIII A.OPG taken in the first week



PRP was placed on the right side. The difference in gray values are noted

VIII B. OPG taken after 2 months

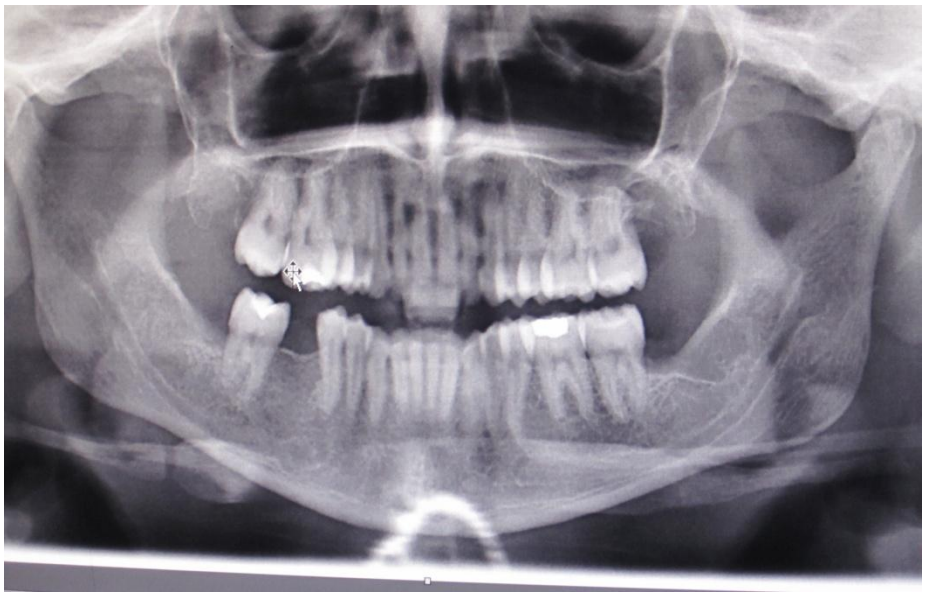


FIGURE IX A. Assessment of swelling (PRP side was on the left while the non PRP side was the right)

Assessment done on the third day

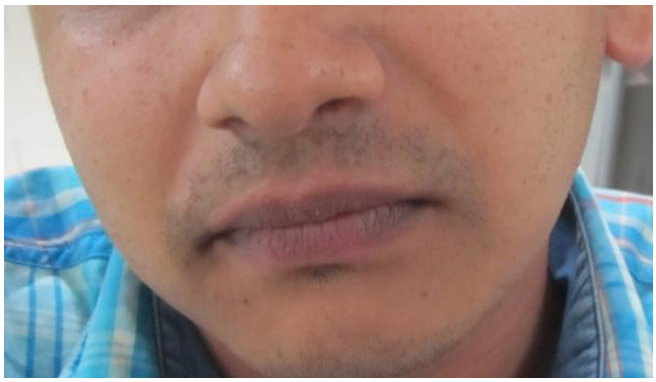
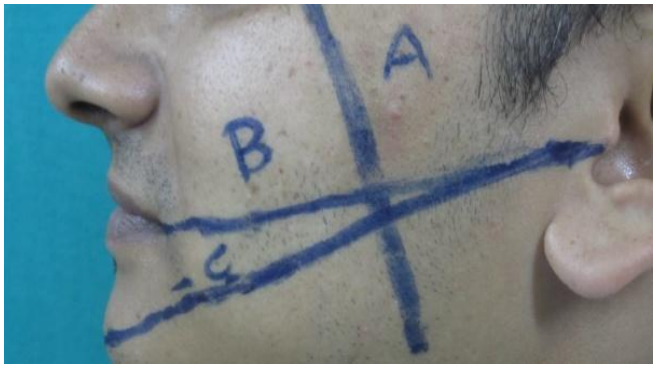


FIGURE IX B. Yakup Ustuns swelling assessment



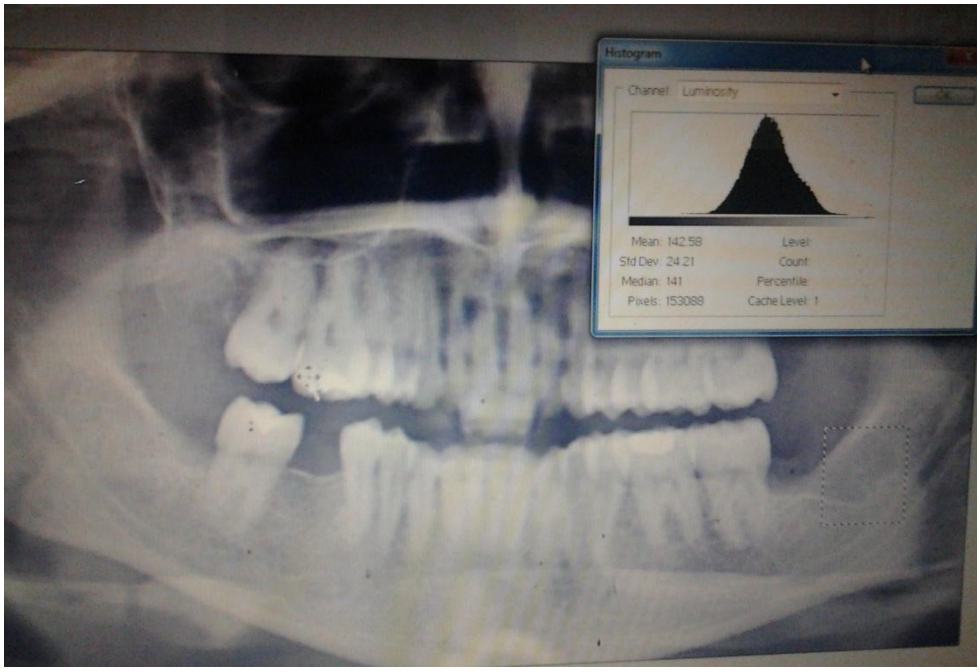


FIGURE X A. gray scale value at the left impacted tooth socket.

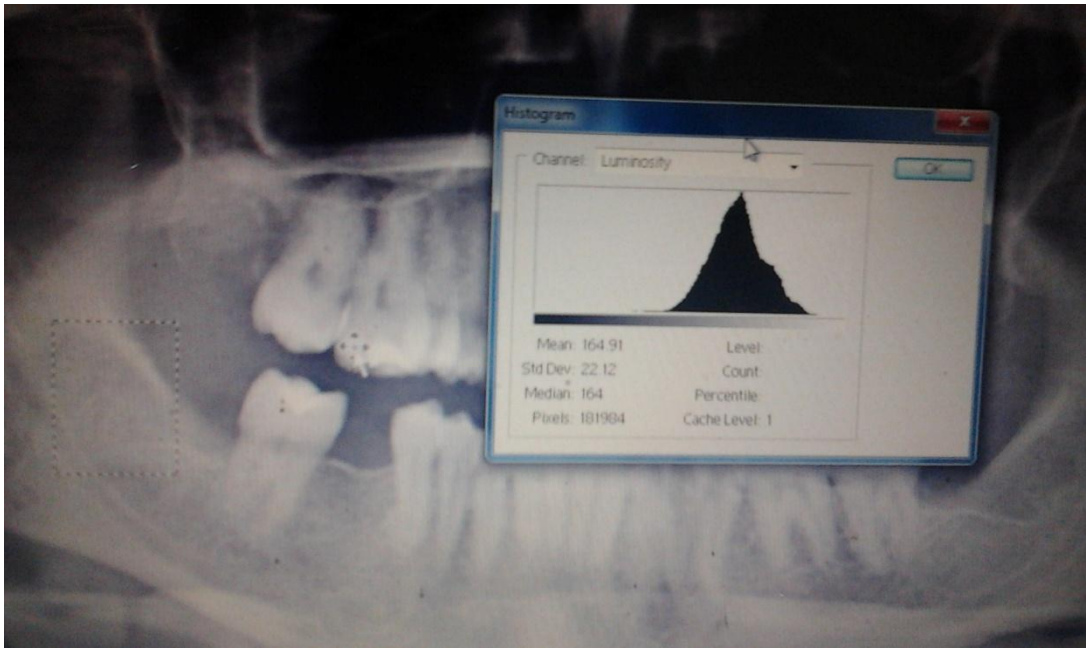


FIGURE X B. Gray scale value at the right impacted tooth socket.

Results

No patient was lost to follow-up. Pain, edema, and limited mouth opening were well controlled in the postoperative period with the drug protocol established. There were spontaneous reports of patients who noticed mild alterations in facial volume at one side, which corresponded to the side of the PRP group. There was no excessive bleeding at any side.

The study consisted of totally 12 patients who reported to Rajas Dental College and Hospital . Following completion of the clinical study ,measurements and data were taken from patients on their post operative follow up. This was a randomized double blinded control trial study. The results and outcomes were checked by another investigator to avoid the bias.

After analysis of the data , the following observations were made. There were 7 male patients and 5 female patients. They were in the age of 18 – 35 years and the mean age was 25.16

Results of clinical assessment

Assessment of pain

Assessment of pain by visual analogue scale on first post operative day showed mean pain score of 2.91 at the test site and 4.16 at the control site (P value – 0.002). On the third day the mean score on the test site was 2.33 while that on the control site was 2.91 (P value 0.091). On the seventh post operative day the mean on the test site was 0.58 while on the control side was 0.91.(P value 0.293).
It was seen that the values obtained for PRP side was statistically significant than non PRP sides in respect to pain. Thus pain was considerably less for the test group than the control post operatively.

Assessment of soft tissue healing

The soft tissue healing assessment was based on the criteria set by Landry and Turnbull. The mean value of the healing assessment was done in the first post operative day and was found to be 3.0 in the control side and 3.08 in the test side 9(P value 0.77). On the third post operative day the mean was 3.91 for the control side and 3.41 for the test side (P value 0.330). One the first week post operatively it was seen that the mean for the test side was 4.75 while that for the control side was 4.6.(P value 0.397). Though the test group exhibited better soft tissue healing , the values obtained showed it was statistically insignificant. This was probably due to the small sample size.

Assessment of gray value scale

This was done using the Adobe Photoshop software. The gray value for this study was done in the apical region of the surgically removed third molars. The mean value obtained for the gray value scale for the control side was 140.91 and for the test side was 128.63 (P value 0.054). The mean value of the gray scale obtained at the end of the first month was 145.58 for the control side and 133.58 for the test side (P value 0.029). The values obtained for the bone density for the control and the test side showed that PRP created a statistically significant difference .

Assessment of swelling

Assessment of swelling as done by Yakup Ustin was done on the second and seventh post operative day. The mean of the swelling values on the second post operative day was 6.33 on the control side and 6.39 on the test side (P value 0.82). On the seventh post operative day it was seen that the mean value on the test side was 3.1 while on the control was 3.2 (P value 0.71). This was statistically significant. Thus PRP did not have any statistical significance on swelling on test side.

Assessment of alveolar osteitis

Examination for dry socket was done on the third day postoperatively. None of the sockets presented with dry socket. Neither the control nor the test side showed dry socket. (Table V). This showed that as per the study, PRP did not have any effect on dry socket. This may be due the limited sample size. But other studies have shown the efficiency of platelet concentrates in the reduction of dry socket [39].

Pain assessment (Table 1)

Days	First day		Third day		Seventh day	
Sl no	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**
1	3	4	2	2	0	2
2	3	4	2	4	1	1
3	4	3	3	4	0	0
4	3	5	2	4	1	1
5	2	5	2	3	0	0
6	2	6	1	4	0	2
7	3	4	2	3	1	2
8	4	3	4	2	1	0
9	3	3	3	2	1	1
10	3	5	2	4	1	1
11	3	4	2	2	0	0
12	2	4	3	2	1	1

Soft tissue healing (Table II)

Sl no	First day		Third day		Seventh day	
	Test	Control	Test	Control	Test	Control
1	3	3	4	4	5	4
2	3	3	4	4	5	5
3	3	3	4	4	5	5
4	4	4	4	4	5	5
5	3	3	4	4	5	4
6	3	3	4	4	5	5
7	3	3	4	4	5	5
8	2	2	3	4	4	4
9	4	3	4	3	4	4
10	4	4	5	3	5	5
11	2	4	2	3	5	5
12	2	2	5	4	4	4

(Table III a)

Gray value scale (at one week)		
Sl no	Test	Control
1	164	141
2	159	132
3	118	98
4	126	120
5	151	143
6	116	130
7	123	116
8	138	112
9	141	130
10	150	141
11	159	141
12	143	132

(Table III b)

Gray value scale (at 2 months)		
Sl no	**Test**	**Control**
1	166	147
2	161	138
3	133	108
4	128	124
5	159	147
6	126	133
7	133	129
8	139	131
9	144	131
10	151	139
11	162	143
12	145	133

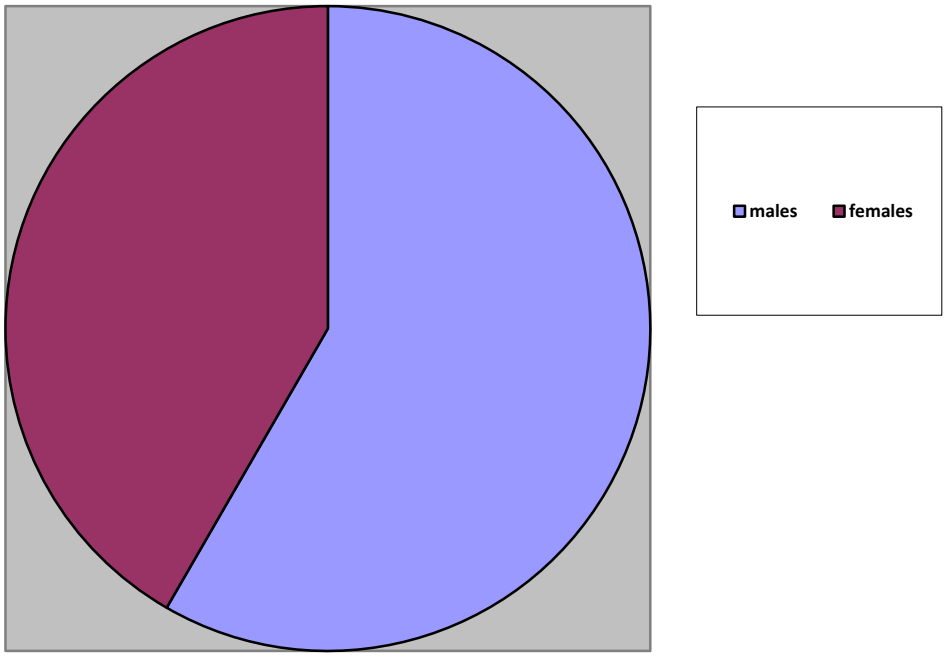
Swelling assessment (Table IV)

Days	Second day		Seventh day	
Sl no	**Test (mm)**	**Control(mm)**	**Test (mm)**	**Control(mm)**
1	7.9	7.9	3.0	3.0
2	6.8	6.5	3.0	3.2
3	5.7	5.9	2.7	3.0
4	6.2	6.5	4.2	3.3
5	6.8	6.9	3.4	3.2
6	5.5	5.4	3.3	3.7
7	5.6	5.2	3.2	3.2
8	5.4	5.9	2.9	2.9
9	7.9	8.0	3.3	3.3
10	7.5	6.6	4.0	3.7
11	6.7	8.0	3.0	2.9
12	7.6	7.9	2.9	2.8

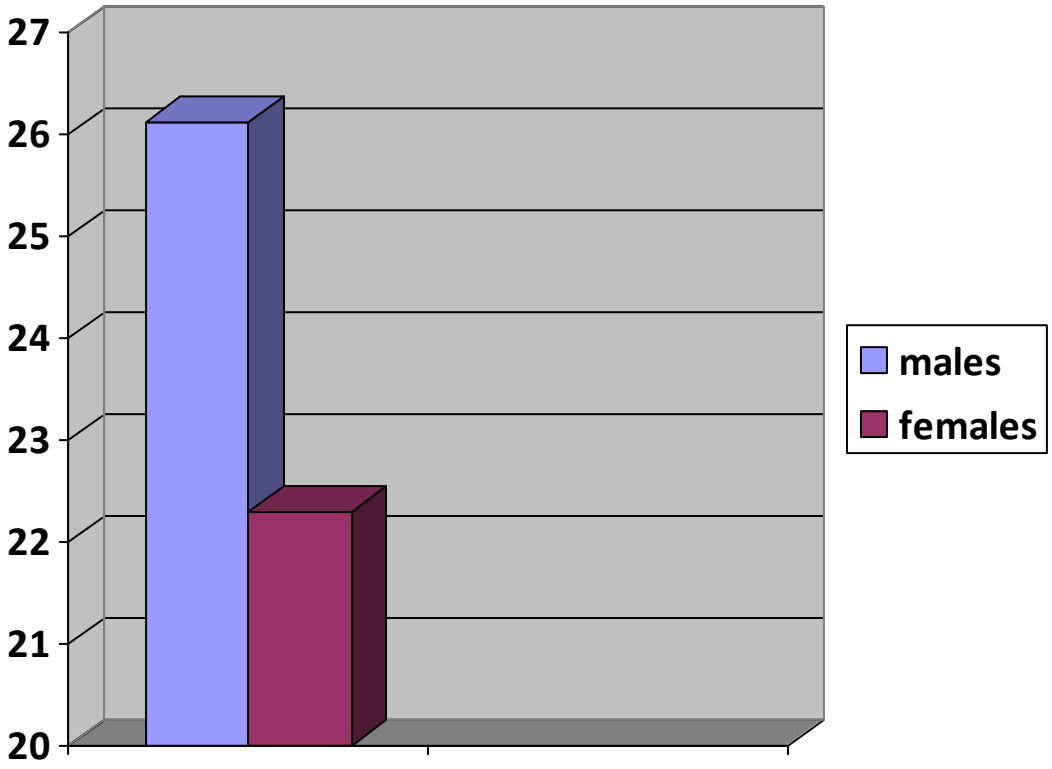
Assessment of alveolar osteitis (Table V)

Sl no	Test	Control
1	Nil	Nil
2	Nil	Nil
3	Nil	Nil
4	Nil	Nil
5	Nil	Nil
6	Nil	Nil
7	Nil	Nil
8	Nil	Nil
9	Nil	Nil
10	Nil	Nil
11	Nil	Nil
12	Nil	Nil

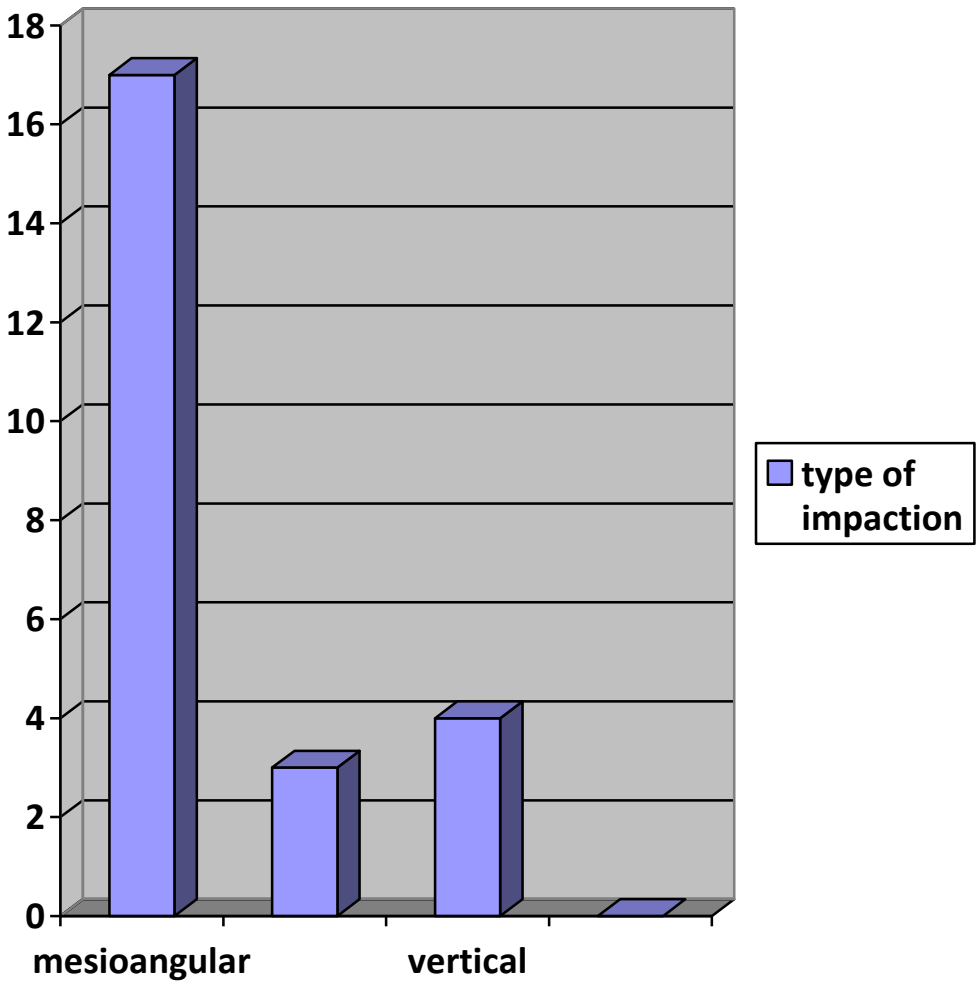
Gender distribution (chart 1)



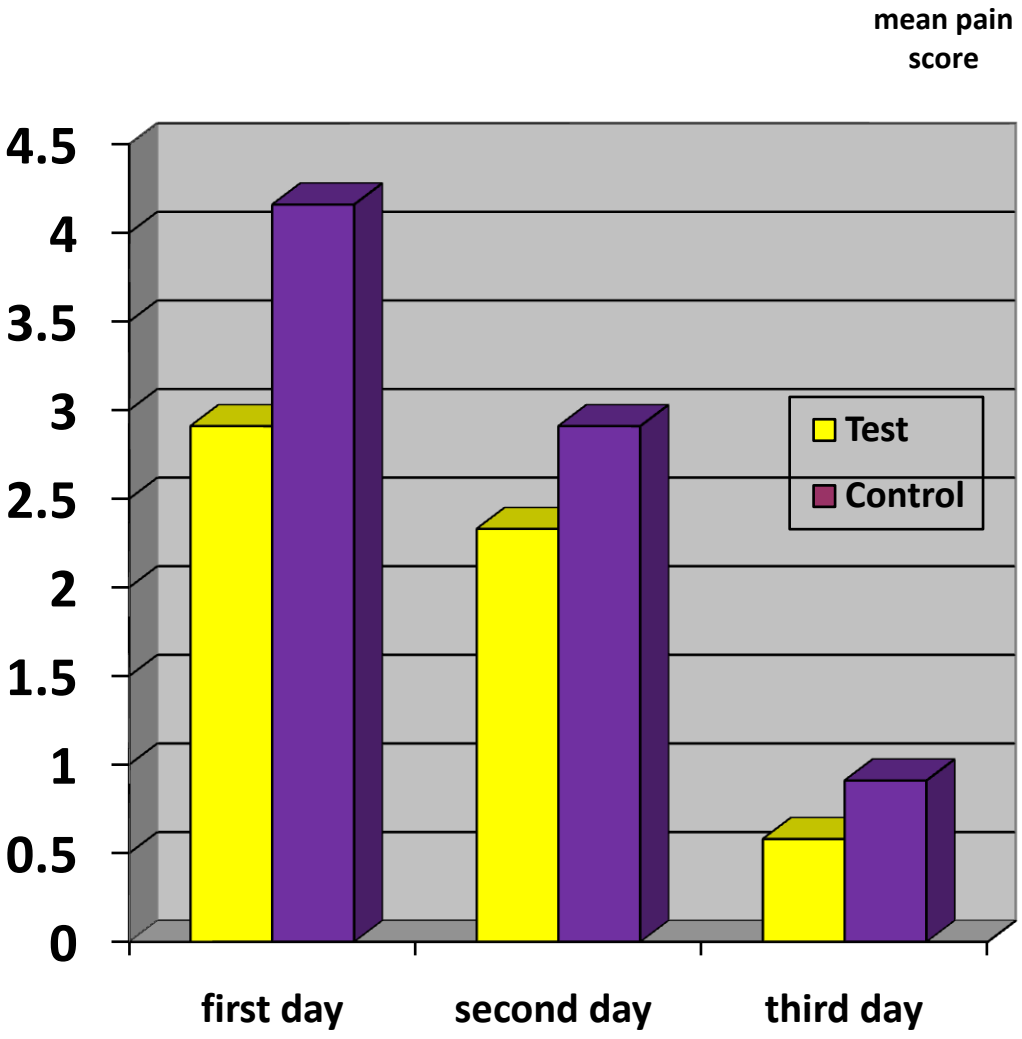
Age distribution (Chart 2)



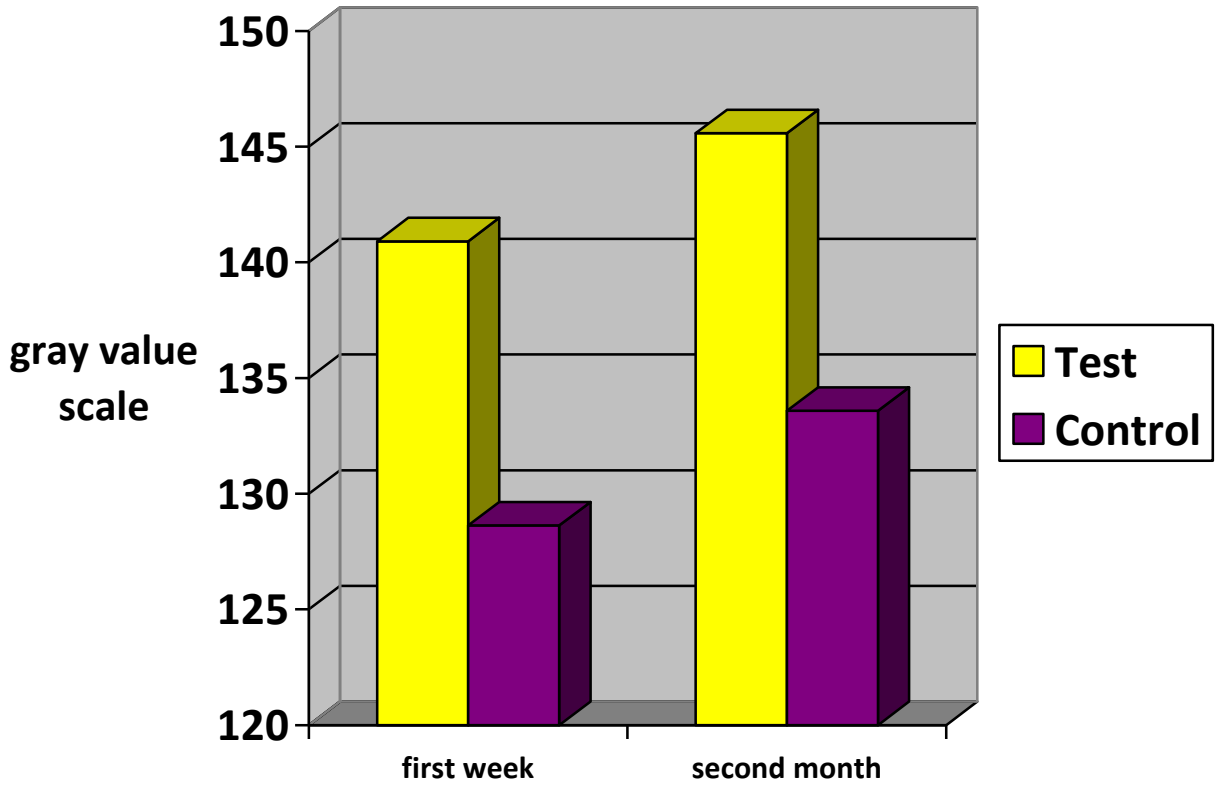
Distribution of impaction (chart 3)



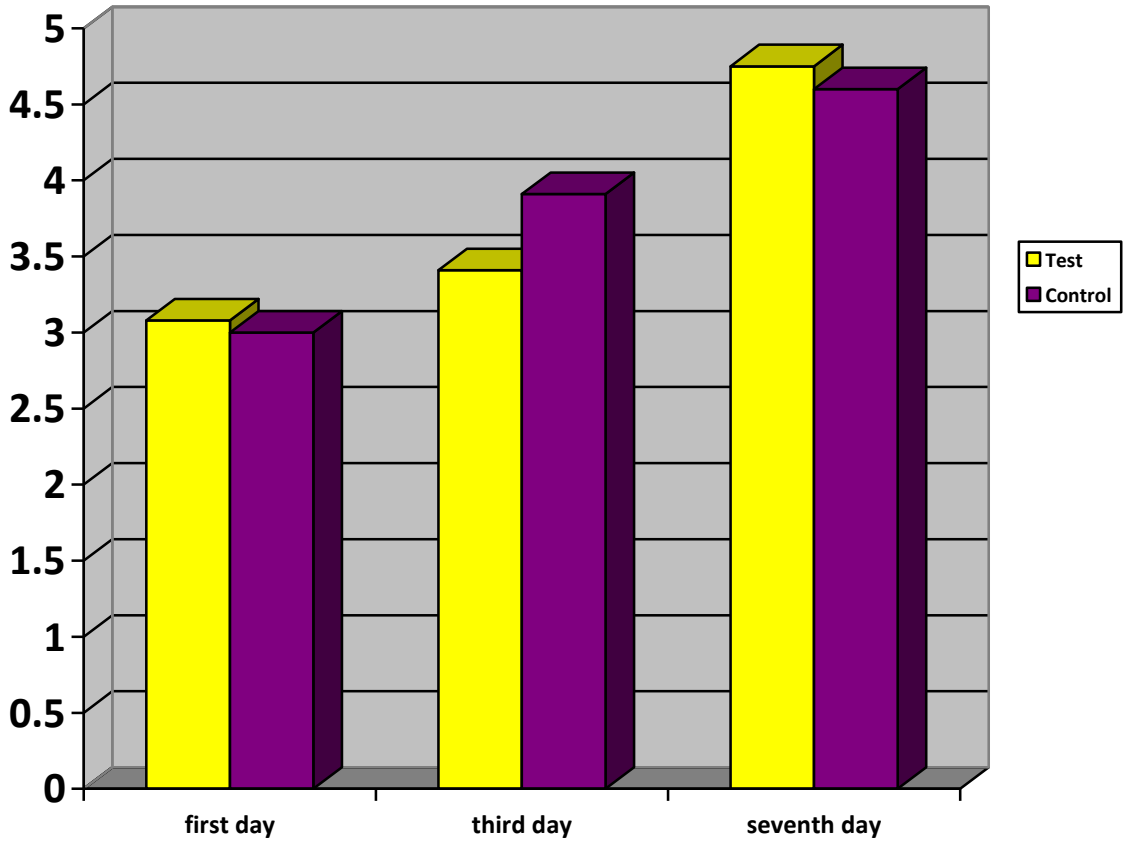
Assessment of pain using visual analogue scale (Chart 4)



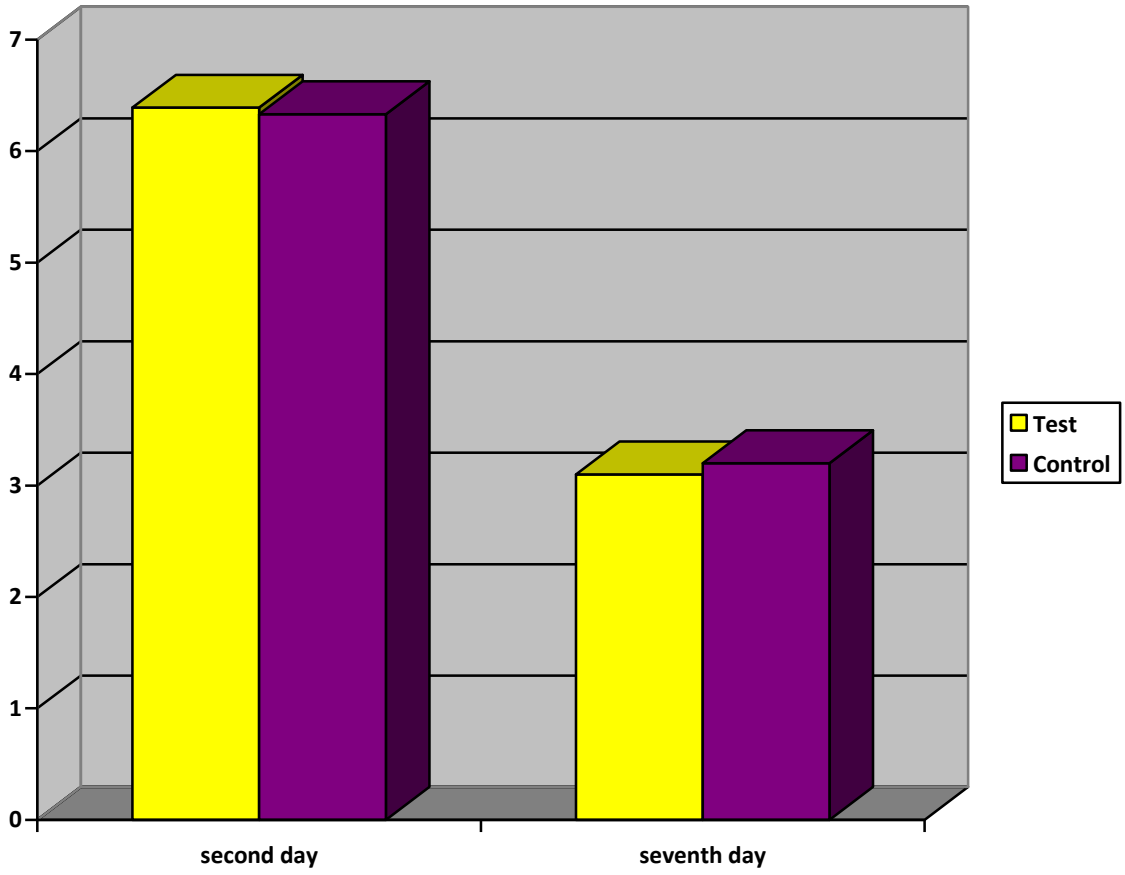
Assessment of bone density using gray value scale (chart 5)



Assessment of soft tissue healing by Landry and Turnbull (Chart 6)



Assessment of swelling (chart 7) by Yakub Ustin



Statistics

Pain assessment

Mann Whitney U test

Test statistics

Test statistics	Pain score (day 1)	Pain score (day 3)	Pain score (day 7)
Mann Whitney U	21.500	45.000	55.500
Asymp. Significance (2 tailed)	0.002	0.091	0.293

P value at day 1 = 0.002, at day 3 = 0.091, at day 7 = 0.293

Non parametric tests

Descriptive statistics

	N	**mean**	**Std. deviation**	**mininum**	**maximum**
Day 1	24	3.5417	1.0206	2.00	6.00
Day 3	24	2.6667	0.916	1.00	4.00
Day 7	24	0.7500	0.675	0	2.00
category	24	0.50	0.511	0	1

Ranks

Category y	N	Mean rank	Sum of ranks
Day 1 0	12	16.71	200.50
1	12	8.29	99.50
total	24		
Day 3 0	12	14.75	177.00
1	12	10.25	123.00
total	24		
Day 7 0	12	13.88	166.50
1	12	11.12	133.50
total	24		

Test statistics [b]

	Day 1	day 2	Day 3
Mann-Whitney U	21.500	45.000	55.500
Wilcoxon W	99.500	123.000	133.500
Z	-3.073	-1.689	-1.051
Asymp. Significance (2 tailed)	0.002	.091	0.293
Exact significance [2*(1 tailed significance)]	0.002[a]	0.128[a]	.347[a]

a -not corrected for ties

b- Grouping variable: category

Soft tissue healing assessment

Mann Whitney U Test

Test statistics

Test statistics	first day	Third day	Seventh day
Mann Whitney U	67.500	58.500	60.000
Asymp . Significance (2 tailed)	0.774	0.330	0.397

Non parametric tests

Descriptive statistics

	N	Mean	Std. Deviation	minimum	maximum
Day 1	24	3.0417	0.69025	2.00	4.00
Day 3	24	3.8333	0.63702	2.00	5.00
Day 7	24	4.6667	0.48154	4.00	5.00
category	24	0.50	0.511	0	1

Ranks

Category y		N	Mean Rank	Sum of ranks
Day 1	**0**	12	12.88	154.50
	1	12	12.12	145.50
	total	24		
Day 3	**0**	12	11.38	136.50
	1	12	13.62	163.50
	total	24		
Day 7	**0**	12	11.50	138.00
	1	12	13.50	162.00
	total	24		

Test statistics[b]

	Day 1	Day 3	Day 7
Mann Whitney	67.500	58.500	60.000
Wilcoxon W	145.500	136.500	138.000
Z	-o.287	-0.974	-0.848
Asymp significance (2 tailed)	0.774	0.330	0.397
Exact significance [2*(1 tailed significance)]	0.799[a]	0.443[a]	0.514[a]

P value =0.774 at day 1, 0.330 at day 3 and 0.397 on day 7

a- Not corrected for ties
b- Grouping variable : category

Assessment of grey scale value

T- test

test statistics

	t	difference	Significance
Grey value at first week	2.032	22	0.054
Grey value at second month	2.341	22	0.029

Swelling assessment

t – test

test statistics

	t	difference	significance
Swelling (day 2)	-0.227	22	0.822
Swelling (day 7)	0.377	22	0.710

Group statistics for grey scale and swelling

T Test

Category y		N	Mean	Std .Deviation	Std error mean
Grey1	**0**	12	1.4067E2	16.49426	4.76148
	1	12	1.2800E2	13.93491	4.02266
Grey 2	**0**	12	1.4558E2	14.10641	4.07219
	1	12	1.3358E2	10.78263	3.11268
Swelling 2	**0**	12	6.6333	0.94804	0.27368
	1	12	6.7250	1.02702	0.29648
Swelling 7	**0**	12	3.2417	0.45017	0.12995
	1	12	3.1833	0.29181	0.08424

P value for soft tissue healing on second day is 0.822 and on the seventh day is 0.710

P value at first week for bone density is 0.05 and at second month is 0.029

Discussion

Platelet rich plasma has been a breakthrough in the stimulation and acceleration of bone and soft tissue healing. It represents a relatively new biotechnology that is part of a growing interest in tissue engineering and cellular therapy today.

Platelet rich plasma gel functions as a tissue sealant and a drug delivery system that contains a host of powerful mitogenic and chemotactic growth factors. Haemostasis is achieved through the formation of a fibrin clot that is initiated by the activation and aggregation of platelets. Beyond maintaining haemostasis,the fibrin clot provides a matrix for the migration of tissue forming cells and endothelial cells involved in angiogenesis and the remodelling of the clot for tissue repair.Using PRP involves, taking sample of patients blood preoperatively , concentrating the autologous platelets and applying the resultant gel to the surgical site. Surgical sites which were treated with PRP has shown to heal 2- 4 times faster than normal sites. PRP is activated to form PRP gel causing degranulation of the α granules to release the growth factors.

PRP can be defined as a volume of autologous plasma that has a platelet concentration above the base line. Normal platelet count in blood ranges from 1,50,000 to 4,00,000 / µL. Since the scientific proof of bone and tissue healing has been shown using PRP with 10,00,000 platelets / µL it is this concentration of platelets in a 5 mL volume of plasma.

Platelet concentration ratio

The effect of platelet rich plasma on wound healing depends on what is likely a function of many variables, which includes the platelet concentration , volume of PRP delivered, extend and type of injury ,and the overall health status of the patient.

The working definition of PRP is 10,00,000/µL with lesser concentrations unable to be relied on to enhance wound healing and greater concentration not known to provide further enhancement. Several authors have stated that the aim is to prepare PRP with a platelet count in excess of 3,00,000 /µL . It was later concluded that the beneficial effects of platelet rich plasma would be well noted if the concentration of platelets ranged between 3- 5 times over the baseline.

Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cell, and are formed in the marrow.They are the smallest of the blood cells, round or oval in shape, and approximately 2 µm in diameter.The cell membrane is trilaminar with glycoprotein receptor surface overlying and partiallyinterspersed with a penetrating bilayer of phospholipids and cholesterol. They lack nuclei but contain organelles and structures such as mitochondria,microtubules, and granules (alpha, delta, and lambda).The α granules[33], bound by a unit membrane, are formed during megakaryocyte maturation. Platelets reside intravascularly, with high concentration in the spleen. The platelets remain in the circulation for an average of about 10 days before removal by macrophages of the reticuloendothelial system.

Activation causes the α granules to fuse to the platelet cell membrane (also called degranulation) where at least some of the secretory proteins (eg, PDGF and TGF-β) are transformed to a bioactive state by the addition of histones and carbohydrate side chains. The active proteins are then secreted, binding to transmembrane receptors of target cells (eg, mesenchymal stem cells, osteoblasts, fibroblasts,endothelial cells, and epidermal cells). These agonist bound transmembrane receptors activate an intracellular signal protein that causes the expression ofa gene sequence that directs cellular proliferation, matrix formation, osteoid production and collagen synthesis. The active secretion of these proteins by platelets begins within 10 minutes after clotting, with more than 95% of the presynthesized growth factors secreted within the first hour.

Since PRP is developed from autologous plasma, it is inherently safe and free from transmissible diseases like HIV and hepatitis . Within PRP the increased number of platelets delivers an increased number of growth factors to surgical area. The growth factors present are[5] :

PDGF αα- Platelet Derived Growth Factor.

PDGF ββ

PDGF αβ

TGF β1- Tissue Growth Factor

TGF β2

VEGF- Vascular Endothelial Growth Factor

EGF – Epidermal Growth Factor

CTGF – Connective Tissue Growth Factor

FBF – Fibroblast Growth Factor

KGF- Keratinocyte Growth Factor

G/MCSF- Granulocyte/Macrophage Colony Stimulating Factor

TNF α – Tumour Necrosis Factor

Thus PRP is a combination of 7 growth factors with the normal clot as carrier. The clot is composed of fibronectin, fibrin and vitronectin which are cell adhesion molecules required for cell migration as well as in osteoconduction ,wound epithelialization and osseointegration.

PRP acts on fibroblasts to increase their number (mitogenesis) and stimulate vascular ingrowth (angiogenesis).PRP is best developed from autogenous whole blood shortly or at the beginning of surgical procedure. Once developed , PRP is stable and remains sterile in the anticoagulated state for 8 hours.

Platelet concentrates may contain small amounts of leukocyte that synthesise interleukins , involved in the non specific immune reaction. In the recent years several regenerative procedures such as grafting the socket with autologous bone or bone substitutes along with platelet

concentrates have been developed. The growth factors are known to emerge from degranulating platelets at the time of injury. Its mechanism is to activate the cell membrane receptors in the target cells, which in turn are thought to develop high energy phosphate bonds on internal cytoplasmic signal proteins to initiate a specific activity within target cells.

The most important specific activities of the growth factors include mitogenesis, angiogenesis (endothelial mitosis into functioning capillaries) and macrophage activation .

Function of each growth factor

The transforming growth factor β ,when released by platelet degranulation acts as a paracrine growth factor (ie growth factor secreted by one cell exerting its effect on the adjacent second cell) affecting mainly fibroblasts, marrow stem cells and preosteoblasts. This growth factor presents a mechanism for long termhealing and bone regeneration molecule and even evolve into a bone remodeling factor over time. In addition TGF β inhibit osteoclast formation and bone resorption, thus favouring bone formation over resorption. The most important function of TGF β1 and β2 are chemotaxis , mitogenesis as osteoblast precursors and have the ability to stimulate osteoblast deposition of collagen matrix for healing of bone.

Platelet derived growth factor (PDGF) is chemotactic for polymorphonucleocytes, macrophages, fibroblasts and smooth muscle cells. PDGF also stimulates cell replication of important stem cells for fibroblasts and endothelialcells (increasing budding of new capillaries), stimulates production of fibronectin a cell adhesion molecule used in cellular proliferation and

migration during healing, including osteoconduction and hyaluronic acid and helps bring about woundcontraction and remodelling. Chondrocytes, osteoblasts and periosteal cell growth is up regulated by this factor. α and β isoforms are potent mitogens for fibroblasts, arterial smooth muscle cells, chondrocytes, and epithelial and endothelial cells. Potent chemoattractant for hematopoietic and mesenchymal cells, fibroblasts,and muscle cells, stimulates chemotaxis toward a gradient of PDGFActivates TGF-β, stimulates neutrophils and macrophages, mitogenesis offibroblasts and smooth muscle cells [5,7].

Insulin like growth factor functions as the key regulator of cell metabolism and growth , stimulates proliferation and differentiation of osteoblasts. Growth factor for normal fibroblasts, mitogenic in vitro for a number of mesodermal cell types.Promotes the synthesis of collagenase and prostaglandin E2 in fibroblasts. Stimulates collagen and matrix synthesis by bone cells, regulating the metabolism of joint cartilage.

Vascular endothelial growth factor (VEGF) regulates angiogenesis. Stimulates the proliferation of macrovascular endothelial cells.A strong angiogenic protein which induces eovascularisation. Induces the synthesis of metalloproteinase, which degrades interstitial collagen type I, II, and III.

Epidermal growth factor (EGF) regulates cell proliferation, differentiation and survival. Stimulates the proliferation of epidermal and epithelial cells, fibroblasts, and embryonic cells. Chemoattractant for fibroblasts and epithelial cells Stimulates re-epithelialization, augments angiogenesis. Influences the synthesis and turnover of extracellular matrix [5].

Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells and angiogenesis . A potent stimulator for the proliferation and differentiation of osteoblasts,stimulates the matrix mineralization.

Tumour necrosis factor is a growth factor for fibroblasts . Promotes angiogenesis

Granulocyte/ macrophage colony stimulating factor stimulates proliferation and differentiation of osteoblasts.Synergizes the proliferation of bone morphogenic progenitor cells. Strong chemoattractant for neutrophils.

Keratinicyte growth factor is the most potent growth factor for skin keratinocytes,playing a role in tissue repair following skin injuries. Promotes wound healing via proliferation, differentiation,angiogenesis, and cell migration.Mitogen for many epithelial cells but not for fibroblasts and endothelial cells

Conventional Processing technique

When anticoagulated blood is centrifuged , three layers become evident. Under all aseptic techniques, blood is drawn intravenously from the anticubital regionof patients forearm using vacutainer needle and vacutainers containing CitratePhosphate Dextrose Anticoagulant (CPDA ,0.8ml each). This anticoagulant is preferred because it preserves the platelet membrane integrity. Growth factors are extruded from the platelets by exocytosis. The protein molecule given off attains a tertiary structure. Fragmented platelets gives off more growth factors into the solution providing higher levels , but if the structure is altered, the effectiveness of these growth factor decreases.

The Vacutainers were thoroughly shaken to ensure maximum mixing of the anti coagulant with the drawn blood. The anticoagulant avoids platelet degranulation and activation. First centrifugation is called as soft spin which allows blood separation into three layers . The layers are bottom most layer (RBC 55% of total volume), top most acellular plasma (40% of total volume) and intermediate layer (5% of total volume) which is called as buffy coat layer. The platelet poor plasma, platelet rich plasma and some RBCs are transferred into another tube without anticoagulant.

This tube will now undergo second centrifugation which is longer and faster than the first. This is called as the hard spin. This allows the platelet to settle at the bottom of the tube with very little RBC, which explains the red tinge of the PRP preparation. The acellular PPP (80 % of the total volume) is found at the top. Most of this platelet poor plasma is removed with the syringe and is discarded and the remaining PRP is shaken well. The PRP is then mixed with bovine thrombin and calcium chloride at the time of application. This results in gelling of

the concentrate. Calcium chloride nullifies the effect of the anticoagulant . The thrombin helps in activating fibrinogen to fibrin and then cross linking .

This forms the basis of preparation of PRP with the yield approximately 10 % of the volume of the whole blood drawn. It is important that the procedure avoids fragmenting the platelets. Because it is this process of activation that results in completion of tertiary structures of some secretory proteins , fragmentation would result in release of high levels but with compromised activity.

Difference between the conventional technique and the technique used in the study

Centrifuge was not used in this method . So it was comparatively less time consuming and cost effective. This can be done as a chair side procedure, and blood handling , pipetting out the platelet poor plasma was avoided . There was also no caution with respect to the asepsis to be followed while centrifuging .The anticoagulant used in both the methods were same.

The end product while using this method yielded a concentrate of platelets , which are lysed , rich in growth factors in an isotonic medium. The plasma has been filtered off. So there was no need to activate the platelets using bovine thrombin. There has been several studies which proved the disadvantages of using bovine thrombin as an activator. Bovine thrombin factor Va may be a contaminant in certain bovine thrombin preparations. Antibodies to bovine thrombin Va may cross react with human factor Va which may lead to coagulopathies and rare bleeding episodes[3].

Only calcium chloride was used here to minimize the effect of the anticoagulant. Sodium chloride was used here to keep the platelet concentrates in an isotonic medium. Thus the end product was activated platelets with the growth factors in an isotonic medium. So this method has been found to be much cost effective, less time consuming , without any use of chemicals for activation and very simple to use.

In various oral surgical procedures, it was highlighted that the advantages of the platelet concentrates on early soft tissue healing might effectively reduce post operative inflammation and pain. In addition there was an increase in epithelialization, and decrease in the rate of alveolar osteitis. PRP also has an important part in the reduction of post operative pain and inflammation.

Uneventful and enhanced wound healing is desirable and critical in ascertaining quality of life after third molar surgery. This study examined the effects of PRP gel on post operative pain and swelling as well as soft tissue healing and bone regeneration potential in third molar extraction sockets.

Some investigators have reported significant improvements in tissue healing and bone formation using PRP, whereas others did not observe improvement. Such discrepancies are probably related to the lack of suitable standardization of definition of the different PRP preparations. The protocols and biological techniques used in the elaboration and administration of PRP differ widely.

Variations in some key properties of PRP, including platelet concentration, type of clot activator, leukocyte content, and the time that the fibrin scaffold is put into place around the tissue after clotting has started, can influence the different biological effects markedly.

Influence of PRP on bone density at the extraction site

Orthopantomograms revealed that the effects of PRP were significantly beneficial for increasing bone density following surgery. The increase in bone density suggests a greater volume of new bone formation with PRP treatment. Moreover, the increase in bone density (presumed as increased volume of new bone formation) was found to occur at earlier time points than non-PRP treated control sites. This corresponds with results from molecular studies that demonstrated PRP treatment of human mesenchymal stem cells induced ,early proliferation of these cells and possibly differentiation into osteoblasts. Accelerated bone formation is in contrast to the drop in bone density (representing bone loss) seen at the control site before bone formation began to take place. It took approximately 6 weeks for the control sites to reach the same bone density that the PRP-treated site had reached by first week.

As per the results in this study it was seen that the bone density at the PRP site was far more than the non PRP site at the end of first week and the fourth week. The values obtained at the end of the first week showed a marked increase in the rate of new bone formation than in the fourth week. Bone density was more in the PRP side when compared with the non PRP side at the end of first week and fourth week respectively. The maximum effect was seen in the first week .This was because the activity of the growth factors take place more in the first week. The maximum growth factors are released during the first week.

The control sites required 16 weeks to reach the same degree of radiographic density for the total extraction socket as the PRP treatment achieved in 8 weeks[56]. This is of clinical significance because it indicates more rapid healing at the PRP treated sites. Patients who undergo complete maxillary or mandibular extractions and are in need of immediate prostheses can benefit from PRP treatment. PRP treatment at the time of tooth extraction may permit dental implants to be placed at a 2 to 4 month time interval, decreasing the time to implant placement by half.

The PRP was placed in the extraction socket (bony defect) with the aim to preserve the bony quantity, treat the periodontal pocket, improve bone vertical dimension for future implant placement and to promote soft tissue healing by using the patients own blood to generate the growth factor. The mechanism by which PRP influences regeneration has been attributed to the presence of PDGF and TGF-β1 . PDGF acts principally on osteoblastic proliferation and TGF-β1 act as

cellular differentiation agents favoring the expression of markers of mineralization when incubated with pre-osteoblastic cells. This suggests that TGF-β1 could favor the differentiation of osteoblasts and the production of fibronectin, a molecule involved in the adhesion of osteoblasts and the angiogenic process. As a result of this fibrin content, the PRP gel permits stabilized coagulation of the blood, thus favoring regeneration of the osseous defect, particularly in the early stages. P value at the end of 1st week was 0.05 and P value at the end of second month was 0.029. Both the values were clinically significant and showed that PRP has beneficial effect on improvement of bone density

The influence of PRP on alveolar osteitis

The beneficial effects of PRP for decreasing the incidence of alveolar osteitis may be related to a number of signaling proteins found in the platelets, including multiple growth factors such as insulin-like growth factor, vascular endothelial growth factor, epidermal growth factor, nerve growth factor, transforming growth factors β1 and β2 (TGF-β1), platelet-derived growth factors, and cytokines such as angiopoietin-2 and interleukin-1.

These factors stimulate cell mitosis and differentiation, increase collagen production, recruit leukocytes and other cells to the surgical site, and initiate vascular in-growth. Growth factors promote both soft , hard tissue healing and angiogenesis. In addition, platelets promote clot formation, which is beneficial in healing. PRP also contains white blood cells that can inhibit bacterial growth. Therefore, PRP application mitigates the currently accepted causes of alveolar

osteitis by facilitation of clot and soft tissue formation and inhibition of bacterial colonization.Platelet concentrates may contain small amounts of leukocytes that synthesize interleukins involved in the non-specific immune reaction.
Antimicrobial activity of platelet concentrates against several bacterial species involved in oral infections has also been reported.

In the present study dealing with 12 patients , neither the control side nor the test side showed any incidence of dry socket. So it can be seen that from this study that PRP did not have any role in the post operative incidence of dry socket.

Furthermore, a recent retrospective study reported that PRP reduced the incidence of alveolar osteitis by 62%[39]. This beneficial effect is thought to be related to high growth factor content of PRP and its ability to initiate and stabilize blood clot in the extraction socket..

PRP for prevention of periodontal defects following third molar surgery[27.49].

The extraction of deeply included third molars may cause multiple periodontal defects at the distal root of the second molar. Platelet-rich plasma can be used in repairing and preventing periodontal complications at the distal root of the second molar adjacent to the extracted third molar. The use of PRP has shown to be a valid technique for promoting bone regeneration at the level of the distal surface of the

mandibular second molar following extraction of a mesioangularly inclined, deeply impacted third molar.

The use of PRP alone to fill the osseous defect has shown to be more valid for periodontal regeneration compared with the control sites, because of both the decrease in the periodontal defect and the attachment gain. The mechanism by which PRP can influence periodontal regeneration is ascribed to the presence of PDGF and TGF-B. PDGF acts principally on osteoblastic proliferation and that, on the other hand, morphogenetic proteins (which are part of the TGF-B superfamily) act as a cellular differentiation agent favoring the expression of markers of mineralization when they are incubated with preosteoblastic cells. This suggests that TGF-B could favor the differentiation of osteoblasts and cementoblasts and the production of fibronectin,a molecule involved in the adhesion of fibroblasts to the radicular surface and in the angiogenic process. As the result of its fibrin content, the PRP gel permits stabilized coagulation of the blood, thereby favoring regeneration of the osseous defect, particularly in the early stages.

Periodontal complications at the distal root surface adjacent to the second molar that follow an impacted mandibular third molar in deep extraction have been judged to be related to the age of a patient and the angulation and severity of the impaction. The possibility to concentrate growth factors in a surgical wound seems to allow activation of cellular events, providing a final gain in quality and time of healing. The use of PRP has been shown to be a useful tool in aiding periodontal and bone regeneration in vivo because of the high content of growth factors.

Rate of soft tissue healing[72]

Soft tissue wound healing and bone growth involve physiologic cascades in which cellular and hormonal factors play pivotal roles. The rationale for applying platelet gel is based on the delivery of platelet growth factors to tissues and on the fact that platelet α-granules, found inside the platelets, contain a variety of growth factors . Platelet gel growth factors are peptides that promote cell proliferation, differentiation, chemotaxis, and the migration of various cells involved in both wound healing[72] .

The numerous proteins secreted by the activated platelets influence many of the aspects of healing; PDGF is chemotactic for macrophages, whereas the combined roles of PDGF, TGF-β, and IGF are chemotaxis and mitogenesis of stem cells and osteoblasts, angiogenesis for capillary in growth, bone matrix formation, and collagen synthesis[73].

The visual analogue scale[69] clearly indicates the reduction of pain in the test group in this study. The pain on the first and third day was comparatively less in the PRP side than the non PRP side. There has not been a valid reason why the pain is less in the PRP side. But there has been many studies indicating that the application of PRP gel substantially reduced the pain postoperatively. P value at the end of first post operative day was 0.002 for pain which was clinically significant.

Many authors have published various articles stating the efficiency of PRP in increasing the bone density, increasing soft tissue healing and decreasing pain. Some in vitro studies on animals showed no benefits of PRP when it was used alone[31,35,40,55]. Because of this uncertainity in literatures, this clinical trial was used to assess the efficiency of PRP , when it was used alone.

Summary and Conclusion

To conclude, from the study conducted we have come to a conclusion that platelet rich plasma has beneficial effects on the improvement of bone density, better soft tissue healing and reduction of pain after mandibular third molar impaction surgery. But from our study we did not find any significant benefit of PRP on prevention of dry socket and reduction of swelling post operatively.

Platelet rich plasma may have benefits in reducing complications such as alveolar osteitis and swelling post operatively. But there were insufficient data to support the use of PRP to enhance the quality of life of the patient after the impaction surgery since the sample size was small to statistically detect any significant difference.

It can be concluded that PRP is a good surgical additive without any side effects and which can be used to improve post operative comfort of the patient. The surgical adhesive thus developed provided adhesiveness and tensile strength for clot stabilization. It was biologically acceptable and was a concentrate of growth factors. It had haemostatic as well as angiogenic properties. Moreover the product showed the potential for wound healing and osteoconduction.

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Annexure I

Informed consent

Why do this study? – we are interested in evaluating the efficiency of platelet rich plasma in healing after third molar impaction surgery. We need to collect data from patients whose extraction sockets have been treated with this platelet concentrate to help us compare the results and conclude whether it is better in clinical trial

What will participation involve? -During this study, in the selected patients with bilaterally impacted mandibular third molars, we treat one side as the test where the platelet rich plasma is placed and the other side is left as control. Postoperatively we determine the bone density, soft tissue healing, pain , swelling and alveolar osteitis reviewing the patient at specific time intervals.

How long will participation take ? -the entire surgical procedure will last one to one and a half hours. There is need for additional visits to the hospital with regard to the research study.

As an informed participant of this study, I understand that:

My participation is voluntary and I may cease to take part in this experiment at any time, without any penalty.

I am aware of what my participation involves

ANNEXURE I

There are no risks involved in the participation of this study.

All my questions about this study and procedures are satisfactorily answered.

I have read and understood the above , and give my consent to participate

Participants signature _____ Date _____

I have explained the above and answered all questions asked by the participant

Researchers signature_____ Date _____

Annexure II

CASE HISTORY PROFORMA

Date:

Name :

Age/Sex :

Address :

Occupation :

Chief Complaint :

History of Presenting Illness :

Past Medical History :

Drug Allergy :

Past Dental History :

Family History :

Personal History :

ANNEXURE II

Vital signs

- BP
- Pulse
- Respiratory rate
- Temperature
- Weight

General examination

- Jaundice
- Anaemia
- Clubbing
- Cyanosis
- Lymphadenopathy
- Oedema

Extra Oral Examination

- Facial asymmetry
- Mouth opening
- Deviation on mouth opening
- Swelling, tenderness

Systemic Examination:

- CNS
- CVS
- RS
- GIT

Intra-Oral Examination

- Soft tissue examination
- Hard tissue examination
- Occlusion

Provisional Diagnosis

Investigations

Final Diagnosis

Treatment Plan

ANNEXURE III

PATIENT DATA FORM

NAME :

AGE :

ADDRESS :

DAY OF REVIEW:

ASSESSMENTS:

1. **PAIN** [69]
2. **SWELLING**[70]
3. **SOFT TISSUE HEALING**[71]
4. **ALVEOLAR OSTEITIS**
5. **RADIOGRAPHY (OPG)**

Annexure IV

Numeric visual analog scale[69]