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Assessment of Posterior Spinal Fusion Using Mixture of Autologous Platelet Rich Plasma and Hydroxyapatite Granules

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Conflicts of interest: None to Declare

Abstract

Background

Spinal fusion is a surgical procedure used to correct problems with the vertebrae. The basic idea is to fuse together the affected vertebrae so that they heal into a single, solid bone.

It also prevents the stretching of nerves and surrounding ligaments and muscles. Originally described for the treatment of tuberculosis of the spine, spinal fusion has become a widely done procedure for various spinal disorders. Among other indications, spinal fusion is used for treatment of degenerative disc disease, congenital and developmental deformities such as scoliosis, and spinal instability secondary to trauma. One of the most common complications of spinal fusion is non-union. Recognizing and treating nonunion of the spine may be crucial in preventing progressive deformity in identifying instrumentation failure, and in relieving persistent pain.

Material and Methods

A total of 30 Patients presented with various diagnosis, who fulfil the inclusion and exclusion criterion and who reported to the Central Institute of Orthopaedics (C.I.O.), Safdarjung Hospital associated with Vardhaman mahavir medical college, New Delhi were included in the study . Written informed consent was obtained from the patients enrolled in the study and they were followed up. Diagnosis was done on the basis of X-Ray, CT Scan and clinical signs/symptoms as per the applicable and widely accepted protocol. A prospective analysis over a period of 18 months from September 2012 to February 2014. .

Preparation of platelet-rich plasma:

20 ml of venous blood of patient undergoing surgery was collected in two anticoagulant citrate dextrose BD vacutainer in equal quantity on the day of surgery under strict aseptic condition. Anticoagulant citrate dextrose-A (ACD-A) works well, as the citrate binds calcium and prevents coagulation, whereas the dextrose and other ingredients support platelet metabolism and viability. The blood sample was sent to blood bank for preparation of plasma-rich protein without any delay. The preparation of PRP was done by REMI centrifuge by centrifugation at 3500rpm for 2 minutes. When anticoagulated blood is

centrifuged, three layers become evident.The bottom layer is comprised of red blood cells (specific gravity = 1.09), the middle of platelets and white blood cells (buffy coat, specific gravity = 1.06), and the top of plasma (specific gravity = 1.03).buffy layer was sepreated. [63] Platelet count of freshly prepared PRP was done to know the level of platelet enrichment. . The PRP was made available immediately after preparation on the day of surgery.

Processing of hydroxyapatite:

10 ml of hydroxyapatite granule of size 5mm (B-OSTIN COMPANY) was taken in bowl under aseptic condition .Hydroxyapatite granules was presoaked with one ml of normal saline to increase effective pore diameter.

Preparation of graft bed:

Soft tissue dissection done in order to reach desired surgical site.Graft bed preparation done prior to rod fixation in case of posterior instrumentation.Unilateral/lateral placement and extent of grafting will depend on availability of the bed. Graft bed made available by decorticating and nibbling facets, posterior arch structure of affected vertebra, base of transverse process.

Freshly prepared PRP and hydroxyapatite was mixed thoroughly and the resultant mixture will be applied evenly to graft bed. Operative site will be sutured in layer wise manner and antiseptic dressing was done.

Results & Discussion- Out of the total 30 patients in this study, 11(36.7%) were in age group of 21 to 30 years. 10 (30.0%) patients were in age group of 31-40 years,5(16.7%) were in age group >40 year age and <20 years. The mean age of the study group was 32 years with range of 16 to 55.

Majority of the patients 20(66.7%) were males whereas 10(33.3%) were females. The distribution of the study group showed a clear male preponderance with male to female ratio of 2:1.

Out of the total 30 patients in this study, 3(10.0%) patients were type of #D/L. 13(43.3%) patients were of Burst type, 2(6.7%) patients were of chance type, 3(10.0%) patients were of Pott's Spine type, 2(6.7%) patients were of Scoliosis type and 7(23.3%) patients were of Wedge type.

out of 30 patients 11(36.7%) patients had at Dorsal level, 12(40.0%) had at Dorsal Lumber and 12(40.0%) patients had at Dorsal Lumber and out of 30 patients 3(10.0%) patients had Intact deficit, 5(16.7%) patients had Paraparesis deficit and 22(73.3%) patients had paraplegia deficit.

In our study during 24 weeks we had found that the mean value of 7.70 of VAS with standard

deviation of 2.037 varies between one to nine in pre-op period, the mean value of 5.0 of VAS with standard deviation of 1.232 varies between two to seven during pre-op to 6 weeks of period, the mean value of 3.33 of VAS with standard deviation of 0.994 varies between one patient to five patients during 6 weeks to 12 weeks of period and the mean value of 1.87 of VAS with standard deviation of 0.86 varies between zero patient to five patients during 12 Weeks to 24 weeks of period . no fusion seen on x-ray after 6 weeks ,After 12 weeks fusion mass seen in 2 cases (6.7%) on x-ray, After 24 weeks on x-ray ,fusion mass seen in 17 cases(56.7%) and after 24 weeks fusion mass seen in 19 cases(63.3%) on CT-scan. comparison between VAS and fusion mass was made, which shows mean VAS with standard deviation at 12 weeks and 24 weeks were 3.39 ± 0.916 and 2.46 ± 0.776 in favour of 2 and 17 cases seen respectively.

Composite grafts permit combination of the osteoinductive and osteogenic capacities of growth factors or autogenous bone with the structural capacity of mineralized matrices. The purpose of the current study was to assess the effect of platelet-rich plasma when added to hydroxyapatite in posterior lumbar fusion. Both

clinical and radiological outcomes were considered. Our first hypothesis was that there would be a clinical benefit that was measured from VAS score difference. Our second hypothesis was that, on radiological assessment, there would be presence of fusion mass.

Conclusions: Combined use of hydroxyapatite granules and platelet rich plasma makes near to ideal bone graft substitute as hydroxyapatite provides osteoconductive property and PRP provides osteogenic and osteoinductive property .This combination is also free from the drawbacks of iliac crest bone graft harvesting as described earlier.

In our study VAS score has been improved with combined use of both PRP and hydroxyapatite and bears statistically significant relation and on radiological assessment there was increase fusion rates .Though this study shows beneficial effect of use of both PRP and hydroxyapatite, we recommend further randomised control to prove efficacy and study in large sample size and long duration of follow up.

Introduction

Spinal fusion is a surgical procedure used to correct problems with the vertebrae. The basic idea is to fuse together the affected vertebrae so that they heal into a single, solid bone. It also prevents the stretching of nerves and surrounding ligaments and muscles.

Originally described for the treatment of tuberculosis of the spine, spinal fusion has become a widely done procedure for various spinal disorders. Among other indications, spinal fusion is used for treatment of degenerative disc disease, congenital and developmental deformities such as scoliosis, and spinal instability secondary to trauma. One of the most common complications of spinal fusion is non-union. The incidence of non-union has been reported as high as 56% in the lumbar spine. Recognizing and treating nonunion of the spine may be crucial in preventing progressive deformity in identifying instrumentation failure, and in relieving persistent pain.

Replacing fractured vertebral bodies with metallic cage implants has become a solid alternative in the treatment of injuries of the thoracic and lumbar spine. On the other hand, a foreign material such as a cage does not provide any osteogenous potential. This can lead to degeneration and correctional loss. In the long run, it is essential to form a biological spondylodesis. This can be reached by adding bone graft, it is inserted in or placed around the cage in order to gain osseous integration and additional stability through bony fusion.

Over the last few decades, there has been a dramatic increase in the spinal fusion surgeries performed. The iliac crest graft which has been used over the years as a novel graft material, due to significant morbidity associated with its harvesting and additional surgical effort now a days less preferred by surgeons worldwide. [1,2,3] This drawback has led to development of newer bone graft alternatives including demineralized bone matrix ,ceramic grafts , mineralised collagen, platelet rich plasma, these alternatives avoid problem due to donor site morbidity however question remain regarding clinical efficacy and safety. [4, 5, 6, 7]

Trying to avoid the problems derived from bone harvesting, different areas of investigation have been developed, which basically follow two ways: bone substitutors (BS) or fusion promoting material (FPM). Up to date, different variants of tricalcium phosphate plus hydroxyapatite (TCP/HA) and Demineralized bone matrix (DBM) are available for surgeons and at least, for TCP/HA, its usefulness has been demonstrated [8, 9] placed around the cage in order to gain fusion.

However,Demineralized bone matrix has risk of disease transmission and immunological response.[10] Ceramic graft

show variable rates of resorption, poor performance and potentially

adverse effects on normal bone remodelling.[11] This led to search of an autologous, immunocompetent, having high osteogenic potential and easily harvestable platelet rich plasma as

an option for spinal fusion surgeries. Once bone morphogenetic proteins (BMPs) were considered to be the gold standard of promoting substances of bone fusion. Its commercial availability, for about one decade, has allowed surgeons to work with a demonstrated osteoinductive product. [12] Because of high risk of disease transmission and immunological response and high cost, have limited its use to a very little amount of patients in our country. [13]

The big amount of growth factors, which are released in the process of platelet degranulation, has opened a new way of work, based on its possible use as FPM. Experience has demonstrated its beneficial effects on soft tissue lesions both if injected or added to the surgical act. The possible beneficial ones on bone pathology are more arguable and literature is scarce and contradictory in results when it's used in lumbar arthrodesis. [14, 15, 16, 17, 18] It is known that in the flow of chemicals steps produced in a fracture healing, platelets degranulation releases a group of proteins, generically known as autologous growth factors. Inside, platelets have three types of granules. The so-called alpha-granules contain more than 30 bioactive proteins, many of which have a fundamental role in haemostasis and tissue healing. The platelet-derived growth factor (PDGF), the insulin-like growth factor (IGF), the transforming growth (TGF) and the vascular endothelial growth factor (VEGF) are some of these proteins. During the last few years, platelet concentrate has been increasingly used in spine surgery, in addition to both autologous bone and osteo-conducive materials such as hydroxyapatite, in order to enhance the density of bone fusion in postero-lateral and inter-transverse fusion procedure. [19]

Platelets and the growth factors released by the platelets are essential for regulating the cellular events that follow tissue damage. They adhere, aggregate, form a fibrin mesh, and subsequently release a large variety of growth factors and cytokines. At least 15 different factors are known to be contained within platelets. [20,21,22] The impact on bone and tissue regeneration of most of these factors has been recognized by many authors. [23,24] As opposed to an artificial composition of recombinant proteins, PRP maintains the natural concentrations within a cocktail of growth factors acting on multiple pathways. [25] Furthermore, artificial recombinant growth factors require further synthetic or animal proteins as carriers. PRP in contrast serves as a natural carrier itself. [26] Thereby PRP can mimic the highly efficient in vivo situation much more closely than a custom designed protein preparation.

The formation of new bone following trauma is initiated by the development of fibrin clots and the aggregation and degranulation of platelets. These platelets and a variety of growth factors contained within them, most notably PDGF and TGF, are known to have a positive influence on bone formation. Platelet-rich plasma (PRP) has a high concentration of these platelets and, therefore a high concentration of growth factors .There has been a wide interest in using PRP as a therapeutic agent to enhance tissue repair or bone formation following fractures or bone grafting. Therefore, when used in addition to a cage implant, PRP combined with autologous bone graft could provide a more efficient bony fusion than a graft alone. The recent literature shows controversial results, when autologous platelet concentrates are used to promotes spinal fusion.

Theoretically, growth factor would stimulate the proliferation and differentiation of mesenchymal stem cells and would facilitate bone formation behaving as histopromotive factors.[14,27] Platelet concentration harvesting is simple, safe, cheap and causes little discomfort to the patient. Its autologous origin avoids inmunologic reactions and disease transmission.

Starting from this previous experience, studies were designed to learn if adding APC to autografts and/or bone substitutes would achieve the improvement of fusion mass (quality and rates) in posterolateral or circumferential arthrodesis in the lumbar segment. The results, although irregular, don`t improve the ones obtained when autograft is used.[14,28,29] The clinical experience is even more contradictory. Its use in intersomatic arthrodesis shows similar results between autograft or allograft plus autologous platelet concentrate. [30] When the analysis of the cage filled with autograft (with or without autologous platelet concentrate) was made by CT, it was observed a quicker fusion in those patients with autologous platelet concentrate added, although the result, at the end of the follow-up, was the same. [31] It is true some authors emphasize on its goodness for lumbar fusion. [32,33] But in literature less fusion rates when adding autologous platelet concentrate to autograft or autograft plus TCP/HA is also reported.[17,34,35,36,37] So far, all clinical reports and case studies and we did not find level-1 data to support the use of autologous platelet concentrate in spinal fusion.[14] While there are numerous case studies and small clinical trials on the clinical applications, knowledge about the underlying effects at the cellular level is limited. Nevertheless, PRP has been shown to stimulate cell proliferation of osteoblasts and fibroblasts and to upregulate osteocalcin in these cells. [38,39] In a recent study demonstrated the differentiation of mesenchymal stem cells (MSC) into bone forming cells in the presence of PRP. [40] An increase in growth and differentiation of PRP treated periodontal ligament cells has been shown by two groups. [16,41] In vivo studies do not support the positive actions of PRP. In fact, in one of the most recent investigations PRP decreased the osteoinductivity of demineralized bone matrix in nude mice. [42] Other researchers performed trials on various animals and reported no beneficial effect of using PRP for bone healing [43] or suggest a low regenerative potential for its use in combination with xenogenic bone grafts. [44] Some studies also show effective augmentation of porous biomaterial in rats [45] and sheep. [46]Careful analysis of these studies reveals that none are scientifically comparable. Therefore, we cannot draw an overall scientific conclusion of PRP actions in animal models.

Unfortunately, as pointed out by Vaccaro [47] at the present time there is an absence of controlled clinical trials evaluating how and to what extent platelete rich plasma increases bone fusion in spinal surgery.

Hydroxyapatite is commonly used as a filler in orthopaedics. Hydroxyapetite has higher osteogenic potential and less biodegrability than tricalcium phosphate [48] Many modern implants e.g. hip replacement and dental implants,are coated with hydroxyapatite. It has been suggested that this may promote osseointegration.[49] Coralline hydroxyapatite conducts bone formation in spine surgery as pointed out by various studies.

In view of various controversies the present study is planned to assess the effect of combined use of hydroxyapatite and PRP on healing of intervertebral body fusion.

Aims And Objectives

1.To assess the time required for spinal fusion using mixture of hydroxyapatite granules and autologous PRP.

2.To assess quality of spinal fusion achieved with the use of autologuous Platelet Rich Plasma and hydroxyapatite granule.

Material And Methods

This study was conducted in Central Institute of Orthopaedics (C.I.O.), Safdarjung Hospital associated with Vardhaman mahavir medical college, New Delhi, which is a tertiary care hospital situated in Delhi and catering to Nursing Homes and Hospital of the region as well as patients from whole of Delhi and areas around Delhi. A prospective analysis over a period of 18 months from September 2012 to February 2014 was carried out in the Department of Central Institute of Orthopedics, VMMC and Safdarjung Hospital.

A total of 30 Patients presented with various diagnosis, who fulfil the inclusion and exclusion criterion and who reported to the Central Institute of Orthopaedics (C.I.O.), Safdarjung Hospital associated with Vardhaman mahavir medical college, New Delhi were included in the study . Written informed consent was obtained from the patients enrolled in the study and they were followed up. Diagnosis was done on the basis of X-Ray, CT Scan and clinical signs/symptoms as per the applicable and widely accepted protocol.

Study Area/Study Centre

The study was conducted at the the Central Institute of Orthopaedics (C.I.O.), Safdarjung Hospital associated with Vardhaman mahavir medical College, New Delhi

Study Design

Tertiary Hospital based prospective, non-randomised, observational study with follow-up. Approval from institutional ethical committee was obtained for the study and all patients signed an informed consent.

Study Population

The study was done on patients admitted through outpatients department and emergency department with a diagnosis of Spinal fusion. Diagnosis was confirmed on the basis of appropriate radiological studies.

Duration of the study

A prospective analysis over a period of 18 months from September 2012 to February 2014 was carried out in the Department of Central Institute of Orthopaedics, VMMC and Safdarjung Hospital.

Sample Size

Total Number of patients: 30

30 cases were accessed on the basis of Cases selection criteria presenting to the OPD or EOT Services of C.I.O, VMMC & Safdarjung Hospital and were included for the study and accordingly followed up. Sample size were based on the availability of the cases in the Hospital as per records of the Hospital.

Sampling Technique

Patients were examined after checking their suitability as per the inclusion and exclusion criteria.

Materials

1) **Bone graft substitutes**

- Autologus platelets with plasma
- Hydroxyapatite

2) **Radiological tools**

- Digital X-rays
- CT Scan

Inclusion Criteria

All cases of spinal fusion with following indications:

- Patients with trauma to the spine requiring spinal fusion
- Spine eformity correction
- Tuderculosis of spine
- All skeletally mature patients of either sex.
- Patients age between 18 and 65 years.
- Given information consent.

Exclusion Criteria

Local infection at the surgical site.

- Uncontrolled diabetes (untreated or not controlled by treatment).

- Long corticosteroid treatment (within last 3 months) for other indications.
- Current Chemotherapy or during the last three months.
- Antecedent regional radiotherapy.
- All the contraindication to hydroxyapatite : severe osteomyelities, bleeding tendencies, necrosis of surgery site.
- Severely altered physical and/or psychological Health, which can affect to patients compliance.
- Unwilling and uncooperative patients.
- Economical constraints.

All the subjects had undergone the following process of recruitment and examination :

- The patients after admission were subjected to the following evaluation.
- Detailed clinical history of the patients were recorded which would include age, personal habit (smoking , drinking, any co morbid serious medical illness)
- The patients would then be subjected to thorough clinical examination including symptoms and signs and detailed neurological examination.
- Investigation were ordered including the relevant blood investigation (serum calcium profile, s. Phosphate, s. Alkaline phosphatise as well as relevant radiological investigation.
- Pre- anaesthetic check-up was done.
- Written informed consent were taken
- Prophylactic antibiotics were given.

Genera Physical Examination:

- Height (in cms)
- Weight (kgs)
- Pulse
- Respiration Rate
- Oral Temperature
- BP
- BMI

Systemic Examination:

- CVS
- RS
- Per Abdomen
- CNS

Base line Blood investigation as follows:

1. Complete blood counts (CBC) including platelet counts
2. Random blood sugar (RBS)
3. Kidney function test (KET) Serum Creatinine, Blood urea
4. Serum electrolytes - Sodium and Potassium
5. Liver function tests (LFT)
6. Serum proteins (albumin and globulin)
7. Coagulation profile

Radiological Investigation

- X-Ray
- CT Scan

Methodology

1. **Preparation of platelet-rich plasma**:

20 ml of venous blood of patient undergoing surgery was collected in two anticoagulant citrate dextrose BD vacutainer in equal quantity on the day of surgery under strict aseptic condition. Anticoagulant citrate dextrose-A (ACD-A) works well, as the citrate binds calcium and prevents coagulation, whereas the dextrose and other ingredients support platelet metabolism and viability. The blood sample was sent to blood bank for preparation of plasma-rich protein without any delay. The preparation of PRP was done by REMI centrifuge by centrifugation at 3500rpm for 2 minutes. When anticoagulated blood is centrifuged, three layers become evident.The bottom layer is comprised of red blood cells (specific gravity = 1.09), the middle of platelets and white blood cells (buffy coat,

specific gravity = 1.06), and the top of plasma (specific gravity = 1.03).buffy layer was sepreated. [50] Platelet count of freshly prepared PRP was done to know the level of platelet enrichment. . The PRP was made available immediately after preparation on the day of surgery.

2. Processing of hydroxyapatite:

10 ml of hydroxyapatite granule of size 5mm (B-OSTIN COMPANY) was taken in bowl under aseptic condition .Hydroxyapatite granules was presoaked with one ml of normal saline to increase effective pore diameter.

3. Preparation of graft bed:

- Soft tissue dissection done in order to reach desired surgical site.
- Graft bed preparation done prior to rod fixation in case of posterior instrumentation.
- Unilateral/lateral placement and extent of grafting will depend on availability of the bed.
- Graft bed made available by decorticating and nibbling facets, posterior arch structure of affected vertebra, base of transverse process.51

4. Freshly prepared PRP and hydroxyapatite was mixed thoroughly and the resultant mixture will be applied evenly to graft bed.

5. Operative site will be sutured in layer wise manner and antiseptic dressing was done.

Pre Operative Evaluation

The patients after admission were subjected to the following evaluation.

- Detailed clinical history of the patient was recorded which will include age, personal habits (smoking, alchoholism, any co morbid serious medical illness).
- The patient was then be subjected to thorough clinical examination including symptoms and signs and detailed neurological examination.
- Investigations was ordered including the relevant blood investigation (serum calcium profile, s. phosphate, s. alkaline phosphatise) as well as relevant radiological investigations.
- Pre-anaesthetic check-up was done.
- Written informed consent was taken.
- Prophylactic antibiotics was given.

Post Operative Care

- Check x-ray standard AP and lateral films to assess fusion and spine stability.
- Rehabilitation started with brace one week postoperatively.
- Stitch removal done after 2 weeks.
- Patient mobilized with wheel chair or any other walking aids depending on neurological status.

Follow-Up Assessment

The patients was followed up at intervals of 6 weeks, 12 weeks and 24 weeks with AP and lateral x ray of the proposed site of spine fusion .At 24 weeks CT scan of Operative site will be done to assess fusion and VAS SCORE at above period.

Clinical Assessment

This was made on the basis of standard clinical parameters such as visual analog score.

Data Collection

The research protocol was reviewed by institutional rewiew board of VMMC and Safdarjung hospital and approval from the board was given.

1. All the patients received detailed information on the procedure and only those who agreed to the study protocol were finally enrolled in the study .The pre-therapeutic imaging included conventional radiograph ,CT scan .

2. Primary data was collected from patient s.

3. Secondary data was taken from earlier treatment records ,lab investigation reports and radiological imaging reports.

Data Analysis

Data was simultaneously entered into proforma and was updated after follw up.Data was entered into Microsoft excel (MS Office 2007) and master chart prepared. Statistical analysis of data was done by

- Nominaldata (such as gender pathology level) were presented as number and percentages.
- Continous data (such as age) were expressed as mean ,standard deviaton and range.
- P value of .05 was considerd as stasistically significant (confidence interval of 95% was taken into account).

Limitations of the study

1. Less number of patients was taken as the single researcher and time constraint.

2. short duration of follw up.

Case Illustrations

CASE 1: 20yrs old female patient presented with back pain and weakness in both lower limbs .She had sustained trauma to back due to fall from height. After investigations and imaging studies she was diagnosed as a case of traumatic fracture of L2 vertebra with paraplegia and bladder bowel involvement. She was operated with posterior decompression and pedicle screw fixation with fusion using hydroxyapatite and autologous PRP. Immediate post-operative period was uneventful without any complication such as wound infection, implant failure, or no graft related complications reported. Patient was followed up for fusion over the period of 6 months.

Pre-operative clinical parameters VAS score 9/10 and no bony fusion. After 6 months follow up: VAS score 2/10 and fusion mass seen on x-ray and CT scan.

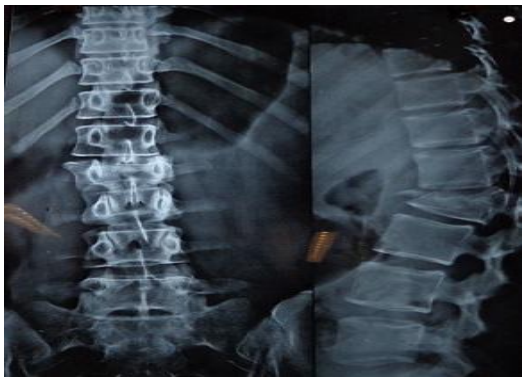


Fig1.1: Pre-operative AP and Lateral x-ray showing wedge compression fracture of l2 vertebra.

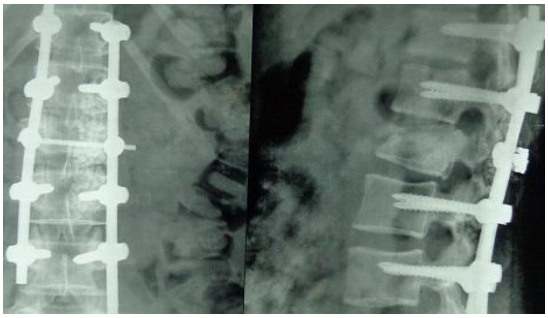


Fig 1.2: AP and Lateral X-ray after 6 weeks

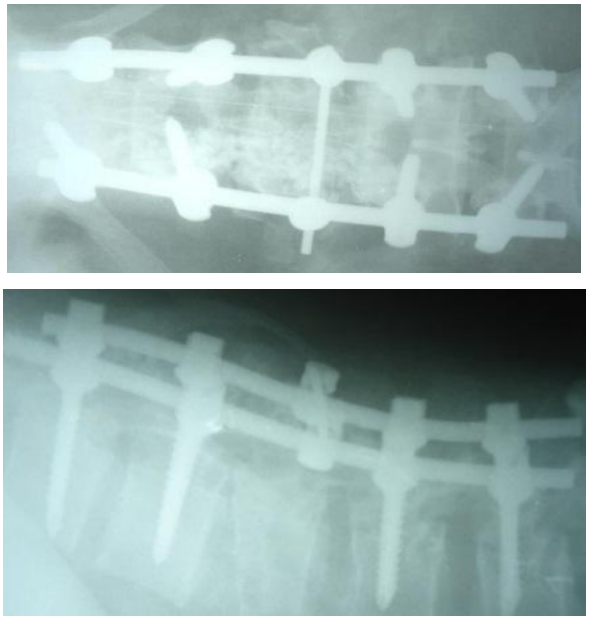
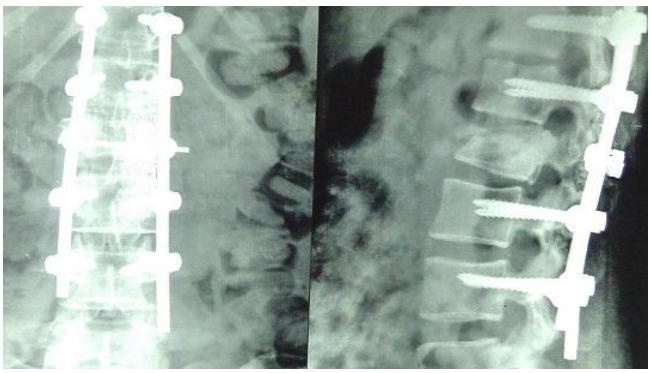


Fig 1.3: AP and Lateral X-ray after 12 weeks

Fig 1.4: AP and Lateral X-ray after 24 weeks showing fusion mass



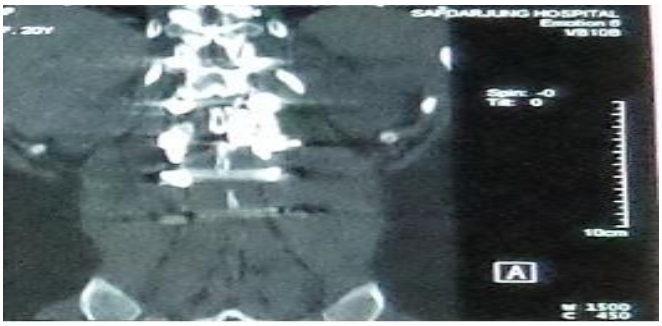


Fig 1.5.1

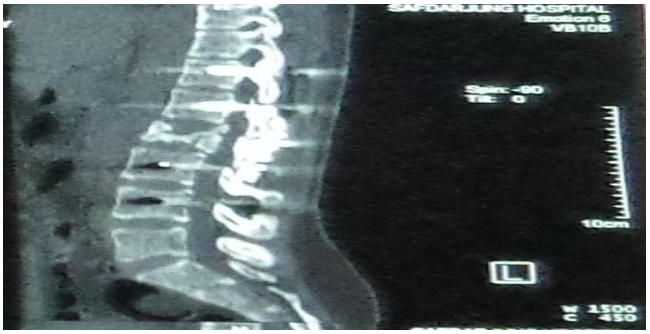


Fig 1.5.2

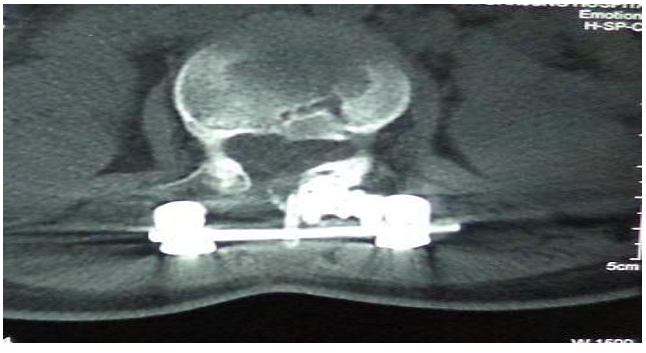


Fig 1.5.3 Sagital section (1.5.1,1.5.2) and axial section CT Scan after 24 weeks showing fusion mass

Results and Discussion

Tissue and mineral grafts in the spine perform mechanical and biologic functions. Their capacity for each function is dependent upon the structural, cellular, and biochemical properties of the particular graft chosen. Autogenous bone offers an optimal balance of osteogenic, osteoinductive, and osteoconductive capacities, structural stability, and biocompatibility. However, the availability of autogenous bone graft is clearly limited, and the complications of autogenous harvest are well known. [52]

Donor site problems, including pain, paresthesias, hematoma,and infection, have been reported in up to 50% of patients in some series.10–12 Up to 60% of patients experience long-term, persistent donor site pain and between 2% and 5% of patients develop wound complications that require re-operation. [52,53,54]

This has led to bone graft alternatives or supplements.Mineralized bone matrices used in spine surgery include corraline hydroxyapatite, and synthetic hydroxyapatite.

Mineralized bone matrices provide an osteoconductive matrix for new bone formation. In isolation, mineralized bone matrix has little osteoinductive or osteogenic potential.

The concept of PRP application is to enhance the healing properties of bone by stimulating osteoinduction and osteogenesis. When multiple growth factors are present at the bone formation site, they may exert a synergistic effect. [26]

Composite grafts permit combination of the osteoinductive and osteogenic capacities of growth factors or autogenous bone with the structural capacity of mineralized matrices. The purpose of the current study was to assess the effect of platelet-rich plasma when added to hydroxyapatite in posterior lumbar fusion. Both clinical and radiological outcomes were considered. Our

first hypothesis was that there would be a clinical benefit that was measured from VAS score difference. Our second hypothesis was that, on radiological assessment, there would be presence of fusion mass.

Age distribution of patient in the study

In our study of total 30 patient. The mean age of the study group was 32 years with standard deviation of 11.64.The minimum age of the patient with spinal fusion was 16 whereas the maximum age of the patient with spinal fusion was 65 years .The range of the age for these was 50 years With 21-30 years age group most frequent being 11 (36.7%). Landi A, Tarantino R et al., in their study mainly had old age population because in their study main indications for fusion were degenerative conditions which are more prevalent in old age. On contrary in our study more young population were include, this may be due to more exposure to outdoor activities of this age group leading to falls, accidents and sports related injuries.

Gender wise distribution of patient

In our study 20(66.66%) patient were male and 10(33.33%) patient were female. As trauma ismore common in young Indian males due to more outdoor activities and risk taking behaviour like driving after alcohol intake. Whereas in another study by. J. Sys • J. Weyler • T. Van Der Zijden evaluated 38 patient ,14 female and 24 male , they used Platelet-rich plasma for mono-segmental posterior lumbar interbody fusion. [67] In another similar study by A. Landi 14 patient ,9 male and 5 female. They use platelet gel in postero-lateral fusion in 14 cases. [19] Hwan T. Hee, Mohammad et al There were 15 women and 8 men in one group in which transforaminal inter body fusion was done with autologous growth factor and in another group 42 male and 69 female in which only transforaminal interbody fusion was done without using autologous growth factor 52 hence it can be said in most there is male preponderance.

Indications for surgery

In our study Out of the total 30 patients in this study, 3(10.0%) patients were type of #D/L. 13(43.3%) patients were of Burst type, 2(6.7%) patients were of chance type, 3(10.0%) patients

were of Pott's Spine type, 2(6.7%) patients were of Scoliosis type and 7(23.3%) patients were of Wedge type. In another similar kind of study by hwan T.Hee , Mohammad et al patient were of

degenerative disc disease (78) ,7 herniated disc(7) ,spinal stenosis (37),spondylolisthesis(18), degenerative scoliosis (6),pseudoarthosis (18) in one group in which only TLIF was done and in another group patient were degenerative disc disease(15),herniated disc (1),spinal stenosis(6) ,listhesis(4),degenerative scoliosis (1),pseudoartosis(7).in this group TLIF was done with AGF.Patient in each can have more than one diagnosis. [55] In another study by Landi et.al 4 patients were traumatic cause and 10 were degenerative. [19] In another study by J.Sys, J.Weyler, indications for surgery were both lytic and degenerative spondylolisthesis, disc degeneration not responding to conservative treatment modalities for at least 6 months, and disc herniation in patients with severe disc degeneration and persisting sciatica despite epidural steroid injections.

In our study there were 2 smoker patient and 5 patient was having co-morbidity like diabetes mellitus(2) and hypertensive (3).

In our study no patient was previously operated for any kind of spinal patholog .

Visual analog scale score

Indirect assessment of fusion has clinically considered to be correlated with gradual subsidence of pain on defined follow up period. VAS score has been one of the reliable

parameter for objective pain assessment in many clinical studies. In our study pre-op VAS was 7.70±2.037 with minimum and maximum value 1-9 and after 6 months follow up VAS was 1.87 ± 0.86 with minimum and maximum value 0-3 and it was found statistically significant.

In study by J.Sys • J.Weyler et al. both groups showed a significant improvement in VAS 2 years post operatively with an improvement of 4.92 points in the study group (p\0.001) and 4.00 point in the control group (p\0.001). The difference in improvement was not statistically significant (p = 0.166).In the study group material used for fusion was autogenous bone graft and PRP while in control group only autogenous bone graft was used. [56]

In study by Hartmann EK, Heintel T, et al. The VAS spine score showed almost the same values in both study and control group. The score dropped by 18.6% in the PRP group and 23.5% in the control group in comparison to the pre traumatic value which revealed to be not significant (*p* > 0.05).In study group patients were initially stabilised using a rigid posterior fixation device. In a second step, the anterior destruction was bridged using a titanium implant. This was an expandable cage in nine cases of bridging or a non-expandable device bridging one segment combined with an anterior plate system in the other six cases. In addition to this rigid fixation, a mixture of bone graft drawn out of the fractured vertebra and PRP had been placed onto the left side of the cage .In control group These patients had a similar treatment consisting of an initial rigid posterior fixation combined with an anterior reconstruction using a titanium cage except no injection of PRP. [31]

Fusion rate

The enhancement of healing by the placement of a supraphysiologic concentration of autologous platelets at the site of surgery is supported by basic science studies. [32] Research has revealed that the role of platelets is much more involved than simply 'plug' formation; they are responsible for actively extruding growth factor, which initiate bone formation. [32]

In our study there was 381 % increase in platelet count from base line.Inferior rates of arthrodesis were reported by Weiner and Walker when AGF was added to autologous bone in posterolateral spine fusion. [37] They performed a retrospective, consecutive series in 59 patients who underwent a single-level fusion. Fusion was assessed by two spine surgeons on dynamic X-rays at 1 and 2 years.

An inhibitory effect of growth factors has been suggested by Castro in spinal interbody fusion. [33] Spinal fusion was assessed in a consecutive series of 62 patients receiving autograft only, followed by a consecutive series of 22 patients receiving autograft and AGF. The arthrodesis rate appeared to decrease in the AGF group with 19%.

Beneficial effects of PRP on spinal fusion were reported by Hee et al. [55] in a prospective study comparing transforaminal lumbar inter-body fusion (TLIF) with autograft and AGF to an historical cohort without AGF. They demonstrated faster fusion but no increase in fusion rates .They included both 1 level and 2 level fusion. Fusion was assessed on x- ray.

Indicators of fusion for radiological assessment include the presence of bony trabeculation across the fusion level with a lack of bony lucency at the graft/vertebral body junction. CT more clearly demonstrates the existence or absence of bridging bone, making bony non-union easier to visualize than on plain radiograph.

In our study no fusion seen on x-ray after 6 weeks .After 12 weeks fusion mass seen in 2 cases(6.7%) on X-ray. After 24 weeks on X-ray fusion mass seen in 17 cases (56.7%) and after 24 weeks fusion mass seen in 19 cases (63.3%) on CT-scan In our study comparison between

VAS and fusion mass was made, which shows mean VAS with standard deviation at 12 weeks and 24 weeks were 3.39 ± 0.916 and 2.46 ± 0.776 in favour of 2 and 17 cases seen respectively. Fusion and vas on 24 weeks found to be statistically significant whereas at 12 weeks it was statistically non-significant.Our study also shows computed tomography is better imaging tool for evaluation of fusion. This fact is supported by study of Ghiselli G, Wharton N, Hipp JA, et al who concluded that CT is now considered the ‘‘gold standard’’ imaging test for assessment of fusion status and evaluating for bridging bone following equivocal plain radiographic findings. [57] In another study by Jordan A. Gruskay et al Based on the data available, thin-cut CT scan and computerized motion analysis of dynamic plain films are the best imaging modalities, whereas surgical exploration remains the gold standard. [58] However, radiation exposure and cost associated with should be taken into account.

Limitation of our study was , failure to evaluate the various confounding variables and co-morbidities associated like effect of smoking , hypertension ,diabetes .Because detailed discussion of this variables was not possible owing to small sample size ,short duration follow-up, another limitations include no use of thin cut section in evaluation of fusion which reduce the effect of artefact .

Conclusions

Combined use of hydroxyapatite granules and platelet rich plasma makes near to ideal bone graft substitute as hydroxyapatite provides osteoconductive property and PRP provides osteogenic and osteoinductive property .This combination is also free from the drawbacks of iliac crest bone graft harvesting as described earlier.

In our study VAS score has been improved with combined use of both PRP and hydroxyapatite and bears statistically significant relation and on radiological assessment there was increase fusion rates .Though this study shows beneficial effect of use of both PRP and hydroxyapatite, we recommend further randomised control to prove efficacy and study in large sample size and long duration of follow up.

TABLES

Table.1: Distribution of patients under study according to Age group

Age Groups	Frequency	%
<=20 yrs	5	16.7%
21 - 30 yrs	11	36.7%
31 - 40 yrs	10	30.0%
>40 yrs	5	16.7%
Total	30	100%
Mean ± SD	32.0 ± 11.64	
Median	30 yrs	
Mode	30 yrs	
Min – Max	16 - 55 yrs	

Out of the total 30 patients in this study, 11(36.7%) were in age group of 21 to 30 years. 10 (30.0%) patients were in age group of 31-40 years,5(16.7%) were in age group >40 year age and <20 years. The mean age of the study group was 32 years with range of 16 to 55.

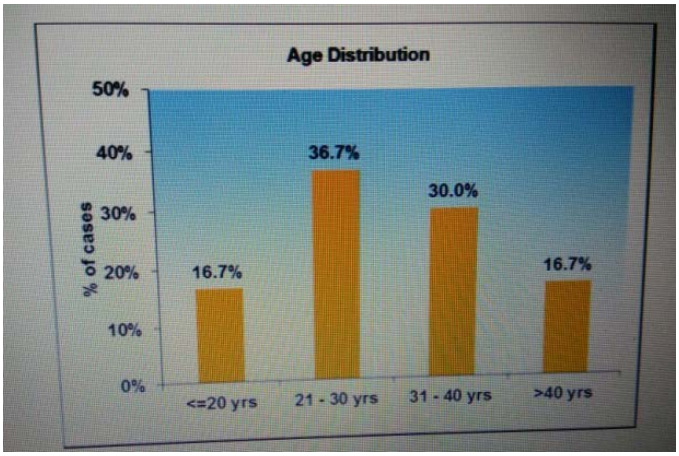


Figure.1: Distribution of patients under study according to Age group

Table 2: Distribution of patient under study according to Gender

Sex	Frequency	%
Male	20	66.7%
Female	10	33.3%
Total	30	100%

Majority of the patients 20(66.7%) were males whereas 10(33.3%) were females. The distribution of the study group showed a clear male preponderance with male to female ratio of 2:1.

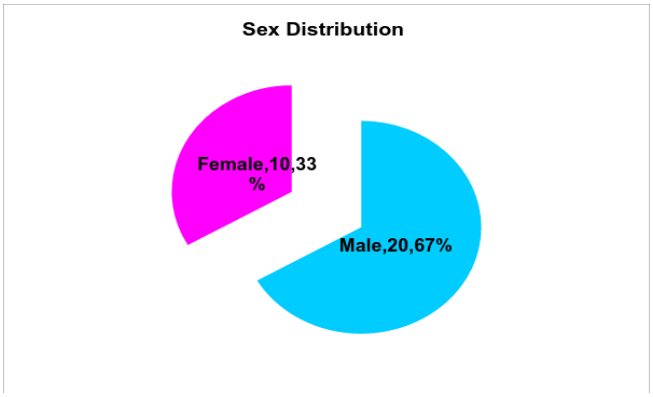


Figure 2: Distribution of patients under study according to Gender

Table 3: Distribution of patient under study according to pathology of spine

Type	Frequency	%
#D/L	3	10.0%
Burst	13	43.3%
Chance	2	6.7%
Pott's Spine	3	10.0%
scoliosis	2	6.7%
wedge	7	23.3%
Total	30	100%

Out of the total 30 patients in this study, 3(10.0%) patients were type of #D/L. 13(43.3%) patients were of Burst type, 2(6.7%) patients were of chance type, 3(10.0%) patients were of Pott's Spine type, 2(6.7%) patients were of Scoliosis type and 7(23.3%) patients were of Wedge type.

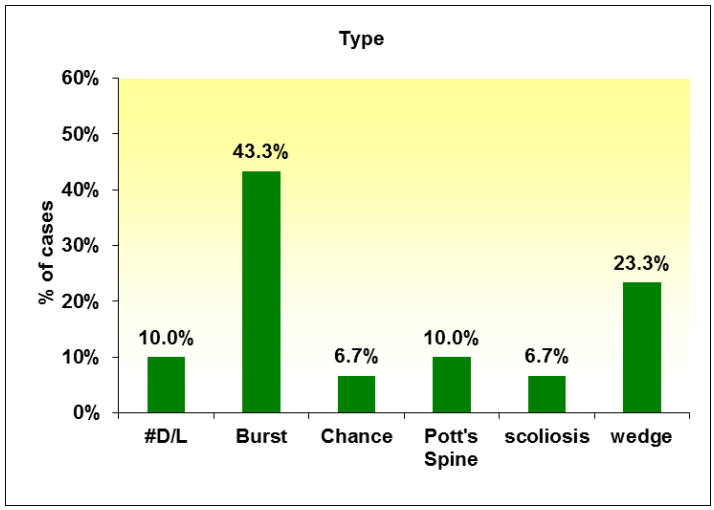


Figure.3: Distribution of patients under study according to different types of spine pathology

Table 4: Distribution of patient under study according to level of vertebra involved

Level	Frequency	%
Dorsal	11	36.7%
Dorsal Lumbar	12	40.0%
Lumbar	7	23.3%
Total	30	100%

In our study of 30 patients 11(36.7%) patients had at Dorsal level, 12(40.0%) had at Dorsal Lumber and 12(40.0%) patients had at Dorsal Lumber.

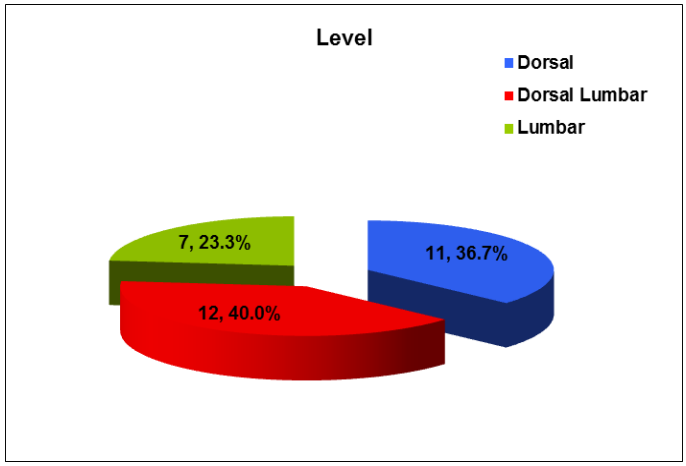


Figure.4: Distribution of patients under study according to vertebral level involved.

Table 5: Distribution of patient under study according to deficit.

Deficit	Frequency	%
Intact	3	10.0%
Paraparesis	5	16.7%
Paraplegia	22	73.3%
Total	30	100%

In our study of 30 patients 3(10.0%) patients had Intact deficit, 5(16.7%) patients had Paraparesis deficit and 22(73.3%) patients had paraplegia deficit.

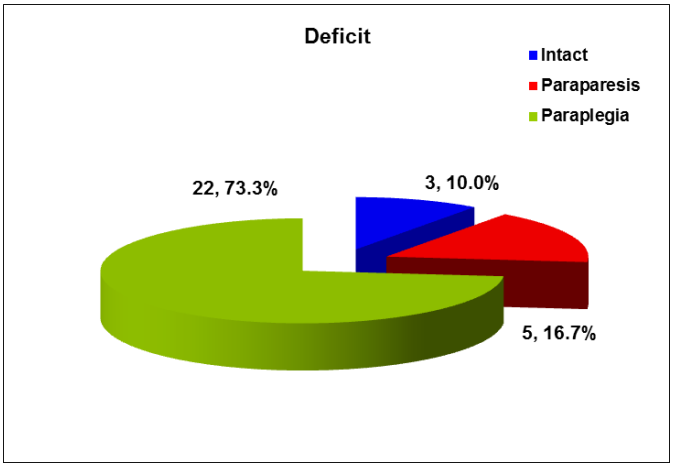


Figure.5: Distribution of patients under study according to neurological deficit

Table 6: Distribution of patient under study according to VAS

VAS	Mean ± SD	Min - Max	P value
Pre - op	7.70 ± 2.037	1 - 9	<0.001*
6 weeks	5.0 ± 1.232	2 - 7	
12 weeks	3.33 ± 0.994	1 - 5	
24 weeks	1.87 ± 0.86	0 - 3	

In our study during 24 weeks we had found that the mean value of 7.70 of VAS with standard deviation of 2.037 varies between one to nine in pre-op period, the mean value of 5.0 of VAS with standard deviation of 1.232 varies between two to seven during pre-op to 6 weeks of period, the mean value of 3.33 of VAS with standard deviation of 0.994 varies between one patient to five patients during 6 weeks to 12 weeks of period and the mean value of 1.87 of VAS with standard deviation of 0.86 varies between zero patient to five patients during 12 Weeks to 24 weeks of period .

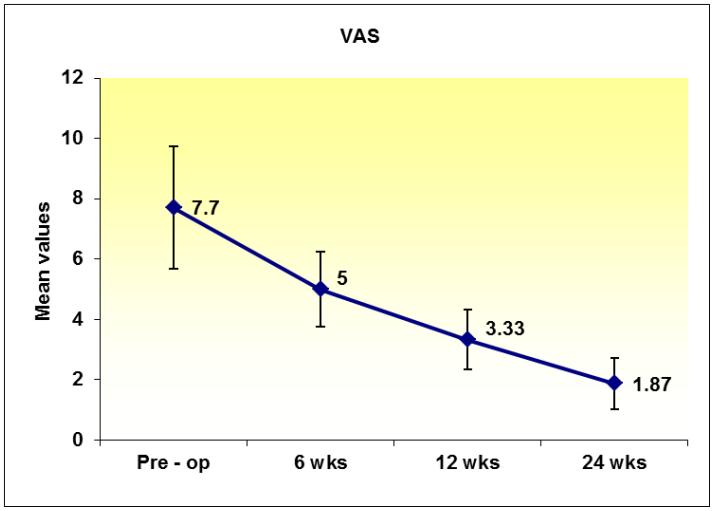


Figure.6: Graph of VAS during different time period of follow up

Table 7: Observations of fusion mass on radiological examination on follow up

	Fusion			
	Seen	**%**	**Not Seen**	**%**
After 6 weeks on x-ray	0	0.0%	30	100.0%
After 12 weeks on x-ray	2	6.7%	28	93.3%
After 24 weeks on x-ray	17	56.7%	13	43.3%
After 24 weeks on CT Scan	19	63.3%	11	36.7%

In this study no fusion seen on x-ray after 6 weeks .After 12 weeks fusion mass seen in 2 cases (6.7%) on x-ray. After 24 weeks on x-ray ,fusion mass seen in 17 cases(56.7%) and after 24 weeks fusion mass seen in 19 cases(63.3%) on CT-scan

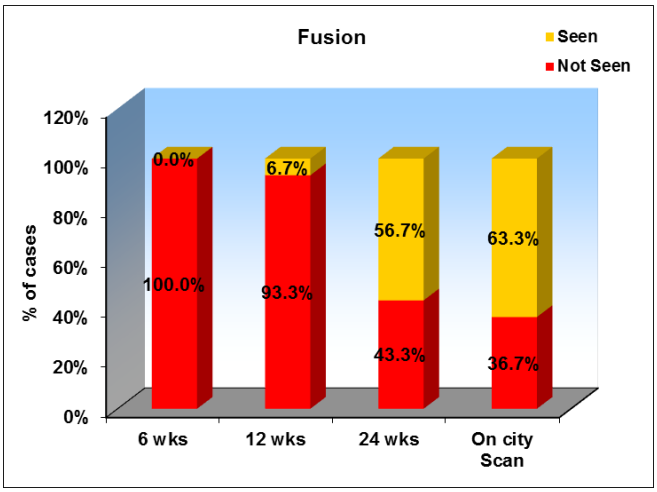


Figure.7: percentage distribution of patient based on Fusions seen or Not seen on X-ray and CT Scan at follow-up

Table 8: Comparison between VAS and fusion seen or not with P value

	Fusion				P value
	Seen	Mean VAS ± SD	Not Seen	Mean VAS ± SD	
6 weeks	0	-	30	5.0 ± 1.232	-
12 weeks	2	3.39 ± 0.916	28	2.50 ± 2.121	0.658
24 weeks	17	2.46 ± 0.776	13	1.41 ± 0.618	<0.001

In our study comparison between VAS and fusion mass was made, which shows mean VAS with standard deviation at 12 weeks and 24 weeks were 3.39 ± 0.916 and 2.46 ± 0.776 in favour of 2 and 17 cases seen respectively.

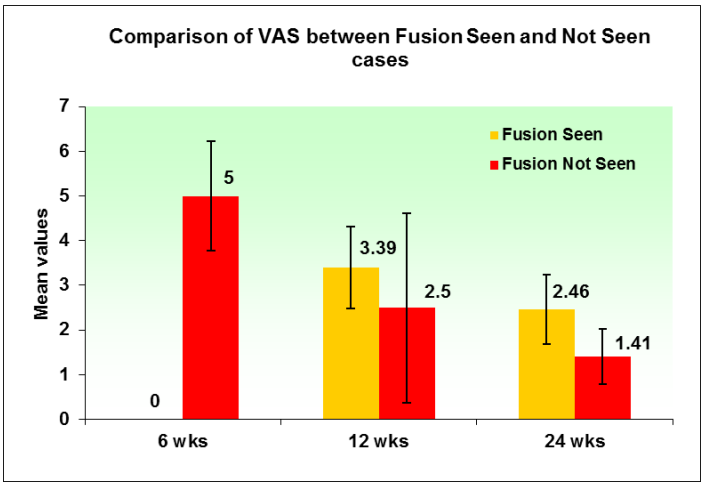


Figure.8: Comparison between VAS and fusion seen or Not during follow-up

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