MATERIALS AND METHOD

Study Design:

This Prospective randomized study for assessment of the clinical effect of platelet rich plasma in Supraspinatus Tendinopathy was conducted in the Department of Orthopaedics, Department of pathology and Transfusion medicine of Acharya Vinoba Bhave Rural Hospital, Wardha, from September 2012 to August 2014.

All the patients who came to Outdoor clinic of the Department of Orthopaedics with shoulder pain were evaluated for the study. Total 146 patients came with the complain of shoulder pain in OPD out of which 100 patients were included in the study who fulfilled the inclusion criterion. The diagnosed as Supraspinatus Tendinopathy was made primarily on the basis of clinical examination and confirmed by Ultrasonography of the shoulder. Fifty patients were assigned to Group PRP in which they received single shot of 2 mL of autologus Platelet Rich Plasma. Other fifty patients were kept in Group MP and treated with local infiltration of 40 mg of single shot of Methylprednisolone. The patients were randomized through lottery system and recruited for the type of infiltration. The patients who did not fulfill the criterion were not included in the study. Independent observers followed all the cases to eliminate observer related bias.

Randomization:

Randomization was done using lottery system

Inclusion criteria:

1) Patient of either sex between age group of 18- 65 years reporting to OPD of AVBRH, Orthopaedics Department.
2) Patient who were clinically diagnosed as Unilateral Supraspinatus Tendinopathy.
3) Acute or sub acute tendinopathy <12 weeks

4) Jobe Test positive with Pain and weakness

Exclusion criteria:

1) Patient with bilateral pathology

2) Patients with Acromioclavicular arthritis or complete rotator cuff tear.

3) Generalized restriction of movements at the shoulder joint.

4) Patients who had negative ultrasonographic findings

5) Patients who had taken breakthrough anti-inflammatory on his own.

6) Patients with neoplastic disease, diabetes mellitus

7) Patient with Platelet dysfunction syndrome, or critical thrombocytopenia.

8) Patients in whom steroid was contraindicated.

9) Patients with Local infection at the site of the procedure.

10) Patients with Platelet count < 105/u.

Protocol and technique

All the patients with shoulder pain were evaluated thoroughly for his/her shoulder, cervical spine and other relevant musculoskeletal pathology. (Annexure I)

Clinical tests for the supraspinatus tendon

Clinical tests were the Jobe and full-can tests. The Jobe test, also known as the empty-can test, was performed with the arm at 90 degrees of abduction, 30 degrees of forward flexion and full internal rotation with the thumbs down (Fig. 1). The full-can test was performed with the same position as the Jobe test except for a 45-degree lateral rotation of the arm (Fig. 1B). For these 2 tests, both pain and weakness against resistance when the physician pulled down on the arm were response criteria.
The patients who had Jobe test response positive for pain and weakness were further underwent Ultrasonographic examination. The relevant ultrasonographic findings were documented. (Annexure III)

![Jobe's Empty Can Test](image)

Figure 7: Jobe’s Empty Can Test

**Ultra Sono Graphic examination**

Additionally, the radiologists determined whether tendinosis, partial- or full-thickness tears, calcific tendinosis, subdeltoid bursitis, and enthesopathic changes were present. Tendinosis was defined as a heterogeneous echo texture with or without thickening of the tendon, a full-thickness tear as discontinuity or nonvisualization of the tendon, a partial tear as a well-defined hypoechoic defect affecting either the bursal or articular surface, calcific tendinosis as focal intrasubstance hyperechogenicity with or without shadowing, subdeltoid bursitis as hypoechogenicity of greater than 2 mm thick situated between the tendon and the peribursal fat stripe, and enthesopathic changes as cortical irregularities manifested by either bony productive or cystic appearances. The patient were classified according to their US finding and correlating with the clinical picture.
### Observations and Results

**Classification of the Supraspinatus Tendinopathies**

<table>
<thead>
<tr>
<th>CLASSIFICATION OF SUPRASPINATUS TENDINOPATHIES</th>
<th>Thickness</th>
<th>Echotexture</th>
<th>Tear/Bursitis</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade 0:</strong></td>
<td>Normal thickn</td>
<td>Normal</td>
<td>No Tear</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>ss 5.9 ± 0.3mm</td>
<td></td>
<td>No Bursitis</td>
<td>ROM</td>
</tr>
<tr>
<td><strong>Grade I:</strong></td>
<td>Increase thickness</td>
<td>Normal</td>
<td>No tear</td>
<td>Pain with and after activity &gt;48 hours</td>
</tr>
<tr>
<td></td>
<td>6.3-6.6 mm</td>
<td></td>
<td>No bursitis</td>
<td>Normal ADL</td>
</tr>
<tr>
<td><strong>Grade II:</strong></td>
<td>Increase thickness</td>
<td>Mild</td>
<td>No tear</td>
<td>Pain with activity and slightly affect ADL</td>
</tr>
<tr>
<td></td>
<td>6.7-7.0 mm</td>
<td>Heterogeneous</td>
<td>Mild fluid around bursal surface</td>
<td></td>
</tr>
<tr>
<td><strong>Grade III:</strong></td>
<td>Increase thickness</td>
<td>Marked</td>
<td>partial tear &lt;2mm of bursal or articular, Moderate bursitis</td>
<td>Pain with activity and markedly affect ADL</td>
</tr>
<tr>
<td></td>
<td>Varying</td>
<td>Heterogeneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grade IV:</strong></td>
<td>Varying thickness</td>
<td>Intrasubstance collection of fluid</td>
<td>Complete tear bursitis</td>
<td>Pain at rest, affected ADL, and Sleep disturbed</td>
</tr>
<tr>
<td><strong>Grade V:</strong></td>
<td>Varying thickness</td>
<td>Intrasubstance collection of fluid</td>
<td>Calcified atrophy</td>
<td>Pain at rest, affected ADL, and Sleep disturbed</td>
</tr>
</tbody>
</table>

*Developed by author, guide and senior radiologist*
P.R.P Preparation

Armamentarium:

1. Digital Centrifugation Machine (REMI Motors Ltd.)
2. Test tubes containing CPDA anticoagulant solution (Borosil company)
3. Test tubes (Borosil company)
4. Anti Coagulant Solution (CPDA)
5. Spinal needle 18 gauge, 78 mm long.
6. Tourniquet
7. Disposable gloves
8. Disposable syringes (10ml, 20 ml, 50ml)

1\textsuperscript{st} STEP: Collection of blood.

Twenty ml of blood was drawn through an anticubital venepuncture containing CPDA anticoagulant solution.

2\textsuperscript{nd} STEP: Preparation of PLATELET RICH PLASMA

The tubes were placed in centrifuge machine and were counter balanced. The first centrifuge cycle was done at 1200 rpm for 10 minutes (Soft spin).

The result was separation of the whole blood into a lower red blood cell region and upper straw colored plasma. This plasma contains relatively low concentration of platelets (Platelet poor plasma) in the uppermost region and higher concentration of platelet in the boundary layer often called as “Buffy Coat”.

The tubes were placed in a rack with their top removed. An equal number of 6 ml tubes without anticoagulant solution were also placed in the rack with their top removed.

A spinal needle (18 gauge and 78 mm long) was attached to 5 ml syringe to withdraw straw coloured plasma from the tubes by moving the needle from top downward as the draw continues.

The draw stopped when a RBC layer was reached or in to the first 1 – 2 mm of that layer. The straw coloured plasma was then expressed into the tube without anticoagulant solution. This process was done gently so as not to damage the platelets. The same procedure was performed with the other tubes. The tubes with straw coloured plasma were again centrifuged at 2000 rpm for 10 minutes.
The tubes were again placed in the rack with their top removed. Now the contents of the tube consist of upper layer of clear supernatant serum containing fibrinogen and very low concentration of platelets. The bottom layer often red tinged consists of highly concentrated platelets.

The upper two third of this liquid was withdrawn with same spinal needle attached to 5 ml syringe leaving behind one third of the serum with concentrated platelets in the tube.

This remaining one third content of the tube was thoroughly mixed to form red coloured concentrated platelet rich plasma.

If multiple patient P.R.P are prepared concurrently, proper labeling of each P.R.P should be completed to ensure no cross contamination or the graft being used on the wrong patient.

Local Infiltration of PRP under USG Guidance

Infiltration Technique: Under all aseptic precaution

Contraindications

Before local corticosteroid injection is administered we identified the possible contraindications and to examine the documentation provided by the patients. These are the main contraindications and the authors’ approach to them:

- Systemic or local infection in progress. Absolute contraindication because of the risk of blood-borne infectious bursitis.
- Diabetes. Relative contraindication. Usually local corticosteroid injection is administered using a delayed-release form of steroid and reducing the dose to
50%; the patient is requested to check blood sugar every day for a week after the injection. In case of doubt the diabetes specialist should be consulted.

- Treatment with anti-coagulant or anti-aggregation drugs. Relative contraindication. If the treatment is well-controlled (adequate International Normalized Ratio (INR) values), local corticosteroid injection is generally well tolerated. The authors never take the initiative to suspend anti-coagulant treatment but prefer contacting the patient’s general practitioner to obtain his/her opinion on the possible need to temporarily suspend the treatment. If the general practitioner consents, local corticosteroid injection is carried out with particular care to avoid adjacent vessels using a 27 gauge needle in order that the injection causes as little damage as possible.

- Allergic reactions to disinfectants (including iodine) and local anesthetics. This phenomenon rarely occurs; the solution is to use products which are well-tolerated by the patient.

- Previous corticosteroid injection. The authors do not administer more than 3–4 corticosteroid injections per patient per year.

After carefully evaluating possible contraindications and after having studied previous images presented by the patient, a complete US examination of the affected shoulder is carried out. If US examination precedes local corticosteroid injection or platelet rich plasma, the usual US gel is substituted by a colorless liquid disinfectant in order to allow the skin to be in contact with the disinfectant during the whole procedure.
Pre-procedure considerations

- The specific indication correlated with physical examination and confirmed with imaging studies viz. ultrasound prior to treatment.
- Appropriate patient education and discussion with an informed consent signed prior to the initiation of the procedure.
- Contraindications to the procedure were reviewed prior to initiation of procedure.

Ultra Sonographic guided local corticosteroid injection/PRP technique

The following material is required:

- Sterile Gauze
- Sterile gloves (not considered essential if the infiltration is done correctly according to the rules indicated below). The gloves are intended to protect the physician rather than the patient. Surgical mask.
- Skin disinfectant.
- 30–70 mm long needles, caliber ranging from 21 to 27 gauge.
- Local anesthetic. We prefer a 1% lidocaine solution.
- Methyl prednisolone 40 mg or platelet rich plasma 2 mL.

Principle of Techniques:

1. Most of the Ultra Sonographic guided corticosteroid injections into the bursal surface of supraspinatus tendon performed in the USG room of department are carried out with the patient sitting on the couch with his/her back turned to the clinician. This technique is particularly useful in anxious patients because the handling of syringes and needles takes place outside the patient’s field of view, thus reducing anxiety.
2. It is important to perform US examination in different positions in order to identify the position in which the fluid tends to collect. In this position it is easier to puncture the fluid-filled bursa. By performing a static and dynamic study, the shoulder is examined in different degrees of rotation so that the fluid is accumulated according to the pressure changes exerted on the supraspinatus and rotator cuff.

3. If there is no fluid collection but a thickening of the bursal wall, or supraspinatus tendon changes the injection should be directed toward the pathological portion of the subacromial-supraspinatus bursal surface. When the most adequate position of the shoulder has been identified, the patient is asked to keep this position while the US probe and skin are disinfected.

4. With the probe in one hand and the syringe in the other, the skin is punctured after application of more disinfectant. The skin is always punctured at a distance of about 2–3 cm from the probe in order to avoid contact between the needle and the probe. As soon as the needle has penetrated the subcutaneous tissues, their progresses were real-time monitored on the US image. When the US beam is perpendicular to the long axis, the needle is visible as a hyperechoic structure with posterior comet-tail artifact.

5. We used a 5 cm long needle caliber 23 Gauge. A local anesthetic (lidocaine) can be associated for an analgesic effect. As a protocol we injected a dose of cortisone mixed with 2–3 cc of 1% lidocaine solution.
Figure 8- Picture showing position of ultrasonographic probe in longitudinal and transverse plane. Normal supraspinatus tendon in both views.

After infiltration of the platelet rich plasma or Methylprednisolone we document the ultrasonographic findings during procedure. Visual analogue score and disability of the arm, shoulder and hand score were noted in subsequent follow up. (Annexure II)
CASE I

A case of 26 year old house wife had excruciating left shoulder pain restricting her movements of the shoulder specially abduction. Jobe empty can test was responsive as pain.

The patient was treated with local infiltration of 2 mL platelet rich plasma. Final follow up showed improvement in VAS score, external rotation and abduction. Her DASH score improved significantly.
Observations and Results

Pre Injection Ultrasonography revealed increased thickness of the supraspinatus tendon without any bursitis. Tendon substance appeared to be mild heterogenic and hence grade as Grade I supraspinatus tendinopathy.

6 months Post injection USG of the same patient revealed isoechoic substance of supraspinatus tendon with normal thickness of the tendon.

Fine Needle aspiration cytology was done after 7 months of infiltration of platelet rich plasma and revealed tendon fibres with reacting tenosynovial cell activity along with fibro collagenous material. (Giemsa x 40x)
CASE II

Clinical picture of 47 year old male labourer had left shoulder pain since 4 weeks. His abduction and external rotation were grossly restricted.

The patient was treated with local infiltration of 2 mL platelet rich plasma. Final follow up showed improvement of in VAS score, external rotation and abduction. His DASH score improved significantly.
Pre infiltration ultrasonography revealed altered echoic pictures and thickening of tendon with associated bursitis and fluid collection around bursal surface of tendon. A tear observed on the bursal surface of the tendon. Grade III type of supraspinatus tear.

Follow up ultrasonography showed improved echoic pictures and thickening of tendon with associated mild bursitis. No fluid collection around bursal surface of tendon were noted.

Histopathology tendon biopsy – shows tenosynovial fibres with little disarraying with proliferating capillaries (neovascularization) suggesting repair phenomenon. (H & E, 40X)
CASE III

A 40 year old male primary school teacher was heaving pain and remarkable disability on his right shoulder. His abduction and external rotation were grossly restricted.

The patient was treated with local infiltration of 2 mL platelet rich plasma. Final follow up showed improvement of in VAS score, external rotation and abduction. His DASH score improved remarkably.
Pre injection ultrasonography of the right shoulder revealed increased thickness, marked heterogeneous of tendon and partial tear of the bursal surface of supraspinatus tendon with moderate bursitis. Grade III supraspinatus tendinopathy.

The final follow ultrasonography showed no signs of bursitis and the tendon substance showed homogeneous and isoechoic on ultrasound. However, thickness remains same.

Histopathology of supraspinatus tendon – of tendinous fibre disoriented for its placement appearing like spikules along with area of a little hyalinization suggestive of reactive fibrosis and healing of the tendon. (H & E 40X)
Plate IV

The USG guided biopsy of the supraspinatus tendon was taken using Biopsy Gun.
Hematoxylin and eosin stain of supraspinatus tendon adjacent to the altered echogenic site on USG in a 46-year-old labourer male who presented with pain over the shoulder since 4 weeks not responding to conservative management. Histopathology supraspinus tendon show reactive spindle cells oriented along the fibres along with mononuclear cell infiltrate suggesting late inflammatory cellular features.
(Not included in the study)

Fine Needle Aspiration Cytology of the different patient who received platelet rich plasma 8 months back for grade II supraspinatus tendinopathy shows reactive fibrocytes, teno synovial cells and fibro collagenous substance.