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Treatment of AVN Using Autologous BM Stem Cells and Activated Platelet-Derived Growth Factor Concentrates

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Abstract

Background: Avascular Necrosis (AVN) of hip is a devastating condition seen in younger individuals. It is the ischemic death of the constituents of the bone cartilage of the hip. The femoral head (FH) is the most common site for AVN. It results from interruption of the normal blood flow to the FH that fits into the hip socket. Earlier studies using autologous bone marrow stem cell concentrate injections have shown encouraging results with average success rates. The current study was designed to improve significantly the cartilage regeneration and clinical outcome. *Methods:* Total of 48 patients underwent autologous bone marrow stem cell and activated platelet-rich plasma derived growth factor concentrate (PRP-GFC) therapy for early and advanced stages AVN of femoral head in a single multi-specialty center. The total treatment was divided into three phases. In the phase I, all the clinical diagnostic measurements such as magnetic resonance imaging (MRI), computed tomography (CT) etc. with respect to the AVN patients and bone marrow aspiration from posterior iliac spine from the patients were carried out. In the phase II, isolation of stem cells and preparation from the patients were performed. Subsequently, in phase III, the stem cells and PRP- GFCs were transplanted in the enrolled patients.
Results: Ninety three percent of the enrolled AVN patients showed marked enhancement in the hip bone joint space (more than 3mm) after combined stem cells and PRP-GFC treatment as evidenced by comparison of the pre- and post-treatment MRI data thus indicative of regeneration of cartilage. The treated patients showed significant improvement in their motor function, cartilage regrowth (3 to 10mm), and high satisfaction in the two-year follow-up.
Conclusion: Combination of stem cell and PRP-GFC therapy has shown promising cartilage regeneration in 45 out of 48 patients of AVN. This study clearly demonstrates the safety and efficacy of this treatment. Larger numbers of patients need to be evaluated to better understand the efficacy of the combined stem cell and PRP-GFC therapy on AVN patients.

Keywords: Avascular Necrosis, Platelet Rich Plasma - Growth Factor Concentrate (PRP-GFC), Femur Head, Hip, Stem Cells, Cartilage

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Article Summary

Article Focus

- Development of an autologous stem cell, point of care, minimally manipulated, method for treatment of AVN.
- Renewal and regrowth of the femoral head shape in AVN patients.
- Renewal or regeneration of the cartilage in the hip joint.
- Development of a minimally invasive procedure as a potential alternative to joint replacement surgeries.

Strengths and Limitations of this Study

- The strength of the study is based on the number of cases we have treated with the unique protocol using BM stem cells plus autologous PRP-GFC. The study establishes that irrespective of the stage of the avascularity, it is possible to regenerate or "replenish" the cartilage with very high success rates. We now know that whether it is stage II, III or IV, and even with the collapse of the femoral head, it is possible to generate the cartilage which gives better gait, improved functionality and a pain free joint.
- The current study was open labeled, unblended and without control groups.

Introduction

AVN is frequently referred to as osteonecrosis, ischemic bone necrosis, or aseptic necrosis. It is a disease of subchondral bone resulting from damaged osseous circulation and/or direct impairment of bone-forming cells. According to the National Institutes of Health (NIH), around 10,000 to 20,000 cases of AVN are reported in the US alone each year. It was reported that around 5% to 18% of 500,000 total hip arthoplastic surgeries were related to the advanced stages of AVN each year [1]. Therefore, the preservation of the femoral head is the objective of diagnostic and treatment strategies [2].

AVN of the hip can occur due to severe trauma, malignant diseases, infections and roentgen therapy. Vast majority of hip AVN are idiopathic, these are confirmed in our results section [Tables 1 and 2]. AVN of the hip is a painful condition where the supply of blood to the femoral head or the hip bone is lost. As a result, it causes death of the bone tissue. Often, this leads to the breakage of the bone and subsequently collapses the femoral head. Final resort is to replace the hip joint that is not always desirable in younger patients.

In an attempt to save natural hip joint in young patients, many hip joint preserving surgeries were designed with limited results. These surgeries include core decompression [3-6], core decompression bone grafting with fibula or vascularized fibula [7], and femoral osteotomies [8]. Lately, stem cell therapy has given promising and long lasting results [9-11] in the treatment of AVN of the femoral head.

Early detection of hip AVN is critical, as all the treatments aiming towards preservation of the femoral head have been more successful in the early stage of the disease progression. There are multiple modalities for diagnosis of osteonecrosis of femoral head. Among all the imaging tests, MRI has been found to be most sensitive means for the diagnosis of AVN [12]. At high magnetic field strength, MRI is more sensitive than CT scanning or nuclear scintigraphy, and is much more sensitive than X-ray diagnosis for detecting AVN. It has been reported that MRI has the specificity of 100% and sensitivity of 90-100% in the diagnosis of osteonecrosis [13]. MRI is beneficial especially in early diagnosis of this disabling condition [14].

As the collagen component of articular cartilage breaks down, water in the cartilage becomes more mobile and results in a prolongation of T2 relaxation times [15-18]. Cartigram (T2 relaxation time mapping) is a validated, non-invasive tool to visualize changes in the composition of cartilage, in some cases before changes in thickness can be seen. Cartigram is a non-invasive imaging technique that is ideal for the early evaluation and assessment of cartilage breakdown, particularly to assess articular cartilage integrity (see legends of Figures 1C, 2C and 3C). It allows better visualization of collagen fibre network loss or degradation. Cartigram provides the radiologist with better diagnostic confidence and the orthopaedic surgeon with the information needed to determine course of treatment, possibly to optimize the timing of a surgical procedure. It also helps with

monitoring the effectiveness of treatment non-invasively, potentially eliminating a "second-look" biopsy.

There are several classification systems that can be used in staging of avascular necrosis on the femoral head. The most commonly used type of classification is the Ficat and Arlet staging system [2, 19]. In this research work, we have utilized Mitchell classification as shown in our results [Table 3]. The Association Research Circulation Osseous (ARCO) system has incorporated the Ficat and Arlet in grading AVN [3]. The Ficat and Arlet staging system do not consider the extent of the necrosis but are based on the radiological findings [20]. The classification system uses a combination of plain film, MRI, and clinical features.

A variety of methods are now used to treat AVN; the most common being the total hip replacement (THR). However, there is a general consensus among orthopedic surgeons that stem cell therapy for AVN is the most promising conservative biologically and physiologically autologous therapy after total hip replacement (THR) [8, 21]. Moreover, total hip replacements have limitations such as long recovery time and short life span of the replacements. Hence, adult bone marrow-mononuclear stem cells, due to its angiogenic and osteogenic properties, have great therapeutic potential in these ailments of AVN patients which are difficult to treat with existing modalities of treatment.

PRP therapy is the one of the treatments that comes under the practice or purview of "regenerative medicine". PRP is typically made from patients' blood and is autologous in nature. It involves concentrating the platelets obtained from patients' blood by centrifugation and thus represents a safe, economical, easy to prepare cellular therapy [22].

PRP is a rich source of various growth factors that are released from activated platelets. Interestingly, numerous growth factors have been shown to have specific activity on cartilage regeneration. About 36 growth factors released from activated platelets initiate and modulate wound healing in both soft and hard tissues [23-25]. Thus PRP-GFC introduction can lead to cartilage regeneration significant reduction of pain and can also improve joint function.

In order to investigate the role of combined BM-SC and PRP-GFC therapy on AVN patients, in this manuscript, we report the overall success of our treatment method and also depicted individualized case studies successfully treated by BM-SC and PRP in patients with AVN.

Materials and Methods

Study Design

The study was carried out between March 2010 and August 2013 with subsequent 2 years follow up. . Total 48 patients underwent stem cell and PRP therapy for early stage AVN of femoral head in a single multi-specialty center. The study had ethical approval and all patients were prospectively enrolled as per the inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

Patients were enrolled with symptomatic early AVN in stage II, III and IV as determined by Ficat and Arlet grading system. Patients with advanced AVN such as in Ficat stage I were excluded from the study.

The total treatment was divided into three phases. In the phase I, all the clinical diagnostic measurements such as magnetic resonance imaging (MRI), computed tomography (CT) etc. with respect to the AVN patients and bone marrow aspiration from posterior iliac spine from the patients were carried out. In the phase II, isolation of stem cells and preparation from the patients were performed. Subsequently, in phase III, the stem cells and PRP-GFCs were transplanted in the enrolled patients.

Stem Cell Treatment

Total 48 patients underwent stem cell and PRP therapy for early stage AVN of femoral head in a single multi-specialty center. Proper informed written consent forms were obtained before the bone marrow procedures from all the patients. Bone marrow aspiration (80 ml) from posterior iliac spine was carried out in the operation theatre.

The patients were pre-screened for infectious disease markers such as HIV, HCV, HBV, CMV, and syphilis. Bone marrow-derived mononuclear cells (BM-MNCs) with its constituent stem cells were separated from blood by the density gradient centrifugation using Lymphoprep™ according to established Standard Operating Protocol/Procedure (SOP). Three infusions of BM-MNCs in the interval of one month were used for each AVN patients.

Preparation of PRP-GFCs

About 10 ml of blood was drawn from each patient in two sterile vacutainer tubes with anticoagulant and then the tubes were placed in a centrifuge. The blood is then centrifuged at 2500 revolutions per minute (rpm) for 10 min to separate the components based on variations in specific gravity. Three layers are obtained, the bottom layer is comprised of red blood cells (RBCs) which are discarded, the middle layer (buffy coat) consists of white blood cells and platelets, and the top layer is the plasma containing fraction. The top and middle layers are collected. After a second softer spin centrifugation at 2000 rpm for 10 min, three layers were obtained; (a) yellow colored acellular plasma in the upper layer, (b) middle Buffy coat and (c) lower layer of remaining red colored RBCs. The upper and middle part containing platelets (platelet rich plasma – growth factor concentrate, PRP-GFC) was subsequently activated by calcium gluconate, 50mg/10ml diluted to 5%, Harson Labs, Vadodara, India. Calcium chloride is also a substitute for calcium gluconate to add to the resultant pellet of platelets and the plasma, inducing platelet activation and release of alpha granule contents.

Stem Cell Preparation Method

The human bone marrow derived mononuclear cells (BM-MNCs) were isolated from 80 to 90ml of bone marrow aspirate using density gradient centrifugation method. The aspirated human bone marrow was diluted at a proportion of 7:1 with a solution containing sterile phosphate-buffered saline (PBS), pH7.2, and 2mM EDTA, kept at 2−8°C. Cells were passed through a strainer (40 µm) to remove bone fragments and cell clusters. Diluted cell suspension of 35 mL was carefully over-layered on 15 mL of Ficoll-Paque in a 50 mL conical tube, centrifuged at 2200 rpm for 10 min at room temperature (RT, 23°C) in a swinging bucket rotor without brake. The upper layer was aspirated leaving the mononuclear cell layer undisturbed at the interphase. The BM MNCs at the interphase were then transferred to a new 50 mL conical tube and the cells were washed by adding up to 40 mL of buffer, mixed gently and centrifuged at 1800 rpm for 10 min at RT. The BM-MNC pellet was resuspended in an appropriate amount of buffer for further usage. Finally, 200 million cells were resuspended in 4 ml of PRP-GFC per injection.

Operation Technique

The following protocol was used to surgically treat all the patients of AVN. We implanted 200 million autologous BM-MNCs along with PRP-GFC obtained from the patient at the point of treatment.

All patients were prepared under epidural anesthesia, positioned in supine and the affected core-decompression was done making sure that the decompression was verified with image intensification. This is performed to confirm that all the cystic cavities created by the effect of AVN were addressed by subjecting to multiple small core decompression using a 3.2mm drill bit the core-decompression holes were sealed with nano-crystalline hydroxyapatite paste.

Cartigram Interpretation

As seen in the cartigrams (Figures 1C, 3C and 5C), the areas marked on the density scales in red indicate the least cartilage presence or the cartilage is in a non-hydrated state. The areas marked in blue indicate the highest cartilage presence or the most hydrated cartilage state. In this case, it shows the quantity of cartilage infused or that has regenerated after stem cell therapy. There are two scales marked from 0-38 and 39-161 in the alternating images, the former lower scoring scale (0-38) set for lack of visible colors and the latter higher scoring scale (39-161) set for visualizing the colors.

Coloured Arrows in the Image Figures

The blue arrows show the cartilage positions post-therapy only in the cartigrams (not applicable to MRIs).The areas pointed by red arrows show loss of cartilage and arrows in green colour show cartilage

that is regenerated post-therapy, in the MRIs. If the red arrow is touching the bone, it indicates that the whole cartilage is lost.

Results

Overview of AVN Patients Admitted and Treated by Surgical and Cellular Interventions

A total of 48 cases were enrolled and studied. The youngest patient was 18 years and the oldest patient's age was 57 years; average age of the patients being 38 years. 42% of the patients were females. More than 70% of the patients had a history of long-term steroid intake for various illnesses they were being treated. In about 20% of the cases, alcohol abuse, smoking and minor trauma were the reasons according to the history that might have caused AVN. About 10% of the cases, the cause was idiopathic.

Among the 48 cases, 67% of the patients were in stage III (Ficat staging), 11% had stage IV and the balance patient number was in stage II. 99% of the cases were of bilateral AVN. The minimum cartilage regeneration was 1.6 mm in a patient with stage III AVN and the highest was 10mm. Some of the initial failures were seen in patients who did not follow the post-operative protocols. The patient at the age of 54 who was treated was not able to continue with exercise and abstain from alcohol for more than 3 months following the treatment. Eventually, 3 out of 48 cases went for total joint replacement.

Table 1. Mitchell classification of avascular necrosis [ref. 26]: A = mild, B = moderate, C = severe

Case	Diagnosis	Unilateral/ Bilateral	Mitchell Classification	
			Left Hip	Right Hip
Case 1	Avascular Necrosis	Bilateral	Class C	Class A
Case 2	Avascular Necrosis	Bilateral	Class C	Class C
Case 3	Avascular Necrosis	Bilateral	Class C	Class C

Table 2. Mitchell classifications as seen in the MRIs are visualized by the signal intensity. The classifications arrived at in Table 1 are derived from these signal intensities

Mitchell's MRI Staging			
Class	T1	T2	Definition
A	Bright	Intermediate	Fat Signal
B	Bright	Bright	Blood Signal
C	Intermediate	Bright	Fluid or Oedema Signal
D	Dark	Dark	Fibrosis Signal

Table 3. Cartilage regeneration post-stem cell therapy (SCT) in combination with platelet rich plasma (PRP) therapy of patients diagnosed with avascular necrosis (AVN). The clinical condition of AVN remains assigned to the affected patients despite the cartilage development and its/their hydration as seen in the cartigram Figures 1C, 2C and 3C. Hence, the "fraying and fissuring" in the prognosis column is not a negative indication of the success of the SCT+PRPT but a clinical condition that has been superseded by the positive results of these advantageous and therapies with no implantation devices

Case	Cartilage Thickness (in mm)		Bone Marrow Oedema		Prognosis	Remarks
	Left Hip Joint	Right Hip Joint	Left Hip Joint	Right Hip Joint		
Case 1	3.3	2.6	Minimally present	Not present	Extensive cartilage seen in left hip joint with focal areas of fraying and fissuring bilaterally	Minimal joint effusion on the left side and minimal asymmetrical joint space reduction on both sides
Case 2	10	10	Not present	Not present	Extensive cartilage seen in both hip joints with focal areas of fraying and fissuring	No evidence of joint effusion bilaterally and minimal asymmetrical joint space reduction, marginal osteophytes and loose bodies bilaterally
Case 3	10	10	Minimally present	Not present	Extensive cartilage seen in both hip joints with focal areas of fraying and fissuring	No evidence of joint effusion bilaterally and minimal asymmetrical joint space reduction noted in left side

In patients with no pain, the Harris Hip Score (HHS) that is a functional measurement of hip strength and its symptoms (maxed out at 100) was measured to be 88. The higher the HHS less is the dysfunction. In patients with occasional pain and minimum limp, the score was 62. In the 2 cases, pain continued with the limp and the hip score was 20. Pre-treatment HHS < 20 led to the diagnosis of this disease and subsequent therapy for the 48 cases as described.

In 15% of the cases in stage III and stage IV of AVN, we noticed the re-modeling of femoral head along with cartilage function. In all the cases, the chondrocytes had covered both articular surfaces with minor fraying in some focal area due to severity of degenerative changes in the femoral head. Most patients complained of pain in the mid-inguinal region and pain down the anterior aspect of the radiating thigh was due to the capsular stretch caused by the increased synovial effusion that eventually got absorbed with increased activity and the pain subsided in all of them.

Case-by-Case Cellular Interventional Study

48 AVN patients were included for the cellular interventional studies and among them 3 patients exhibited noticeable improvements in terms of disease management and progression. Individualized case studies are described below:

Case 1

Symptoms: The 22 years old female was reported with limited mobility in both right and left hip with pain. She was suffering from SLE and was prescribed with steroidal drugs and its continual usage caused for AVN to develop. She was advised THR for AVN of R/L hip in the year 2011. Investigations revealed no abnormality in metabolic profile.

Clinical diagnosis: Sclerosis of the head of the left femur and acetabular brim is noted likely due to AVN (X-rays, not shown). Flattening of the head is also noted with suspected CDH. Right hip joint appears almost normal with slight sclerosis of the femoral head. SI joints are normal.

MRI scan protocol used was non-contrast T1 and T2 weighted spin echo sequence in axial, sagittal and coronal plane (Figure 1A). STIR sequence was in coronal plane. *Right hip joint*: The head of femur is normal in bulk and has normal articular margin. There is a geographical lesion in head of femur which has signal intensity corresponding to marrow fat and is bordered by a hypointense rim in both T1 and T2 weighted image. Minimal marrow edema is seen in femoral head surrounding the lesion as well as in neck of femur. The acetabular margin is unremarkable. The hip joint space is preserved and no effusion is seen in hip joint. The soft tissue around hip joint is normal. *Left hip joint*: There is mild subchondral collapse of with mild irregularity of its articular margin. There is geographic region in head of femur appearing hypointense in T1 weighted image and hypointense in T2 weighted and STIR sequence. Mild marrow edema is also seen in femoral head around the lesion and neck of femur.

A small subchondral cyst is seen in postero-superior part of the acetabulam with minimal surrounding marrow edema. The articular surface of acetabulum however is smooth and hip joint space is preserved. No effusion is seen in hip joint. The soft tissue around hip joint is normal.

Results post-treatment: Stem cells and PRP combination therapy exhibited marked regeneration of cartilage in the hip of this patient. As shown in Figures 1B and 1C, MRI data clearly revealed noticeable enhancement of joint space (by 3 mm) that was indicative of regeneration of cartilage (Figures 1B and 1C). Evidence of re-arrangement of radiolucent shadow of hip joint indicates some modification of bone adjacent to the hip joint. Dramatically, significant improvements in movements of hip indicate remodeling of degenerated cartilage through redistributive regeneration to yield functional hip joint.

Thus even though the case 1 patient have had a lower grade of regeneration post-therapy (Figure 1) is however able to maintain the levels of cartilage regeneration to-date (Figures 2A and 2B) and thus for extended periods of time which is a promising prognosis and supportive of the therapeutic strategy.

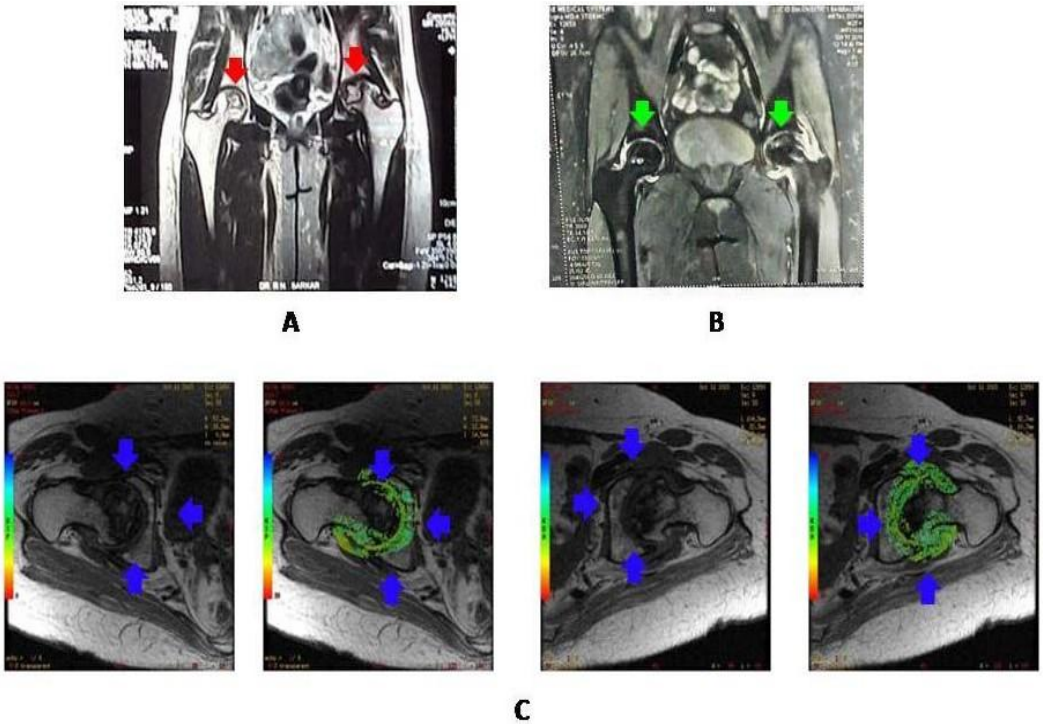

Figure 1. Pre and post treatment cartigrams showing improved cartilage thickness for patient case 1: A (Top Left) - Pre-therapy MRI; B (Top Right) - Post-therapy MRI; C (Bottom 4 images) - Post-therapy cartigrams consisting of alternating images of those with no colors for the lack depiction of hydration within the cartilage and those with colors of cartilage depiction of hydration. Red arrows indicate the location of loss of cartilage and Green arrows show the location of the regeneration of cartilage, respectively, in the pre- and post-therapy MRIs; blue arrows show the cartilage location in the cartigrams

Case 2

Symptoms: The 45 year old male was reported with restricted movement in both right and left hip with extreme pain. He exhibited a marked limp while walking. His range of motion was limited. He was treated conventionally with non-steroidal anti-inflammatory drug (NSAID) and physiotherapy. He was advised THR for AVN of R/L hip in the year 2013. Investigations revealed no abnormality in metabolic profile.

Clinical diagnosis: A 45-year old male patient was presented with focal serpiginous low signal intensity line with fatty center noted in both hip joints involving subchondral regions (osteochondral fragmentation) with 'double line' sign and bone remodeling (Figure 2A). These are hyperintense on T2 Wt and intermediately hyperintense on T1 Wt images. No significant bone marrow oedema in both hip joints. No evidence of joint effusion bilaterally. Minimal asymmetrical joint space reduction, marginal osteophytes and loose bodies bilaterally. Subchondral cyst measuring 21 x 19 mm in superior aspect of right acetabulum was indicated. Cartigrams show extensive cartilage in both hip joints (>10mm in maximum width) with focal areas of fraying and fissuring.Greater and lesser tronchanters are normal. Sacro-iliac joints are normal. No evidence of abnormal signal changes. Muscles surrounding the hip joints are normal. Psoas muscles also appear normal.

Results post-treatment: BM Stem cells and PRP-GFC combination therapy exhibited marked regeneration of cartilage in the hip of this patient. As shown in Figures 3B and 3C, MRI data clearly revealed noticeable enhancement of joint space (by 10 mm) that was indicative of regeneration of cartilage (Figures 3B and 3C). Evidence of re-arrangement of radiolucent shadow of hip joint indicates some modification of bone adjacent to the hip joint. Dramatically, significant developments in movements of hip indicate remodeling of degenerated cartilage through redistributive regeneration to yield a functional hip joint.

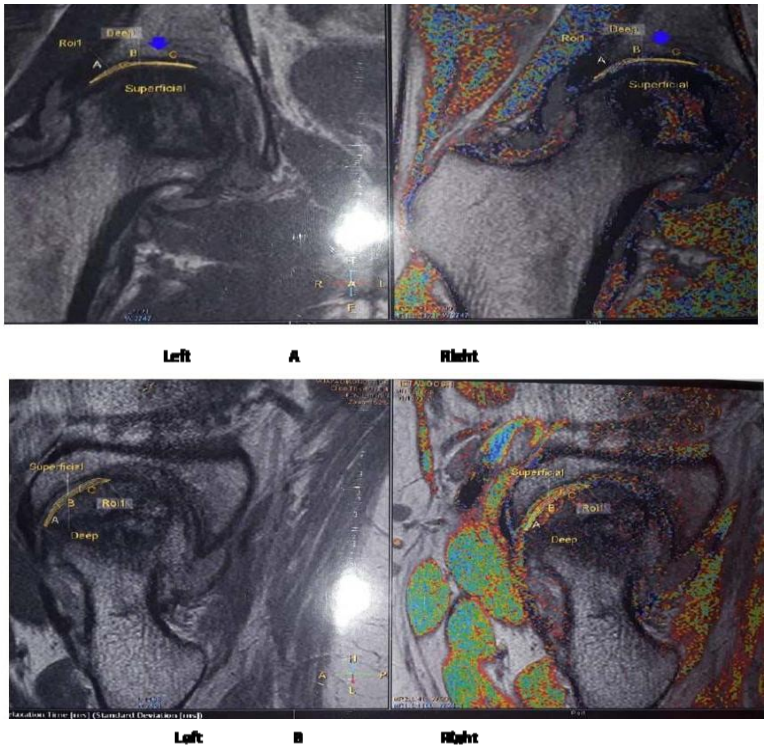

Figure 2. (Case 1): Bilateral cartigram images 32 weeks post-therapy without (Left) and with (Right) depiction of the staining. A. Top - Right hip joint. B. Bottom - Left hip joint. These suggest this patient is able to retain the intactness of the regenerated cartilage for extended periods of time thus suggesting that the prognosis is quite optimistic. Blue arrows show the cartilage location in the cartigrams.

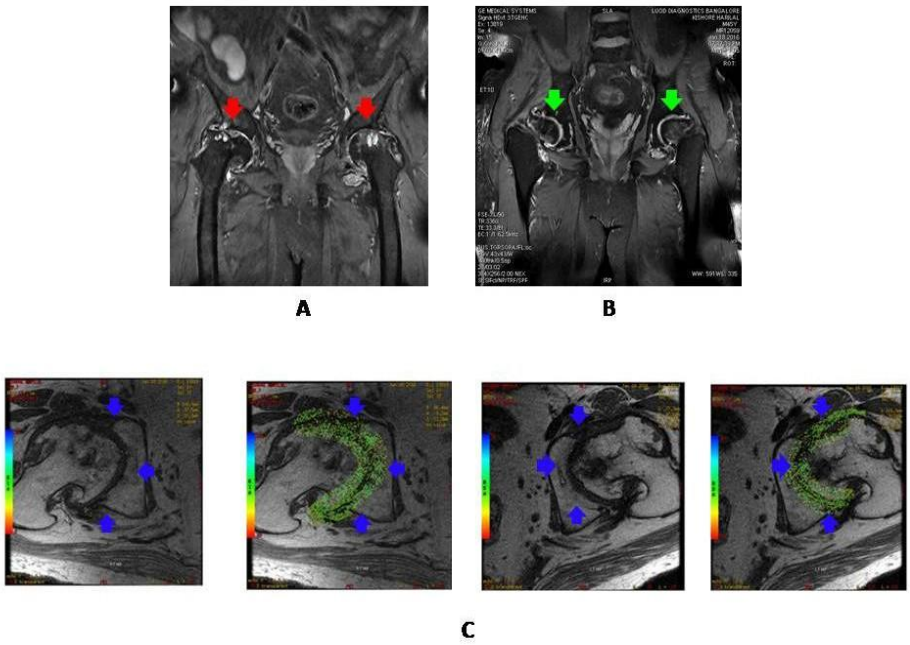

Figure 3. Case 2: MRIs pre- and post-therapy. A (Top Left) - Pre-therapy MRI; B (Top Right) - Post-therapy MRI; C (Bottom 4 images) - Post-therapy cartigrams consisting of alternating images of those with no colors for the lack depiction of hydration within the cartilage and those with colors of cartilage depiction of hydration. Red arrows indicate the location of loss of cartilage and green arrows show the location of the regeneration of cartilage, respectively, in the pre- and post-therapy MRIs; blue arrows show the cartilage location in the cartigrams.

Extended prognosis: Figure 4 and Table 4 could suggest that this case 2 patient shows some treatment based behavioral results vis-à-vis case 1 patient in the extended post-therapy retention of the regenerated cartilage and hence the therapeutic strategy may be individual patient oriented benefit over long term. However it is unclear if this can be attributed to the patient or the therapeutic strategy. Quantitative correlation between the results in Figure 4 and Table 4 may not be achievable to decipher but the Figure 4 is favorably suggestive for the therapy and the reasons for the "decline" in the Table 4 must be examined at the clinical level.

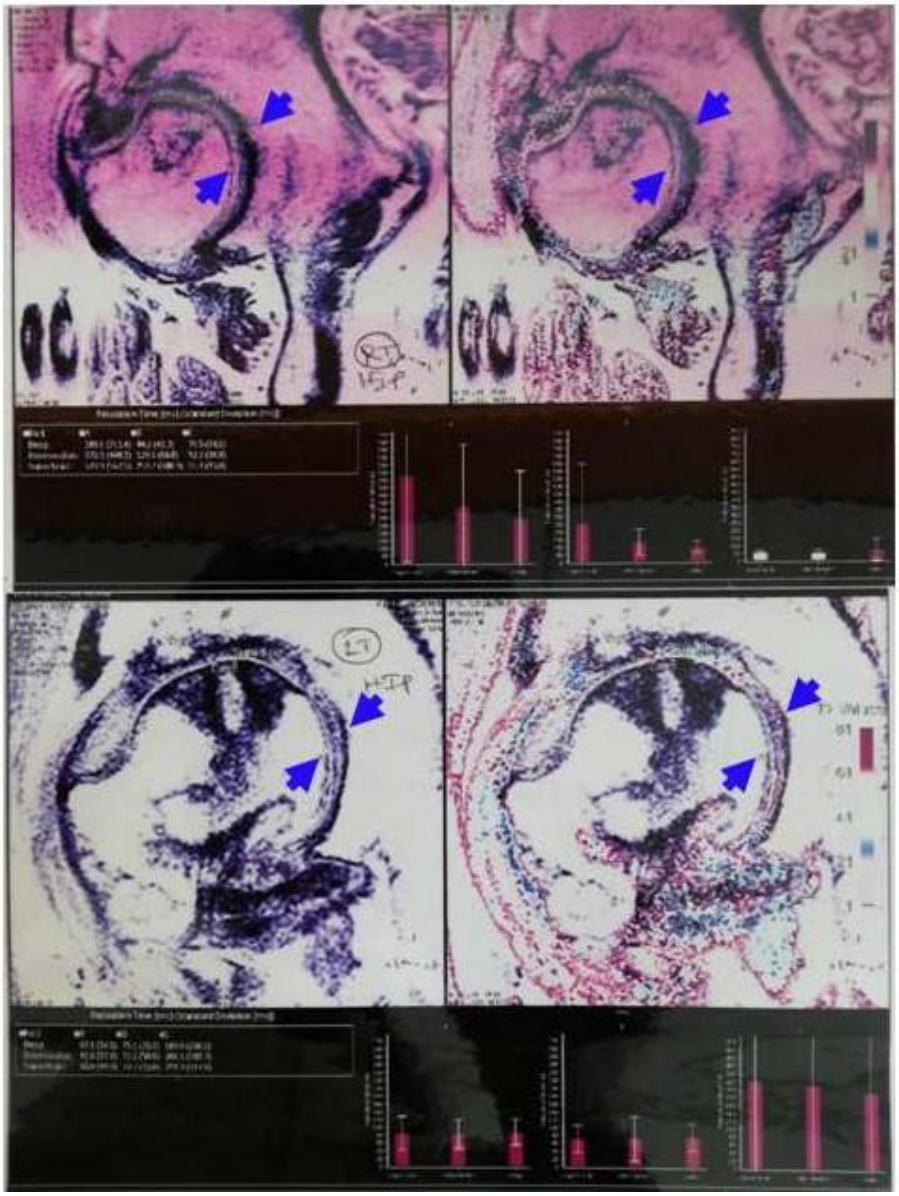

Figure 4. Case 2 Cartigram image 12 weeks post-treatment: Top Left – Right hip joint image with a higher colour contrast that varies across the regenerated cartilage. Top Right – Right hip joint image with lower colour intensity contrast but yet consistent with the density or intensity trend. The error bars of the colour intensities in the panel below the top images is the background intensity levels at different sites of the images. Bottom Left –Left hip joint image without staining. Bottom Right – Left hip joint image with staining. The left and middle error bar panels below these bottom images are baseline intensities and the extreme right panel depicts error bars of the captured stains. Staining for all the images could be set for detection, confirmation and quantization, as needed. Blue arrows show the cartilage location in the cartigrams.

Table 4. Post-treatment prognosis: The data in this table could be suggestive of non-compliance of the clinical advice on post-treatment care by the patient, or need for continued monitoring and possible future repeat therapy, due to a receding preservation or intactness of the newly formed cartilage. This however could be patient dependent and also preservation methods including customization for sustenance of the cartilage post-therapy could be undertaken

Case	Cartilage Thickness (in mm)		Ace Tabular Cartilage (in mm)		Remarks
	Right Femoral Head	Left Femoral Head	Right	Left	
Case 2	Anterior – 3.3	Anterior – 3.1	Anterior – 4.3 Mid – 2.0 Posterior – 2.6	Anterior – 3.1 Mid – 2.1 Posterior – No measurable cartilage	Minimal effusion is seen bilaterally and minimal asymmetrical joint space reduction, marginal osteophytes and loose bodies seen at left hip at antero-inferior and posterior aspect of the joint
	Anterosuperior – 2.8	Anterosuperior – 2.9			
	Posterosuperior – 3.2	No measurable cartilage			
	Posterior – 2.1	No measurable cartilage			

Case 3

Symptoms: The 37 year old male suffered with increased pain in the left hip joint. He developed AVN as he was a chronic alcoholic and smoker. He was treated conventionally with non-steroidal anti-inflammatory drug (NSAID) and physiotherapy. He was advised THR for AVN of the left hip in the year 2013. Investigations revealed no abnormality in metabolic profile.

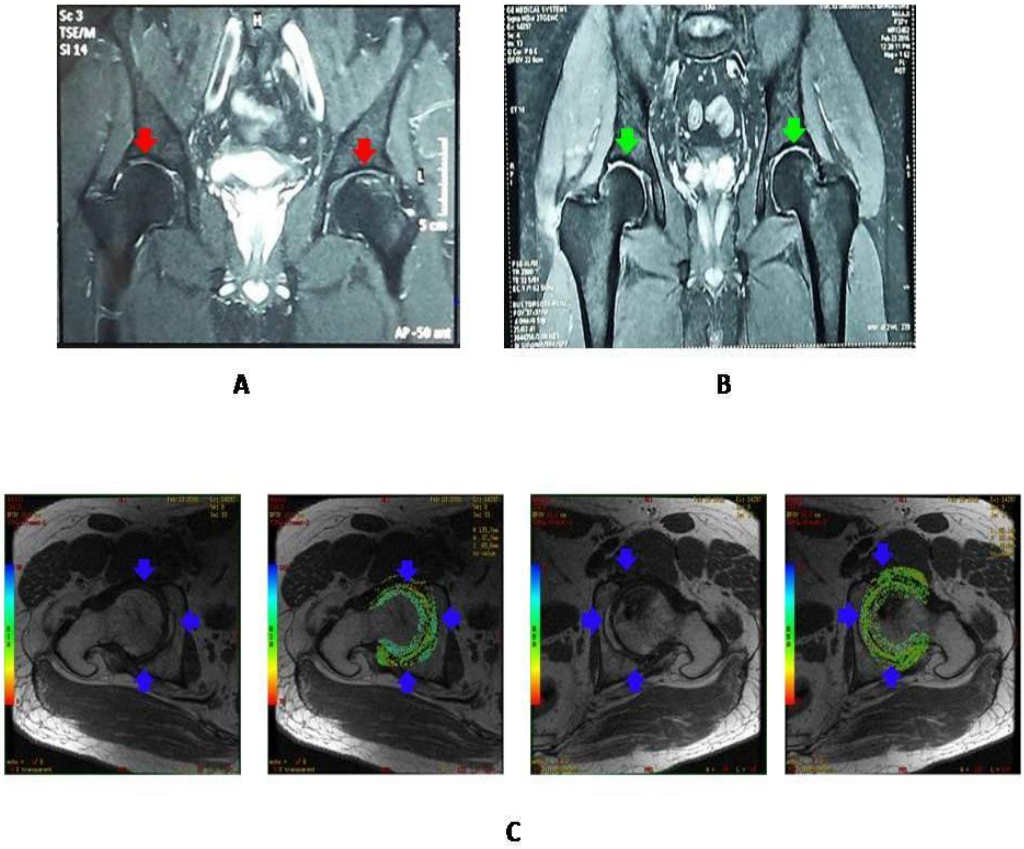

Figure 5. Case 3: MRIs pre- and post- therapy. A (Top Left) - Pre-therapy MRI; B (Top Right) - Post-therapy MRI; C (Bottom 4 images) - Post-therapy cartigrams consisting of alternating images of those with no colors for the lack depiction of hydration within the cartilage and those with colors of cartilage depiction of hydration. Red arrows indicate the location of loss of cartilage and green arrows show the location of the regeneration of cartilage, respectively, in the pre- and post-therapy MRIs; blue arrows show the cartilage location in the cartigrams.

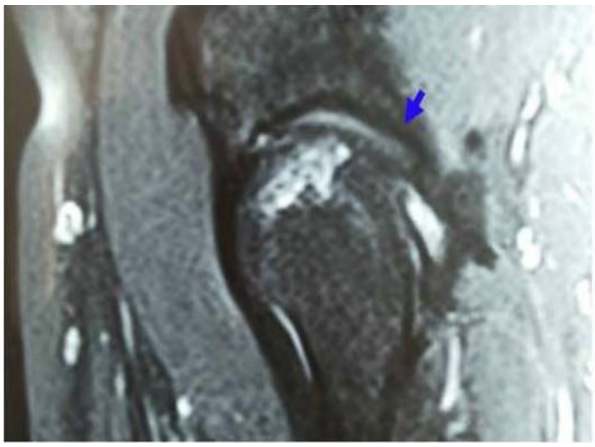

Figure 6. Case 3: Loss of cartilage as shown by MRI. The complete loss of joint hip bone cartilage in the femur head is shown by this MRI at the diagnosis stage and prior to the decision to perform and subject the patients to the stem cell and platelet rich plasma therapies. Comparison of this MRI to Figures 5B and 5C post SCT + PRPT show the marked contrast in the cartilage development. Blue arrow shows the cartilage location in the cartigram.

Clinical diagnosis: MRI of both hip joints was performed in multi-planar and multi-echo sequences (Figure 5A). The MRI for diagnosis of AVN in this case 3 shows the destroyed hip joint with significant loss of cartilage (Figure 6). Left hip: Focal serpiginous low signal intensity line with fatty center noted in the left hip joint involving subchondral regions (osteochondral fragmentation) with 'Double line' sign. These are sustained hyper-intense on T2 Wt and intermediately hyper-intense on T1 Wt images. Minimal bone marrow oedema seen in left hip joint. No evidence of joint effusion bilaterally. Minimal asymmetric joint space reduction between both hips is noted in left side. Greater and lesser tronchanters are normal. Acetabular margins and acetabular labra are normal bilaterally. Sacro-iliac joints are normal. No evidence of abnormal signal changes. Muscles surrounding the hip joints are normal. Psoas muscles also appear as normal. Cartigram shows extensive cartilage seen in left hip joint (>10mm in maximum width) with focal areas of fraying and fissuring.

Results post-treatment: BM-MNC and PRP-GFC combination therapy exhibited marked regeneration of cartilage in the hip of this patient. As shown in Figures 5B and 5C, MRI data clearly revealed noticeable enhancement of joint space (by greater than 10 mm) that was indicative of regeneration of cartilage (Figures 5B and 5C). Evidence of re-arrangement of radiolucent shadow of hip joint indicates some modification of bone adjacent to the hip joint. Most importantly, substantial improvements in movements of hip indicate remodeling of degenerated cartilage through redistributive regeneration to yield functional hip joint.

Discussion

Osteonecrosis of the femoral head is often the devastating side effect of steroid treatments [27]. Epidemiologically, AVN is the operative cause for 10% of all cases of total hip replacements [28]. Pathophysiology of AVN has not been fully understood [29]. Vascular occlusion, intravenous coagulation, healing process, and primary cell death are some of the factors that may be responsible for AVN [30]. The typical radiographic features include; relatively more radiopaque area due to reactive hyperemia resulting from resorption of nearby living bone [31]. In late stage of disease, also seen are radiolucent area following collapse of subchondral bone, the crescent sign and ringed regions of radio density [32].

CT scan of AVN has typically shows clumping and distortion of the central trabeculae and an adjacent low density region representing the reparative zone. It is caused by the micro-fractures subsequently from the condensed mechanical load of dead bone trabeculae, which alters the shape of asterisk [33]. The lucent cystic region is due to the new bone formation on the dead trabeculae. MRI shows classic AVN 'double line sign' of AVN that is made of two concentric low and high signal bands and the high-signal-intensity line represents hyper-vascular granular tissue.

The routine approaches to treat AVN of hip are conservative measures such as limited weight bearing with use of crutches and statin therapy. Surgical interventions include core decompression, bone graft osteotomy and total hip arthroplasty [34]. However, all these approaches limit movements significantly affecting quality of life available to the affected patients [3]. Stem cell based therapies offers the regeneration of bone and cartilage without limitations of surgery and conservative management.

This has been evidenced by the results in Table 3 wherein case 1 reached an average cartilage thickness of 3mm in 9 weeks when the MRI (Figure 1B) and cartigram (Figure 1C) evaluations were performed post therapy. However, case 2 took only 3 weeks to reach a thickness of sizable 10mm. The contrast in time and thickness difference between the two cases 1 and 2 may be due to the steroid prescription prior to this BM-MNC + PRP-GFC + treatment of case 1, whereas case 2 patient developed a rapid cartilage formation or regeneration. Case 3 who had also reached a cartilage thickness of 10 mm had delayed the clinical evaluation for personal reasons, and since he was an alcoholic prior-treatment, it is not clear if that might have contributed to any delayed clinically relevant response which has since been masked by the passage of time by the time post-therapy MRIs and cartigrams were taken.

Case 1 due to previous history of regular intake of drugs such as steroids for example, which leads to serious side effects contributing to but has not been beneficial to relieve the osteonecrosis of the hip, showed a modest cartilage regeneration of 3 mm post-therapy (Table 3). However this patient has subsequently due to discontinuation of the steroids and among other possible lifestyle modifications, been able to retain the levels of cartilage thickness. A future selective imaging by the patient could throw light on further extended prognosis.

Case 2 suggests that alcoholism is less deleterious than steroid intake (case 1) for a quick regeneration of the cartilage of 10 mm thickness post-therapy (PRP-GFC) but for unknown reasons has not sustained that level of cartilage thickness (comparison of Tables 3 and 4). However the thickness reduction is by no means alarming at this stage but any future physical discomfort would be suggestive of a need for clinical re-evaluation of the condition.

Case 3 has positively responded post-therapy as in case 2 but has not felt the need to undergo any further re-examination and hence no additional clinical data is available. All the three cases when compared suggest the success if this SCT-GFC therapy is performed. The potential causes for variations between cases 1 and 2 in terms of post-therapeutic effects during the short and long terms have been discussed above. Extended prognosis clinical data is not available. Sustained therapeutic benefit requires consultations and examinations whenever new medications are prescribed. Moreover, adherence to proper dietary intake and avoidance of un/self-prescribed medications without awareness of the side effects of the ingredients in these medications is necessary, since they may have indirect effects on the sustenance of the regenerated cartilage.

Table 5. Summary of the data on the 48 cases treated to-date since August 2010. The regeneration of cartilage as seen in the MRIs and cartigrams post-therapy have not been subjected to further invasive procedures due to unnecessary risks involved and also the lack of subsequent adverse complaints of symptoms by the patients on their life comfort with respect to the hip bone joints

Cartilage Growth Thickness (in mm)	Number of Cases	Remarks
3	15	Minimal joint effusion on the left side and minimal asymmetrical joint space reduction on both sides
3 – 10	30	No evidence of joint effusion bilaterally and minimal asymmetrical joint space reduction noted in left/right side
Less than 3	2	Associations of bilateral femoral head AVN in the form of marrow oedema of femoral head-neck-upper diaphysis and moderate effusions of both the hip joints
Number of cases that did not show any response	1	Destruction of bilateral femoral heads with sclerosis

Since the patients who have been administered the above discussed stem cells therapeutics as summarized in Table 5 have not required such a renewed treatment /procedure, we can conclude that these procedures have largely and in general successful to promote regeneration of the hip bone joint cartilage and alleviation from the symptoms and sufferings of AVN. Greater chances for reoccurrence of AVN following BM-MNC + PRP-GFC + treatment and cartilage degeneration may not be ruled out for an eventual adverse or relapse condition in the event that non-adherence to clinically based contraindicative medications or other adverse oral intake habits are adopted. However, a strict discipline in observance of avoidance of causes for deleterious condition could well prove beneficial with this therapy.

Treatment using stem cells for the avascular necrosis of the femoral head have been discussed [10]. Bone necrosis is the result of steroid induced depletion of bone marrow cells due to their stimulation of differentiation into adipocytes. Autologous bone marrow transplantation was proposed for treatment of osteonecrosis with achievement of good results [35]. It was also found that avascular necrosis generally occurs following allogeneic but not autologous stem cell transplantation [36]. This is because patients of allogeneic stem cell transplantations are more susceptible to chronic graft versus host disease and thus require steroid prescriptions [36]. Allogeneic SCT recipients are much larger in numbers (10 patients) than those who received autologous SCT (2 patients). The combination of concentrated autologous bone marrow and platelet-rich plasma were used for minimally invasive decompression to treat patients with early stage avascular necrosis [37]. After the femoral head is decompressed, adult mesenchymal stem cells obtained from the iliac crest and platelet-rich plasma were injected into the area of osteonecrosis. A Mayo Clinic team recently performed a retrospective analysis of 60 patients who were treated in this manner. Of the 73 hips receiving this treatment, 16 hips (22 percent) progressed to further stages of osteonecrosis, ultimately requiring total hip replacement. This treatment resulted in significant pain relief and halted the progression of disease in a reasonable amount of patients [37].

Conclusion

The proposed treatment is found to be safe for treatment of the patients. The efficacy of our autologous BM MNC + GFC treatment protocol was found to be better than previous protocols with about 93% success rates.

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