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A PROSPECTIVE MONOCENTRIC REPORT ON THE USE OF AUTOLOGOUS ADULT LIVE CULTURED OSTEOBLASTS (**OSSGROW®**) FOR THE TREATMENT OF PATIENTS WITH **AVASCULAR NECROSIS OF THE HIP**

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Introduction

Avascular Necrosis **(AVN)**, also known as Osteonecrosis, is a pathologic process of bone death that results from the interruption of blood supply to the bone. **AVN** causes deprivation of the structural integrity of the bone leading to functional deterioration. People between the age group of 20 to 40 years are at higher risk of developing **AVN**. The incidence of **AVN** is higher in the male population with a ratio (M:F) of 5:1 [1]. Several factors are associated with **AVN** including alcohol abuse, excess corticosteroid intake, and physical trauma. Idiopathic **AVN** is a common type of **AVN** where the cause of occurrence is unknown. Recent evidence proves **AVN** to be a post-COVID-19 complication due to an increase in the use of steroids for disease management [2]. **AVN** is a progressive disease and, in its advanced stages, **AVN** culminates in arthritis of the hip joint. In 2019, the Indian Society of Hip and Knee Surgeons (ISHKS) reported that 49% of Total Hip Replacement surgeries were caused due to **AVN** [3].

Cellular basis of **AVN**

Pathogenesis involves both biological and mechanical characteristics. The biological aspect of the disease includes inhibition of osteoblastogenesis along with an inadequate repair process of revascularization which leads to the eventual collapse of the femoral head. The continuous cell apoptosis and inadequacy of the repair processes cause disruption in the bone at the cellular level. This initiates necrosis in the bone which forms a lesion due to prolonged ischemia. The constant pressure affects the mechanical characteristics of bone including its structural integrity. This leads to functional deterioration of the bone and loss of biomechanical strength. The sphericity of the femoral head is altered due to compromised bone remodeling that causes flattening of the head. Advancement of **AVN** leads to subchondral fracture, which causes joint irregularity resulting in arthritis of the hip joint [4].

AVN can be broadly classified into two types- Non-Traumatic and Traumatic. Non-Traumatic **AVN** occurs due to excessive intake of alcohol and steroids. Alcohol and steroids induce abnormal cell populations in the bone marrow. This leads to intravascular coagulation in the bones and gives rise to ischemia. Traumatic **AVN** occurs when fractures damage the femoral head medial circumflex femoral artery (MCFA)- the main source of blood supply to the femoral head. Fractures cause articular bleeding into the joints. This induces blood clots around the MCFA and its nutrient vessels. As a result, the flow of blood inside the artery is compromised due to increased hydrostatic pressure in its surrounding area. This phenomenon gives rise to ischemia. All the risk factors along with idiopathic **AVN** result in osteoblastic dysfunction [5]. Other factors associated with **AVN** include diseases such as COVID-19, hemoglobinopathies, organ transplantation, Rheumatoid arthritis, and treatments that require high corticosteroids, immunosuppressants during chemotherapy and radiation[6-9].

Treatment scenario for **AVN**

Bisphosphonates (BPs) such as alendronate or zoledronic acid have been useful to delay structural femoral head damage/collapse and reduce levels of pain, but this use is off-label and there is no definitive recommendation on the duration of treatment and therapeutic dose. Several studies report the ineffectiveness of BPs in **AVN** treatment. They are reported to cause atypical femoral fractures, upper gastrointestinal adverse effects, hypocalcemia, and severe suppression of bone turnover [10-12]. BPs are one of the major causes of **AVN** of the jaw. Current estimates of oral bisphosphonates related to **AVN** of the jaw are

approximately 1 in 10,000 to 1 in 100,000 patient-years [13-15]. Other treatments include surgical options such as core decompression, bone grafts, and tantalum rods which may help to delay the progression of **AVN**. Core decompression is a surgical procedure performed in the initial stages to decrease the intraosseous pressure in the joint. It re-establishes blood flow but, does not heal the necrotic fragment present in the bone. To further improve the results, augmented techniques like bone grafting are implemented post-core decompression. However, the eventual outcome of most of the additional techniques led to the need for Total Hip Replacement (THR) [16]. There is an unmet medical need to address the root cause of **AVN**- Bone Necrosis.

An ideal treatment of bone necrosis would be revascularization of the femoral head, removal of the necrotic area, and implantation of healthy osteoblasts into the femoral head. Osteoblasts form new and healthy bone in the presence of adequate blood supply. **OSSGROW**® is an autologous regenerative cell therapy intended for early to mid-stages of **AVN** that treats the disease on a cellular level by regeneration of bone tissue. The treatment has been scientifically validated and medically proven through clinical trials in India and has received a commercial license from the Indian FDA (DCGI). It focuses on improved vasculature in the femoral head along with new bone formation that preserves the sphericity, and structural integrity of the femoral head, avoiding further treatment.

This white paper is based on the results of a survey to determine the clinical efficacy outcomes of Autologous Adult Live Cultured Osteoblasts (**OSSGROW**®) in the treatment of Avascular Necrosis of the hip in 12 patients. It discusses key findings and highlights the safety and efficacy of **OSSGROW**® in detail.

Materials and methods

Surgical technique and follow-up

Under local or general anaesthesia, 4 ml for unilateral and 8 ml for bilateral bone marrow from the posterior/ superior iliac crest or in some cases from the sternum was collected as per the individual discretion of the surgeon. The bone marrow harvest specimens were then sent to the GMP-certified cell processing facility (Regrow Biosciences® Pvt. Ltd.) in a sterile tube containing a culture medium to manufacture **OSSGROW**®.

For administration, an incision was made on the lateral side of the affected hip joint, and a standard core decompression method was performed in the necrotic area in the femoral head under C-Arm guidance. The necrotic bone was gently debrided with the help of long surgical scoops and osteoblasts were implanted into the defect site. Later, patients followed a rehabilitation program under the expert guidance of the treating orthopedic surgeon medical need to address the root cause of **AVN**- Bone Necrosis.

Patients were followed up post-surgery. The average time of follow-up was 7.5 months. The shortest follow-up was 3 months, and the longest follow-up was 26 months.

Results

Patient demographics

A total of 12 patients (1 female and 11 males) diagnosed with **AVN** were selected for the study. The details of each subject were recorded during screening (Table 1). As for occupation, 50.0% of patients belonged to a service job followed by teachers (8.3%), photographers(8.3%), students(8.3%), doctors(8.3%), engineers(8.3%), and homemakers(8.3%). Most of the **AVN** cases were idiopathic (41.66%). Other factors include steroid use (33.3%), smoking (8.3%), accidents (8.3%), and chemotherapy(8.3%). An equal number of unilateral and bilateral surgeries were performed (6 each).

Before **OSSGROW**®, 2 patients (16.6%) were on oral medications and 1 patient (8.3%) underwent core decompression for **AVN**

treatment. None of the patients reported additional surgeries after **OSSGROW**® at the follow-up time. 2 patients (16.7%) rated their overall physical and mental health as excellent, 5 patients (41.6%) rated good followed by 3 patients (25%) with average outcome, and 2 patients (16.6%) with not good outcome.

Safety assessment

There were no Adverse Events or Serious Adverse Events reported during the study in any patient. Overall, 58.3% reported better health after **OSSGROW**® treatment

Efficacy assessment

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a widely used, proprietary set of standardized questionnaires used by health professionals to evaluate the condition of patients with osteoarthritis of the knee and hip, including pain, stiffness, and physical functioning of the joints[17]. It is widely used in the evaluation of Hip and Knee Osteoarthritis and other rheumatic conditions such as rheumatoid arthritis, juvenile rheumatoid arthritis, fibromyalgia, systemic lupus erythematosus, low back pain, and **AVN**. Patient-reported outcomes were based on pain improvement, ability to perform physical activities, and overall development in the condition.

Statistical analysis

Paired t-test (Student t-test) was performed on continuous variable WOMAC scores, and the calculated value of test statistics of the continuous variable was compared with the tabulated value of test statistics with (N-1) df (degree of freedom) at a significant level of 0.001.

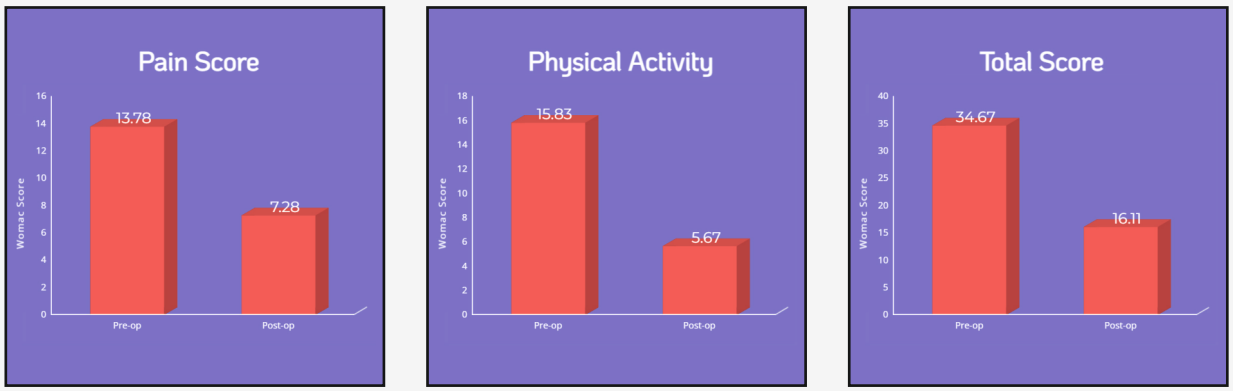

Figure 1: WOMAC scores of patients comparing pain, physical activity and the total scores before and after **OSSGROW**® cell therapy

1 PAIN IMPROVEMENT

The pain score (mean) before and after OSSGROW® treatment was 13.7 ± 3.5 and 7.28 ± 2.5 respectively indicating a decrease in the pain. From the calculation, the p-value was 0.000003, thus H0 was rejected at a significant level of 0.001 as there is a significant mean difference between the initial and final assessments.

2 ABILITY TO PERFORM PHYSICAL ACTIVITIES

Factors such as walking, stair climbing, nocturnal pain, pain while resting, and weight bearing were considered. The mean score of patients before the treatment was 15.83 ± 3.5, which was reduced to 5.67 ± 3.1 during the follow-up. The p-value was 0.00002 thus H0 was rejected at a significant level of 0.001 as there is a significant mean difference between the initial and final assessments. This indicates a significant decrease in pain while performing physical activities.

3 OVERALL DEVELOPMENT OF THE CONDITION

The score changed from 34.67 ± 8.3 (pre-op) to 16.11 ± 6.8 (post-op). From the calculation, the p-value was 0.000001, thus H0 was rejected at a significant level of 0.001, as there is a significant mean difference between the initial and final visit. The pain scores reduced significantly in the patients.

PATIENT	AGE	GENDER	BM1	PROFESSION	AVN LATERALITY	CAUSE OF AVN	FOLLOW-UP PERIOD (MONTHS)	WOMAC SCORE BEFORE OSSGROW	WOMAC SCORE POST OSSGROW	SURGERY BEFORE OSSGROW	SURGERY POST OSSGROW	OUTCOME
1	35	Male	24.1	Teacher	Unilateral	Steroids	26	40	2	-	-	Excellent
2	39	Male	25.88	Photographer	Unilateral	Smoking/ Accident	17	26	16	-	-	Excellent
3	24	Female	22.5	Student	Unilateral	Idiopathic	11	39	7	-	-	Good
4	30	Male	24.7	Service	Bilateral	Idiopathic	11	62	38	Core Decompression	-	Not Good
5	25	Male	22	Service	Bilateral	Chemotherapy	9	90	26	-	-	Good
6	25	Male	34.5	Engineer	Unilateral	Idiopathic	7	36	13	-	-	Good
7	33	Male	21.6	Service	Unilateral	Others	6	35	18	-	-	Average
8	33	Male	25.7	Service	Bilateral	Steroids	6	48	40	-	-	Average
9	30	Male	22.5	Service	Unilateral	Steroids	5	34	16	Pills/Medication	-	Good
10	25	Male	28.7	Doctor	Bilateral	Steroids	3	44	24	Pills/Medication	-	Average
11	56	Male	27.2	Home Maker	Bilateral	Idiopathic	3	82	30	-	-	Good
12	33	Male	21.1	Service	Bilateral	Idiopathic	4	88	60	-	-	Not Good

Table 1: Patient demographics

Radiology assessment

Pre-operative and post-operative X-rays were compared to assess the differences in the femoral head after undergoing osteoblast implantation. Figure 2 shows the differences between the Pre-Op X ray and Post-Op X ray of a male patient, aged 23 years after 9 months post osteoblast implantation. Pre-operative X-ray shows pelvis with signs of Stage II Avascular Necrosis of both hip- mixed osteopenia and sclerosis without any subchondral collapse. Post-operative X-ray post 9 months shows both hips showing well preserved contour of the joint.

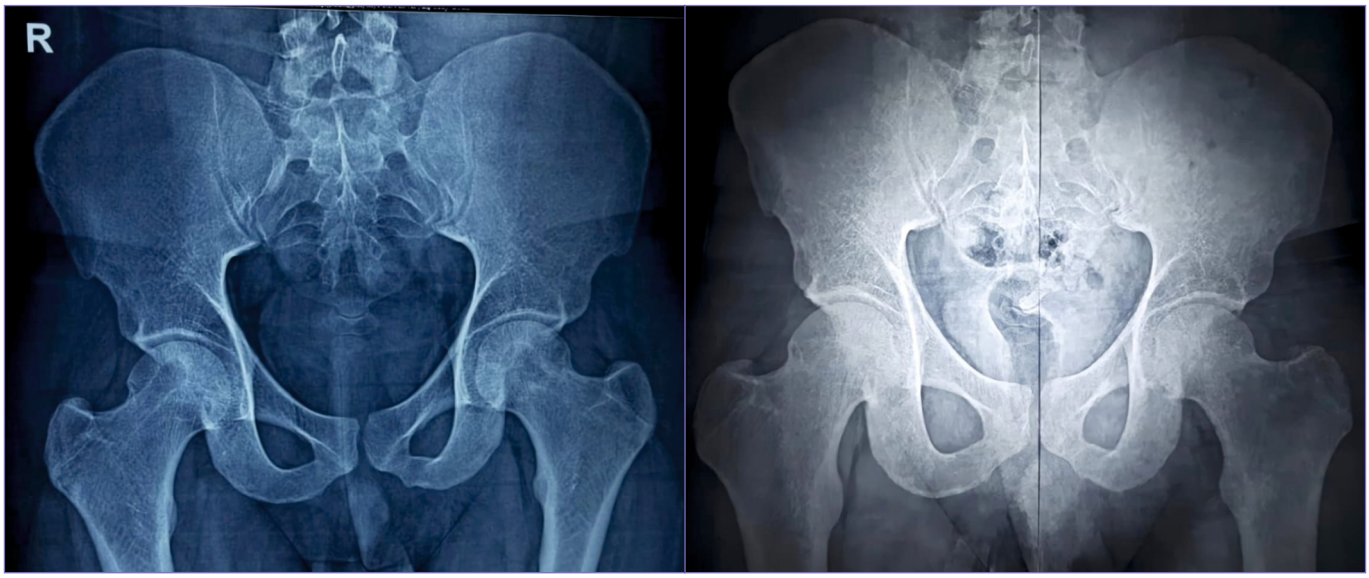

Figure 2: Pre-Op and Post-Op X-ray images before and after **OSSGROW**® cell therapy.

Discussion

The objective of this study was to determine the therapeutic effects of **OSSGROW**® bone cell therapy in patients with **AVN** of the femoral head. Mean age of patients was 32.33 ± 8.8 years. The results showed that most of the patients (91.7%) were aged between 20 to 40 years. This corresponds to the prevalence data from scientific literature confirming a higher occurrence of **AVN** in the middle-aged population. The literature also suggests a higher risk of **AVN** in the male population compared to female [1]. This is also confirmed by the results of this study as there was only one female patient. Most of the patients possessed a service job which may indicate a lack of an active lifestyle due to prolonged sitting hours. In this study, idiopathic **AVN** and steroid-led **AVN** were the most common type of **AVN**. Hence, attention should be paid to informing people about the importance of an active lifestyle and the uncontrolled use of corticosteroids. In **OSSGROW**® bone cell therapy, the implanted osteoblasts replace the damaged bone with a new regenerated bone. Natural remodeling of the bone requires several months (6-12 months). Hence, patients with longer follow-up times had far better results than patients with lesser follow-up. Patients who underwent core decompression before the surgery did not have a positive outcome indicating that patients with previous surgeries require more time (>6-12 months) for new bone formation. Platelet-rich plasma, growth factors, and bone marrow aspirate concentrate (BMAC) are widely used along with traditional techniques such as core decompression or bone grafts for **AVN** of the femoral head today. None of these treatment options have been approved by any regulatory authority for **AVN**. A Randomized Controlled Single-Blind Study carried out by Ganji et al., concluded that autologous osteoblastic cells are more efficacious than bone marrow concentrate in the early stages of **AVN** of the femoral head [18]. Hernandez et al. showed that core decompression combined with implantation of autologous bone marrow concentrate and tricalcium phosphate will not prevent radiographic progression of early-stage **AVN** of the hip. They observed higher failure rates in the second and third stages of **AVN**[19]. Camp et. al., reported core decompression to be an ineffective procedure with significant morbidity.

The sensitivity and specificity of the intraoperative functional diagnostic tests (venograms, stress tests, and determinations of pressure) were uniformly disappointing throughout the study [20]. Overall, the follow-up of patients who underwent osteoblast implantation (**OSSGROW**®) showed statistically significant results with reduced pain and improved functional activities. Therefore, surgery to reduce the pressure on the hip joint and implantation of osteoblasts can significantly improve patients' condition in the long term. This also restores the structure and function of the joint and prevents the progression of the disease. Patients with longer follow-up times had the best treatment outcomes. Moreover, none of the patients underwent any additional surgeries or a hip replacement post **OSSGROW**® treatment.

Conclusion

Based on the short-term safety and efficacy results of the **OSSGROW**® treatment in 12 patients in a real-world setting, it can be concluded that **OSSGROW**® is a safe and effective treatment option in preserving the hip and improving the quality of life. The treatment effectively addresses the root cause of **AVN** by delivering healthy autologous bone making cells that recovers the lost bone mass. The blood supply to the bones resumed via the core-decompression procedure provides a viable environment for the cells to sustain and further start the bone remodeling cycle naturally. The goal of the targeted and personalized **OSSGROW**® bone cell therapy is the formation of new bone to preserve the structural-functional integrity of the joint. Recent publications indicate long-term safety and efficacy with excellent patient benefits such as reduction of hip pain, improvement in functional activity, and prevention of the need for additional medical intervention/ surgery.

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