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ANALYSIS OF RESULTS AFTER SURGICAL APPLICATION OF BONE MARROW ASPIRATE STEM CELL CONCENTRATE IN THE TREATMENT OF AVASCULAR NECROSIS OF THE FEMORAL HEAD

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ABSTRACT

Introduction: Avascular necrosis or osteonecrosis of the femoral head is defined as a pathological process that results in a critical reduction in the blood supply to the hip head with increased intraosseous pressure. Avascular necrosis is a multifactorial disease. The treatment used so far consists of transosseous decompression of the femoral head and is the most commonly used method in the early stages of osteonecrosis.

Aim: The aim of this study was to evaluate the results after application of bone marrow stem cells obtained from the pelvic ridge, which was applied to the necrotic zone after previous decompression.

Material and methods. The study is of prospective character and included 30 patients with first, second, and third degrees of AVN according to the Ficat classification, determined with X-ray. The range of motion in the hip was examined preoperatively by using a goniometer, a radiological evaluation of the degree of AVN was performed, according to the Ficat classification, and the VAS scale and the Harris Hip Score were examined preoperatively and postoperatively.

Results: The average value for the Harris Hip Score (HHS) preoperatively was: for Ficat I -66.32±5.1, for Ficat II - 49.19±3.4 and for Ficat III - 33.71±2.1. At the 3-month postoperative follow-up, the average HHS values were: 87.92±4.3 for Ficat I, 78.64±6.6 for Ficat II and 76.48±2.6 for Ficat III. The same values for HHS were obtained in the control examinations at the 6th and 12th month postoperatively, indicating the fact that good bone regeneration was achieved and the progression of the condition was prevented. A decrease in the HHS value was observed at the control examination at 1 year after the surgical treatment, in 3 patients according to the Ficat classification of grade III, in whom a total hip replacement surgery was performed.

Conclusion: The use of stem cells in the treatment of avascular necrosis of the femoral head has achieved good functional results and reduced pain in operated patients. Radiographically, good bone regeneration was achieved and the progression of necrosis to a higher degree was prevented.

Keywords: avascular necrosis, decompression, stem cells, Harris Hip Score

INTRODUCTION

Avascular necrosis (AVN) of the femoral head, also known as osteonecrosis, occurs when trabecular bone cells die spontaneously as a result of a critical reduction in the blood supply to the femoral head and elevated intraosseous pressure, resulting in collapse of the femoral head, and in-

cludes subsequent degenerative changes.(1, 2) The incidence in the United States is 10,000-20,000 patients per year, usually between the ages of 20-60 years, with a peak incidence in the younger population [1, 3, 4]. After the onset of a femoral head collapse bone, severe pain occurs and regression rarely occurs during disease [5, 6, 7, 8].

The pathophysiological mechanism for the occurrence of avascular necrosis remains unclear despite many attempts to provide a theoretical model [9, 10]. Some conditions and ecological components are known that may lead to avascular necrosis of the femoral head and are divided into two groups: traumatic or direct injuries which include: neck/head fracture of the femur and hip dislocation. The second group incudes non-traumatic factors which include: corticosteroid use, alcohol abuse, idiopathic, sickle cell disease, Cayson's disease, systemic lupus erythematosus, Cushing's disease, smoking, pregnancy, previous radiotherapy, organ transplantation, chronic pancreatitis, chronic renal failure, coagulopathies, and disorders of fat metabolism. Although these factors are thought to increase the risk of avascular necrosis of the femoral head, some authors believe that this condition is a result of a coagulation disorder or a genetic abnormality that leads to vascular compromise [11]. Another working hypothesis suggests that cell death is due to increased intramedullary pressure in the femoral head, leading to reduced blood flow and, consequently, to cell death through a mechanism similar to compartment syndrome after a traumatic event. Cases in which the underlying cause of the condition cannot be determined are considered idiopathic and are generally attributed to coagulation abnormalities or collagen mutations [11, 12]. Jones and colleagues found in their study that approximately 82% of the patients included in their study had at least one abnormality in coagulation factors [11]. Liu and colleagues found the presence of a COL2A1 genetic mutation in certain families predisposed to developing avascular necrosis of the femoral head [13].

Avascular necrosis tends to affect patients between the ages of 20 and 40, with the average age of patients being 38 years [3, 14, 15]. Patients' symptoms vary, as there is a wide range of symptoms depending on the stage. In the early stages of the disease there is an insidious onset of pain, without a clear cause or triggering event, and patients often have a normal range of motion, which can be limited by the pain itself, especially the internal rotation of the hip. As the condition progresses, discomfort in the groin area may be followed by a sudden onset of severe pain, an event that signals the collapse of the femoral head, leading to degenerative changes in the final stage. The development of the disease itself in the late stage is typically marked by increased mechanical symptoms that include a reduced and painful range of motion, a Trendelenburg sign, and crepitations [16].

Avascular necrosis of the femoral head is diagnosed by native radiography; in the early stages, high-resolution three-dimensional imaging, such as magnetic resonance imaging or computed tomography, is usually necessary. Imaging characteristics are of particular importance in order to determine the exact stage of the disease which is essential for further treatment. In our study we used the Ficat classification [16, 17].

Existing treatment of decompression of the femoral head is a commonly used method in the early stages of avascular necrosis. The purpose of decompression is to reduce the intraosseous pressure of the femoral head and to establish normal vascular circulation while reducing hip pain. The decompression method is often combined with a nonvascular graft (allograft bone or demineralized bone matrix) or a vascular bone graft from a fibula or iliac bone [18].

Table 1. *Clinical and radiographic features of the four degrees of avascular necrosis*

	X-ray	MRI	Bone scan	Symptoms
Stage 0	Normal	normal		None
Stage I	Normal or minor osteopenia	oedema	Increased uptake	Pain typically in the groin
Stage II	Mixed osteopenia &/or sclerosis	Geographic defect	Increased uptake	Pain and stiffness
Stage III	Crescent sign & eventual cortical collapse	Geographic defect	Pain and stiffness	Radiation to knee and limp
Stage IV	End stage with evidence of secondary degenerative changes	Geographic defect		Pain and limp

Free vascular fibula graft is used to prevent collapse of the femoral head with the possibility of bone vascularization in that region. This preventive method is recommended for younger patients as prevention of femoral head collapse.

Non-vascular bone grafts have a more theoretical advantage in the precancerous and early post-collapsing state of osteonecrosis of the femoral head, a condition in which articular cartilage is relatively undamaged. There is no consensus on the use of the neural graft, and the results are twofold [18].

Osteotomy is used to displace the necrotic segment below the load surface in the early stage of osteonecrosis of the femoral head. It provides worse end functional results in younger people.

Other methods of treatment are titanium porous wedges, in order to incorporate bone tissue, thus preventing collapse.

Total hip arthroplasty is reserved for the advanced progressive stage in the development of avascular necrosis of the femoral head. The indications are individual and variable for cement or non-cement prosthesis or unipolar or bipolar prosthesis [16].

Stem cells are cells that have the ability to self-replicate and give rise to new specialized cells through mitosis. Stem cells can be found at different stages of fetal development and are present in different tissues of adults. Many of the terms used to distinguish stem cells are based on their origin or the path of their origin. These cells are found in most, if not all, multicellular organisms.(19)

The aim of this study was to evaluate the results after application of bone marrow stem cells obtained from the iliac crest which were applied to the necrotic zone after previous decompression.

MATERIALS AND METHODS

The study was conducted at the University Clinic for Orthopedic Surgery in Skopje. It is a prospective study and includes patients diagnosed with avascular necrosis of the femoral head with first, second and third degrees, according to the Ficat classification. All patients were treated with decompression of the femoral head and application of iliac bone marrow stem cells. This is a new method, applied in our country for the first time, in the preservation of the femoral head. To date, 30 unselected cases with first, second, and third degree avascular necrosis of the femoral head have been reported. Patients with a fourth degree, according to the Ficat classification, were excluded from the study. Fourth degree represents complete destruction of the femoral head with inclination and deformity, indicating hip replacement surgery. Each group included 10 patients, in the age group of 20-55 years. Preoperatively in patients the range of motions in the hip was measured using a goniometer.

Patients were operated on under spinal anesthesia. The iliac bone stem cells were first prepared, followed by decompression of the osteonecrotic area and application of stem cells.

Assessment of the patients was made by clinical examination, Visual Analogue scale (VAS) for pain, Harris Hip Score (HHS) and ROM of the hip was measured with goniometer before surgery and after 3, 6 and 12 months.

For statistical analysis, the following tests were used: Difference test, Analysis of Variance test, ANOVA test, post hoc test - Tukey HSD.

Short description of the method

Bone marrow is aspirated from the iliac crest. The patient is in a lateral position after receiving spinal anesthesia. After insertion with a 6-8 cm long and 1.5 cm inner diameter trocar needle, 180 ml of bone marrow blood is aspirated into 3 anticoagulant syringes. The aspirate is placed in a plastic container with the addition of an anticoagulant. It is then centrifuged and filtered to separate the cell substrate and the fat. Separation of bone marrow stem cells is performed using the Angel Artrex System. The mononuclear fraction, the stem cells are then separated for a standard injection procedure. Decompression of the necrotic area is performed by a percutaneous approach by burping the area. The separated stem cells are then injected with a small trocar into the osteonecrotic area and intraarticularly. All patients underwent antibiotic therapy after surgery, during 3 days after surgery, and anticoagulant therapy during 3 weeks after surgery.

The first follow-up examination was scheduled at 6 weeks after surgery, then at 3 months, at 6 months, and then at 12 to 18 months after surgery. The values of the Harris Hip Score and the VAS scale were analyzed in the interest of the study. The obtained data were statistically analyzed.

Table 2. *Demographic data of the patients*

Gender	Number	%
Male	19	63.3
Female	11	36.7
Diagnosis		
AVN of the right hip	13	43.3
AVN of the left hip	17	56.7
Risk factors		
Alcohol abuse, cigarettes	1	3.3
Idiopathic	10	33.3
Corticosteroids	7	23.3
Medicaments	5	16.7
Obesity	5	16.7
Coagulopathy	1	3.3
Age average±SD	42.5±7.5	
Minimum	25.0	
Maximum	55.0	

Table 3. *VAS assessment of pain*

Pain duration			
Ficat1	**4.65±2.8**	**2.0**	**12.0**
Ficat2	**9.9±3.6**	**6.0**	**18.0**
Ficat3	**12.25±2.8**	**9.0**	**18.0**
VAS before surgery		**average±SD**	**N**
Ficat1		**2.3±0.5**	**10**
Ficat2		**5.5±0.5**	**10**
Ficat3		**7.6±0.5**	**10**
VAS 6 weeks after surgery		**average±SD**	**N**
Ficat1		**0.6±0.7**	**5**
Ficat2		**2.6±0.7**	**10**
Ficat3		**3.9±0.7**	**10**
VAS 3 months after surgery		**average±SD**	**N**
Ficat1		**0**	**10**
Ficat2		**0.4±0.5**	**4**
Ficat3		**0.8±0.6**	**7**
VAS 6 months after surgery		**average±SD**	**N**
Ficat1		**0**	**10**
Ficat2		**0**	**10**
Ficat3		**0.8±0.6**	**7**
VAS 12 months after surgery		**average±SD**	**N**
Ficat1		**0**	**10**
Ficat2		**0**	**10**
Ficat3		**2.4±3.1**	**7**

Graphic 1. *VAS assessment of pain*

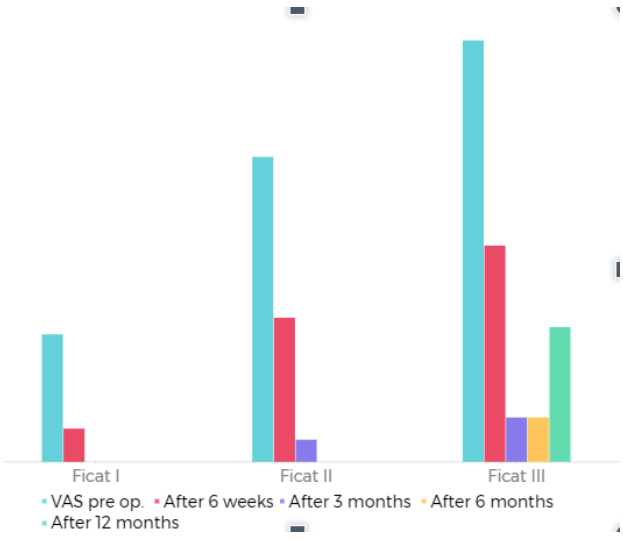
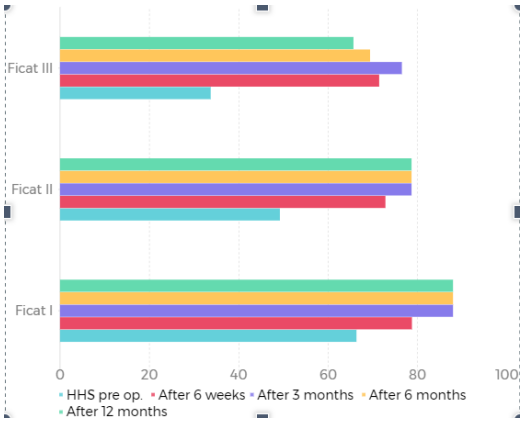


Table 4. *Harris Hip Score*

HHS before surgery	***average±SD***	***N***
Ficat1	**66.32±5.1**	**10**
Ficat2	**49.19±3.4**	**10**
Ficat3	**33.71±2.08**	**10**
HHS 6 weeks after surgery	**average±SD**	**N**
Ficat1	**78.73±1.4**	**10**
Ficat2	**72.80±5.4**	**10**
Ficat3	**71.40±5.8**	**10**
HHS 3 months after surgery	**average±SD**	**N**
Ficat1	**87.92 ± 4.3**	**10**
Ficat2	**78.64±6.6**	**10**
Ficat3	**76.48±2.6**	**10**
HHS 6 months after surgery	**average±SD**	**N**
Ficat1	**87.92 ± 4.3**	**10**
Ficat2	**78.64 ± 6.6**	**10**
Ficat3	**69.37±10.9**	**10**
HHS 12 months after surgery	**average±SD**	**N**
Ficat1	**87.92 ± 4.3**	**10**
Ficat2	**78.64 ± 6.6**	**10**
Ficat3	**65.65±16.6**	**10**

Graphic 2. *Harris Hip Score*



RESULTS

A total of 30 patients were included in the study, including 19 (63.3%) men and 17 (36.7%) women, the percentage difference is statistically significant at p<0.05 (Difference test, p=.0394). The average age of patients was 42.5±7.5 years, the youngest patient was 25 years old, while the oldest was 55 years old. 50% of the patients were older than 44.0 years with an interquartile rank 38-48g (Me=44.0 IQR (38-48). 13 patients were diagnosed with avascular necrosis of the right hip, 17 of the left. Regarding the etiology and risk factors for the development of osteonecrosis in 10 (33.3%) patients avascular necrosis was idiopathic, 7 (23.3%) patients had developed avascular necrosis due to corticosteroids, 5 (16.7%) due to obesity and medications, and 1 (3.3%) patient had alcoholic etiology and confirmed coagulopathy (Table 2).

An equal number of patients were included in each of the three groups according to the Ficat classification, 10 in each group. According to the anamnestic data for the duration of the pain, in the patients from the group with Ficat I, the pain started 2-12, at an average of 4.65 months before the first examination, in the patients according to Ficat II anamnestic data were obtained for 6-18 months, with an average of 9.9 months before the first examination. In the last group, Ficat III, the pain dates from 9- 18 months, with an average of 12.25 months before the first examination (Table 2). According to the Analysis of Variance test, the difference between average time duration is statistically significant p<0.05(F=15.8. p=.000029). There is a large selection of so-called post hoc tests that are performed after the ANOVA test, giving statistically significant results. These are also called multiple comparison tests. The purpose of these tests is to discover the difference between multiple samples which is "responsible" for the overall statistically significant result. According to the post hoc test, the Tukey HSD test, between the duration and Ficat, the overall significance is due to the statistical significance between the average duration in the Ficat 1 group versus the average values of Ficat 2 and Ficat 3 for p<0.05 (p=0.002245, p=0.000145)

In the interest of this study, the range of motion of the hip was determined preoperatively in patients, as follows: flexion, external rotation, abduction and adduction, where the following values were obtained. In 7 (23.3%) patients, the hip flexion range was between 55-65 degrees, in 10 (33.3%) patients, there was hip flexion between 65-70 degrees, in 7 (23.3%) patients, flexion was measured between 70-75 degrees, in 5 (16.7%) patients, there was hip flexion between 75-80 degrees, and flexion between 80-90 degrees was measured in only 1 (3.3%) patient. In all patients, the volume of external rotation was 5-10 degrees. Regarding the adduction, 10 (33.3%) patients had an adduction of 0-5 degrees, and 20 (66.7%) patients were at 5-10 degrees. Regarding the abduction, 10 (33.3%) respondents had a value of 0-5 degrees, 11 (36.7%) respondents had a value of 5-10 degrees and 9 (30%) respondents had a value of 10-15 degrees.

In the interest of this study, patients were also examined with the VAS scale to determine the degree of pain at all time points analyzed in the study. The Harris Hip Score was used for assessment of disability.

The mean values for the VAS scale preoperatively were: for patients with Ficat I – 2.3±0.5,

Ficat II -5.5±0.5 and for patients with Ficat III – 7.6±0.5. At the first control, 3 months postoperatively after finished physical therapy or balneotherapy, patients with avascular necrosis with Ficat I did not provide a history of pain, while 4 patients from the Ficat II group rated the pain with a score of 1. Six patients from the Ficat III group rated the pain with a score of 1, and only 1 patient rated it with a score of 2. At the control examination, 6 months postoperatively, patients with Ficat classification I and II had no pain. This stands in contrast to patients with Ficat III: 6 had a pain score of 1, and 1 patient rated the pain with a score of 2. At the control examination, after 1 year of surgical treatment, only a few of the patients with Ficat III had persisted pain as a symptom, 4 of them rated the pain with a score of 1, 1 patient rated the pain with a score of 5, 1 with a score of 7 and 1 with a score of 8. Patients with a score of 5, 7 and 8 were also those in whom, after 1 year from the surgical treatment, avascular necrosis progressed and their definitive method of treatment was total hip replacement surgery.

The difference between the average values of VAS and patients with Ficat 1, preoperatively and during the control, is significant at $p<0.05$ (Friedman ANOVA and Kendall Coeff. of Concordance, ANOVA Chi Sqr. (N = 10, df = 4) = 35.38462 p = .00000)

The difference between the average values of VAS and patients with Ficat 2, preoperatively and during the control is significant for $p<0.05$ (Friedman ANOVA and Kendall Coeff. of Concordance ANOVA Chi Sqr. (N = 10, df = 4) = 38.32558 p = .00000)

The difference between the average values of VAS and patients with Ficat 3, preoperatively and during the control is significant for $p<0.05$ (Friedman ANOVA and Kendall Coeff. of Concordance ANOVA Chi Sqr. (N = 10, df = 4) = 33.44578, p = .00000)

The average value for the Harris Hip Score (HHS) preoperatively was: for Ficat I -66.32±5.1, for Ficat II - 49.19±3.4 and for Ficat III - 33.71±2.1. At the 3-month postoperative follow-up, the average HHS values were: 87.92±4.3 for Ficat I, 78.64±6.6 for Ficat II and 76.48±2.6 for Ficat III. The same values for HHS were obtained in the control examinations at the 6th and 12th month postoperatively, thus indicating the fact that good bone regeneration was achieved and the progression of the condition was prevented. A decrease in the HHS value was observed at the control examination at 1 year after the surgical treatment, in 3 patients according to the Ficat classification of grade III, in whom a total hip endoprosthesis was implanted.

DISCUSSION

In 1993, Hernigou and Beaujean were the first who described the technique of injecting mesenchymal stem cells combined with standard decompression as a biological introduction to the necrotic area. The first results were published in 2002. This study included 116 patients, in which mesenchymal stem cells were implanted from the iliac crest, injected after decompression of the necrotic area. After 7 years of follow-up, 145 operated hips, classified according to Ficat 1 and 2, only 9 needed hip replacement surgery. Of the 44 patients classified as Ficat III and IV, only 25 patients underwent joint replacement surgery. He believes that in those patients who apply a larger amount of progenitor cells, transplanted into the necrotic zone, have better results [19, 20, 21, 22, 23, 24, 25, 26, 27, 28].

Gangii (2011) refers to the treatment of patients with AVN. After 60 months, 8 of 11 patients with AVN in the decompression control group ended up with a higher degree of hip head necrosis and articular cartilage fracture, while only 3 patients out of 13 who had mesenchymal stem cells applied had progression to that stage. The analysis showed a significant difference between the two groups [29, 30, 31].

Papakostidis (2016), in his study, points out that by researching the literature by Hernigou, he concluded that the implantation of autologous stem cells in the decompressed femoral head provides better clinical and radiological results, compared only with decompression of the femoral head [32].

Daltro (2015), in his study of 89 patients with AVN, demonstrated significant relief of pain and improvement of function measured by the Harris Hip score, which was less than p-0.0005. After mesenchymal stem cell implantation, radiographic stabilization of the necrotic zone was detected, and only 3.7% of treated patients did not achieve the desired clinical results [33].

CONCLUSION

This study proved that after the application of the stem cells in the necrotic zone of the femoral head and after the intraarticular application, we have achieved good functional results. Radiographically, good bone regeneration was achieved and the progression of necrosis to a higher degree was prevented. The key role in achieving good results postoperatively is the age of the patients, the degree of damage to the femoral head as well as the qualitative and quantitative ratio of bone marrow stem cells.

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Резиме

АНАЛИЗА НА РЕЗУЛТАТИТЕ ПО ОПЕРАТИВНА АПЛИКАЦИЈА НА МАТИЧНИ КЛЕТКИ ОД КОСКЕНА СРЦЕВИНА ВО ТРЕТМАНОТ НА АВАСКУЛАРНА НЕКРОЗА НА ГЛАВАТА НА ФЕМУРОТ

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Вовед: Аваскуларна некроза (АВН), или остеонекроза на главата на бутната коска, се дефинира како патолошки процес што резултира со критична редукција на крвоснабдувањето на главата на колкот со зголемен интраосеален притисок. Аваскуларната некроза е мултифакториелно заболување. Утврдениот досега употребуван третман се состои во трансосеална декомпресија на главата на бутната коска и е најчесто употребуваната метода во раниот стадиум на остеонекрозата.

Цел на студијата: Целта на оваа студија беше да се направи евалуација на резултатите по апликација на матични клетки од коскена срцевина добиена од гребенот на карличната коска, која беше аплицирана во некротичната зона по претходна декомпресија.

Материјал и методи: Студијата е од проспективен карактер и опфати 30 пациенти со прв, втор и трет степен на АВН според класификацијата на Ficat, а од студијата беа исклучени пациентите со четврт степен на АВН. Предоперативно се мереше обемот на движења во колкот со помош на гониометар, се направи радиолошка евалуација на степенот на АВН според класификацијата на Ficat, а се испитуваа и VAS-скалата и Harris Hip Score, предоперативно и постоперативно.

Резултати: Просечната вредност за Harris Hip Score (HHS) предоперативно беше: за Ficat I -66,32±5,1, за Ficat II -49,19±3,4 и за Ficat III -33,71±2,1. На тримесечното постоперативно следење, просечните вредности на HHS беа: 87,92±4,3 за Ficat I, 78,64±6,6 за Ficat II и 76,48±2,6 за Ficat III. Истите вредности за HHS се добиени и при контролните прегледи во шестиот и во дванаесеттиот месец постоперативно, што укажува на фактот дека е постигната добра коскена регенерација и спречена прогресија на состојбата. Намалување на вредноста на HHS е забележано на контролниот преглед на 1 година од хируршкиот третман, кај 3 пациенти според класификацијата Ficat од III степен, кај кои е имплантирана тотална ендопротеза на колк.

Заклучок: Примената на стем-клетки во третманот на аваскуларната некроза на главата на бутната коска даде добри функционални резултати и намалување на степенот на болка кај оперираните пациенти. Радиографски се покажа дека е постигната добра коскена регенерација и спречена е прогресијата на некрозата во повисок степен.

Клучни зборови: аваскуларна некроза, декомресија, матични клетки, Harris Hip Score