EFFECTS AND SAFETY OF PLATELET-RICH PLASMA IN COMBINATION WITH HUMAN MESENCHYMAL STEM CELLS FROM AUTOLOGOUS ADIPOSE TISSUE FOR KNEE OSTEOARTHRITIS

Cao Truong Sinh *, Le Thi Thanh Huyen *, Dinh Nhan Duong *

ABSTRACT:

Objective: To evaluate the efficacy of platelet-rich plasma in combination with human mesenchymal stem cells from autologous adipose tissue for knee osteoarthritis treatment. **Research object and method:** In the study, there are 30 patients including 26 females and 4 males; correspondingly, 60 knee joints were diagnosed with osteoarthritis according to the American College of Rheumatology (ACR) who are at the mean age of 58,63 ± 11.11, disease duration is from 5,3± 4,6 years which is respectively of stages II - III according to Kellgren and Lawrence. All were injected with autologous platelet-rich plasma that was extracted by PRP set, APC 30 PRP PRCEDURE PRAK and autologously extracted mesenchymal stem cells from abdominal adipose tissue using the ADI-25-01 ADIPOSEPRCEDURE PRAK set with the Harvest smart stem cell separator of TERUMO BCT- USA. **Results:** After 12 months of treatment: the pain level according to VAS score at the right knee joint was decreased from 6.0 ± 1.28 before treatment to 1.9 ± 0.3; VAS score at the left knee joint was decreased from 6.43±1.19 to 2.25±0.43. Total Liquesce score at right knee joint was decreased from 16.04±1.57 before treatment to 4.31±1.04, at left knee joint was decreased from 17.52±1.74 before treatment to 5.15. ±1.48. Total WOMAC score at right knee joint was decreased from 55.93±5.56 to 10.37±1.56; at left knee joint was decreased from 53.97±5.57 to 10.07±1.59. There were 52/60 (86.77%) joints with cartilage thickness change and the patellar cartilage thickness was increased from 1.56 ± 0.09 mm before treatment to 1.65 ± 0.09 mm. The rate of complications at injection site, abdominal adipose removal is low, there are no complications of local infection and systemic reactions. **Conclusion:** The treatment of knee osteoarthritis with platelet-rich plasma in combination with mesenchymal stem cells from autologous adipose tissue is effective in reducing pain, improving patient's mobility and walking function comparing with before treatment; reforming articular cartilage thickness on magnetic resonance image which therefore improves the mobility function of the knee joint. This therapy is safe.

Vinh Medical University Hospital
Responsible person: Cao Truong Sinh
Email: caotruongsinh@gmail.com
Date of receipt: 21/2/2022
Date of scientific judgment: 09/5/2022
Reviewed date: 23/5/2022

I. INTRODUCTION

Osteoarthritis of the knee is a degenerative disease which can be classified into primary and secondary knee osteoarthritis. Primary knee osteoarthritis is a disease of unknown cause which is mainly related to the aging process. Secondary knee osteoarthritis with identifiable etiology includes endocrine disorders, anatomical abnormalities, post-traumatic arthritis, and septic arthritis [1]. Osteoarthritis of the knee is characterized by the reduction of basal joint cells and destruction of the joint base [2]. Particularly, symptoms of osteoarthritis include the damage of continuous articular cartilage base [3], loss of articular chondrocytes in joints [4], subchondral microosteal damage [5], exposure of subchondral bone [6], articular surface edge and subchondral bone hyperplasia [10]. Clinically, the patients developed slow-growing knee osteoarthritis,

including joint pain, stiffness, joint swelling, decreased joint mobility and joint deformity [2,7]. Epidemiological statistics show that the overall rate of primary knee osteoarthritis in people over 40 years old is 46.3%, male is 41.6% and female is 50.4%. Furthermore, the prevalence in the middle-aged population can be up to 40% and the elderly population can be up to 80%. Osteoarthritis of the knee affects the quality of life, in severe cases, it can lead to disability. The disability rate can be as high as 50% or higher [8], [9]. Therefore, knee osteoarthritis has become a serious health problem.

Currently, the treatment of knee osteoarthritis is limited. There are several conventional therapies for knee osteoarthritis which include physical therapies, non-steroidal anti-inflammatory pain relievers, drugs which slow down the degenerative process such as glucosamine, joint lubricant supplements such as hyaluronic acid, platelet-rich plasma (PRP) or intra-articular corticosteroid injections, traditional medicine measures and arthroscopic knee surgery. All of the above-mentioned treatments can only relieve symptoms which do not restore articular cartilage. When degeneration is more severe, total knee replacement surgery is required [11], [12].

Autologous intra-articular platelet-rich plasma (PRP) injection is a new method in the treatment of osteoarthritis. Platelet-rich plasma has been shown to contain growth factors, in which, transforming growth factor-β (TGF-β) plays an important role to increase the base for chondrocytes' growth, the proliferation of chondrocytes and the regulation of proteoglycan synthesis [13].

In the world, in the past few years, many studies have shown that autologous intra-articular PRP injection is an effective therapy in the treatment of knee osteoarthritis [14]. This has been proven by many studies around the world.

Mesenchymal stem cells (MSCs) have the potential for self-renewal and multidirectional differentiation [15] which can exert therapeutic effects on various diseases through directed differentiation [16], regulate immune system [17], anti-inflammatory, progenitor [17], improve microenvironment [18] and promote regeneration [19]. MSCs have been used in the treatment of various diseases [20], such as ovarian failure, Parkinson's disease, nervous system damage and amyotrophic lateral sclerosis (ALS). MSCs therapy can be applied in the treatment of knee osteoarthritis and has shown encouraging results [16], [20].

Recent researches have shown the presence of stem cells in adipose tissues which are known as adipose-derived stem cells (ADSCs). These cells are referred to as mesenchymal stem cells (MSCs) that expose a number of special characteristics. They participate in the fibroblast-like surface formation and differentiate into osteoblasts, cartilage, and adipocytes [21]. Many researches have been conducted over the last few years which include preclinical and clinical trials to perform the treatment of cartilage injuries and knee osteoarthritis.

Stem cell therapy is a milestone in regenerative medicine for the treatment of knee osteoarthritis. MSCs have not been widely adopted because of cell source problems and expensive cell cultures. Furthermore, its efficacy and safety are being explored [22].

Therefore, the combination therapy of platelet-rich plasma with mesenchymal stem cells from autologous adipose tissue will promote both types of effects: (1) closed TGF-β increases the substrate for chondrocyte's growth, proliferation of

chondrocytes, regulation of proteoglycan synthesis; (2) mesenchymal stem cells increase cartilage regeneration, repair the organization of damaged cartilage, regenerate subchondral bone to heal joints.

Therefore, the aim of this study was to evaluate the efficacy and safety of platelet-rich plasma therapy combined with autologous adipose tissue mesenchymal stem cells in the treatment of knee osteoarthritis.

II. RESEARCH OBJECT AND METHOD

2.1. Research design

The research is a randomized clinical trial design which evaluates the results by comparing those before and after treatment 1 week, 1 month, 3 months, 6 months and 12 months according to clinical rating scales and changes of cartilage thickness on magnetic resonance images.

The study was implemented at Vinh Medical University Hospital, from June 2020 to November 2021.

2.2. Research objects

Bệnh nhân được siêu âm và chụp cộng hưởng từ để đánh giá đầu vào trước khi tiêm huyết tương giàu tiểu cầu phối hợp với tế bào gốc trung mô từ mô mỡ tự thân.

2.3. Research object and method

In the study, there are selected 30 patients including 26 females and 4 males; correspondingly, 60 knee joints were diagnosed with osteoarthritis according to the American College of Rheumatology (ACR) [23] who are at the mean age of 58,63 ± 11.11, disease duration is from 5,3± 4,6 years which is respectively of stages II - III according to the classification standard of Kellgren and Lawrence with a normal platelet count (≥150,000/mm^3).

The design is excluded patients who are at stage I, stage IV of degeneration; have severe anemia and platelets <150,000/mm^3, have secondary knee osteoarthritis, post-traumatic, infectious degeneration, cancer or have contraindications to surgery.

Patients received ultrasound and magnetic resonance imaging to evaluate input data before injecting platelet-rich plasma in combination with mesenchymal stem cells from autologous adipose tissue.

2.4. Material and method

Autologous platelet-rich plasma was extracted from 30ml of peripheral blood according to the process of PRP set, APC 30 PRP PRCEDURE PRAK and the Harvest smart extraction system of TERUMO BCT-USA, following an 8-step process with a count of 5ml (platelet count is up to 1.5 million/ml).

Autologous mesenchymal stem cells were obtained from the patient's abdominal adipose tissue and was extracted by a set of ADI-25-01 ADIPOSEPRCEDURE PRAK (2 syringes, 25 ml in each syringe) and by the Harvest Smart Stem Cell Extractor of TERUMO BCT-USA company according to the 10-step process, the obtained count is 10 ml.

Two products with platelet-rich plasma and stem cells were mixed together (15ml) and then were equally divided into 2 single-use syringes, each 7.5ml syringe was injected into a knee joint.

All procedures are carried out in the operating room, extraction room, and sterile injection room.

2.5. Criteria for evaluating treatment results

1) Evaluation of improvement in pain symptoms and knee function: before treatment, in the first week, in 1 month, 3 months, 6 months and 12 months after injection.

Using the rating scale: VAS (Visual Analog Scale); LEQUESNE; WOMAC

(Western Ontario and McMaster Universities Osteoarthritis);

+ VAS scale is with 4 pain levels, in which from 1 to 3 is mild pain, from 4 to 6 is moderate pain, from 7 to 9 is severe pain (very painful), 10 is unbearable pain.

+ LEQUESNE scale has totally 24 points: Severe ≥ 14 points; Very heavy 11-13 points; Heavy: 8-10 points; Average 5-7 points; Mild: 0-4 points.

+ WOMAC scale includes general WOMAC, pain WOMAC, stiffness WOMAC, mobility WOMAC. The maximum score of WOMAC scale is 96 points, in which pain WOMAC is 20, stiffness WOMAC is 8, mobility WOMAC is 68.

2) Evaluation of the results on magnetic resonance image after 12 months of follow-up in comparison with prior treatment.

- There is a number of patients with changes in cartilage thickness on magnetic resonance image.

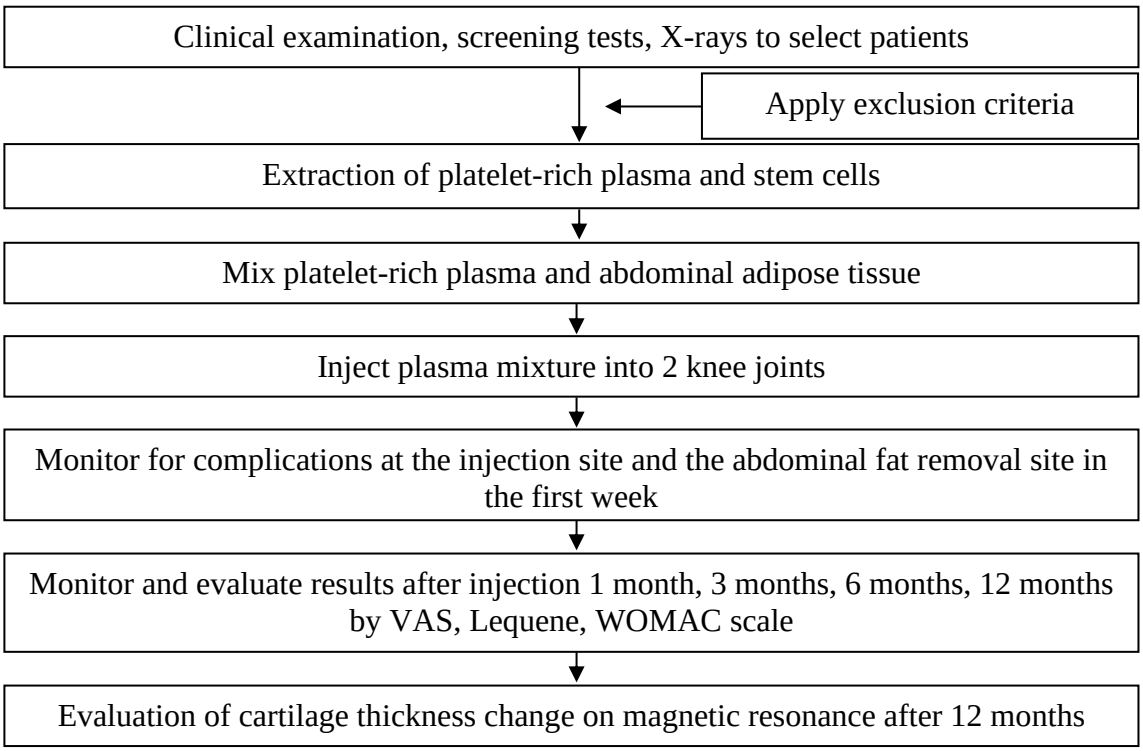
- The cartilage thickness is changed after treatment: the thickness of articular cartilage is measured at the positions of internal condyle, external condyle of the femur, intercondylar, medial tibial plateau, lateral tibial plateau, middle tibial plateau, patella.

3) Evaluation of safety

- In joints: Pain persists after injection; bleeding; swelling; infection; effusion

- At the place of abdominal adipose removal: pain, bleeding; prolonged congestion; infection.

RESEARCH CHART



6) Statistical processing: The data was processed using SPSS 20.0 software; qualitative variables are expressed as frequency and proportions, quantitative variables are expressed as mean ± standard deviation. The Chi^2 test was used to compare the 2 proportions and the T test was to compare the 2 means. The difference was statistically significant when $p < 0.05$.

III. RESULTS

3.1. Characteristics of the study sample

Table 3.1. Characteristics of patient input data

Variable	Male	Female	Total
Number of patients	4 (13.3%)	26 (86.7%)	30 (100%)
Number of joints	8 (13.3)	52 (86.7)	60 (100%)
Age	54.25 ± 9.57	59.31 ± 11.34	58.63 ± 11.11
Disease duration	3.00±1.15	5.69±4.88	5.3 ± 4.6
Height	1.63±0.03	1.55±0.05	1.56±0.05
Weight	64.25±7.27	53.5±7.2	54.93±8.00
BMI (kg/m^2)	24.0 ±2.33	22.1 ±2,2	22.3 ± 2.28
Obesity	0	2 (6,7%)	2 (6,7%)
Diabetes	1 (3.3%)	2 (6.7%)	3 (10%)
Hypertension	2 (6.7%)	4 (13.3%)	6 (20%)
Degenerative stage according to Kellgren and Lawrence classification			
Stage II	2 (3.3%)	10 (16.7%)	12 (20%)
Stage III	6 (10%)	42(70%)	48 (80%)
Platelet count	220.25±53.7	275.23±90.44	267.9±87.81

In the study sample, the number of male patients including 4 people accounted for 13.3% and the number of joints with stage III damage accounted for 80%.

3.2.Evaluation of treatment results

3.2.1. Evaluation of treatment results through the VAS scale

Table 3.2. Evaluation of treatment results of bilateral knee joints through the VAS scale

Variable	Before treatment	After treatment					p(t-s12)
		1 week	*1 month*	*3 months*	*6 months*	*12 months*	
Knee joint R	6.0 ±1.28	6.37±1.16	5.5±0.68	4.1 ±0.48	2.7±0.46	1.9±0.3	0.00
Knee joint L	6.43±1.19	6.73±0.58	5.9±0.55	4.4±0.62	3.5±0.51	2.25±0.43	0.00

The average VAS pain score of right knee before treatment which was 6 ± 1.28 decreased to 2.70 ± 0.46 after 6 months of treatment and to 1.90 ± 0.30 after 12 months of treatment. This improvement is statistically significant with $p < 0.001$. The mean VAS pain score of the left knee joint before treatment was 6.43 ± 1.19, decreased to 3.5 ± 0.51 after 6 months of treatment and to 2.25 ± 0.43 after 12 months of treatment. This improvement is statistically significant with $p < 0.001$.

3.2.2. Evaluation of treatment results using the Lequesne scale

Table 3.3. Evaluation of treatment results of knee joints by Lequesne scale

Variable	Before treatment	After treatment					p(t-s)
		1 week	*1 month*	*3 months*	*6 months*	*12 months*	
Knee joint R	16.04±1.57	17.03±0.73	15.51±1.64	12.53±2.17	6.0±1.38	4.31±1.04	0.00
Knee joint L	17.52±1.74	18.13±2.02	16.35±1.81	13.55±1.95	7.02±1.27	5.15±1.48	0.00

After 12 months of treatment, the mean LEQUESNE scale of right knee joint was improved, decreasing from 16.04 ± 1.57 to 4.31 ± 1.04 with statistical significance with $p<0.001$. After 12 months of treatment, the average LEQUESNE scale of the left knee joint was improved, decreasing from 17.52±1.74 to 5.15±1.48 with statistical significance with $p<0.001$.

3.2.3. Evaluation of treatment results by WOMAC scale

Table 3.4. Evaluation of treatment results of the right knee joint through the WOMAC scale

Variable		Before treatment	After treatment					p(t-s)
			1 week	1 month	3 months	6 months	12 months	
General WOMAC	$\overline{X}$	55.93	54.00	43.93	30.53	25.67	10.37	0.00
	SD	5.56	5.60	4.64	3.39	3.44	1.56	
Pain WOMAC	$\overline{X}$	12.47	12.17	10.03	6.30	4.20	2.17	0.00
	SD	2.47	2.90	2.70	2.17	1.52	0.95	
Mobility WOMAC	$\overline{X}$	40.00	38.37	31.70	23.17	20.57	7.33	0.00
	SD	3.86	3.99	2.72	2.27	2.40	0.99	
Stiffness WOMAC	$\overline{X}$	3.47	3.47	2.20	1.07	0.90	0.87	0.00
	SD	1.14	1.14	0.61	0.45	0.40	0.34	

Overall WOMAC scale of right knee joint was decreased from 55.93 ± 5.56 to 10.37 ± 1.56 after 12 months of treatment. There was a remarkable improvement in the scales of pain WOMAC, mobility WOMAC, and stiffness WOMAC after the treatment.

Table 3.5. Evaluation of treatment results of the left knee joint through the WOMAC scale

Variable		Before treatment	After treatment					p(t-s)
			1 week	1 month	3 months	6 months	12 months	
General WOMAC	$\overline{X}$	53.97	55.00	42.93	33.90	19.43	10.07	0.00
	SD	5.57	5.60	4.64	4.51	3.51	1.59	
Pain WOMAC	$\overline{X}$	12.17	12.17	11.03	9.30	4.20	3.17	0.00
	SD	2.90	2.90	2.70	2.16	1.51	0.95	
Mobility WOMAC	$\overline{X}$	38.33	39.37	29.70	23.53	14.57	6.33	0.00
	SD	3.95	3.99	2.73	3.44	2.40	0.99	
Stiffness WOMAC	$\overline{X}$	3.47	3.47	2.20	1.07	0.67	0.57	0.00
	SD	1.14	1.14	0.61	0.45	0.55	0.50	

Overall WOMAC scale of left knee joint was decreased from 53.97±5.57 to 10.07±1.56 after 12 months of treatment. There was a remarkable improvement in the scales of pain WOMAC, mobility WOMAC, and stiffness WOMAC after the treatment.

3.2.4. Evaluation of the results based on the change of cartilage thickness on magnetic resonance image

Table 3.6. Change in thickness of articular cartilage on MRI

Position	Time	Measurement position (mm)					
		External condyle	p(t-s)	Internal condyle	p(t-s)	Intercondylar	p(t-s)
Lower end of femur	Before treatment	1,40±0,10	0.00	1,35±0,16	0.00	1,57±0,11	0.00
	After treatment	1,46±0,11		1,42±0,15		1,64±0,12	
Upper end of femur	Before treatment	1,39±0.10	0.00	1,35±0,16	0.00	1,56±0,12	0.00
	After treatment	1,45±0,12		1,43±0,16		1,63±0,12	

Position	Time	Measurement position (mm)					
		External condyle	p(t-s)	Internal condyle	p(t-s)	Intercondylar	p(t-s)
Patellofemoral joint	Before treatment	1,56 ±0,09					0.00
	After treatment	1,65±0,09					

p(t-s): P-value before and after treatment

The surface of articular cartilage on magnetic resonance in the above mentioned positions were improved after 12 months of treatment, the difference was statistically significant.

Table 3.7. Number of joints with changes in cartilage thickness on MRI

	Stage II		Stage III		Overall	
	n	%	n	%	n	%
Unchanged joints	0	0	8	100	8	13,33
Changed joints	12	23,07	40	76,92	52	86,77

After 12 months of treatment, there were 8 joints (13.33%) that did not change the total thickness of cartilage at the measured locations (lower end of femur, upper end of tibial, patella joint), found in degenerative joints of stage III.

3.3. Evaluation of the safety of therapy

Table 3.8. Complications at the injected knee joint

Symptoms		n	Rate %
Knee joint pain after stem cell injection	Pain after injection 0 - 3 hours	45	75
	Pain after injection 3h - < 6 hours	4	6,7
	Pain after injection 6h - <12h	3	5
	Pain after injection 12h - < 24h	2	3,3
	Pain after injection ≥ 24h	6	10
Joint effusion		6	10
Joint infection		0	0,0
Soft tissue infections around joints		0	0,0

The rate of knee pain after 3 hours of injection is 15/60 joints (accounting for 25%), of which 6 cases of pain persisted for more than 24 hours, 10% of the knee joints effused after injection. There were no patients who exposed joint infections or soft tissues around the joints after injection.

Table 3. 9. Complications at abdominal adipose removal site

Characteristics			*n*	*%*
Prolonged pain at the site of abdominal adipose removal	Pain gone after 3 hours		22	73,3
	Pain persists after abdomen adipose removing >3h	Overall	8	26,7
		Pain 3- < 24h	7	23,3
		Pain > 24h	1	3,3
Hemorrhage at subcutaneous abdomen	No		22	73,3
	Yes		8	26,7
	Average time		1,8±3,3	

- There were 73.3% of patients without prolonged pain after adipose removal and having no hemorrhage at subcutaneous abdomen after adipose removal.

- The rate of prolonged pain in the abdomen after adipose removal was 26.7%, of which the majority of patients had pain within 1 day. There is only one patient who had pain lasting more than 24 hours.

- There are 26.7% of patients with congestion in the abdomen after adipose removal. The longest congestive time was 1.8±3.3 days.

IV. DISCUSSION

4.1. Evaluation of treatment results

4.1.1. Clinical evaluation of treatment results

After 12 months of treatment with a mixture of platelet-rich plasma in combination with mesenchymal stem cells from autologous adipose tissue, we found that the patients had reduced pain, improved motor function, and improved signs of knee stiffness which was as follows:

The patient's joint pain relief was shown by the mean of VAS score at the right knee, the left knee joint decreased significantly compared to before treatment. This improvement started after 1 month, 6 months and was most pronounced after 12 months.

Patients improved signs of stiffness, the ability to walk, go up and down stairs, squat as shown by the mean of LEQUESNE score of the right knee and the left knee all decreased significantly compared to before treatment with p<0.001.

The WOMAC score of both right and left knee decreased significantly after 12 months of treatment. In which, there was a significant improvement in the WOMAC pain scale, WOMAC movement score, and WOMAC joint stiffness score after the treatment.

In this regard, some studies also showed similar results:

In the study by Bui et al (2013) on 21 patients with stage II-III knee osteoarthritis treated with adipose tissue stem cell therapy combined with platelet-rich plasma. The results showed that all patients had improved knee function after 8.5 months of treatment. The VAS score of pain decreased from 7.6 ± 0.5 to 3.5 ± 0.7 after 3 months and 1.5 ± 0.5 score after 6 months [24].

Similarly, Tran et al (2016) evaluated the effect of autologous adipose stem cell therapy on 42 patients with stage I-II knee osteoarthritis compared with the control group treated with intramuscular hyaluronic acid, the results showed that the intervention group had lower VAS and WOMAC scores than the control group. After 12 months of follow-up, VAS score decreased from 6.16 ± 1.06 to 2.26 ± 1.04 and WOMAC decreased from 54.26 ± 10.61 to 16.7 ± 9.47 points with p< 0, 05 [25].

Another study treated knee osteoarthritis with autologous adipose tissue stem cell therapy by Pham (2017) showing that before treatment all the patients had pain when climbing stairs (100%). Then, after 6 months of treatment, the rate of knee pain when climbing stairs was 69.4% and reduced to 48.6% after 1 year of treatment. Furthermore, before treatment 83.3% of patients felt pain of their knees when standing for more than 30 minutes, but after 1 year of treatment, there were no cases of pain when standing [26].

Recent meta-analysis by Yancheng Song and colleagues (2020) in China for the treatment of knee osteoarthritis with

mesenchymal stem cells. The study based on the results of 15 controlled clinical trials, 2 longitudinal follow-up studies, and 2 cohort studies with a total of 484 joints. The results showed that stem cell therapy for knee osteoarthritis significantly reduced the VAS score after 12 months of treatment and the WOMAC score significantly decreased after 6 months [22].

4.1.2. Evaluation of treatment outcomes based on MRI

The results of our study depicted that all positions of the surface of articular cartilage on MRI has improved. Specially, the lateral condyle of the lower femoral increased from 1.40 ± 0.10 mm before treatment to 1.46 ± 0.11 mm after 12 months of treatment. The surface of articular cartilage of the medial condyle in the lower femoral bone increased from 1.35 ± 0.16 to 1.42 ± 0.15 and the surface of the condyle in the head of the tibia increased from 1.35 ± 0.16 to 1.43 ± 0 ,16 after 1 year of treatment. The surface of inferior femoral condylar increased from 1.57 ± 0.11 to 1.64 ± 0.12 after 1 year of treatment. The surface of supracondylar on the tibia increased from 1.56 ± 0.12 to 1.63 ± 0.12 after 1 year of treatment. All 3 measurement positions of the kneecap, there was an improvement from 1.56 ± 0.09 to 1.65 ± 0.09 after 1 year of treatment (table 6). After 12 months of treatment, remaining 8 stage-III knees (13.33%) had no change in total cartilage thickness at measurement sites at the lower head of femoral, the upper tibia and the patellar joint (Table 3).

Several studies have used parameters on MRI such as the change of cartilage thickness to evaluate the effectiveness of autologous adipose tissue stem cell therapy or platelet-rich plasma therapy in the treatment of knee osteoarthritis.

Study by Tran et al (2016) showed that there was an improvement in articular cartilage thickness on MRI at 4 positions which were medial tibial plateau, lateral tibial plateau, medial condyle, and lateral condyle of the femur at the time of 6 months and 1 year follow-up but there was no statistical significance ($p > 0.05$). This may be because the majority of study subjects are mild knee osteoarthritis (stage I-II), so the difference was not significant [26].

Another study by Pham (2017) on treatment of primary osteoarthritis with autologous adipose stem cell therapy, the findings depicted that: the thickness of articular cartilage at the femoral condyle increased from 1, 52 ± 0.57 mm before treatment to 1.61 ± 0.59 mm after 6 months and to 1.65 ± 0.56 mm after 1 year. The thickness of articular cartilage at the tibial plateau from 1.59 ± 0.59 mm before treatment to 1.68 ± 0.59 mm after 6 months and to 1.75 ± 0.57 mm after 1 year [25].

In 2012, Yong-Gon Koh and colleagues evaluated the clinical and imaging results in 18 patients with knee osteoarthritis treated with autologous adipose tissue stem cells at the lower-kneecap. The authors evaluated the improvement of articular cartilage by the WORMS (whole organ magnetic resonance imaging score) scale, the findings showed that the WORMS score decreased from 60.0 to 48.3 points ($p<0.01$). In particular, this study also showed that there was a positive correlation between the improvement of clinical symptoms and images of knee osteoarthritis with the amount of injected stem cells. The authors concluded that adipose tissue stem cells were a valuable source of cells in the treatment of articular cartilage damage [27], [28].

In 2016, a study by Liang-jing Lu and

colleagues on 18 patients with bilateral knee osteoarthritis who were treated with adipose tissue stem cell therapy with 3 injections: before treatment, after 3 weeks and after 48 weeks, the results showed that the volume of articular cartilage of the femur, tibia and patella increased steadily during the entire follow-up period, which was statistically significant after 6 months, 12 months and 18 months [29].

In summary, all above researches have depicted that the adipose tissue stem cells were effective in repairing cartilage damage.

4.2. Safety of the therapy

The ratio of knee pain after < 3 hours injections were 45/60 joints (accounting for 75%). There were 6 cases of pain which lasted more than 24 hours, accounting for 10%. There was 10% effusion of the knee joint after injection and there was no case of infection at the joints or soft tissue around the knee joint.

The majority of patients had no pain after 3 hours of adipose removal (accounting for 73.3%) and no bleeding under the skin of the abdomen after adipose removal (accounting for 73.3%). In our study, the pain ratio after abdominal adipose removal accounted for 26.7%. There are 26.7% patients with bleeding in the abdomen after adipose removal. The longest bleeding time was 1.8±3.3 days.

The pain rate and complications after abdominal adipose removal and after injection was low, in addition, our study did not experience any undesirable systemic effects such as headache, dizziness, rash, bleeding or state of shock. It is likely that our study used autologous adipose tissue stem cells which has many advantages in many aspects such as: easy to obtain, minimal intervention, thus it minimized complications in comparison with method of bone marrow mesenchymal stem cell extraction.

Khanh Hong Thien Bui (2013) found that 100% of patients had no side effects or complications which were related to the treatment process such as infections or presence of tumors in the joints [24].

Tien Tran Viet et al in 2016 showed that this is a quite safe technique, the rate of complications is very low. There is no patient with complications of knee infection. In terms of joint exudation reaction, there were only 2 patients with this manifestation at a mild level and completely resolved after 3-5 days of taking anti-edematous drugs (Alpha-chymotrypsin) and no longer expressed in the following months [26]].

In 2012, the author Yong-Gon Koh et al. conducted a study to evaluate the clinical results and medical imaging on 18 patients with knee osteoarthritis who were treated with autologous adipose tissue stem cells at the lower part of the patella which has shown a few cases of mild knee pain after injection lasting 2-3 days. However, there was 1 patient with severe pain accompanied by swelling of the knee joint after injection, these symptoms resolved spontaneously after 2 weeks [28], [29].

In 2016, Liang-Jing Lu et al. reported a study on 18 patients with bilateral knee osteoarthritis who were treated with adipose tissue stem cell therapy with 3 injections: before treatment, after 3 weeks and after 48 weeks of treatment. There were no deaths or serious adverse events during the study [29].

Centeno's study was conducted for 2 years on 339 osteoarthritis patients [30] who used mesenchymal stem cells showed that this is a safe therapy. No cases of infection or cancer have been found after treatment.

Yancheng Song et al (2020) in China

conducted a meta-analysis of clinical trials for the treatment of knee osteoarthritis with mesenchymal stem cells. The study came to a conclusion that stem cell therapy is effective in improving motor function in patients with knee osteoarthritis, and is a safe and potential therapy for patients with knee osteoarthritis [22].

V. CONCLUSION

The treatment of knee osteoarthritis with platelet-rich plasma in combination with mesenchymal stem cells from autologous adipose tissue is effective in reducing pain, improving patient's mobility and walking function through scale reductions of VAS, LEQUENE, WOMAC comparing with before treatment, improving the thickness of articular cartilage thickness on magnetic resonance thereby improving the mobility function of the knee joint. This therapy is safe with a low rate of local complications which has no systemic complications.

REFERENCE

1. **Koh YG, Choi YJ(2012),** Infrapatellar adipose pad-derived mesenchymal stem cell therapy for knee osteoarthritis, *The Knee 2012*;19(6):902-7.
2. **Ghosh P, Smith M (2002),** Osteoarthritis, genetic and molecular mechanisms, *Biogerontology 2002*;3(1-2):85-8.
3. **Vega Aurelio, Martín-Ferrero Miguel Angel, Canto Francisco Del, Alberca Mercedes, García Veronica, Munar Anna, et al (2015),** Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells. *Transplantation 2015*;99(8): 1681-90.
4. **Pers YM, Rackwitz L, Ferreira R, Pullig O, Delfour C, Barry F, et al (2016),** Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial, *Stem Cells Transl Med* 2016;5:847-56. sctm.2015- 0245.
5. **Wang YL, Jin WX, Liu HY, Cui YH, Mao QC, Fei ZY, et al (2016),** Curative effect of human umbilical cord mesenchymal stem cells by intra-articular injection for degenerative knee osteoarthritis. Zhongguo xiu fu chong jian wai ke za zhi Zhongguo xiufu chongjian waike zazhi *Chinese journal of reparative and reconstructive surgery* 2016;30(12):1472.
6. **Espinoza F, Matas J, Orrego M, Tapia R, Infante C, Khoury M, et al (2017),** Allogeneic mesenchymal stromal cell (MSC) therapy for knee osteoarthritis (OA): a phase I/II randomized controlled trial, *Cytotherapy* 2017;19(5):24.
7. **Hootman Jennifer M, Helmick Charles G, Barbour Kamil E, Theis Kristina A, Boring Michael A(2016)** Updated projected prevalence of self-reported doctor-diagnosed arthritis and arthritis-attributable activity limitation among US adults, 2015-2040, *Arthritis Rheum* 2016;68(7):1582-7.
8. **Bastos Ricardo, Mathias Marcelo, Andrade Renato, Bastos Raquel, Balduino Alex, Schott Vinicius, et al (2018),** Intra-articular injections of expanded mesenchymal stem cells with and without addition of platelet-rich plasma are safe and effective for knee osteoarthritis. *Knee Surgery, Sports Traumatology, Arthroscopy*; 2018.
9. **Qingyun X, Kunzheng W, Fuxing P, Hanmin Z, Dadi J, Tianzun T, et al (2015),** The survey of the prevalence of primary osteoarthritis in the population aged 40 years and over in China. *Chin J Orthop* 2015;35(12):1206-12.
10. **Zhu Yu, Yuchen Wang, Bizeng Zhao, Xin Niu, Bin Hu, Qing Li, et al (2017),** Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. *Stem Cell Res Ther* 2017;8(1):64.
11. **Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, et al (2016),**

Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy -a review. *BMC Muscoskel Disord* 2016;17(1):230.

12. **Jones IA, Togashi R, Wilson ML, Heckmann N, Vangsness CT (2019),** Intra-articular treatment options for knee osteoarthritis. *Nat Rev Rheumatol* 2019;15(2):77-90.
13. **El-Sharkawy H, Kantarci A, Deady J. et al (2007).** Platelet-rich plasma: growth factors and pro-and anti-inflammatory properties. *J Periodontol*, 78 (4), 661-669.
14. **Sampson S, Reed M, Silvers H. et al (2010),** Injection of platelet-rich plasma in patients with primary and secondary knee osteoarthritis: a pilot study. *Am J Phys Med Rehabil*, 89 (12), 961-969.
15. **Coulson-Thomas VJ, Coulson-Thomas YM, Gesteira TF, Kao WY (2016),** Extrinsic and intrinsic mechanisms by which mesenchymal stem cells suppress the immune system, *Ocul Surf* 2016;14(2):121-34.
16. **Ryu Jae-Sung, Jung Yeon-Hwa, Cho Mi-Young, Yeo Jee Eun, Choi Yun-Jin, Kim Yong Il, et al (2014),** Co-culture with human synovium-derived mesenchymal stem cells inhibits inflammatory activity and increases cell proliferation of sodium nitroprusside-stimulated chondrocytes, *Biochem Biophys Res Commun* 2014; 447(4):715-20.
17. **Barry F, Murphy M (2013),** Mesenchymal stem cells in joint disease and repair, *Nat Rev Rheumatol* 2013;9(10):584-94.
18. **Mamidi MK, Das AK, Zakaria Z, Bhonde R (2016),** Mesenchymal stromal cells for cartilage repair in osteoarthritis, *Osteoarthritis Cartilage* 2016;24(8):1307-16.
19. **Caplan AI (2010),** Adult mesenchymal stem cells for tissue engineering versus regenerative medicine, *J Cell Physiol* 2010;213(2):341-7.
20. **Susanne G, Julia L (2014),** Tissue-engineering strategies to repair chondral and osteochondral tissue in osteoarthritis: use of mesenchymal stem cells, *Curr Rheumatol Rep* 2014;16(10):452.
21. **Patricia A. Zuk et al (2001),** Multilineage Cells from Human Adipose Tissue*: Implications for Cell-Based Therapies*. 2001 Apr;7(2):211-28
22. **Yancheng Song et al (2020),** Mesenchymal stem cell in knee osteoarthritis treatment: A systematic review and meta-analysis, *Journal of Orthopaedic Translation* 24 (2020)121-130.
23. **Thitinan Srikulmontree (2012).** *Osteoarthritis*. The American College of Rheumatology.
24. **Khanh Hong-Thien Bui, Triet Dinh Duong, Nhan Thanh Nguyen et al (2014),** Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study, *Biomedical Research and Therapy*, 1, 02-08.
25. **Phạm Hoài Thu (2017)** *"Nghiên cứu kết quả điều trị bệnh thoái hóa khớp gối nguyên phát bằng liệu pháp tế bào gốc mô mỡ tự thân"*. Luận án tiến sỹ Y học, Trường đại học Y Hà Nội.
26. **Trần Viết Tiến và cộng sự (2015).** *Nghiên cứu ứng dụng tế bào gốc tự thân trong điều trị bệnh thoái hóa khớp.* Đề tài độc lập cấp nhà nước, Học viện quân Y.
27. **Yong-Gon Koh, Seung-Bae Jo, Oh-Ryong Kwon et al (2012),** Mesenchymal Stem Cell Injections Improve Symptoms of Knee Osteoarthritis, *The Journal of Arthroscopic and Related Surgery*, 29 (4), 748-755.
28. **Yong-Gon Koh, Yun-Jin Choi, Sae-Kwang Kwon et al (2013),** Clinical results and second-look arthroscopic findings after treatment with adipose- derived stem cells for knee osteoarthritis, *Knee Surg Sports Traumatol Arthrosc*.
29. **Liang-jing Lu et al (2016),** Treatment with human adipose- derived mesenchymal stem cells for knee osteoarthritis. *NCT 021626693, The 18th Congress of Asia Pacific League of Association for Rheumatology* (APLAR 2016). Shanghai, China.
30. **Centeno CJ, Schultz J, Cheever M. et al(2010).** Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique, *Curr Stem cell Res Ther*, 5 (1), 81-93.