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RESEARCH ARTICLE

The intra-articular injection of adipose-derived stem cells decreases pain and reduces inflammation in knee osteoarthritis, with or without the addition of platelet-rich plasma also improves functionality

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Abstract

The increase of individuals with Osteoarthritis (OA) has generated an increase in public spending in the treatments, which are still not that effective. So, the purpose of this study was to analyze and compare four types of interventions: platelet-rich plasma (PRP), adipose-derived stem cells (ADSCs), ADSCs + PRP and the standard surgical video arthroscopy (All groups passed through standard arthroscopy before intervention). The evaluation was performed by applying the questionnaires Western Ontario McMaster Universities, Short Form Health Survey 36 and Visual Analog Pain Scale, also by analyzing the synovial fluid (inflammatory cytokines, enzymatic, colorimetric and viscosity analysis), this evaluation happened in two moments: before the surgical procedures and after 6 months of the interventions and also was made a comparison to standard arthroscopy. The questionnaires results showed a greater improvement in the scores of the domains analyzed in the ADSCs + PRP group, followed by the ADSCs and PRP group. In the evaluation of inflammatory cytokines, there was a significant reduction in the cytokine IL-1b only in the ADSCs + PRP group (46%) and ADSCs (31%), of IL-6 in the ADSCs + PRP group (72%), of IL-8 in the ADSCs + PRP group (50%) and ADSCs (31%), and TNF in the ADSCs + PRP group (46%). There was also a significant increase in the amount of

total proteins (79%) in the control group and polymorphonuclear cells (47%) in the ADSCs + PRP group. Taking all the results into account, we infer that therapies with ADSCs + PRP and only ADSCs are safe and effective over 6 months for the improvement of pain, functional capacity and joint inflammation in volunteers with OA. It is also considered that the use of ADSCs + PRP, particularly, is a promising alternative to help manage this disease, due to the better results presented among the four propose interventions.

KEYWORDS
arthralgia, cell therapy, mesenchymal stem cells, regenerative medicine

1 | INTRODUCTION

Osteoarthritis (OA) is currently considered the fourth disease that most promotes functional disability in the world (Lapuente, Dos-Anjos, & Blázquez-Martínez, 2020). In Brazil, if the aging factor of the population is considered it is expected to reach 64 million individuals with OA in 2050. So it is reveal a relevant world level socioeconomic problem and affects the gross domestic product of developed countries. This pathology can cause severe limitations on the individual's activities of daily living (ADLs), which seriously affect their quality of life, their well-being and their ability to carry out their work.

The options of conservative treatments with medications, intra-articular injections, orthosis, supplementation and physiotherapy exists. Unfortunately, these options have limitations due to the low regenerative capacity of the articular cartilage, so there is still no standard treatment that is completely efficient. These facts increase the number of volunteers waiting in the lines for assistance in Orthopedics services, in order to solve their problem and return to their ADLs. So, given all this ineffective therapeutic proposal and high patient demand, high costs are generated in the world annually.

Recent advances in science have provided a new understanding of the pathophysiology of OA, in which inflammatory mediators, growth factors, chondrocyte apoptosis, and imbalance between anabolic and catabolic mechanisms play an important role in the onset and progression of the disease (Woodell-May & Sommerfeld, 2020). Therefore, the compression of the inflammatory process of the joint elements gained attention. Currently, mesenchymal stem cells (MSCs) highlighted as an effective therapy, because of their immunomodulatory and trophic effects related to the production of cytokines and growth factors acting to modulate the inflammatory and degenerative aspects of OA (Najar, Martel-Pelletier, Pelletier, & Fahmi, 2020; Pers et al., 2018). Due to higher yield after extraction and ease of access, the adipose-derived stem cells (ADSCs) are being used more. Another proposed therapy is the use of platelet-rich plasma (PRP), which consists of a high concentration of autologous platelets, growth factors and cytokines. Its use accelerates the healing process and promotes cartilage repair. Acting in the control of inflammation and catabolic events (Shahid & Kundra, 2017). It is believed that the combination of these two therapies would provide better results in relation to the progression of the degenerative process of OA (Bennell, Hunter, & Paterson, 2017).

In view of this problem, to optimize the treatment of OA in a public hospital which our research group belongs, we focus our attention on research related to the topic. Our preclinical results allowed us to conclude that ADSCs therapy is beneficial for tissue regeneration of OA in an experimental model, the effect was via paracrine and chondrogenic differentiation (Hermeto et al., 2016). Thus, the present research is the first proposal for cell therapy in humans in our state, therefore is so important to start the investigation about the resolution of the OA in humans.

2 | MATERIAL AND METHODS

2.1 | Casuistry

The volunteers were triages from June/2018 to June/2019. These volunteers were already reffered for knee surgery. Included were adult individuals, of both genders, aged between 40 and 75 years, with a diagnosis of OA grade II to IV (Kellgren & Lawrence, 1957), diagnosis was performed by means of X-Ray. Volunteers should still not have been responsive to conservative treatment (physical and drug) commonly used in the Unified Health System (SUS) for at least 6 months. Excluded from volunteers were individuals with hemophilia, anemia, gastrointestinal diseases, cancer, thyroid disorders, liver, previous nephrology and Acquired Immunodeficiency Syndrome (AIDS).

This is an open clinical trial. The initial number of participants approved to receive treatment in this research by the ethics committee was 40 individuals. However, we had some difficulty of performing elective surgeries, since the work was performed in a public hospital and, in part, during the Covid-19 pandemic period. So, we decided to analyze the results of a total of 26 volunteers only that participated in this period and were randomly distributed into four groups by the responsible doctor, as soon that the volunteers entered in the service of the Orthopedics outpatient clinic/HUMAP. All groups passed through standard arthroscopy before the proposed intervention, and received only one application of the cell therapy proposed in the group which belonged. Each group contained at least

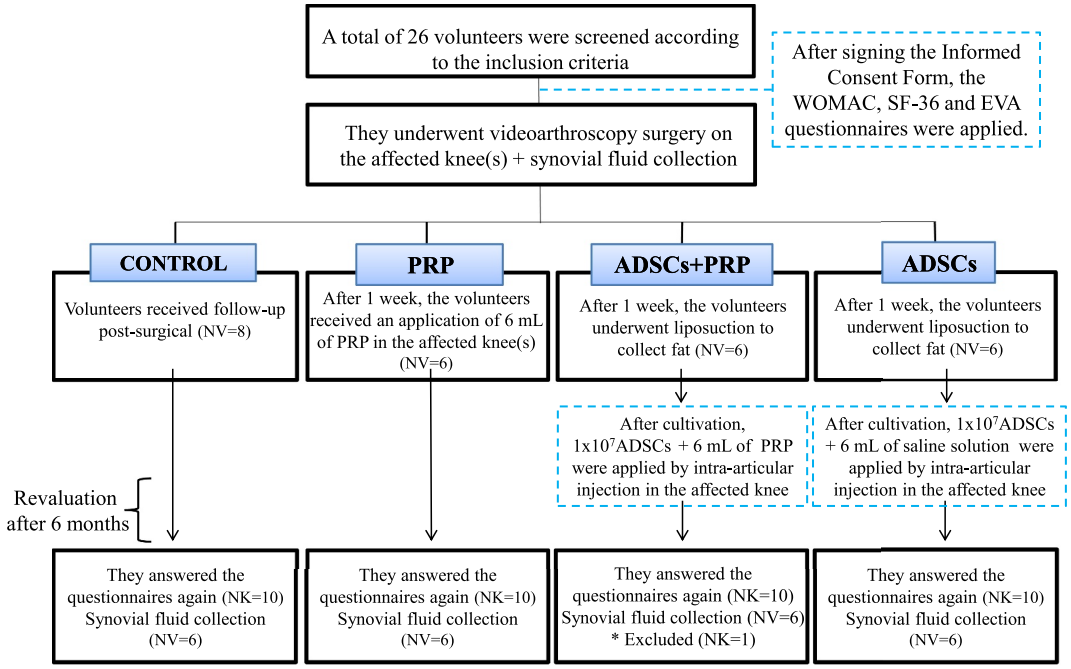

FIGURE 1 Flowchart of the stages of screening volunteers and their respective groups. ADSCs = Adipose-derived stem cells; NK = number of knees; NV = number of volunteers; PRP = platelet-rich plasma

10 treated knees, all data shown here were calculated based on the unique findings of each knee (Figure 1).

The present study was not carried out blindly, due to the requirements of the ethics committee for its performance. Clearly, the steps proceeded with the following details. Volunteers were informed of all procedures they would undergo in each group, this was a requirement of the ethics committee. Thus, only was collected adipose tissue from volunteers who would receive ADSCs transplants. Only volunteers who would receive PRP had their blood collected. Therefore, the volunteer knew which treatment he would be submitted to as instructed by the Ethics Committee. Also, due the transplanted material had different colors, the doctors who made the applications knew when they applying ADSCs (transplanted fluid was colorless) or PRP (transplanted fluid was red). However, they did not known whether the PRP was pure or in association with ADSCs. However, just the first authoress, responsible for handling the biological material and cultivating the ADSCs, knew for sure, the contents of the syringes and which group the volunteers belonged to in this research. It is also noteworthy that all other researchers who performed clinical or biological assessments were not aware of which treatment each patient was submitted to.

2.2 | Surgical procedures

2.2.1 | Knee video arthroscopy

For the pre-surgical evaluation, the parameters evaluated were blood count, potassium, urea, creatinine, blood glucose, prothrombin activity time, activated partial thromboplastin time, thrombin time and C-reactive protein. After these tests, volunteers were forwarded to video arthroscopy surgery. All video arthroscopy surgeries were performed according to the hospital routine. The surgeries were performed by the same orthopedist. Only joint cleaning and light debridement of the affected cartilage was performed. No microfracture or aggressive debridement was performed. Antibiotics and analgesics were prescribed for the first week after surgery and the use of crutches if the volunteer still felt pain.

2.2.2 | Procedure of liposuction, extraction of ADSCs and cultivation

Volunteers in the ADSCs + PRP and ADSCs groups returned 1 week after video arthroscopy for liposuction. The surgery was performed without the addition of any vasoconstrictor substance in Klein's solution (100 ml) that were applied subcutaneously in the chosen region for fat emulsification. After 15 min the liposuction was performed with a 3.25 d medium gauge cannula attached to a 60 ml syringe. An average of 200 ml of biological material were extracted from the lower abdomen region and deposited in a sterile container, containing 200 ml of phosphate buffer solution (PBS) and 1% of antibiotic (Penicilin, Streptomycin, Amphotericin - Sigma-Aldrich® - A5955, Lot#017M47). Subsequently, the material was sent to CeTroGen to the processing of the cells. It was performed by enzymatic digestion with Collagenase type 1 (Gibco®; N° 9001-12-1; 290 U/mg) as described (Markarian et al., 2014; Schweich-Adami et al., 2021) (Figure 2). For every 1 ml of fat, 3 ml of collagenase (1 ml of PBS: 0,87 mg de collagenase) was used. The culture medium used was low glucose Dulbecco's modified Eagles medium (DMEM)

(Sigma®, D5523, Lot#SLBT7950) with 2,5 g of Free Acid HEPES (Sigma®, H3375, Lot#SLCDE799), 3,7 g of sodium bicarbonate (Dinâmica®,1005-1, P.10.0159.003.00.30) and 10% of bovin fetal sérum (BFS) (Gibco®, Sigma-Aldrich®, F2561, Lot#SPBB2353V67 V).

The fractionated vascular stromal pellet acquired at the end of the process, was resuspended in 5 ml of DMEM and seeded in a culture 25 cm^3 flask. After 3 days the culture was washed 3x with 3 ml PBS to eliminate non-adherent cells and debris. DMEM culture medium, 10% BFS and 1% antibiotic (Penicilina/Estreptomicina/Amphotericin, Sigma-Aldrich®, A5955, Lot#017M47) it was changed every 72 h (5 ml). Upon reaching 70%–80% confluence in the flasks, they were trypsinized (0,25% trypsin/3 min), divided and seeded in new larger flasks (75 cm^2), until the amount of cells stipulated for the treatment of each knee was obtained (1×10^7). A separate aliquot of cells was cultured for immunophenotyping by flow cytometry (CD105, CD90, CD34 and CD133) and cell differentiation (adipogenic, osteogenic and chondrogenic). All biological materials used for transplantation were previously submitted to cell viability analysis, using trypan blue, and conventional microbiological routine (aerobiosis and microaerophilia), for 72 h of incubation.

2.2.3 | Preparation and transplantation of platelet-rich plasma

Volunteers in the PRP group returned after 1 week of video arthroscopy. For each treated knee, 18 ml of peripheral blood were collected in tubes containing 3,2% sodium citrate. Vials were centrifuged at 1500 rpm/10 min. The plasma and the adjacent pellet formed were then separated in another tube and centrifuged at 2000 rpm/15 min. After discarding the upper two-thirds, the PRP was aspirated by a syringe. Then, with the same syringe that already contained the PRP, 0,3 ml of 10% calcium gluconate was aspirated. The product was then homogenized and immediately transplanted. Before transplantation, the patient's knee was submitted to asepsis and then the intra-articular injection of PRP was performed (Figure 2). A bandage was applied around the treated knee in order to avoid limb flexion in the first 12 h. Orientations regarding rest in the first week and post-transplant care were duly explained to the volunteers.

2.2.4 | Preparation and transplantation of ADSCs associated or not with platelet-rich plasma

After reaching the amount of ADSCs needed for transplantation in each knee (1×10^7), volunteers in the ADSCs + PRP and ADSCs groups were contacted and returned for the transplant.

For the syringe preparation, the ADSCs vials were first washed with 3 ml of PBS (3x), then they were trypsinized (0,25% trypsin/3 min) and the contents were centrifuged (1200 rpm/5 min) in a falcon tube. The supernatant was then discarded and the cell pellet was resuspended in 3 ml PBS. Afterward, falcon tubes were centrifuged again (1200 rpm/5 min). This process was repeated 3x. To ADSCs Group this pellet received 6 ml of saline solution. After this preparation, the syringe with the ADSCs was sent to the Orthopedics Outpatient Clinic/HUMAP. At the clinic, asepsis of the affected knee was performed. The patient received local anesthesia (2% lidocaine) in the lateral interarticular region of the knee. After 10 min, transplantation was performed through an intra-articular injection (21G needle) of ADSCs in the affected knee (Figure 2). A bandage was applied around the knee in order to avoid limb flexion in the first 12 h. Orientations regarding rest in the first week and post-transplant care were duly explained to the volunteers.

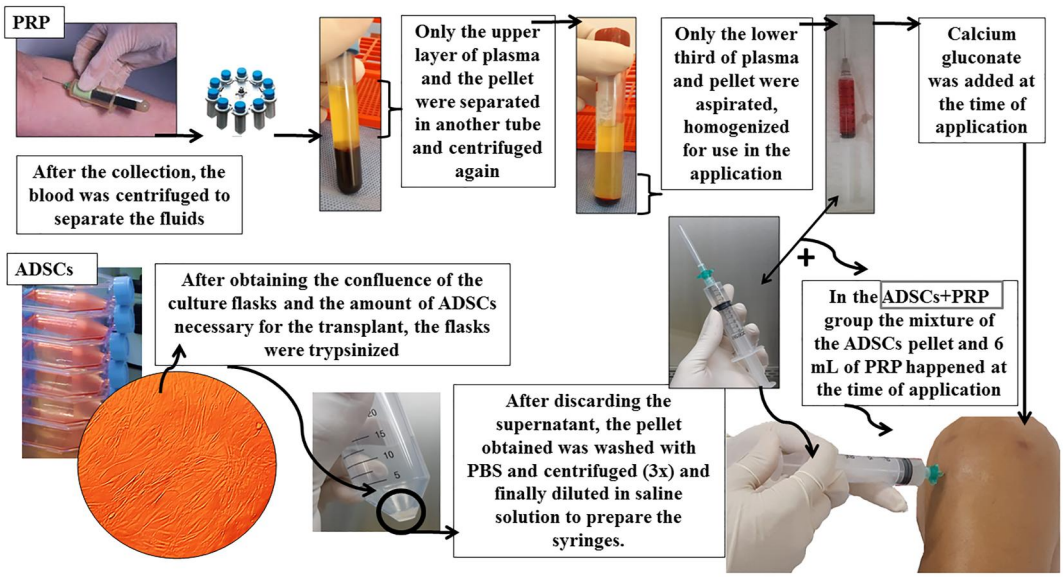

FIGURE 2 Flowchart illustrating the steps of handling biological materials in groups of platelet-rich plasma (PRP), adipose-derived stem cells (ADSCs) and ADSCs + PRP

For volunteers in the ADSCs + PRP Group, during the preparation of the ADSCs for transplantation, blood was collected and the preparation of the PRP was performed as described above. Both the pellet of ADSCs and PRP were mixed with intersyringe connector for total homogenization. However, 10% calcium gluconate was not added to gel the material. The application and care were carried out in the same way as described above. All volunteers received the same guidelines.

2.2.5 | Synovial fluid evaluation

Synovial fluid collection occurred in 2 moments. The first was still in the operating room before the start of video arthroscopy surgery. After antisepsis and preparation for asepsis and patient sedation, 15 ml of saline solution was injected intra-articularly into the affected knee. Ten passive knee flexions were performed to homogenize with the synovial liquid, subsequently, arthrocentesis was performed to collect 5 ml of the intra-articular fluid. One ml were destined for laboratory analysis in a sterile falcon tube, and 2 ml were cryopreserved in a cryogenic tube in the deep-freezer (−80°C). After all the samples was removed together and sent for cytometer analysis. The second collection was performed after 6 months of cell therapy. After knee asepsis, local anesthesia (lidocaine 2%) was applied in the lateral interarticular region of the knee, after 10 min, 15 ml of saline solution was injected intra-articularly in the knee and the procedures were continued as described above.

The fresh samples was analyzed blindly about the groups by the hospital professionals, according routine procedures. Macroscopically, was analyzed quality of viscosity (fluidity), if there were fragments present in the sample and color quality (clear, translucent, cloudy, straw yellow or opalescent yellow). Also, microscopically, was analyzed by them the enzymatic colorimetric assays, fractions of total protein and albumin/globulin (ALB/GLOB) were evaluated with the Total Protein Gen.2 kit, and glucose with Glucose HK kit, both were analyzed by Cobas 6000® according fabricant instructions. The analysis of global cytology and differential cytology were analyzed in an automated Sysmex XN-3000™ according fabricant instructions.

Cytokine analysis was performed in cryopreserved samples. For this purpose, the Human Inflammatory Cytokine CBA KIT (BDBiosciences – Cat: 551811) was used, according to the manufacturer's instructions. Analysis were performed by flow cytometry (Cytoflex – Beckman Coulter).

2.2.6 | Questionnaires

To assess the volunteers, the Short Form Health Survey 36 (SF-36) and Western Ontario McMaster Universities (WOMAC) specific quality of life questionnaire for OA were used. These questionnaires were applied in 2 moments: before the surgical procedures and after 6 months of the different interventions carried out in the groups proposed here. Similarly, the Visual Analog Pain Scale (VAS) was used to measure pain at these same times.

2.2.7 | Statistical analysis

Results were expressed as mean ± standard deviation of the mean and percentages. Statistical analysis was performed by comparing before and after data with Student's *t*-test. For parametric data and comparisons between pre-treatment data and between post-treatment data, ANOVA/Bonferroni was used, and for non-parametric data, the Kruskal Wallis/Dunn test was used. Absolute numbers and percentages were compared by chi-square. Differences were considered significant when $p \leq 0.05$ (GraphPad InStat 5 software).

3 | RESULTS

The socio-demographic data and clinical characteristics of the different groups are shown in Table 1. There were no statistical significant differences ($p > 0.05$) for any of the parameters analyzed between the different experimental groups. On average, volunteers in the Control, PRP and ADSCs groups were overweight by body mass index. The ADSCs + PRP group had grade 1 obesity. Among the most cited concomitant clinical characteristics altered are hypertension, hypercholesterolemia, depression and hyperinsulinemia.

3.1 | Proof of multipotency capacity of ADSCs

The cells used in this study had the capacity to differentiate into adipogenic, osteogenic and chondrogenic cells, which were confirmed by Oil red O, Alizarin red S and Alcian Blue stains (Figure 3A1-4). These same cells expressed in the immunophenotyping assay the markers CD105 and CD90 and did not express CD34 and CD133 (Figure 3b). These characteristics confirm that the cells used were MSCs derived from adipose tissue.

The average time of cultivation of ADSCs to reach the amount needed for transplantation was 56,33 ± 15,08 days.

4 | WESTERN ONTARIO McMASTER UNIVERSITIES

The results of the WOMAC questionnaires showed that in the groups control and PRP do not change ($p > 0.05$) scores for the pain intensity domain. However, there was a decrease in this domain by 23% and 28%, respectively. The ADSCs + PRP and ADSCs groups presented a significant decrease ($p < 0.05$) (initial x final evaluation), the reduction percentages of pain intensity score were 50% and 41%, respectively. For this domain, the best treatment was ADSCs+ PRP ($p < 0.05$; different letter in the bar) (Figure 3c).

For the domain stiffness, there was a statistical difference ($p < 0.05$) between the control group and all other groups in the initials values of pre-treatment. In the post-treatment the Control group and PRP did not change ($p > 0.05$) the percentages of improvement were only 5% and 29%. For the ADSCs + PRP and ADSCs groups there was a

TABLE 1 Characteristics of volunteers collected during the anamnesis and interview

		Control	PRP	ADSCs + PRP	ADSCs
N° of volunteers		8	6	6	6
Socio-demographic data					
Gender[1]	**Male**	6 (75%)	4 (66.7%)	3 (50%)	4 (66.7%)
	Female	2 (25%)	2 (33.3%)	3 (50%)	2 (33.3%)
Age (years)[2]		41 ± 4.67	48 ± 3.51	52.66 ± 4.60	58.5 ± 6.17
Height (cm)[2]		1.72 ± 0.02	1.72 ± 0.04	1.68 ± 0.04	1.71 ± 0.03
Starting weight (kg)[2]		78.75 ± 5.0	80.33 ± 4.0	87.5 ± 6.42	89.33 ± 8.0
BMI starting [2]		26.48 ± 1.51	27.30 ± 1.74	31.08 ± 2.66	29.98 ± 1.69
N° of volunteers with concomitant clinical characteristics altered[1]	**Yes**	2 (25%)	4 (66.7%)	3 (50%)	4 (66.7%)
	No	6 (75%)	2 (33.3%)	3 (50%)	2 (33.3%)
Duration of symptoms in the affected knee (Years)[2]		6.87 ± 2.08	5.16 ± 1.04	11.5 ± 2.56	9.16 ± 2.50
Time of financial assistance from the government received during absence from work (Years)[2]		4 ± 1.85	3.8 ± 0.75	3.8 ± 2.63	2.8 ± 2.56
Waiting time for elective orthopedic surgery by SUS (Years)[2]		4 ± 1.85	3.8 ± 0.75	5 ± 1.79	4.8 ± 0.72
Characteristics of treated knees					
N° of treated knees		10	10	11	10
N° of volunteers who had both knees treated [1]		2 (25%)	4 (66.7%)	5 (83.33%)	4 (66.7%)
N° of volunteers who had only 1 knee treated[1]		6 (75%)	2 (33.3%)	1 (16.6%)	2 (33.3%)
N° of volunteers who had primary OA (Aging)[1]		4 (40%)	4 (40%)	8 (80%)	8 (80%)
Number of volunteers who had secondary OA (Accident or trauma)[1]		6 (60%)	6 (60%)	2 (20%)	2 (20%)
Classification of OA by X-ray (Kellgren Lawrence)					
Degree 2 [2]		5 (50%)	4 (40%)	3 (27.27%)	2 (20%)
Degree 3 [2]		5 (50%)	6 (60%)	5 (45.45%)	5 (50%)
Degree 4 [2]		0 (0.0%)	0 (0.0%)	3 (27.27%)	3 (30%)
Previous treatments ineffective					
Pharmacological [2]		8 (100%)	6 (100%)	6 (100%)	6 (100%)
Surgery [2]		2 (25%)	0 (0.0%)	2 (33.4%)	0 (0.0%)
Physiotherapy [2]		2 (25%)	2 (33.4%)	2 (33.4%)	2 (33.4%)

The values for age, weight, height, initial weight and initial BMI were expressed as mean ± standard deviation of the mean. BMI – overweight: values from 25 to 29,5 and obesity 1: values from 30 to 34,9. The individual was considered elderly with ≥60 years old. In the items with number about writing 1, the chi-square test was used, and with number 2 about writing, ANOVA/Bonferroni was used, the differences were considered significant when $p \leq 0, 05$.

Abbreviations: ADSCs, adipose-derived stem cells; BMI, body mass index; OA, Osteoarthritis; PRP, platelet-rich plasma; SUS, Unified Health System.

significant decreased ($p < 0.05$), the percentages were 67% and 47%, respectively. For this domain, the best treatment was ADSCs + PRP ($p < 0.05$; different letter in the bar) (Figure 3c).

For the physical activity domain, the Control group and PRP did not change ($p > 0.05$) the decrease percentages were only 3% and 25%, respectively. For the ADSCs + PRP and ADSCs groups there was a significant improvement ($p < 0.05$), the decrease percentages were 41% and 38%, respectively. For this domain, the best treatments were ADSCs + PRP and ADSCs (Figure 3c; different letter in the bar).

The total WOMAC indicated that the Control group and PRP did not decrease significantly ($p > 0.05$) and the percentages were 7% and 26%. For the ADSCs + PRP and ADSCs groups there was a significant ($p < 0.05$) global improvement and the percentages were 46% and 39%, respectively. For this domain, the best treatments were ADSCs + PRP and ADSCs (Figure 3c; different letter in the bar).

5 | VISUAL ANALOG PAIN SCALE

The values pre-treatment of control group showed statistical difference ($p < 0.05$) between the ADSCs and ADSCs + PRP groups. Visual Analog Pain Scale values decrease in all groups. However, was only

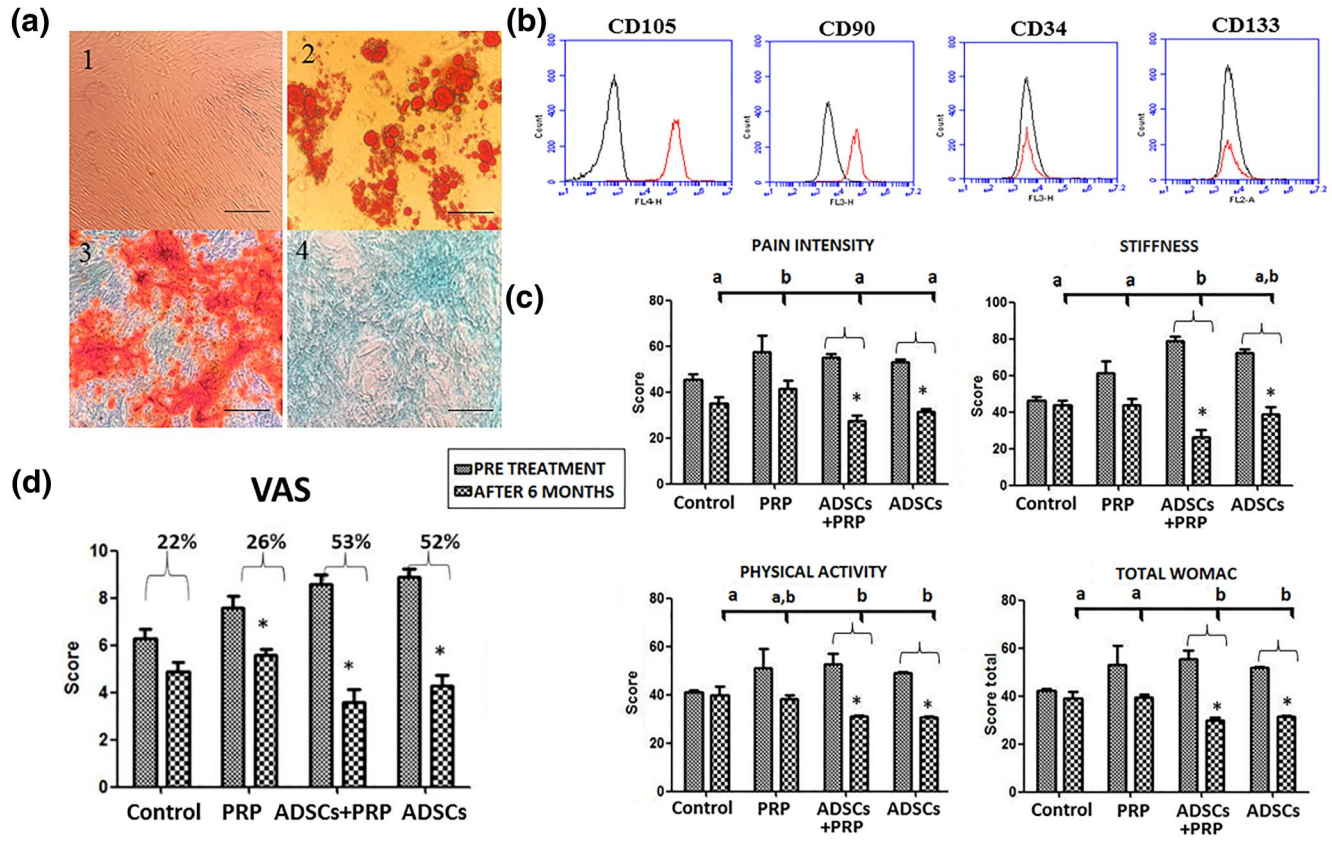

FIGURE 3 (a) Morphology, immunophenotyping and differentiation potential of adipose-derived stem cells (ADSCs): (1) Undifferentiated culture - cells with fibroblast characteristics; (2) adipogenic differentiation culture – presence of lipid vacuoles stained with Oil Red O; (3) osteogenic differentiation culture – presence of calcium deposits stained with Alizarin red S; and (4) chondrogenic differentiation culture – presence of extracellular matrix rich in glycosaminoglycans stained with Alcian Blue. Bars represent 50 μm; (b) Immunophenotypic profile of mesenchymal stem cells (MSCs) derived from adipose tissue, the cells expressed the markers CD105 and CD90 and did not express CD34 and CD133; (c) Score of domains of the Western Ontario McMaster Universities (WOMAC) questionnaire applied before (initial) and 6 months after the interventions (final). The score ranged from None = 0 (best), Little: 25, Moderate: 50, Severe: 75, Very intense: 100 (worst) and (d) Visual Analog Pain Scale (VAS) score applied before and 6 months after the interventions. All values were shown as mean ± standard deviation. Statistical Analysis: *Statistically significant difference between initial and final assessments (t-Student; $p < 0.05$). Different letters indicate statistically significant differences between the different groups in the final assessment (Anova/Bonferroni; $p < 0.05$)

significant for PRP, ADSCs + PRP and ADSCs groups. In Control group, the initial score was 6.3 ± 1.15 and decreased to 4.9 ± 1.19 (22%) ($p > 0.05$); in PRP it decreased from 7.6 ± 1.57 to 5.6 ± 0.69 (26%) ($p < 0.05$); in ADSCs + PRP it decreased from 8.6 ± 1.17 to 3.6 ± 1.71 (53%) ($p < 0.05$); and in ADSCs it decreased from 8.9 ± 1.10 to 4.3 ± 1.41 (52%) ($p < 0.05$) (Figure 3d).

6 | SHORT FORM HEALTH SURVEY (SF-36)

The results of the SF-36 questionnaire showed that the functional capacity increased significantly ($p < 0.05$) in all groups. Control group increased the domain by 229%, PRP by 318%, ADSC + PRP by 343% and ADSCs by 318% (Figure 4a).

The limitation by physical aspect did not change in the Control group and the percentage of improvement was only 29%. However, this domain increased significantly ($p < 0.05$) in PRP by 71%, in ADSCs + PRP by 154% and in ADSCs by 138% (Figure 4b).

For the pain domain there was a statistical difference ($p < 0.05$) between the control group and all other groups in the initials values of pre-treatment. In the post-treatment the control group did not result in significant improvement ($p > 0.05$) despite having an improvement of 17%. The increase significant ($p < 0.05$) for PRP was 63%, for ADSCs + PRP was 197% and for ADSCs was 200% (Figure 4c).

For the general health domain, the control group showed a statistical difference ($p < 0.05$) in the initials values between the ADSCs and ADSCs + PRP groups. In the post-treatment all groups showed improvement ($p < 0.05$). The increase percentages were in the order of 38%, 63%, 142% and 110% for the Control, PRP, ADSCs + PRP and ADSCs groups, respectively (Figure 4d).

For the vitality domain, there was a statistical difference ($p < 0.05$) between the control group and all other groups in the

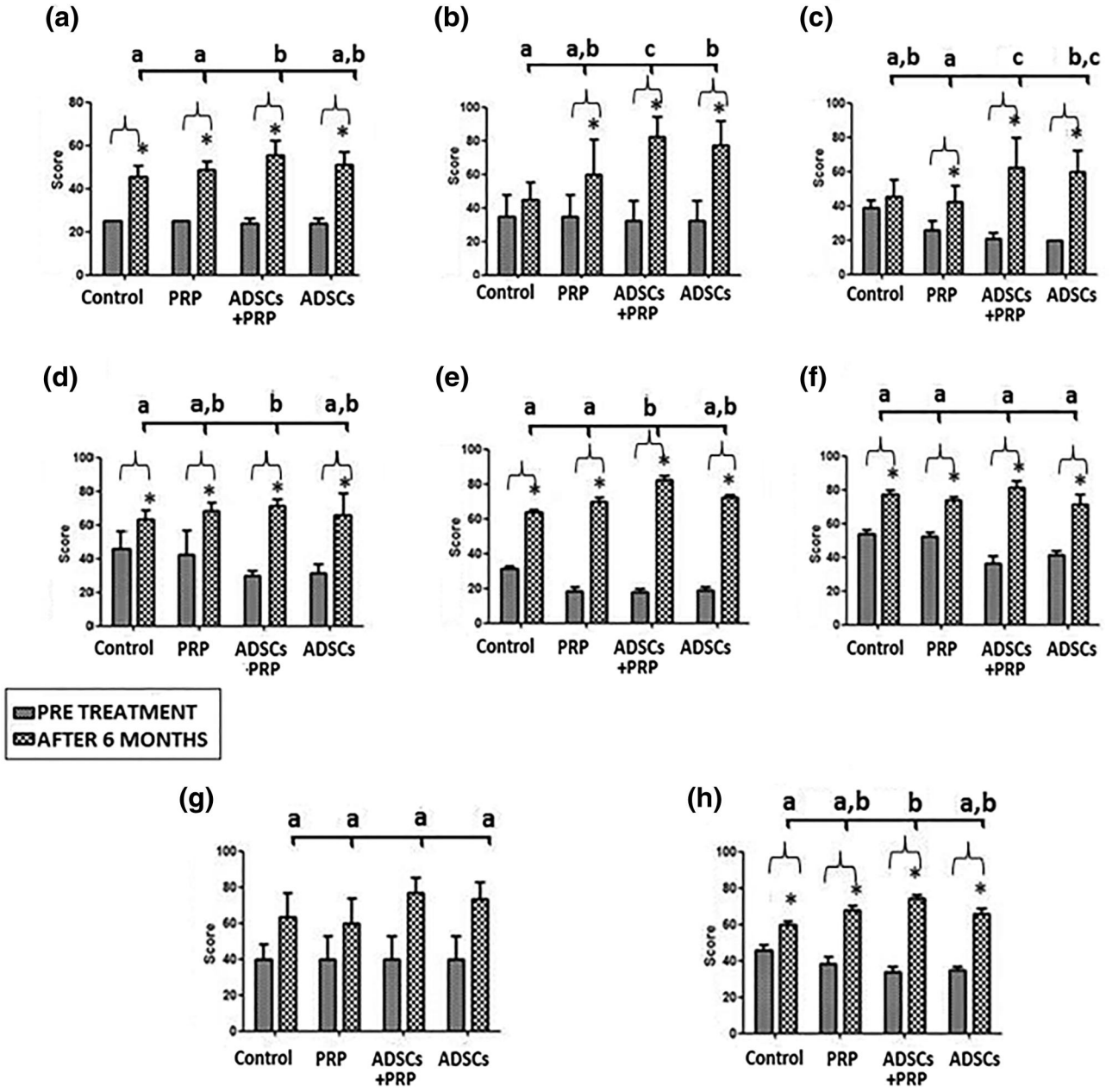

FIGURE 4 Score of domains of the Short Form Health Survey 36 (SF-36) questionnaire applied before and 6 months after the interventions (a) Functional capacity; (b) Limitation due to physical aspects; (c) Pain; (d) General health status; (e) Vitality; (f) Social aspects; (g) Limitations due to emotional aspects; (h) Mental health. The scores ranged from 0 (zero) to 100 (one hundred), where 0 = worst and 100 = best for each domain, were shown as mean ± standard deviation. Statistical Analysis: *Statistically significant difference between initial and final assessments (t-Student; $p < 0.05$). Different letters indicate statistically significant differences between the different groups in the final assessment (Anova/Bonferroni; $p < 0.05$)

initials values of pre-treatment. In the post-treatment all groups showed improvement ($p < 0.05$). The percentages of improvement were 103%, 278%, 358% and 282% for the Control, PRP, ADSCs + PRP and ADSCs groups, respectively (Figure 4e).

For the social aspects domain, the control group showed a statistical difference ($p < 0.05$) between the ADSCs and ADSCs + PRP groups. In the post-treatment all groups showed improvement ($p < 0.05$). The percentages of improvement were 44%, 40%, 124% and 73% for the Control, PRP, ADSCs + PRP and ADSCs groups, respectively (Figure 4f).

For the domain limitation by emotional aspects, all groups showed improvement ($p > 0.05$). The improvement percentages were 58%, 50%, 92% and 83% for the Control, PRP, ADSCs + PRP and ADSCs groups, respectively (Figure 4g).

For the mental health domain, all groups showed improvement ($p < 0.05$). The increase percentages were 30%, 77%, 119% and 90% for the Control, PRP, ADSCs + PRP and ADSCs groups, respectively (Figure 4h).

6.1 | Synovial fluid analysis

6.1.1 | Inflammatory cytokines

The quantification of inflammatory cytokines present in the synovial fluid showed in Figure 5. In the Control group there was a maintenance of the cytokine IL-1b and an increase ($p > 0.05$) of IL-6 (30%), IL-8 (113%), IL-10 (90%), IL- 12p70 (99%) and Tumor necrosis factor (TNF) (38%).

In the PRP group there was a slight increase, although not significant ($p > 0.05$), in the cytokine IL-1b (11%), IL-10 (17%) and IL-12p70 (13%). A decrease ($p > 0.05$) of 42% in IL-6, 16% in IL-8 and 5% in TNF was also observed.

In the ADSCs + PRP group all cytokines decreased their concentrations (Figure 5a–g). However, only was considered significant ($p < 0.05$) the decreases in IL-1b (46%), IL-6 (72%), IL-8 (50%) and TNF (46%).

In the ADSCs group, there was a decrease of IL-1b (31%), IL-6 (36%), IL-8 (31%), IL-10 (29%), IL-12p70 (30%) and TNF (18%). Only IL-1b and IL-8 reductions were statistically significant ($p > 0.05$).

6.1.2 | Enzymatic, colorimetric and viscosity analysis

It was also analyzed macroscopically, the viscosity, clarification of color and purity of the synovial fluid, it was observed that there was a higher improvement in the samples collected from the group ADSCs + PRP, followed by ADSCs and PRP.

Regarding the results of the parameters analyzed by enzymatic and colorimetric assays of the synovial fluid collected (Table 2), in the Control group there was a significant increase ($p < 0.05$) of total proteins (79%). The others parameters also increased ($p > 0.05$) of Albumin/Globulin (26%), glucose (79%), polymorphonuclear cells (34%), lymphocytes (13%) and leukocytes (8%).

In the PRP group, there was a decrease ($p > 0.05$) total protein (25%), of Albumin/Globulin (9%), glucose (7%), of

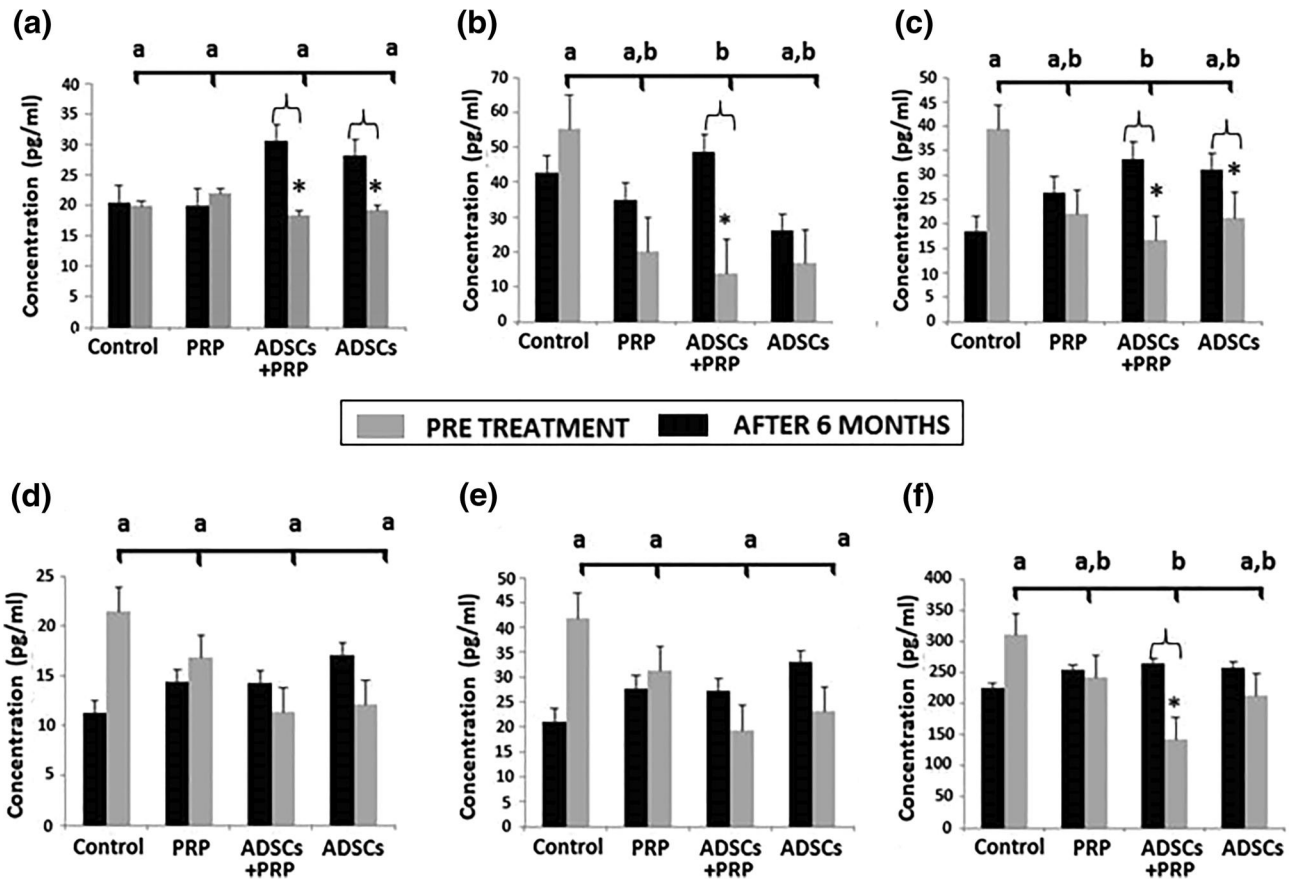

FIGURE 5 Cytokine quantification values in synovial fluids collected before and 6 months after the interventions. (a) IL-1b; (b) IL-6; (c) IL - 8; (d) IL-10; (e) IL-12p70 and (f) Tumor necrosis factor (TNF). Values are presented as mean ± standard deviation. Statistical Analysis: *Statistically significant difference between initial and final assessments (t-Student; $p < 0.05$). Different letters indicate statistically significant differences between the different groups in the final assessment (Anova/Bonferroni; $p < 0.05$)

TABLE 2 Parameter data analyzed by enzymatic and colorimetric assays of collected synovial fluid

Parameter data analyzed		Control	PRP	ADSCs + PRP	ADSCs
Total proteins (g/dl)	**Pre-treatment**	0.86 ± 0.56	1.66 ± 1.52	1.00 ± 0.54	0.87 ± 0.81
	After 6 months	1.54 ± 0.42[a]	1.24 ± 0.99[a]	1.1 ± 0.44[a]	1.29 ± 0.45[a]
	p	0.024*	0.830	0.051	0.072
Albumin/Globulin (g/dl)	**Pre-treatment**	1.38 ± 0.60	1.82 ± 1.06	1.83 ± 0.95	2.02 ± 0.75
	After 6 months	1.74 ± 0.41[a]	1.66 ± 0.45[a]	1.47 ± 0.27[a]	2.55 ± 1.77[a]
	p	0.450	0.061	0.371	0.562
Glucose (mg/dl)	**Pre-treatment**	29.8 ± 13.21	80.83 ± 1.52	32.4 ± 21.12	35.87 ± 16.68
	After 6 months	53.20 ± 25.84[a]	75.5 ± 57.35[a]	57.16 ± 14.52[a]	50.12 ± 35.15[a]
	p	0.015*	0.630	0.073	0.141
Polymorphonuclear cells (%)	**Pre-treatment**	19.33 ± 11.99	60.83 ± 11.05	25.26 ± 28.33	43.62 ± 26.15
	After 6 months	29.26 ± 11.58[a]	41.57 ± 8.74[a]	47.75 ± 13.04[a]	27.85 ± 21.95[a]
	p	0.859	0.290	0.015*	0.153
Lymphocytes (%)	**Pre-treatment**	69.66 ± 31.99	44.28 ± 25.72	73.09 ± 30.85	63.15 ± 15.61
	After 6 months	80 ± 13.69[a]	57 ± 20.38[a]	54.83 ± 11.32[a]	59.28 ± 18.17[a]
	p	0.983	0.395	0.721	0.561
Leukocyte (/mm3)	**Pre-treatment**	50.37 ± 33.62	47.28 ± 23.17	43.10 ± 28.01	65.16 ± 28.40
	After 6 months	54.66 ± 35.91[a]	32.85 ± 27.65[a]	31.89 ± 28.03[a]	40 ± 31.05[a]
	p	0.812	0.333	0.409	0.378

Note: Informations in bold correspond to the values of the samples in the pre-treatment moment and after 6 months of the intervations, follow by *p* that means the result of Statistical Analysis: *Statistically significant difference between initial and final assessments (t-Student; $p < 0.05$). Different letters indicate statistically significant differences between the different groups in the final assessment (Anova/Bonferroni; $p < 0.05$).

Abbreviations: ADSCs, adipose-derived stem cells; PRP, platelet-rich plasma.

polymorphonuclear cells (46%) and leukocytes 44%, and increase ($p > 0.05$) lymphocytes (22%).

In the ADSCs + PRP group, there was a significant increase ($p < 0.05$) of total proteins (10%) and polymorphonuclear cells (47%). The other parameters had a decrease ($p > 0.05$) of Albumin/Globulin (20%), lymphocytes (33%) and leukocytes (35%).

In the ADSCs group, there was an increase ($p > 0.05$) of total protein (48%), Albumin/Globulin (26%), glucose (40%). Also a decrease ($p > 0.05$) of polymorphonuclear cells (43%), lymphocytes (7%) and leukocytes (63%).

7 | DISCUSSION

Cell therapies with ADSCs, due to their regenerative potential, present interesting results in the treatment of OA and are a hope for controlling the progression of this disease (Biazzo, D'Ambrosi, Masia, Izzo, & Verde, 2020). It is known that the inflammatory process in OA is directly linked to pain, edema, redness and joint stiffness (Allen & Golightly, 2015). These symptoms, are associated with the worsening of the clinical characteristics, which reflects in difficulties to made their ADLs and worsening in quality of life. In our study, we observed that the use of the therapy with ADSCs + PRP intra-articular showed significant improvements in the WOMAC and VAS questionnaires, with higher percentages of improvement than the group that received the isolated ADSCs. We can also affirm that these kind of cell therapy has improved the functionality of these individuals, helping their return to ADLs. This feedback also allowed for an improvement in the indices observed in the SF-36 quality of life questionnaire, especially in relation to social aspects, general health status, vitality and mental health.

For the SF-36, there was an improvement in the indices for all groups when comparing the before and after each group. It is suggested that this occurred because the wait list for this elective surgery in the Brazilian Unified Health System (SUS) is so long (up to 5.00 ± 1.79 years) that all interventions performed here represented beyond of the improvement of the OA, but also the improvement in front of the receiving care attention of these patients. Moreover, cell therapy with ADSCs + PRP and/or ADSCs are an important strategies to treat OA and the improvements promoted by theses therapies positively impact in mental health and quality of the individuals life. With these physical and psychological benefits, individuals with OA will not need as much the usual care that normally are provide by the SUS. They are living with frequent returns, frustrated therapies (since they are not responsive to medications), thus generating even more costs for the SUS.

The video arthroscopy surgery is the gold standard for OA treatment, offered by most hospitals of SUS. This technique improves pain, promotes cartilage leveling and resolution of the acute inflammatory process (Felson, 2010). However, this technique only promotes satisfactory results in individuals with initial grade OA (Law et al., 2019; Totlis et al., 2021). It is known that OA is associated with the physiological imbalance of chondrocyte degradation and synthesis, which results in changes in the composition of the local extracellular matrix (Nelson, Allen, Golightly, Goode, & Jordan, 2014). Therefore, when synovial fluid production returns after video arthroscopy, it still contains inflammatory cytokines and the new tissue that will be stimulated to be produced will not promote the regeneration of cartilage tissue, but its healing with a different tissue (fibrocartilage). So, it will not prevent the return of the OA (Cooper, Javaid, & Arden, 2014), since this newly formed tissue is not of sufficient quality and the patient will continue to be susceptible, and also the pain probably will return in the near future. Our results in this group justifies this information, as there was an increase in all cytokines in the post treatment analysis.

The therapeutic effects of ADSCs are due to the presence of trophic factors that include granulocytes C; interleukins−6, −7, −8 and −11; and growth factors such as HGF, VEGF, PGF, TGFβ and FGF-2 (Brown, 2018). The presence of these components evidences its ability to self-replicate and differentiate into other cell types (Fuchs et al., 2013), in certain degenerative diseases, such as OA, local MSCs are depleted and have reduced proliferative capacity and cell differentiation capacity (Murphy et al., 2002). Therefore, the local delivery of healthy ADSCs to these individuals may improve the regeneration capacity, the functional capacity of the joint and promote the reconstitution of cartilage in the joint affected by OA (Jo et al., 2017; W. S.; Lee, Kim, Kim, Kim, & Jin, 2019; Song et al., 2018). However, it was emphasized that the larger size of the meniscal lesion and degeneration in a advance state, less effective the therapy was, requiring more applications or a higher number of ADSCS, for a long-lasting and someones result (Y. G. Koh, Choi, Kwon, & Kim, 2014; Song et al., 2018). We observed this same situation in our study, the majority of volunteers who reported complete satisfaction with the treatment had initial degrees of OA.

The OA joints have high concentrations of VEGF, the accumulation of this factor favors angiogenesis and hinders the differentiation of resident stem cells. Chondrogenic tissues are avascular and therefore the increase in angiogenesis is inversely proportional to chondrogenic differentiation (Takano et al., 2018). As ADSCs, they have an angiogenic capacity that is mediated by an increase in VEGF. Thus, if ADSCs are transplanted into cartilage with OA there will be an major increase in VEGF, therefore the cartilage will have even more difficulty being repaired (C. S. Lee et al., 2012). However, when transplanting ADSCs in association with PRP, there will be a better paracrine targeting, that will favor differentiation rather than angiogenesis. Another point to be considered, is that the components present in PRP are nothing more than bioactive molecules secreted by hematopoietic stem cells, normally present in the blood, which have the function of promoting intracellular and cell-environment communication, for its proper homeostasis (Sundman et al., 2014). The fact that we not activated the PRP with gluconate of calcium in the ADSCs + PRP group, somehow made the PRP components available more easily, improving cell-environment-cell interaction would cause local homeostasis and prevent apoptosis of transplanted ADSCs and chondrocytes present, greater synthesis of type II collagen, aggregation (Rashid & Kwoh, 2019), better organization of the extracellular matrix (Bennell et al., 2017) and chondrogenic differentiation. This combination of therapies act better, first in the control of local inflammation and after that it provides the necessary specific cell differentiation (Van Pham, Hong-Thien Bui, Quoc Ngo, Tan Khuat, & Kim Phan, 2013). Our results also demonstrated a trend toward greater improvement in volunteers of ADSCs + PRP group, when compared to the other interventions. However, more evaluations with imaging and/or biopsy could improve the knowledge about this process.

Regarding the pathogenesis of OA, the literature highlights the predominance of IL-6, IL-1b and TNF (Wang & He, 2018). These cytokines have the ability to activate multiple inflammatory pathways and may increase the severity of the disease, causing joint swelling and/or cartilage destruction, all through increased secretion of matrix metalloproteinases, prostaglandins and inhibition of proteoglycan and collagen synthesis type II (Wang & He, 2018). The ADSCs + PRP group showed higher decrease in the levels of these cytokines. Enabling the reduction of all these cytokines in the joint environment interrupts the pro-degenerative vicious circle, slowing disease progression and restoring tissue homeostasis (Siqueira et al., 2017). The IL-8 also has a better decrease in this group, however during the blinded analysis of the synovial liquid in a closed system, a significant increase in polymorphonuclear cells was observed. In view of this, we raise the hypothesis that the machine assumed the ADSCs still present in the samples (due its size) as this cell type (polymorphonuclear), fact that is justify by the support of more time of survival that de PRP gives to the ADSCs. This same biochemical and cellular analysis showed an increase in total proteins and glucose in the control group when comparing the two times of analysis. This increase only in the control group suggests that the cartilage still continues to be degraded. The groups treated with ADSCs, PRP or ADSCs + PRP did not present this same profile. This fact suggests that these three therapies improve the anti-inflammatory effect and, therefore, are consistent with the clinical improvements observed. Since this liquid is directly in contact with all joint structures and instantly reacts to some disorder, changing its physical-chemical characteristics (Huang, 2018).

8 | LIMITATIONS OF THE PRESENT STUDY

The first limitation of this study was the small number of participants. This happened due to difficulties in scheduling elective surgeries in na public hospital during the proposed research period. Second, the

failure to perform the MRI exam due to the lack of service at the Hospital. The lack of a second X-Ray analysis was due to the difficulty of selectively scheduling the appointments of our volunteers in the services of the public hospital where our research was carried out. Regarding the use of ADSCs, we can mention the long processing time from the harvesting of the adipose tissue until reaching the necessary number of cells. No histological proposals were made of the cartilage, treated because the ethics committee to which this work was submitted did not approve this second intervention. The reduced number of patients after collecting synovial fluid 6 months after the interventions was because of the difficulty of moving patients to the capital, as many of them are from another cities. Because of this, the applications of the questionnaires in these volunteers was made by telephone. In addition, it was not possible to follow up the treated patients for a period longer than 6 months, due to the restriction of return visits to the Hospital during the SARS-CoV-2 pandemic in 2020.

9 | CONCLUSION

Taking all the results into account, we infer that therapies with ADSCs + PRP and only ADSCs are safe and effective over 6 months for the improvement of pain, functional capacity and joint inflammation in the volunteers with OA who were unresponsive to conservative treatment. It acts as a preventive therapy for the progression of this disease in cases of OA with initial degrees and in more advanced degrees, this therapy could be used to postpone the placement of total knee prostheses, but analyzes with a longer follow-up time should be performed.

AUTHOR CONTRIBUTIONS

Laynna de Carvalho Schweich-Adami performed the literature search, cultivated the ADSCs, made the assays, data analysis and write the article; Roberto Antoniolli da Silva performed the screenings of patients, surgeries and application of proposed therapies; Jovino Nogueira da Silva Menezes made the liposuction surgeries; Adrivanio Baranoski, Candida Aparecida Leite Kassuya and Luana Bernardi made the assays in the flow cytometry; Rodrigo Juliano Oliveira and Andréia Conceição Milan Brochado Antoniolli-Silva participated in the conception of the article, data analysis and reviewed the writing of the article.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

Allen, K. D., & Golightly, Y. M. (2015). Epidemiology of osteoarthritis: State of the evidence. *Current Opinion in Rheumatology*, *27*(3), 276. https://doi.org/10.1097/bor.0000000000000161

Bennell, K. L., Hunter, D. J., & Paterson, K. L. (2017). Platelet-rich plasma for the management of hip and knee osteoarthritis. *Current Rheumatology Reports*, *19*(5), 24. https://doi.org/10.1007/s11926-017-0652-x

Biazzo, A., D'Ambrosi, R., Masia, F., Izzo, V., & Verde, F. (2020). Autologous adipose stem cell therapy for knee osteoarthritis: Where are we now? *The Physician and Sports medicine*, *48*(4), 1–8. https://doi.org/10.1080/00913847.2020.1758001

Brown, L. L. (2018). Adipose-derived stromal stem cells. In *Advanced procedures for pain management* (pp. 489–507). Springer.

Cooper, C., Javaid, M. K., & Arden, N. (2014). Epidemiology of osteoarthritis. In *Atlas of osteoarthritis* (pp. 21–36). Springer.

Felson, D. T. (2010). Arthroscopy as a treatment for knee osteoarthritis. *Best Practice & Research Clinical Rheumatology*, *24*(1), 47–50.

Fuchs, Y., Brown, S., Gorenc, T., Rodriguez, J., Fuchs, E., & Steller, H. (2013). Sept4/ARTS regulates stem cell apoptosis and skin regeneration. *Science*, *341*(6143), 286–289.

Hermeto, L., DeRossi, R., Oliveira, R., Pesarini, J., Antoniolli-Silva, A., Jardim, P., Santana, A., Deffune, E., Rinaldi, J., & Justulin, L. (2016). Effects of intra-articular injection of mesenchymal stem cells associated with platelet-rich plasma in a rabbit model of osteoarthritis. *Genetics and Molecular Research*, *15*, 3. https://doi.org/10.4238/gmr.15038569

Huang, X. (2018). *Multi-signals involved in human joint homeostasis*. University of Twente.

Jo, C. H., Chai, J. W., Jeong, E. C., Oh, S., Shin, J. S., Shim, H., & Yoon, K. S. (2017). Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: A 2-year follow-up study. *The American Journal of Sports Medicine*, *45*(12), 2774–2783.

Kellgren, J., & Lawrence, J. (1957). Radiological assessment of osteo-arthrosis. *Annals of the Rheumatic Diseases*, *16*(4), 494. https://doi.org/10.1136/ard.16.4.494

Koh, Y. G., Choi, Y. J., Kwon, O. R., & Kim, Y. S. (2014). Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. *The American Journal of Sports Medicine*, *42*(7), 1628–1637.

Lapuente, J. P., Dos-Anjos, S., & Blázquez-Martínez, A. (2020). Intra-articular infiltration of adipose-derived stromal vascular fraction cells slows the clinical progression of moderate-severe knee osteoarthritis: Hypothesis on the regulatory role of intra-articular adipose tissue. *Journal of Orthopaedic Surgery and Research*, *15*, 1–9. https://doi.org/10.1186/s13018-020-01664-z

Law, G. W., Lee, J. K., Soong, J., Lim, J. W. S., Zhang, K. T., & Tan, A. H. C. (2019). Arthroscopic debridement of the degenerative knee–Is there still a role? *Asia-Pacific Journal of Sports Medicine, Arthroscopy, Rehabilitation and Technology*, *15*, 23–28. https://doi.org/10.1016/j.asmart.2018.11.003

Lee, C. S., Burnsed, O. A., Raghuram, V., Kalisvaart, J., Boyan, B. D., & Schwartz, Z. (2012). Adipose stem cells can secrete angiogenic factors that inhibit hyaline cartilage regeneration. *Stem Cell Research & Therapy*, *3*(4), 35. https://doi.org/10.1186/scrt126

Lee, W. S., Kim, H. J., Kim, K. I., Kim, G. B., & Jin, W. (2019). Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: A phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Translational Medicine*, *8*(6), 504–511. https://doi.org/10.1002/sctm.18-0122

Markarian, C. F., Frey, G. Z., Silveira, M. D., Milani, A. R., Ely, P. B., Horn, A. P., Nardi, N. B., & Camassola, M. (2014). Isolation of adipose-derived stem cells: A comparison among different methods. *Biotechnology Letters*, *36*(4), 693–702.

Murphy, J. M., Dixon, K., Beck, S., Fabian, D., Feldman, A., & Barry, F. (2002). Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis & Rheumatism*, *46*(3), 704–713.

Najar, M., Martel-Pelletier, J., Pelletier, J.-P., & Fahmi, H. (2020). Mesenchymal stromal cell immunology for efficient and safe treatment of osteoarthritis. *Frontiers in Cell and Developmental Biology*, *8*. https://doi.org/10.3389/fcell.2020.567813

Nelson, A. E., Allen, K. D., Golightly, Y. M., Goode, A. P., & Jordan, J. M. (2014). A systematic review of recommendations and guidelines for the management of osteoarthritis: The chronic osteoarthritis management initiative of the US bone and joint initiative. In *Paper presented at the seminars in arthritis and rheumatism*.

Pers, Y.-M., Quentin, J., Feirreira, R., Espinoza, F., Abdellaoui, N., Erkilic, N., Cren, M., Dufourcq-Lopez, E., Pullig, O., Noth, U., Jorgensen, C., Louis-Plence, P., & Nöth, U. (2018). Injection of adipose-derived stromal cells in the knee of patients with severe osteoarthritis has a systemic effect and promotes an anti-inflammatory phenotype of circulating immune cells. *Theranostics*, *8*(20), 5519. https://doi.org/10.7150/thno.27674

Rashid, H., & Kwoh, C. K. (2019). Should platelet-rich plasma or stem cell therapy be used to treat osteoarthritis? *Rheumatic Disease Clinics*, *45*(3), 417–438.

Schweich-Adami, L. d. C., Bernardi, L., Baranoski, A., Rodrigues, T. d. A. F., Antoniolli-Silva, A. C. M. B., & Oliveira, R. J. (2021). The enzymatic disaggregation by trypsin does not alter cell quality and genomic stability of adipose-derived stem cells cultivated for human cell therapy. *Cell and Tissue Banking*. https://doi.org/10.1007/s10561-021-09958-0

Shahid, M., & Kundra, R. (2017). Platelet-rich plasma (PRP) for knee disorders. *EFORT Open Reviews*, *2*(2), 28–34.

Siqueira, M. B., Frangiamore, S., Klika, A. K., Gajewski, N., Barsoum, W. K., & Higuera, C. A. (2017). Comparison of synovial fluid cytokine levels between traumatic knee injury and end-stage osteoarthritis. *Journal of Knee Surgery*, *30*(02), 128–133.

Song, Y., Du, H., Dai, C., Zhang, L., Li, S., Hunter, D. J., Lu, L., & Bao, C. (2018). Human adipose-derived mesenchymal stem cells for osteoarthritis: A pilot study with long-term follow-up and repeated injections. *Regenerative Medicine*, *13*(3), 295–307.

Sundman, E. A., Cole, B. J., Karas, V., Della Valle, C., Tetreault, M. W., Mohammed, H. O., & Fortier, L. A. (2014). The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. *The American Journal of Sports Medicine*, *42*(1), 35–41.

Takano, S., Uchida, K., Inoue, G., Matsumoto, T., Aikawa, J., Iwase, D., Mukai, M., Miyagi, M., & Takaso, M. (2018). Vascular endothelial growth factor expression and their action in the synovial membranes of patients with painful knee osteoarthritis. *BMC Musculoskeletal Disorders*, *19*(1), 204. https://doi.org/10.1186/s12891-018-2127-2

Totlis, T., Fermín, T. M., Kalifis, G., Terzidis, I., Maffulli, N., & Papakostas, E. (2021). Arthroscopic debridement for focal articular cartilage lesions of the knee: A systematic review. *The Surgeon*, *19*(6), 356–364. https://doi.org/10.1016/j.surge.2020.11.011

Van Pham, P., Hong-Thien Bui, K., Quoc Ngo, D., Tan Khuat, L., & Kim Phan, N. (2013). Transplantation of none xpanded adipose stromal vascular fraction and platelet-rich plasma for articular cartilage injury treatment in mice model. *Journal of Medical Engineering*, 1–7. https://doi.org/10.1155/2013/832396

Wang, T., & He, C. (2018). Pro-inflammatory cytokines: The link between obesity and osteoarthritis. *Cytokine & Growth Factor Reviews*, *44*, 38–50. https://doi.org/10.1016/j.cytogfr.2018.10.002

Woodell-May, J. E., & Sommerfeld, S. D. (2020). Role of inflammation and the immune system in the progression of osteoarthritis. *Journal of Orthopaedic Research*, *38*(2), 253–257.

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