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ORIGINAL ARTICLE

Increased Cellular Dosage of Bone Marrow Aspiration Concentrate Does Not Translate to Increased Clinical Effectiveness in Knee Osteoarthritis: A Phase I Dose Escalation Study

Sathish Muthu[1,2] · Karthikraja Ramanathan[2] · Sangilimuthu Alagar Yadav[3] · Saurabh Kumar Jha[1,4] · Rajni Ranjan[5]

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Abstract

Introduction Knee osteoarthritis (KOA), a chronic degenerative disease, significantly impairs quality of life due to pain and mobility limitations. Traditional treatments focus on symptom management without addressing the underlying disease progression, leading to a growing interest in regenerative medicine approaches. Bone marrow aspirate concentrate (BMAC), rich in mesenchymal stem cells and growth factors, has shown potential for cartilage repair and symptom relief in KOA. Despite promising outcomes, the optimal BMAC dosage for knee OA treatment remains undetermined. This study aims to evaluate the clinical efficacy and safety of varying BMAC dosages in knee OA treatment.

Methods This prospective controlled dose–escalation study involved 75 patients with early-stage knee OA, categorized into three groups based on BMAC dosage administered 10×10^6 cells (low-dose group), 50×10^6 cells (medium-dose group), or 100×10^6 cells (high-dose group). All the patients underwent a single intra-articular injection of BMAC and were monitored over a year. The primary outcomes include Visual Analog Scale (VAS) for pain and the Knee Injury and Osteoarthritis Outcome Score (KOOS) for joint function recorded at baseline, 1, 3, 6, and 12 months post-intervention. Adverse events were also documented.

Results Significant clinical improvements in VAS and KOOS scores were noted across all groups at all time points compared to the baseline. However, these improvements did not significantly differ between dosage groups throughout the follow-up period. Adverse effects were minimal and primarily consisted of transient post-injection pain and effusion, with no dose-dependent increase in complications.

Conclusion BMAC treatment for knee OA is safe and demonstrates potential for significant pain relief and functional improvement, irrespective of the dosage administered within the tested range. The lack of significant differences among varying dosages suggests a plateau in therapeutic efficacy beyond a certain threshold. Further research is necessary on the long-term outcomes to optimize the dosing strategy.

Keywords BMAC · Bone marrow aspirate concentrate · Clinical outcome · Standardization · Dose–escalation study

✉ Sathish Muthu
drsathishmuthu@gmail.com

1 Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh 201310, India

2 Department of Orthopaedics, Government Medical College, Dindigul, Tamil Nadu 624001, India

3 Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu 641021, India

4 Department of Zoology, Kalindi College, University of Delhi, New Delhi 110008, India

5 Department of Orthopaedics, School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh 201310, India

Introduction

Osteoarthritis (OA) of the knee, a progressive degenerative condition marked by the breakdown of articular cartilage, osteophyte formation, and periarticular bone changes, significantly impacts joint biomechanics and quality of life, leading to disability and pain [1]. Management of knee osteoarthritis (KOA) poses a substantial challenge to the field of regenerative medicine, urging the exploration of disease-modifying treatments capable of offering regenerative solutions beyond the palliative measures currently available [2]. Despite advances in surgical and molecular strategies aimed at alleviating inflammation and protecting cartilage,

treatments remain largely symptomatic, with joint replacement being the final recourse in advanced stages [2, 3].

In recent years, bone marrow aspirate concentrate (BMAC), rich in mesenchymal stem cells (MSCs) and growth factors, has emerged as a promising alternative in the landscape of regenerative therapies [4]. Despite the BMAC's potential for disease modification and cartilage regeneration, the intervention lacks standardization in the form of determination of an optimal dosage, understanding the mechanisms of action, and evaluating long-term efficacy and so [5]. The clinical studies conducted thus far on BMAC therapy for KOA have demonstrated safety and potential effectiveness in providing symptomatic relief and improving joint function [4, 6]. However, these studies exhibit a notable heterogeneity in methodologies, dosages, and patient follow-up durations, leading to inconclusive results regarding the treatment's efficacy and standardization [7, 8]. Moreover, the rapid increase in publications on BMAC treatments necessitates clarification of the optimal use of this therapy on procedure variables, such as cellular dosage, aspiration techniques and processing methods [5, 9]. Further, standardization of these process variables along with the optimal usage scenarios would largely benefit the patients and clinicians to obtained the maximum benefit out of the procedure.

This background underscores a critical need to elucidate the optimal conditions for BMAC's application in the management of KOA. Previous studies have highlighted that patient factors, such as age, comorbid conditions and procedure factors, such as aspirate volume significantly affect the concentration of MSCs in BMAC. [5, 9] However, no attempts were made to understand the effect of varying doses of cells in BMAC on its clinical effectiveness. Therefore, this study seeks to address these knowledge gaps by investigating the safety and correlation between the dosage of BMAC and its clinical effectiveness in knee OA treatment.

Methods

The study was conducted following approval of the protocol of conduct by the Institutional Ethical Committee (DMC/IEC/2021/E2/38). This is a prospective controlled dose–escalation study conducted in patients with early osteoarthritis with a single dose of intra-articular bone marrow aspiration concentrate injection between Nov 2022 and March 2023.

Inclusion Criteria

The study enrolled patients between 30 and 80 years of both sexes presenting with radiologically diagnosed primary osteoarthritis of knees (Kellgren–Lawrence grade 1, 2). Patients with severe pain and under anti-inflammatory treatment without improvement > 3 months were included. Patients who have given consent for treatment as per our protocol using a single intra-articular injection of bone marrow aspiration concentrate and agreed to the regular follow-up visits as per the protocol were included.

Exclusion Criteria

We excluded patients aged less than 30 and more than 80 years and those presenting with advanced primary osteoarthritis Kellgren–Lawrence (KL) grade 3, 4 or secondary knee osteoarthritis. We excluded patients who have undergone prior corticosteroid injection to the affected knee within 3 months of presentation. We also excluded patients diagnosed with rheumatoid arthritis, inflammatory arthritis, and autoimmune diseases.

Treatment Allocation

Patients considered for inclusion into the study were sequentially allocated to either one of the three treatment groups with different cellular concentrations of bone marrow aspiration concentrate 10×10^6 cells (low-dose group), 50×10^6 cells (medium-dose group), or 100×10^6 cells (high-dose group). The medium dose of 50×10^6 cells is obtained from the previously conducted systematic review of literature by the authors analyzing the minimal effective cellular concentration for knee osteoarthritis [9].

Patient Screening

All the patients who consented to participate in the study were subjected to preliminary pre-surgical screening. Patients deemed fit for the procedure based on the pre-surgical workup were enrolled on the study, and their baseline visual analog scale (VAS) and knee injury and osteoarthritis outcome score (KOOS) were recorded [10].

Surgical Procedure

We followed the standardized procedure for bone marrow aspiration and processing to obtain the bone marrow aspirate concentrate in a sterile fashion in the operating theater as described [5]. Briefly, the procedure involves the collection of 80–120 ml of bone marrow from the anterior superior iliac spine with the patient in supine position under local anesthesia. The aspirated bone marrow was diluted with plain medium (MesenPRO RS™, Gibco® Life Technologies™, Grand Island, NY, USA) at a ratio of 5:2. The mixture was rinsed well and sieved through a 100 mm cell strainer (Gibco, Life Tech, Grand Island, NY, USA) to dissolve the remaining cell aggregates. We used hypotonic ammonium chloride buffer (Himedia R075, India) to lyse

the RBCs present in the solution through short-term incubation for 30 s. This solution was layered over HiSep 1.077 (Himedia R075, India) to isolate human mononuclear cells, and then centrifuged at 400×*g* for 40 min. The bone marrow mononuclear cell (BMNC) buffy coat layer was then collected and washed in the plain medium. The final aspirate subjected to cell counts to titrate the dosage required. Cell counting is done using a dedicated 6-part differential hematology analyzer (XN-350™, Sysmex, India). The final intra-articular injection is administered in the same operative session in a sterile fashion.

Post-Operative Protocol

Patients were refrained from taking anti-inflammatory drugs and paracetamol was the only rescue drug prescribed for post-operative pain refractory to hot fomentation. Three sessions of quadriceps strengthening exercises were advised every day in the post-operative period until 12 weeks. Serial follow-ups were made at 1, 3, 6, and 12 months with subsequent recording of their VAS and KOOS scores that included sub-sections for pain, symptoms, quality of life and function [11].

Statistical Analysis

We used mean and standard deviation to present continuous variables and percentage to present categorical variables. We analyzed the improvement compared to the baseline using a paired *t* test and between the groups using a one-way analysis of variance. A *p* value less than 0.05 was considered statistically significant. The statistical analysis was conducted using IBM SPSS Version 25 (Armonk, USA).

Results

Characteristics of Patients

All three patient cohorts had comparable baseline characteristics, such as age, sex, body mass index and distribution of KL grades, of osteoarthritis as shown in Table 1. Similarly, the baseline outcome variables, such as VAS and KOOS scores, were comparable among the three cohorts. We included 75 patients in total with 25 patients in each dosage group. However, we had 23 patients in the low-dose group, 24 patients in the medium-dose group, and 21 patients in the high-dose group with complete follow-up till 1-year time point as shown in Fig. 1.

Clinical Outcome

Compared to the baseline VAS, the change in the score at serial follow-up is presented in Table 2. We noted statistically significant improvement in the VAS and KOOS scores compared to the baseline levels at subsequent follow-up time points across all three patient groups as shown in Table 2. Although the clinical improvement was sustained over 1-year period across all the patient groups, no significant differences in the clinical outcome could be noted between the three dosage groups analyzed as shown in Table 3. Upon analysis of the individual subscores of KOOS, we did not

Table 1 Characteristics of patients included in the study

Characteristic	Low-dose cohort (*n* = 23)	Medium-dose cohort (*n* = 24)	High-dose cohort (*n* = 21)
Age (year)	48.8 (±5.4)	49.3 (±5.8)	47.5 (±4.7)
Women	12 (52.2%)	14 (58.3%)	14 (66.7%)
Body mass index	24.5 (±3.4)	26.5 (±2.8)	25.8 (±3.2)
Kellgren–Lawrence grading			
Grade I	4 (17.4%)	9 (37.5%)	7 (33.3%)
Grade II	19 (82.6%)	15 (62.5%)	14 (66.7%)
Side (R:L)	5:18	6:18	10:11
VAS Score (0–10 score)	6.6 (±1.1)	6.8 (±1.3)	6.3 (±1.5)
KOOS (0–100 scale)			
Symptom subscore	56.3 (±4.3)	55.5 (±11.6)	56.7 (±4.8)
Pain subscore	56.1 (±6.1)	55.9 (±7.8)	54.1 (±9.4)
ADL subscore	72.2 (±2.5)	67.3 (±3.4)	66.1 (±4.1)
Recreational activities subscore	65.6 (±3.2)	64.3 (±3.4)	69.3 (±3.7)
QOL subscore	49.8 (±6.2)	52.4 (±5.8)	48.8 (±5.2)
Total KOOS score	59.6 (±2.4)	62.5 (±4.0)	55.4 (±7.1)

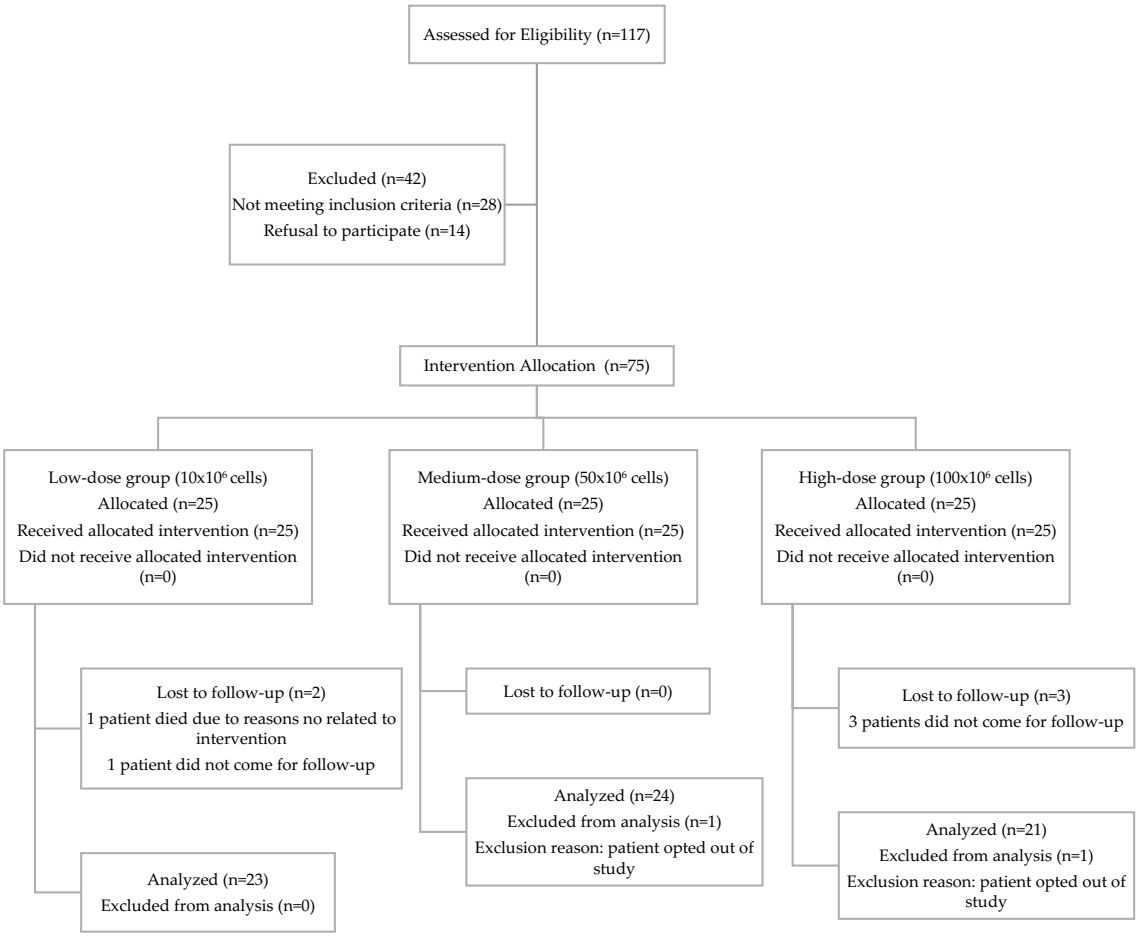

Fig. 1 CONSORT flow diagram of assessment, inclusion, allocation, follow-up, and analysis of patients included in this prospective cohort study

note any significant change in scores among the three patient groups as shown in Fig. 2.

Complications

We noted increased pain and mild effusion in the immediate post-injection period that lasted for 48 h in 12 (16%) of the patients without any preference for the dosage injected. The pain settled with a rescue analgesic without any further discomfort. No dose-dependent increase in complications was noted. One patient in the medium dosage group and one in the high-dosage group continued to have increased pain and opted out of the study at 3 and 5 months respectively following injection.

Discussion

The exploration of BMAC dosage in knee OA is motivated by the need to refine regenerative therapies for improved clinical outcomes [9]. Given the limitations of conventional treatments, which often fail to halt the progression of OA or offer lasting symptom relief, BMAC presents a viable alternative with its potential for cartilage regeneration and symptom management [12]. The focus on dosage is particularly relevant, as it may determine the extent of clinical effectiveness and the sustainability of treatment outcomes. In our study, we standardized the patient characteristics and analyzed their clinical outcomes and complications across three dosage cohorts. The cohorts were well-matched for baseline characteristics, including age, sex, body mass index, KL grades of osteoarthritis, baseline VAS, and baseline KOOS. Despite initial equal patient allocation, follow-up was completed for 23, 24, and 21 patients in the low-, medium-, and high-dose groups, respectively. Hence, the analysis was based only on those who completed the one-year follow-up. The key clinical findings noted in the study are as follows:

1. Significant clinical improvements in VAS and KOOS scores were noted across all groups at all time points compared to the baseline. However, these improve-

Table 2 Summary of clinical improvement noted in the patients included in the study

Outcome	Δ, 3 months	*p* value	Δ, 6 months	*p* value	Δ, 12 months	*p* value
Low-dose cohort (10×10^6 cells)						
VAS Score	−2.03 (±1.2)	< 0.001	−2.2 (±2.6)	< 0.001	−5.1 (±1.8)	< 0.001
KOOS Total	9.3 (±1.6)	< 0.01	16.5 (±5.9)	< 0.001	21.3 (±2.8)	< 0.001
Symptom subscore	11.4 (±4.2)	0.027	24.4 (±6.2)	< 0.001	29.4 (±5.2)	< 0.001
Pain subscore	3.4 (±6.8)	< 0.001	12.6 (±8.6)	< 0.001	26.6 (±8.2)	< 0.001
ADL subscore	4.5 (±2.2)	< 0.001	6.9 (±2.7)	< 0.001	8 (±3.5)	< 0.001
Recreational activities subscore	9.7 (±8.2)	< 0.001	11.2 (±4.8)	< 0.001	12.5 (±3.2)	< 0.001
QOL subscore	18.6 (±6.2)	< 0.001	27.6 (±7.4)	< 0.001	31.6 (±4.5)	< 0.001
Medium-dose cohort (50×10^6 cells)						
VAS score	−3.7 (±1.2)	< 0.001	−2.7 (±2.9)	< 0.001	−5.6 (±1.4)	< 0.001
KOOS total	18.3 (±3.9)	< 0.01	16.4 (±3.9)	< 0.001	25.4 (±3.3)	< 0.001
Symptom subscore	24.4 (±5.2)	0.027	24.4 (±8.2)	< 0.001	31.4 (±5.2)	< 0.001
Pain subscore	14.1 (±7.3)	< 0.001	12.5 (±14.7)	< 0.001	29.6 (±14.7)	< 0.001
ADL subscore	9.6 (±3.5)	< 0.001	6.6 (±3.5)	< 0.001	11.62 (±4.4)	< 0.001
Recreational activities subscore	13.4 (±4.2)	< 0.001	11.2 (±5.4)	< 0.001	15.3 (±3.8)	< 0.001
QOL subscore	29.6 (±3.2)	< 0.001	27.4 (±8.1)	< 0.001	29.5 (±4.8)	< 0.001
High-dose cohort (100×10^6 cells)						
VAS Score	−1.8 (±1.1)	< 0.001	−2.0 (±3.6)	< 0.001	−5.1 (±1.4)	< 0.001
KOOS Total	9.6 (±2.6)	< 0.001	17.2 (±2.9)	< 0.001	23.5 (±3.6)	< 0.001
Symptom subscore	12.4 (±4.1)	0.044	26.4 (±8.2)	< 0.001	28.4 (±6.2)	< 0.001
Pain subscore	2.3 (±4.8)	< 0.001	15.6 (±11.1)	< 0.001	29.6 (±11.2)	< 0.001
ADL subscore	5.7 (±9.2)	< 0.001	7.5 (±5.1)	< 0.001	9.38 (±4.3)	< 0.001
Recreational activities subscore	9.0 (±11.2)	< 0.001	11.4 (±3.2)	< 0.001	15.0 (±7.2)	< 0.001
QOL subscore	24.4 (±8.2)	< 0.001	25.6 (±7.4)	< 0.001	23.5 (±3.5)	< 0.001

Table 3 Intergroup comparison in clinical outcome between three cohorts analyzed

Outcome (mean ± SD)	Low-dose cohort (10×10^6 cells)	Medium-dose cohort (50×10^6 cells)	High-dose cohort (100×10^6 cells)	*p* value
1 month				
VAS score	3.3 ± 1.6	3.1 ± 1.4	3.9 ± 2.4	0.497
KOOS Total	70.09 ± 4.49	76.87 ± 7.85	72.25 ± 3.04	0.416
3 months				
VAS score	4.5 ± 1.7	4.3 ± 2.6	5.2 ± 1.4	0.485
KOOS total	68.05 ± 4.04	67.67 ± 3.85	62.89 ± 2.42	0.425
6 months				
VAS score	3.9 ± 2.4	4.2 ± 1.7	4.7 ± 1.9	0.521
KOOS total	76.10 ± 2.44	74.65 ± 3.44	71.95 ± 4.91	0.535
12 months				
VAS score	1.9 ± 2.9	1.5 ± 1.4	2.1 ± 1.3	0.427
KOOS total	80.90 ± 2.44	84.93 ± 5.41	76.92 ± 4.64	0.225

ments did not significantly differ between dosage groups throughout the follow-up period.

2. Adverse effects were minimal and primarily consisted of transient post-injection pain and effusion, with no dose-dependent increase in complications.

Clinical outcomes showed significant improvements in VAS and KOOS from baseline at all follow-up time points across all patient cohorts. However, these improvements did not consistently progress at subsequent follow-up points significantly between the follow-up time points but

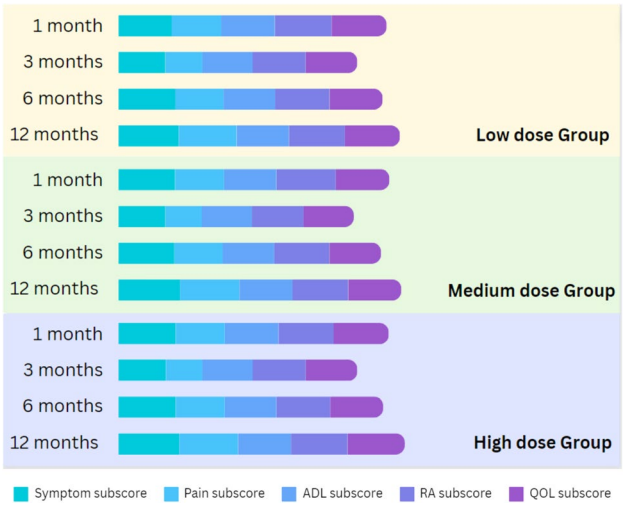

Fig. 2 Comparison of KOOS outcome subscores across three cohorts

the intervention reached a plateau response that was maintained over the 1-year follow-up period. This suggests that varying the dosage does not significantly alter the clinical trajectory over a year. Regarding safety, a small subset of patients experienced increased pain shortly after injection, resolving with analgesics. Notably, two patients had sustained increase in pain and opted out of the study, suggesting the need for careful patient selection and monitoring for adverse outcomes.

A systematic review provides a broad overview of BMAC's safety and efficacy, highlighting the need for high-quality research to better define its role in OA treatment, with a recommended dosage of $5–10 \times 10^7$ cells for optimal benefits [6]. The investigation into the effectiveness of varying dosages of BMAC for knee OA yields several key insights when compared with existing literature. Our study's findings, indicating no significant difference in clinical outcomes among different dosage groups over one-year period, align with broader discussions within the field about the efficacy and optimization of BMAC treatment for OA.

Regenerative modalities, such as platelet-rich plasma and BMAC, have now been accepted as a first-line injectable therapy in the management of knee osteoarthritis [13, 14]. The importance of BMAC dosage in the management of knee OA emerges from the heterogeneous results reported in the literature [7]. The rationale behind investigating BMAC dosage efficacy lies in its potential to modulate the complex pathophysiology of knee OA through the delivery of a rich mixture of mesenchymal stem cells (MSCs), growth factors, and cytokines directly into the affected joint [4]. These components contribute to the modulation of inflammation, repair and regeneration of cartilage, and improvement of joint function, which are crucial in the management of knee OA. The clinical effectiveness of BMAC in knee OA is underscored by its ability to harness the body's intrinsic healing mechanisms [15]. BMAC's rich content of MSCs and growth factors play a pivotal role in mitigating inflammation and promoting the regeneration of damaged cartilage. There is a lack of clear consensus in identifying the critical dosage of cells to be administered to achieve optimal results [9]. The concentration of MSCs and the volume of BMAC injected can influence the clinical outcomes in patients with knee OA [5]. The variability in BMAC preparation techniques, the concentration of MSCs, and the volume administered across studies pose challenges in drawing definitive conclusions regarding dosage efficacy [8, 16]. Nonetheless, the evidence suggests a dose-dependent relationship between BMAC volume and clinical outcomes, either higher or lower doses potentially offering greater therapeutic benefits [17, 18].

Pers et al.[18] in their clinical trial upon analyzed dosage groups including low (2×10^6 cells), medium (10×10^6 cells), and high (50×10^6 cells) doses of cultured adipose-derived MSCs in the management of severe KOA. They analyzed clinical outcomes at 6-month follow-up and found the intervention to be safe without serious adverse events. However, they noted statistical significance only in the low-dose cohort. The main limitation of the above study was heterogeneity among the cohorts compared. Their concern with the noted difference in the treatment response was due to the priming of the injected MSCs in the inflammatory milieu. Gupta et al.[19] while analyzing the dosage of adult cultured allogeneic MSCs for OA among cohorts using 25, 50, 75, and 150 million cells found clinical effectiveness from a minimal dose of 25 million cells which is similar to the results of this study. They concluded that a cell dose of 25 million cells was sufficient to demonstrate clinical effectiveness with allogeneic cultured MSCs. The study by Matas et al.[17] showed their dose–escalation trial to assess the safety and efficacy of umbilical cord-derived MSCs in mild symptomatic knee osteoarthritis noted significant improvement in the low- (2×10^6 cells) and medium-dose (20×10^6 cells) group compared to the high-dose (80×10^6 cells) cohort. Moreover, they noted injection-related swelling in all the patients of high-dose cohort while in our study, we noted post-injection pain and effusion that lasted for 48 h in 12 (16%) of the patients without any preference for the dosage injected.

The overall literature on the dosage analysis is limited and all the analyzed studies were on cultured MSCs rather than BMAC, which is a more cost-effective intervention that could be administered in a single surgical setting with minimal manipulation as required by the regulatory norms [20]. Hence, the current study sheds some light into analyzing the effect of cellular volume in the BMAC injected for early OA knee. One possible explanation for the lack of significant changes in the clinical outcomes noted in the current study could be due to the shorter duration of follow-up or

early disease state included in the current study. Although Pabinger et al. [6] analyzed a 4-year outcome in severe knee osteoarthritis in 37 patients, they did not mention the dosage used in their patient group. However, they noted a 95% success rate and significant improvement in walking distance which adds to the clinical effectiveness of BMAC in severe disease scenarios as well.

This study on BMAC for knee OA has notable limitations. One significant constraint is the absence of radiological outcomes to corroborate clinical findings, relying solely on subjective assessments, such as the VAS and KOOS. The study's design as a dose–escalation study lacks a placebo or control group, potentially biasing patient-reported outcomes. Additionally, the relatively small sample size and the short one-year follow-up duration limit generalizability and long-term assessment of treatment sustainability and adverse effects. The clear benefit of the variable dosage may be elucidated by following up the patients for longer periods. Variability in BMAC preparation techniques poses challenges, necessitating strict protocol adherence. Future research should prioritize larger, longer-term randomized trials with standardized protocols, incorporating radiological outcomes to objectively assess treatment efficacy and structural changes in the knee joint. Furthermore, investigating BMAC's synergistic potential with other therapies and identifying predictive biomarkers for treatment response could enhance personalized treatment approaches for KOA management.

Conclusion

This prospective controlled dose–escalation study on the use of BMAC for the treatment of early KOA demonstrates that while BMAC treatment is safe and associated with significant improvements in pain and functional outcomes compared to the baseline, these benefits do not exhibit a clear dose-dependent relationship. However, the absence of significant differences in clinical outcomes among the dosage groups suggests that increasing the concentration of BMAC beyond a certain threshold does not necessarily enhance therapeutic benefits in knee OA treatment. These results call for further research to refine BMAC dosage guidelines, optimize treatment protocols, and explore the mechanisms underlying its regenerative potential. A deeper understanding of these aspects could significantly advance the application of BMAC and other regenerative therapies in managing knee OA, ultimately improving patient care and outcomes in this prevalent condition.

Author Contribution Dr SM: conceptualization, data curation, analysis, visualization, management, writing—original draft, writing—review and editing; Dr KR: management, writing—review and editing; Dr SAY: data curation, writing—review and editing; Dr SKJ: conceptualization, data curation, analysis, writing—review and editing and; Dr RR: conceptualization, data curation, management, writing—review and editing.

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Data availability The data generated from the study are available upon reasonable request to the corresponding author.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval The study was conducted following approval of the protocol of conduct by the Institutional Ethical Committee (DMC/IEC/2021/E2/38).

Informed Consent Informed consent is obtained from all patients included in the study.

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