Safety and efficacy of umbilical cord tissue-derived mesenchymal stem cells in the treatment of patients with aging frailty: a phase I/II randomized, double-blind, placebo-controlled study

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Research Article

Keywords: aging frailty, human umbilical cord derived mesenchymal stem cells, safety and efficacy, randomized controlled trial, quality of life, physical performance, chronic inflammation

Posted Date: January 19th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3847704/v1

Abstract

Background: Mesenchymal stem cell (MSC) based therapy holds great promise for cell-based therapy in regenerative medicine. In this study, we aimed to evaluate the safety and efficacy of intravenous infusion of human umbilical cord-derived MSCs (HUC-MSCs) in patients with aging frailty.

Methods: In this randomized, double-blind, placebo-controlled trial, participants diagnosed with aging frailty were randomly assigned to receive intravenous administrations of HUC-MSCs or placebo. All of serious adverse events (SAEs) and AEs were monitored to evaluate the safety of treatment during the 6-month follow-up. The primary efficacy endpoint was alteration of physical component scores (PCS) of SF-36 qualities of life at 6 months. The secondary outcomes including physical performance tests and pro-inflammatory cytokines, were also observed and compared at each follow-up visits. All evaluations were performed at 1 week, 1, 2, 3 and 6 months following the first intravenous infusion of HUC-MSCs.

Results: In the MSCs group, significant improvements in PCS of SF-36 were observed from first post-treatment visit and sustained throughout the follow-up period, with greater changes compared to the placebo group (p=0.042). EQ-VAS scores improved significantly at 2 month (p=0.023) and continued until the end of the 6-month visit (p=0.002) in comparison to the placebo group. The timed up and go (TUG) physical performance test revealed significant group difference and showed continual enhancements over 6 months (p 0.05). MSC transplantation improved the function of four-meter walking test (4MWT) compared with the placebo group with a decrease of 2.05s at 6 months of follow-up (p=0.21). The measurement of grip strength revealed group difference with MSCs group demonstrating better performance, particularly at 6 months (p=0.002). Inflammatory cytokines (TNF-a, IL-17) exhibited declines in MSCs group at 6 months compared to the placebo group (p=0.034 and 0.033, respectively). There was no difference of incidence of AEs between the two groups.

Conclusion: Intravenous transplantation of HUC-MSCs is a safe and effective therapeutic approach on aging frailty. The positive outcomes observed in improving quality of life, physical performance and reducing chronic inflammation, suggest HUC-MSC therapy may be a promising potential treatment option for aging frailty.

Trial Registration: Clinicaltrial.gov; NCT04314011;

Registered 10 December, 2019;

URL: https://clinicaltrials.gov/ct2/show/NCT04314011.

Background

Aging frailty is a clinical geriatric syndrome with multiple contributors and causes, which develops as the cumulative declines in function and progressive loss of physiologic reserve across multiple systems, leading to increased vulnerability to stresses. (1, 2). Frailty places the elderly at high risk of adverse health outcomes, including fall, disability, hospitalization and even death (2, 3). Aging frailty is highly prevalent with an estimated prevalence of 10.7% in community-dwelling participants aged 65 years and older worldwide (4). In the context of aging population, frailty is more prominent among the older adults, which has emerged as a globally public health challenge due to its associated economic and social burden (5). Thus, the strategies that can manage aging frailty are of great significance. Currently, guidelines strongly recommend that the elderly with frailty should take physical activity and withdraw inappropriate medications. The guidelines also conditionally recommend sufficient protein and vitamin D supplementation (6). However, the evidence to develop the guidelines is relatively limited due to lack of well-designed clinical studies with large samples. Also, the efficacy of these interventions for managing frailty is controversial (7), and no approved medical therapies for frailty are available till now.

The individuals with frailty may present the phenotypes of weakness, including unintentional weight loss; self-reported exhaustion, slow walking speed; low grip strength and decreased physical performance (1). It has been hypothesized that the capacity of endogenous stem cell to regenerate and differentiate may decline with advancing age, which is characterized by diminished homeostasis and reduced organ function in individuals due to exhaustion and depletion of endogenous stem cells (8). At such, stem cell therapy holds great promise in frailty treatment (8–10). Human umbilical cord-derived mesenchymal stem cells (HUC-MSCs) are groups of stromal cells presenting in umbilical cord tissues. HUC-MSCs have biological properties of stem cells with self-renewal and multipotency. Compared to the sources from bones, adiposes and others, HUC-MSCs are easily accessible, and have low immunogenicity and no ethical issues, which have remarkable advantages in the field of regeneration medicine (11, 12). Clinical applications of HUC-MSCs are very extensive due to their trophic, immunomodulatory, and anti-inflammatory effects of MSCs (13, 14). To date, numerous studies have illustrated the therapeutic benefits of HUC-MSCs for many diseases, including chronic graft-versus-host disease (15), heart failure (16), type 1 diabetes (17), demonstrating that the intravenous administration of HUC-MSCs is safe and effective. Based on current evidence, we conducted the randomized, double-blinded, and placebo-controlled study among older adults with aging frailty, with an aim to evaluate the efficacy and safety of HUC-MSC transplantation in aging frailty.

Methods

Study Design

The study was a phase 1/2 randomized, double-blind, placebo-controlled clinical trial. This trial was conducted at the clinical research center of Shanghai East hospital, China between July 3, 2020 (data that first participant enrolled) and January 6, 2022 (date that last participant completed follow-up visit). The study was designed to enroll a total of 30 participants, who will be randomly assigned into the HUC-MSCs treatment group or placebo group. The interventions include intravenous infusions of HUC-MSCs at a dose of 10^6 cells/kg or placebo once a month for twice. The efficacy and safety assessment will be performed at 1 week, 1 month, 2 months, 3 months, and 6 months after the first treatment (Fig. 1). This trial protocol has been approved by the Human Cell Clinical Research Ethics Committee of Shanghai East Hospital and was supervised by an independent data and safety monitoring board. All participants had provided written informed consent prior to enrollment, as mandated by the Declaration of Helsinki. The study has been registered in ClinicalTrials.gov (NCT04314011).

Study Population

Participants between ages of 60 and 80 years were screened in the communities across Mainland of China. All the participants have provided written informed consent prior to any study procedures. Inclusion criteria for this study were as follows, (1) aged from 60 and 80 years old; (2) meeting the diagnostic criteria of frailty evaluated via the Fried frailty phenotype scale and scored 1–4 (1); (3) expected to live more than 12 months. Participants were excluded if they had an allergic constitution or positive history of drug allergy, advanced liver disease or renal failure, class III/IV congestive heart failure, myocardial infarction, unstable angina, stroke, uncontrolled hypertension or hyperglycemia, drug or alcohol abuse, had history or presence of malignant tumors, had active autoimmune diseases, had any active infection (including positivity for hepatitis BsAg, hepatitis C antibody, or HIV antibody, or positive PPD test), poor compliance, or planned organ transplantation; a history of participating in another clinical trials within the previous 3 months or surgeries within 6 months, receipt of MSC-based therapy within the previous 4 weeks.

Screening

In the study, a screening visit was performed after participants gave written informed where a focused medical examination including the assessment of inclusion and exclusion criteria was conducted. After screening, the eligible participants attended a baseline visit and five subsequent 6-month follow-up visits (scheduled at 1-week, 1-month, 2-month, 3-month and 6-month after the first intravenous transplantation of HUC-MSCs). The baseline assessment took place within one month from the screening visit and were completed prior to random assignment and intervention, then the baseline data on demographic, clinical characteristics of participants were collected.

Randomization

The randomization sequence was obtained using a random number generator by a statistician external to study. A block randomization method with a block size of six was applied to ensure a balanced intergroup assignment. The allocation was sequentially numbered, and the sequence was concealed by sealed opaque envelopes. All the participants were assigned the unique randomization number, and were randomly allocated to the HUC-MSC treatment group or placebo group at a ratio of 1:1 in accordance with randomization sequence. Both clinicians and research assistants were blinded to allocation status.

Intervention

Eligible participants were randomly assigned in a 1:1 ratio to receive either HUC-MSCs or placebo. In the HUC-MSCs treated group, HUC-MSCs were intravenously infusion at a dose of 1 × 10^6 /kg at the fifth passage, and subsequently administrated at 1-month interval. The placebo group participants received the same volume of 0.9% normal saline twice with same intervention procedure. Both products were matched in size, packaging, appearance, and texture.

Preparation of HUC-MSCs

Clinical-grade HUC-MSCs were obtained from umbilical cord of healthy donors after full-term delivery with the written informed consent. The procedure of processing the samples and culturing HUC-MSCs were conducted fully compliant with current good manufacture practice (GMP) guidelines in GMP laboratory. The umbilical cord tissues were diced into cubes of approximately 0.5cm^3 following removal of vessels, which were subsequently cultured and collected between the third and fourth passages for intravenous infusion. HUC-MSCs were identified based on surface marker characteristics detected with flow cytometry. Moreover, the differentiation capacity including osteogenesis, adipogenesis, and chondrogenesis were tested by muti-differentiation assay (Supplemental Fig. 1). HUC-MSC release criteria also included absence of all tested contaminants (bacteria, mycoplasma, syphilis, and fungi) and endotoxin ≤ 0.5 EU/mL.

Study Outcomes

The primary efficacy outcome for this study was the general quality of life measured by physical component scores (PCS) of Short Form 36 Health Survey (SF-36) at 6 months after the first intravenous infusion. The secondary efficacy outcomes assessed the overall physical performance which encompassed grip strength, four-meter walking test (4MWT), timed up and go (TUG) test, five times sit to stand test (FTSST), which examined the ability to stand or movement. The serum levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), interleukin-8 (IL-8) and interleukin-17 (IL-17) were also measured and analyzed between the two groups. Sleep quality was assessed via Pittsburgh sleep quality index (PSQI) questionnaire. The mental composite score (MCS) of SF-36 was used to measure the mental health status. The EuroQol visual analogue scale (EQ-VAS) of 0 to 100 were also utilized to assess quality of life in this study, by which the higher scores indicate better health status. The evaluations were conducted at baseline, 1-week, 1-month, 2-month, 3-month, and 6-month follow-up. The safety end point was the difference in the incidence of reported serious adverse events (SAEs) or adverse events (AEs) following first intravenous infusion in the MSCs and placebo arms, including rates of death, thromboembolic events, hospitalization, and significant abnormal laboratory test results.

Anthropometry

Height in stocking feet and weight in light clothing were measured by the trained staff using a digital tester (Tsinghua Tongfang, China). The grip strength of dominant hand was measured using a hand dynamometry (Tsinghua Tongfang, China). 4MWTs were measured by timing participants walking over four meters at usual pace. TUG test required participants to stand up from a seated position in a chair (seat height 46 cm), walk three meters in a straight line, turn around, walk back, and sit down. The time needed to complete TUG test was recorded in seconds (s). FTSST measured the time needed to rise from a seated position and sit down for five repetitions as quickly as possible without using arms.

Blood samples

At each follow-up visit, about 20 ml peripheral blood samples of every participant were drawn into ethylenediaminetetraacetic acid (EDTA) coated vacuum tubes between 7:00 am and 9:00 am. The fresh blood samples were processed within three hours. First, the samples were centrifuged at 1000 × g for 10 min to separate plasma at 4 °C.

Questionnaires

For each participant, participants filled in validated translations of questionnaires that included SF-36, composed of PCS and MCS; the EQ-VAS as well as PSQI regarding quality of life, health and well-being, and sleep quality. Participants were required to fill in the questionnaires at baseline and at each scheduled follow-up visit, and they were required not to change lifestyles during the intervention period.

Statistical methods

Sample size

To determine the required sample size, it was estimated based on a well-established MSCs anti-frailty study conducted by the University of Miami (18). The sample size for difference between two independent samples of quantitative data was calculated using a two-sided t test and Mann-Whitney test, with a significance level (α) of 0.05 and a power of 80%. A sample size of 15 patients in each group would achieve 80% power to detect a difference in term of effect size (PASS 15.0). We chose to include a total of 30 participants from the community hospital and the outpatient geriatric center to increase the precision of the estimate.

Statistical Analysis

All statistical analyses were based on the intention-to-treat (ITT) patient population and were performed using SPSS (26.0), Prism (9.2.0) and R software (4.1.2). Comparison between two groups at baseline was analyzed using the independent-sample t test or Mann-Whitney U test according to data distribution. Intraindividual comparison of continuous variables at baseline with those at follow-up was performed with paired t test or Wilcoxon rank sum test according to data distribution. For comparisons of effects on various post-treatment evaluations of MSCs treatment, Bonferroni alpha correction was applied, and statistical significance was set as a value of $p < 0.01$. Categorical data were presented as n/N (%) and tested by Chi-square test. The differences between two groups over different time points were analyzed through a mixed effect maximum likelihood regression. Statistical significance was assumed at a value of $p < 0.05$. The safety analysis was performed on ITT population, including all the participants received the treatment in this study.

Results

Study population

From June 2020 through January 2022, a total of 110 participants were consecutively screened for eligibility in Shanghai East Hospital, and 80 patients were excluded according to the inclusion and exclusion criteria. Finally, 30 patients were randomly assigned in a 1:1 ratio to receive

either HUC-MCSs or matching placebo (Figure 2). Of the thirty participants enrolled in the study, there were 24 participants (12/15 in MSCs group, and 12/15 in placebo group) assessed at month 2, 25 participants (12/15 in MSCs group, and 13/15 in placebo group) at month 3, 27 participants (12/15 in MSCs group, and 15/15 in placebo group) at month 6. One patient in MSCs group was lost to follow-up, who withdrew consent 2 months after the first treatment. Compliance of this trial was excellent with only 14 of scheduled 180 visits (7.77%) missed. The mean age of participants in the MSCs group and placebo group was 67.27 ± 5.23 and 69.27 ± 5.02 years, respectively. At baseline, the two groups showed no statistically significant differences in the demographic and clinical characteristics, including chronic diseases, medications, laboratory tests, physical performance. The baseline demographic characteristics of participants were presented in Table 1.

Table 1. Baseline characteristics of participants

Characteristics	MSCs group (N=15)	Placebo group (N=15)
Age (years)	67.27 ± 5.23	69.27 ± 5.02
Sex (n, %)		
Male	5 (33.33)	7 (46.67)
Female	10 (66.67)	8 (53.33)
BMI (kg/m^2)	24.03 ± 2.89	24.95 ± 3.67
Fried frailty phenotype scale	2 (2, 2)	2 (2, 3)
Chronic conditions		
Hypertension (%)	4 (26.67)	7(46.67)
Dyslipidemia (%)	4(46.67)	1(6.67)
Type 2 diabetes mellitus (%)	3(20)	5(33.33)
Ischemic cardiomyopathy (%)	2(13.33)	2(13.33)
Medication		
Aspirin (%)	2(13.33)	1(6.67)
ACEI/ARB (%)	2(13.33)	4 (26.67)
Calcium antagonists (%)	2(13.33)	1(6.67)
Metformin (%)	1(6.67)	2(13.33)
Other oral antidiabetics (%)	2(13.33)	3(20)
Insulin (%)	2(13.33)	2(13.33)
Stain (%)	5(33.33)	1(6.67)
Laboratory		
Hemoglobin (g/L)	135.80 ± 7.72	132.47 ± 13.71
White blood cell count (10^9/L)	5.89 ± 1.37	5.69 ± 1.45
Platelet count (10^9/L)	203.2 ± 42.75	206.4 ± 73.50
AST (U/L)	23.07 ±14.26	23.73 ± 8.68
ALT (U/L)	22.73 ± 18.18	21.00 ± 12.62
GFR (mL/min per 1.73 m^2)	70.73 ± 13.98	75.93 ± 16.35
FBG (mmol/L)	5.94 ± 1.41	5.89 ± 1.26

Data are median (interquartile range, IQR), mean (standard deviation, SD), or n (%).

Safety

No serious adverse event was observed during the 6 months of the follow-up in this study, the incidences of adverse events did not differ between the two groups, which occurred in 2 (13.33%) participants in the placebo group (one had black pain, and one had lower extremity

edema) and 1 (6.77%) participant in the MSCs group (one had dizziness). All of adverse events were transient and were considered as unrelated to treatment. No patients were withdrawn from this study due to adverse events. There were no clinically important differences between two groups for the outcomes of laboratory tests and vital signs at all the time points. The summary of adverse events is presented in Table 2.

Table 2. Summary of adverse events

Adverse events (n, %)	MSCs (n=15)	Placebo (n=15)	P	Related to treatment
Back pain	0 (0)	1 (6.67)	NS	No
Dizziness	1 (6.77)	0 (0)	NS	No
Lower extremity edema	0 (0)	1 (6.67)	NS	No

Data are shown as n (%).

Clinical outcomes

Quality of life were evaluated throughout the study and are depicted in Table 3. The primary end point was the changes in PCS of SF-36. Compared with baseline, there were improvements in the changes of PCS in the MSCs group that began at 1 week of follow-up (+75.53 ± 23.02; p=0.003), and continued at 1 month (+97.27 ± 23.02; p 0.001) and till to 6 months (+96.41 ± 24.70, p 0.001). The changes of PCS from baseline to month 6 were significantly greater in the MSCs group than in the placebo group (p=0.042) (Fig.3A). The enhancements were observed in the MCS of SF-36 within the MSCs group, showing a significant increase from 287.81 ± 72.44 at baseline to 365.29 ± 19.81 at 6 months (p=0.0001), however, there was no statistically significant difference between MSCs group and placebo group (Fig.3B). The EQ-VAS serves as an indicator of perceived quality of life of individuals. In this study, the MSCs group exhibited an enhancement in EQ-VAS scores at the 2-month follow-up (p=0.023) in comparison to the placebo group, with the improvement persisting until the end of the visit. The most substantial improvements in EQ-VAS were noted at the 6-month follow-up (p=0.002). In the MSCs group, scores of EQ-VAS were significantly improved compared with baseline, from a group average of 79.67± 10.77 at 1 month (p=0.007) to 82.92 ± 8.38 at the end of 6 months (p=0.001). However, the placebo group showed no differences in these variables over 6 months (Fig.3C). Also, we found no difference in the changes of PSQI among the two groups (Fig.3D).

To compare the function status between the two groups of patients, we continued to conduct the physical performance tests, including TUG, 4MWT, grip strength, and FTSST at week 1, months 1, 2, 3 and 6 of follow-ups. Notably, compared with the placebo group, greater TUG improvement was observed in the patients injected with HUC-MSCs from initial visit to all follow-up points (p 0.05)(Fig.4A). There were substantial improvements in 4MWTs performance in the HUC-MSC group at 6 months (p=0.021) (Fig.4B). Patients treated with HUC-MSCs experienced enhanced physical performance as measured by the grip strength at several visits of follow-up, and the most significant increased grip strength was at 6 months of follow-up (p=0.002) (Fig.4C). However, no statistically significant differences in the FTSST of participants tested before, during and at the end of any follow-up points were found. The physical performance between the two groups of patients were summarized in Table 3.

It has been reported that the expression of inflammatory cytokines was associated with frailty in the elderly. In this study, the serum levels of cytokines (TNF-a, INF-g, IL-8, IL-17) were measured six times consecutively in both groups, and the results were presented in Table 3. Of note, in comparison to controls, the decline in the concentrations of TNF-a was observed in patients receiving HUC-MSCs infusion at the month 6 (p=0.034). Consistent with the reduced changes of TNF-a, the levels of IL-17 in MSCs treated group exhibited a significant decrease compared with the placebo group at month 6 (p=0.033). However, HUC-MSCs did not significant decrease the levels of IL-8 and IFN-γ at the any of point of time visit.

Table 3. Efficacy of endpoints at baseline and follow-up points

Variables	Group	Baseline	1 week	1-month	2-month	3-month	6-month
PCS	MSCs	239.87±72.15*	315.40±68.25**	337.13±66.44**	353.75±42.81**	293.00±153.86**	351.83±68.35**,†
	Placebo	213.27±98.27	227.07±100.10	249.87±81.22	263.92±92.71	234.46±98.05	240.27±116.08
MCS	MSCs	287.81±72.44**	348.27±47.09**	361.65±27.76**	363.11±25.62**	296.33±154.75**	365.29±19.81**
	Placebo	262.18±88.17	256.76±97.73	275.46±91.12	281.18±102.73	280.32±88.34	307.53±77.12
EQ-VAS	MSCs	66.67 ± 13.58	76.0 ± 13.65	79.67 ± 10.77*	84.58 ± 11.76**,†	83.17 ± 6.83**, ‡	82.92 ± 8.38**, ‡
	Placebo	67.33 ± 10.50	71.4 ± 10.86	75.33 ± 9.72	76.42 ± 8.39	73.46 ± 8.51	72.87 ± 11.70
PSQI	MSCs	8.60 ± 3.99	6.13 ± 3.07	6.20 ± 3.55	6.17 ± 4.55	6.33 ± 3.85	7.33 ± 4.23
	Placebo	9.60 ± 5.05	8.80 ± 3.17	8.33 ± 3.20	9.0 ± 4.0	8.23 ± 4.23	8.0 ± 4.11
TUG(s)	MSCs	10.20 ± 2.75	8.36 ± 1.70†	8.25 ± 1.34†	7.66 ± 1.46**,†	7.92 ± 1.35*,†	7.78 ± 1.63*,†
	Placebo	10.98 ± 2.46	11.17 ± 3.75	11.05 ± 4.36	11.08 ± 4.66	10.77 ± 3.46	10.97 ± 5.27
4MWT(s)	MSCs	5.04 ± 0.96	4.15 ± 0.69	4.18 ± 0.52*	3.89 ± 0.53**	3.94 ± 0.67**	4.04 ± 0.45**,†
	Placebo	5.90 ± 1.49	5.82 ± 2.37	5.68 ± 2.46	5.47 ± 2.18	5.68 ± 1.87	6.09 ± 3.39
Grip strength (Kg)	MSCs	17.99 ± 7.31	21.92 ± 7.54	22.27 ± 7.29	23.63 ± 7.13†	24.96 ± 6.56†	25.44 ± 5.44 ‡
	Placebo	17.04 ± 9.65	18.89 ± 9.06	18.76 ± 8.41	17.33 ± 9.28	19.08 ± 9.38	18.33 ± 10.11
FTTST(s)	MSCs	15.10 ± 8.13	11.89 ± 3.41	11.37 ± 3.28	10.33 ± 3.45	10.69 ± 3.47	11.27 ± 3.07
	Placebo	17.37 ± 10.71	17.25 ± 13.67	16.81 ± 13.45	15.68 ± 12.22	16.87 ± 12.26	17.50 ± 9.44
IL-8(pg/mL)	MSCs	14.30 ± 4.52	12.72 ± 3.14	11.40 ± 4.57	14.27 ± 7.51	9.59 ± 6.06	10.21 ± 3.32
	Placebo	21.29 ± 14.62	15.70 ± 6.28	15.29 ± 5.38	20.28 ± 20.63	13.43 ± 4.72	13.14 ± 6.33
IL-17(pg/mL)	MSCs	19.25 ± 27.76	12.70 ± 2.63	14.69 ± 6.11	21.82 ± 14.60	17.22 ± 10.06	18.50 ± 22.70†
	Placebo	11.78 ± 3.36	13.79 ± 3.86	17.66 ± 19.41	19.05 ± 6.70	14.50 ± 4.35	32.76 ± 42.96
IFN-γ(pg/mL)	MSCs	2.70 ± 0.40	3.00 ± 0.34	2.63 ± 0.22	3.10 ± 0.54	3.17 ± 0.73	2.79 ± 0.50
	Placebo	2.60 ± 0.22	3.00 ± 0.38	2.64 ± 0.27	3.19 ± 0.46	3.04 ± 0.64	3.12 ± 0.58
TNFα(pg/m)	MSCs	2.53 ± 0.10	2.59 ± 0.14	2.51 ± 0.04	2.68 ± 0.22	2.76 ± 0.39	2.61 ± 0.25†
	Placebo	2.53 ± 0.08	2.68 ± 0.20	2.51 ± 0.03	2.73 ± 0.42	2.60 ± 0.25	3.13 ± 1.22

*p 0.01 vs baseline; **p 0.002 vs baseline; † p 0.05 vs placebo; ‡ p 0.01 vs placebo.

PCS: Physical component scores; MCS: Mental composite score; EQ-VAS: EuroQol visual analogue scale; PSQI: Pittsburgh sleep quality index;

TUG: Timed Up and Go test; 4MWT: Four-meter walking test; FTTST: Five times sit to stand test; IL-8: Interleukin-8; IL-17: Interleukin-17;

INF-g: Interferon-γ; TNF-a: Tumor necrosis factor-α.

Discussion

This study is the first randomized, double-blind, placebo control clinical trial with intravenous delivery of HUC-MSCs in the elderly individuals with frailty. With an aim to investigate the efficacy and safety of transplantation of allogeneic HUC-MSCs in aging frailty, our study has revealed findings pertaining to predetermined primary end points. HUC-MSCs have been demonstrated to be safe and feasible in the context of aging related chronic diseases, as evidenced by the data of several randomized controlled clinical trial (18–21). In line with the numbers of previous trials, intravenously infused allogenic HUC-MSCs did not lead to any severe adverse events or complications, indicating the safety profile of this novel therapeutic approach. Furthermore, the HUC-MSCs treatment in this study induced no adverse immune responses among aging frail individuals, indicating the well-tolerance and feasibility of HUC-MSC-based therapy.

Regarding the primary endpoint defined as the physical component of SF-36 quality of life, our data showed notable improvement in the PCS scores of SF-36 within the group subjected to MSC treatment. This improvement was observed starting from one week after MSC transplantation and persisted until the final follow-up assessments. Additionally, the administration of HUC-MSCs have led to a significant amelioration in EQ-VAS exclusively at 2-, 3-, and 6-month follow-up intervals. Furthermore, the mental component of SF-36 quality of life exhibited significantly enhancement within the MSCs group during the 6-month follow-up periods. In contrast, no differences were observed between the two groups in terms of sleep quality, as evaluated through the PSQI at any of the follow-up time points.

The results in this study have suggested that HUC-MSC therapy produced clinically significant improvement in the quality of life and functional performance outcomes. The findings are in line with previous clinical trials that investigated the therapeutic potential of MSCs administration for aging frailty. A phase I clinical trial conducted by Golpanian et al.(22) has investigated the effects of intravenous infusion of allogenic BM-MSCs on the frail elderly individuals and reported the significant improvements in quality of life and 6-minute walk distance as well as the reduction levels of TNF-α. The consecutive phase II study was a randomized, double-blind, placebo controlled clinical trial conducted by Tompkins et al.(18), which has demonstrated the efficacy of allogenic BM-MSCs in improving quality of life in older adults with frailty. Collectively, these studies support the contention that MSCs-based therapy holds considerable promise as a novel approach for ameliorating and preventing the development of aging frailty. As for the physical component of the SF-36 quality of life, set as a primary endpoint, out data showed that a significant improvement in the PCS of SF-36 were observed at month 6 with patients receiving MSCs compared with placebo. For patients subjected to HUC-MSCs treatment, the greater PCS were reported started from one weeks after the procedure and remained until the end of follow-ups. Furthermore, the MSC treatments leaded to a significant amelioration in the health self-evaluation assessed via EQ-VAS exclusively at the 3- and 6-month follow-ups. In addition to the physical component, the mental composite quality of life was noteworthy enhanced in the MSCs-treated group during the 2-, 3-, and 6-month follow-up periods. However, in this study, there was no significant difference in PSQI score the change between two groups, indicating HUC-MSCs did not exert a beneficial effect on ameliorating sleep quality. This finding is consistent with prior research that patients underwent the transplantation of hematopoietic stem cells experiencing significant sleep disturbances (23). It is noteworthy that sleep quality is influenced by various external factors, such as environmental conditions, psychological diseases and lifestyle choices (24), which may impact the response to HUC-MSC infusion in the context of sleep quality. Also, a longer follow-up period may provide a more comprehensive understanding of MSC therapy on sleep quality.

In this study, intravenous administration of MSCs was considered beneficial for the elderly individuals with frailty. We observed the substantial improvements in the physical performance capacity following the administration of HUC-MSCs. In terms of grip strength, the MSCs group exhibited greater enhancement at the 2-, 3- and 6-month follow-up compared to the control group, which indicated enhanced muscle strength in upper arms. This finding aligns with a preclinical study utilizing MSCs infusion (25), as well as two clinical studies reported by Golpanian et al. (22) and Tompkins et al.(18). In addition, the improved performance in TUG tests, assessing the mobility as well as balance ability (26), exhibited continuous improvement in patients treated with HUC-MSCs during each post-treatment visit. This finding suggested an overall enhancement in physical function among MSCs group. Notably, the result indicated an increase in 4MWT performance at the 6-month follow-up in the MSCs group compared to the placebo group. Aging frailty is an aging related condition, accompanied with the declines in physical capacity and exert negative effects on the quality of life (27), the findings of this trial may highlight the effects of HUC-MSCs in ameliorating physical decline associated with aging frailty. However, it is worth noting that there were no significant differences between the two groups in the performance of the FTSST during each follow-up visit. The negative results of FTSST may be attributed to the relatively small sample size of the study population and short duration of follow-up. Consequently, investigations involving larger sample sizes and longer-term follow-up are warranted to elucidate the therapeutic effect of MSC-based therapy on these outcomes among older adults with frailty.

In the present study, we also observed that MSC treatment could lead to the decrease in the levels of TNF-α as well as IL-17 at the 6-month follow-up. However, there were no significant differences in the levels of IL-8 and IFN-γ between the MSCs group and placebo group. Several explanations for our findings warrant consideration. The reduction in the levels of TNF-α and IL-17 following MSC therapy confirm the anti-inflammatory and immunomodulatory properties of HUC-MSCs. As reported by multiple studies, MSCs have been shown to possess anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines, thereby further attenuating various degenerative and inflammatory disorders, including aging frailty (9, 10, 28). The declines in both TNF-α and IL-17 levels in older adults treated with HUC-MSCs typically support the current evidence that MSCs can alleviate the systemic chronic low-grade inflammation and thus prevent the procession of aging frailty (10, 18, 22). However, there was no significant difference in the levels of IL-8 and IFN-γ between the MSCs and placebo groups in our study. It is well acknowledged that IL-8 and IFN-γ are pro-inflammatory cytokines involved in inflammation and innate immune responses, playing prominent roles in the recruitment, activation and survival of neutrophils at sites of inflammation (29, 30). The findings of this study may be partially attributed to the small sample sizes. Besides, it is possible that MSCs may exert context-specific effects on specific signaling pathways to regulate inflammation (31). Thus, further investigations are still needed to elucidate the specific mechanisms by which MSCs regulate the production and secretion of pro-inflammatory cytokines. Our findings indicated that intravenous administration of MSCs may mitigate the chronic inflammatory state via reducing the levels of TNF-α and Il-17, the potential mechanisms underlying the anti-inflammatory role of MSCs have not been thoroughly elucidated and thus further investigations are still needed.

MSCs have been shown to possess regenerative and differentiation properties that can contribute to the tissue repair process (32, 33). Our data in this study indicated that HUC-MSCs may exert their beneficial effects through enhancing physical performance and suppressing chronic inflammation. Our study also demonstrated that MSC therapy in aging frailty resulted in increased quality of life. In addition to the anti-inflammatory effects, it is conceivable that MSCs are capable of promoting tissue regeneration, muscle strength as well as the overall physical function. The potential mechanisms underlying the improved physical performance and quality of life following administration of HUC-MSCs may be partially attributed to the regenerative capacity of MSCs. The therapeutic benefit of MSC therapy may be derived from the paracrine action of MSCs such as secretion of growth factors and cytokines that are responsible for modulating the cellular microenvironment, promoting tissue repair and regeneration (34).

Of note, the present study has certain limitations. The relatively short duration of follow-up and the specific characteristics of the study population may influence the immune and inflammatory responses. Additionally, the sample size in our study may have limited our ability to detect small differences, especially in immune parameters. Future studies with larger sample sizes and longer follow-up periods are needed to confirm and further explore the anti-inflammatory and immunomodulatory effects of MSC therapy in aging frailty.

Conclusions

In conclusion, this randomized controlled clinical trial provides evidence supporting the safety and feasibility of HUC-MSCs therapy for aging frailty. The significant decrease of TNF-α and IL-17, the observed improvements in quality of life as well as physical performance, including TUG tests, grip strength and 4MWT, highlight the potential of HUC-MSCs as a therapeutic option for intervention and prevention of the physical decline associated with aging frailty. However, further research is warranted to elucidate the effects of HUC-MSCs therapy on other functional measures and to unravel the potential mechanisms underlying. These findings contribute to the growing body of literature supporting the use of MSCs-based interventions in the treatment of aging-related diseases.

Abbreviations

HUC-MSCs: Human umbilical cord-derived mesenchymal stem cells

SAEs: Serious adverse events

AEs: Adverse events

PCS: Physical component scores

MCS: Mental component scores

SF-36: Short Form 36 Health Survey

EQ-VAS: EuroQol visual analogue scale

PSQI: Pittsburgh sleep quality index

TUG: Timed up and go

4MWT: Four-meter walking test

FTSST: Five times sit to stand

TNF-a: Tumor necrosis factor-α

INF-g: Interferon-γ

IL-8: Interleukin-8

IL-17: Interleukin-17

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. The clinical study of umbilical cord tissue-derived mesenchymal stem cells in the treatment of patients with aging frailty: a phase I/II randomized, double-blind, placebo-controlled study

was approved by the Human Cell Clinical Research Ethics Committee of Shanghai East Hospital (EC.D(BG).020.02.0-2019-008) on October 8, 2019. All patients have provided written informed consent before the beginning of the study.

Consent for publication

All participants enrolled in this study have provided written informed consent for the publication of their anonymized data and related information.

Availability of data and material

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no competing interest.

Funding

This work was also supported by Major Program of Development Fund for Shanghai Zhangjiang National Innovation Demonstration Zone (Stem Cell Strategic Biobank and Stem Cell Clinical Technology Transformation Platform) (No. ZJ2018-ZD-004); Shanghai Pudong New District Health and Family Planning Commissions (No. PW2022B-13); Peak Disciplines (Type IV) of Institutions of Higher Learning in Shanghai; National Key Research and Development Program of China (No. 2022YFC3601504) and Shanghai Municipal Health Commission (No.202140248).

Authors' contributions

HJ, ZML and HLL initiated and designed the study. YQZ and CH carried out the study. YQZ took the lead in drafting the manuscript with CH. YQZ, CH and LZ curated data and conducted statistical analysis. QQL, JLG, SSG, XC, HXY, YL collected the data. WWJ was responsible for the quality control of the clinical-grade stem cells. KPS, YL, TY and ZMZ contributed to sample preparation. HJ, ZML and HLL revised the manuscript. All authors approved the final manuscript.

Acknowledgements

We acknowledge and express our gratitude to the individuals who consented to participate, and our colleagues in Institute for Regenerative Medicine, National Stem Cell Translational Resource Center of Shanghai East Hospital for their huge contribution to making this project possible.

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Figures

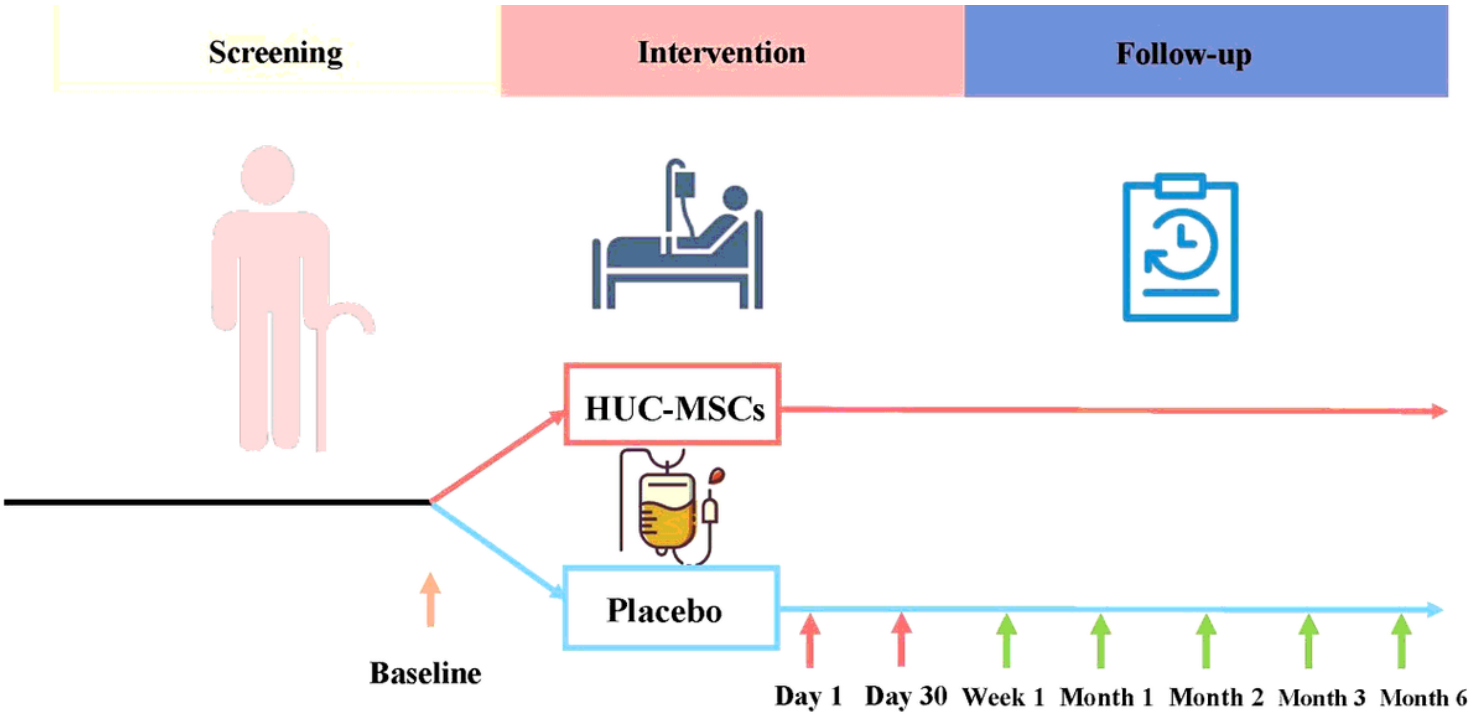

Figure 1

The schedule of study. The intravenous infusions of HUC-MSCs at a dose of 10^6 cells/kg or placebo were intravenously infused twice at day 1 and day 30. The quality of life, physical function, clinical laboratory parameters and inflammatory cytokines were assessed at baseline and follow-up visits. SAEs and AEs were collected within 6 months following the first treatment.

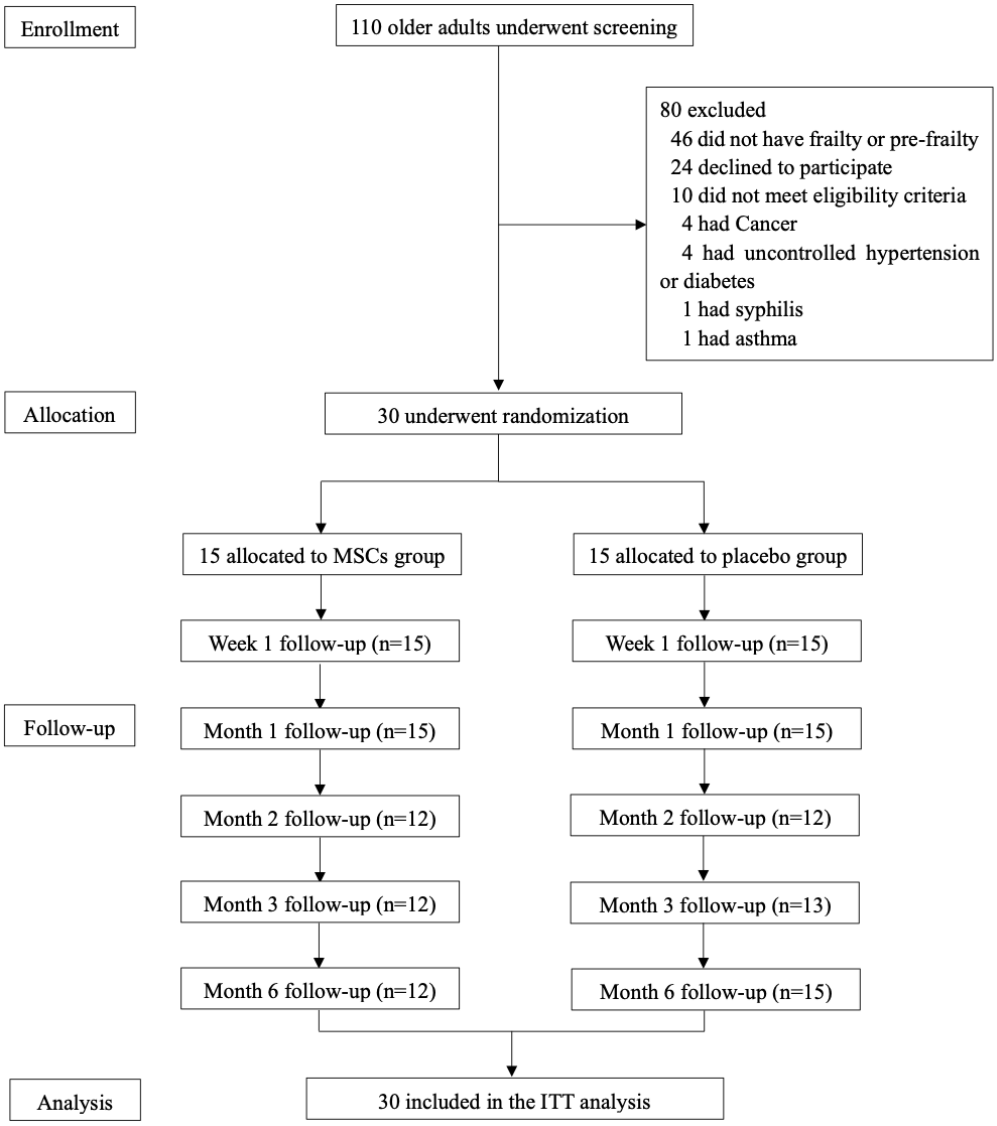

Figure 2

The flow chart of trial. Patient enrollment, allocation, follow-up and analysis.

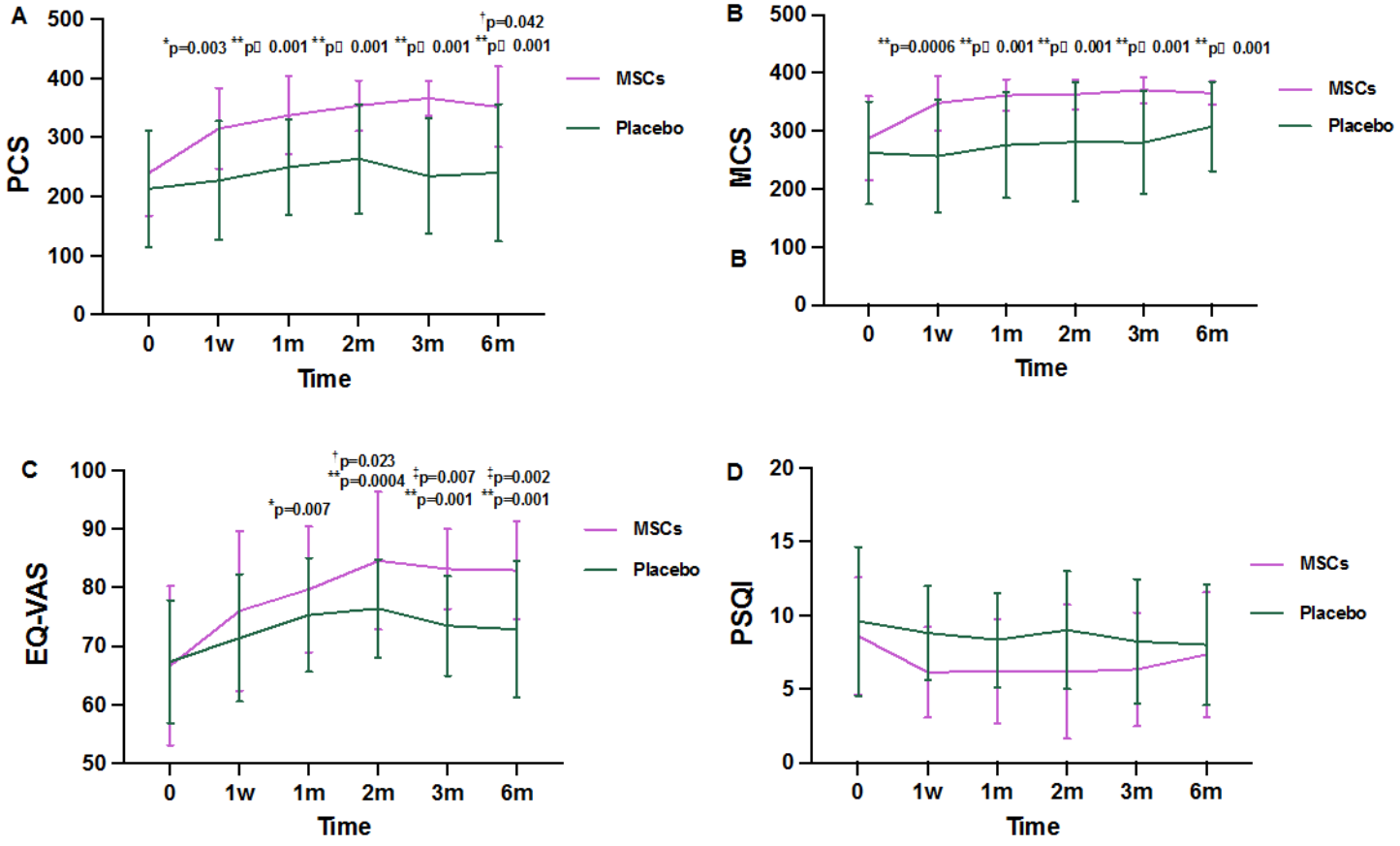

Figure 3

Changes in the scores of qualities of life from baseline to 6-month after intravenous infusion in the MSCs group and placebo group. A. Physical component scores (PCS); B. Mental composite score (MCS); C. EuroQol visual analogue scale (EQ-VAS); D. Pittsburgh sleep quality index (PSQI). *p 0.01 vs baseline, **p 0.002 vs baseline; †p 0.05 vs placebo, ‡ p 0.01 vs placebo.

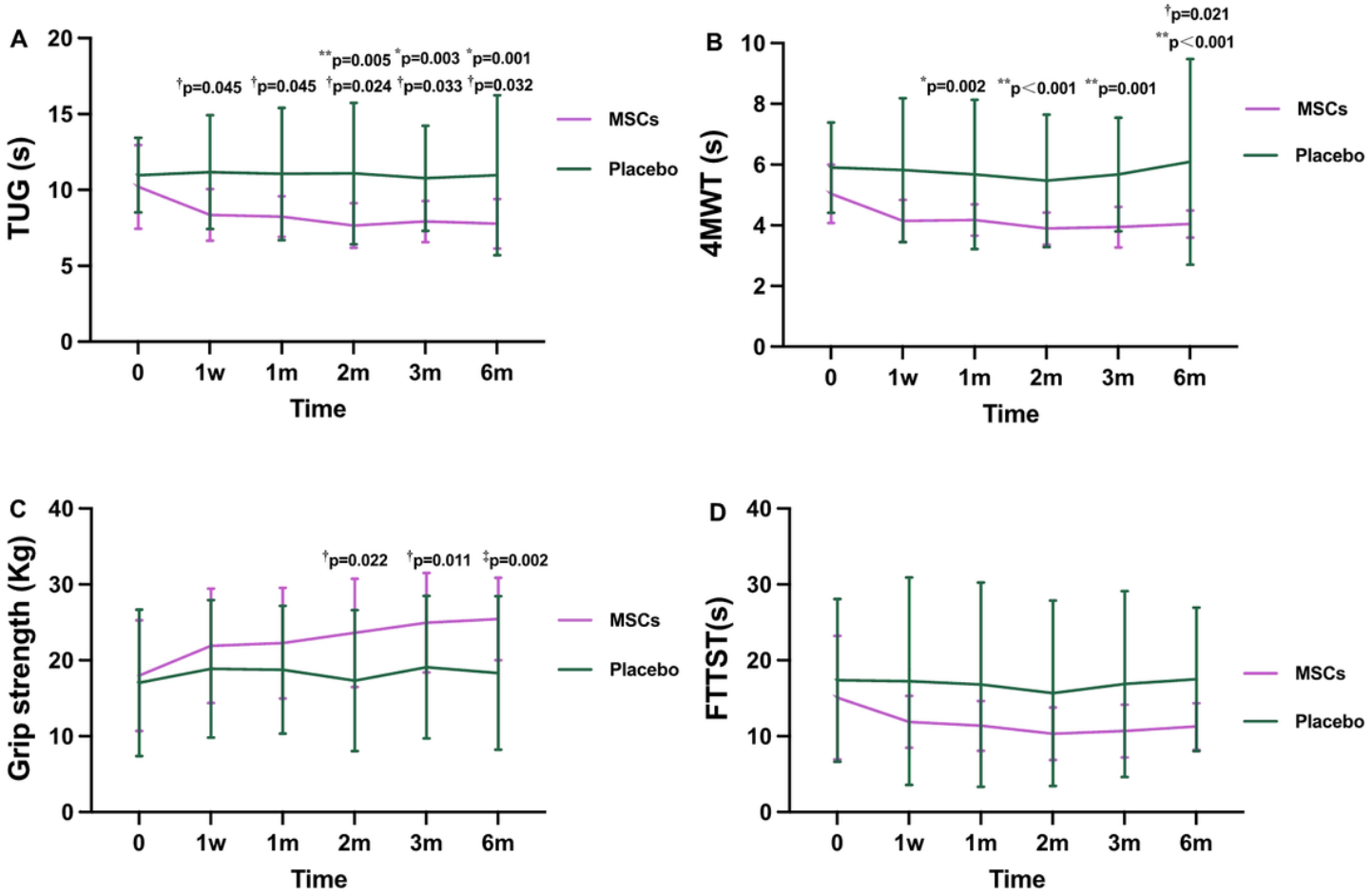

Figure 4

Changes in the levels of physical performance from baseline to 6-month after intravenous infusion in the MSCs group and placebo group. A. Timed Up and Go (TUG); B. Four-meter walking test (4MWT); D. Five times sit to stand test (FTTST). *p 0.01 vs baseline, **p 0.002 vs baseline; †p 0.05 vs placebo, ‡ p 0.01 vs placebo.

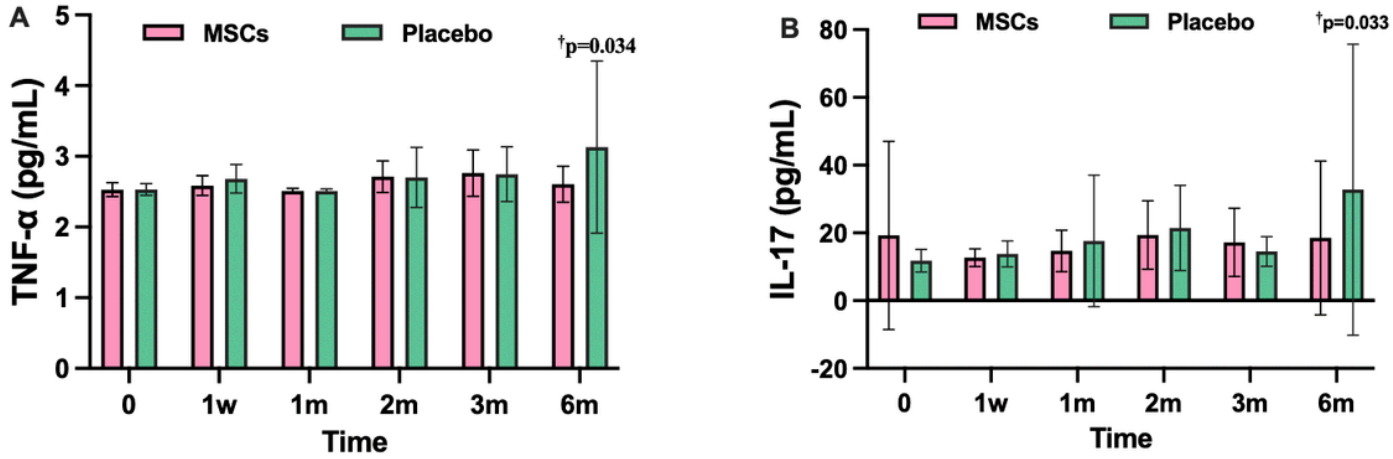

Figure 5

Changes in the levels of inflammatory cytokines from baseline to 6-month after intravenous infusion in the MSCs group and placebo group. A. Tumor necrosis factor-α (TNF-a); B. Interleukin-17 (IL-17). † p 0.05 vs placebo.