Title: Intranasal Delivery of Wharton's Jelly Mesenchymal Stem Cell-Derived Conditioned Medium in Patients with Alzheimer's Disease: A Phase 1 Safety and Volum-Escalation Study

Authors: Setareh Akbari[1,2], Mostafa Almasi-Dooghaee[3,4], Alireza Amanollahi[5], Mohammad Taghi Joghataei[6,7], Saeed Oraee-Yazdani[2,8], Alireza Zali[2,8],*, Soraya Mehrabi[6,7,*], Naser Amini[7,9,*]

1. *Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences (IUMS), Tehran, Iran.*
2. *Functional Neurosurgery Research Center, Research Institute of Functional Neurosurgery, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
3. *Department of Neurology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
4. *Firoozgar Clinical Research Development Center (FCRDC), Iran University of Medical Sciences, Tehran, Iran.*
5. *Trauma and Injury Research Center, Critical Care Department, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
6. *Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.*
7. *Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*
8. *USERN Office, Functional Neurosurgery Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
9. *Institutes of Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.*

***Corresponding Author**: Naser Amini, Department of Neuroscience, Institutes of Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran. Email: Amini_ot@yahoo.com

***Co-corresponding authors**: Soraya Mehrabi; Cellular and Molecular Research Center, Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran. Email: soraya.mehrabi@gmail.com

Alireza Zali, Functional Neurosurgery Research Center, Research Institute of Functional Neurosurgery, Shohada Tajrish Comprehensive Neurosurgical Center of Excellence, USERN Office, Functional Neurosurgery Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: alirezansmd@gmail.com

To appear in: **Basic and Clinical Neuroscience**

Received date: 2026/05/9

Revised date: 2026/08/21

Accepted date: 2026/09/1

Please cite this article as:

Akbari, S., Almasi-Dooghaee, M., Amanollahi, A., Joghataei, M.T., Oraee-Yazdani, S., Zali, A., et al. (In Press). Intranasal Delivery of Wharton’s Jelly Mesenchymal Stem Cell-Derived Conditioned Medium in Patients with Alzheimer’s Disease: A Phase 1 Safety and Volum-Escalation Study. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.8588.1

DOI: http://dx.doi.org/10.32598/bcn.2026.8588.1

Abstract

Purpose of the study: Alzheimer's disease is missing proven disease-modifying therapies; Mesenchymal stem cell-derived conditioned medium (MSC-CM) provides a without using living cells anti-inflammatory and neuroprotective strategy. This phase 1 trial examined how safe and acceptable it is to administer intranasal Wharton's jelly MSC-CM in mild-to-moderate AD.

Methods: We designed a phase trial that was open-label, single-arm; nine patients (4 males, 5 females; aged 65-89) with mild-to-moderate AD took part. Participants received intranasal WJMSC-CM at escalating s (1.2, 1.8 and 3.0 mL/day) over three consecutive days. Daily vital signs, physical checks and adverse checks were conducted to assess adverse. Baseline and 3-month brain MRI were used to test structural neuroanatomical safety. The baseline and day 4 serum biomarkers (IL-1β, IL-6, TNF-α, CRP, TGF-β, BDNF) were quantitatively determined by ELISA. The MMSE, MoCA, ADL, and IADL scales were used to assess cognitive and functional statuses.

Results: All nine patients finished both parts of the study. No major side effects or neuroimaging abnormalities were found. The side effects of two people in the medium group were mild and temporary-cough and tingling of the lips-went away on their own. No significant decrease in pro-inflammatory cytokines was found from serum biomarker analysis (IL-6, TNF-α, IL-1β, CRP; $p > 0.05$). Interestingly, BDNF levels increased significantly ($p = 0.01$), mainly in the low- cohort. Although this study was not powered to prove efficacy, exploratory analyses indicated numerical improvements for the treatment group. There was a trend for improvement in cognitive and functional scores during follow up. The results of this study should be interpreted with care, as they may be hypotheses under test and need to be confirmed by properly powered studies.

Conclusion: After the experiments, we determined that the WJMSC-CM could be safely administered via the nasal route. Patients with mild and moderate Alzheimer's had no major problems with it. These initial results indicate possible biological activity and warrant further investigation. Further investigation in larger RCTs to assess clinical efficacy.

Keywords: Alzheimer's Disease; Mesenchymal Stem Cells; Conditioned Medium; Intranasal Administration; Safety; Phase I Clinical

Highlights

- The three s of intranasal WJ-MSC-CM (around 1.2, 1.8 and 3.0 mL/day) were well tolerated.
- No severe adverse events or limiting toxicities were noticed.
- All nine participants completed the planned 3+3 escalation protocol.
- These results help to pave the way for further testing in a Phase II clinical trial.

Introduction

Background and Rationale

Worldwide, no other dementia is as common as Alzheimer's. In addition, it also contributes to the damage of nerve cells. The clinical course of AD manifests with a gradual cognitive decline with severe memory loss and progressive loss of functional autonomy. AD currently affects more than 55 million people worldwide, and by 2050, this number is expected to rise to around 139 million as people are living longer and longer (Organization, 2019). Although the socioeconomic impact of AD is overwhelming, current treatments are only capable of providing symptomatic relief for short periods of time, and have shown very little success in slowing or stopping the underlying pathological processes (Cummings, Lee, Zhong, Fonseca, & Taghva, 2021). The modest cognitive benefit of standard drugs used in clinical practice, such as drugs which increase acetylcholine (cholinesterase inhibitors) or inhibit NMDA receptor, does not change the underlying disease course (Yiannopoulou & Papageorgiou, 2013). In addition, the limited permeability of the blood-brain barrier (BBB) to these agents, and their systemic side effects, highlight the need for novel, non-invasive, and disease-modifying therapeutic approaches (Pardridge, 2005).

AD is a multifactorial pathobiology with complex relationship between amyloid-β (Aβ) aggregation, hyper-phosphorylation of tau protein, loss of synapses, oxidative stress, and chronic neuroinflammation. Importantly, the neuroinflammatory pathways play a major role in mediating disease progression, much of which is driven by microglial and astrocytic reactivity to Aβ deposition (Heneka, Golenbock, & Latz, 2015). These activated glial cells release a variety of pro-inflammatory cytokines such as TNF-alpha, IL-1beta, and IL-6, which further damage the neurons and contribute to abnormal signaling from synapses and apoptotic pathways (Heppner, Ransohoff, & Becher, 2015). At the same time, peripheral and central blood markers of inflammation, like C-reactive protein (CRP), are increased in AD patients (A. Ng et al., 2018). Furthermore, a deficiency of neurotrophic factors, such as BDNF and TGF-β, is associated with impaired neuroprotection and reduced neurogenesis (T. K. S. Ng, Ho, Tam, Kua, & Ho, 2019). Such mechanisms involve both neurodegenerative and inflammatory processes, creating a vicious circle that leads to an accelerated cognitive decline.

In recent years, MSCs, particularly those derived from Wharton's jelly, have been the subject of great interest as a novel approach to the treatment of neurodegenerative disorders. The power of Wharton's jelly derived MSCs is largely their capacity to modulate immune responses, reduce inflammation and promote growth of nerve cells, all via signals they send to neighboring cells rather than through the replacement of the damaged neurons themselves (Galipeau & Sensébé, 2018). These effects are thought to be mediated by a mixture of cytokines, growth factors and extracellular vesicles released into the surrounding growth environment, called secretome or conditioned medium (CM) (Vizoso, Eiro, Cid, Schneider, & Perez-Fernandez, 2017). So far, there are some benefits to CM based treatments compared to those involving live cells. They are less likely to change over time, have a normal dosage, are less likely to cause immune reactions, and do not have any of the tumor or clogged blood vessel side effects (Toh, Lai, Zhang, & Lim, 2018).

WJ-MSC-derived CM contains high levels of anti-inflammatory cytokines (such as IL-10 and TGF-beta), neurotrophic factors (such as BDNF, NGF and IGF-1) and regulatory microRNAs that regulate neuroinflammation and neuronal survival (Mendes-Pinheiro et al., 2018). Preliminary investigations have shown that CM has beneficial effects on reducing microglial activation, oxidative stress, and improving synaptic plasticity, which helps reduce the core features of AD including Aβ deposition (Mita et al., 2015). WJ-MSC-CM is thus a novel cell-free neuroprotective modality with the potential to target the multiple mechanisms involved in the pathophysiology of AD.

Getting past the BBB is still one of the toughest problems when treating diseases of the central nervous system (CNS). The nasal route of delivery is a popular method of circumventing this barrier because is effective, and requires no surgery and can exploit the olfactory and trigeminal nerve pathway to reach the brain (Lochhead & Thorne, 2013). The advantages of this route are: fast onset of action, limited systemic exposure and good compliance by the patient, especially elderly people (Per G Djupesland, Messina, & Mahmoud, 2014). Preclinical data indicate intranasal

delivery of bioactive secretomes is effective for controlling microglial activation and normalizing synaptic performance in rodent AD models (Lochhead & Thorne, 2012; Mita et al., 2015). In addition, intranasal insulin and oxytocin administration has been demonstrated to exert cognition-enhancing effects in early AD (Reger et al., 2008), confirming the potential of this method to deliver CNS therapeutics.

Although there is clearly good preclinical data that supports the delivery of MSC-derived CM (Alidoust et al., 2023; Tolstova, Dotsenko, Luzgina, & Rusanov, 2024), there are no obvious clinical trials that assess the safety and efficacy of CM delivered via the nose in humans. Although the use of systemic MSC transplantation has been widely studied, CM has not yet been thoroughly explored for clinical translation as a non-invasive approach (Morita et al., 2024; Xu et al., 2018; Yun et al., 2013).

Rationale and Hypothesis

Our main goal in this study was to find out whether giving WJ-MSC-CM through the nose is safe and practical for people with mild to moderate Alzheimer's disease. Considering the preclinical findings, we hypothesized that WJ-MSC-CM can suppress pro-inflammatory cytokines (TNF-alpha, IL 6 and IL-1beta) and upregulate neurotrophic cytokines (BDNF, TGF-beta), which lead to neuroprotection while not causing immune rejection (Mehrabadi, Sadr, & Hoseini, 2019; Xie et al., 2016). In this study, the intranasal route is explored as a possible and safe delivery platform for this novel cell-free therapeutic in a clinical setting (Morita et al., 2024; Vizoso et al., 2017).

The present first-in-human Phase I clinical trial was designed to evaluate the safety and tolerability of intranasally administered WJ-MSC-CM in patients with mild-to-moderate Alzheimer's disease, while providing preliminary insights into associated biological and clinical changes to inform the design of future clinical studies.

Materials and Methods

Study Design and Participants

This research was meticulously structured and executed in strict adherence to the CONSORT 2025 statement and its specific extensions for early-stage (phase I) clinical trials (Hopewell et al., 2025a, 2025b).

Patient and Public Involvement (PPI)

In accordance with the standard protocols for phase I -escalation studies, patients and the public were not directly involved in the design, recruitment, or reporting phases of this trial (Hopewell et al., 2025a, 2025b).

Trial Design

We used a phase I design that included measurements before and after treatment, single-arm, non-randomized, -escalation, and was open-label. The went up stepwise using the usual 3+3 plan. Our aim was to figure out the highest people could take without serious side effects (that's the MTD) and also to get a first look at whether the treatment had any biological effect. (Le Tourneau, Lee, & Siu, 2009; Storer, 1989). This design was selected for its methodological transparency and established safety track record in first-in-human (FIH) studies where prior clinical safety data are sparse (Le Tourneau et al., 2009; Rahma, Gammoh, Simon, & Khleif, 2014).

Changes to Trial Protocol

Following the commencement of the trial, no modifications were made to the original protocol, eligibility criteria, or pre-specified outcome measures.

Trial Setting

The clinical phase was conducted at the Dementia Clinic of Firoozgar Hospital (affiliated with Iran University of Medical Sciences, Tehran, Iran), under the supervision of a multidisciplinary committee of clinical and translational

neuroscientists. Centralization of laboratory analysis in Shohada Tajrish hospital and Sorna Research laboratory. All procedures were performed in compliance with Good Clinical Practice (GCP) and ISO 15189 guidelines (Barton, 2007).

Participant Eligibility Criteria

Potential subjects were males or females aged 65 to 89 years who have a confirmed diagnosis of mild to moderate AD (according to the criteria of the NIA-AA, neuroimaging, and using the MMSE and MoCA scores 10-24).

Exclusion criteria were:

1. Non-AD dementias or major depressive disorder (HAMD > 17).

2. Metabolic (or hormonal) disorder that cannot be controlled.

3. Deficiency of B12 vitamin or active malignancy/infection.

4. History of stroke within the past 3 months or concurrent enrolled in clinical trials.

We ensured that everyone who participated in the study and their legal guardian read and signed a consent form explaining all aspects of the trial.

Participants and Recruitment

The patient recruitment was carried out in Firoozgar hospital, in the dedicated dementia clinic during February 2024 and until January 2025. Patients with cognitive disorders are regularly assessed at the clinic, but for this Phase I safety study, recruitment was done subject to stringent criteria. A patient was eligible if he or she had mild to moderate Alzheimer's disease and met stipulated inclusion criteria and if the patient was willing to attend repeated visits. Those patients with severe disease stages, mixed dementia, significant neurological comorbidities or who were unable to complete follow-up assessments were excluded. Besides, patients from outside Tehran were not included in the study, as they would need to be monitored carefully during the intervention period.

Site and Personnel Eligibility

Comprehensive facilities for cognitive assessment and management of neurodegenerative disorders were provided at the trial site. All clinical staff received intensive training in a standardized intranasal administration method and had a wealth of experience in the care of geriatric dementia patients.

Procedures

Intervention and Administration

This was performed by intranasal delivery of WJ-MSC-derived CM via a calibrated nasal spray device. The participants were given progressive tric s of the CM according to a 3+3 escalation. Patients received a standard diet prior to the first (Day 0), and their current medications, such as cholinesterase inhibitors and memantine, were not changed during the study period to prevent the effects of these medications (Govind, 2020; Kishi et al., 2017) (see Table 1).

No protocol deviations occurred for all participants who were assigned to receive the predetermined intranasal of conditioned medium derived from WJ-MSC. 100% adherence to the treatment/intervention protocol.

[Please insert Table 1 here].

The study protocol outlines the safety monitoring procedures.

WJ-MSC-CM Preparation and Characterization

Quality Control and Manufacturing Conditions of WJ-MSC-CM

WJ-MSCs were cultured for expansion in DMEM media containing 10% human serum albumin (HSA), at standard culture conditions (37°C, 5% CO_2, humidified) in vitro. Donors of the umbilical cord were screened using the recommendations of institutional donor criteria including serology for HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and human T-cell lymphotropic virus type I/II (HTLV-I/II). Informed consent was obtained in writing prior to sampling. Donor characteristics and screening procedures are summarized in Supplementary Table 1.

[Please insert Supplementary Table 1 here].

WJ-MSC identity was confirmed according to the minimal criteria established by the International Society for Cell and Gene Therapy (ISCT). Flow cytometric characterization was performed before conditioned medium production using MSC-associated positive markers (CD73, CD90, CD105, and CD44) and hematopoietic lineage negative markers (CD34 and CD45). The expanded cells demonstrated the expected MSC immunophenotype, with high expression of positive markers (CD73: 98.22%, CD90: 99.72%, CD105: 97.67%, and CD44: 98.44%) and low expression of negative markers (CD34: 2.3% and CD45: 3.12%) (Supplementary Table 2). Chromosomal stability was evaluated by karyotype analysis, which demonstrated a normal chromosomal profile, with chromosomal abnormalities reported to be below 10%. Cell viability before conditioned medium production was greater than 92%, as assessed by trypan blue exclusion.

[Please insert Supplementary Table 2 here].

WJ-MSC identity characterization

Conditioned medium was generated from passage 4 WJ-MSC cultures. Passage 4 WJ-MSCs were seeded at an initial density of approximately 8×10^4 cells/cm² in T75 tissue culture flasks and cultured under standard conditions until approximately 80% confluence. Before serum-free conditioning, to reduce proteins from serum, the cell monolayer was washed three times with sterile phosphate-buffered saline (PBS; 5 mL per T75 flask) for about 60 seconds. Serum-free DMEM medium was subsequently added, and cultures were maintained for 48 hours at 37°C and 5% CO_2 to allow accumulation of MSC-secreted factors. The conditioned medium was then collected under aseptic conditions for further processing.

The collected CM was centrifuged at 4000 × g for 10 minutes and passed through a 0.22 µm sterile membrane filter. The processed CM underwent predefined quality-control release testing before clinical administration. Sterility testing was performed using culture-based methods according to USP <71>. No bacterial or fungal growth was detected after 14 days of incubation, indicating that the CM met the predefined sterility release criterion. Endotoxin levels were quantified using the Limulus Amebocyte Lysate (LAL) assay, with a predefined acceptance limit of <0.2 EU/mL. Mycoplasma contamination was evaluated using PCR-based detection with a reported analytical sensitivity of < 0.06%.

Only CM preparations fulfilling predefined release criteria were used for clinical administration. The final product was aliquoted under aseptic conditions and cryopreserved at -80°C according to institutional SOPs developed for cell-derived biological products. These procedures were consistent with previously described approaches for the preparation and characterization of MSC-derived conditioned media (Ghasemi et al., 2023; Vizoso et al., 2017). A single manufacturing batch generated from passage 4 WJ-MSCs was used for all nine participants. Following completion of quality-control release testing, the batch was divided into sterile single-use aliquots and stored at -80°C until administration, thereby minimizing variability associated with inter-batch differences in CM production.

The isolation, expansion of WJ-MSCs, and preparation of CM were performed at the Comprehensive Stem Cell and Regenerative Medicine Center, Shahid Motahari Burn Hospital, Iran University of Medical Sciences. Manufacturing procedures were conducted within a validated ISO Class 5 cleanroom environment. All open aseptic manipulations were performed using a Class II Type A2 biological safety cabinet located within the ISO Class 5 cleanroom (Azotech Company). Environmental monitoring, including temperature, humidity, pressure differential, particulate monitoring, and microbiological surveillance, was routinely performed according to institutional quality-control procedures.

Supplementary Table 1 provides details of key manufacturing parameters, quality assurance procedures, MSC characterization data, and CM production specifications.

Total protein concentration and some bioactive factors were quantified prior to clinical administration by Bradford assay and ELISA (R&D systems, USA). Characterization of 1 mL of CM showed that the factors BDNF and TGF-β are measurable and can be classified as neurotrophic and immunomodulatory, respectively (Table 2).

[Please insert Table 2 here].

Preparation of Intranasal Administration Procedure and Delivery Device

The intranasal delivery system was a commercially available metered nasal spray system (Naxonella®; Koushan Pharmed, Iran; registration number: 8551640591472004). The original bottle/pump assembly was kept and the commercially available budesonide formulation was removed and replaced in the same bottle with WJ-MSC derived conditioned medium in aseptic conditions.

While preparation of the device, the components were treated with institutional aseptic preparation procedures prior to filling. Under a biological safety cabinet, surface decontamination of components was carried out using 96% ethanol and ultraviolet irradiation. Sterilization was performed by either heat or autoclaving the components if applicable at 121°C for 15 minutes. The prepared components were then moved to the controlled clean room for the aseptic assembly and filling.

Each actuation of the metered- nasal spray device was specified to deliver approximately 100 µL of formulation. The labeled of 64 µg per actuation corresponds to the original budesonide-containing Naxonella® formulation and did not apply to the WJ-MSC-derived conditioned medium preparation used in this study.

Following assembly, the devices were filled with the assigned WJ-MSC-CM formulation under controlled aseptic conditions and individually packaged. Each participant received a dedicated nasal spray device according to the assigned -escalation group, which was discarded after completion of the administration period.

The administration protocol consisted of 2, 3, or 5 actuations per nostril every 8 hours for the low-, intermediate-, and high- groups, respectively. The daily administered CM s were calculated according to the nominal actuation of approximately 100 µL: 2 actuations/nostril/8 hours (low- cohort), 3 actuations/nostril/8 hours (medium- cohort) and 5 actuations/nostril/8 hours (high- cohort). If more than one actuation was necessary for each nostril, at least 30 seconds was left between actuations in the same nostril to ensure sufficient deposition time and avoid potential loss of formulation due to nasal drainage.

Biomarker Analysis

Peripheral blood samples (10-15 mL) were taken at baseline (day 0) and on day 4. Serum was isolated and stored at -80°C for subsequent analysis. Concentrations of BDNF, TGF-beta, TNF-alpha, IL-1beta, IL-6, and CRP were determined using high-sensitivity ELISA kits (R&D Systems), following validated protocols for early-phase trials (Ermann et al.). We ran every test twice, and we kept the variation within each run (that's intra-assay) and between runs (inter-assay) under 10% - that's how we made sure our measurements were reliable.

Outcome Measures

Primary Outcome Measures: Safety and Tolerability

The primary endpoint was the safety and tolerability of the intervention, adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 (Shah, 2022). Causality for any AEs was adjudicated using the WHO-UMC causality assessment system (Center & Organization, 2018).

Secondary Outcome Measures Included serum biomarkers, cognitive assessment, functional status, and MRI-based safety assessment.

Serum Biomarkers. Quantitative changes in pro-inflammatory cytokines (IL-1beta, TNF-alpha, IL-6, CRP) and neurotrophic factors (BDNF, TGF-beta) were evaluated at day 4 relative to baseline.

Cognitive Assessment. Global cognitive function was evaluated at baseline and at the 3-month follow-up using the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). The MMSE is a 30-point screening instrument that evaluates orientation, immediate and delayed recall, attention and calculation, language, and visuoconstructional abilities, and is widely used to assess global cognitive status in patients with Alzheimer's disease. Because educational attainment can influence performance on cognitive screening instruments, scores were interpreted considering participants' educational backgrounds, and the MoCA education adjustment was applied according to standard recommendations. The MoCA is also a 30-point cognitive screening tool but provides greater sensitivity for detecting mild cognitive impairment by assessing visuospatial and executive function, attention, language, abstraction, delayed recall, memory index score (MIS), and orientation (Creavin et al., 2016; Nasreddine et al., 2005).

In order to reduce practice effects from repeated cognitive testing, different validated English versions of the MoCA (version 8.1, 8.2, and 8.3) were administered at each study visit, following the official administration guidelines. The assessments were conducted using specified procedures by trained personnel of the study. In keeping with the MoCA administration manual, one extra point was awarded to those who reported ≤12 years of formal education. Cognitive scores were also adjusted for literacy and educational level for exploratory comparison between the various groups.

Functional Status. Functional status was assessed at baseline and at the 3-month follow-up using validated Persian versions of the Activities of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) questionnaires. The ADL questionnaire comprises seven items evaluating independence in basic daily activities, including eating, dressing, walking, personal grooming, bathing, transferring in and out of bed, and toileting. The IADL questionnaire consists of nine items assessing more complex activities required for independent living, including telephone use, transportation, shopping, meal preparation, housekeeping, laundry, minor household repairs, medication management, and financial management. Each item was scored on a three-point scale (2 = independent, 1 = requires assistance, 0 = unable to perform). Total ADL scores ranged from 0 to 14, and total IADL scores ranged from 0 to 18, with higher scores indicating greater functional independence. The complementary use of these instruments enabled comprehensive evaluation of both basic and instrumental functional abilities in patients with Alzheimer's disease (Katz, Ford, Moskowitz, Jackson, & Jaffe, 1963; Lawton & Brody, 1970).

Activity of Daily Living (ADL) and Instrumental Activities of Daily Living (IADL) questionnaires were validated Persian versions and used to determine functional status at baseline and at 3-month follow-up. The ADL questionnaire consists of seven items measuring the ability to perform activities in everyday life, such as eating, dressing, walking, self-grooming, bathing, getting in and out of bed and using the restroom. The IADL questionnaire includes nine items for more challenging activities needed for independent living, such as using the telephone, travelling by transport, shopping, preparing food and meals, housekeeping, laundry, minor home repairs, taking medicines and managing finances.

MRI-based safety assessment. Structural safety was evaluated using a 3-T brain MRI, including T1-weighted, T2-weighted, FLAIR, and diffusion-weighted imaging (DWI) sequences, with and without gadolinium contrast as specified in the study protocol. Images obtained at baseline and 3 months were independently reviewed by a board-certified neuroradiologist blinded to treatment allocation to identify any neoplastic transformation or other structural abnormalities. (Abdi, Javanmehr, Ghasemi-Kasman, Bali, & Pirzadeh, 2022; Liu et al., 2025; Wilson, de Natale, & Politis, 2022).

Safety and Adverse Events (Harms)

Active surveillance of patient safety was performed by active surveillance of drug Safety Events using the Medical Dictionary for Regulatory Activities (MedDRA v26.1) for event coding, and the Common Terminology Criteria for Adverse Events (CTCAE v5.0) for the severity grading. Allergic reactions and systemic symptoms were assessed and a daily evaluation of vital parameters and of the integrity of the mucosa of the nasal passages was conducted (see Table 3).

[Please insert Table 3 here].

A mild AE of tingling around the mouth (Grade 1) and mild cough were noted in the medium group. Similar to previous reports of a spontaneous resolution of both events after intranasal administration of the MSC-secretome (Aguilera et al., 2021; Morita et al., 2024). Notably, no serious adverse events (SAEs), -limiting toxicities (DLTs), or structural abnormalities on MRI were identified, confirming the safety profile of the intervention.

Sample Size and Trial Design

This exploratory phase I trial enrolled a total of nine participants (three subjects per cohort), adhering to the conventional 3+3 -escalation paradigm (Julious, 2005). The sample size was not determined by formal power calculations but was selected to assess feasibility, safety, and preliminary biological signals.

Escalation and Interim Analysis

escalation proceeded only after a 14-day DLT-free observation period for each cohort (Iasonos & O'Quigley, 2014). The protocol mandated trial discontinuation if two or more subjects in any cohort experienced DLTs or treatment-related SAEs. All escalation decisions were adjudicated by the principal investigator based on pre-specified safety criteria.

Randomization and Blinding

This was an open-label, non-randomized, single-arm study. Participants were enrolled serially into three escalating groups (low, medium, and high) upon satisfactory safety clearance of the preceding cohort. While the clinical team and participants were aware of the regimens (open-label design), analytical blinding was maintained; laboratory technicians and the board-certified neuroradiologist remained masked to the clinical data and -based cohorts to ensure the objectivity of the outcome measurements.

Ethical Considerations and Registration

The study protocol was approved by the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.REC.1402.885) and registered with the Iranian Registry of Clinical Trials (IRCT20240131060870N1). The trial was conducted in strict accordance with the Declaration of Helsinki and the ICH-GCP guidelines. Any minor protocol deviations, primarily minor shifts in follow-up scheduling, were documented and did not impact the integrity of the primary outcomes.

Statistical Analysis

Data analysis followed the CONSORT 2025 guidelines for phase I trials (Ellison et al., 2023; Hansen, Graham, Pond, & Siu, 2014). IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Due to the exploratory, first in human (phase I) design and the small study population, analyses were mostly descriptive in nature and aimed to describe the safety, tolerability, and early changes in clinical and laboratory parameters. Data for continuous variables were reported as mean ± standard deviation (SD) and categorical variables as frequencies and percentages.

For continuous outcomes, changes from baseline were calculated as the post-intervention value minus the corresponding pre-intervention value (post-intervention- baseline). Within-participant changes were evaluated using the paired-samples t-test for approximately normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. The choice of test was based on assessment of the distribution of the data, taking into account the limited sample size.

For categorical variables, the McNemar test was used for paired binary outcomes, where applicable. Chi-square testing was used for comparisons of independent categorical data when the assumptions of the test were met. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant. Given the exploratory nature of this phase I study and the absence of a formal hypothesis-testing framework for efficacy, p-values were interpreted cautiously and primarily as descriptive indicators of potential within-participant changes rather than as confirmatory evidence of treatment efficacy. In the SPSS output, "Sig. (2-tailed)" was interpreted as the two-sided p-value. The abbreviation "ns" denotes a non-significant result.

Exploratory Nature: No multiplicity adjustments were carried out as a result of the exploratory and hypothesis generating nature of the study. Analysis was performed on a per protocol basis and there was little missing data that was handled through listwise deletion.

Ancillary Analyses

No subgroup or sensitivity analysis was conducted owing to the small sample size and exploratory nature of the Phase 1 design.

Results

3.1. Participant Characteristics and Trial Flow

The progression of participants through the trial is summarized in the CONSORT flow diagram (Figure 1). At the start, we checked 19 people to see if they could be part of the study. Of these, 10 patients were excluded before enrollment, primarily due to declining participation or failure to provide written informed consent.

The remaining nine eligible participants were successfully enrolled and assigned to one of three -escalation cohorts in accordance with the 3+3 design:

Cohort I (Low). 1.2 mL/day (n = 3)

Cohort II (Medium). 1.8 mL/day (n = 3)

Cohort III (High). 3.0 mL/day (n = 3)

All participants received the prescribed intranasal WJ-MSC-CM intervention and completed the full treatment protocol without any attrition or dropouts.

3.1.1 Flow Diagram:

Study design and -escalation protocol of intranasal WJMSC-CM administration can be seen in Figure 1.

[Please insert Fig. 1 here]

Fig. 1. The CONSORT flow diagram illustrates the screening, enrollment, allocation, follow-up, and analysis of participants across the three groups in the phase 1 intranasal WJ-MSC-CM trial.

3.2. Safety and Protocol Adherence

Notably, no SAEs, treatment-related complications, or -limiting toxicities (DLTs) were observed. Consequently, no dosage adjustments or treatment discontinuations were required. Full datasets for serum biomarkers, neuroimaging, and cognitive/ functional assessments were obtained for all nine participants at all pre-specified time points. The final analysis was conducted on a complete-case basis, including all nine recruited patients.

3.3. Baseline Demographic and Clinical Characteristics

Baseline demographic characteristics are summarized in Table 4, while baseline cognitive/ functional measures are presented in Table 5 and serum biomarkers are presented in Table 6.

[Please insert Table 4 here].

As shown in Table 4, the participants were 70.33 (SD= 4.44) years old on average (65 to 89 years). The mean ages in the low-, medium-, and high- cohort groups were 70.67 ± 2.08, 69.33 ± 3.06, and 71.00 ± 7.93, respectively; none of these values were statistically significant ($p > 0.05$, ANOVA).

As indicated in Table 4, the gender distribution (4 males, 5 females) was similar in the dosing groups, and all groups did not show any inter-group differences ($p > 0.05$, Chi-square test).

According to Table 4, regarding educational attainment, 44.4% of participants completed primary education, 22.2% secondary, 22.2% tertiary, and 11.1% had no formal schooling; no significant differences were found between cohorts ($p > 0.05$).

The homogeneity of baseline variables across the dosing cohorts enhances the internal validity of the study and minimizes potential confounding bias.

3.4. The analysis and interpretation of clinical data and biological markers

3.4.1. Cognitive and Functional Outcomes

All 9 participants were analyzed both per protocol and intention-to-treat analysis. Participants had mild to moderate cognitive impairment as confirmed by baseline assessments. Exploratory pre-post intervention analyses were performed to evaluate preliminary clinical signals following intranasal WJ-MSC-CM administration.

Changes in cognitive and functional outcomes, including MMSE, MoCA, IADL, and ADL scores, are summarized in Table 5.

[Please insert Table 5 here]

Cognitive composite scores increased from 50.35 ± 11.11 at baseline to 60.93 ± 9.84 after intervention; however, this change did not reach statistical significance ($p = 0.32$). The greatest numerical improvement was observed in the high-volume cohort (+17.28 ± 13.26), whereas the medium-volume cohort showed a smaller change (+3.33 ± 11.54).

IADL scores increased from 57.71 ± 21.48 to 74.53 ± 18.46 after intervention (mean change: +16.82 ± 7.83, p = 0.74). Numerical improvements were observed across all volume cohorts, with the largest change observed in the lower and intermediate volume groups.

ADL scores showed no statistically significant changes after intervention, consistent with the relatively preserved baseline functional status of participants.

Standardized changes in cognitive and functional measures across volume groups are presented in Figures 2-4.

[Please insert Figures 2-4 here]

Although numerical improvements were observed, particularly in cognitive composite scores, no definitive volume-response relationship could be established in this small exploratory Phase I study.

3.4.2. Changes in serum biomarkers

Changes in serum biomarkers following intranasal WJ-MSC-CM administration are summarized in Table 6, whereas biomarker-specific changes are illustrated in Figures 5-10. Serum concentrations of BDNF, CRP, TGF-β, IL-1β, IL-6, and TNF-α were measured before and after intervention in all volume groups. Data are presented as mean ± SD, and changes (Ch) were calculated as the difference between post- intervention and baseline values. Statistical comparisons were performed using paired t-tests or Wilcoxon signed-rank tests, with a two-sided P value <0.05 considered statistically significant.

[Please insert Table 6 here]

Among the evaluated biomarkers, BDNF demonstrated the most pronounced intervention- related effect. Mean serum BDNF increased significantly after the intervention (Ch = +5.03 ± 3.60 pg/mL, P = 0.01), with the largest numerical increase were observed in the low-volume group (+8.83 ± 0.17 pg/mL). In contrast, reductions in the pro-inflammatory cytokines IL-6, TNF-α, IL-1β, CRP, and TGF-β were observed across all volume groups; however, none of these changes reached statistical significance.

The largest reduction in IL-6 occurred in the low-volume group (Ch = -5.16 ± 6.01 pg/mL), whereas the greatest decreases in IL-1β and TGF-β were observed in the medium-volume (Ch = -9.68 ± 7.36 pg/mL) and high-volume (Ch = -7.68 ± 10.51 pg/mL) groups, respectively. CRP and TNF-α also demonstrated downward trends following the intervention, with the largest numerical reductions observed in the low-volume group (CRP: Ch = -1.63 ± 1.53 mg/L; TNF-α: Ch = -1.80 ± 2.25 pg/mL), although these findings were not statistically significant**.** Representative changes in IL-6, TNF-α, BDNF, TGF-β, IL-1β, and CRP are shown in Figures 5-10.

[Please insert Fig. 5 here]

[Please insert Fig. 6 here]

Please insert Fig. 7 here]

[Please insert Fig. 8 here]

[Please insert Fig. 9 here]

[Please insert Fig. 10 here]

3.5. Harms: Safety and Tolerability

3.5.1. Safety and Adverse Events (AEs/ SAEs)

As shown in Table 3, no SAEs were detected in any of the three volume groups. No AEs were reported in either the low-volume (1.2 ml) or high-volume (3.0 ml) group. In the medium-volume group (1.8 mL), there were two mild AEs, namely, momentary scattered cough in one male participant and upper lip tingling in one female participant. Both events were categorized as grade 1 (mild, CTCAE 5.0), self-limited, and resolved without any intervention.

Overall, CM formulated using Wharton's Jelly MSCs intranasally was tolerated at every volume. No SAEs occurred in any of the groups. AEs were mild (grade 1), self-limiting, and resolved without medical intervention.

The intervention was monitored to prove that it did not lead to any severe harm and that any less-harsh incidents were self-limiting. These minor AEs were noted to occur in the medium-volume group, and it is possible that they were due to individual differences in mucosal sensitivity and not volume-related toxicity. These results verify the positive safety and tolerability of CM in this first-in-human phase 1 trial and justify the transition to further trial stages.

4. Discussion

4.1 Principal Findings

This Phase I trial provides preliminary evidence that intranasal administration of WJ-MSC-derived conditioned medium is feasible and may induce biologically relevant changes in patients with Alzheimer's disease. The principal finding was a significant increase in serum BDNF levels, accompanied by consistent, although statistically non-significant, reductions in IL-6, TNF-α, IL-1β, and CRP. Taken together, these findings suggest that WJ-MSC-CM may exert both neurotrophic and anti-inflammatory effects, the observed shift toward an anti-inflammatory biomarker profile is consistent with previous experimental and preclinical studies demonstrating the immunomodulatory and regenerative properties of MSC-derived conditioned medium (da Silva, Serrenho, Araújo, Carvalho, & Baltazar, 2023; Hambarsari et al., 2024; Santamaria et al., 2021). Furthermore, the more pronounced biomarker modulation observed in the low-volume group raises the possibility of a non-linear volume-response relationship that warrants further investigation in adequately powered randomized controlled trials.

4.2 Biological Interpretation of Biomarker Changes

While not statistically significant, the steady decrease in the amount of IL-6 and TNF-α in all the groups is well aligned with the immunomodulatory effects that have been shown for the MSC secretome. By looking at the cytokine levels of Interleukin-1β, one notes a continuing drop down indicating that WJ-MSC-CM may modulate inflammatory systemic signaling. The simultaneous reduction in CRP supports this systemic downregulation of inflammation as well, but it needs to be proven in sufficiently powered studies.

It is biologically relevant that the marked elevation in serum BDNF is known to contribute to a wide range of processes, including formation of memory, survival of neurons and their synapses, and synaptic plasticity. Increased expression of BDNF observed in the circulation could be one way WJ-MSC-CM exhibits its potential to be neuroprotective, a role that is consistently related to reduced expression of BDNF in Alzheimer's disease (Jaberi & Fahnestock, 2023; Numakawa & Kajihara, 2023; Park & Poo, 2013).

Paradoxically, the decrease in the transforming growth factor-β (TGF-β) has to be interpreted with caution. TGF-β also has anti-inflammatory properties, but dysregulated TGF-β signaling has been implicated in Alzheimer's disease, and may have different biological effects at different disease stages and in different tissue compartments.

4.3 Hormetic Dose-Response and Pharmacokinetic Constraints

One of the main findings was that it is a biphasic, non-linear -response curve. The effects of the biomarkers were greatest in the low group (1.2 ml/day) and were smaller in the higher group (1.8-3.0 ml/day). This effect can be accounted for as a result of two alternative mechanisms:

Hormesis. As described by Calabrese (2008), neurotrophic agents often exhibit a U-shaped response, in which submaximal volumes optimize adaptive signaling and synaptic plasticity, whereas excessive levels trigger receptor desensitization or negative feedback loops (Calabrese, 2008; Wan et al., 2024).

Anatomical and Volumetric Limitations. The nose cavity of humans has an absorptive capacity of limited extent. In most cases, more than 0.15-0.20 ml per nostril will lead to overflow and/or posterior runoff into nasopharyngeal area (Madden, Carrazana, & Rabinowicz, 2023; Vizoso et al., 2017). Thus, the higher of the 3.0 ml cohort may have contributed to lower bioavailability in the CNS because the increased size of the 3.0 ml cohort results in more rapid mucociliary clearance and swallowing (Per Gisle Djupesland, 2013; Wu, Li, Yuan, Zhao, & Li, 2025).

4.4 Comparison with Previous Studies

Our findings are parallel to recent clinical data, including a study conducted by Morita et al. (2024) in Japan which indicated that intranasal administration of MSC-secretome is effective in supporting cognitive functions and safe to use (Morita et al., 2024). In addition, the protective signaling we reported is consistent with preclinical studies reporting on a restoration of hippocampal function and decrease in amyloid pathology following intranasal EVs (extracellular vesicles) (Losurdo et al., 2020; Santamaria et al., 2021). The fact that we have a cell-free intervention addresses the most important safety concerns associated with stem cell therapy (tumorigenicity and embolic risk) and suggest that WJ-MSC-CM could be considered a viable disease-modifying therapy that needs to be continued further studied.

5. Strengths and Limitations

There are a number of interesting strengths to this study. To date, it is believed to be the first human trial of intranasal administration of WJ-MSC derived conditioned medium and used a prospective, Phase I, escalation study aimed chiefly at safety and feasibility. The study included multimodal outcome measures such as cognition testing, functional evaluation, neuroimaging and peripheral inflammatory and neurotrophic biomarkers as well as comprehensive clinical evaluations, which was an integrated assessment of the biological effects of the intervention.

There are also some caveats. First, that a modest-sized first-in-human Phase I study naturally was not powerful enough to draw clear-cut conclusions about efficacy. Second, performance and observer bias could have occurred for exploratory clinical outcomes in the open-label, non-randomized design. Third, the three-month follow-up might not have been long enough to assess the long-term treatment efficacy and disease modification benefits. In addition, biomarker analysis was limited to peripheral blood biomarker concentrations only, which may not accurately reflect CNS changes. Lastly, there was no direct evidence of central target engagement by non-imaging cerebrospinal fluid (CSF) biomarkers or PET imaging with amyloid or tau. They should be investigated in larger, longer follow-up, formal delivered-Volume uniformity testing in future Phase II safety studies, comprehensive device performance validation will be necessary in future studies with greater clinical development.

Findings should be interpreted with caution due to the long recruitment period, and small number of samples. This Phase 1 study was very much a study of safety aspects of this design and there was some stringent participant selection and monitoring. Broader eligibility criteria and potentially multicenter patient population recruitment strategies in future Phase II studies could allow for larger, more representative patient populations.

6. Clinical Inferences and Future Directions

The results of the current study indicate that clinical benefit of higher CM is not necessarily proportional. Future trials of a Phase II/III nature should:

1. Use randomized double-blinded and placebo-controlled designs.
2. Add to the above the mechanistic pathways to be understood using neuroimaging biomarkers (multimodal neuroimaging / MRS) and CSF proteomics.
3. Study the increase of the nasal retention time for mucoadhesive formulations/nanotechnology.
4. Identify the secretome profile with advanced OMICs based QC (Jeyaraman et al., 2023).

7. Conclusion

Overall, intranasal administration of the WJ-MSC conditioned medium was well-tolerated and feasible in this Phase I, small Volume-escalation first-in-human study. The results of these observations offer preliminary data about the safety profile of this cell-free experimental product and the nature of its effect, although due to the exploratory nature of the design and the small sample size, conclusions about safety or efficacy cannot be drawn. The results observed here regarding the biological markers (BDNF) and some inflammatory markers should be taken as hypothesis generating and further validated in larger controlled clinical studies.

Open Science Statement

Abbreviations:

Acknowledgements:

Funding

This study was supported by the Iran University of Medical Sciences (Project code: 24908). The funding organization had no role in the study design, data collection, analysis, interpretation, or manuscript preparation.

Declarations

Trial Registration, Ethics Approval, and consent to participate

This clinical trial was approved by the Ethics Committee of Iran University of Medical Sciences (Approval No. IR.IUMS.REC.1402.885), and the clinical trial was registered at the Iranian Registry of Clinical Trials (IRCT20240131060870N1; https://irct.behdasht.gov.ir/trial/75444). Before enrollment, patients were fully informed of the experimental nature of the study intervention, all potential outcomes, and possible complications or intervention-related AEs. Written informed consent, including for all study procedures, was obtained from all patients. This study was conducted in accordance with the principles of the Declaration of Helsinki and the ICH-GCP guidelines.

Consent for publication

Written informed consent for the publication of individual data was obtained from all patients.

Competing interests

The authors declare no competing interests.

CRediT authors'contribution statement

S.A: Investigation, Data Curation & Acquisition, Formal Analysis, Writing-Original Draft; M. A.-D: Clinical Diagnosis, Patient Recruitment, Validation; AR. A: Statistical Analysis, Methodology, Data Interpretation, Writing-Review & Editing; MT. J: Conceptualization, Supervision; AR. Z: Supervision; S. O-Y: Supervision; S. M: Supervision, Writing- Review & Editing, Project Administration; N.A: Supervision, Funding Acquisition, Conceptualization, Writing- Review & Editing.

All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Informed consent

Before performing any study-related procedures, including the intervention, specimen analysis, and written informed consent was obtained from all participants or their legally authorized representatives before enrollment in the study. They received a detailed explanation of the intervention, its experimental nature, potential adverse events, and consented to the publication of all the data and images in a scientific article.

Trial Registration, Ethical Approval, and consent to participate

This clinical trial was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1402.885) and registered in the Iranian Registry of Clinical Trials (IRCT20240131060870N1; https://irct.behdasht.gov.ir/user/trial/75444/view) before participant enrollment. The study followed the principles of the Declaration of Helsinki and ICH-GCP guidelines.

Data Availability Statement and Materials

The datasets generated and analyzed during the current study are not publicly available due to ethical and patient privacy considerations, but are available from the corresponding author upon reasonable request.

Trial registration

This clinical trial was registered in the Iranian Registry of Clinical Trials (IRCT) under the identifier IRCT20240131060870N1, available at https://irct.behdasht.gov.ir/user/trial/75444/view. The trial was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1402.885) and registered on 1402.10.16 before participant enrolment. No trial results have been publicly posted or preprinted before this publication.

Protocol and statistical analysis plan

The datasets analyzed during this study are available from the corresponding author upon reasonable request.

A summary of the trial protocol is accessible through the Iranian Registry of Clinical Trials (IRCT ID: IRCT20240131060870N1) and includes detailed descriptions of the eligibility criteria, intervention procedures, and safety monitoring framework.

Data sharing

De-identified individual participant data (including a data dictionary), ELISA raw data, and statistical analysis code (SPSS) are available from the corresponding author upon reasonable request.

Data sharing will be granted for academic and non-commercial purposes after the approval of a data access agreement. No publicly available repository is currently used because of the early phase nature of the study.

Conflict of interest

The authors declare that they have no financial or non-financial conflicts of interest related to this study. All authors had full access to the study data and took responsibility for the integrity and accuracy of the analysis.

Research Involving Animals

This article does not contain any studies involving animals performed by any of the authors.

Declaration of generative AI and AI-assisted technologies in the writing process

No AI-generated text was used in the preparation of this manuscript, nor was any AI credited as an author. However, large language models have occasionally been employed for AI-assisted copy-editing.

References:

Abdi, S., Javanmehr, N., Ghasemi-Kasman, M., Bali, H. Y., & Pirzadeh, M. (2022). Stem cell-based therapeutic and diagnostic approaches in Alzheimer's disease. *Current Neuropharmacology, 20*(6), 1093-1115.

Aguilera, Y., Mellado-Damas, N., Olmedo-Moreno, L., López, V., Panadero-Morón, C., Benito, M., . . . Capilla-González, V. (2021). Preclinical safety evaluation of intranasally delivered human mesenchymal stem cells in juvenile mice. *Cancers, 13*(5), 1169.

Alidoust, L., Akhoondian, M., homayoun Atefi, A., Keivanlou, M.-H., Ch, M. H., & Jafari, A. (2023). Stem cell-conditioned medium is a promising treatment for Alzheimer's disease. *Behavioural Brain Research, 452*, 114543.

Barton, A. (2007). Handbook for good clinical research practice (GCP): guidance for implementation: BMJ Publishing Group Ltd.

Calabrese, E. J. (2008). Hormesis: why it is important to toxicology and toxicologists. *Environmental Toxicology and Chemistry, 27*(7), 1451-1474.

Center, U. M., & Organization, W. H. (2018). The use of the WHO-UMC system for standardised case causality assessment. *Accessed August, 30*, 2021.

Creavin, S. T., Wisniewski, S., Noel-Storr, A. H., Trevelyan, C. M., Hampton, T., Rayment, D., . . . Milligan, R. (2016). Mini-Mental State Examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations. *Cochrane Database of systematic reviews*(1).

Cummings, J., Lee, G., Zhong, K., Fonseca, J., & Taghva, K. (2021). Alzheimer's disease drug development pipeline: 2021. *Alzheimer's & Dementia: Translational Research & Clinical Interventions, 7*(1), e12179.

da Silva, A. V., Serrenho, I., Araújo, B., Carvalho, A. M., & Baltazar, G. (2023). Secretome as a tool to treat neurological conditions: are we ready? *International journal of molecular sciences, 24*(22), 16544.

Djupesland, P. G. (2013). Nasal drug delivery devices: characteristics and performance in a clinical perspective—a review. *Drug delivery and translational research, 3*(1), 42-62.

Djupesland, P. G., Messina, J. C., & Mahmoud, R. A. (2014). The nasal approach to delivering treatment for brain diseases: an anatomic, physiologic, and delivery technology overview. *Therapeutic delivery, 5*(6), 709-733.

Ellison, T. S., Cappa, S. F., Garrett, D., Georges, J., Iwatsubo, T., Kramer, J. H., . . . Merrilees, J. (2023). Outcome measures for Alzheimer's disease: a global inter-societal Delphi consensus. *Alzheimer's & Dementia, 19*(6), 2707-2729.

Ermann, N., Trojanowski, J. Q., Karaca, I., Wagner, H., Oeckl, P., van Doorn, L. v. W., . . . Farotti, L. Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry.

Galipeau, J., & Sensébé, L. (2018). Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell stem cell, 22*(6), 824-833.

Ghasemi, M., Roshandel, E., Mohammadian, M., Farhadihosseinabadi, B., Akbarzadehlaleh, P., & Shamsasenjan, K. (2023). Mesenchymal stromal cell-derived secretome-based therapy for neurodegenerative diseases: overview of clinical trials. *Stem cell research & therapy, 14*(1), 122.

Govind, N. (2020). Donepezil for dementia due to Alzheimer's disease. *British journal of community nursing, 25*(3).

Hambarsari, Y., Suroto, S., Mirawati, D. K., Soetrisno, S., Wasita, B., Widyaningsih, V., . . . Hamidi, B. L. (2024). A Systematic Review of Secretome-Based Therapies for Alzheimer's Disease: Bridging the Preclinical and Clinical Gap.

Hansen, A. R., Graham, D. M., Pond, G. R., & Siu, L. L. (2014). Phase 1 trial design: is 3+ 3 the best? *Cancer control, 21*(3), 200-208.

Heneka, M. T., Golenbock, D. T., & Latz, E. (2015). Innate immunity in Alzheimer's disease. *Nature immunology, 16*(3), 229-236.

Heppner, F. L., Ransohoff, R. M., & Becher, B. (2015). Immune attack: the role of inflammation in Alzheimer disease. *Nature Reviews Neuroscience, 16*(6), 358-372.

Hopewell, S., Chan, A.-W., Collins, G. S., Hróbjartsson, A., Moher, D., Schulz, K. F., . . . Berlin, J. A. (2025a). CONSORT 2025 explanation and elaboration: updated guideline for reporting randomised trials. *bmj, 389*.

Hopewell, S., Chan, A.-W., Collins, G. S., Hróbjartsson, A., Moher, D., Schulz, K. F., . . . Berlin, J. A. (2025b). CONSORT 2025 statement: updated guideline for reporting randomised trials. *The Lancet, 405*(10489), 1633-1640.

Iasonos, A., & O'Quigley, J. (2014). Adaptive dose-finding studies: a review of model-guided phase I clinical trials. *Journal of Clinical Oncology, 32*(23), 2505-2511.

Jaberi, S., & Fahnestock, M. (2023). Mechanisms of the beneficial effects of exercise on brain-derived neurotrophic factor expression in Alzheimer's disease. *Biomolecules, 13*(11), 1577.

Jeyaraman, M., Rajendran, R. L., Muthu, S., Jeyaraman, N., Sharma, S., Jha, S. K., . . . Lana, J. F. S. D. (2023). An update on stem cell and stem cell-derived extracellular vesicle-based therapy in the management of Alzheimer's disease. *Heliyon, 9*(7).

Julious, S. A. (2005). Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics: The Journal of Applied Statistics in the Pharmaceutical Industry, 4*(4), 287-291.

Katz, S., Ford, A. B., Moskowitz, R. W., Jackson, B. A., & Jaffe, M. W. (1963). Studies of illness in the aged: the index of ADL: a standardized measure of biological and psychosocial function. *jama, 185*(12), 914-919.

Kishi, T., Matsunaga, S., Oya, K., Nomura, I., Ikuta, T., & Iwata, N. (2017). Memantine for Alzheimer's disease: an updated systematic review and meta-analysis. *Journal of Alzheimer's Disease, 60*(2), 401-425.

Lawton, M., & Brody, E. M. (1970). Assessment of older people: self-maintaining and instrumental activities of daily living. *Nursing research, 19*(3), 278.

Le Tourneau, C., Lee, J. J., & Siu, L. L. (2009). Dose escalation methods in phase I cancer clinical trials. *JNCI: Journal of the National Cancer Institute, 101*(10), 708-720.

Liu, J., Cheng, L., Ma, C., Wang, X., Ji, X., Li, Y., & Liu, J. (2025). Clinical trials and advanced MRI techniques with stem cell therapy for ischemic stroke: present and future perspectives. *Journal of Translational Medicine, 23*(1), 1-28.

Lochhead, J. J., & Thorne, R. G. (2012). Intranasal delivery of biologics to the central nervous system. *Advanced drug delivery reviews, 64*(7), 614-628.

Lochhead, J. J., & Thorne, R. G. (2013). Intranasal drug delivery to the brain *Drug delivery to the brain: physiological concepts, methodologies and approaches* (pp. 401-431): Springer.

Losurdo, M., Pedrazzoli, M., D'Agostino, C., Elia, C. A., Massenzio, F., Lonati, E., . . . Bresciani, E. (2020). Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease. *Stem cells translational medicine, 9*(9), 1068-1084.

Madden, S., Carrazana, E., & Rabinowicz, A. L. (2023). Optimizing absorption for intranasal delivery of drugs targeting the central nervous system using alkylsaccharide permeation enhancers. *Pharmaceutics, 15*(8), 2119.

Mehrabadi, S., Sadr, S. S., & Hoseini, M. (2019). Stem cell conditioned medium as a novel treatment for neuroinflamation diseases. *International journal of medical investigation, 8*(3), 1-12.

Mendes-Pinheiro, B., Teixeira, F. G., Anjo, S. I., Manadas, B., Behie, L. A., & Salgado, A. J. (2018). Secretome of undifferentiated neural progenitor cells induces histological and motor improvements in a rat model of Parkinson's disease. *Stem cells translational medicine, 7*(11), 829-838.

Mita, T., Furukawa-Hibi, Y., Takeuchi, H., Hattori, H., Yamada, K., Hibi, H., . . . Yamamoto, A. (2015). Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer's disease. *Behavioural Brain Research, 293*, 189-197.

Morita, Y., Izawa, H., Ohga, H., Sugie, H., Yamamoto, T., Hirano, A., . . . Yonei, Y. (2024). Safety and clinical efficacy on intranasal administration of mesenchymal stem cell-derived secretome in patients with Alzheimer's disease and its future prospect. *Glycative Stress Research, 11*(3), 103-110.

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., . . . Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society, 53*(4), 695-699.

Ng, A., Tam, W. W., Zhang, M. W., Ho, C. S., Husain, S. F., McIntyre, R. S., & Ho, R. C. (2018). IL-1β, IL-6, TNF-α and CRP in elderly patients with depression or Alzheimer's disease: systematic review and meta-analysis. *Scientific reports, 8*(1), 12050.

Ng, T. K. S., Ho, C. S. H., Tam, W. W. S., Kua, E. H., & Ho, R. C.-M. (2019). Decreased serum brain-derived neurotrophic factor (BDNF) levels in patients with Alzheimer's disease (AD): a systematic review and meta-analysis. *International journal of molecular sciences, 20*(2), 257.

Numakawa, T., & Kajihara, R. (2023). Neurotrophins and other growth factors in the pathogenesis of Alzheimer's disease. *Life, 13*(3), 647.

Organization, W. H. (2019). World Health Organization dementia factsheet.

Pardridge, W. M. (2005). The blood-brain barrier: bottleneck in brain drug development. *NeuroRx, 2*(1), 3-14.

Park, H., & Poo, M.-m. (2013). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience, 14*(1), 7-23.

Rahma, O. E., Gammoh, E., Simon, R. M., & Khleif, S. N. (2014). Is the "3+ 3" dose-escalation phase I clinical trial design suitable for therapeutic cancer vaccine development? A recommendation for alternative design. *Clinical Cancer Research, 20*(18), 4758-4767.

Reger, M., Watson, G., Green, P., Wilkinson, C., Baker, L., Cholerton, B., . . . DeGroodt, W. (2008). Intranasal insulin improves cognition and modulates ß-amyloid in early AD. *Neurology, 70*, 1-9.

Santamaria, G., Brandi, E., Vitola, P. L., Grandi, F., Ferrara, G., Pischiutta, F., . . . Uccelli, A. (2021). Intranasal delivery of mesenchymal stem cell secretome repairs the brain of Alzheimer's mice. *Cell Death & Differentiation, 28*(1), 203-218.

Shah, S. (2022). Common terminology criteria for adverse events. *National Cancer Institute: USA, 784*, 785.

Storer, B. E. (1989). Design and analysis of phase I clinical trials. *Biometrics*, 925-937.

Toh, W. S., Lai, R. C., Zhang, B., & Lim, S. K. (2018). MSC exosome works through a protein-based mechanism of action. *Biochemical Society Transactions, 46*(4), 843-853.

Tolstova, T., Dotsenko, E., Luzgina, N., & Rusanov, A. (2024). Preconditioning of Mesenchymal Stem Cells Enhances the Neuroprotective Effects of Their Conditioned Medium in an Alzheimer's Disease In Vitro Model. *Biomedicines, 12*(10), 2243.

Vizoso, F. J., Eiro, N., Cid, S., Schneider, J., & Perez-Fernandez, R. (2017). Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *International journal of molecular sciences, 18*(9), 1852.

Wan, Y., Liu, J., Mai, Y., Hong, Y., Jia, Z., Tian, G., . . . Liu, J. (2024). Current advances and future trends of hormesis in disease. *npj Aging, 10*(1), 26.

Wilson, H., de Natale, E. R., & Politis, M. (2022). Recent Advances in Neuroimaging Techniques to Assist Clinical Trials on Cell-Based Therapies in Neurodegenerative Diseases. *Stem Cells, 40*(8), 724-735.

Wu, H., Li, C., Yuan, H., Zhao, J., & Li, S. (2025). Brain Delivery Strategies for Biomacromolecular Drugs: Intranasal Administration. *International Journal of Nanomedicine*, 6463-6487.

Xie, Z.-H., Liu, Z., Zhang, X.-R., Yang, H., Wei, L.-F., Wang, Y., . . . Bi, J.-Z. (2016). Wharton's Jelly-derived mesenchymal stem cells alleviate memory deficits and reduce amyloid-β deposition in an APP/PS1 transgenic mouse model. *Clinical and experimental medicine, 16*(1), 89-98.

Xu, Z., Nan, W., Zhang, X., Sun, Y., Yang, J., Lu, K., . . . Mao, W. (2018). Umbilical cord mesenchymal stem cells conditioned medium promotes Aβ25-35 phagocytosis by modulating autophagy and aβ-degrading enzymes in BV2 cells. *Journal of Molecular Neuroscience, 65*(2), 222-233.

Yiannopoulou, K. G., & Papageorgiou, S. G. (2013). Current and future treatments for Alzheimer's disease. *Therapeutic advances in neurological disorders, 6*(1), 19-33.

Yun, H., Kim, H., Park, K., Shin, J., Kang, A., Song, S., . . . Hong, J. (2013). Placenta-derived mesenchymal stem cells improve memory dysfunction in an Aβ1–42-infused mouse model of Alzheimer's disease. *Cell death & disease, 4*(12), e958-e958.

Figure Legends

Figure 1.

The CONSORT flow diagram illustrates the screening, enrollment, allocation, follow-up, and analysis of participants across the three Volume groups in the phase 1 intranasal WJ-MSC-CM trial.

Figure 2.

Standardized cognitive scores (mean ± SD) pre and post intranasal WJ-MSC-CM administration for the 3 -escalation cohorts. CM regimens were low, medium or high and each regimen had three participants. Values are exploratory within-participant change from Baseline to the Post intervention assessment. Standard deviations (SD) are shown as error bars. These results should be considered descriptive and hypothesis-generating, in view of the limited sample size and Phase I design.

Figure 3.

Changes in IADL scores before and after administration of intranasal WJ-MSC-CM within every WJ-MSC-CM group. Data is shown as mean ± SD. There were three individuals in each cohort. Standard deviations (SD) are represented as error bars. These observed changes are exploratory functional outcomes of this Phase I safety and feasibility study.

Figure 4.

Changes in ADL scores pre-to-post intranasal administered of each of the three groups (low, medium and high) of WJ-MSC-CM. Data are given as mean ± SD. Standard deviations (SD) are shown as error bars. No significant ($p < 0.05$) within-participant effects were observed. Results must be interpreted due to the exploratory design and the small sample size.

Figure 5.

Changes in serum levels of IL-6 (pg/mL) pre and post intranasal treatment with conditioned medium from WJ-MSCs (CMs) in each of the three escalation groups (n=3/ escalation cohort, n=9/total). The data are expressed as mean ± SD. Standard deviations (SD) shown for error bars. The within-participant difference was not statistically significant.

Figure 6.

Changes in serum TNF-α concentrations (pg/mL) before and after intranasal administration of WJ-MSC-derived conditioned medium across the three -escalation cohorts (n=3 per group). The data is presented as Mean ± SD. Standard deviations (SD) are shown as error bars. There was no statistical difference between the pre- and post-intervention results within the same participant.

Figure 7.

Serum BDNF changes (ng/mL) with intranasal administration of WJ-MSC-CM in the three -escalation groups (n=3, each; n=9, total). All values are given as mean ± SD. The standard deviations (SD) are represented using error bars. The increase in the overall BDNF levels was statistically significant (p=0.01) in within-participant analysis following intervention. Results must be interpreted due to the exploratory design and the small sample size.

Figure 8.

Changes in serum TGF-β level (ng/mL) before and after intranasal administration of conditioned medium from WJ-MSC in the three escalation groups (n=3 for each group; overall n=9). All data are mean ± SD. Standard deviations (SD) are represented by error bars. There were no statistically significant within-participant differences noted.

Figure 9.

Changes in serum levels of IL-1β (pg/mL) in the three escalation groups (n=3 per group, total = 9) before and after IN administration of the conditioned medium prepared from WJ-MSC cells. The data are expressed as mean ± SD. Standard deviation (SD) are represented by error bars. Within-participant changes were not statistically significant.

Figure 10.

Changes in serum concentrations of CRP (mg/L) were measured before and after intranasal administration of WJ-MSC derived conditioned medium in the three cohorts examined (n=3 per group, total n=9). Data shown as mean ± SD. Data shown as error bar are standard deviations (SD). There was no evidence that there were statistically significant within-participant differences.

Table 1. -escalation protocol and intranasal administration parameters of WJ-MSC[1]-derived conditioned medium.

cohort	Puffs	Frequency	Duration	Calculation	Delivered / actuation	Actual daily CM
Low	2 puffs in each nostril, session	Every 8 h	3 days	2 × 0.1 × 2 × 3	0.1 mL	1.2 mL/day
Medium	3 puffs in each nostril, session	Every 8 h	3 days	3 × 0.1 × 2 × 3	0.1 mL	1.8 mL/day
High	5 puffs in each nostril, session	Every 8 h	3 days	5 × 0.1 × 2 × 3	0.1 mL	3.0 mL/day

[1] WJ-MSC: Wharton's Jelly-Derived Mesenchymal Stem Cells

Supplementary Table 1. Manufacturing environment, quality assurance procedures, WJ-MSC characterization, and conditioned medium production parameters.

Parameter	Description
A. Manufacturing Environment and Quality Assurance	
Manufacturing facility	Comprehensive Stem Cell and Regenerative Medicine Center, Shahid Motahari Burn Hospital, Iran University of Medical Sciences
Facility status	Validated cleanroom facility (not GMP-certified)
Cleanroom classification	ISO Class 5 cleanroom conditions
Aseptic manipulation equipment (processing)	Class II Type A2 Biological Safety Cabinet located within the cleanroom
Environmental monitoring	Routine particle-count monitoring and microbiological surveillance according to institutional quality-control procedures
Standard operating procedures	Manufacturing performed according to validated institutional SOPs for cell-derived biological products
Quality assurance	Standard contamination- prevention measures throughout cell culture, CM collection, filtration, storage, and transportation
B. WJ-MSC Source and Characterization	
Donor characteristics	Maternal age: 30-35 years; mode of delivery: caesarean section
Donor screening	Serological screening for HIV, HBV, HCV, syphilis, and HTLV-I/II
Tissue source and Cell type	Human Wharton's Jelly- derived mesenchymal stromal cells
Tissue procurement	Umbilical cords obtained following written informed consent under institutional ethical approval
Product type	Wharton’s Jelly MSC-derived conditioned medium (cell-free product)
MSC identity	Confirmed according to International Society for Cell & Gene Therapy (ISCT) minimal criteria
Positive surface markers	CD73, CD90, CD105, CD44 (97-99%)
Negative surface markers	CD34, CD45 (<3%)
Flow cytometry	Multiparametric immunophenotypic characterization performed before CM production
Cell morphology	Typical spindle-shaped fibroblast-like morphology

Cell viability	>92% before conditioning
Karyotype analysis	Normal chromosomal profile; chromosomal abnormalities <10%
Passage used for CM production	Passage 4
C. Conditioned Medium Production and Processing	
Cell culture medium	DMEM supplemented with 10% Human serum albumin (HSA) during expansion
Cell culture conditions	37°C, 5% CO_2, humidified incubator
Cell confluence before conditioning	Approximately 80% before serum deprivation
Washing before conditioning	Three PBS washes before serum-free conditioning
Conditioning medium	Serum-free DMEM
Serum-free conditioning period	Serum-free medium exposure for 48 hours
CM collection	Supernatant harvested under aseptic conditions
Processing (Centrifugation & Sterile filtration)	Centrifugation at 4000 × g for 10 min followed by 0.22 µm membrane filtration
Sterility testing	Culture-based sterility testing according to USP <71>; no bacterial or fungal growth detected after 14 days of incubation
Endotoxin testing	LAL assay; acceptance limit <0.2 EU/mL
Mycoplasma testing	PCR-based detection; analytical sensitivity <0.06%
Release quality-control testing	Sterility, mycoplasma, and endotoxin testing performed before clinical administration
Batch information	Single manufacturing batch used for all enrolled participants
Batch strategy	Single-batch production approach to minimize inter-batch variability during Phase I evaluation
Aliquoting procedure	Product aliquoted after quality-control release testing
Storage condition	-80°C until clinical administration
Release quality-control testing	Sterility, mycoplasma and endotoxin testing performed before clinical administration
Aliquoting	Sterile single-use aliquots
Storage condition	Aliquoted and stored at -80°C until clinical administration
CM characterization	Total protein quantified by Bradford assay; selected bioactive factors measured using ELISA (see Table 2)

Supplementary Table 2. Flow cytometric characterization of passage 4 WJ-MSCs used for conditioned medium production according to ISCT minimal criteria.

Marker	**Cell surface category**	**Expected ISCT profile**	**Expression (%)**
CD73	MSC positive marker	Positive	98/72
CD90	MSC positive marker	Positive	99/65
CD105	MSC positive marker	Positive	97/67
CD44	MSC positive marker	Positive	98/44
CD34	Hematopoietic marker	Negative	2/3
CD45	Hematopoietic marker	Negative	3/12

Table 2. Protein content and cytokine profile of Wharton's Jelly MSC-derived conditioned medium (CM) quantified by Bradford assay and ELISA.

IL-6 (pg/mL)	IL-1β (pg/mL)	TNF-α (pg/mL)	TGF-β (pg/mL)	BDNF (pg/mL)	Total Protein (µg/mL)	Sample
241.8	4.32	9.38	860.5	163.73	112	CM[2]
–	–	–	–	–	0.00	Primary (Culture) Medium

[2] Values represent concentrations per 1 mL of conditioned medium.

Table 3. Safety profile and adverse events (AEs) in participants receiving intranasal CM across groups.

After (%)[3]	Before (%)	Outcome	CTCAE Grade[4]	Reported AE[5]	SAE	Group
0	0	–	–	None	No	Low (1.2 mL)
2 (67%)	0	Self-limited	Grade 1 (Mild)	Transient cough (male), lip tingling (female)	No	Medium (1.8 mL)
0	0	–	–	None	No	High (3.0 mL)

[3] Data are presented as n (%) and based on the number of participants per cohort (n = 3).
[4] Graded according to CTCAE v5.0.
[5] Adverse events were coded using MedDRA v26.

Table 4. Baseline demographic characteristics of the study participants (n = 9).

Variable[6]	Mean ± Standard Deviation (SD)[7]			Total (n = 9)
	Low (n = 3)	Medium (n = 3)	High (n = 3)	
Age (years)	70.67±2.08	69.33±3.05	71±7.93	70.33±4.44
Sex				
Male	(٢)١	(٢)٢	(١)١	(4)1
Female	(١)٢		(٢)٢	(٥)٢
Education level:				
Illiterate 0			(١)٠	(١)٠
Primary 1	(١)١	(٢)١	(١)١	(٤)١
Secondary 2	2(2)			2(2)
Diploma 3				
University 4	(١)٤	(١)٤	(٢)٤	(١)٤

[6] One-way analysis of variance (ANOVA) was applied for comparisons of continuous variables, and the Chi-square test was used for categorical variables. Results that were not significant are indicated by the abbreviation ns ($p>0.05$).

[7] Descriptive statistics of the continuous variables are reported as mean ± standard deviation (SD), frequencies, and percentages of reported data.

Table 5. Changes in cognitive and functional outcomes before and after intranasal administration of conditioned medium across groups.

Mean ± Standard Deviation (SD)						
p-value[10]	**Mean Change[9]**	**After**	**Before**	**n**	**Group**	**Variable[8]**
	11.11±3.85	53.33±8.81	42.22±6.94	3	Low	Cognitive Tests
۰/۳۲	3.33±11.54	65.55±3.85	62.22±10.18	3	Medium	
	17.28±13.26	63.91±12.69	46.63±3.27	3	High	
	10.57±10.84	60.93±9.82	50.35±11.11	9	**Total**	
	18.05±6.36	80.09±18.85	62.03±12.52	3	Low	IADL
۰/۷۴	18.75±6.25	68.75±25	50±28.64	3	Medium	
	13.65±11.95	74.76±16.93	61.11±27.11	3	High	
	16.82±7.83	74.53±18.46	57.71±21.48	9	**Total**	
1.00	0.00	14.00±0.00	14.00±0.00	9	**All groups**	ADL

Table 6. Changes in serum biomarkers (BDNF, CRP, TGF-β, IL-1β, IL-6, TNF-α) in all three Volume groups (n=9) before and after intranasal administration of WJ-MSC-derived conditioned medium.

[8] This phase 1 study was not powered to detect efficacy differences.

[9] Mean change = After – Before.

[10] P-values are exploratory and were calculated using non-parametric paired tests due to the small sample size.

Mean ± Standard Deviation (SD)[12]					Variable[11]
p-value[14]	**Mean Change**[13]	**After**	**Before**	**Group**	**Biomarkers**
0.30	-5.16±6.01	4.16± 0.47	9.33 ± 5.90	Low	**IL-6** (Pg/ml)
	-1.46±1.61	2.33 ± 0.57	3.79 ± 2.16	Medium	
	-0.33±0.50	3.29 ± 1.25	3.63 ± 0.76	High	
	-2.32±3.81	**Total**			
0.57	-1.80±2.25	4.50 ± 0.60	6.30 ± 2.04	Low	**TNF-α** (Pg/ml)
	-0.60±0.87	5.06± 0.23	5.66 ± 1.02	Medium	
	-0.66±0.89	4.40 ± 0.69	5.06 ± 1.58	High	
	-1.02±1.41	**Total**			
0.01	8.83±0.18	24.09 ± 3.27	15.26 ± 3.10	Low	**BDNF** (ng/ml)
	4.77±1.95	16.31 ± 3.06	11.54 ± 4.40	Medium	
	1.50±2.77	17.49 ± 1.99	15.98 ± 1.08	High	
	5.03±3.60	**Total**			
0.40	-1.61±1.07	3.64 ± 2.75	5.26 ± 2.47	Low	**TGF-β** (ng/ml)
	-1.30±0.19	5.58 ± 1.48	6.89 ± 1.45	Medium	
	-7.68±10.51	4.09 ± 3.53	11.77± 8.69	High	
	-3.53±6.13	**Total**			
0.64	-5.96±5.23	14.22 ± 6.37	20.18 ± 4.05	Low	**IL-1β** (ng/ml)
	-9.68±7.36	10.28 ± 1.24	19.96 ± 8.10	Medium	
	-3.38±10.61	5.12 ± 3.78	8.50 ± 6.85	High	
	-6.34±7.48	**Total**			

[11] Statistical analyses were performed using paired t-tests or Wilcoxon signed-rank tests as appropriate.
[12] Values are presented as mean ± SD.
[13] Indicates the change between post- and pre-treatment values (post-pre).
[14] Two-tailed p-values <0.05 were considered statistically significant.

	-1.63±1.53	1.43 ± 0.86	3.06 ± 1.85	Low	
0.46	-0.23±0.11	0.5 ± 0.4	0.76 ± 0.53	Medium	**CRP** (mg/L)
	-1.06±1.67	0.80 ± 0.43	1.86 ± 1.53	High	
	-0.97±1.29	**Total**			