Efficacy of Placental-Derived Mesenchymal Stem Cell Exosome Therapy in Treating Androgenetic Alopecia: A Clinical Trial Study

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Abstract

Background: Androgenetic alopecia (AGA) is characterized by the miniaturization of hair follicles, leading to hair thinning and loss. Existing treatments are limited, and stem cell-derived exosome therapy has emerged as a potential alternative. This study aimed to evaluate the efficacy of placenta-derived mesenchymal stem cell (P-MSC) exosomes for treating AGA.

Methods: This phase I/II clinical trial included 12 alopecia patients aged 18-60. Exosomes were isolated from mesenchymal stem cells harvested from placentas of consenting donors and injected into the scalp every 14 days for 2 months. Outcome measures included hair density, hair diameter, and hair loss count, assessed at baseline, 3 weeks, and 6 weeks post-intervention. Data were analyzed using the repeated measure ANOVA with STATA version 14.2.

Results: At baseline, the mean hair density was 96.5 hairs/cm², hair diameter was 0.049 mm, and hair loss count was 200 hairs. Post-intervention, hair density significantly increased to 111.7 hairs/cm² at 3 weeks and 163.5 hairs/cm² at 6 weeks. Hair diameter also increased significantly to 0.058 mm at 3 weeks and 0.059 mm at 6 weeks. Mean hair loss count decreased significantly to 80 hairs at 6 weeks. No significant adverse effects or changes in clinical laboratory findings were observed.

Conclusions: Placenta-derived mesenchymal stem cell exosome therapy demonstrated significant improvements in hair density, diameter, and reduced hair loss in patients with androgenetic alopecia. Further controlled trials with larger sample sizes and longer follow-up periods are warranted to validate these findings and explore the molecular mechanisms involved.

Highlights

Exosome therapy shows potential as an effective treatment for AGA.

Hairloss was decreased by Exosome treatment.

Exosome has beneficial effects on density, regrowth and thickness of hair.

Introduction

Androgenetic alopecia (AGA) is a hair condition characterized by the miniaturization of hair follicles, converting terminal hair to vellus hair and reducing follicle count. The shortened anagen phase in the hair growth cycle contributes to hair thinning. AGA's pathogenesis is largely affected by genetics, androgen hormones like dihydrotestosterone (DHT), and various environmental factors.(1, 2). AGA can significantly affect individuals' psycho-emotional well-being and quality of life, particularly when it occurs early.(3). The main aim of treatment is to restore thinning areas and prevent progression. Currently, only topical minoxidil and oral finasteride are FDA-approved for this condition.(4).

Stem cell therapies for hair follicle regeneration use cells from adipose tissue, hair follicles, blood, bone marrow, and Wharton's jelly. Transplants can be autologous or allogeneic. Anudeep et al. classified stem cells into adult (like ADSCs, HFSCs, BMSCs) and perinatal types (from umbilical cord and placenta). A combination of skin epithelial and mesenchymal stem cells can promote hair growth. HFSCs shift between active and inactive states,

which is essential for follicle stability but decreases with age.(5–7). Stem cell therapy has limitations, including tumor risk, immune rejection, short shelf-life, and costly, complex administration.(8, 9). Stem cells, like all cells, release exosomes for intercellular communication. These vesicles, ranging from 40 to 160 nm, are secreted through the endosomal pathway. Their diverse contents—nucleic acids, proteins, lipids, and metabolites—reflect their cellular origins and present potential therapeutic benefits, paving the way for cell-free therapies.(10). So, exosome therapy has been proposed as alternative method in terms of alopecia management. This study aimed to examine the efficacy of placental derived mesenchymal stem cells exosome for treatment of androgenetic alopecia.

Method

Protocol

This phase I/II clinical trial aimed to investigate the efficacy of stem cell therapy for the treatment of alopecia. The trial was registered at clinicaltrial.gov under the code NCT05658094. Ethical approval was obtained from the Azad Najafabad University Research Ethics Committee, with the approval code IR.IAU.NAJAFABAD.REC.1401.029.

Patients

The study population consisted of 12 randomly selected alopecia patients who met the following criteria. Inclusion Criteria included people with Chestnut brown, dark, or black hair color, age 18-60-years-old, AGA for female Ludwig grade I to II and for men Norwood-Hamilton grade III to IV, Hair density 60 to 190 hairs/cm², Vertical region hair length more than 3 cm at the start and during the follow-up period. Exclusion Criteria was Pregnant or females without effective contraception, Use of any topical or systemic medications within 3 months before study and Systemic or dermatological scalp diseases. Patients also were excluded if they experienced adverse effects such as fever or gastrointestinal issues (e.g., stomachache, nausea, vomiting, GI bleeding) or any adverse effects not present before treatment.

Data Collection

Demographic features were collected with a standardized questionnaire at the study's start. Laboratory tests including LDH, ALT, AST and HS-CRP were measured.

Laboratory Procedures

Mesenchymal stem cells were harvested from the placentas of consenting pregnant mothers and processed at Gandhi Hospital following GMP guidelines. After achieving 70% homogeneity in the third passage, the cells were cultured in serum-free medium (CTS Knockout Sigma-12861-01) for 72 hours. Exosomes were isolated based on Thery(11) et al. protocol and underwent identification tests. Stem cells were sent for patient treatment after identity confirmation and quality control.

Donors were screened for HIV, HBV, HCV, and COVID-19. Bacterial and fungal infections were tested using capillary electrophoresis. Quality control tests for stem cells included:

- Exosome concentration and size (electron microscopy, DLS, TNA)
- Exosome surface factors (flow cytometry)
- Infection screening (culture and direct smear for bacteria and fungi, PCR for mycoplasma, viral analysis of placental source)
- Toxin detection (MTT test)

Phenotype analysis of cells was conducted at the third passage. Surface antigens were analyzed by flow cytometry for positive CD-29, CD-44, and CD-105 markers and negative for CD-45 and HLA-DR. Centrifugation steps were performed to remove cells, dead cells, and debris, followed by exosome concentration and identification through TEM/SEM, flow cytometry for CD markers, and size measurement by dynamic light scattering.

Clinical Procedure

Exosomes were injected by an experienced dermatologist under local anesthesia and sterile condition using an insulin syringe and nappage technique. The injection was done intradermally with a 24-gauge insulin syringe. Injections were focused on the frontal, parietal and temporal regions. The distance of each point of injection was 1 cm and the amount of injection was 0.1 ml. Treatment sessions were performed every 14 days up to a maximum of 4 sessions, with patients receiving 100×10^9 exosomes per session.

Outcome Assessment

Outcomes were measured through hair density, hair diameter, and hair loss at baseline, 3 weeks, and 6 weeks after the first injection. Hair density was assessed by phototrichogram and reported in hairs per cm^2. Hair diameter was evaluated using surface electron microscopy. Hair loss was counted by patients who were instructed not to wash or brush their hair the day before the test, collecting and counting every fallen hair.

Statistical Analysis

Data analysis was performed using STATA version 14.2. The aim of this study was the impact of the MSC exosome intervention on hair density, hair diameter, and hair loss at three different time points (baseline, three weeks, and six weeks after the first injection). To identify any differences in these outcomes over time, we conducted a repeated measures ANOVA. Mean differences (MD) and 95% confidence interval (95% CI) were reported for each comparison. Graphical representations of the data were created using Stata to provide better visualization of the intervention effects over time. Statistical significance was set at p less than 0.05.

Results

Seventy-five patients were assessed for eligibility, with 40 excluded based on inclusion and exclusion criteria. Twelve patients were enrolled (Fig. 1), predominantly male (n = 7 (58.3%)) with a median age of 37.5 years (Q1-Q3: 30–39).

All Laboratory tests including LDH, ALT, and AST were in normal range. Serum HS-CRP level also was negative in all patients.

Mean differences between 3-week and baseline, 6-week and baseline, and 6-week and 3-week and their 95%CIs in hair density were 15.2 (6.7 to 23.6), 67.0 (58.8 to 75.5), and 51.8 (43.3 to 60.3) hairs/cm², respectively. Hair diameter also increased to 0.058 mm and 0.059 mm at 3- and 6-weeks post-intervention, respectively, with significant increases in values. Mean hair loss counts decreased from 200 to 80 hairs at 6 weeks, with significant reductions in values (Table 1, Fig. 2).

Table 1
Outcome measurements in patients receiving mesenchymal stem cell exosomes

Variable	Mean $_{base}$ (SD)	Mean $_{sec}$ (SD)	Mean $_{third}$ (SD)	MD $_{sec - base}$ (95% CI)	p	MD $_{third - base}$ (95% CI)	p	MD $_{third - sec}$ (95% CI)	p
Density	96.5 (2.3)	111.7 (5.04)	163.5 (13.6)	15.2 (6.7 to 23.6)	< 0.0001	67.0 (58.5 to 75.5)	< 0.0001	51.8 (43.3 to 60.3)	< 0.0001
Diameter	0.049 (0.0013)	0.058 (0.0013)	0.059 (0.0004)	0.0087 (0.0076 to 0.0097)	< 0.0001	0.0093 (0.0082 to 0.0104)	< 0.0001	0.0007 (-0.0004 to 0.0018)	0.35
Hair loss	200 (37)	135 (87)	80 (33)	-65 (-126 to -4)	0.035	-120 (-181 to -59)	< 0.0001	-55 (-116 to 6)	0.09

MD; mean difference, Base; baseline, Sec; second, 95% CI; 95% confidence interval

Discussion

The results of this single-arm study revealed the efficacy of placenta-derived mesenchymal stem cells (P-MSC) therapy for the treatment of androgenetic alopecia. Patients receiving stem cell therapy showed significant improvements in hair density, hair shaft diameter, and hair loss count. While the efficacy of stem cell therapy and stem cell exosomes has been examined in various studies, to the best of our knowledge, no study has specifically investigated the efficacy of placental-derived mesenchymal stem cell exosomes.

Stem cell therapy has been used for androgenetic alopecia, with previous studies demonstrating the efficacy of different stem cell therapies; however, the safety of stem cell therapy remains controversial(5). The emergence of new clinical studies has significantly increased the documentation of negative occurrences and secondary impacts associated with MSC-based treatments. (12) There are instances where the efficacy of cellular therapy seems exaggerated, potentially resulting in severe consequences for patients. Stem cell-derived exosomes offer several benefits over stem cells, including lack of immune response, absence of infusion-related toxicity, convenient accessibility, simple preservation, and avoidance of tumorigenic risks and ethical concerns. These exosomes can mimic the therapeutic properties of their precursor cells, such as embryonic and adult stem cells, by transferring their pluripotency or multipotency vertically(14). In this study conducted by us, no patient suffered any secondary complications from treatment by these exosomes, which was consistent with other studies.

Exosomes, ranging from approximately 40 to 100 nanometers, represent small lipid bilayer vesicles of biological origin released by cells. These vesicles exhibit a buoyant density of 1.13 to 1.19 grams per milliliter when placed in a sucrose density gradient medium(11, 15–18). The term "exosomes" was introduced by Trams and colleagues in 1981 to describe vesicles derived from the plasma membrane. (19) They postulated that exosomes, possessing 5'-nucleotide enzyme activity, could serve specific physiological roles and emerge from the extrusion of diverse cell line cultures(20, 21).

Different animal and in-vivo studies have examined the efficacy of exosomes in the treatment of androgenetic alopecia, but there are no published human clinical trials. These studies have underscored various mechanisms for exosome therapy in terms of hair regrowth. A recent study by Wang et al. examined the efficacy of dermal papilla cell exosomes for stimulating hair regrowth in mice, revealing that these exosomes caused hair regrowth by upregulating AKT1 and VEGF. The results suggested a probable therapeutic role of dermal papilla cell exosomes in AGA by regulating signaling pathways in cell growth, proliferation, and apoptosis(22). Another study by Shi et al. demonstrated higher efficacy of micro-needling with adipose-derived stem cell exosomes combined with chitosan lactate compared to minoxidil treatment in mice. The authors proposed the regulation of hair follicle cycling by increasing the expression of MMP-3 and β-catenin in DPCs(23).

A study by Wu et al. also showed the efficacy of adipose-derived stem cells in a mouse model, revealing higher regeneration of follicles in the exosome group due to higher expressions of PDGF and vascular endothelial growth factor compared to the control group(24). According to the point of difference and also the advantage of our study compared to other studies, this procedure is performed on human samples, which is more accurate due to the physiological and anatomical differences between humans and other animals.

Another study by Hu et al. showed higher efficacy of dermal papillary-derived exosomes in progressing the hair follicle cycle from telogen to anagen compared to minoxidil. This study also highlighted the role of miR-218-5p in regulating follicular development by suppressing the WNT signaling inhibitor SFRP2(25). There is also supporting evidence for the efficacy of dermal papilla exosomes in hair growth, with proposed mechanisms involving improved expression of growth factors such as IGF-1, KGF, and HGF(26). Further supporting evidence indicates the role of β-catenin and sonic hedgehog in dermal papilla cell exosome therapy for follicular growth regulation(27) and the activation of mitochondria and β-catenin in the hair follicle growth cycle. However, the exact mechanism of exosome function in hair growth remains controversial(28).

This study was not without limitations. The limited sample size and lack of a control group were significant limitations. Additionally, the lack of cellular studies to explore the molecular basis of the intervention and the short follow-up period, as well as the absence of molecular examination of growth factors as outcome assessments, were other limitations. Therefore, further controlled trials with larger sample sizes and longer follow-up durations are needed.

In conclusion, P-MSC exosome therapy shows potential as an effective treatment for AGA, offering a novel approach that leverages the regenerative capabilities of stem cells in a safer, more manageable form. Continued research and controlled clinical trials are essential to confirm these initial findings and optimize the therapeutic protocols for broader clinical application.

Abbreviations

DPC
Dermal papillary cell
P-MSC
Placental derived mesenchymal stem cell
IQR
Inter quartile range
ADSC
Adipose derived stem cell
AGA
Androgenetic Alopecia
HFSC
Hair follicle stem cell
BMSC
Bone marrow stem cell

Declarations

ETHICS STATEMENT

This study has obtained ethical approval from the Isfahan Azad University of Najafabad.

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The authors received no financial support for this study

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy

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None

COMPETING INTERESTS

The authors declare that they have no competing interests

Disclosures: The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

THE AUTHORS' CONTRIBUTION

DATA AVAILABILITY STATEMENT

All data used and analyzed during this study are available from the corresponding author upon

reasonable request.

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Figures

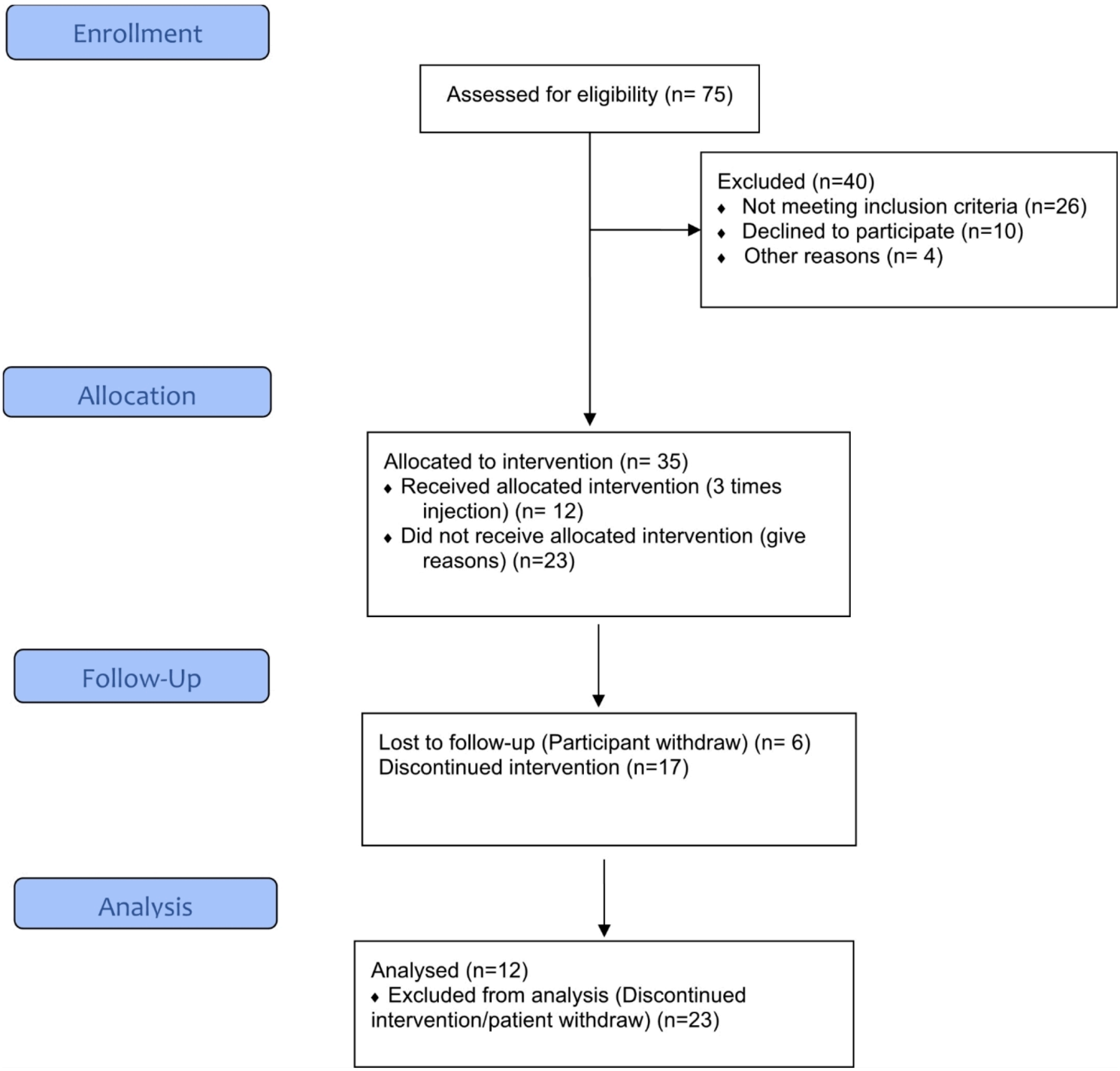

Figure 1

CONSORT Flow Diagram

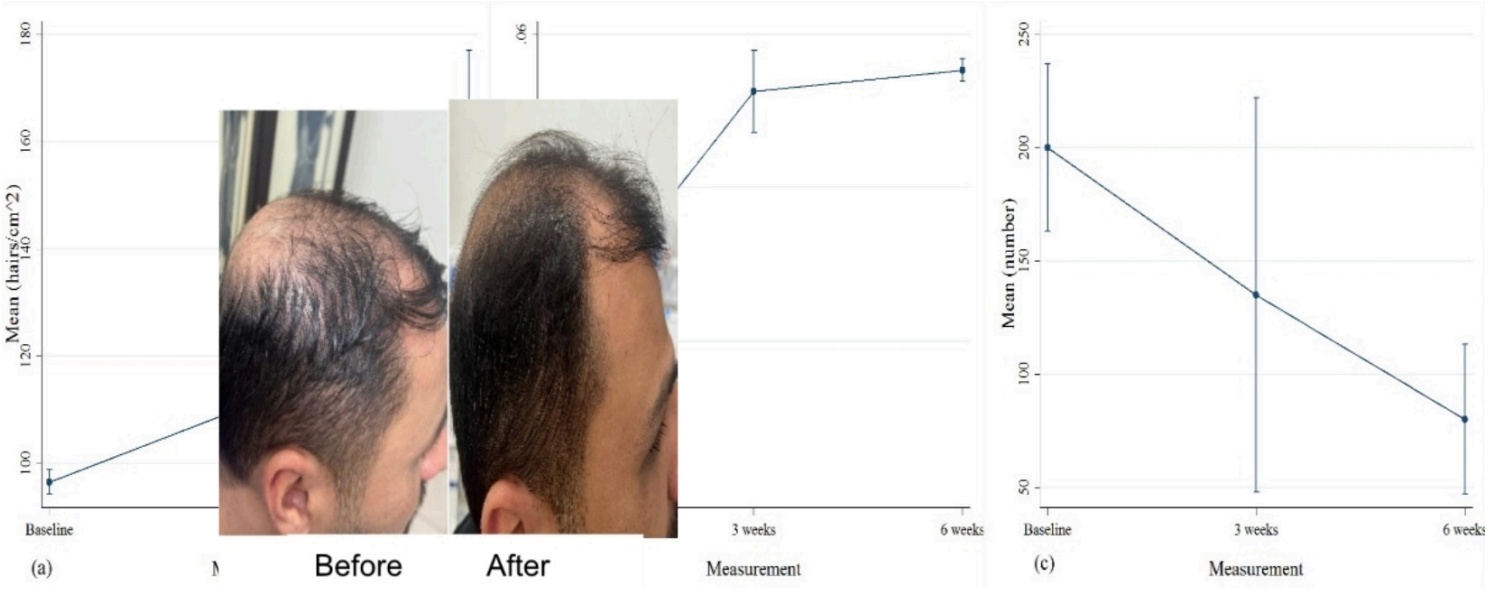

Figure 2

Mean (SD) in hair density (a), hair diameter (b), hair loss (c) at baseline, 3 weeks and 6 weeks after first injection (d) Before/After