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Mesenchymal Stem Cell-derived Exosome: An Interesting Nanocarrier to Improve Allergen-specific Intranasal Immunotherapy

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ABSTRACT

Increasing the efficacy of allergen-specific intranasal immunotherapy (INIT) has recently been the main goal of several studies to establish this route as a safe delivery method through mucosal pathways. In this case, the present study evaluated the potential of INIT using ovalbumin (OVA)-loaded mesenchymal stromal/stem cell (MSC)-derived exosomes (Exo-OVA) in an allergic asthma mouse model.

Together with control groups, sensitized Balb/c mice underwent intranasal immunotherapy with Exo-OVA (10 μg OVA per dose) for three consecutive weeks. Serum-specific immunoglobulin E (IgE) levels, transforming growth factor-beta (TGF-β), interleukin (IL)-4, and interferon-gamma (IFN-γ) production by cultured spleen cells, lung histopathologic analysis, and nasopharyngeal lavage fluid cellular examinations were then conducted.

The results showed that INIT using Exo-OVA significantly increased IFN-γ and TGF-β secretion, while allergen-specific IgE and IL-4 production were dramatically decreased compared to the control group receiving phosphate-buffered saline. In addition, the eosinophil and total cell counts in the nasopharyngeal lavage fluid were reduced, and inflammatory conditions and cell accumulation in lung tissue were ameliorated.

In conclusion, the Exo-OVA improved the INIT efficacy compared to free OVA. Therefore, this formulation could be introduced as an effective approach for immunomodulatory purposes with a shorter treatment duration and reduced side effects.

Keywords: Allergen immunotherapy; Exosomes; Immunomodulation; Mesenchymal stromal cell; Nanoparticle drug delivery system

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INTRODUCTION

Allergic asthma is considered a heterogeneous disease with variable clinical and pathologic characteristics and an increasing prevalence rate worldwide. It triggers a variety of symptoms including wheezing, shortness of breath, cough, and tightness of the chest leading to airflow limitation and bronchial hyperresponsiveness against various environmental stimuli.[1] Both innate and acquired immunity have a direct impact on disease pathogenesis, and dendritic cells (DC) and airway epithelial cells are key players in activating the humoral and cellular immune responses through antigen presentation and cytokine secretion.[2,3]

Inhaled corticosteroids and long-acting β-agonists are the primary drug therapy agents that alleviate symptoms and restore respiratory function without modifying disease pathogenesis.[4] Allergen-specific immunotherapy (AIT) is the gold standard used as a therapeutic modality that progressively induces immune tolerance against allergens and is clinically effective in reducing symptoms and preventing new allergic sensitization and disease progression. Subcutaneous injection is the common route of gradual dose-increasing administration of allergen extracts, which requires 30-80 injections over 3 to 5 years and has a high potential for anaphylaxis and other side effects.[5,6] Mucosal-based immunotherapies, such as intranasal, oral, and sublingual, are among the noninvasive approaches that have beneficial properties, including the elimination of injection-related risks, ease of treatment, and increased patient adherence to the therapeutic regimen.[7,8] While intranasal allergen-specific immunotherapy (INIT) is considered an interesting local approach to the control of allergic airway inflammation, direct intranasal administration of the allergen leads to allergic nasal symptoms after repeated nasal reactions to the allergen. In this case, encapsulation of the allergen in nano-sized vesicles/particles to increase the delivery efficiency could reduce the risk of side effects by stabilizing and increasing its immunogenicity even at lower doses.[9,10]

Exosomes (Exo) are biological nanoparticles capable of delivering cargoes such as small RNAs and proteins. Various cells release these vesicles in the endosomal network and play important roles in intercellular interactions while possessing beneficial properties, such as biocompatibility, low clearance, low long-term accumulation, loss of toxicity, and accelerated cellular uptake.[11,12] Various studies have claimed that mesenchymal stromal/stem cell (MSC)-derived exosomes exert immunomodulatory effects and potentially induce tolerance and immune hemostasis while regulating immune responses.[13,14]

In the present study, we evaluated the efficacy of ovalbumin (OVA)-loaded MSC exosomes for INIT in a mouse model of allergic airway inflammation.

MATERIALS AND METHODS

Adipose Tissue (AD)-MSCs Isolation, Characterization, and Differentiation

Under sterile conditions, the adipose tissues of female Balb/c mice were isolated and minced into small sections and digested with 0.01% type I collagenase (Merck, Germany) for 1 hour at 37°C. Then, the 10% fetal bovine serum (FBS) (Bioidea Co, Iran) supplemented with Dulbecco's modified Eagle's medium (DMEM) media (Bioidea Co, Iran) was used to neutralize the enzyme activity and centrifuged (15 min at 500 g) to obtain a stromal vascular fraction at the bottom and adipocytes at the top section. The cell pellet was resuspended in DMEM media supplemented with 10% FBS, and 1% penicillin/streptomycin (Bioidea Co, Iran) and cultured in T-25 flasks under the following incubation conditions: 37°C, 5% Co_2, and 95% humidity. After 48 hours, the media was replaced and the non-adherent cells were removed. Media replecement for adherent cells was continued every 3-days to reach 70-90% confluent flasks; then 0.25% trypsin/EDTA (Bioidea Co, Iran) was administered to the passage cells.

To characterize the membrane expression of specific markers, five microliters of specific anti-CD31, anti-CD45, anti-CD73, and anti-CD105 antibodies and isotype control antibody (Abcam, Cambridge, UK) were added to 1×10^5 cells resuspended in staining buffer and incubated at 4◦C for half an hour. The washed cells were then analyzed using a flow cytometer (BD Bioscience, USA) and Flow Jo analysis software (San Jose, USA).

In addition, to assess the osteogenic differentiation capacity, 2×10^4 cells were seeded at passage two and cultured in osteogenesis-inducing media for three weeks. The media consisted of DMEM supplemented with 10 mM β-glycerol phosphate, 5nM dexamethasone, and 50 μg/mL ascorbic acid biphosphate. Finally, a paraformaldehyde solution was applied to fix the

differentiated cells and then stained with alizarin red to visualize the mineralized matrix.

Exosome Harvest and Characterize

To isolate exosomes from MSCs, FBS-free media was added to confluent passage two cells for 72 hours. The conditioned supernatant media was then harvested, centrifuged (2500 g, 15 min), and filtered through a 0.22μ membrane. The solution was then isolated using the exosome isolation kit (Exospin, USA).

To characterize the purified exosomes, the size and shape of the exosome were evaluated by scanning electron microscopy (SEM). Also, DLS Zetasizer Nano-ZS (Malvern, UK) was used to assess the size of exosomes.

Surface markers of exosomes were also assessed by flow cytometry. The exosome solution was incubated with 50 μL beads at room temperature (RT). Next, 5 μL anti-CD9 and anti-CD63 antibodies (Abcam, UK) were added to the washed beads and incubated (30 min, 4°C). Finally, the beads were washed and analyzed using a flow cytometer (BD Bioscience, USA) and Flow Jo analysis software (San Jose, USA).

In addition, a bicinchoninic acid (BCA) protein assay kit (Parstous, Iran) was used to quantify the protein content of exosomes.

Preparation and Confirmation of OVA-loaded Exosomes

To prepare OVA-enriched exosomes, the incubation method was implicated based on previous studies [15-17]. The exosome solution (200 μg/mL) was added to the 500 μg/mL of OVA solution (Sigma, Germany) (1:1 ratio). The mixture was incubated for 6 hours at 22°C with gentle shaking. Then, an exosome isolation procedure was performed to remove free OVA.

The successful incorporation of OVA into exosomes was primarily evaluated using the BCA protein assay kit (Parstous, Iran). In this case, the loaded OVA concentration is equal to the subtraction of the initial OVA concentration from the OVA concentration in the supernatant.

The OVA concentration in the OVA-exosome pellet was also determined directly by HPLC. In this case, the pellet was lysed using radioimmunoprecipitation assay (RIPA) buffer (Kiazist, Iran), and samples were analyzed by UV-HPLC (Santa Clara, USA).

Animals

Twenty-five female Balb/c mice (6-8 weeks old) were obtained from the Pasteur Research Institute, Tehran, Iran. Animals were housed according to the local animal care guidelines. All animal handlings were approved by the Institutional Animal Ethics Committee of AJA University of Medical Sciences, Tehran, Iran (IR.Ajaums.Rec.1400.223).

Induction of the Allergic Asthma Model

Allergic asthma induction was performed as previously described.[18,19] Briefly, mice received two intraperitoneal (IP) injections consisting of 10 μg OVA absorbed on 2 mg aluminum hydroxide, Al $(OH)_3$, (Sigma, France) in 100 μL PBS at a two-week interval. Controls received 100 μL PBS/Alum. On days 21-24, mice were challenged with 1% w/v OVA solution for twenty minutes via an aerosol nebulizer (Zenith Nebulizer, Switzerland). On day 25, the retro-orbital sinus blood samples were collected to measure the serum levels of specific IgE and to verify the sensitization (Figure 1).

Intranasal Immunotherapy Procedure

Mice were divided into five groups (n=5), including non-sensitized untreated (negative control), sensitized PBS-treated (positive control), sensitized free OVA-treated, sensitized free MSC-Exo-treated, and sensitized OVA-enriched MSC-Exo-treated (Exo-OVA) (10 μg/dose OVA) groups. The intranasal immunotherapy was administered every 3 days for 3 consecutive weeks (10 μL per nostril). Two days after the last dose, mice were nebulized with 1% w/v OVA for twenty minutes for two days. After 24 hours, the mice were sacrificed, and cardiac punction-isolated blood samples, nasopharyngeal lavage fluids (NALF), and spleen and lung tissues were isolated for further investigations (Figure 1).

Evaluation of the INIT Consequences

Specific IgE Response

Blood samples were obtained by the cardiac puncture, and serums were used to assess allergen-specific IgE levels by enzyme-linked immunosorbent assay (ELISA) kit (East Biopharm, China).

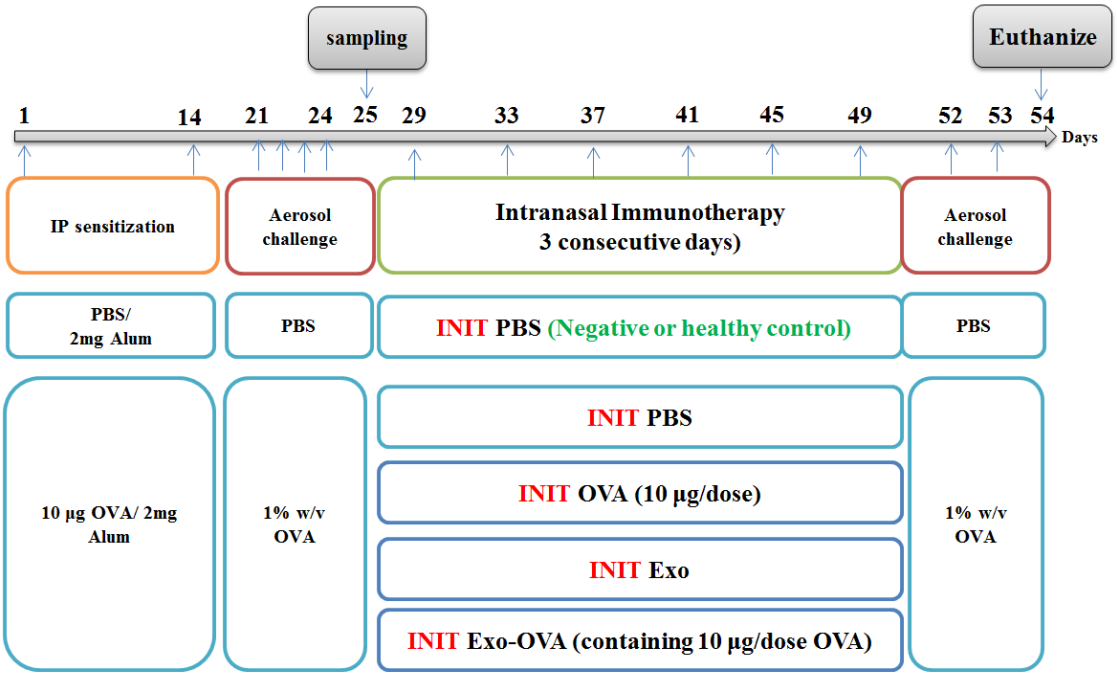

Figure 1. Schematic design of asthma induction and immunotherapy process for sensitization, mice received two intraperitoneal (IP) injections containing 10 µg ovalbumin (OVA) absorbed on 2mg Alum followed by four days of 1% w/v OVA aerosol challenge. Blood sampling was conducted on day 25, and serum OVA-specific immunoglobulin E (IgE) levels as measured to confirm sensitization. The intranasal specific immunotherapy (INIT) was performed every 3 days for 3 consecutive weeks in four treatment groups, including phosphate-buffered saline (PBS) (1), OVA (2), exosome (Exo) (3), and Exo-OVA (4). Also, a healthy control group (5) received PBS/Alum as IP sensitization and PBS as aerosol challenges and immunotherapy process. At the immunotherapy endpoint, animals received 1% w/v OVA aerosol challenge for two days. Then, animals were sacrificed.

OVA-specific Cytokine Secretions

The production of transforming growth factor-β (TGF-β), interleukin-4 (IL-4), and interferon-γ (IFN-γ) cytokines in the supernatant of cultured splenocytes was evaluated using ELISA kits (Karmania Pars Gene, Kerman, Iran). In this case, after lysis of red blood cells, cells were collected and washed with PBS. Then 2×10^6 cells were suspended in FBS-supplemented Roswell Park Memorial Institute (RPMI 1640) (Bioidea Co, Iran) media in 24-well plates and stimulated with 10 µg OVA as the allergen or 1µg PHA as positive control or free media as a negative control for 72 hours. The supernatants were then collected for evaluation of cytokine levels using ELISA kits.

Cellular Analysis of NALF

The upper part of the trachea was resected, and 1 mL of PBS was injected into the nostrils and collected via the nares. To obtain the cell pellets, the collected NAL fluid was centrifuged (10 min, 1500 rpm) and cell pellets were suspended in 500 µL PBS. The total cell count was performed using a hemocytometer. In addition, an air-dried and Wright-Giemsa-stained microscope slide was prepared to count the eosinophils in the NALF.

Lung Histological Analysis

The formaldehyde-fixed lung tissues were subjected to histopathologic procedures for sectioning and staining with hematoxylin and eosin (H&E) staining. Finally, the pathological findings were examined by an animal pathologist using a light microscope (Zeiss, Germany).

Data Analysis

GraphPad Prism software version 6 (GraphPad, CA, USA) was used for statistical analysis. In this case, unpaired t-tests and One-way ANOVA tests were applied and $p<0.05$ was considered significant.

RESULTS

AD-MSC Characterization

Following flow cytometric analysis, most of the cells expressed high levels of CD105 and CD73 and low levels of CD45 and CD31 were observed (Figure 2a). Microscopic observation confirmed the typical morphology of MSCs (Figure 2b). Also, extracellular calcium deposition proved the *in vitro* differentiation potential of MSCs into osteocytes (Figure 2c).

Characterization of Exosomes

Spherical morphology and estimated size of both free and OVA-loaded exosomes were validated by DLS and SEM analysis (Figure 3a, b). Also, high expression of CD9 and CD63 was observed by flow cytometric analysis (Figure 3c).

OVA-loaded Exosome Verification

BCA and UV-HPLC were performed to quantify the concentration of exosome-loaded OVA. Both indirect and direct methods showed approximately 20 μg OVA content in the exosomes. Appropriate dilutions with PBS were performed to reach the 10μg OVA per dose for immunotherapy sessions.

Consequences of INIT

Sensitization Elevated the Allergen-specific IgE Concentration

Evaluation of serum IgE levels after the sensitization showed that sensitized mice had significantly increased OVA-specific IgE levels ($p<0.0001$) (Figure 4).

Effects of INIT on Serum IgE Levels

As expected, INIT with PBS does not affect the elevated levels of OVA-specific IgE due to sensitization. Intranasal administration of free OVA also showed similar results with an insignificant reduction in IgE levels.

A dramatic reduction in IgE levels was observed with Exo INIT treatment compared to PBS and OVA INIT ($p<0.0001$) and with Exo-OVA INIT compared to PBS and OVA INIT ($p<0.0001$). A dramatic difference was observed between Exo INIT and Exo-OVA INIT ($p<0.01$), with Exo-OVA INIT showing greater efficacy in reducing specific IgE levels. Notably, IgE levels were still higher in the Exo-OVA INIT group than in healthy mice ($p<0.001$) (Figure 5).

Effects of INIT on Cytokine Secretions by Splenocytes

Figure 6 summarizes the production of TGF-β, IL-4, and IFN-γ in the supernatant of cultured splenocyte.

The IFN-γ secretion was significantly elevated in Exo INIT than both PBS-INIT and OVA-INIT groups ($p<0.001$), and in the Exo-OVA-INIT than both PBS-INIT and OVA-INIT groups ($p<0.0001$). In other words, INIT OVA showed insignificant differences from than PBS group. Also, a remarkable difference was observed between Exo-OVA INIT and Exo INIT ($p<0.001$), and Exo-OVA INIT showed higher efficacy in elevating the IFN-γ levels (Figure 6a).

The results of IL-4 indicated that reduced the IL-4 cytokine levels were observed in the Exo INIT treatment ($p<0.05$) and in the Exo-OVA INIT than PBS ($p<0.0001$), while negligible difference was reported between OVA INIT and PBS INIT. The differences between Exo-INIT and Exo-OVA-INIT and between OVA INIT and Exo-INIT were also insignificant (Figure 6b).

In the case of TGF-β, significantly increased levels of this cytokine were observed in the Exo INIT group compared to both the PBS INIT and OVA INIT groups ($p<0.0001$) and in the Exo-OVA INIT than both PBS INIT and OVA INIT groups ($p<0.0001$), while the OVA INIT treatment showed no therapeutic efficacy. In addition, a dramatic difference was observed between the Exo INIT and Exo-OVA groups ($p<0.001$) (Figure 6c).

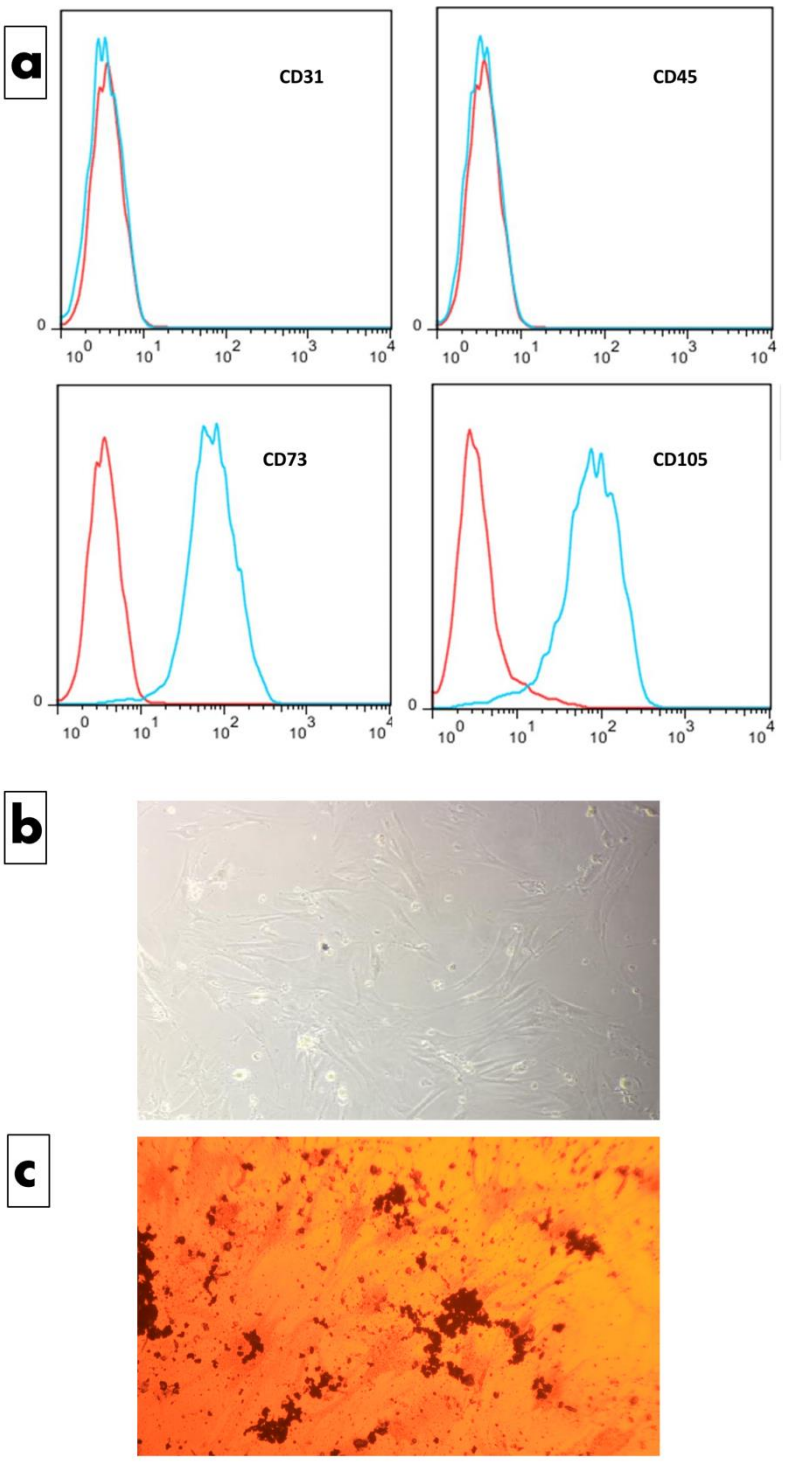

Figure 2. Characterization of mesenchymal stromal/stem cells (MSC) isolated from adipose tissue (a) flow cytometric analysis for surface expression of CD31, CD45, CD73, and CD105 markers (blue graphs) in the presence of isotype control results (red graphs); (b) microscopic view of MSCs; (c) microscopic view of osteogenesis differentiation

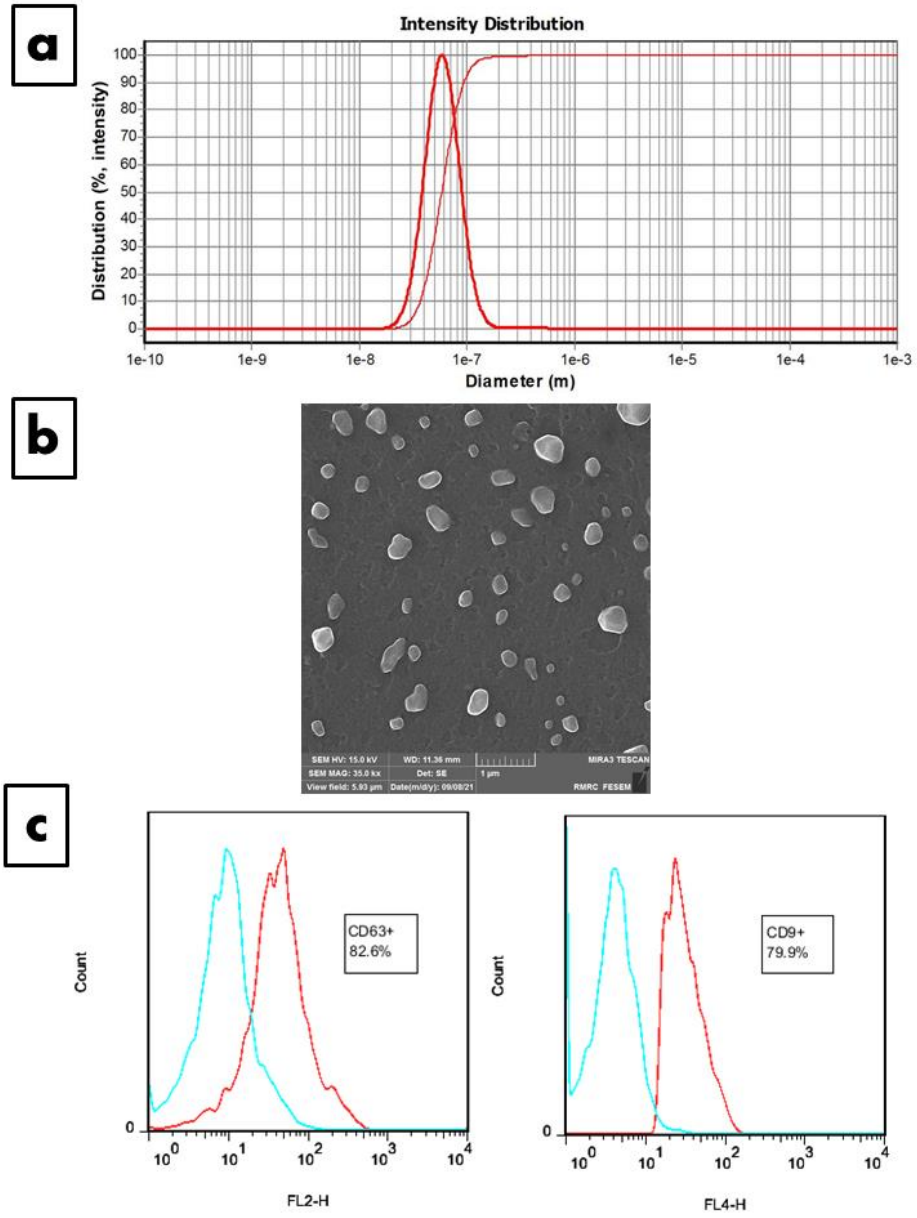

Figure 3. Characterization of mesenchymal stromal/stem cell (MSC)-isolated exosomes (a) Dynamic light scattering (DLS) analyzed the size distribution of exosomes; (b) Scanning electron microscopy (SEM) verified the spherical shape and size of exosomes; (c) Surface expression of CD63 and CD9 markers (red graphs) were analyzed by flow cytometry (blue graphs are unstained results)

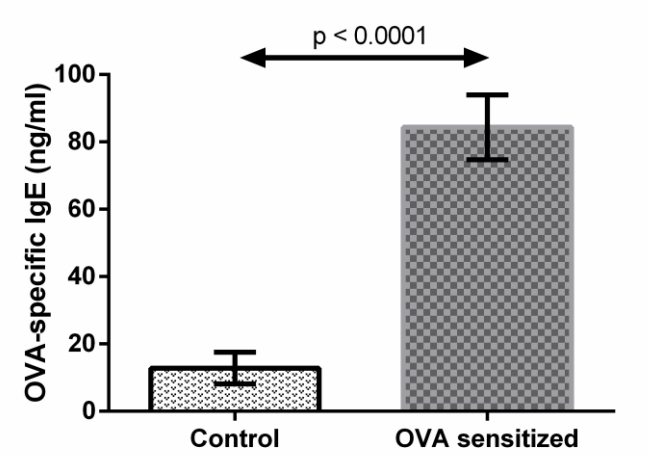

Figure 4. The ovalbumin (OVA)-specific immunoglobulin E (IgE) serum levels in the OVA-sensitized mice compared to the control group Significant difference was observed between OVA-sensitized mice and the healthy control group which received phosphate-buffered saline (PBS)/Alum (Control; healthy mice that received PBS/Alum, OVA-sensitized; mice sensitized with OVA/Alum) (negative control or healthy control group).

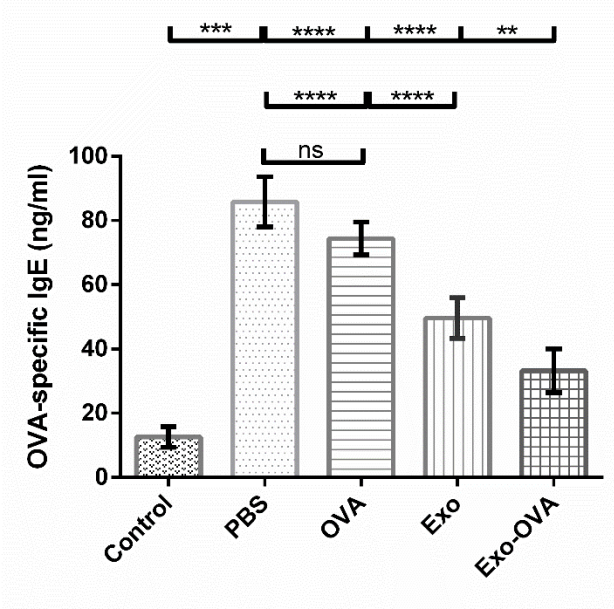

Figure 5. Effects of various intranasal specific-immunotherapy (INIT) formulations on ovalbumin (OVA)-specific immunoglobulin E (IgE) serum levels (Control; negative or healthy control that sensitized with phosphate buffered saline (PBS)/Alum, PBS; mice sensitized with OVA and received intranasal PBS as treatment, OVA; mice sensitized with OVA and received intranasal OVA (10µg/dose) as treatment, exosome (Exo); mice sensitized with OVA and received intranasal Exo as treatment, Exo-OVA; mice sensitized with OVA and received intranasal Exo-OVA (containing 10µg/dose OVA) as treatment) (ns indicates not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$)

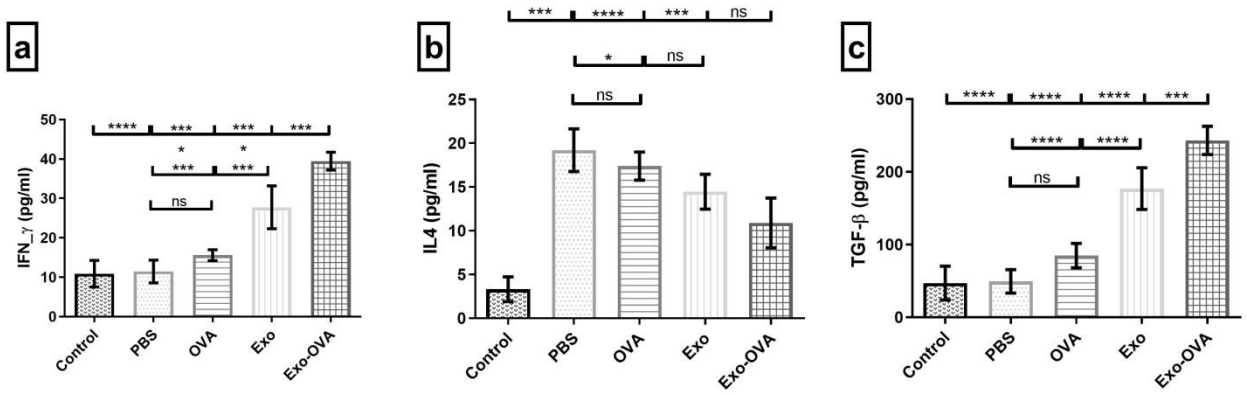

Figure 6. Interferon-gamma (IFN-γ) (a), interleukin (IL)-4 (b), and transforming growth factor-beta (TGF-β) (c) production in the supernatants of cultured splenocytes in treatment and control groups

Control; negative or healthy control that sensitized with phosphate-buffered saline (PBS)/Alum, PBS; mice sensitized with ovalbumin (OVA) and received intranasal PBS, OVA; mice sensitized with OVA and received intranasal 10 µg/dose OVA, exosome (Exo); mice sensitized with OVA and received intranasal Exo, Exo-OVA; mice sensitized with OVA and received intranasal Exo-OVA (containing 10 µg/dose OVA). (ns indicates not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$)

Effects of INIT on NALF Cellular Analysis (Figure 7)

The NAL fluid analysis showed that Exo-OVA INIT significantly reduced the total cell count compared to both the PBS-treated and OVA INIT groups ($p<0.0001$). in addition, Exo INIT showed a significantly reduced total cell count than both PBS-treated and OVA INIT groups ($p<0.0001$). In other words, negligible change was observed after OVA INIT treatment. Exo-OVA INIT also showed higher efficacy than Exo INIT treatment ($p<0.05$). In addition, the differences between Exo-OVA INIT and healthy control mice were insignificant, indicating that this formulation considerably improved the recruitment of inflammatory cells to the NALF to reach the negative control levels (Figure 7a).

In the case of eosinophil count, Exo-OVA INIT significantly reduced eosinophil count than both PBS-treated and OVA INIT groups ($p<0.0001$). A dramatic reduction in eosinophil count was also observed in the Exo-OVA INIT group compared to both the PBS and OVA INIT groups ($p<0.0001$). In addition, the eosinophil count was lower in Exo-OVA INIT than in the Exo INIT group ($p<0.05$), and a slight difference was reported between Exo-OVA INIT and the control, showing the efficacy of this treatment in reducing eosinophils in the NALF (Figure 7b).

Effects of INIT on Lung Histopathology

A summary of the histopathologic evaluation of the lungs in different therapeutic groups is shown in Figure 8. Healthy lung tissue was normal in the peribronchial and perivascular regions without infiltrates and inflammation (Figure 8a). In the OVA-sensitized PBS INIT mice, extensive bronchiolar epithelial degeneration was observed along with severe immune cell infiltration in most lung regions (Figure 8b). Similar observations were also reported in the OVA-sensitized OVA INIT group. Bronchiolar epithelial degeneration and inflammatory cell infiltration were observed in most lung regions in this group (Figure 8c). In the OVA-sensitized Exo INIT group, lung tissues showed moderate cellular infiltration in peribronchial and perivascular areas. Mononuclear and polymorphonuclear inflammatory cell infiltration was also observed in moderate lung areas (Figure 8d). In addition, analysis of the main therapeutic group, Exo-OVA INIT, showed local cellular infiltrations in peribronchial areas, and minimal septal thickening was observed (Figure 8e).

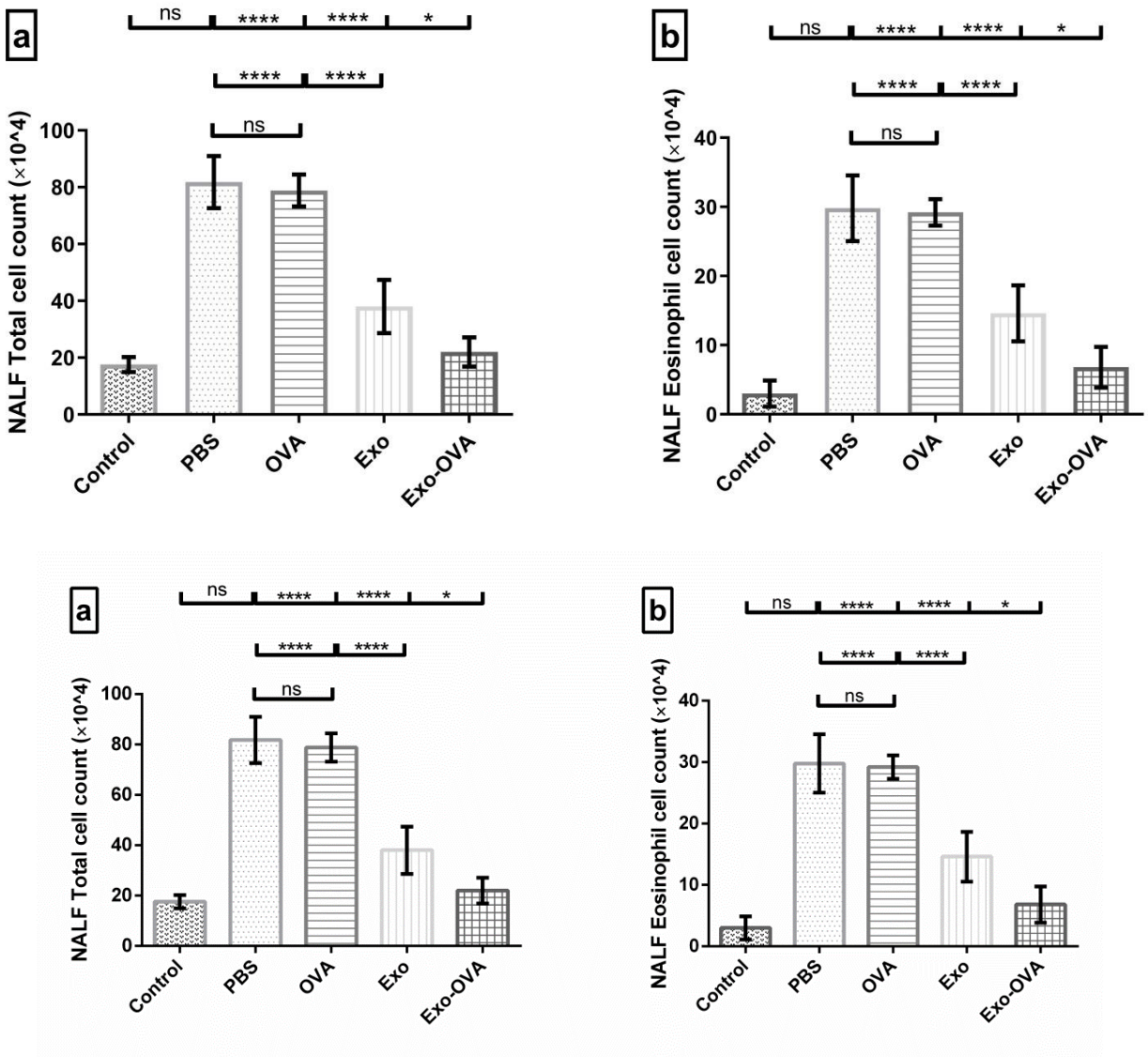

Figure 7. Analysis of nasopharyngeal lavage fluid (NALF) in case of the total cell (a) and eosinophil (b) counts between treatment and control groups

Control; negative or healthy control that sensitized with phosphate-buffered saline (PBS)/Alum, PBS; mice sensitized with ovalbumin (OVA) and received intranasal PBS, OVA; mice sensitized with OVA and received intranasal 10µg/dose OVA, exosome (Exo); mice sensitized with OVA and received intranasal Exo, Exo-OVA; mice sensitized with OVA and received intranasal Exo-OVA (containing 10µg/dose OVA). (ns indicates not significant, * p<0.05, ** p<0.01, *** p<0.001, and **** p<0.0001)

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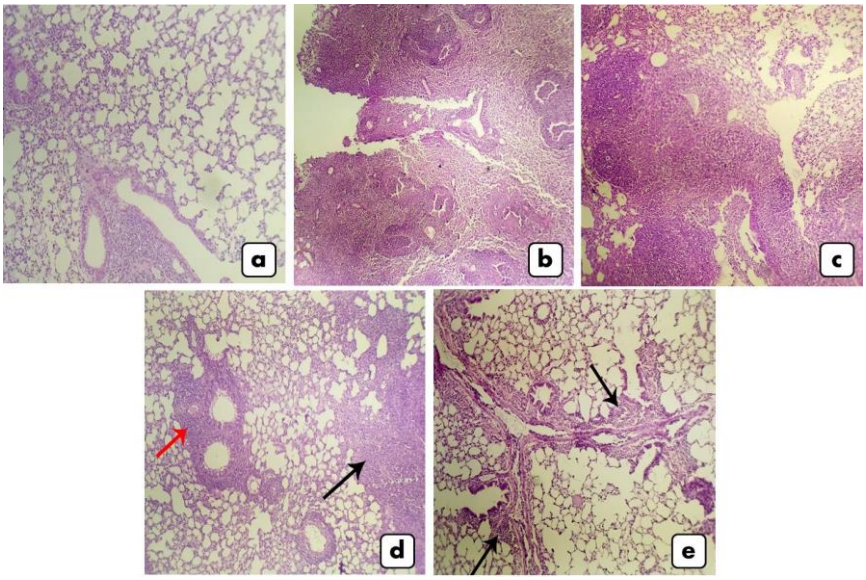

Figure 8. Histopathological analysis of lung tissues in various therapeutic and control groups (a) normal healthy control, (b) ovalbumin (OVA)-sensitized phosphate-buffered saline (PBS) intranasal specific-immunotherapy (INIT); bronchial epithelial degeneration and severe inflammatory cells infiltration into extensive areas, (c) OVA-sensitized OVA INIT; bronchial epithelial degeneration and severe inflammatory cells infiltration into extensive areas, (d) OVA-sensitized exosome (Exo) INIT; moderate peribronchial and perivascular cell infiltrations (red arrows) and inflammatory cell infiltrates into moderate areas of lung (black arrow), and (e) OVA-sensitized Exo-OVA INIT; local peribronchial and perivascular infiltrate of inflammatory cells (black arrows) and thickening of septa in minimal areas (Hematoxylin and Eosin (H&E) stained sections, ×100 magnification microscopy)

DISCUSSION

The present study evaluated the efficacy of MSC exosomes as an immunoregulatory delivery system with immunomodulatory properties through specific intranasal immunotherapy in the allergic asthma mice model. The results showed that Exo-OVA improved lung pathological circumstances and reduced eosinophil infiltration in the NALF. When serum IgE and splenocyte cytokine secretion were analyzed, increased productions of TGF-β and IFN-γ levels were reported to be consistent with decreased IL-4 secretion and serum levels of OVA-specific IgE.

The administration of dosage forms suitable for mucosal membranes, especially nasal mucosa, is of great of interest for AIT procedures and has shown desirable results.[7,20] In this case, Moitra et al, reported that INIT significantly induced variable Treg phenotypes, including Tr1, Th3, and CTLA4+ cells and modulated the expression of regulatory molecules.[21] Nevertheless, several studies reported that direct intranasal administration of crude allergens can potentially lead to local manifestations such as itching, swelling, coughing, and nasal congestion, as well as systemic consequences such as hives, fever, and dyspnea due to repeated nasal reactions to allergens.[22] These results are consistent with our findings that intranasal administration of free OVA showed similar results to the sensitized group and no improvement in allergic inflammation was reported. Therefore, different delivery systems were evaluated to improve the efficacy of INIT and to avoid adverse reactions. Various nanoscale carriers including chitosan,[23,24] poly (lactide-co-glycolide) (PLGA),[25] polypropylene sulfide (PPS),[26] gold nanoparticles (GNP),[27] and also liposome vesicles,[28,29] were used to deliver allergens and showed promising results in controlling allergic airway inflammation and inducing Th2-to-Th1 shift without side effects. Similar results were observed in our study. The Exo-OVA INIT dramatically suppressed eosinophil infiltration in NALF and reduced perivascular and peribronchial inflammation in lung tissue compared with free OVA INIT. Significant differences between the levels of specific IgE and secretion of TGF-β, IL-4, and IFN-γ also highlighted the efficacy of Exo-OVA INIT compared to free OVA INIT.

MSCs and their derivatives have been widely used for immunomodulatory and regenerative purposes in various experimental and clinical studies.[30,31] These nanovesicles act as a paracrine arm of MSCs by carrying various bioactive molecules, including functional mRNAs, miRNAs, proteins, enzymes, cytokines and other components that mimic the immunomodulatory benefits of the parental cells.[32] Cho et al, reported that AD-MSCs dramatically suppressed allergic symptoms and airway hyper-responsiveness through Treg induction and Th2 inhibition in bronchial alveolar fluid and lung-draining lymph nodes.[33] In addition, several studies have demonstrated that MSC-derived exosomes exert modulatory effects similar to those of MSCs, but with greater safety and efficacy. For instance, Fang et al, showed that MSC exosomes significantly suppressed inflammatory cell infiltration into the airways after the sensitization phase and before the allergen challenge in a mouse model of allergic asthma,[34] and Du et al, showed that these nanoscale vesicles induce Tregs proliferation via the induction of TGF-β and IL-10 secretion in the peripheral blood mononuclear cells (PBMCs) isolated from asthmatic patients.[35] Similar results were also reported by de Castro et al,[36] and Cruz et al,.[37] De Castro et al, demonstrated that MSC exosomes significantly increased lung TGF-β levels and reduced the eosinophil population in the BAL fluid and lung tissue similar to MSCs in an allergic asthma murine model.[36] In addition, Cruz et al, demonstrated that systemic administration of human BM-MSCs and derived exosomes at the onset of allergen challenge significantly ameliorated lung inflammation and Th2 phenotype.[37] Our findings showed promising results. In this case, MSC-derived exosomes effectively decreased specific IgE serum levels and elevated IFN-γ and TGF-β production in splenocytes. These findings were consistent with improved lung tissue inflammatory conditions and reduced eosinophil and inflammatory cell infiltration in the NALF and airways.

Collectively, our results can be explained from several perspectives. First, we showed that intranasal administration of free MSC-isolated exosomes could effectively ameliorate allergic inflammation and modulate both cellular and humoral responses due to their immunomodulatory properties. These findings are similar to previous studies. In this case, Mun et al, reported that intranasal MSC-derived exosomes significantly reduced airway hyper-responsiveness and bronchial alveolar fluid eosinophil count and decreased the levels of total and specific IgE and IL-4 secretion, while Treg populations were dramatically increased.[38] Furthermore, Ren et al, reported that intranasal MSC-derived exosomes efficiently ameliorate allergic airway inflammation through increased IL-10 production.[13] Another important aspect is the loading of allergens into these nanoscale vesicles. Liu et al,[39] showed that OVA-loaded exosomes had a higher potential to reduce allergic symptoms and serum IgE levels, while their exosomes were not from an MSC source. In this case, our findings showed that the Exo-OVA formulation had the highest efficacy compared to other groups. These results are associated with both immunomodulatory and delivery properties of MSC exosomes.

In conclusion, our results demonstrated both the immunomodulatory and delivery potential of exosomes when comparing the differences between Exo-OVA and free OVA or free Exo therapeutic regimens in suppressing airway inflammation and cellular infiltration along with modulating humoral and cellular immune responses. Therefore, it was suggested that the use of MSC-derived exosomes as a delivery system could improve intranasal allergen-specific immunotherapy. Although concerns associated with exosome isolation and preparation methods are limited and provide potential challenges to the MSC-derived exosome-based therapeutic approaches in the clinical administration future in-depth studies are required to reach the desired results.

STATEMENT OF ETHICS

All animal handlings were approved by the Institutional Animal Ethics Committee of AJA University of Medical Sciences, Tehran, Iran (IR.Ajaums.Rec.1400.223).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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