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Original Article

Umbilical cord as a source of mesenchymal stem cells improves melasma in parturients: a clinical randomized trial

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ABSTRACT

Introduction: Melasma is a common skin problem in pregnant women that appears to be related to physiological changes. Mesenchymal stem cells (MSCs) have been used to treat skin disorders because of their direct cell-to-cell contact or release of several biomolecules. The umbilical cord, which is available at birth, is a rich source of MSCs. This study was undertaken to evaluate the effect of the umbilical cord as a natural face mask for treating women's melasma after parturition.

Methods: This randomized clinical trial was carried out on parturients suffering from melasma. Fifty pregnant women were randomly categorized into two groups of control and treated with the umbilical cord. A section of the umbilical cord (10-20 cm) was cut immediately after parturition and used as a face mask on the face of each mother in the hyperpigmentation areas for one hour. Melasma Area Severity Index (MASI) was used to measure hyperpigmentation and calculated for each participant before and four weeks after intervention.

Results: A significant decrease in MASI was observed in the women treated with the umbilical cord ($p<0.05$).

Conclusion: Our findings suggested that a fresh umbilical cord, as a natural face mask containing MSCs, can improve the melasma in the parturients.

Keywords:
Melasma
Umbilical cord
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Introduction

Hyperpigmentation during pregnancy is a common skin problem that appears to be related to physiological alterations, particularly endocrinological changes, including the increased level of estrogen, progesterone, and melanocyte-stimulating hormones (Hughes and Saleh, 2017; Urasaki, 2010). The nipple and areola, axillae, anus, perineum, genitalia, neck, inner thighs, and lentigines are the areas that develop hyperpigmentation during pregnancy. Linea alba often darkens, which is called the linea nigra, and areola often turns darker. The most evident pigmentation change during pregnancy is called melasma or chloasma or mask of pregnancy, which remains a cosmetic concern (Geraghty and Pomeranz, 2011).

Melasma is characterized by an excess of melanin in the epidermis and upper dermis, which is the consequence of the increased number of melanocytes, elevated expression of the a-melanocyte-stimulating hormone in the keratinocytes, and overexpression of the stem cell factor in the fibroblast and its receptor C-kit in the melanocytes (Ortonne and Bissett, 2008). Melasma may follow a centrofacial pattern, the asymmetric darkening of the skin on the forehead, cheeks, upper lip, chin, and nose. Malar pattern includes darkening of the cheeks and nose, and mandibular pattern in which the ramus of the mandible becomes darker (Barankin et al., 2002). Melasma occurs in about 75% of pregnant women with various extents and a wide spectrum of pigmentation intensities. This condition often disappears completely in the first year after birth; however, about 30% of women, especially those who have used oral contraceptives and those who have been exposed to solar radiation, suffer from some blemishes. As the treatment of melasma is mostly difficult, combination therapy, e.g. using bleaching creams, retinoid, hydroquinone, corticosteroids, chemical peels, laser therapy, microdermabrasion, sunscreen, and also the avoidance of sun exposure, are suggested to prevent additional skin darkening (Geraghty and Pomeranz, 2011; Gupta et al., 2006; Urasaki, 2010). In this respect, Lakhdar and associates exhibited the efficiency of the well-tolerated broad-spectrum sunscreen for inhibiting the development of melasma during pregnancy (Lakhdar et al., 2007).

Recently, there is a rising interest in the use of stem cells to treat recalcitrant diseases. Based on accumulating pieces of evidence, the application of mesenchymal stem cells (MSCs) for treating inflammatory skin diseases can be helpful owing to the immunomodulation effect of MSCs. Different animal models of inflammatory skin conditions, such as psoriasis, atopic dermatitis, and autoimmune disorders affecting the skin (e.g. graft versus host disease and systemic lupus erythematosus), have been improved by MSC therapy. Moreover, many clinical trials have currently confirmed the efficacy and safety of this therapy (Shin et al., 2017). Among the sources of MSCs, the umbilical cord is a rich source that is available at birth. The umbilical cord is comprised of two arteries and one vein, which are surrounded and supported by mucoid connective tissue, known as the Wharton's jelly, and enclosed by epithelial cells derived from the enveloping amnion. In the umbilical cord, there are stromal cells that are characterized by a fibroblast-like morphology, the ability of differentiation to other cell lineages such as adipocytes, osteoblasts, and chondrocytes, and expression of MSC surface markers viz CD44, CD105, CD29, CD51, SH2, and SH3. However, hematopoietic differentiation markers such as CD34 and CD45 do not exist (Wagner et al., 2005; Wang et al., 2004).

MSCs are potent cells for the treatment of skin disorders, and umbilical cord jelly is an available source of MSCs for all mothers, even those who intend to save umbilical cord blood. With this in mind, this study was undertaken to evaluate the effect of the umbilical cord on the treatment of a mother's melasma following parturition.

Materials and methods

Trial and study design

This randomized clinical trial was carried out in the midwifery wards of different hospitals, in Abadan, Iran, between November 2018 and May 2019 and registered in the Iranian registry of clinical trials (IRCT registration number: IRCT20180116038386N1). A total of 50 pregnant women, aged 18-40 years, were selected and randomly categorized into two (treated and control) groups. Inclusion criteria included mothers who suffer from melasma. However, those who were exposed to direct sunlight and underwent systemic steroid therapy, as well as mothers suffering from skin diseases such as rash and acne and also suffering a systemic disease with dermatological signs, i.e. hepatitis C and lupus erythematosus, were excluded. Consent forms were received

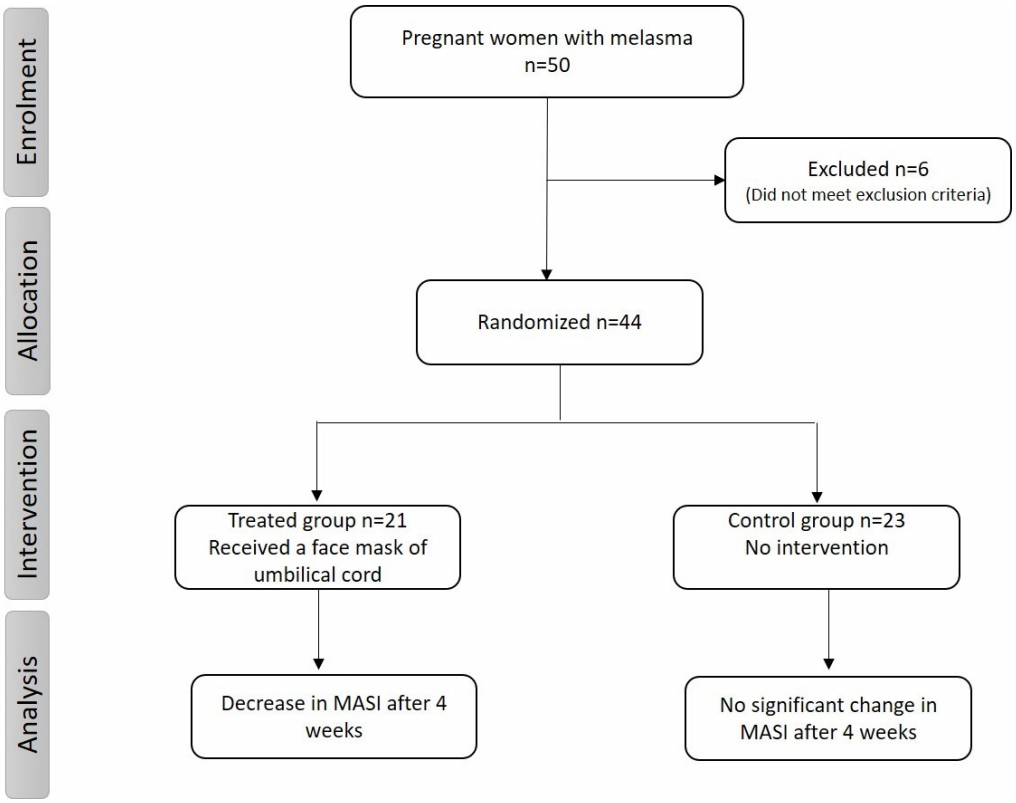

FIGURE 1. Clinical trial flow chart

from all participants before the initiation of the intervention. The detailed information of the participants is represented in Figure 1. The protocols of this study were approved by the Research Ethics Committee of Abadan University of Medical Science (IR.ABADANUMS.REC.1396.245).

Preparation of umbilical cord face mask

Immediately following parturition, a section (10-20 cm) of the baby's umbilical cord was cut horizontally using a scalpel blade, placed in a Petri dish, and washed with normal saline, all in sterile conditions. The pieces of flattened cord, as face masks, were placed on the hyperpigmentation area on the face of mothers in the treated group for one hour. After massaging onto the face using sterile gloves, the mask was removed, and the remaining Wharton's jelly was spread on the skin until its complete dryness. Just a single intervention was carried out for the treated group; however, another group, the control, received no intervention. The time interval for evaluating the improvement of melasma in the aforementioned mothers was four weeks. All subjects were visited before and four weeks after the parturition.

Clinical evaluations

Melasma area severity index (MASI) was used to measure hyperpigmentation, which was assessed by two research assistants independently. For calculating MASI, the face was divided into four regions, including forehead, right molar, left molar, and chin. Moreover, a numeric value of 0-6 was assigned to each region based on the area of involvement. The severity of darkness and homogeneity of melasma related to each region were also graded on a scale from 0 to 4. Then MASI was calculated for all participants before and four weeks after intervention according to the following formula:

$$MASI = 0.3\ (DF + HF)\ AF + 0.3\ (DMR + HMR)\ AMR + 0.3\ (DML + HML)\ AML + 0.1\ (DC + HC)\ AC$$

Where F, MR, ML, and C denote forehead, right malar, left malar (all 30%), and chin (10%), respectively; D indicates darkness, with a scale from 0 (absent) to 4 (severe); H implies homogeneity, with a five-point scale ranging from 0 (absent) to 4 (maximum); A shows area involvement, from 0 (no involvement) to 6 (90–100% involvement) (Lee et al., 2006). In the end, the value of the total facial score achieved was between 0 and 48.

Statistical analysis

The following standard formula recommended for the clinical trial was used to calculate the sample size by considering type one error (α) of 0.05 and type two error (β) of 0.2 (power = 80%).

$$n = \frac{(z_{1-\alpha/2}+z_{1-\beta})^2[p_1(1-p_1)+p_2(1-p_2)]}{(p_1-p_2)^2}$$

Based on previous studies, P1 = 0.2 and P2 = 0.56 were considered, and a sample size of 25 subjects per group was calculated.

All data were analyzed using SPSS software (version 16, USA). Kolmogroph-Smirnoph test was employed to determine the normality of the data in each group. Using a paired t-test, the value of MASI before and after treatments was compared. Also, the MASI score between the control and treated groups was compared, and an independent t-test was used to compare the MASI score between the control and treated groups. A significant difference was defined at a P-value <0.05.

Results

Characteristics of the subjects

The average age of the volunteers was 27.93 ± 4.69 years (age range: 20-40 years). All participants confirmed the avoidance of direct sunlight exposure one month after the parturition. None of the subjects was under any therapy for hyperpigmentation. Six participants dropped out of the study, and the survey was carried out with 46 participants.

Effect of umbilical cord face mask on melasma

The effects of the umbilical cord containing MSCs on the melasma in the parturients were determined using MASI. Of 50 subjects included, 44 completed the study. In addition, six participants dropped out of the study owing to either an intercurrent illness or failure to meet protocols. The mean of MASI in the control group (n = 23) and treated group (n = 21) in the time of parturition were 4.26 ± 0.69 and 5 ± 0.66, respectively [IC95% -1.21 to 2.7, P>0.05]. There was no significant difference between the women treated with the umbilical cord and those who received no treatment. The MASI of participants with no intervention was 4.1 ± 0.68, while that of those treated with umbilical cord was 0.77 ± 0.22 one month after giving birth [IC95%: -4.8 to -1.88, P= 0.000]. Also, no significant difference was observed between the control group and the group treated with umbilical cord one month after delivery. The result of the t-test demonstrated a significant difference between the subjects of the treated group before and after treatment with umbilical cord (P < 0.05; Figure 2). No side effect

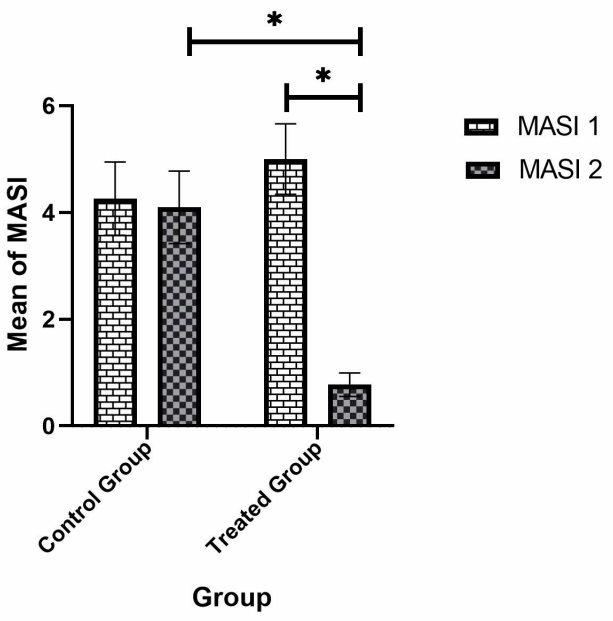

FIGURE 2. Effect of umbilical cord mask on melasma
There is no significant difference between the mean of MASI in the treated and the control groups at the time of parturition. However, there is a significant difference between the control group and the group treated with umbilical cord one month after giving birth (P< 0.05). Also, there is a significant difference between the treated and control groups, as well as in the value of MASI before and after intervention in each group (P< 0.05). MASI 1 = MASI before intervention, MASI 2 = MASI one month after intervention, *P<0.05 considered as significantly different between different groups. Results are represented as mean ± SEM of MASI obtained from all participants in each group.

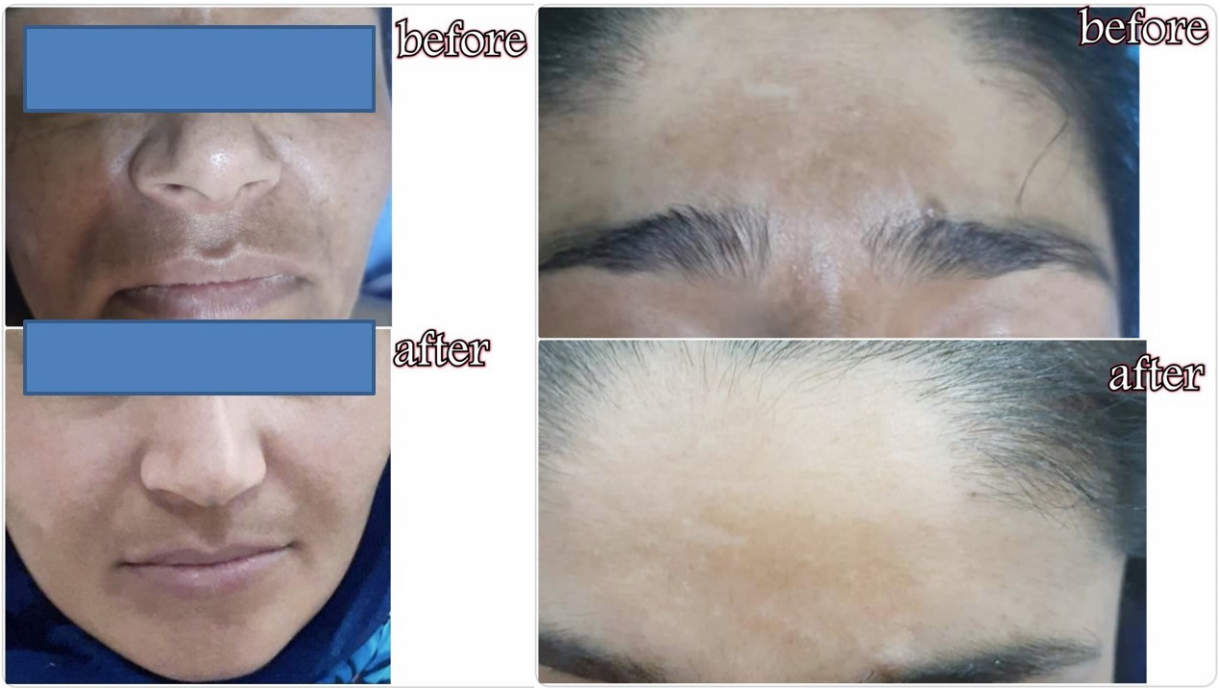

FIGURE 3. Image of participants treated with umbilical cord mask
The hyperpigmentation of patients treated with umbilical cords decreased one month after the intervention. The top left photo is the picture of a 30-year-old mother before the intervention, and the bottom left is the same mother four weeks after intervention. The top right photo is the picture of a 27-year-old mother before the intervention, and the bottom right is the same mother four weeks after intervention.

FIGURE 4. Image of participants with no intervention (control group)
The hyperpigmentation of a patient in the control group showed no difference one month after parturition. At the top, the picture of a 29-year-old mother with no intervention is shown. The picture at the bottom illustrates the same mother after four weeks without intervention.

or complication was reported in the treated group.

Discussion

Since the umbilical cord is discarded as a waste material after birth, various parts of the cord are considered for MSC isolation in clinical applications (Sivakumaran et al., 2018; Teresa Conconi et al., 2011). Melasma is a chronic pigmentary disorder that occurs in partu-

rients and can benefit from the therapeutic potential of the umbilical cord. Pregnant women have a higher risk of developing melasma, which is often characterized by asymmetrical brown patches on different areas of the face, including the cheeks, forehead, upper lip, nose, and chin (Grimes et al., 2019; McKesey et al., 2019).

For the first time in the present study, umbilical cords were used to accelerate the treatment of melasma in pregnant women. As a semi-quantitative index, MASI was employed to measure melasma. The results showed a significant decrease in MASI of patients treated with umbilical cord as compared to the untreated group (Figs. 2 and 3). Reduction in MASI after treatment with an umbilical cord can be probably due to the presence of MSCs in fresh samples obtained immediately after parturition. MSCs can affect the body's cells through varied mechanisms, comprising cell-to-cell contact, material transfer, integration and differentiation, release of soluble factors, and exosome secretion (Bajetto et al., 2017). Recently, MSCs have been used in the treatment of skin disorders in animal models and clinical trials. In this regard, Chen et al. have reported the treatment of two *Psoriasis vulgaris* cases by umbilical cord-derived MSCs. According to their results, there were no symptoms of psoriatic relapse after about five years (Chen et al., 2016). Moreover, Shin et al. have reviewed the effect of MSCs on inflammatory skin diseases and indicated that MSCs have regulatory effects on immune responses under inflammatory conditions (Shin et al., 2017). Jackson and colleagues have also utilized bone marrow-derived MSCs in treating dermal fibrosis in a mouse model. Their finding displayed inhibiting the formation process of bleomycin-induced skin fibrosis, alleviating inflammation, and remodeling extracellular matrix by bone marrow MSCs therapy (Wu et al., 2014).

Various methods have been established to enhance the efficiency of MSC-based therapy for skin disorders. Using scaffolds, MSC preconditioning, and genetic modification are some approaches applied to increase the delivery, proliferation, and migration of MSCs (Ezquer et al., 2017; Shojaei et al., 2019). In the current study, we used a fresh umbilical cord, as a natural scaffold containing alive MSCs, immediately after giving birth to treat melasma in women. We also used for the first time the umbilical cord as a face mask for the treatment of melasma in parturients.

The use of MSC isolated from umbilical cord jelly or cord blood can provide more information on the beneficial effects of this treatment. This clinical trial demonstrates that the umbilical cord is not only safe but also well-tolerated in parturients suffering from melasma. One limitation of our study was the absence of the placebo group because of the inability to prepare a face mask similar to the umbilical cord in terms of appearance. Although there are some other limitations in this study, such as the lack of long-term follow-up, small sample size, and the lack of a quantitative index, the achieved data showed the beneficial effect of this treatment.

Conclusions

The findings of the current investigation suggest that the fresh umbilical cord, as a natural face mask containing MSCs, can accelerate the amelioration of melasma. However, more research is needed to determine the molecular mechanism of the MSC effect on hyperpigmentation and also the efficiency enhancement of MSC-based therapy.

Acknowledgments

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Ethical statement

This study was registered in the Iranian registry of clinical trials (IRCT registration number: IRCT20180116038386N1) and approved by the Research Ethics Committee of Abadan University of Medical Science (IR.ABADANUMS.REC.1396.245). This study was financially supported by Abadan University of Medical Sciences (Grant No. 96ST-496)

Conflict of interest

The authors declared they have no potential conflict of interest.

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