

# A systematic review of intracavernosal injection of mesenchymal stem cells for diabetic erectile dysfunction

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pISSN: 0853-1773 • eISSN: 2252-8083  
<https://doi.org/10.13181/mji.oa.204475>  
**Med J Indones. 2021;30:96–105**

**Received:** January 06, 2020  
**Accepted:** September 03, 2020  
**Published online:** May 19, 2021

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#### ABSTRACT

**BACKGROUND** As current erectile dysfunction (ED) treatments are limited, other treatment such as stem cells should be explored. Hence, this study aimed to review the sources, method of administration, and therapeutic effect of mesenchymal stem cells (MSCs) for diabetic ED treatment.

**METHODS** All relevant articles regarding the use of MSCs for diabetic ED were searched in PubMed and Google Scholar databases from December 15, 2019 to January 1, 2020 published in the past 10 years. The keywords were “mesenchymal stem cells” and “diabetic ED”. The selection and critical appraisal of the studies were discussed. Diabetic ED was evaluated for functional and structural outcome. Functional outcome in animal studies was assessed by intracavernosal pressure/mean arterial pressure (ICP/MAP) ratio, meanwhile the structural outcome was done microscopically. In human study, the assessments were done using international index of erectile function score (IIEF-5) to erection hardness score and penile Doppler ultrasonography.

**RESULTS** There were 10 animal studies and 3 human studies. The studies used MSCs from adipose (n = 6), bone marrow (n = 4), placenta (n = 1), umbilical cord (n = 1), and muscle tissue (n = 1). The MSCs were administrated through intracavernosal injection in all studies. In all animal studies, functional outcome was improved, shown in higher ICP/MAP ratio. Microscopically, there were an increase of cavernosal endothelial cells, vascular endothelial growth factor, nitric oxide synthase, and smooth muscle cells. In human studies, IIEF-5 and erection hardness score were improved. Peak systolic velocity was also higher.

**CONCLUSIONS** MSCs may be a promising therapy for diabetic ED; however, long-term safety concerns still need further investigations.

**KEYWORDS** diabetes mellitus, erectile dysfunction, mesenchymal stem cells

Erectile dysfunction (ED) is a complex condition of persistent inability to attain and maintain an erection sufficient for satisfactory sexual performance.<sup>1</sup> The risk of ED is 2-fold to 3-fold higher in men with diabetes mellitus (DM) than in healthy men.<sup>2,3</sup> Moreover, it has become the second-most common diabetes-related complication.<sup>4</sup> Hence, a comprehensive management should comprise treatments of diabetic ED.

ED is mainly treated with various modalities, such as oral therapy, vacuum devices, penile intracavernosal

(IC) injection, shockwave therapy, and penile prosthesis implantation surgery.<sup>5</sup> Despite the wide range of efficacy and satisfaction rate, these well-established treatments have limitations.<sup>6</sup> Phosphodiesterase type 5 (PDE5) inhibitors, the first-line oral therapy, have safety issues for patients with cardiovascular problems. Several studies showed PDE5 inhibitors are ineffective.<sup>5</sup> Vacuum devices also have low satisfaction rates (35–50%)<sup>7,8</sup> with several adverse events, including the inability to ejaculate, bruising, and numbness.<sup>9</sup>

Occasionally, it also causes extensive damage which reduces response to oral or local pharmacotherapy in diabetic ED patients.<sup>10</sup> Nevertheless, novel and promising treatments should be sought.

Stem cell therapy has been proposed as a promising treatment for ED that can regenerate damaged penile neurovascular and endothelial tissues. Several studies have investigated the potential therapeutic effects of stem cells from various origins and populations,<sup>6</sup> but mesenchymal stem cells (MSCs) are more advantageous because they are multipotential, easily accessible, and expandable; however, they pose genomic stability and ethical issues.<sup>11,12</sup> Several experimental studies in China and South Korea have demonstrated that MSCs therapy improves erectile function.<sup>12</sup>

Studies on the efficacy of MSCs in diabetic ED treatment are still limited. Therefore, research and advancement on MSCs may contribute to the establishment of a better diabetic ED treatment. This study aimed to systematically review the sources, transplantation method, and therapeutic effect of MSCs injection for diabetic ED treatment.

## METHODS

The population of this review was subjects (human or animals) with diabetic ED, and the intervention was

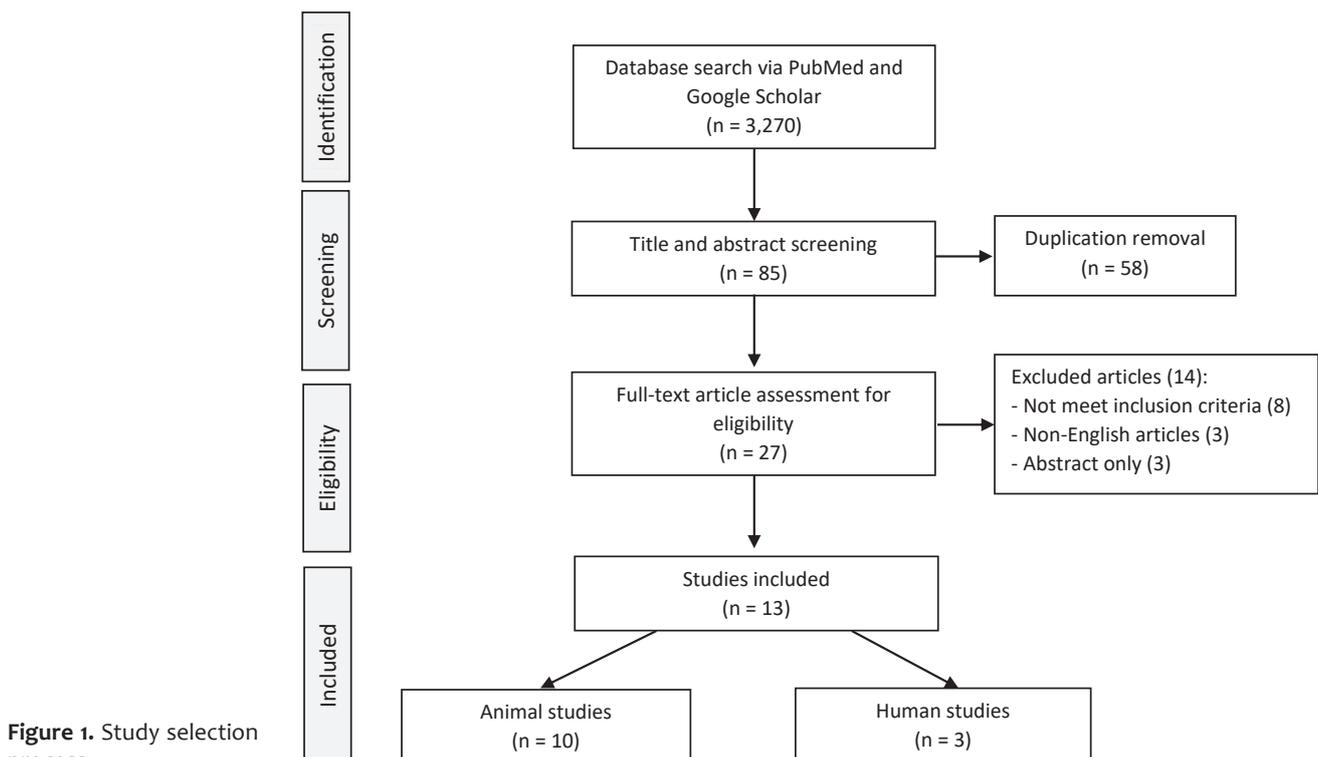
MSCs injection. The comparison was subject with no intervention or placebo. The outcome was divided into functional and structural outcome. The functional outcome was the improvement of erectile function which was assessed by international index of erectile function (IIEF) score, intra corporal pressure and mean arterial pressure (MAP) evaluation, and peak systolic velocity (PSV) evaluated by penile ultrasonography, meanwhile structural outcome was assessed using biomolecular or histochemistry parameters which included vascular endothelial growth factor (VEGF) expression, neuronal nitric oxide synthase (nNOS), and cavernosal smooth muscle cells (CSMCs)/collagen contents.

### Literature search strategy

A literature search was performed on electronic databases, including PubMed and Google Scholar from December 15, 2019 to January 1, 2020. The search was limited to articles written in English and published from 2010 to 2020. The keywords used were “mesenchymal stem cells” and “diabetic ED”.

### Study selection

The articles were independently appraised by two reviewers (F and GA), and a discussion was conducted to determine the selected articles for this review. The



**Figure 1.** Study selection process

Table 1. Summary of animal studies

First author, year	Models/number of samples/treatment/control	Type of ED	Stem cell source/dose of injection cell numbers	Methods of administration	Follow-up	Function	Outcome
Garcia, <sup>13</sup> 2010	Rats/20/10 ADSC group/10 control with PBS injection	ZDF-induced diabetic ED	Autologous adipose tissue/ $1 \times 10^6$ cells	Intracavernosal	3 weeks	Mean ICP/MAP ratio* in the ADSC group (0.55) vs. control group (0.24), $p = 0.0004$  No inflammatory changes in the local injection site	Decreased apoptosis evaluated by mean number of TUNEL positive cells in the ADSC group (36.7) vs. control group (156.4), $p < 0.0001$  Lower caspase-3 expression level <sup>†</sup> in the ADSC group (5) vs. control group (7), $p < 0.05$  Mean nNOS staining <sup>‡</sup> of the dorsal nerve in the penile midshaft specimen in the ADSC group (484.0 pixels) vs. control group (301.8 pixels), $p < 0.0001$
Qiu, <sup>14</sup> 2011	Rats/30/9 MSC group /10 DM control with PBS and 8 nondiabetic control	STZ-induced diabetic ED	Bone marrow/ $4 \times 10^5$ cells	Intracavernosal	4 weeks	Mean ICP/MAP ratio* in the DM control group (0.4) vs. nondiabetic control group (0.8), $p < 0.01$ ; vs. MSC group (0.55), $p < 0.05$	Mean vWF positive area/total area <sup>§</sup> in the DM control group (0.0034) vs. nondiabetic control group (0.0069), $p < 0.01$ ; vs. MSC group (0.0047), $p < 0.05$  Mean $\alpha$ -SMA positive area/total area <sup>¶</sup> in the DM control group (0.040) vs. nondiabetic control group (0.087), $p < 0.01$ ; vs. MSC group (0.059), $p < 0.05$
Sun, <sup>15</sup> 2012	Rats/40/10 MSC group/10 DM control, 10 with MSC-conditioned medium group, 10 nondiabetic group	STZ-induced diabetic ED	Bone marrow/ $5 \times 10^5$ cells	Intracavernosal	12 weeks	Mean ICP/MAP ratio* in the DM control group (0.3) vs. MSC group (0.65) vs. medium group (0.45) vs. nondiabetic control group (0.8), $p < 0.05$	Mean nNOS/area <sup>†</sup> of dorsal nerve in the DM control group (0.5 pixels) vs. MSC group (0.9 pixels) vs. medium group (0.6 pixels) vs. nondiabetic control group (1.4 pixels), $p < 0.05$
Liu, <sup>16</sup> 2013	Rats/60/12 ADSC group/12 with lentivirus-VEGF, 12 with VEGF/GFP expressing ADSC, 12 PBS group, 12 normal group	STZ-induced diabetic ED	Allogeneic adipose tissue/ $1 \times 10^6$ cells	Intracavernosal	4 weeks	Mean ICP/MAP ratio* in the PBS group (0.2) vs. ADSC group (0.5) vs. lentivirus-VEGF group (0.5) vs. VEGF expressing ADSC group (0.7) vs. normal group (0.8), $p < 0.05$	Mean VEGF/beta-actin expression** in PBS group (0.1) vs. ADSC group (0.4) vs. lentivirus-VEGF group (0.3) vs. VEGF expressing ADSC group (0.7) vs. normal group (1.2), $p < 0.05$

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Table 1. (continued)

First author, year	Models/number of samples/treatment/control	Type of ED	Stem cell source/dose of injection cell numbers	Methods of administration	Follow-up	Function	Outcome
He, <sup>17</sup> 2014	Rats/35/9 KCNMA1-MSC group/9 with empty vector lentivirus transfected MSC/ null-MSC, 9 with nontransfected MSC, 8 control with PBS	STZ-induced diabetic ED	KCNMA1 gene modified bone marrow/1 x 10 <sup>6</sup> cells in 200 µl PBS	Intracavernosal	2 weeks	Mean ICP/MAP ratio* in PBS control group (0.34) vs. MSC group (0.46) vs. null-MSC (0.47) vs. KCNMA1-MSCs group (0.65), p<0.05	Mean grey value of positive expression of KCNMA1 <sup>+</sup> in DMED + KCNMA1-MSCs group (141.62), p<0.05; DMED + MSCs (103.26) bp; DMED + null-MSCs group (97.34), p>0.05; DMED+PBS group (67.18) bp, p<0.05
Liu, <sup>18</sup> 2015	Rats/40/10 ADSC group/10 with ADSC + HGF group, 10 with PBS, 10 sham/normal group	STZ-induced Diabetic ED	Autologous adipose tissue/2 x 10 <sup>6</sup> cells	Intracavernosal	4 weeks	Mean ICP/MAP ratio* in sham group (0.86) vs. PBS group (0.26), p<0.05; ADSC group (0.44) vs. ADSC + HGF group (0.74), p<0.05	Increased in mean SMCs/collagen ratio <sup>††</sup> in ADSC + HGF group (0.5) vs. ADSC group (0.35) vs. PBS group (0.15) vs. sham group (0.6), p<0.05 Decreased mean apoptotic index in ADSC + HGF group (0.125) vs. ADSC group (0.175) vs. PBS group (0.28) vs. sham group (0.05), p<0.05
Kovanecz, <sup>19</sup> 2016	Rats/40/8 obese with early diabetic MDSC group/8 obese with early diabetic under high glucose condition stem cells, 8 obese with late diabetic MDSC, 8 obese untreated group, 8 non-obese untreated group	Diabetic ED	Muscle derived/1 x 10 <sup>6</sup> cells	Intracavernosal	8 weeks	Increased mean ICP* in early diabetic MDSC (70 mmHg) vs. early diabetic under high glucose condition group (50 mmHg) vs. late diabetic group (10 mmHg) vs. obese untreated group (10 mmHg) vs. non-obese untreated control group (80 mmHg), p<0.01	Increased mean SMCs/collagen ratio <sup>††</sup> in early diabetic MDSC (0.12) vs. early diabetic under high glucose condition group (0.11) vs. late diabetic group (0.09) vs. obese untreated group (0.07) vs. non-obese untreated control group (0.12), p<0.001
Zhu, <sup>20</sup> 2017	Rats/36/10 ADSC group/10 magnetic field application ADSC group, 8 diabetic with PBS group, 8 normal group	Diabetic ED	Adipose tissue/1 x 10 <sup>6</sup> cells	Intracavernosal	4 weeks	Increased mean ICP/MAP ratio* in ADSC group (0.6) vs. magnetic field application ADSC group (0.75) vs. diabetic with PBS group (0.3) vs. normal group (0.9), p<0.05	Mean VEGF release/field** in ADSC group (6%) vs. magnetic field application ADSC group (7.5%) vs. diabetic with PBS group (1%) vs. normal group (4%), p<0.05

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Table 1. (continued)

First author, year	Models/number of samples/treatment/control	Type of ED	Stem cell source/dose of injection cell numbers	Methods of administration	Follow-up	Function	Outcome
Zhou, <sup>21</sup> 2016	Rats/45/10 DM + ADSC group/10 DM + ADSC + insulin group, 10 DM + insulin group, 10 DM+PBS injection, 5 untreated control group	Diabetic ED	Autologous adipose tissue/1 × 10 <sup>6</sup> cells	Intracavernosal	4 weeks	Mean ICP/MAP ratio* in the DM + ADSC group (0.55) vs. DM + ADSC + insulin group (0.75) vs. DM + insulin group (0.58) vs. DM + PBS group (0.35) vs. control group (0.85), p<0.05	Increased mean VEGF/GAPDH ratio** in the DM + ADSC group (0.2) vs. DM + ADSC + insulin group (0.3) vs. DM + insulin group (0.18) vs. DM + PBS group (0.1) vs. control group (0.3), p<0.05  Increased nNOS/GAPDH* in the DM + ADSC group (0.58) vs. DM + ADSC + insulin group (0.77) vs. DM + insulin group (0.56) vs. DM + PBS group (0.3) vs. control group (0.8), p<0.05
Chen, <sup>22</sup> 2017	Rats/32/8 ADSC group/8 ADSC derived EXO group, 8 control with PBS, 8 normal control group	Diabetic ED	Adipose tissue/1 × 10 <sup>6</sup> cells	Intracavernosal	4 weeks	Mean ICP/MAP ratio* in the ADSC group (0.6) vs. ADSC derived EXO group (0.6) vs. PBS group (0.3) vs. normal control group (0.08), p<0.05	Increased mean SMCs/collagen <sup>††</sup> in the ADSC group (8.5%) vs. ADSC derived EXO group (9%) vs. PBS group (4%) vs. normal control group (14%), p<0.05  Increased mean CD31(+) area/tissue area <sup>§§</sup> in the ADSC group (2.4%) vs. ADSC derived EXO group (2.5%) vs. PBS group (0.5%) vs. normal control group (5%), p<0.05

α-SMA=alpha smooth muscle actin; ADSC=adipose derived stem cells; DM=diabetes mellitus; DMED=diabetes mellitus-induced erectile dysfunction; ED=erectile dysfunction; EXO=exosome; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; GFP=green fluorescent protein; HGF=hepatocyte growth factor; MAP=mean arterial pressure; MDSC=muscle derived stem cells; MSC=mesenchymal stem cell; nNOS=neuronal nitric oxide synthase; PBS=phosphate buffer saline; SD=standard deviation; SMC=smooth muscle cell; STZ=streptozocin; TUNEL=terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; VEGF=vascular endothelial growth factor; vWF= von Willebrand factor; ZDF=Zucker diabetic fatty  
\*High ICP/MAP ratio means better erectile function; †lower caspase-3 level expression means decrease apoptosis; †high vWF positive area/total area means increased content of endothelial cell; †high α-SMA/total area means increased content of cavernous smooth muscle cell; \*\*high VEGF means increased content of endothelial cell; ††high KCNNMA1 means better erectile function; ††high SMC/collagen ratio means better erectile function; †††increased CD31(+) area/tissue area means increased content of endothelial cell

**Table 2.** Summary of human studies

First author, year	Number of samples (experimental/control)	Type of ED	Stem cell source/cell numbers	Method of administration	Follow-up	Outcome
Bahk, <sup>23</sup> 2010	10 (7 experimental group/3 control with normal saline injection)	Diabetic ED	Allogeneic human umbilical cord/1.5 x 10 <sup>7</sup> cells	Intracavernosal	11 months	<p>IIEF score.* (UC-MSC vs. control group)            Question "How often get erection"            Baseline: 1 vs. 1; 6<sup>th</sup> month: 2–3 vs. 1; 11<sup>th</sup> month: 1–3 vs. 1            Question "How often able to penetrate"            Baseline: 1 vs. 1; 6<sup>th</sup> month: 1–3 vs. 1; 11<sup>th</sup> month: 1–3 vs. 1</p> <p>GAQ "Did stem cell improve your erection?"            Without sildenafil citrate: "Yes" 3 experimental subjects vs. "No" 4 experimental subjects &amp; 3 controls            With sildenafil citrate: "Yes" 5 experimental subjects vs. "No" 2 experimental subjects &amp; 3 controls</p> <p>SEP question "Insert penis into vagina" in the 5<sup>th</sup> month (UC-MSC vs. control group):<sup>†</sup>            1–4 times successful penetration in 4–8 times attempts of sexual intercourse vs. zero/no successful penetration from 2–3 times attempts of sexual intercourse</p>
Levy, <sup>24</sup> 2016	8/8 experimental group/none	Organic ED	Placental matrix derived/1 ml of PM-MSCs in 2 ml of isotonic saline	Intracavernosal	6 months	<p>Improved PSV (range):<sup>‡</sup>            Baseline: 23.1–49.3 cm/s; 6<sup>th</sup> week: 25.5–56.5 cm/s, <math>p &lt; 0.05</math>; 3<sup>rd</sup> month: 32.5–66.7 cm/s, <math>p &lt; 0.05</math>; 6<sup>th</sup> month: 50.7–73.9 cm/s, <math>p &lt; 0.01</math></p> <p>Stretched penile length (range):            Baseline: 12.7–20.3 cm; 6<sup>th</sup> week: 13.3–21 cm, <math>p &gt; 0.05</math>; 3<sup>rd</sup> month: 15–19.5 cm, <math>p &gt; 0.05</math>; 6<sup>th</sup> month: 15.5–19.5 cm, <math>p &gt; 0.05</math></p> <p>IIEF score (range):.*            Baseline: 21–54; 6<sup>th</sup> week: 21–61, <math>p &gt; 0.05</math>; 3<sup>rd</sup> month: 11–52, <math>p &gt; 0.05</math>; 6<sup>th</sup> month: 23–67, <math>p &gt; 0.05</math></p>
Al Demour, <sup>25</sup> 2018	4/4 experimental group/none	Diabetic ED	Bone marrow autologous/30 x 10 <sup>6</sup> cells, twice injections on baseline point and 1 month	Intracavernosal	12 months	<p>IIEF-15 total score (range):.*            Baseline: 15–25; 3<sup>rd</sup> month: 40–70; 12<sup>th</sup> month: 25–60</p> <p>EHS (range):.*            Baseline: 0–1; 3<sup>rd</sup> month: 4; 12<sup>th</sup> month: 1–4</p>

ED=erectile dysfunction; EHS=erection hardness score; GAQ=global assessment questions; IIEF=International Index of Erectile Function; PM-MSC=placental matrix derived-mesenchymal stem cell; PSV=peak systolic velocity; SEP=sexual encounter profile; UC-MSC=umbilical cord mesenchymal stem cells

\*Higher IIEF score and EHS means better erectile function; †higher frequency of successful penetration shows better erectile function; ‡higher PSV means better penile vascularization and erectile function

inclusion criteria were as follows: clinical trials, studies on humans or animals with MSCs to treat diabetic ED. Articles were then identified by removing duplicate studies and screening the remaining articles. The study selection process is presented in Figure 1.

#### Data extraction

Data extraction from the articles was performed by two authors (FB and GA), and any disagreement was settled by consensus. The relevance of the articles was determined by screening through titles and abstracts. Variables extracted from the articles included the first author's name, year of publication, characteristics, intervention, control, method of administration, and outcome of the study. In case of missing data, original authors of the included articles were contacted for further details or additional data. A critical appraisal was made based on Oxford's Center for Evidence-Based Medicine.

## RESULTS

The titles or abstracts of 3,270 initially identified articles were screened, and duplicate articles were eliminated. After the studies were selected, 13 most relevant full-text papers of human clinical trials ( $n = 3$ ) and animal studies ( $n = 10$ ) were analyzed thoroughly (Figure 1). All animal studies involved streptozocin-induced diabetic rat models. Two human studies included patients with diabetic ED, and one study covered patients with diabetic ED and vascular or neurological disorders. The follow-up duration ranged from 1 week to 12 months after the stem cells were injected. The source of MSCs used were derived from the umbilical cord (UC) ( $n = 1$ ), placenta ( $n = 1$ ), adipose tissue ( $n = 6$ ), bone marrow ( $n = 4$ ), and muscle ( $n = 1$ ). All kind of sources of MSCs showed improvement in both functional and structural outcomes.

The methods of administration were IC in all human studies. Both animal and human studies showed erectile function improvement after MSCs were injected into the ED models. Erectile function was measured through objective evaluation parameters, such as intracavernosal pressure/mean arterial pressure (ICP/MAP) ratio, PSV by Doppler ultrasound, personal questionnaire IIEF, erection hardness score (EHS), sexual encounter profile, and global assessment questions. All animal studies revealed an improvement in the ICP/MAP ratio. Three human

studies found an increased IIEF score after the stem cell therapy compared with that in the control groups. One study indicated a decrease in blood glucose levels. The characteristics and results of each study are summarized in Table 1 and 2.

## DISCUSSION

The current systematic review examined 10 animal studies and 3 human studies. Overall, all the selected studies showed similar functional outcomes on the improvement of erectile function after the intervention. This improvement was mostly evaluated by the penile ICP/MAP ratio in animal studies, and the increase was ranged 2–3-fold compared with control group. High blood glucose is known to cause dysfunction and reduction in the cavernous nerve, endothelial cells (ECs), and CSMCs because of a decrease in VEGF.<sup>26</sup> These combined mechanisms promote the reduction of the ICP/MAP ratio in diabetic ED patients. The concept of MSCs therapy is based on the transplanted stem cells that may stimulate the regeneration of hosts' own ECs and SMCs or restore proper interactions between ECs and SMCs through a paracrine effect, thus finally improved the erectile function which showed in reported studies.<sup>27-31</sup>

All kind of MSCs' sources used in the included studies showed an improvement in functional and structural outcomes. Adipose tissue was the most used stem cell sources in present study. The multipotency of adipose-derived stem cells (ADSCs) are useful for repairing muscle tissues and improving wound vascularization. However, studies reported that only a few ADSCs could be found after IC injection for ED even the function was improved in the subjects.<sup>13,16,18,20-22</sup> Several studies have discussed that the source of stem cells is selected based on the safety, ethical issues, proliferation rate, immunomodulatory properties, immunosuppressive cytokine secretion, and potential immunogenicity.<sup>32</sup> Thus, human UC is a better MSCs source. The collection of umbilical cord MSCs (UC-MSCs) as a residual waste is safe and noninvasive, so its usage has no ethical issues. Furthermore, it is also easily accessible and cost-effective.<sup>33,34</sup>

UC-MSCs can be produced in large amounts and have high proliferation rates compare with bone marrow MSCs (BM-MSCs). They grow 1.33- and 1.75-fold faster than adipose tissue stem cells.<sup>33,34</sup> However, the use of UC-MSCs requires an observation of the donor's

health condition through genomic and chromosomal evaluation. In addition, preventive measures involving the screening of infectious diseases (HIV, hepatitis B, and cytomegalovirus) should be implemented in a standardized laboratory to avoid infection transmission risk from the UC donor. Meanwhile, the other sources of MSCs allowed physicians to directly examine the health condition of a donor and make a decision.<sup>33,34</sup>

Kovanecz et al<sup>19</sup> implanted muscle derived stem cells (MDSC) to ameliorate ED in rat models. It showed that MDSCs are also promising because of the easy access and capacity to differentiate into neural, angiogenic, and other lineages via paracrine and juxtacrine. However, it reported that repair capacity of MDSC depended on the noxious tissue milieu affecting the skeletal muscle source. Long-term hyperglycemia and severe dyslipidemia might impair the quality of stem cells. Besides, the immune rejection risks should be concerned.<sup>22</sup> In addition, Levy et al<sup>24</sup> showed that placental matrix derived MSC (PM-MSC), which came from the chorionic layer of amniotic sac, also caused a vasculogenesis increase and improvement of blood flow to penis, and the effect sustained for 6 months.

Modified stem cells were used recently to improve the outcome of stem cells therapy. An included study used KCNMA1 gene which expression can cause functional ion channel-mediated intracellular K<sup>+</sup> outflow, membrane hyperpolarization, and cell excitability reduction. The effect of MSC modified by KCNMA1 into penile tissue for treatment of diabetic ED showed greater improvement in erectile function and longer effect compared with MSC therapy.<sup>20</sup> Liu et al<sup>16</sup> used lentivirus expressing VEGF as one of the treatments in their study. Infectious units of lentivirus-VEGF were added and incubated with ADSCs; then, the virus-containing medium was replaced with a normal culture medium. Erectile function improvement was shown in subjects injected with lentivirus-VEGF. However, the treatment effect was still lower compared with the modified ADSCs-VEGF injection. Random insertional mutagenesis should also become safety concerns associated with lentivirus.<sup>19</sup> Despite the variety of stem cells sources, the personal preferences and previous experiences on certain sources may play a greater role.<sup>32,34</sup>

IC injection is the most common method of administration. All animal and human studies used IC as the stem cell delivery route. The efficacies of different routes for stem cell transplantation include

intraperitoneal, intravenous (IV), or IC injection which have also been discussed in other studies.<sup>30,35,36</sup> A study demonstrated that the intraperitoneal infusion of stem cells was less efficacious than IC injection, although the erectile function of both groups was improved compared with baseline values.<sup>37</sup> It was also shown that less than 1% of stem cells infused via the IV route, reached the target tissue, and dissipated after a few days.<sup>38</sup> IV transplantation of stem cells worked through paracrine effects by triggering the endogenous mechanisms of regeneration rather than trans-differentiation into different cell types.<sup>39,40</sup>

Bahk et al<sup>23</sup> conducted a clinical trial and administered UC-MSCs in patients with type 2 DM and ED via IC injection. They injected  $15 \times 10^6$  cells to each patient. In their procedure, the base of the penis was clamped with a band, the cells were then injected, and the clamp was opened after 30 min. The condition of seven patients improved when the treatment was combined with PDE5-inhibitors.<sup>23</sup> Al Demour et al<sup>25</sup> applied the IC injection of BM-MSCs and showed that the IIEF-15 ( $p < 0.05$ ) and EHS improved ( $p < 0.05$ ) after the patients were given injections twice; each injection was composed of  $30 \times 10^6$  cells/4 ml of 0.9% NaCl and placed into a 1 cc sterile syringe. These studies were well tolerated and reported no adverse events. However, none of the included studies compare different transplantation methods all at once in one study.

Qiu et al<sup>14</sup> conducted an *in vivo* animal study and demonstrated that MSCs can differentiate into cells that express smooth muscle and endothelial markers, such as alpha-smooth muscle actin, calponin, von Willebrand factor, and CD31, 4 weeks after IC injection. Garcia et al<sup>13</sup> evaluated erectile function for ICP/MAP ratio in diabetic ED rats and showed that MSC treatment helped improving erectile function which possibly mediated by the decreased intracorporeal apoptosis and the increased number of ECs. It is suggested that MSCs can exert a paracrine effect on the surrounding penile tissue and release cytokines and growth factors.<sup>41</sup> Liu et al<sup>18</sup> also found that the concentrations of VEGF significantly increased after MSCs were transplanted in diabetic rats; consequently, the recovery of endothelial and erectile function was promoted.

The VEGF enhances the ability of ECs to produce nitric oxide synthase (NOS) isoforms, which regulate the relaxation of cavernous smooth muscle and induce the significant recovery of erectile function.<sup>42</sup> In a study, the staining of the neurofilament and

nNOS showed that the neuroregeneration or nerve preservation of the group that received stem cell injection was greater than that of the control group.<sup>43</sup> Liu et al<sup>18</sup> also demonstrated that eNOS was significantly expressed in diabetic rats with improved erectile function after MSC injection. Another experimental study involved IC BM-MSCs showed that the erectile function improved as a result of an increase in endothelial and IC smooth muscle and an increase in neuronal markers for nNOS and neurofilament in the dorsal penis nerve.<sup>15</sup>

Bahk et al<sup>23</sup> performed a human clinical trial on UC-MSC IC injection in seven patients with diabetic ED and found that the patients regained morning erections within 3 months; their erections were maintained for more than 6 months. In addition, blood glucose and glycosylated hemoglobin levels were decreased.<sup>23</sup> Al Demour et al<sup>25</sup> also conducted a human clinical trial and reported that the erectile function, sexual desire, intercourse satisfaction, and overall satisfaction of four patients with diabetic ED significantly improved after the IC injection of BM-MSCs, as indicated by their IIEF score. Furthermore, the VAS pain score ranged from 2 to 4 during IC injection, indicating that the procedure was well tolerated. No severe adverse events related to nervous, cardiovascular, respiratory, and gastrointestinal systems were reported.<sup>25</sup>

Most studies on stem cell therapy for ED have reported positive structural and functional outcomes. Multiple mechanisms likely occur simultaneously to achieve both outcomes. They consist of increased VEGF, eNOS, and nNOS expression; increased cytokines and chemokines, paracrine effects, and differentiation into various cell types in the corpora cavernosa; and increased CSMCs. Moreover, NO stimulates and activates the NO/cyclic guanosine monophosphate pathway.<sup>13,14,41,42</sup> The production of cytokines and chemokines may prolong the life span of cavernosal component cells under pathological conditions and decrease cell apoptosis.<sup>16,24,44</sup>

The limitation of this study includes the possibility of other available data on animal and human studies that have not yet been reported or those that are not available in English or on online databases. There was also heterogeneity observed among the studies, which may be explained by study design, different inclusion criteria, confounding risk factors, various follow-up times, and the different locations of the

studies. Thus, no quantitative analysis was done, resulted to the possibility of incomplete outcomes evaluation and potential bias.

In conclusion, this systematic review reveals that the IC injection of MSCs is a promising and novel therapy for diabetic ED. Short-term follow-up studies have shown its beneficial outcomes for improving erectile function and being well-tolerated therapy without any severe complications. However, long-term studies are still limited. Similar to other biological therapies, the IC injection of MSCs poses safety concerns, such as immunogenicity, tumorigenicity, and other complications. Therefore, these aspects should be explored further through large and multicenter investigations to apply this treatment in clinical practice.

#### Conflict of Interest

The authors affirm no conflict of interest in this study.

#### Acknowledgment

None.

#### Funding Sources

None.

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