Effect of Mesenchymal Stem Cells on Diabetic Nephropathy in Experimental Animals

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Abstract

Background: Diabetic nephropathy is the most common cause of end-stage renal disease in the world, and could account for disability and high mortality rate in patients with diabetes. MSCs have several advantages for therapeutic use such as ability to migrate to the sites of tissue injury. Successful MSC treatment of diabetic nephropathy could be explained by MSCs competence to differentiate into insulin-producing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells. Taken together, these data indicate that MSC transplantation prevents the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals.

Aim: The present study aimed to clarify the effect of mesenchymal stem cells on an animal model of diabetic nephropathy.

Material and Methods: The study was conducted on 30 female albino rats divided into five groups: Group I (n=10) healthy female albino rats as a control group, Group II (n=10) female albino rats with induced diabetic nephropathy. Group III (n=10) female albino rats with induced diabetic nephropathy treated with MSCs. Serum levels of TGFβ & KIM-1 were estimated by ELISA. Histopathology was performed to examine kidney tissue. TGFβ & CD24 were detected immunohistochemically. TGFβ gene expression was detected by real time PCR.

Results: There was improvement of kidney functions and pathology, decreased TGFβ expression and KIM-1 levels after administration of MSCs.

Conclusions: Administration of MSCs exerts a therapeutic effect on diabetic nephropathy.

Key Words: Diabetic nephropathy – MSCs – TGFβ – KIM-1 – CD24.

Introduction

DIABETIC nephropathy is a major complication of diabetes mellitus and is one of the main causes of death among diabetics [1]. In diabetes mellitus the abnormal metabolic environment has a major influence on renal pathologic alterations including tubulo-epithelial cell atrophy, tubulo-epithelial cell hypertrophy, interstitial fibrosis, proliferation of mesangial cells and thickening of glomerular basement [2].

Transforming growth factor-beta 1 (TGF-beta 1) is a fibrogenic growth factor involved in the pathogenesis of kidney damage and is locally produced in the kidney. It has been shown that TGF-beta 1 induces apoptosis of tubular epithelium cells and contributes to progressive renal tubular atrophy [3].

TGF β has been implicated in diabetic nephropathy in both animal models and human. On detection of amount of TGF β protein and TGF β mRNA, they are increased in the glomeruli and in the proximal tubules. Elevated glucose stimulates the production of TGF β in murine mesangial cells, and treatment with anti-TGF β antibodies prevented the effect of hyperglycemia in both vivo and vitro [4].

Kidney injury molecule-1 (KIM-1), originally identified as hepatitis A virus receptor 1 (HAVCR1, also known as Tim-1), is a type 1 transmembrane protein strongly induced by ischemic and toxic insults to kidney. It also plays diverse roles in T and B cell biology [5].

KIM-1 is expressed in chronic kidney disease [6], where it colocalizes with areas of fibrosis and inflammation, and its expression correlates directly with interstitial fibrosis in human allografts [7].

During the past several years, a great deal of attention has been focused on the plasticity of bone marrow–derived mesenchymal stem cells (BMMSC). Traditionally, stem cells were believed
to be lineage restricted and organ specific. However, one study demonstrated that bone marrow-derived stem cells have the ability to cross lineage boundaries and to form functional components of other tissues \[8\].

MSCs administration can prevent and treat diabetic nephropathy, MSCs have been used for the treatment of diabetic nephropathy in nonobese diabetic/severely compromised immunodeficient (NOD/SCID) rats, which develop DM after application of multiple low doses of STZ. About 30-60 days after STZ injection, kidneys of treated mice showed the presence of abnormal glomeruli characterized by increased deposits of ECM protein in the mesangium, hyalinosis, and increased number of macrophages in the glomeruli \[9\].

Successful MSC treatment of diabetic nephropathy could be explained by MSCs competence to differentiate into insulin-producing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells \[9\].

Taken together, these data indicate that MSC transplantation prevents the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals.

**Aim of the work:**

The present study aims to clarify the effect of mesenchymal stem cells on an animal model of diabetic nephropathy.

**Material and Methods**

This work was performed at Medical Biochemistry Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

The study was carried on 30 female albino rats, of an average weight 150-200gm. obtained from an inbred colony at the Kasr Al-Aini Animal Experimental Unit, Faculty of Medicine, Cairo University.

**Experimental animals:**

Rats were bred and maintained in an air-conditioned animal house with specific pathogen-free conditions, and were subjected to a 12:12-h daylight/darkness and allowed unlimited access to chow and water. All the ethical protocols for animal treatment were followed and supervised by the animal facilities, Faculty of Medicine, Cairo University. All animal experiments received approval from the institutional animal care committee. The 30 female albino rats were divided into 3 groups as follow:

- **Group I (Control group):** It consisted of 10 healthy female albino rats.
- **Group II (Diabetic nephropathy group):** It consisted of 10 female albino rats with induced diabetic nephropathy by streptozotocin.
- **Group III (Diabetic nephropathy with MSCs group):** It consisted of 10 female albino rats with induced diabetic nephropathy treated with mesenchymal stem cells.

**Preparation of the animal model:**

**Group II (diabetic nephropathy):** Was induced by a single intra peritoneal injection of streptozotocin (STZ) 60mg/kg body weight dissolved immediately before administration in freshly prepared 0.1mol/L citrate buffer (pH 4.5). Diabetes was defined as random blood glucose reading of >16.7 mmol/L at 3 continuous days after 72 hours of STZ injection \[10\]. Diabetic nephropathy was confirmed after 12 weeks by measuring serum urea and creatinine in blood and also by histopathological changes.

**Group III (diabetic nephropathy + MSCS):** The diabetic nephropathy rats were treated with MSCs in a dose of 106 cells of Bone marrow derived mesenchymal stem cells (BMMSCs) per rat \[11\] given by Intravenous infusion at the rat tail vein.

Four weeks after MSCs injection, each group was subjected to blood sampling.

**Blood sampling:**

Blood samples were collected from 12-14 hours fasting rats, from retro-orbital sinus by heparinized capillary tubes under light ether anaesthesia. Blood was collected in a centrifuge tubes and allowed to clot at room temperature and then centrifuged at 3000 xg for 15 minutes then sera were separated and stored at −20°C up to time of use. The separated sera were analyzed for:

1- Biochemical analysis of glucose, urea and creatinin using Hitachi 917 analyser.
2- Estimation of TGFβ and KIM-1 in serum by ELISA techniques.

**Tissue studies:**

At the planned time of sacrifice, the animals were anaesthetized by light ether anaesthesia. A dorsal midline incision was done with dissection of the muscle to reach renal bed and expose the kidney. Careful dissection was done to the renal
pedicle and it was cut rapidly. The kidney was dissected and washed with phosphate buffer saline then:

The kidney tissue was divided into:
- 1st part was examined histopathologically by haematoxylin and eosine.
- 2nd part was examined for TGFβ and CD24 genes expression by immunohistochemistry.
- 3rd part was examined for gene expression of TGFβ by real time PCR.

Preparation of BM-derived mesenchymal stem cells from rats:

A- Isolation of BM-derived MSCs from rats:

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male white albino rats with Dulbecco’s modified Eagle’s medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine medium (GIBCO/BRL). Nucleated cells were isolated with a density gradient and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL).

Cells were incubated at 37°C in 5% humidified CO₂ for 12-14 days as primary culture or upon formation of large colonies. When large colonies developed (80-90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 minutes at 37°C. After centrifugation (at 2000 xg for 20 minutes), cells were resuspended with serum-supplemented medium and incubated in 50cm² culture flask (Falcon). The resulting cultures were referred to as first-passage cultures[12].

B- Identification of BM-derived MSCs:

MSCs in culture were characterized by their adhesiveness and fusiform shape.

C- RT-PCR Detection of CD29 gene expression:

Total RNA was extracted from cells using RNeasy Purification Reagent (Qiagen, Valencia, CA), and then a sample (1µg) was reverse transcribed with M-MLV (Moloney – Murine Leukemia virus) reverse transcriptase (RT) for 30 minutes at 42°C in the presence of oligo-dT primer. Polymerase chain reaction (PCR) was performed using specific primers (UniGene Rn.25733) forward: 5’-AA TGGTTCAGTGCA GA GC-3’ and reverse: 5’- TTGGGGAT GA TGTCGGGAC-3’. PCR was performed for 35 cycles, with each cycle consisting of denaturation at 95°C for 30 seconds, annealing at 55°C to 63°C for 30 seconds, and elongation at 72°C for 1 minute, with an additional 10-minute incubation at 72°C after completion of the last cycle. To exclude the possibility of contaminating genomic DNA, PCRs were also run without RT. The PCR product was separated by electrophoresis through a 1% agarose gel, stained, and photographed under ultraviolet light.

Quantitation of TGFβ and KIM-1 in the rat serum by ELISA techniques:

A- Quantitation of human TGFβ level in serum:

The TGF-B was extracted from the serum by using Human TGF-B ELISA kit provided by ID labs Biotechnology London, ON, Canada.

B- Quantitation of KIM-1 in serum:

Detection of human KIM-1 in serum was done using ELISA kit. The ELISA kit was provided by Aviscera Bioscience Inc, Santa Clara, USA (Cat No SK00186-01).

Detection of TGFβ gene expression by Real time-Polymerase Chain Reaction (real time-PCR):

- Total RNA was extracted from kidney tissue using SV Total RNA Isolation system. The yield of total RNA obtained was determined by nanodrop.

- The extracted RNA was reverse transcribed into cDNA using RT-PCR kit. Three µl of random primers were added to the 10µl of RNA which was denatured for 5 minutes at 65°C in the thermal cycler. The RNA primer mixture was cooled to 4°C. The cDNA master mix was prepared according to the kit. Total volume of the master mix was 19µl for each sample. This was added to the 13µl RNA-primer mixture resulting in 32µl of cDNA. The last mixture was incubated in the programmed thermal cycler one hour at 37°C followed by inactivation of enzymes at 95°C for 10 minutes, and finally cooled at 4°C. Then RNA was changed into cDNA.

qPCR (quantitative real time PCR):

The gene-specific forward and reverse primer pair was normalized. Each primer (forward and reverse) Table (1) concentration in the mixture was 5pmol/µl.

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>Oligonucleotide primers sequence of studied genes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ gene</td>
<td>Forward primer: 5’- AATGTCAGCTCAGGA ACATCCA -3’</td>
</tr>
<tr>
<td></td>
<td>Reverse primer: 5’- GTTCCGTGACACATGA ACCCTTG -3’</td>
</tr>
</tbody>
</table>
The experiment and the following PCR program was set up:
1- 50°C 2min., 1 cycle.
2- 95°C 10min., 1 cycle.
3- 95°C 15sec. → 60°C 30sec. → 72°C 30sec., 40 cycles.
4- 72°C 10min., 1 cycle.

A real time-PCR reaction mixture was 20µl.

The following mixture was prepared in each optical tube:
- 10µl SYBR Green Mix (2x).
- 0.5µl kidney cDNA.
- 2µl primer pair mix (5pmol/µl each primer).
- 7.5µl H2O.

Data analysis:
At the end of a qPCR running with SYBR Green chemistry, the relative quantification was used. This frequently used method presents expression levels in number of folds as compared to expression level of calibrator which is usually the biological control sample (taken as expression of "1").

Quantification is calculated by Livak method [18]. The actual operation of these quantification methods was performed by qPCR software.

Detection of TGFß and CD24 by immunohistochemistry:
Kidney sections (5mm) were collected from formalin-fixed, paraffin-blocks on microscopic slides coated with 3-amino propyl triethoxy silane (Sigma). We used the standard streptavidin-biotin peroxidase complex method (APC) method. Following deparaffinization and rehydration, endogenous peroxidase activity was blocked using 3% solution of hydrogen peroxide in methanol for 30 minutes at room temperature then antigen retrieval was performed by microwaving in 10mM citrate buffer, pH 6.0 for 15 minutes. Non-specific antibody binding was prevented by pre-incubation with 100mL blocking serum for 30min at room temperature.

Sections were incubated overnight with the primary antibodies: TGFß (146, Santa Cruz, USA) and CD24 monoclonal antibody (sc 7034, Santa Cruz, USA) at the optimal working dilution of 1:100 both after thorough washing in buffer. We used substrate chromogen mixture (a biotinylated secondary anti-immunoglobulin (IgG) LSAB® System (k0679), a preformed streptavidin biotinylated horseradish peroxidase complex and the chromogen used were 3-3' diaminobenzidine tetrahydro-chloride (D.A.B.) (DAKO, Denmark) and sections were counterstained with Mayer’s hematoxylin before mounting.

Positive control was done using colon cancer. Negative control slides included a blank control and omission of primary antibody. Positive and negative controls were stained in the same settings (battery) of stain to standardize our technique.

Biochemical analysis of glucose and renal functions:
Estimation of serum glucose, urea and creatinine was done by automated method (using Hitachi autoanalyzer).

Histopathological examination of kidney tissues:
Kidney samples were collected into PBS and fixed overnight in 40g/L paraformaldehyde in PBS at 4°C. Serial 5-µm sections of the cortex and the medulla of the kidney were stained with hematoxylin and eosin (H&E).

Statistical analysis:
Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test. Correlations were done to test for linear relations between quantitative variables by Pearson correlation coefficient. Receiver operator characteristic (ROC) curves were derived and area-under-the curve (AUC) analysis performed to get the best cutoff values for detecting diabetic nephropathy cases. p-values less than 0.05 were considered as statistically significant.

Results

Serum levels of biochemical parameters among different groups Table (2):
Glucose level of diabetic nephropathy group (365.57±60.93) was significantly higher than that of the control group (97.00±21.60) (p<0.05), Glucose level of diabetic nephropathy with MSCs group (172.00±34.58) was significantly higher than that of the control group (97.00±21.60) and showed a significant decrease when compared to diabetic nephropathy group (365.57±60.93) (p<0.05).

Urea level of diabetic nephropathy group (81.71±4.15) was significantly higher than that of the control group (32.57±1.99) (p<0.05), Urea level of diabetic nephropathy with MSCs group (42.86±3.80) was significantly higher than that of the control group (32.57±1.99) (p<0.05), and
showed a significant decrease when compared to diabetic nephropathy group (81.71±4.15) (p<0.05).

Creatinine level of diabetic nephropathy group (0.68±0.22) was significantly higher than that of the control group (0.31±0.08) (p<0.05), Creatinine level of diabetic nephropathy with MSCs group (0.49±0.05) showed no significant differences compared to other groups (p>0.05).

TGFβ level of diabetic nephropathy group (207.74±10.14) was significantly higher than that of the control group (90.39±13.46) (p<0.05), TGFβ level of diabetic nephropathy with MSCs group (141.10±15.84) was significantly higher than that of the control group (90.39±13.46) (p<0.05), and showed significant decrease when compared to diabetic nephropathy group (207.74±10.14) (p<0.05).

KIM-1 level of the diabetic nephropathy group (278.29±38.72) was significantly higher than that of control group (131.71±11.66) (p<0.05), KIM-1 level of diabetic nephropathy with MSCs group (169.86±10.04) was significantly higher than that of control group (131.71±11.66) (p<0.05), but showed significant decrease when compared to diabetic nephropathy group (278.29±38.72) (p<0.05).

Table (2): Serum levels of biochemical parameters among different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Diabetic nephropathy group</th>
<th>Diabetic nephropathy with MSCs group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>97.00±21.60</td>
<td>365.57±60.93*</td>
<td>172.00±34.58*#</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>32.57±1.99</td>
<td>81.71±4.15*</td>
<td>42.86±3.80#</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.31±0.08</td>
<td>0.68±0.22*</td>
<td>0.49±0.05</td>
</tr>
<tr>
<td>TGFβ (pg/ml)</td>
<td>90.39±13.46</td>
<td>207.74±10.14*</td>
<td>141.10±15.84*#</td>
</tr>
<tr>
<td>KIM-1 (pg/ml)</td>
<td>131.71±11.66</td>
<td>278.29±38.72*</td>
<td>169.86±10.04*#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.
*: Statistically significant compared to control group (p<0.05).
#: Statistically significant compared to diabetic nephropathy group (p<0.05).

Table (3): TGFβ gene expression in kidney tissues among different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Diabetic nephropathy group</th>
<th>Diabetic nephropathy with MSCs group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ (RQ)</td>
<td>0.97±0.18</td>
<td>1.69±0.48*</td>
<td>0.82±0.13#</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.
*: Statistically significant compared to control group (p<0.05).
#: Statistically significant compared to diabetic nephropathy group (p<0.05).

TGFβ gene expression of diabetic nephropathy group (1.69±0.48) was significantly higher than that of the control group (0.97±0.18) (p<0.05). TGFβ gene expression of diabetic nephropathy with MSCs group (0.82±0.13) showed significant decrease compared to diabetic nephropathy group (1.69±0.48) (p<0.05).

A significant positive correlation was found between TGFβ in serum and TGFβ gene expression where p-value <0.05, r-value=0.451 (Fig. 1).

A significant positive correlation was found between KIM-1 and TGFβ in serum p-value <0.01 r-value=0.866 and TGFβ gene expression (RQ) p-value <0.05 r-value=0.615 Figs. (2,3).
Results of histopathology and immunohistochemistry:

Are summarized in (Figs. 4-6).

**Group I (Control group):**

![Fig. (4-A): Histopathological picture of Group I: Showed normal cortical tubules](image)

![Fig. (4-B): IHC of TGFβ in Group I: Kidney section showed mild positive expression (20%) for TGFβ 1 in the tubular cells (IHC x400).](image)

![Fig. (4-C): IHC of CD24 in Group I: Kidney section negative for CD24 (IHC x400).](image)

**Group II (Diabetic nephropathy group):**

![Fig. (5-A): Histopathological picture of Group II: Kidney section showed mesangial proliferation (black arrow) and thickening of glomerular basement membrane (red arrow) & Fibrin in Bowman’s space (green arrow) hyaline degeneration of the tubules (yellow arrow) (HE x400).](image)

![Fig. (5-B): IHC of TGFβ in Group II: Kidney section showed highly positive (90%) for TGFβ 1 in the tubular cells (black arrow) and few in mesangial cells (red arrow) (IHC x400).](image)

![Fig. (5-C): IHC of CD24 in Group II: Kidney section negative for CD24 (IHC x400).](image)

**Group III (Diabetic nephropathy with MSCs):**

![Fig. (6-A): Histopathological picture of Group III: Showed minimal changes (HE x400).](image)

![Fig. (6-B): IHC of TGFβ in Group III: Kidney section showed highly positive (90%) for TGFβ 1 in the tubular cells (black arrow) and few in mesangial cells (red arrow) (IHC x400).](image)

![Fig. (6-C): IHC detection of CD24 in Group III: Kidney section positive for CD24 in epithelial cells in Bowman’s capsule (black arrow) and to tubular cells (red arrow) (IHC x400).](image)
Diabetic nephropathy is the most common cause of end-stage renal disease in the world, and could account for disability and high mortality rate in patients with diabetes. MSCs have several advantages for therapeutic use such as ability to migrate to the sites of tissue injury. Successful MSC treatment of diabetic nephropathy could be explained by MSCs competence to differentiate into insulin-producing beta cells followed by decrease of glycemia and glycosuria, factors important for damaging renal cells.

Taken together, these data indicate that MSC transplantation prevents the pathological changes in the glomeruli and enhances their regeneration resulting in improved kidney function in diabetic animals.

The results of the present study showed that there was a significant increase in the serum urea and creatinine concentrations of the diabetic nephropathy group compared to the control group. This agrees with results obtained from a study done by Blessing et al. [14] but on human cases. They showed that in addition to elevated blood sugar level in type 2 diabetes mellitus, plasma creatinine and urea concentration are also significantly increased in male and female diabetics compared with their levels in apparently healthy non-diabetic male and female controls.

Also, Abdel Aziz et al. [15] coincided with our results, they worked on 30 inbred strains of white albino female rats with chronic renal failure and showed that serum urea and creatinine concentrations were significantly elevated in chronic renal failure (CRF) group compared to control group.

Our results showed that MSCs when infused into the rats with induced diabetic nephropathy there was a significant decrease in the serum urea and creatinine concentration compared to the diabetic nephropathy group. These findings agreed also with Abdel Aziz et al. [18] who worked on 30 inbred strains of white albino female rats to study the protective effect of bone marrow derived mesenchymal stem cells on chronic renal failure. They found that injection of MSCs ameliorated the renal function.

TGF-β, which has a role in the pathogenesis of diabetic nephropathy and was examined in this study, showed a significant increase of serum TGF-β and its gene expression in diabetic nephropathy group compared to that of the control group. This agrees with Yamamoto et al. [16] who reported that normal glomeruli express or contain detectable levels of TGF-β. Based on the fact that TGF-β generally functions as a differentiation factor, the basal levels of TGF-β could be contributed to the maintenance of normal glomerular structure and function. In various pathological conditions, TGF-β is upregulated in the glomerulus.

Our results were confirmed also by Cairong et al. [17] who worked on Sprague-Dawley rats with diabetic nephropathy induced via STZ 60mg/kg body weight and showed that TGF-β gene expression was higher in diabetic rats than in control rats. Also, Sharma et al. [18] work coincided with our results as they evaluated in vivo the functional role of the renal TGF-β system in diabetic kidney disease by treatment of streptozotocin-induced diabetic mice with neutralizing monoclonal antibody against TGF-β1, -β2, and -β3. There was significantly increased mRNAs encoding TGF-β in diabetic group compared to control group.

The elevation of TGF-β, which is a powerful stimulus of extra cellular matrix production, may be due to elevated glucose [19]. High levels of glucose were significantly increasing the levels of TGF-β and lead to fibrosis of these organs and induction of diabetic nephropathy [20].

At the same time, Hellmich et al. [21] investigated whether circulating TGF-β associated with diabetic kidney disease by measuring serum levels of active and total TGF-β in type 2 diabetic patients with nephropathy or without and normoglycemic controls, they found that serum levels of circulating active TGF-β were significantly higher in patients with diabetic nephropathy compared with diabetic patients without renal involvement and healthy controls.

In the present work, after injection of mesenchymal stem cells there was a significant decrease in TGF-β gene expression compared to the diabetic nephropathy group. These results agreed with Semedo et al. [22] who reported a significant reduction in gene expression of TGF-β in MSCs-treated animals for three doses of MSCs injection every other week compared with untreated rats.

In contrast to the previous recorded data, Stokman et al. [23] recorded that stem cells treatment could not improve TGF-β and did not alter progression of fibrosis in obstructive nephropathy in mice.

KIM-1 is a type 1 transmembrane protein, with an immunoglobulin and mucin domain, and whose expression is markedly up-regulated in the proximal
tubule in the post-ischemic rat kidney [24]. In our study KIM-1 level in diabetic nephropathy group was significantly higher than that of control group. This agreed with van timmeren et al. [7] who reported that KIM-1 is upregulated in renal disease and is associated with renal fibrosis and inflammation. KIM-1 is also associated with inflammation and so, KIM-1 can be used as a non-invasive biomarker in renal disease.

The present study was supported by Ichimura et al. [25] who reported that in healthy kidney, KIM-1 is undetectable, but after injury, it is induced more than any other protein, in which case it localized to the apical surface of surviving proximal tubule epithelial cells.

At the same time, our results were coincided with Waanders et al. [6] who stated that, KIM-1 was localized with areas of fibrosis and inflammation, and its expression correlates directly with interstitial fibrosis in human allografts [7].

Following stem cells injection, there was a significant decrease in KIM-1 expression compared to diabetic nephropathy group. This was confirmed by Park et al. [26] who reported that KIM-1 a well-known kidney-specific biomarker enhanced the in vitro migration capacity of MSCs as a potent kidney-specific chemo-attractant or an inducer.

Our study showed that there was a significant decrease in KIM-1 expression when MSCs were infused into the rats compared to diabetic nephropathy group which coincided with the previous combined results of Huseynova et al. [27] and Park et al. [26].

In the current study renal tissue was examined histopathologically by staining them with hematoxylin and eosin (H&E). In kidney samples of diabetic nephropathy group, the most evident findings were, exaggerated mesangial proliferation and thickening basement membrane, thickening of glomerular capillary walls and arterioli & early nodularity, sclerosis of glomerular tuft (focal or diffuse), hyaline and atrophic changes in tubules with dilatation of lumen & casts, interstitial infiltrate by lymphocytes. These results agreed with Zhou et al. [10] who worked on 70 male Sprague-Dawley rats that received a single intraperitoneal injection of streptozotocin (STZ) (60mg/kg) and showed a glomerulus with glomerulosclerosis, diminution of capillary lumen, predominance of dense hyaline matrix and peripheral capillaries of thick stiff wall.

Also, Kang et al. [28] found that beside tubular destruction and interstitial fibrosis, there was a decrease in number of peritubular capillaries and that there is a good inverse correlation between the severity of interstitial fibrosis and the number of peritubular capillaries as well as between renal function and the number of interstitial capillaries.

In the current study when MSC was administered there were peritubular stem cell collections, minimal changes, minimal mesangial accentration & no thickening of capillary wall, increased cellularity of glomerular tuft & absence of sclerosis.

These findings also agreed with those of Li et al. [29] who recorded similar perivascular and periglomerular infiltration. In addition, they reported cell fusion, with occurrence of binucleated cells. Similar findings were obtained by Hauger et al. [30] who used MRI to detect homing of donors' cells into the injured kidneys.

Our results showed an increased expression of TGFß in diabetic nephropathy group compared to control group, but following administration of either MSCs there was decrease in expression of TGFß by immunohistochemistry. These results coincided with Taneda et al. [31] who reported that there was an increase in TGF-beta 1 expression in animal model of chronic DN when detected by IHC. At the same time, Shasha et al. [32] reported that by IHC, the expression of TGFß was downregulated by MSCs.

Our results also revealed that there was detection of CD24 by IHC with administration of MSCs (homing of MSCs) this was confirmed by de Becca et al. [33] who reported that, CD24 is the most consistently used biomarker. To identify and characterize the breast cancer stem cell (CSC) phenotype. These findings also agreed with Paola and Raghu [34] who stated that CD24 renal progenitors also represent a common progenitor of tubular cells and podocytes during renal development.

**Conclusion:**

Our current study showed improvement of kidney functions and decreased TGFß and KIM-1 levels after administration of MSCs compared to diabetic nephropathy group. And a significant positive correlation was found between serum (KIM-1 and TGFß).

Also, improvement of histopathological picture of the kidney after administration of MSCs compared to diabetic nephropathy group was detected. Decreased TGF-ß expression after administration of MSCS compared to diabetic nephropathy group...
when detected by Immunohistochemistry. Immunohistochemistry detection of CD24 in kidney tissue detected homing of MSCS. TGFβ and KIM-1 can be used as markers for diagnosis of diabetic nephropathy and for follow up after administration of MSCS.

So; the study suggests that administration of MSCs exerts a therapeutic effect on diabetic nephropathy.

References


Effect of Mesenchymal Stem Cells on Diabetic Nephropathy


مرتبة الكلي السكري هو السبب الأكثر شيوعاً للمرحلة الأخيرة لمرض الكلى في العالم، وهو المسؤول عن معدل الازدياد وارتفاع معدل الوفيات في المرحلة التي يعاني من مرض الكلى. بين الخلايا الجذعية، الخلايا الجذعية الوسيطة التي لها العديد من الطرق للاستخدام الدي_fee.php. المثل القديمة على الهجرة إلى مكان إصابة الأنسجة أو مثبطات قوية للمناعة. وقد أظهرت المراجعة أن هناك حاجة إلى أن يمتلك العلاج أن يكون بشكل ماجن أنه يوفر بعض انتهاز الأوعية الدموية معقولة ككيبيات الخلايا.

تهدف هذه الدراسة إلى توضيح تأثير العلاج بالخلايا الجذعية الوسيطة على نموذج كبيبيات مصاب بمرض الكلى السكري.

وقد أجريت هذه الدراسة على 50 من الجرذان الإناث من متوسط وزن 150-200 جرام. تم الحصول عليها من وحدة حيوانات التجارب في القصر البيئي، كرية الطب، جامعة القاهرة. تم تقسيم الجرذان الإناث إلى 3 مجموعات على النحو التالي:

1- المجموعة الأولى (مجموعة التحكم): تتكون من 10 من الجرذان الإناث والأصابع السليمة.
2- المجموعة الثانية (مجموعة علاج): تتكون من 10 من الجرذان الإناث مصابون بمرض الكلى السكري.
3- المجموعة الثالثة (مجموعة علاج): تتكون من 10 من الجرذان الإناث مصابون بمرض الكلى السكري بجراحة الخلايا الجذعية الوسيطة.

تعرضت كل مجموعة للآتي:

1- تحليل مستوى الجلوكوز والكليوت.
2- ELISA قياس مستوى TGFβ و KIM-1 بواسطة تقنية 
3- تحليل نسبة الكلييات بواسطة (Eosine) و haematoxylin.
4- فحص نسبة الكلييات بواسطة PCR و TGFβ CD24.
5- فحص نسبة التعبير الجيني ل TGFβ في جسم الجرذان بواسطة PCR.
6- فحص الاضطرابات البصرية في ظل الجرذان بواسطة تحليل عدد TGFβ.

وقد أظهرت النتائج:

تحسين وظائف الكلي، انخفاض مستويات TGFβ1، بعد استخدام الخلايا الجذعية الوسيطة مقارنة مع مجموعة مرض الكلى السكري KIM-1 وا TGFβ1.

تظهر هذه النتائج تحسين في فحص الإصابة في الجراحة بعد استخدام الخلايا الجذعية الوسيطة مقارنة مع مجموعة مرض الكلي السكري KIM-1 وا TGFβ1.

تظهر هذه النتائج تحسين في فحص الإصابة في الجراحة بعد استخدام الخلايا الجذعية الوسيطة مقارنة مع مجموعة مرض الكلي السكري KIM-1 وا TGFβ1. الكشف الجنسي للأنسجة في نهاية المطاف بعد استخدام الخلايا الجذعية الوسيطة مرتبط بمرض الكلي السكري. الكشف الجنسي للأنسجة في نهاية المطاف بعد استخدام الخلايا الجذعية الوسيطة مرتبط بمرض الكلي السكري. الكشف الجنسي للأنسجة في نهاية المطاف بعد استخدام الخلايا الجذعية الوسيطة مرتبط بمرض الكلي السكري.