



Role of human umbilical cord blood derived-mononuclear cells-versus CD34 cells on chronic renal failure in adult male albino rats

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ABSTRACT

Chronic renal failure (CRF) has become a public health problem. The therapeutic approaches have not been successful in limiting disease progression. The therapeutic potential of adult stem cells for the treatment of chronic diseases is becoming increasingly evident over the last few years as the current therapeutic opportunities to slow the progression of the disease are limited. We attempted to assess the therapeutic effectiveness of mononuclear cells (MNCs) versus CD34+ cells derived from human umbilical cord blood stem cells (HUCBSCs) in chronic renal failure rat model. The 5/6 nephrectomy model was used in rats to induce chronic renal failure. Forty inbred strain adult albino male were allocated randomly to 4 equal groups: A control group G1, CRF groups (CRF with saline infusion group) G2, CRF/MNCs group G3 and CRF/HUCB CD34+ cells group G4. Systolic blood pressure, renal functioning tests, histological examination and immunohistochemistry for laminin expression were assessed. Treatment with MNCs and CD34+ cells significantly decreased serum creatinine and urea as well as systolic blood pressure with concomitant decrease in the degree of fibrosis indicated histologically by decreased renal laminin expression. There was a significant difference in these parameters between MNCs and CD34+ stem cells treated groups. There was positive reaction for antibody anti-rat laminin in 80% of animals in MNCs treated rats versus 60% in CD34+ cells treated rats. HUCB derived CD34+ and MNCs accelerate regeneration of renal tubular epithelial cells and lead to reduction of progressive renal injury in induced renal failure rats.

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INTRODUCTION

Chronic renal failure (CRF) is a common and severe state leading to a considerable increase in mortality in addition to reduced quality of life, and high costs for renal replacement therapy. It represents a crisis for healthcare world-wide, being the 12th cause of mortality and the 17th cause of disability worldwide (Couser et al., 2011).

Majority of patients with CRF entering dialysis programs develop complications including type 2

diabetes, chronic glomerulonephritis, or hypertension. This rendered dialysis impractical as a therapy choice for CRF patients (Dirks et al., 2006). Moreover, the lack of suitable transplantable organs has prevented kidney transplantation from becoming a practical solution for most cases of CRF. Consequently, the need for a new type of therapy, for renal failure with the capacity to replace a wider range of the kidney's functions, thereby reducing morbidity, mortality, and the overall economic impact associated with this condition is mandatory (Kurokawa et al., 2002; Yokoo et al., 2007).

Stem cells have been proposed as a new opportunity

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for the treatment of many diseases, Human umbilical cord blood (HUCB) as a source of stem cells has a number of significant advantages over other stem cell sources (Tögel and Westenfelder, 2007; Gajkowska et al., 2006). It has advantages of easy procurement, no risk to donors, low risk of transmitting infections, immediate availability and immune tolerance allowing successful transplantation despite HLA disparity and it could be cryopreserved. Moreover, cord blood can be obtained non-invasively and frequently in contrast to invasive bone marrow aspiration (Gajkowska et al., 2006).

Being attractive for renal repair; stem cells have been used in experimental acute renal failure that could lower renal injury, accelerate tubular proliferation and improve renal function with a dramatic repopulation of the mesangium (Parker, 2011).

Human umbilical cord blood stem cells were found differentiated into cells that improve a variety of disease conditions in animals (Ende, 2000). Mononuclear cells derived from human umbilical cord blood were also found to differentiate into cells that improve a variety of disease conditions in animals (Manotham et al., 2004). They contain a relatively high number of CD133 and CD34 progenitor cells. They can be easily obtained, can be enhanced for self-renewal and differentiation, and can be stored for future use (Ende, 2000; Manotham et al., 2004).

The CD34 antigen is highly expressed in pluripotent cells and its expression gradually reduces as the level of maturation of hematopoietic cell lineages increases, to the point of becoming completely absent in fully mature cells. HSCs can be mobilized into the circulation in response to multiple cytokines, chemokines and adhesion molecules (McGuckin et al., 2003; Zhanga and Lodishb, 2008).

Laminin is implicated in cell migration, remodeling of the extracellular matrix (ECM) and basement membrane. Therefore, laminin deposition could be of concern in the progression of renal diseases (Aumaille, 2005).

Therefore, the present study was designed to assess whether the infusion of human umbilical cord-derived mononuclear cells and CD34 cells could reduce/stabilize the rate of progression and improvement of renal impairment of CRF in rats. Also, the regenerative effect of MNCs was compared to that of CD34+hematopoietic stem cells to suggest the better cell type.

MATERIALS AND METHODS

Ethical consideration

All experimental procedures were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA). They were left for acclimatization for one week

prior to the start of the study. All efforts were made to minimize animal suffering and to reduce the number of animals used. All animals were anesthetized before sacrifice. Animals were handled gently, housed in suitable environmental conditions, and allowed free access to water and rat chow.

In brief, the purpose of the study was explained and a written consent was taken from the study participants (cordblood donors). The used methods were safe and had no harm to mothers or neonates. The samples were used only in this study and then discarded.

Experimental animals

Forty inbred Sprague-Dawley albino male rats, 8 weeks old, weighing between 150 and 200 g supplied by the Modern Veterinary Office for Laboratory Animals (Cairo, Egypt) were used in this study. Rats were housed in groups of ten in polyethylene cages under controlled laboratory conditions, normal dark/light cycle and allowed unlimited access to chow and water ad libitum. The morphological and behavioral changes of rats were monitored every day.

Experimental induction of chronic renal failure

After the one-week acclimatization period, rats were divided randomly into the following equal groups: Control group: involved 10 healthy rats (normal control), CRF groups: 30 rats that underwent modified 5/6 nephrectomy. They were anaesthetized with sodium thiopental by intramuscular injection. Laparotomy was done followed by surgical excision of one kidney and cauterization of the other to excise 5/6 of its tissue. The wound was then sealed by continuous 6/0 stitches in 2 layers. Finally, antibiotic ointment (teramycin) and powder (neomycin) were applied to the wound. CRF was determined by elevated blood creatinine.

Experimental protocol

Animals

Rats were divided into four equal groups each consisting of ten animals:

- Group 1: *Normal control group*: they were healthy group received nothing, so they served as normal control group.
- Group 2: *Renal failure control group*: chronic renal failure rats received iv saline. So they served as renal failure control group.
- Group 3: *Chronic renal failure group treated with MNCs*:

Chronic renal failure rats infused with a single dose of syngenic MNCs (150×10^6) cells per rat (intravenous route) in rat tail vein

Group 4: *Chronic renal failure group treated with CD34+ cells*: Chronic renal failure rats infused with a single dose of syngenic CD34+ cells 1×10^6 cells per rat (intravenous route) in rat tail vein.

Investigation done at the beginning of the study, then at 1st week, 4th week and 8th week after administration of MNCs and CD34+ cells. Investigations include serum creatinine, urea and measuring systolic blood pressure.

Biochemical assessment

Level of serum creatinine and urea were assessed using conventional available kit. Venous blood was collected from the retro-orbital vein.

Measurement of serum urea and creatinine levels:

Blood samples were centrifuged at 3000 rpm for 10 min and the supernatant was stored at -70°C for later determination of serum urea and creatinine which were done by vitro Scient colorimetric kits (Vitro Scient, Inshas Industrial Zone, Belbis, Sharkia, Egypt) according to manufacturer's manual.

Measurement of systolic blood pressure

Arterial systolic blood pressure was measured by rat-tail cuff method using an electronic electro sphygmomanometer after the rat was pre-warmed to 37°C for 15 min. The average of 3 pressure readings was recorded for each measurement while the animals were quietly resting.

Collection and isolation of MNCs:

UCB was collected in the presence of anticoagulant and was diluted 1:1 in isolation buffer. Seven ml of diluted cord blood was transferred onto 3 ml ficoll and was centrifuged 20 min at $800 \times g$. The interface which contains the low density mononuclear cells was collected and suspended in equal volume of isolation buffer and was centrifuged 20 min at $500 \times g$. Then, the pellet was resuspended in isolation buffer again and was centrifuged 20 min at $300 \times g$ and maintained at $2-8^\circ\text{C}$ (Jaatinen and Laine, 2007).

Collection and isolation of HUCB CD34+ cells:

Sterile collection tubes (50 ml) containing citrate

phosphate dextrose adenine-1 (CPDA-1) as anticoagulant (5 ml) was used for collection of the human umbilical cord blood. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (Sigma). Separation of CD34 positive progenitor cells was carried out with immunomagnetic separation technique by Dynal CD34+ progenitor cell selection system) (Hasein et al., 2011).

Histopathological examination

Eight weeks after initiation of injection treatment, rats were sacrificed by CO_2 narcosis. Their abdomens were opened and both kidneys of each animal were extracted for histopathological and immunohistochemical examination. Kidney specimens were collected and fixed 10% neutral buffered formalin saline for 24 h. Specimens were processed to paraffin sections. Serial $5\text{-}\mu\text{m}$ sections the kidneys were stained with hematoxylin and eosin (H&E) stain for kidneys general architecture evaluation and periodic Acid Schiff (PAS) stain for evaluation of the basement membranes and tubular brush borders and were scored histopathologically according to Lloberas et al. (2001). Other sections were unstained for laminin immunohistochemistry.

Immunohistochemistry and image analysis

Briefly, immunostaining was performed using streptavidin-biotin immunoperoxidase complex method with 4-mm thick sections which have been deparaffinized and heated in 0.01 M citrate buffer solution (pH= 6) for 15 min for antigen retrieval. Sections were then incubated overnight with rabbit polyclonal antibody anti-rat laminin (Dako Company, Egypt).

After conjugation with streptavidin-biotin peroxidase complex (broad spectrum LAB-SA detection system, Invitrogen), 3, 3'-diaminobenzidine (DAB, Sigma-Aldrich, MO, USA) was used as a chromogen and Mayer's hematoxylin was used as a counterstain. Then, tissue sections were examined using a light microscope and photomicrographs were captured and analyzed using the Image J software developed by the National Institute of Health (Bethesda, Maryland, USA). Briefly, the positive DAB stained area, which represent the positive area, in each digital photomicrograph was automatically separated from hematoxylin, which represent the total area, using color deconvolution plugin. Images were then processed into binary color image (black and white).

The percentage of positively stained area (represented by the black color) was then determined. Immunoreactivity for laminin was evaluated in ten consecutive sections representative to the whole tissue section in each.

Table 1. Creatinine levels in in the different groups.

Weeks	G1	G2	G3	G4	P-value
0 week	0.92±0.07	0.80±0.16	0.83±0.34	0.82±.16	NS
After 1 week	0.67±0.10	6.07±1.13"	3.03±1.12*	2.66±1.72*	0.004
After 4 weeks	0.98±0.12	7.39±0.33"	2.476±0.80*	2.26±1.15*	0.000
After 8 weeks	0.65±0.06	7.76±0.33"	2.005±0.75*	1.94±0.63*	0.000

n = 10, Values are the mean ± SD; G1, Negative control; G2, renal failure control; G3, MNCS treated group; G4, CD 34+ treated group; ", Represents a statistically significant difference between the given group and group 1; *, represents a statistically significant difference between the given group and group 2.

Table 2. Urea levels in the different groups.

weeks	G1	G2	G3	G4	P value
0 week	18.5 ±2.24	17.5±2.24	19.36±2.24	18.94±2.13	NS
After 1 week	16.87±1.17	65.2±8.79"	30.45±7.18*	30.2±10.48*	0.000
After 4 weeks	19.87±1.42	84.23±4.43"	36.5±8.28" *	31.74±9.75*	0.000
After 8 weeks	19.23±1.40	93.21±7.90"	33.53±26.95*	32.14±10.90*	0.000

n = 10, Values are the mean ± SD; G1, negative control; G2, positive control; G3, MNCS treated group; G4, CD 34+ treated group; ", represents a statistically significant difference between the given group and group 1; *, represents a statistically significant difference between the given group and group 2.

Table 3. Systolic blood pressure (mm/Hg) in the different groups.

weeks	G1	G2	G3	G4	P value
0 week	100±5	100±4	101±4	102±5	NS
After 1 week	102±4	116±5	110±3	108±4	.000
After 4 weeks	103±3	132±4"	117±3*	105±3*	.000
After 8 weeks	100±2	141±4"	111±2*	103±3*	.000

n = 10, Values are the mean ± SD; G1, negative control; G2, positive control, G3: MNCS treated group, G4: CD 34+ treated group; ", represents a statistically significant difference between the given group and group 1; *, represents a statistically significant difference between the given group and group 2.

Laminin was detected by rabbit polyclonal antibody anti-rat laminin (Dako Company, Egypt) using the heat-induced antigen retrieval technique. Intensity of laminin stain was performed on scale based on Taneda scale: 0 – no staining; 1– mesangial staining involving less than 25% of the area examined; 2 – segmental mesangial staining involving 25 to 50% of mesangial areas present; 3 – mesangial staining involving 50 to 75% of the areas examined; 4 – diffuse mesangial staining involving more than 75% of are as examined (Taneda et al., 2003).

Statistical analysis

Results were collected and expressed as the mean ± SD. Results were analyzed using The Statistical Package for the Social Sciences, version 15 (SPSS Software, SPSS Inc., Chicago, USA). One-way analysis of variance

(ANOVA) followed by Duncan's *post-hoc* test were used to test the significance of the difference between quantitative variables; p value<0.05 was considered to be statistically significant.

RESULTS

Blood chemistry

Renal failure control group showed significant (p<0.05) increase in serum creatinine (Table 1), blood urea (Table 2) compared with normal control group, suggesting a significant degree of glomerular dysfunction. These functional changes were associated with a significant elevation in systolic blood pressure (p<0.05) in comparison with normal control group especially in the end of 8 weeks (Table 3).

These deleterious effects associated with induced renal failure were significantly improved by treatment with MNCs and CD34+ cells in comparison with renal failure control group ($p < 0.05$). Meanwhile CD34+ cells treated group exhibited more improvement in all studied parameters ($p < 0.05$) as compared to MNCs treated group, indicating that CD34+ cells more effective in improving renal function.

Histopathology and immunohistochemistry results

Group1: Normal control group

Sections of the kidney stained by H&E in normal control group showed the malpighian renal corpuscles surrounded by proximal and distal convoluted tubules. Each malpighian corpuscle was made up of a tuft of blood capillaries (the glomerulus) surrounded by the Bowman's capsule, which was lined by a single layer of flat epithelium. Few pyramidal cells lined each proximal convoluted tubule. The cytoplasm was acidophilic and nuclei were round and near the base of the cells. The cells had a free striated boarder (Figure 1A). The distal convoluted tubules were lined by a relatively large number of cuboids epithelial cells. The lumens of the distal tubules were wider than the proximal tubules. The cytoplasm was less acidophilic and the nuclei were round and tend to be located in the apical region (Figure 1B).

Morphometric evaluation revealed a significant difference between treated groups (G3 & G4) and the positive control group (G2). Kidneys of the control group (G1) showed normal renal architecture of both renal glomeruli, proximal and distal renal tubules (Figures 1E and 1F) and reduced intensity for laminin staining (Figures 2B and 3C).

Group 2 showed marked obliteration and sclerosis of glomeruli, infiltration by macrophages, dense interstitial, periglomerular, perivascular and diffuse interstitial fibrosis. There was also atrophy of renal tubules with thickened blood vessels wall with narrowed lumina (Figures 1C and D). Glomerulosclerosis was characterized by segmental or global increase of glomerular matrix, adhesions to Bowman's capsule, or crescent formation (Figure 1C) with increase in PAS+ material in both brush borders and basement membranes (Figure 3C) and marked positive reaction for laminin (Figure 2B). The severity of glomeruloclerosis and inflammatory infiltrates were associated with significant ($p < 0.05$) increase in the expression of renal laminin in this group indicating excessive deposition and accumulation of extracellular matrix than in other two groups that received treatment (G3 & G4). These deleterious effects were ameliorated by treatment with either CD34+ cells or MNCs (Figure 2). However, CD34+ cells treated group showed better improvement.

Group 3 and Group 4: There were histopathological improvements (Figures 1E and F) that were associated with significant ($p < 0.05$) reduction in renal laminin expression and accumulation (Figures 2B and C). Both groups showed less sclerotic changes of glomeruli and less inflammatory cellular infiltrate than the chronic renal failure control group (G1) (Figures 1E and F), mild decrease in PAS+ material (Figures 3D and E) and moderate positive reaction for laminin in 80 and 60% of animals (Figures 2B and C) respectively.

It was obvious that CD34+ cells administration was associated with significant reduction in laminin stain intensity scores (Figure 10) in comparison to that afforded by MNCs treatment ($p < 0.05$) (Figure 9); indicating that CD34+ cells offered more corrective effects than MNCs.

Both G3 and G4 showed reduced optical density for laminin compared to G2. Moreover, chronic renal failure group treated with CD34+ cells showed significant decreases in immunostaining for laminin renal tissues compared to their corresponding group treated with MNCs (Figure 4).

DISCUSSION

In the current study subtotal (5/6) nephrectomy was performed on laboratory animals to achieve a model for chronic renal failure (Grossman, 2010). The remaining kidney of the (5/6) nephrectomized animals was utilized as a model for renal fibrosis and a model for glomerular sclerosis (Gava et al., 2012).

In the subtotal nephrectomy group, kidney functions assessment showed significant elevations of urea and creatinine levels as well as significant elevation of systolic blood pressure compared to the control group. These results are in accordance to studies by (Ali et al., 2014; Tadesse et al., 2013; El Aggan et al., 2013).

Thickness of Bowman's capsule is linked to the deposition of fibrous tissue usually secondary to chronic inflammation. Glomerular mesangial tissue cellularity indicates infiltration with inflammatory cells thereby linking it with later development of glomerulosclerosis. The functional correlates of these quantitative changes include deterioration in creatinine clearance with significant increase in water intake, urine production, creatinine and urea concentrations as observed in adenine- CKD. Accumulation of the uremic toxins is known to cause several structural changes in the kidneys such as inhibition of tubular epithelial cell proliferation, probably mediated by stress in the endoplasmic reticulum (Ali et al., 2014).

Stem cells are attractive candidates for renal repair leading to differentiation of both nephrons and collecting ducts. Indeed, they have been used in experimental acute renal failure, which could lower renal injury,

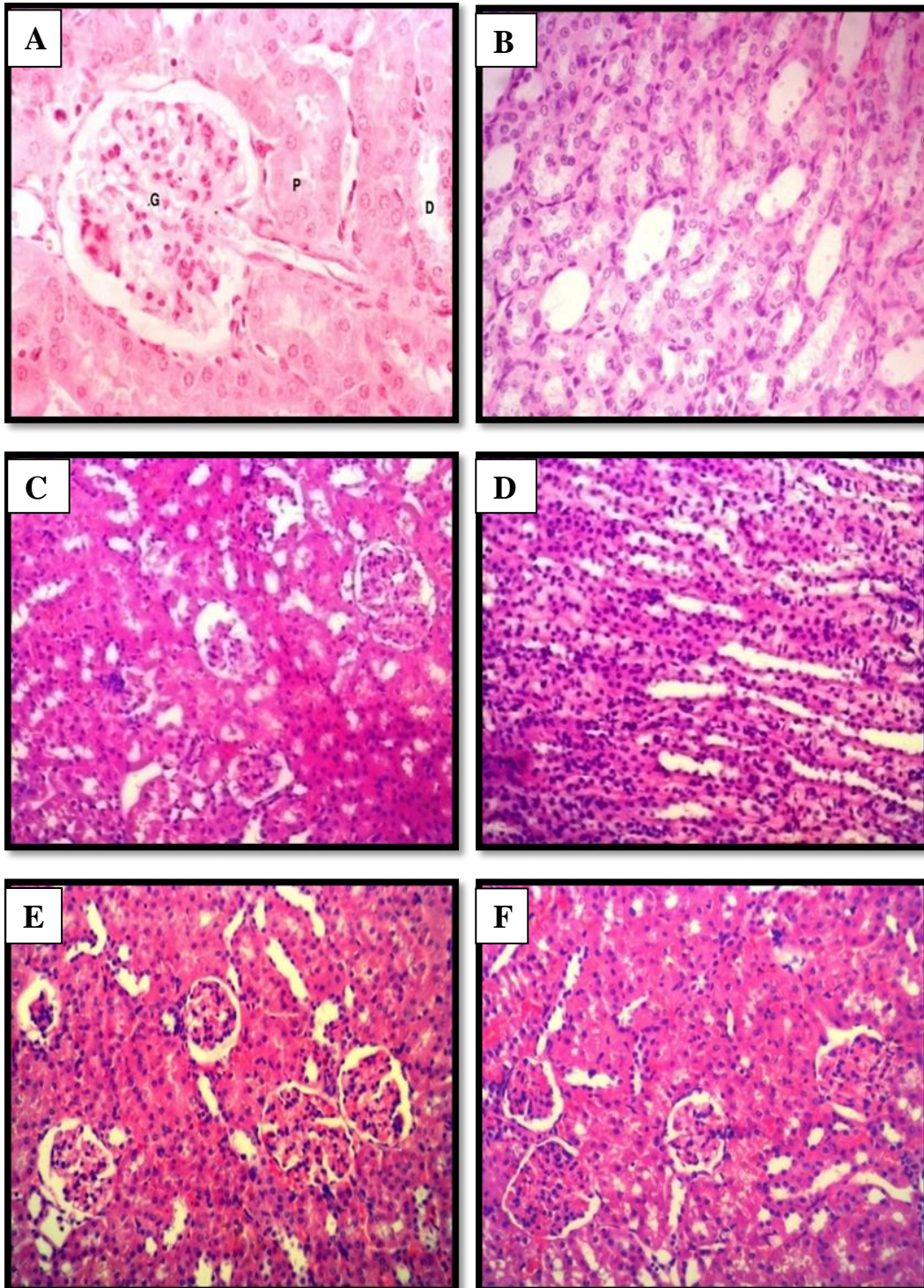


Figure 1. H&E (X400) stained sections. **A and B**, normal control group: **A**, showing renal glomerulus (G), surrounded by proximal (P) and distal (D) convoluted tubules in normal control group. **B**, the distal convoluted tubules; the lumens were wider than the proximal tubules. The cytoplasm was less acidophilic and the nuclei were round. **C and D**, renal failure control group: the kidney showed marked obliteration and sclerosis of glomeruli, infiltration by macrophages, and interstitial fibrosis. There was also atrophy of renal tubules. **E**, chronic renal failure group treated with MNCs, the kidney showed less marked sclerosis of glomeruli, few inflammatory cellular infiltrate compared to the control renal failure group. **F**, chronic renal failure group treated with CD34+ cells, the kidney showed sclerosis of glomeruli and inflammatory cellular infiltrate but less than the chronic renal failure group treated with MNCs.

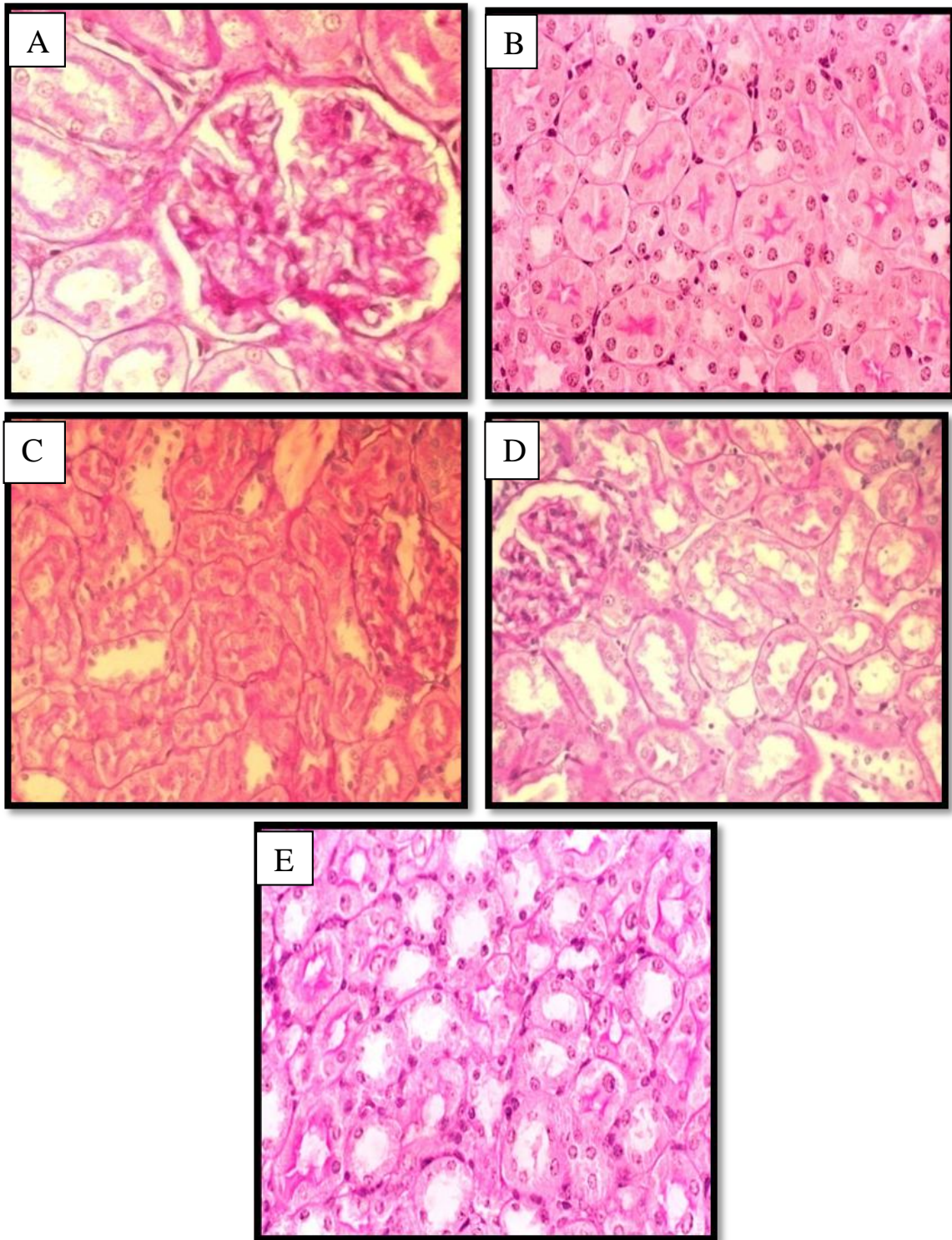


Figure 2. PAS stained sections (X400). **A and B**, normal control group: showing normal PAS+ material in the brush border and basement membranes of both P and D convoluted tubules in normal control group. The glomeruli showed deeply stained basement membrane of the capillary loops. The proximal tubules showed a bright red colored basement membrane and deeply stained brush border. **C**, renal failure control group: showing increase in PAS+ material in both brush borders and basement membranes. **D and E**, showing mild decrease in the PAS+ material in the brush borders with normal basement membranes in MNCs and CD34 treated groups, respectively.

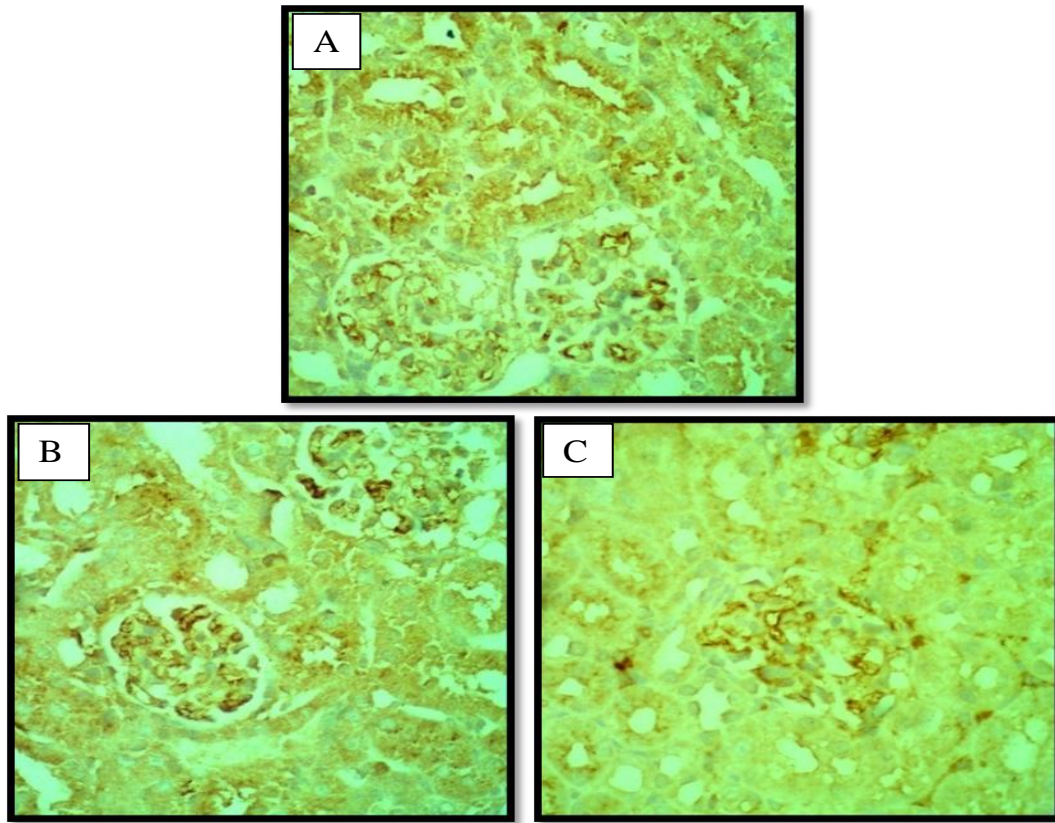


Figure 3. IHC for human anti-Laminin (X400). **A**, renal failure control group: the kidney showed positive reaction in the form of brown pigmentation that indicates increase in the expression of renal laminin indicating excessive deposition and accumulation of extracellular matrix. **B and C**, positive reaction in Chronic renal failure group treated with CD34+ cells and Chronic renal failure group treated with MNCs, respectively showed marked decrease in the expression of renal laminin indicating less interstitial fibrosis when compared to chronic renal failure group.

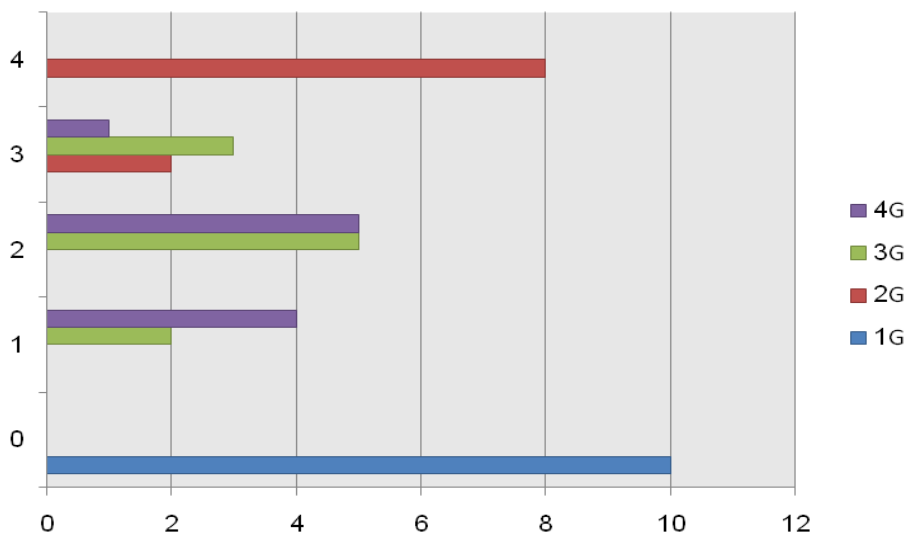


Figure 4. Laminin immune-reactivity expression in the different groups. G1, Control group; G2, chronic renal failure group received no treatment; G3, chronic renal failure group treated with CD34 cells; G4, chronic renal failure group treated with MNCs.

accelerate tubular proliferation and improve renal function with a dramatic repopulation of the mesangium (Parker, 2011). Parker (2011) have demonstrated in experimental acute renal failure that they may lower renal injury, accelerate tubular proliferation and improve renal function with a dramatic repopulation of the mesangium.

The existing study was designed to compare between the renal response to HUCB derived MNCs versus CD34+ stem cells transplantation in CRF. We found significant improvement of renal function tests in both treated groups (UCB MNCs and UCB CD34+ cells) in comparison to the renal failure control group. Renal function tests in both treated groups were not significantly different from each other. Histopathological examination revealed a significant difference in regaining a normal nephronal architecture in both treated groups and they were less reactive to the human specific anti-laminin antibody compared to chronic renal failure group. Moreover, The CD34+ cells treated group showed less deposition and accumulation of extracellular matrix than MNCs treated group.

In this present study, the improvement of the rats' renal function after injection of UCB derived MNCs and CD34+, was in match to the results reported by Kirpatovskii et al. (2007) who found similar improvement after 2 weeks of kidney cells or bone marrow stromal cells (MSCs) injection. They also establish that kidney function remained normal during the remainder of the study period which also is in accordance with our study (Kirpatovskii et al., 2007).

Bussolati and Camussi (2007) explained that the administered stem cells (SCs) may modify the microenvironment either by inducing dedifferentiation and proliferation of tubular cells surviving from injury or by allowing expansion of resident SC.

Furthermore, our results are in agreement with those of Cao et al. (2010), who reported that the administration of UCB MSC leads to improvement in both renal function and histological alterations after reperfusion in comparison to that in control animals in UCB MSC treated rats. This is also supported by Morigi et al. (2010) who observed that infusion of UCB MSCs in immune-deficient mice with cisplatin-induced acute kidney injury ameliorated both renal function and tubular cell injury, and prolonged the survival (Cao et al., 2010; Morigi et al., 2010).

The exact mechanism through which MSCs mitigate renal damage is not fully elucidated. One possible explanation is provided by Humphreys and Bonventre, (2008) those MSCs produce cytokines and growth factors that promote anti-inflammatory, immunosuppressive, anti-apoptotic and proliferative effects. In addition to this, Huls et al. (2010) revealed that, bone marrow derived MSCs may have the capacity to migrate to the injured kidney and contribute to tubule epithelium regeneration and renal function repair without fusing with resident tubular

cells (Huls et al., 2010). Also, Tögel and Westenfelder (2011) proposed that MSCs may provide paracrine and/or endocrine factors that have positive effects on kidney repair.

Theoretically MSCs have a better capacity for self renewal while maintaining their multipotency (Engler et al., 2006). They are also a better candidate for renal repair, because nephrons are of mesenchymal origin (Ryan et al., 2005). MSCs have a better immune-modulatory effect by avoiding allo-recognition, interference with T cell function and generation of local immunosuppressive microenvironment through cytokines secretion. Ryan et al. (2005) also confirmed that the immune-modulatory function of MSCs is enhanced when the cells are exposed to an inflammatory environment. All of these may explain the absence of rejection to HUCB stem cells (Castillo et al., 2007).

The results of the ongoing study showed that all of the examined parameters had significant improvement following MNCs injection compared to the renal failure group. Creatinine, urea and SBP showed statistically significant improvement following MSC and CD+ cell injection. These results are in match with Villanueva et al. (2011) who reported significant improvement in kidney functions in CRF after Mesenchymal stem cells infusion.

Histopathological examination of renal tissue samples stained with PAS showed dense interstitial, periglomerular, perivascular and diffuse interstitial tissue infiltrates. Semedo et al. (2009) studied the effect of MSCs on renal inflammation and fibrosis in a rat model of chronic renal failure and suggested that MSC therapy can modulate the inflammatory response that follows the initial phase of chronic renal injury. The immunosuppressive and remodeling properties of MSCs may be involved in the decreased fibrosis in the kidney. Morigi et al. (2004) explained the effect of MSCs derived from male mice in treating cisplatin induced ARF and their results offered a strong case for exploring the possibility that MSCs could have the renotropic property and tubular regenerative potential that may have a role in the treatment of acute renal failure in humans.

Kunter et al. (2006) studied the reparative role of MSCs in a rat model of glomerulonephritis through infusion of MSCs in the rat renal artery after induction of GN, then detection of these intraglomerular cells and correlation with glomerular healing. Reparative role of MSCs in rat model of glomerulonephritis was considered using infusion of MSCs in rat renal artery after generation of GN. These intraglomerular cells were detected and interrelated with glomerular healing. Acute renal failure was ameliorated by MSC injection into the left renal artery on day 2 after disease induction. On second day after disease induction, MSC injection was given in the left renal artery to alleviate the acute renal failure). Moreover, MSC led to more rapid recovery from mesangiolytic, increased glomerular cell proliferation,

and reduction of proteinuria by 28%. Furthermore, it was observed that MSC led to more rapid recovery from mesangiolytic, increased glomerular cell proliferation, and reduction of proteinuria by 28%.

Chen et al. (2008) studied the ability of kidney derived MSCs to produce endothelial and smooth muscle like cells under the influence of angiogenic factors (VEGF) both *in vivo* and *in vitro* and stated that kidney mesenchymal stem cells are capable of differentiation toward endothelial and smooth muscle cell lineages *in vitro* and *in vivo*, support new blood vessel formation in favorable conditions and promote functional recovery of an ischemic kidney. The capability of kidney derived MSCs to generate endothelial and smooth muscle like cells under the influence of angiogenic factors (VEGF) both *in vivo* and *in vitro* has been demonstrated by Chen et al. (2008). He declared that kidney mesenchymal stem cells are capable of differentiation toward endothelial and smooth muscle cell lineages *in vitro* and *in vivo*, support new blood vessel formation in favorable conditions and promote functional recovery of an ischemic kidney (Chen et al., 2008).

Tögel et al. (2007) studied the positive effect of MSC infusion in the setting of AKI and concluded that MSC and endothelial cells interact and that these interactions are likely responsible, at least in part, for the kidney-protective effects of MSC in AKI, mediated by complex paracrine actions that are able to significantly protect and regenerate the damaged vasculature in AKI. In the same study by Tögel et al. has shown positive effect of MSC infusion in the setting of AKI and reached to the decision that MSC and endothelial cells interact with each other. These interactions may be accountable, at least in part, for the kidney-protective effects of MSC in AKI, mediated by complex paracrine actions that are able to considerably protect and regenerate the damaged vasculature in AKI.

CD34 was detected obviously around blood vessels and within the interstitium, this exceeds the limits of renal endothelial cells and presents the up-regulation of HSCs and their homing in the transplanted kidney in patients with chronic allograft injury and may be a part of the setup of renal repair. Di Marco et al. (2011) detected an increased level of endothelial progenitor cells which are CD34 + ve, and VEGFR2 + ve in renal transplant patients who had endothelial dysfunction than matched controls and assumed that it has a reparative role. BM derived stem cells have been reported by Rookmaaker et al. (2002) to contribute to glomerular endothelial repair following thrombotic microangiopathy.

Therefore our research may establish that intravenous transfusion of human umbilical cord blood cells in nephrectomized rats has a beneficial effect on renal dysfunction. Two major mechanisms of the HUCBSCs effect can be explained. Human umbilical blood stem cells transfusion might play an important role in the

angiogenesis, nephron regeneration or nephron proliferation and apoptosis. Collection of HUCBSCs can take place at any hospital or birthing center. The procedure takes about 5 min and poses no risk to mother or baby. Human umbilical cord blood banking provides enough CD34+ cells needed to clinically benefit humans. In addition, CD34+ cells are safe to use and are associated with few ethical issues. Thus, it seems that CD34+ cell therapy is one of the potentially useful strategies for treating renal failure.

In conclusion, HUCB derived MNCs or CD34+ cells have induced renal improvement to a comparable degree in nephrectomized rats induced chronic renal failure. Accordingly, HUCB can provide a novel source of cells for stem cell-based therapies. It may help develop improved therapies for renal failure with the capacity to replace a wide range of the kidney's functions, thereby reducing morbidity, mortality, and the economic impact associated with this disease.

Conflict of interest

It is declared that the authors on this manuscript have no conflicts of interest. This work was not supported by any funds from any organization.

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