Biochemical and histopathological evaluations of chronic renal failure rats treated with pluripotent human stem cells

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Regeneration of damaged kidney cells using stem cells is the current research approach in the treatment of chronic renal failure (CRF). In the present study, the histopathological and biochemical techniques were used to evaluate stem cells' (SCs) role in treatment of CRF. Sixty-four rats were divided into eight groups. Group I (GI): rats were injected with doxorubicin (15 mg/kg) to initiate CRF. GII-GVII: rats were injected with doxorubicin and treated with SCs (1×10^6 MSCs or/and 2×10^4 HSCs/rat) with/without growth factors extract (200 µL/rat) and/or immunosuppressor (cyclosporine A, 5 mg/kg/day). GVIII: rats treated with PBS (100 µL/kg/day). Levels of creatinine, urea and uric acid were increased in rats sera after injection with doxorubicin, while blood electrolyte levels of Na, K, P and Mg were decreased. Also, histopathological abnormalities such as hyalinized blood vessels, degenerated hyalinized glomerulus tubules and cell debris in the lumen and degeneration of renal tissues were observed in these rats. After treatment with SCs, all these parameters restore their normal values with regeneration of the damaged cells as demonstrated in histopathology of the treated groups. It can be concluded that, the use of SCs in treatment of kidney diseases is a promising approach and needs more efforts.

Keywords: Electrolytes. Growth factor. Hematopoietic stem cells. Immunosuppressor. Mesenchymal stem cells. Regeneration.

INTRODUCTION

The kidney is a complex organ. Repair and regeneration of damaged kidney cells are still under investigation (Little, 2006). Regardless of progress in the management of chronic kidney disease (CKD) with prescriptions and renal dialysis or transplantation therapy, CKD still a major health problem. In CKD, fibrosis is chronic since renal regenerative capability is not enough and ineffective. The presently available medicines cannot repair the damaged tissue. Therefore, morbidity and mortality in CKD are still high (Saad *et al*., 2018a). Renal dialysis is a solution, but the mortality rate of patients requiring chronic dialysis is high. Although kidney transplantation is the most effective treatment option for the majority of end-stage renal disease, the shortage of donors is a limiting factor. Considerable efforts have been done for identification of innovative therapies to delay renal damage in CKD. The animal model research on cell-based therapies in CRF is little (Little, 2006). Confirmation for improvement of kidney function and structure through applying cell-based therapies in preclinical models of CKD was introduced (Saad, EL-Demerdash, Abd EI-Fattah, 2019).

The reported results about the effectiveness of mesenchymal stem cells (MSCs) in treating renal disease has been investigated in animals, and in pre-clinical models the results are promising (Papazova *et al*., 2015). Multiple mechanisms have been impacted in the therapeutic potential of MSCs. These mechanisms include activation of regulatory immune cells, immunomodulatory effects through secretion of regulatory cytokines and the

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capacity to increase cellular repair through secretion of anti-apoptotic, anti-fibrotic, and up-regulation of renal development markers (Morigi, Rota, Remuzzi, 2016).

SCs are classified as totipotent, pluripotent and multipotent, and can differentiate into diverse specialized cell types and self-renew to produce more SCs (Mimeault, Batra, 2006; Saad *et al.*, 2018b). Experimental evidence revealed that tubular, glomerular, and interstitial kidney compartments might be structurally recovered by MSCs, in addition to the improvement in the function of chronic kidney injury (Asanuma, Meldrum, Meldrum, 2010). After injury, damaged cells secrete a group of cytokines called chemokines that act as attractants to recruit immune and SCs to the damaged cells to start the process of repair (Chavakis, Urbich, Dimmeler, 2008).

The mechanisms proposed to explain renal tissue regeneration include trans-differentiation, a process by which a MSC differentiates into an adult cell from another tissue, cell fusion between bone marrow MSC with cells from the affected organ, and paracrine action of MSC on remaining tissue due to secretion of a variety of anti-inflammatory cytokines and growth factors that modulate the inflammatory response (Little, 2006; Saad, EL-Demerdash, Abd EI-Fattah, 2019). Insulin-like growth factor-1 (IGF-1) is one of the key cytokines secreted by MSCs post-transplantation that participates in proliferation and differentiation of SCs (Tao *et al.*, 2010).

Several tissue-originating MSCs display significant differences in their proliferative capacities. For example, human umbilical cord blood (hUCB)-derived MSCs (hUCB-MSCs) have better proliferative potential than bone marrow and have lower risk of graft-versus-host disease (Morigi *et al.*, 2010). The mean doubling time of the UC-MSCs revealed about 24 hours and persisted almost constantly for up to 10 cell passages. On contrary, the doubling time of bone marrow (BM)-MSCs reached about 40 hours and considerably increased already after 6 cell passages (Lu *et al.*, 2006). Likewise, adipose tissue-derived MSCs also demonstrated an elevated growth rate as compared to BM-MSCs (Kern *et al.*, 2006).

Therefore, the present work is a participation in the efforts to exploit the characteristics of SCs, and how to use them in regeneration of damaged kidney cells during CRF using histopathological and biochemical techniques

in rat model. To investigate if the immune suppressor has effect or not on the homing of stem cells, we used cyclosporine A as an immunosuppressor in our model.

MATERIAL AND METHODS

Stem cells (SCs)

Umbilical cord samples (n=4) were collected from women with healthy pregnancies, after obtaining their informed consent at the Department of Obstetrices and Gynecology, Al-Azhar University Hospital, Faculty of Medicine, Al-Azaher University, Damietta, Egypt. Umbilical cord tissue was enzymatically digested for obtaining MSCs and its heparinized blood was withdrawn for immuno-magnetic separation of hematopoietic stem cells (HSCs). Institutional ethics committee approval was obtained for all procedures. Hematopoietic stem cells (HSCs) ($CD34^+$) and MSCs ($CD34^-$) were isolated and identified using immune-magnetic selection method (Manual EasySep® Protocol Using Purple EasySep® Magnet (StemCell Technologies)). SCs were then cultured and passaged. Trypan blue viable cells count was carried out by trypan blue exclusion.

Growth factor extract

Umbilical cord tissue as a source for insulin-like growth factor-1 was used. Tissue was weighed, homogenized in 10 mM Tris-HCl buffer pH 7.4, sterilized and filtered using filter syringe o.4 μm in sterilized fume hood. Growth factor presence was confirmed via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970), followed by radial immunodiffusion test (Graham, 1996) application. Growth factor extract activity was examined microbiologically via its effect on *Klebsiella pneumoniae* growth. Bacterial growth was estimated by turbidity measurement (Mackintosh, Watson, O'Grady, 1973) where absorbance is proportional to the number of bacterial cells. In addition, the bacterial number in culture media after 24 hours was counted. The bacterial inoculum was kindly provided by Prof. M. I. Abou Dobara, Botany

Department, Faculty of Science, Damietta University, Egypt and cultured on nutrient-agar medium.

Experimental animals of the study

All experiments were performed on 64 adult male Sprague Dawley albino rats purchased from Theodore Bilharzia Institute, Giza, Egypt, with body weights of 80 to 110 g. Rats were fed on commercial standard diet and tap water and housed in steel mesh cages (8 rats/cage) and maintained for two weeks acclimatization periods following guidelines of National Institute of Health (NIH, 1996). The rats were divided into eight groups, 8 animals each.

Experimental groups

Group I: Chronic renal failure (CRF) rats: injected once i.p. with 15 mg doxorubicin (adriamycin®)/kg (Mansour, El-kashef, Al-Shabanah, 1999).

Group II: CRF rats treated with MSCs: after one week from i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with MSCs (1×10^6 cells) (Kim *et al.*, 2012).

Group III: CRF rats treated with MSCs and an immunosuppressor; and after two days of i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with MSCs (1×10^6 cells) and 5 mg cyclosporin A (Sigma)/kg/day as immunosuppressor for 30 days (Tanabe *et al.*, 2000).

Group IV: CRF rats treated with HSCs: after one week from i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with HSCs (2×10^4 cells) (Habib *et al.*, 2020).

Group V: CRF rats treated with HSCs and an immunosuppressor, and after two days of i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with HSCs (2×10^4 cells) and 5 mg cyclosporin A/kg/day as immunosuppressor for 30 days.

Group VI: CRF rats treated with MSCs and HSCs, and after one week of i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with MSCs (1×10^6 cells) and HSCs (2×10^4 cells).

Group VII: CRF rats treated with MSCs, HSCs, growth factors extract and an immunosuppressor; after two days from i.p. injection with 15 mg doxorubicin/kg, rats were injected once i.p. with MSCs (1×10^6 cells), HSCs (2×10^4 cells), and daily with sterilized growth factor crude extract (200 μL) (Habib *et al.*, 2020) and 5 mg cyclosporin A/kg/day as immunosuppressor for 30 days.

Group VIII: Negative control rats; rats were injected i.p. with 100 μL of physiological saline solution/kg/day for 30 days.

The study protocol was approved by the Chemistry Department, Faculty of Science, Damietta University.

Following–up of HSCs and MSCs

After 30 days of treatment, the rats of different groups were euthanized using a cutter after being anesthetized. Blood samples were obtained then and the biochemical and histopathological changes were tested to follow-up the injected SCs (MSCs&HSCs). A part of each collected blood sample was centrifuged for obtaining serum and the other part was collected using EDTA as anticoagulant for obtaining plasma. Kidney samples were quickly excised, rinsed with isotonic saline solution, and dried with filter paper. Kidney samples were weighed and stored at -20 ºC in plastic vials containing 0.5 mL of ice cold sterile isotonic saline solution. 0.14 g of each kidney was homogenized in 1 mL phosphate buffered saline pH 7.4 using a Teflon pestle connected to a homogenized motor (25 strokes per minute at 1000 rev/min) and the homogenate was diluted to become 10% (w/v). The homogenate was centrifuged at 13000 rpm for 30 min at 4ºC. The supernatant was used for biochemical analysis.

Biochemical analysis

Creatinine was determined by the alkaline picrate the method of Brod and Sirota (1948). Urea was determined by the method of Chaney and Marbach (1962). Uric acid

was determined by the method of Henry, Sobel, Kim (1957). Serum sodium and potassium were determined by the method of Maruna (1958). Serum phosphorous and magnesium were determined according to the method of Vassault *et al.* (1986). Protein fractionation was done using one-dimensional SDS polyacrylamide gel electrophoresis according to the method of Laemmli (1970).

Histopathological examination

For the histopathological examination, tissues were stored in 10% formalin and embedded in paraffin. Sections (4 μm) were stained routinely with Hematoxylin and eosin stain (H&E) (Puchtler, Meloan, Waldrop, 1986).
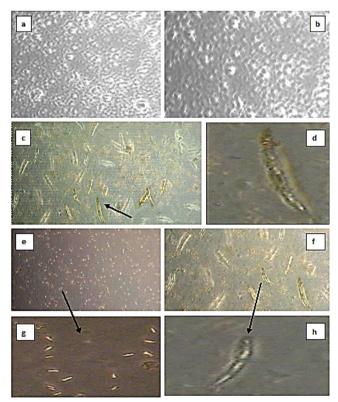
Statistical analysis

Statistical analysis was carried out by SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The data were expressed as mean±SD. Correlation tests were made by Graph pad prism 5. The p values ≤0.05 was said to be significant.

RESULTS

HSCs and MSCs

Figure 1 shows successful culturing of separated and identified MSCs with their characteristic fibroblast shape with a comparison between MSCs after culturing for 4 days and 7 days, in addition to the successful growth of separated and identified HSCs isolated from umbilical cord blood in culture media after 7 days incubation.
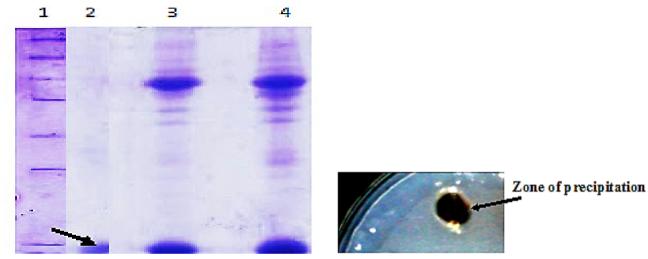

FIGURE 1 - Growth of CD34 positive (CD34⁺) cells (hematopoietic stem cells (HSCs)) in culture after 7 days incubation using inverted microscope (**a & b**, 200X). Umbilical cord mesenchymal stem cells (MSCs) (CD34 negative (CD34⁻) cells) isolated from Wharton's jelly after 7days of incubation in culture media (fibroblast-like morphology) (**c**, 200X). A large magnification of MSC (**d**, 400X). MSCs after 4 days of incubation of culture media (**e**, 400X). MSC after 7 days of incubation of culture media (**f**, 200X). MSCs have the fibroblast cell shape with Magnification 400X (**g & h**)
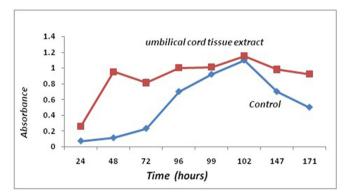
Growth factor extract

Separated bands, at 7.9 kDa, from the umbilical cord tissue extract samples suggested that the extract may contain insulin-like growth factor-1 (Figure 2, Left). This is further ascertained via application of a radial immunodiffusion test which showed the precipitation zone due to insulin-like growth factor-1 and insulin-like growth factor-1 antibody (concentrations 25 µg/dL protein) reaction evidencing the presence of insulin-like growth factor-1 in the umbilical cord tissue extract (Figure 2, Right). Next, the biological activity for this growth factor tissue extract was examined using *Kelibsella pneumoniae* as a bacterial model. The bacterial growth was enhanced by the addition of the growth factor tissue extract to the culture media as seen in Figure 3. After 24 hours of bacterial culturing, the mean bacterial number in presence of the growth factor tissue extract was significantly increased compared to that of the control (in absence of the growth factor tissue extract) (4231×10^6 and 3465×10^6, respectively, $p < 0.05$) (data not shown). This indicates the supportive role of the growth factor tissue extract on cellular growth.

FIGURE 2 - SDS-PAGE (16%) pattern for growth factor tissue extract [Left]. Lane 1: Marker (175, 80, 58, 46, 30, 25, and 7 kDa), Lane 2: Standard Insulin-like growth factor-1 in a concentration of 8 µIU/mL (obtained from Faculty of Medicine, Al-Azaher University, Damietta, Egypt, Lane 3: Sample of umbilical cord extract, and Lane 4: Another sample of umbilical cord extract. Arrow shows band of standard insulin-like growth factor (7.9 kDa). Radial immunodiffusion test for umbilical cord tissue extract and insulin-like growth factor-1 with anti-insulin-like growth factor-1 antibody [Right]. Arrow shows the precipitation zone after 24 hours

FIGURE 3 - Effect of growth factor on bacterial growth using *Kelibsella pneumoniae* as a bacterial model in culture media with and without growth factor tissue extract from umbilical cord using absorbance at wavelength 600 nm

Drug-induced CRF in rat model

In rats with induced-CRF, the most important observations were recorded including the reduction in the physical activity and loss of appetite in addition to bleeding during urination. Besides, the detected significant increases in levels of serum creatinine (16.27 mg%), uric acid (95.81 mg%), and urea (114.59 mg%) compared to 6.43 mg%, 61.11 mg%, and 44.67 mg%, respectively in normal rats (Table I). In addition to the detected significant decreases in levels of sodium, phosphorous and potassium compared to normal (Table II).

Histopathological studies for rats with CRF revealed that doxorubicin drug induced renal damage as shown in Figure 4a-c; glomeruli showed hyalinosis and tubules became ecstasies with protein cast compared to control (Figure 4j).

TABLE I - Mean levels of creatinine (mg%), urea (mg%), uric acid (mg%) and creatinine/urea ratio in serum of rats with chronic renal failure (GI-VII) and control rats (GVIII)

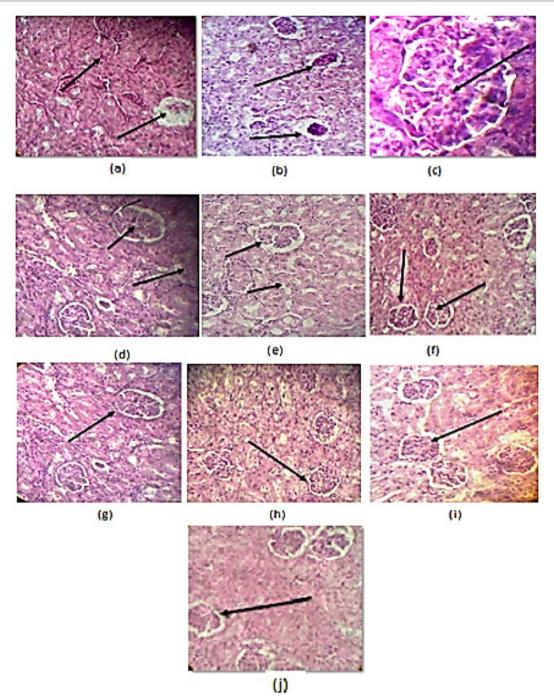| Groups	Creatinine (mg%)	Urea (mg%)	Uric acid (mg%)	(Creatinine/ urea) ratio
Group I	$16.27\pm 6.54^{**}$	$114.59\pm30.57^{**}$	$95.81\pm 6.90^{**}$	$0.23\pm0.09^{*}$
Group II	$6.58\pm 2.99^{ns,\dagger\dagger}$	$49.19\pm8.22^{ns,\dagger\dagger}$	$62.50\pm 5.74^{ns,\dagger\dagger}$	$0.13\pm0.07^{ns,\dagger}$
Group III	$6.98\pm 1.65^{ns,\dagger\dagger}$	$50.36\pm6.88^{ns,\dagger\dagger}$	$70.77\pm 15.44^{ns,\dagger\dagger}$	$0.11\pm 0.05^{ns,\dagger}$
Group IV	$7.69\pm 0.99^{ns,\dagger\dagger}$	$38.63\pm 3.09^{ns,\dagger\dagger}$	$64.92\pm 6.96^{ns,\dagger\dagger}$	$0.15\pm0.01^{ns,\dagger}$
Group V	$5.70\pm 0.28^{ns,\dagger\dagger}$	$60.51\pm8.44^{ns,\dagger\dagger}$	$68.32\pm43^{ns,\dagger\dagger}$	$0.11\pm0.03^{ns,\dagger}$
Group VI	$6.59\pm2.07^{ns,\dagger\dagger}$	$65.09\pm3.54^{ns,\dagger\dagger}$	$62.07\pm3.44^{ns,\dagger\dagger}$	$0.15\pm0.23^{ns,\dagger}$
Group VII	$8.32\pm3.43^{ns,\dagger\dagger}$	$41.38\pm 6.47^{ns,\dagger\dagger}$	$69.63\pm 14.32^{ns,\dagger\dagger}$	$0.17\pm0.07^{ns,\dagger}$
Group VIII	6.43 ± 0.12	44.67 ± 12.6	61.11 ± 4.37	0.10 ± 0.12

The values represented as mean±SD, (n=8). Group I: Rats with chronic renal failure (CRF). Group II: Rats with CRF treated with MSCs. Group III: Rats with CRF treated with MSCs and cyclosporin A. Group IV: Rats with CRF treated with HSCs. Group V: Rats with CRF treated with HSCs and cyclosporin A. Group VI: Rats with CRF treated with HSCs and MSCs. Group VII: Rats with CRF treated with MSCs, HSCs, growth factor and cyclosporin A. Group VIII: Kidney of control rats. ns, *, **: non-significant (p>0.05), p<0.05, p<0.001, respectively when compared with Group VIII. †, ††: p<0.05, p<0.001, respectively when compared with Group I.

TABLE II - Mean levels of sodium (mmole/L), potassium (mmole/L), phosphorous (mg%) and magnesium (mg%) in serum of rats with chronic renal failure (groups I – VIII) and control rats (group VIII)

Groups	Sodium (mmole/L)	Phosphorous (mg%)	Magnesium (mg%)	Potassium (mmole/L)
Group I	$130.75\pm1.50^{**}$	$7.12\pm0.67^{**}$	$1.18\pm0.13^{**}$	$3.57\pm1.91^{**}$
Group II	$151.20\pm7.40^{*,\dagger\dagger}$	$9.57\pm1.18^{ns,\dagger\dagger}$	$3.07\pm.39^{ns,\dagger\dagger}$	$5.06\pm.30^{ns,\dagger}$
Group III	$151.52\pm8.99^{ns,\dagger\dagger}$	$8.75\pm1.24^{ns,\dagger\dagger}$	$2.05\pm.94^{*,\dagger}$	$4.88\pm.20^{ns,\dagger}$
Group IV	$155.12\pm10.08^{ns,\dagger\dagger}$	$8.11\pm.79^{ns,\dagger}$	$2.26\pm0.34^{*,\dagger}$	$4.97\pm1.09^{ns,\dagger}$
Group V	$140.43\pm9.57^{*,\dagger}$	$7.41\pm0.51^{ns,\dagger}$	$2.03\pm0.22^{*,\dagger}$	$4.83\pm0.32^{ns,\dagger}$
Group VI	$137.23\pm12.32^{*,\dagger}$	$7.94\pm0.04^{*,\dagger}$	$2.65\pm0.23^{*,\dagger}$	$4.66\pm0.45^{ns,\dagger}$
Group VII	$150.54\pm3.07^{ns,\dagger\dagger}$	$8.55\pm1.04^{ns,\dagger\dagger}$	$2.06\pm0.30^{*,\dagger}$	$4.77\pm0.43^{ns,\dagger}$
Group VIII	160.12 ± 3.59	$9.77\pm.31$	$4.11\pm.68$	5.29 ± 1.00

The values represented as mean±SD, (n=8). Group I: Rats with chronic renal failure (CRF). Group II: Rats with CRF treated with MSCs. Group III: Rats with CRF treated with MSCs and cyclosporin A. Group IV: Rats with CRF treated with HSCs. Group V: Rats with CRF treated with HSCs and cyclosporin A. Group VI: Rats with CRF treated with HSCs and MSCs. Group VII: Rats with CRF treated with MSCs, HSCs, growth factor and cyclosporin A. Group VIII: Kidney of control rats. ns, *, **: non-significant (p>0.05), p<0.05, p<0.001, respectively when compared with Group VIII. †, ††: p<0.05, p<0.001, respectively when compared with Group I.

FIGURE 4 - Histopathological examination of kidney tissue (E&H, 400 X). **Group I**: (**a, b, c**) Rats with chronic renal failure (CRF). **Group II**: (**d**) Rats with CRF treated with isolated MSCs. **Group III**: (**e**) Rats with CRF treated with isolated MSCs and cyclosporin A. **Group IV:** (**f**) Rats with CRF treated with isolated HSCs. **Group V:** (**g**) Rats with CRF treated with isolated HSCs and cyclosporin A. **Group VI:** (**h**) Rats with CRF treated with isolated HSCs and MSCs. **Group VII:** (**i**) Rats with CRF treated with MSCs, HSCs, growth factor and cyclosporin A. **Group VIII**: (**j**) Kidney of control rats.

Treatments of drug-induced CRF in rat model

The highly significantly-increased serum levels of creatinine, urea, uric acid, and creatinine/urea ratio in CRF rats (Group I) were nearly normalized after treatment with MSCs and/or HSCs either in the presence or absence of growth factor and cyclosporin A as immunosuppressor drug (Group II-VII) (Table I).

The highly significantly-decreased levels of sodium, phosphorous and potassium in CRF rats became higher, near to the control, after treatment with MSCs and/or HSCs either in the presence or in the absence of growth factor and cyclosporin A as immunosuppressor drug (Group II-VII). Also, magnesium levels were re-elevated after treatment, but its levels still in the treated groups (Group II-VII) lower than its level in the normal control (Group VIII) (Table II).

Histopathology

Figure 4 shows the histopathological examination of kidney tissues of rats with CRF and treated rats for comparison. Renal tissues of CRF showed a reduction in Bowman's capsule spaces, focal segmental, glomerulosclerosis, tubular atrophy, interstitial expansion, loss of glomerular attachments and mesangial stroma, widened urinary space, melting of cell margination of renal tubules, appearance of intertubular inflammatory cells, vacuolation of tubules with necrotic lesions in some areas and red blood cells in between the interstitial tissue due to doxorobcin effect (Figure 4a-c). After 30 days of treatment, the histopathology of kidney tissues showed interlobulation of glomerular tufts into finger-like projections and cloudy swelling and vacoulation of the proximal tubules indicating renal tissue regeneration. This means that, MSCs and HSCs administration nearly normalize the abnormal changes in CRF, in nearly all the treated groups (Figure 4d-i).

Correlations

Serum creatinine in CRF was negatively correlated with levels of sodium, phosphorous, and potassium while positively correlated with levels of urea and uric acid (Figure 5).
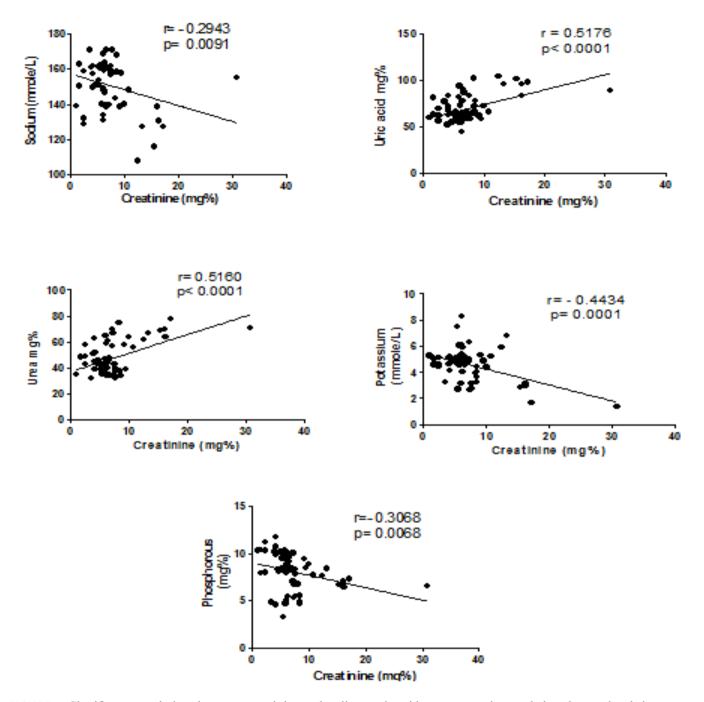
FIGURE 5 - Significant correlations between creatinine and sodium, uric acid, urea, potassium and phosphorous levels in serum

DISCUSSION

Renal toxicity may arise as a complication of certain chronic conditions including cancer, or arises as a characteristic adverse effect of certain anticancer drugs limiting their clinical use (Saad, Hassanien, Elneely, 2017a; Saad *et al.*, 2017b; Saad *et al.*, 2018c; Elsayed, Saad, Mostafa, 2019; Saad, Waly, 2019). Among these anticancer drugs, doxorubicin is accompanied by high incidence of renal tubular damage due to nephrotoxicity

resulting in impaired renal function, as measured by blood urea and creatinine between 4 and 7 days after doxorubicin administration in the murine model (Kawaida *et al.*, 1994).

In the present study, the obtained results clearly showed that the injection of the doxorubicin drug resulted in an increase in levels of creatinine, urea, and uric acid ($p<0.001$). They all are indications of renal damage as supported here by the detected positive correlations between them ($p<0.0001$). MSCs and/or HSCs, with or without immunosuppressor and growth factor administration, normalized these changes ($p>0.05$ compared to normal control). These results indicate both human umbilical cord and (hUC)-derived SCs were of benefit in the treatment of renal damage in our model. Earlier, Kunter *et al.*, (2006) stated that MSCs contribute to regeneration of endothelial and mesangial cells in a CRF model, and MSCs display low immunogenic potential as they express small amounts of antigen-expressing molecules (major histocompatibility class I (MHC-I) and class II (MHC-II)) (Kode *et al.*, 2009) in addition to the immunosuppressive properties of MSCs (Mosanya, Isaacs, 2019). HSCs have a capacity for maintaining the immune system throughout life via generating all blood cells (Lee *et al.*, 2019).

Ma *et al.* (2013a) reported that cisplatin and doxorubicin increase blood urea and creatinine levels. These elevations are indications of impaired renal function (Toson *et al.*, 2014; Saad *et al.*, 2017b). These reports confirm data in the current study; doxorubicin injection resulted in elevated urea and creatinine levels because of their impaired renal elimination, indicating a drop in glomerular filtration rate (Saad *et al.*, 2018a). Besides, the present study, in agreement with Saad *et al.* (2018c), showed that induced-CRF caused loss of appetite together with the increase in catabolism resulting in acidosis, which was accompanied by anorexia. Numerous investigations showed that cisplatin/doxorubicin injection resulted in nephrotoxicity provoked by oxidative stress and caused damage to renal tubular epithelial cells (Chirino, Pedraza-Chaverri, 2009; Saad *et al.*, 2020).

The present data showed that single injection of doxorubicin in rats resulted in deterioration of renal function as indicated by the disturbance in blood electrolyte levels (Na, Mg, P, and K), along with the negative correlations detected between Na, K, and P and serum creatinine ($p<0.01$- $p<0.0001$). These disturbances may be due to an abnormal transport system throughout renal failure condition (Saad, EL-Demerdash, Abd EI-Fattah, 2019). These results are consistent with other studies (Behling *et al.*, 2006). The present results also revealed that Na, P and K returned approximately to the normal levels (except Mg was still less than normal) when the animals were treated with SCs. Also, a significant improvement in spinal cord entrapment and kidney failure was observed after implantation with umbilical cord MSCs (Rahyussalim *et al.*, 2017).

The histopathological abnormalities, such as hyalinized blood vessels, degenerated hyalinized glomerlus tubules, cell debris in the lumen and degeneration of renal tissues in rats with CRF because of doxorubicin administration were observed in the current study (GI). These pathological changes are in the same line with those observed by Ma *et al.* (2013a) and Saad, EL-Demerdash, Abd EI-Fattah (2019) who reported that either cisplatin or doxorubicin enhances apoptosis, proximal tubules necrosis, and abundant tubular protein casts generation in rats. These abnormalities were repaired in the groups treated with MSCs and HSCs in the presence or absence of cyclosporine A and insulin-like growth factor-1 (GII-VII), verifying a regeneration of renal glomeruli when compared to normal kidney tissue injected intraperitoneally with physiological saline solution as a negative control (GIII), with slight differences between the treated groups. Other previous studies demonstrated the role of SCs in regeneration of damaged renal tissues, like the study of Ma *et al.* (2013b) and of Saad, EL-Demerdash, Abd EI-Fattah (2019). This indicates that SCs have a prospective protective influence versus cisplatin and doxorubicin-induced nephrotoxicity.

Compared to the normal kidney tissue section, 30 days of different SCs treatments to CRF showed interlobulation of glomerular tufts into finger like projections, and cloudy swelling and vacoulation of the proximal tubules, indicating renal tissue regeneration. Like MSCs in the previous work (Saad, EL-Demerdash, Abd EI-Fattah; 2019), the present experiment illustrated

that the HSCs too, have the ability to interact with injured renal cells and regenerate renal tissue and restore their functionality. Zhang, Qin, Zhou (2007) reported that MSCs have the ability to home to the injured kidney and to speed up morphological and functional regeneration, possibly by paracrine or even endocrine pathways. The production of growth factors and cytokines with immunosuppressive, anti-inflammatory, anti-apoptotic and proliferative effects is the major mechanism in the effect of SCs (Kazi, Mubarak, 2011).

Comparing the effects of different treatments on doxorubicin-induced CRF, administration of MSCs and/or HSCs with or without immunosuppressor and growth factor exhibited approximately similar actions. These results indicate that each single type, MSCs or HSCs, of human umbilical cord (hUC)-derived SCs can do the job; gathering of both did not show a more valuable effect than single ones did. Moreover, addition of the immunosuppressor cyclosporine to treatment did not augment an amelioration role played by either MSCs and/or HSCs, as evidenced here. This proposes that SCs have the ability to stimulate secretion of growth factors and immunosuppressors, so there is no need to add any exogenous growth factor or immunosuppressor during the use of SCs for treatment.

In conclusion, the results of the present work explored the nearly-equal ability of both human umbilical cord HSCs and MSCs to repair doxorubicin-injured rat renal cells and to restore their function. Their capacity to improve renal dysfunction and repair tubular injury may be attributed to the ability to initiate renal tubular cell proliferation and to differentiate into tubular cells. This property suggests the production of growth factors and anti-inflammatory with immunosuppressive cytokines during the mechanism. Therefore, they do not need to use exogenous growth factors or immunosuppressors.

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CONFLICT OF INTEREST

Authors have no conflict of interest regarding this study.

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