

## Follow-up the Human Umbilical Cord Stem Cell Injected Into Rats with renal Impairment

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**Abstract:** Umbilical cord (UC) is a source of a population of pluripotent, mesenchymal-like stem cells, UC is rapidly gaining attention for its therapeutic value their low incidence of rejection after UC transplantation. UC transplantation does not require perfect antigen matching. The aim of this study to follow-up human Umbilical Cord Stem Cell injected Into Rats With renal Impairment by biochemical and histochemical parameters and show the effect of mesenchymal stem cell (MSC) on kidney generation. After cells isolation and then they were cultured in optimum growth media. Twenty four albino male rats used in this study, Sixteen rats were injected with cisplatin drug (5 mg /kg body weight) single dose to obtain rats with acute renal failure, then they were divided into two groups, group I: contains eight rats with a cute renal failure as a positive control, group II: contains eight rats were injected with a mesenchymal stem cell (MSC) (100 µl of cell suspension (1x10<sup>6</sup> cell) intraperitoneally injected single dose. Group III: contains eight rats were intraperitoneally injected with saline solution (100 µl /kg body weight). Biochemical and histochemical studies to show significantly ( $p < 0.05$ ) renal tissue recovery. This work showed that these cells have the therapeutic potential for cell therapy and biological treatment which can be easily obtained.

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**Key words:** Mesenchymal stem cell, Acute renal failure, Wharton's jelly, tissue engineering.

### 1. Introduction:

Mesenchymal stem cells (MSCs) are an important source for tissue engineering. Embryo-derived tissues, such as umbilical cord (UC), represent attractive sources of MSCs because their use is not related to any ethical and technical issues (Igura *et al.*, 2006).

The UC matrix is considered to be a rich, non-controversial and inexhaustible source of primitive MSCs (Weiss *et al.*, 2006). Human umbilical cord Wharton's jelly-derived MSCs (WJMSCs) could be differentiated *in vitro* into adipocytes, chondrocytes, osteocytes, neurons and myogenic cells (Igura *et al.*, 2006) and they did not express major histocompatibility complex class II antigens (Sarugaser *et al.*, 2005).

These cells have more potency in differentiation into osteogenic and chondrogenic cells compared with bone marrow-derived MSCs (Karahuseyinoglu and Cinar, 2007). Therefore, WJ-MSCs could become a useful alternative source of MSCs for cell therapy and tissue repair in the field of regenerative medicine.

Mesenchymal stromal cells (MSCs), as defined by the International Society for Cellular Therapy, are plastic-adherent cells with a specific surface phenotype that have the capacity to self-renew and to differentiate into various lineages including bone, cartilage, and adipose. Such cells can be derived from several different sources, such as trabecular bone,

adipose tissue, skeletal muscle, dermis, blood, and bone marrow.

Cord stem cells are extracted from either cord blood or the Wharton's jelly of umbilical cords through enzyme digestion (Harris *et al.*, 2007) and have a number of critical advantages over other MSCs: (1) the tissue is routinely discarded; therefore, the tissue is available for cord stem cell extraction; (2) the collection process of the tissue is non-invasive; and (3) there is no donor risk to an organ or tissue.

Mesenchymal stem cells (MSCs) are capable of self-renewal and differentiation into multiple cell lineages. MSCs are multipotent and are easily derived from a variety of tissues, including fat, skin and bone marrow. Presently, bone marrow is considered as a prime source of MSCs and also gingival (Tomaret *et al.*, 2010). These cells are fibroblasts in appearance and can be expanded for many passages. Most importantly, population of mesenchymal stem cells (MSCs) is strongly adherent, therefore can be isolated by culturing marrow on an appropriate substrate and washing other cells off. Mesenchymal stem cells can give rise to many kinds of connective tissue cells including those responsible for remodeling of cartilage, bone, fat, and vascular tissue (Pittenger and Martin, 2004; Chen *et al.*, 2008). MSCs are likely to participate in the maintenance of the essential microenvironment necessary to support the

hematopoietic stem cells in the bone marrow (**Dennis and Caplan, 2004**).

Human adult mesenchymal stem cells (MSCs) are non-hematopoietic, adherent fibroblast-like cells with an intrinsic ability of self-renewal and potential for multilineage differentiation. Immunosuppressive properties of MSC most probably also depend on environmental factors. Human and murine MSC have been shown to express toll-like receptors (TLRs) and the ligation of TLR3 (Toll-like receptor 3) and TLR4 (Toll-like receptor 4) by their respective natural ligands, double-stranded RNA and LPS (lipopolysaccharide), prevented the MSC from inhibiting T cell responses by the down-regulation of Jagged-1 expression on MSC (**Tomchuck et al., 2008**).

UCMSCs (Umbilical cord mesenchymal stem cells) have surfaced phenotype differentiation capability and immune properties similar to MSCs derived from bone marrow and adipose. UCMSCs are more similar to fetal MSCs in terms of their in vitro expansion potential. MSCs were isolated from the Wharton's jelly of umbilical cord segments and defined morphologically. UC-MSCs was then tested for their ability to regenerate Kidney tissues in rat infected with acute renal failure.

Acute renal failure represents a rapid decline in renal function sufficiently to increase blood levels of nitrogenous wastes and impair fluid and electrolyte balance.

It is a common threat to seriously ill persons in intensive care units, with a mortality rate ranging from 42% to 88% (**Levy et al., 1996**). Although treatment methods such as dialysis and renal replacement methods are effective in correcting life-threatening fluid and electrolyte disorders, the mortality rate associated with acute renal failure has not changed substantially since the 1960s (**Thadhani et al., 1996; Brady et al., 2000**). This probably is because acute renal failure is seen more often in older persons than before, and because it frequently is superimposed on other life-threatening conditions, such as trauma, shock, and sepsis.

The aim of this study to follow-up human Umbilical Cord Stem Cell injected Into Rats With renal Impairment by biochemical and histochemical parameters and show the effect of mesenchymal stem cell (MSC) on kidney generation

## 2. Materials and Methods:

Our study was made on 24 adult male albino rats. Sixteen rats were injected intraperitoneally with cisplatin drug single dose (5mg/kg body weight) to obtain rates with Acute renal failure then they were divided into two groups (n=8 rat/group). Group I: Rats with Acute renal failure. Group II: Rats were

injected intraperitoneally with isolated Mesenchymal stem cell (MSC) (100µl of cell suspension (1x10<sup>6</sup> cell). Group III: Contains 8 rats, Rats were injected intraperitoneally with saline.

### Isolation of Mesenchymal stem cell (MSC):

Umbilical Cord samples were collected from women with healthy pregnancies, after obtaining their informed consent, at the Department of Obstetrics and Gynecology, Al-Azhar University hospital, New Damietta.

According to (**Bakhshiet al., 2008**) portion of the umbilical cord was then cut into approximately 3-cm-long segments. The segments were then placed immediately into 25 ml of phosphate-buffered saline without calcium and magnesium (PBS) supplemented with antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 0.025µg/mL amphotericin B). Each 3-cm umbilical cord segment was dissected longitudinally utilizing aseptic technique. The tissue was carefully undermined and the umbilical vein and both umbilical arteries were removed. The remaining segment was sutured inside out and incubated in 25 mL of PBS, antibiotics, and 1 mg per mL collagenase at room temperature for 2 hours. The cell suspension was separated equally into two tubes, and the cells were washed three times by diluting with PBS to yield a final volume of 50 ml per tube and then centrifuged.

Red blood cells were then lysed using a hypotonic solution. Finally, cells were plated onto 25-T flask at a concentration of  $5 \times 10^6$  to  $20 \times 10^6$  cells in 1 ml of medium. UC-MSCs were cultured in low-glucose Dulbecco's modified Eagle medium (DMEM) with 10 percent FBS (Hyclone, Logan, UT), 2 mmol per L L-glutamine, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin, 0.025 µg/ml amphotericin B). Cells were washed 48 hours after the initial plating with PBS and given fresh medium. Cell culture medium was subsequently changed twice a week through half medium changes. After 7 days, 70 to 80 percent confluence, cells were then passed using non mammalian dissociation solution (HyQTase, Hyclone). Cells were then regularly packaged with a 1:2 dilution every 7 days or reaching 80 percent Confluence. Aliquots of cultures were frozen at - 80°C for 30 days beginning with Passage 1 in medium supplemented with 10 percent DMSO and 50 percent FBS. After thawing for injection, Cells were counted and viability determination using trypan blue carefully from any contamination through days of culture according to (**Maclimanset et al., 1957**).

### Animals:

All experiments were performed with adult male albino rats purchased from Theodore Bilharz

Institute, Giza, Egypt, with an average body weight of 70 to 120 g. Rats were housed in a steel mesh cage and maintained for two weeks acclimatization periods on commercial standard diet and tap water *ad libitum*. Then, rats were divided into three groups, 8 animals per each group, as following:

sixteen adult male albino rats were injected intraperitoneal with cisplatin drug single dose (5mg/kg body weight) to obtain rats with Acute renal failure method by (Yadav *et al.*, 2010) (positive control) then they were divided into two groups (n=8 rat/group):

Using **Group I:** Acute renal failure (ARF) model (5 mg of cisplatin drug was injected intraperitoneal per kg body weight) according to (Priyadarsini *et al.*, 2012).

**Group II:** were injected intraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100  $\mu$ l of cell suspension ( $1 \times 10^6$  cells) according to (Kim *et al.*, 2012), and **Group III:** Contains 8 rats (negative control) were treated intraperitoneally with 100  $\mu$ l physiological saline solution as negative control.

### 2.3. Biochemical and histochemical Monitoring:

After 30 days of injection Mesenchymal stem cell (MSC) into rats, rats were sacrificed, heparanized blood samples were obtained then Biochemical, and histochemical parameters were investigated for all groups to follow up the injected cells as following:

#### Biochemical parameters:

Serum urea was determined by the method of (Chaney *et al.*, 1962) using Bio MED-Urea Enzymatic and Colorimetric kit, Serum Creatinine was determined by Alkaline picrate using the kinetic method by (Brod and Sirota, 1948), Serum total proteins were determined in serum by the method of (Lowery *et al.*, 1951), using the Bio MED Diagnostic-Total protein kit, and (creatinine/urea) ratio was calculated by creatinine and urea results. Kidney of studied groups after scarifying removed and was weighted also body weight was taken.

#### Histochemical study:

For the Histochemical examination, tissues were fixed in 10% formalin and embedded in paraffin. Sections (4- $\mu$ m) were stained with H&E (Hematoxylin and eosin stain) staining.

#### Statistical analysis:

Statistical analysis was conducted with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Results were expressed as mean  $\pm$  SD. Correlation tests were made by Graph pad prism 5 which were conducted using the Pearson test, all comparisons being two-

tailed. The two tailed *P*- values tables were used for statistical analyses.

### 3.Results:

In our study, Mscs (Mesenchymal stem cells) isolated from umbilical cord tissue and after their cultured in media with low glucose, Cells were harvested and were counted using inverted microscope, Rats with acute renal failure obtained after injection with cisplatin drug (5mg/kg body weight), As shintraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100  $\mu$ l of cells suspension ( $1 \times 10^6$  cells). Functional recovery in acute renal failure is well known, and the adult kidney is generally recognized to have the capacity to regenerate and repair. Umbilical cord tissue -derived cells have role in regenerative process by producing by directly differentiating to replace damaged cells. Therefore, for clinical regenerative medicine in kidney disease, the focus of stem cell biology will shift from multiple differentiation of cells or cell-therapy to multiple functions of the cells. the total loss of renal structure, kidney function can be restored by reactivating quiescent renal stem cells and supplying renal stemcells expanded sufficiently *in vitro*. Such stemcells may contribute to kidney regeneration, leading to recovery from organ failure. We believe that emerging knowledge of kidney stem cell biology and developmental biology will enable the development of new therapeutic strategies for kidney regeneration that aim to regain damaged components of the kidney or restore kidney function.

Table (1) Show Rats with acute renal failure enlarged kidney weight significantly ( $p < 0.05$ ) and kidney weight was restored after treatment with MSC significantly. Creatinine increased significantly ( $p < 0.001$ ), also it was shown in urea ( $p < 0.001$ ), mass body index was shown by kidney (weight / body weight). Total protein In issue in serum and renal tissue restored significantly.

Figure (1) Show the effective role of mesenchymal stem cell for studied group. Figure (2) Show different correlation curves between different parameters using Graphpad prism 5, Creatinine with urea showing  $p < 0.0001$ ,  $r$  (person correlation). ( $r = 0.6212$ ), highly correlated also for kidney weight with creatinine ( $p < 0.0001$ ), ( $r = 0.4764$ ), urea and total protein in serum ( $p < 0.0001$ ), ( $r = 0.7533$ ).

After Histopathological examination of renal tissues in different studies groups, Rats with Acute renal failure as shown in figure (3) cisplatin drug caused renal tissue damage, hyalinized tubules and degenerated glomerulus and blood vessels and tissue was restored its morphology after MSC treatment.

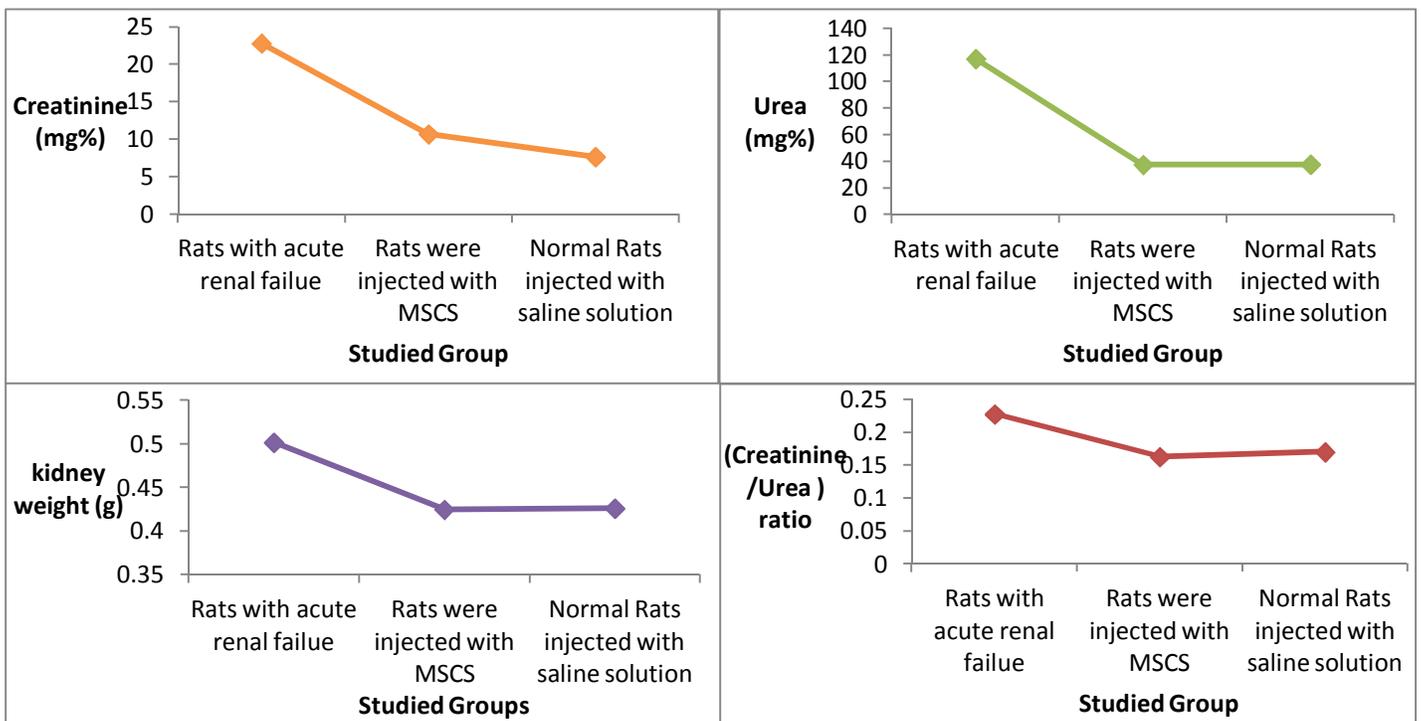
Parameters	Kidney weight(g)	Total protein In issue(mg%)	Creatinine (mg %)	(Creatinine/urea) ratio	Urea (mg %)	Total protein In srum (mg %)	Kidney wt/body wt Ratio
<b>Group I</b>	0.50 ±0.11*	126 ±18.84*	22.79±1.65**	9.04 ±3.90**	116.94±58.98*	19.42±9.07**	135.73±55.92*
<b>Group II</b>	0.43 ±0.04 <sup>ns,a</sup>	149.88 ±7.53 <sup>ns,a</sup>	10.70±2.75 <sup>ns,b</sup>	2.63±11.38 <sup>ns,b</sup>	37.61±4.23 <sup>ns,b</sup>	65.42 ±2.08 <sup>ns,b</sup>	144.77 ±20.34 <sup>ns,a</sup>
<b>Group III</b>	0.42 ±0.04	149.75 ±7.70	6.10± 1.64	1.03 ±1.66	37.72± 2.65	64.45±1.72	175.01 ±15.37

Table 1:Show Kidney weight(gm),Total protein In issue(mg%),Creatinine (mg %),(Creatinine/urea) ratio,Urea(mg %),Total protein In srum (mg %) and Kidney wt/body wt Ratio. the values represented as mean ± SD, Each group contains Number of Rat (n=8) ns non significant ( $P > 0.05$ ) when compared to group IV; \*significant ( $P < 0.05$ ) when compared to group IV; \*\*highly significant ( $P < 0.001$ ) when compared to group IV; where a significant ( $P < 0.05$ ),b highly significant ( $P < 0.001$ ),c non significant ( $P > 0.05$ ) when compared to group I,Significance was determined using ANOVA analysis.

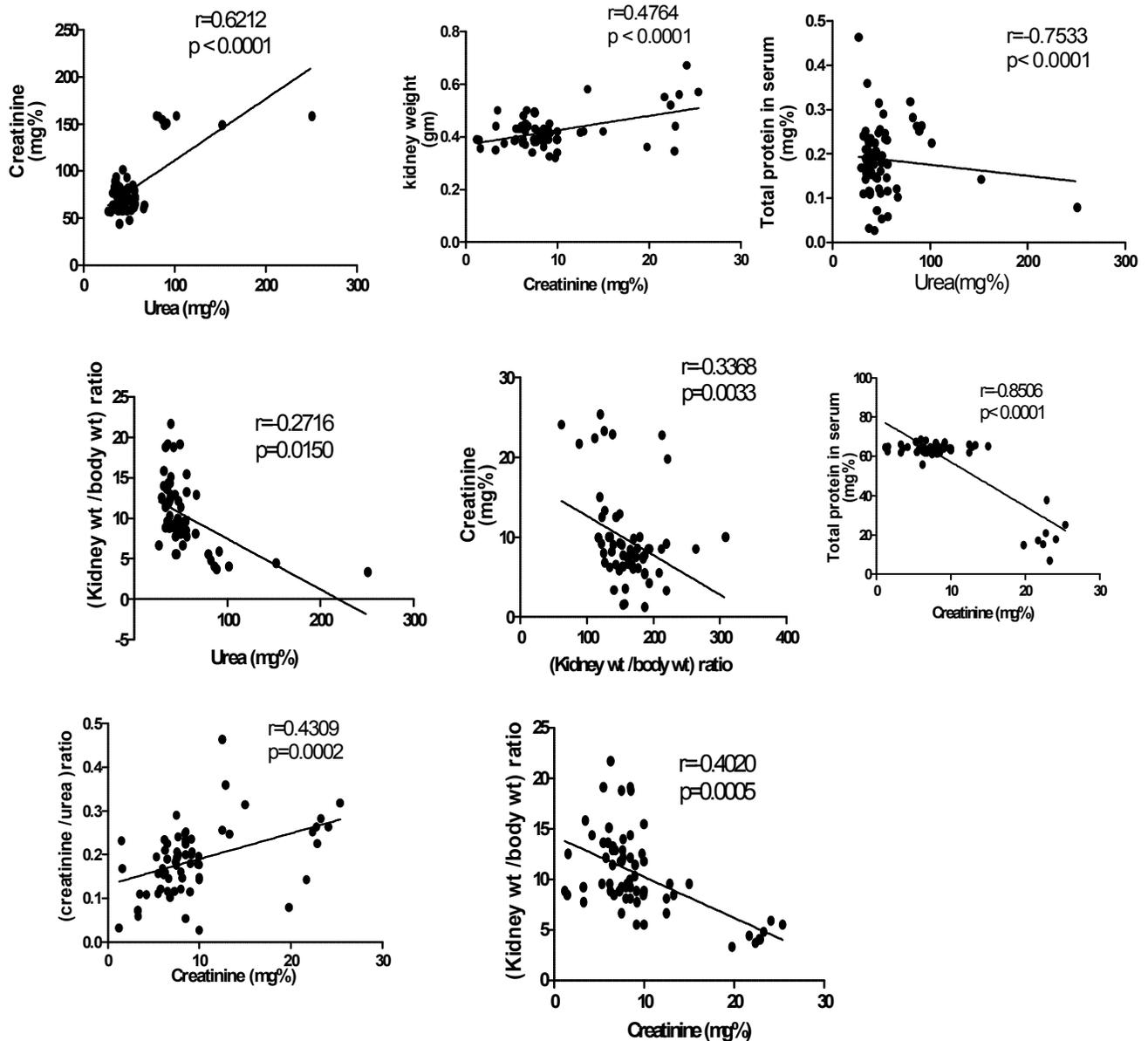
**Group I:** Acute renal failure (ARF) model (5 mg of cisplatin drug were injected intraperitoneal per kg body weight).

**Group II:** were injected intraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100 µl of cells suspension ( $1 \times 10^6$  cells).

**Group III:** (negative control) rats were treated intraperitoneally with physiological saline solution as negative control.



**Figure 1:** Show kidney weight,(creatinine/urea), creatinine and Urea (mg%) for studied groups: **Group I:** Acute renal failure (ARF) model (5 mg of cisplatin drug were injected intraperitoneal per kg body weight). **Group II:** were injected intraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100 µl of cells suspension ( $1 \times 10^6$  cells). **Group III:** (negative control) rats were treated intraperitoneally with physiological saline solution as negative control.

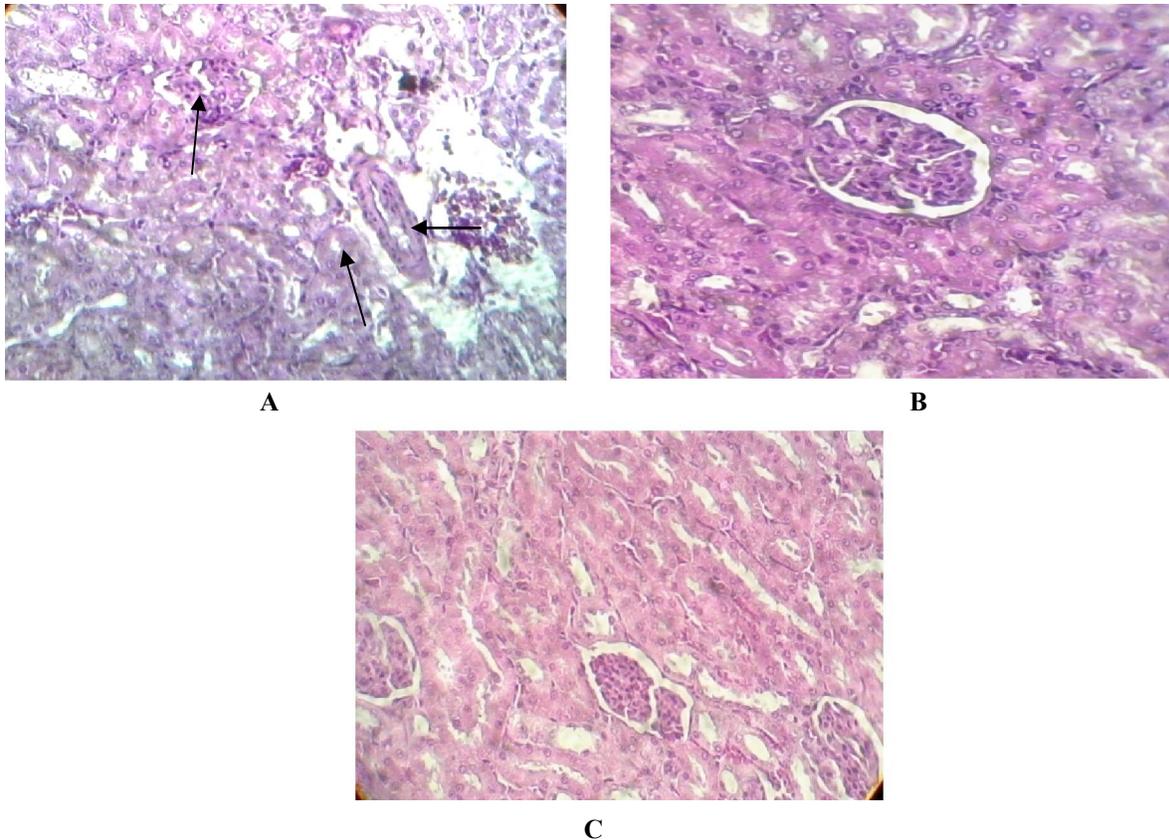


**Figure 2:** Show different correlation curves between different parameters using Graphpad prism 5. Where significance  $P<0.05$  significant correlation,  $P<0.001$  highly significant correlation;  $r$  (correlation (person correlation)) (where  $r$  near 1 indicated highly correlated). Where studied groups as following:

**Group I:** Acute renal failure (ARF) model (5 mg of cisplatin drug was injected intraperitoneal per kg body weight).

**Group II:** were injected intraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100  $\mu$ l of cells Suspension ( $1 \times 10^6$  cells).

**Group III:** (negative control) rats were treated intraperitoneally with physiological saline solution as negative control.



**Figure 3:** Show Histopathological examination of renal tissues in different studies groups as following: Group I: Acute renal failure (ARF) model (5 mg of cisplatin drug were injected intraperitoneal per kg body weight). Group II: were injected intraperitoneal with isolated Mesenchymal stem cell (MSC) single dose (100  $\mu$ l of cells suspension ( $1 \times 10^6$  cells). Group III: (negative control) rats were treated intraperitoneally with physiological saline solution as negative control. Show Histopathological examination of group (I) Rats with acute renal failure injected with cisplatin showing hyalinized tubules and degenerated glomeruli and blood vessels as indicated arrow, (B) Show renal tissue regeneration after treatment with mesenchymal stem cell isolated from umbilical cord for group (II), (C) Show normal renal tissue of group (III).

#### 4. Discussion

Mesenchymal stem cells (MSCs) are an important source for tissue engineering. Embryo-derived tissues, such as umbilical cord (UC), represent attractive sources of MSCs because their use is not related to any ethical and technical issues (Igura *et al.*, 2006). The UC matrix is considered to be a rich, non-controversial and inexhaustible source of primitive MSCs (Weis *et al.*, 2006). WJ-MSCs could become a useful alternative source of MSCs for cell therapy and tissue repair in the field of regenerative medicine.

Recent advances in stem cell research have brought the possibility of using somatic stem cells for organ regeneration one step closer to realization. Newly developed organs can then be used in clinical organ replacement. However, anatomically complicated organs such as the kidney have proven

more refractory to stem cell-based regenerative techniques.

The kidney retains the potential to regenerate if the damage is not too severe, and the kidney structure remains intact. It was previously believed that bone marrow-derived stem cells could differentiate into renal-resident cells and participate in kidney regeneration after renal ischemia/reperfusion injury (Kaleet *et al.*, 2003; Lin *et al.*, 2003); however, recent studies have suggested that the number of bone marrow-derived cells that engraft injured tubules and develop into functional renal tissue is very low and, thus, their overall contribution to renal repair would be minor in the setting of acute kidney injury (Lin *et al.*, 2005; Duffield *et al.*, 2005) Tögel (Tögelet *et al.*, 2009) and Lange (Lange *et al.*, 2005) *et al.* reported that MSC treatment resulted in significant functional improvement and injury score recovery in the absence of tubular transdifferentiation in a rat model of

ischemia/reperfusion injury. Morigi et al noted that exogenous human MSCs decreased proximal tubular epithelial cell injury, ameliorated renal dysfunction and decreased mortality in a mouse model of cisplatin induced acute renal failure (**Morigi et al., 2008**).

The effect of exogenous MSCs on glomerular healing during experimental glomerulonephritis was also investigated. Kunter et al observed that injected MSCs accelerated glomerular healing in rat (**Kunter et al., 2007**).

In contrast, Behr et al reported that autologous MSCs localized to the kidney but did not provide any beneficial effect on renal function or histology in an ovine model of bilateral renal ischemia/reperfusion injury (**Behr et al., 2009**).

This finding suggests that differences in SC behavior and regenerative potential exist between species and may account for discrepancies between rodent and large animal models. Only a few groups have examined the clinical applications of SC therapy for neurological and urological disease. A recent case study showed that SCs (stem cells) may provide some beneficial effects for the growing kidney (**Sharma et al., 2007**).

Westenfelder et al reported the preliminary results of a phase I clinical trial in which patients undergoing open heart surgery and known to be at high risk of postoperative acute kidney injury were treated with allogeneic MSCs (**Westenfelder et al., 2009**). Postoperative length of stay and rehospitalization in MSC treated patients was decreased by 50%. At discharge hormonal function in study patients remained at baseline while acute kidney injury developed in about 20% of historical controls. Three to 6 months postoperative renal function in study patients remained normal but progressively deteriorated in historical controls. In this study, Cisplatin is an antitumor drug also used for acute renal failure model according to (**Priyadarsini et al., 2012**). Rats were injected intraperitoneal with 100  $\mu$ l of cell suspension ( $1 \times 10^6$ ) showing the regeneration of renal tissue after one month of treatment, we follow-up umbilical Cord Stem Cell Injected Into Rats With renal Impairment with biochemical as creatinine and urea also histochemical study show restoring the tubules and glomerulus structure. So we need further in vivo studies to show the effective role of stem cell especially from source like an umbilical cord.

#### **Conclusion:**

Acute kidney injury is associated with poor short- and long-term outcomes and has a serious impact on patient health and cost of health care. The current therapies for kidney injury are supportive and do not facilitate regeneration. With the improved understanding of kidney stem cells, we expect developing that will facilitate regeneration. Such therapies may include exogenous administration of

kidney stem cells, kindling of endogenous stem cells for accelerated renal recovery, replacement of specific mature kidney cell types, such as products, by directing differentiation of stem cells and nephrogenesis using stem cells. However, before one can make a rapid stride in the field of kidney regeneration, there are several limitations that need to be overcome, such as identification of definitive kidney stem cell markers, niches and differentiation pathways.

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