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POST-TREATMENT AUGMENTATION OF ANGIOGENESIS AND ANTI-OXIDATIVE INDEX OF ADULT STEM CELLS DERIVED FROM WHARTON'S JELLY

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ABSTRACT

Objectives: The umbilical cord is proven as a potential source of adult stem cells, due to its abundance, harvesting, deficiency of ethical issues and wider potential of differentiation. Mesenchymal stromal cells (MSCs) derived from umbilical cord Wharton's jelly (WJ) are called as Wharton's jelly mesenchymal stromal cells (WJMSCs). Chronic kidney diseases (CKD) are categorized by functional loss and progressive damage of the parenchymal tissue of kidney which results into chronic kidney failure. Present study was planned to examine the potential role of WJ-MSCs for angiogenesis, wound healing and anti-oxidative index after their pre-treatment. **Methods:** The treatment of 2nd passage WJ-MSCs was done by using serum from CKD patients (unhealthy serum) and normal humans (healthy serum). To estimate the vascular endothelial growth factor (VEGF) levels in various treatment groups sandwich ELISA and immunocytochemistry was done. Wound healing capacity and anti-oxidative enzymes were performed independently groups. Anti-oxidants were projected by SOD, CAT, APOX and GSH assay. **Results:** VEGF levels are found to be increased in post-treatment group. Moreover, improved angiogenesis and enhanced wound healing expression show a reduction in injured group (post-treatment

group). Whereas increased levels of anti-oxidative enzymes in post-treatment group result in decrease of reactive oxygen species (ROS). **Conclusion:** In conclusion our study reveals that serum treatment significantly enhanced the repair potential of WJMSCs by its anti-oxidative and angiogenic capacities. The study emphasizes the efficacy and feasibility of serum treated WJMSCs and may be promising in its application and transplantation in CKD in vivo.

KEYWORDS: Mesenchymal stromal cells, chronic kidney disease, angiogenesis, wound healing, Wharton's jelly mesenchymal stromal cells.

INTRODUCTION

Chronic kidney disease (CKD) is a globally public health problem, with unfavorable effects of renal failure, premature death and cardiovascular disease (CVD) (Levey et al. 2005). CKD is a problem with an expanding rate and prevalence, poor results and high cost. After effects of CKD comprise not only kidney failure but also a risk factor for other diseases (Chinda et al. 2012; Kumai et al. 2012; Miwa et al. 2014; Nakayama et al. 2007; Oksala et al. 2010; Tsagalis et al. 2009; Yahalom et al. 2009). CKD Patients are also prone for endothelial dysfunction and are at risk from both thrombotic and hemorrhagic events (Cho et al. 2009; Ito et al. 2009; Khatri et al. 2007; Kumai et al. 2012; Oksala et al. 2010; Tsagalis et al. 2009). CKD is also associated with cerebral small vessel diseases because they share similar anatomical and physiologic characteristics (Cho et al. 2009; Ikram et al. 2008; Khatri et al. 2007; Miwa et al. 2014; Otani et al. 2010; Ryu et al. 2012). Existing evidence recommends that some of these adverse outcomes can be prohibited or delayed by early recognition and treatment.

Human Wharton's jelly mesenchymal stromal cells (WJ-MSCs) which are class of pluripotent stem cells developed from mesoderm and a kind of adult mesenchymal stromal cells. They have the capability of multi-directional differentiation and self-renewal. Multipotent mesenchymal stromal cells or mesenchymal stromal cells (MSCs) contain a capable tool for renewing stem cell behavior due to their capability to differentiate into numerous tissues, self-renew (Pittenger et al. 1999) and immuno modulatory properties (Le Blanc 2003). WJ-MSCs are highly proliferative so have additional self-renewal capacities than adult tissue-derived MSCs. Further, they also display a wide array of surface markers related to MSCs and regeneration, thus, can be differentiated into wider lineages (Troyer and Weiss 2008).

Angiogenesis is the development of new capillaries from pre-existing capillaries and circulating endothelial precursors. Vascular endothelial growth factor (VEGF) is a crucial regulator of physiological angiogenesis during reproductive functions, skeletal growth and embryogenesis. Vascular endothelial growth factor (VEGF) is a mostly precise mitogen for endothelial cells (Neufeld et al. 1999). Five VEGF isoforms are formed as a result of alternative splicing from a single VEGF gene (Keck et al. 1989; Leung et al. 1989). These isoforms differ in their biological properties and in molecular mass such as their ability to bind to cell-surface heparan-sulfate proteoglycans (Gospodarowicz et al. 1989; Neufeld et al. 1999). Derestricted VEGF expression encourages the progression of solid tumors by stimulating tumor angiogenesis and to the etiology of many other diseases that are categorized by abnormal angiogenesis (Adamis et al. 1994; Aiello et al. 1994). Oxidative stress is linked to the progression of CKD (Crawford et al. 2011). In CKD patients oxidative stress is prevalent and is considered to be significant pathogenic mechanism (Small et al. 2012). Increased concentrations of reactive oxygen species result in oxidative stress and/or a reduction in antioxidants. The antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase form the primary defense system against reactive species and oxidative stress (Crawford et al. 2011).

Oxidative stress develops from an imbalance between free radical production frequently increased through dysfunctional mitochondria formed with increasing age, inflammation, type 2 diabetes mellitus and reduced anti-oxidant defenses. Perturbations in cellular oxidant handling influence downstream cellular signaling and, in the kidney they encourage renal cell apoptosis and senescence, reduced regenerative ability of cells, and fibrosis. These factors have a random harmful effect on kidney function. In CKD patients the majority of studies examining anti-oxidant treatments show a reduction in oxidative stress and many show improved renal function (Small et al. 2012). Although existing data suggest a prominent role of CKD associated oxidative stress in uremic toxicity (Massy et al. 2009b). Therefore in this study therapeutic potential of WJMSCs for the reduction of oxidative stress in CKD patients has been investigated in vitro.

In the present study, we used blood serum from CKD patients (injured serum) and normal subjects (normal serum) for the pre-treatment of 2nd passage WJMSCs to explore their potential for the injury reduction of kidney via reduced angiogenesis and injury. After pre-treatment, WJMSCs were also analyzed for improved antioxidant levels via SOD, CAT,

APOX and GSH assays. Thus, this study was designed to investigate the potential role in improved angiogenic ability and injury reduction via improved anti-oxidant status of WJMSCs after their pre-treatment with serum from CKD patients and normal subjects.

MATERIALS AND METHODS

Sample Collection

All the procedures were approved by the Biosafety Board at The University of Lahore, Lahore, Pakistan. Serum samples from the patients diagnosed with CKD were randomly collected from Agha Khan Laboratory, Lahore, Pakistan.

Mothers selected for the study were Hepatitis B and C virus (HBV and HCV) negative. Umbilical Cords were obtained from 30 normal mothers with the informed consent of the mother from full term caesarian sections. Umbilical cord tissue was taken in a 50 ml falcon tube containing sterile normal saline, 100 U/ml penicillin and 100 ug/ml streptomycin (Gibco, Grand Island, NJ) sealed tightly and brought in tissue culture laboratory.

Isolation and culturing of WJMSCs

Explant method is used to acquire umbilical cords. The cord was taken from the hospital in a sterile container of normal saline containing 100 U penicillin and 100µg/ml streptomycin. The cord was taken out and washed with normal saline solution to remove the contamination and the umbilical veins were also washed with the same solution.

The cord piece was placed in a petriplate, containing the normal saline solution and cut down into pieces of 1-2 inches in size. The minced pieces were plated in the culturing flask with the help of forcep and medium was added. The medium used for culturing is Dulbacco Modified Eagle Medium low glucose (DMEM LG) and 10% serum and 100 U of penicillin and 100µg/ml streptomycin at 37ºC and 5% CO_2. The medium was changed from the culturing flask after 3 days.

Sub culturing of WJMSCs

Sub culturing of Wharton jelly mesenchymal stem cells was conducted when the cultures achieved 70-80% confluence. The cells that are attached to the walls of the culturing flask were washed with normal saline solution and incubated in 0.5ml/10cm^2 of 0.05% trypsin and 0.53 Ethylene diamine tetra acetic acid (EDTA) for 5 minutes for the detachment of cells from the culturing flask. When the flask was observed under the microscope, the detachment

of the cells was assured. 10% serum and equal volume of DMEM were added to the jars and mix well by stirring. The mixture was centrifuge for centrifugation the mixture was added to a 15 ml tube and centrifuged at 12000 rpm for 10 minutes. After centrifugation the supernatant was removed and only pellet was left. The pallet was re-dissolved in medium and cells were plated into culture plate. Same protocol was repeated for 2nd passage culturing but this time cells were plated onto six well plates containing cover slips in it, so that the cells would get attached on the six well plate surface as well as onto cover slips.

Pre-treatment of the WJMSCs

Cells attached to six well plates were subjected to pre-treatment with medium containing 10% serum from diseased kidney patients (injured serum group) and serum from normal subjects (normal serum group). This constitutes the post-treatment group of the experiment. The pre-treatment group consisted of only the medium containing 10% serum from respected group that was not given to cells yet. All the experiments were repeated four times. Evaluation of pre-treatment was done afterwards.

Wound Healing

For evaluation of the wound healing potential of preconditioned WJMSCs (P-WJMSCs), a cut was marked with the help of a blade onto the surface of six well plate before cell plating and pre-treatment. Degree of cell accumulation around this mark/wound was assessed.

Evaluation of Angiogenic Ability By Immunocytochemistry And ELISA

After pre-treatment, WJMSCs were analyzed for angiogenic ability via immunocytochemistry and ELISA.

Immunocytochemistry of WJ-MSCs

Cells were placed in a 24-well plate for characterization by immunocytochemistry. Wharton's jelly Mesenchymal Stem Cells was taken and those cells were grown on cover slips. With TBS-T, cells were washed three times for 5 minutes for the removal of the extra media in which they were cultured. Medium was removed from all wells for staining. Cells were fixed in 4% Para formaldehyde at room temperature for 30 minutes. Then cells were again washed five times with TBS-T. The permeability of cells increased with TBS-T. Treat those cells with 5% BSA for 25 minutes to block the nonspecific binding. Cells were again washed five times with TBS-T and incubated with primary antibody of VEGF. Then samples were place in incubator at 37 C for 1 and half hour. Then cells were washed again for 5 times with TBS-

T. Then incubate the cells with FITC conjugated secondary antibody at 37C for 1 and half hour. Then cells were washed 5 times with TBS-T. Cells were incubated with DAPI stain for 15 minutes at room temperature for the staining of nucleus. Then cells were washed 5 times with TBS-T. The cells were mounted in mounting media vecta shield. A drop of vecta shield was placed on slide for this purpose. Apply cover slips on which cells were grown inverted on the slide on which cells were grown. Results show nucleus of cell was stained with blue color; cells are spindle shaped and appear to be in green color.

ELISA

The Sandwich ELISA measures the amount of antigen between two layers of antibodies. Solid phase sandwich ELISA for VEGF was performed. Determined the number of the wells needed including samples, standards, blanks and/or controls should be analyzed in duplicate. Dilute the capture antibody to a concentration of 1:50 then transfer 100 mcl of capture antibody that will be diluted in coating buffer to each well of the plate. Then coated plate was incubated for 1 day (24 hours). Removed the Capture Antibody solution from each well with the help of dropper after incubation. Then each well was washed three times with washing solution (1X TBS). For this purpose, each well was fixed with wash solution and then removed wash solution with dropper. Repeat washing for 5 times. For blocking, Adding 200mcl of blocking solution (BSA) in each well of the plate and incubate for 30 minutes. After the incubation, removed the Blocking solution and washed each well 5 times for 5 minutes. Add 100mcl of culturing medium to each well and plate was incubated for 6 hours. After incubation, medium was removed and each well was washed three times for 5 minutes. 100 ul of HRP (secondary antibody) were added to each well and incubate for 2 hours. After incubation, the secondary antibody was removed and three times washing was given to each well for 5 minutes.

For Enzyme substrate reaction, we prepared the substrate solution according to the manufacturer's recommendation. TMB is the most popular and highly recommended chromogenic substrate for HRP detection in ELISA. 100 mcl of TMB solution (chromogenic substrate) were added to each well then the plate was incubated for 60 minutes. To stop the TMB reaction, applied 100ul of 0.18 M H2SO4 to each well. Then used a micro titer plate reader, read the plate at the appropriate wavelength for the substrate (450nm for TMB).

Evaluation of Antioxidative Stress

Glutathione reductase: Glutathione Reductase assay was performed by the techniques of Saba Shamim and Israr et al. Assay was performed in 96 well plate with a reaction mixture of 250ul in each well. Reaction mixture containing 0.5 mM oxidized glutathione, 1 mM EDTA, 100 mM KH_2PO_4 buffer (PH of buffer is 7.5) and the remaining quantity was post treatment media. Before starting the reaction, 0.2 mM NADPH was put at the near end of spectrophotometer to initiate the reaction. The reaction was permitted to run for 3 minutes. The absorbance was taken at 340 nm. Absorbance was plotted in the graph to show the differences in glutathione reductase activity between treated and control groups.

Catalase assay

Catalase assay was performed as reported by the technique of Luck and Saba Shamim. Two buffer solutions were used in this assay. Buffer 1 solution consists of 50 mM KH_2PO_4. The PH of KH_2PO_4 buffer is 7.0 and buffer 2 consists of 12.5 mM H_2O_2 in 50 mM KH_2PO_4. Its pH is also 7.0. Twenty micro liters post treatment media was taken into the wells of 96 well plate. One well consist of 900 ml of buffer 1(50mM KH_2PO_4) served as control, while the second well for the same sample consist of 900 ml of buffer 2 (12.5 mM H_2O_2 in 50 mM KH_2PO_4 served as sample. Plate was kept in dark after the addition of enzyme extract. After that absorbance was taken at 240 nm after 45 sec and 60 sec. We used the difference in absorbance at 45 and 60 sec to calculate the activity of CAT.

Superoxide dismutase

The assay of superoxide dismutase was performed by techniques of Israr et al and Saba Shamim. Assay was performed in 96 well plate. Reaction mixture (250 ul) containing riboflavin 60 mM, 100 mM KH_2PO_4 buffer (PH of buffer is 7.8), 2.25 mM Nitro Blue Tetrazolium (NBT), 0.1 mM EDTA, 13mM methionine and the remaining quantity was post treatment media. The plate containing all reaction components was exposed to light for 10 min. The reaction mixture in the plate with enzyme extract kept in the dark served as blank, reaction mixture without enzyme extract kept in light served as control. The absorbance was taken at 560 nm against the blank via Ultra Violet spectrophotometer. Absorbance was plotted in the graph.

APOX: The assay of APOX was done by the methods of Israr et al and Nakano and Asada. One milliliter reaction mixture contained 100 mM KH_2PO_4 buffer (pH 7.0), 0.5 mM ascorbate, 0.3 mM H_2O_2 and remaining was post treatment media. OD290 was taken after 3

min (as oxidation of ascorbic acid was measured as decrease in absorbance at 290 nm for 3 min). The enzyme activity was expressed as enzyme units per gram fresh weight (U g-1 FW). One unit of enzyme is the amount necessary to decompose 1 μmol H_2O_2 of per minute at 25°C.

RESULTS

Pre-conditioning of WJMSCs

In order to preconditioned WJMSCs, the cells at 2nd passage were preconditioned with serum from kidney patients and normal subjects, the preconditioned medium comprised of DMEM-HG with 10% serum (diseased/normal), 100μg/ml penicillin G, 100μg/ml streptomycin for three days. After pre-treatment WJMSCs were analyzed for improved wound healing and reduced angiogenesis via immunocytochemistry and ELISA. WJMSCs were also analyzed for anti-oxidant level.

Wound Healing

A cut was marked on the surface of the plate for wound healing assay. Then the cells were plated onto this plate. Cells were cultured in preconditioned medium. It is observed that cells cultured in the medium containing serum from injured kidney accumulate more around the wound as compared to the cells that were cultured in the medium that contained serum from normal subjects (Figure 1).

Increased Angiogenesis After Treatment Of CKD Serum

After pre-treatment, WJMSCs were analyzed for reduced angiogenesis via immunocytochemistry and ELIZA.

Immunocytochemistry

Anti VEGF antibody was used to stain the cells and DAPI was used to stain the nuclei of the cells. Two groups, normal group and treated group were subjected to immunocytochemistry. WJMSCs in normal group showed low expression of VEGF in the cells while WJMSCs in treated group showed high expression of VEGF in the cells (Figure 2A-C).

ELISA

After pre-treatment WJMSCs were analyzed for reduced angiogenesis via ELISA. WJMSCs cells medium is used for ELISA. In pre-treatment group VEGF levels of injured serum were low (0.045± 0.0015) than normal serum levels (0.068±0.0018) in the same group. Whereas,

in the post-treatment group VEGF levels of injured serum were higher (0.091 ± 0.003) than normal serum levels (0.066±0.003) in the same group. VEGF levels were high in injured serum of post-treatment group (0.091 ± 0.003) as compared to injured serum of pre-treatment group (0.045± 0.0015) (Figure 2D).

Evaluation of Anti Oxidant Level

Estimation of APOX, CAT, SOD and GSH Activity in different experimental groups was done.

APOX

WJMSCs culture medium was used for estimation. APOX Activity was significantly different in normal serum of post-treatment group as compared to normal serum of pre-treatment group. While, APOX activity of injured serum of post treatment was higher than injured serum of pre-treatment group .On the other hand, injured serum of pre-treatment group shows high percentage activity of APOX (32.43±0.35) as compared to normal serum of same group (18.62±0.19). Moreover, injured serum of post-treatment group show high percentage activity of APOX (39.8±0.9) as compared to normal serum (20.35±0.1) of the same group.

SOD

Estimation of SOD in different experimental groups was done. After preconditioning WJMSCs were analyzed for superoxide dismutase (SOD) activity. WJMSCs culture medium was used for estimation. SOD activity was significantly increased in normal serum of post-treatment group as compared to normal serum of pre-treatment group. While, SOD activity was significantly three fold higher in injured serum of post-treatment group as compared to injured serum of pre-treatment group. On the other hand, injured serum (58.64±0.96) of pre-treatment group show high percentage activity of SOD as compared to normal serum (28.46±1.4) of the same group. Moreover, injured serum (179.67±4.45) of post-treatment group show high percentage activity of SOD as compared to normal serum (81.06±1.01) of the same group.

GSH

Estimation of GSH Activity in different experimental groups was done. After preconditioning, WJMSCs were analyzed for glutathione reductase (GSH) activity. WJMSCs culture medium was used for estimation.

GSH activity was significantly increased in normal serum of post-treatment group as compared to normal serum of pre-treatment group. While, GSH activity of injured serum of post treatment is higher than injured serum of pre-treatment .On the other hand, injured serum of post treatment group show high activity of GSH (81.04±1.5) as compared to normal serum of the same group (34.06±4.3). Moreover, injured serum of pre-treatment group show high percentage activity of GSH (50.83±1.05) as compared to normal serum (25.45±1.6) of the same group.

CAT

Estimation of CAT activity in different experimental groups was done. After preconditioning, WJMSCs were analyzed for catalase activity. WJMSCs culture medium was used for estimation. CAT activity was significantly increased in normal serum of post-treatment group as compared to normal serum of pre-treatment group. While, CAT activity was significantly two fold higher in injured serum of post-treatment group as compared to injured serum of pre-treatment group. On the other hand, injured serum of pre-treatment group show high percentage activity of CAT (13.14±0.22) as compared to normal serum (7.55±0.28) of the same group. Moreover, injured serum of post-treatment group show high percentage activity of CAT (20.99±0.36) as compared to normal serum (11.76±0.24) of the same group.

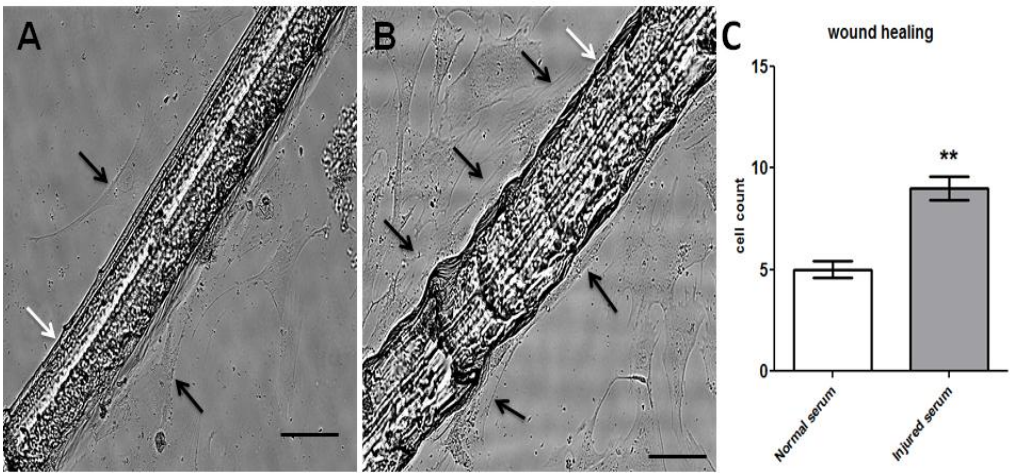

Figure. 1.

Figure 1: Wound healing ability of UCMSCs after pre-conditioning. A) Wound healing ability of normal UCMSCs, **B)** Wound healing of UCMSCs pre-conditioned with serum derived from CKD patients (200X). **C)** Graphical representation of wound healing ability of pre-conditioned UCMSCs. All values are expressed as mean ± SEM. p-value < 0.05 was considered significant. * *p<0.05* for injured serum group vs. normal serum group.

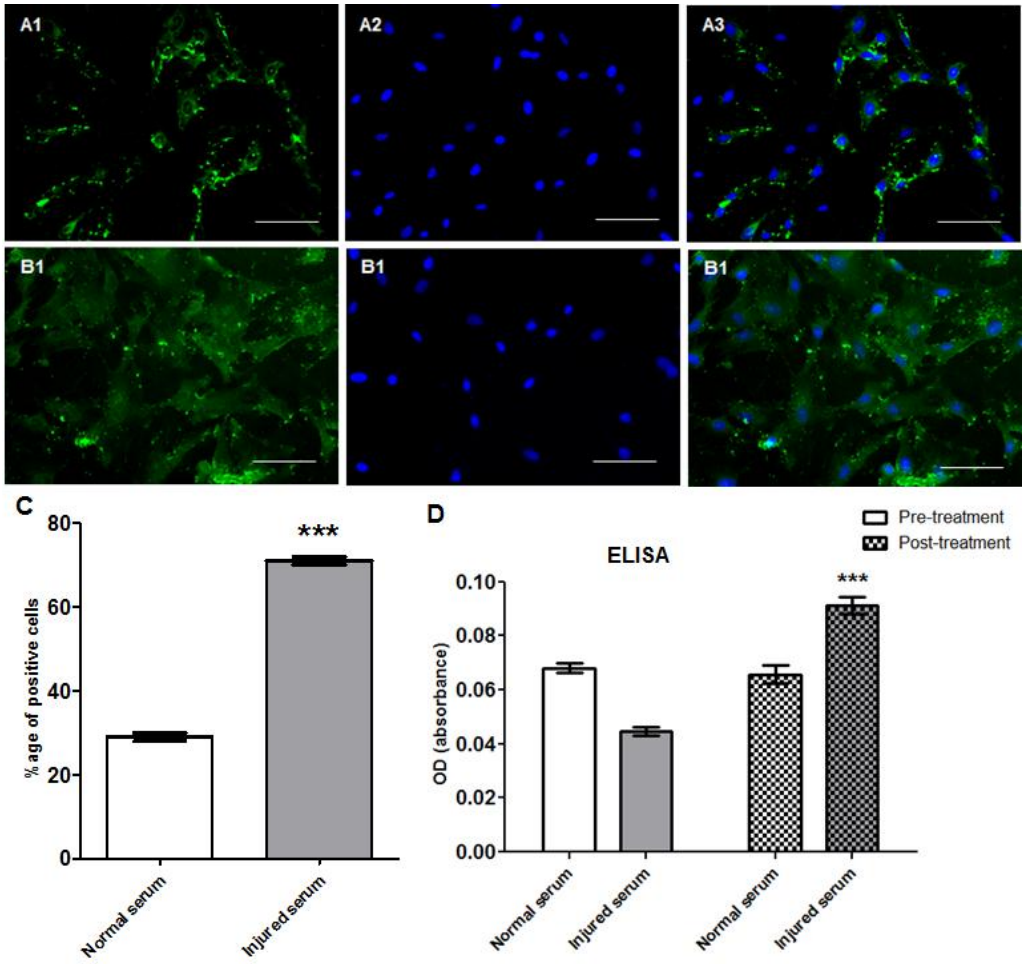

Figure. 2.

Figure 2: Expression of VEGF in normal and pre-conditioned UCMSCs groups. (A1-A3) normal UCMSCs (A1: anti-VEGF, A2: DAPI, A3: merge anti-VEGF/DAPI). **(B1-B3)** UCMSCs pre-conditioned with serum derived from CKD patient (B1: anti-VEGF, B2: DAPI, B3: merge anti-VEGF/DAPI) (200X). **C)** Graphical representation of VEGF positive cells in pre-conditioned UCMSCs. All values are expressed as mean ± SEM. p-value < 0.05 was considered significant. * $p<0.05$ for injured serum group vs. normal serum group. **D)** Graphical representation of ELIZA for VEGF in different treatment groups of UCMSCs. Values are expressed as mean ± SEM. p-value < 0.05 was considered significant. * $p<0.05$ for post-treatment group vs. pre-treatment group, $^{\alpha}p<0.05$ for injured serum group vs. normal serum group.

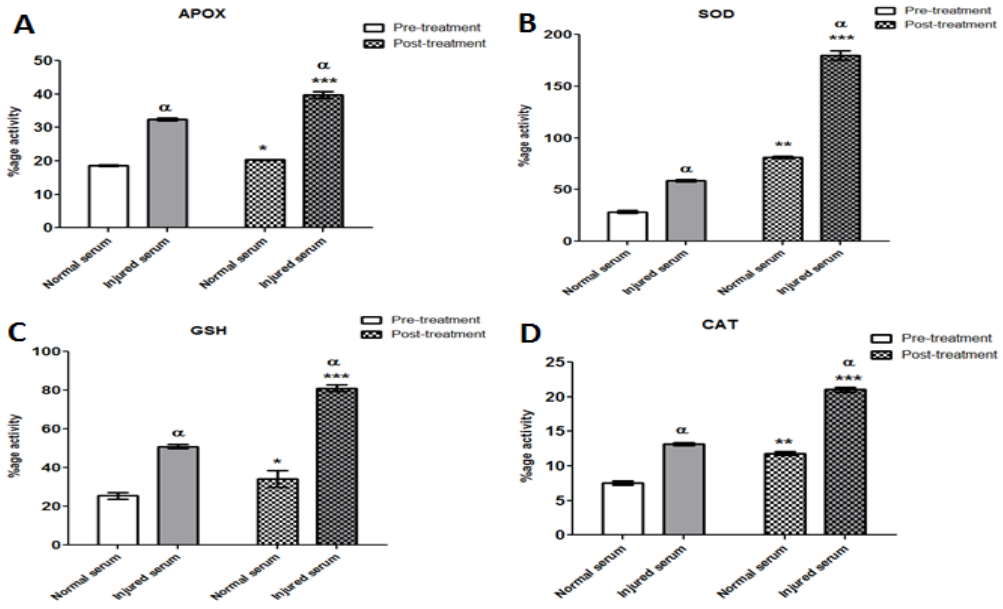

Figure. 3.

Figure 3: (A) Percentage analysis of APOX Activity by different groups pre-treatment and post-treatment, **(B)** Percentage analysis SOD Activity by different groups pre-treatment and post-treatment, **(C)** Percentage analysis of GSH activity by different groups pre-treatment and post-treatment **(D)** Percentage analysis of Catalase activity by different groups pre-treatment and post-treatment. Values were expressed as mean ±SEM (injured serum vs. normal serum $^{\alpha}p < 0.05$ and pre-treatment vs. post-treatment $^{*}p < 0.05$).

DISCUSSION

Chronic kidney disease (CKD) is a condition characterized by a loss of kidney function and it is associated with cardiovascular mortality, diabetes, hypertension and glomerulonephritis, leading to end-stage renal disease (ESRD) (Beck and Salant 2008; Bosan 2007; Freedman and Sedor 2008; Gilbertson et al. 2005; Golgert et al. 2008; Hauser et al. 2008; Jha et al. 2013; Oldrizzi 2007). Transplantation of MSCs has been demonstrated by different groups which show that injured kidney tissues can be reversed by MSCs .However, transplanted MSCs have limited and delayed paracrine effects which reduces their therapeutic potential which can be overcome by pretreating MSCs for their enhanced effect (Bartosh et al. 2010; Castaneda et al. 2003; Chen et al. 2011; Zhuo et al. 2010). As in view about promising role of stem cell therapy, in this study we demonstrated, in vitro, that preconditioning to WJMSCS may be a solution for better treatment in CKD. In previous reports it has demonstrated that pre-conditioning has enhanced therapeutic effect of MSCs in vivo and in vitro against other diseases (Bartosh et al. 2010; Khan et al. 2011; Khan et al. 2013; Nasir et al. 2013; Zhang et

al. 2012). We demonstrated that serum preconditioning significantly enhanced secretion of different factors from MSCs, which contributed to antioxidant effect and angiogenesis.

Angiogenesis is controlled by the balance between pro-angiogenic and anti-angiogenic factors. Progressive renal disease is marked by reduced expression of angiogenic growth factors and elevated expression of antiangiogenic factors (Kang et al. 2001a; Kang et al. 2001b; Shulman et al. 1996; Tufro et al. 1999). Angiogenesis associated factors are concerned with the nephrogenesis and vascularization leading to development of the kidney (Kitamoto et al. 1997). Administration of the pro-angiogenic, vascular endothelial growth factor (VEGF) has recently been shown to enhance glomerular capillary repair and accelerate renal recovery or prevent progression of renal disease in several experimental models (Kim et al. 2000; Masuda et al. 2001; Woolf and Yuan 2001). VEGF levels were also reported to be increased in hydrogen peroxide pretreated WJMSCS in myocardial infarction treatment (Zhang et al. 2012). in vitro, immunocytochemistry and ELIZA results (Figure 2) shown in this study revealed that the enhanced levels of VEGF in pre-treatment with injured serum indicating role of serum in enhancing the WJMSCs potential in repairing CKD. This result is consistent with the previous demonstrated results.

In terms of anti-oxidant effect of WJMSCs Glutathione reductase, catalase, APOX and SOD levels increased in post treatment with injured serum as compared to normal serum Figure 3 (A,B,C,D) Oxidative stress is changing ratio between pro- oxidants to depressed anti-oxidation system leading to immune system dysregulation accompanied by complication in CKD. These levels are on high in CKD is due to production of ROS and its poor clearance from kidney and are considered major contributor in aggravating the situation (Massy et al. 2009a; Rebholz et al. 2012). Antioxidant mediation on oxidative stress has examined by many workers and studies confirmed the reducing myocardial infarction, ischemic stroke and ESRD patients receiving vitamin E (Boaz et al. 2000). Oxidized glutathione is converted to reduced glutathione by glutathione reductase. In hemodyalic patients administration of GSH prevented uremic anemia of RBCs (Usberti et al. 1997). CRF is also reported to be caused due to at least one reason of glutathione and catalase in sufficiency (Sindhu et al. 2005). Moreover, in rats, renal insufficiency cause CRF was resulted in significant increase in superoxide and melanoaldehyde concentration in plasma (Vaziri 2004). This was also accompanied by down regulation of superoxide dismutase resulting in superoxide abundance (Vaziri et al. 2003). Ascorbate, a substrate used in down regulation of hydrogen peroxide,

represents as antioxidants, facilitating beneficial effects by reducing endothelial dysfunction and inhibition of lipid peroxidation (Deicher and Hörl 2003). The results obtained in our study show that oxidative stress in CKD may be, in part, due to down-regulation of catalase and glutathione and treatment with injured serum significantly enhanced the levels of glutathione reductase, catalase, apox and sod .

In vitro, scratch essay, is designed to demonstrate the behavior if cell migration in vivo (Liang et al. 2007). Rodriguez- Menocal and his colleagues suggested that wound healing may have improved by MSCs due to their paracrine factors and microenvironment (Rodriguez-Menocal et al. 2012). Our findings with WJMSCs are consistent with the previous study as pre-treated WJMSCs have significant effect in wound healing demonstrated in scratch essay (figure 1) and this result may become valuable in designing treatment of wounds with MSCs (Chen et al. 2014; Miyahara et al. 2006).

In conclusion our study reveals that pre-treatment significantly enhanced the repair potential of WJMSCs by its antioxidative and angiogenic capacities. The study emphasizes the efficacy and feasibility of pre-treated WJMSCs and may be promising in its application and transplantation in CKD in vivo.

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