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RESEARCH ARTICLE

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# Effect of Mononuclear Cells on Morphometric of Kidney parameters in Hyperglycemic Rat

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**ABSTRACT**

*In recent years have attracted much attention that hyperglycemia may exert its negative effect on the kidney. The objective of this practical work is to investigate the mononuclear cells(MNCs) protective effect against the hyperglycemic renal complication. Male rats (170–230 g) were made hyperglycemic by I.P. injection of alloxan monohydrate . The hyperglycemic state of the animal was confirmed by the demonstration of blood glucose levels 250 mg/dL one week after alloxan injection. Cord blood MNCs were isolated by a conventional centrifuge method through a Ficoll-density gradient. For characterization of MNCs in bloods was determined using flow cytometry. Seven days after alloxan injection hyperglycemic rats were assigned to either an untreated group or a MNCs treated group. In the MNCs -treated group, rats were implanted with to deliver 2-3*10$^6$ of cells on day 10 and again on day 20. The MNCs cells transplanted to the rats through the tail vein. Sixteen weeks after treatment, the rats were sacrificed, and kidneys were rapidly removed and weighed. Our most important finding was that kidney volume, and medulla volume no significant difference were observed between treated rats with mononuclear cells and untreated hyperglycemic rats. In contrast, the results obtained here indicate that the volume density of capillaries and serum urea level significant differences were observed between the two groups. MNC cells may be exerts a protective effect against renal injury.*

**Key words:** Cord blood **,** hyperglycemic ,renal , mononuclear cells , medulla volume

## INTRODUCTION

A number of evidence suggest that hyperglycemia is an important contributor to the development of diabetic nephropathy (1).It is widely known that increased glucose initiates changes in the mesangial cell structure and glomerular hemodynamic (2). Evidence from many animal studies and some experimental research suggest that diabetic nephropathy is a leading cause of renal failure(3). There is , in fact a growing body of literature on hyperglycemia and glomerular hypertension in diabetes development and progression of diabetic nephropathy(3). Earlier studies have reported that mesangial enlargement is accompanied by a decrease in the surface area available for capillary filtration (4). Growing evidence from several studies indicates that diabetic nephropathy is

characterized by an expansion of the glomerular mesangium and an excessive accumulation of extracellar matrix proteins, eventually leading to renal failure (5, 6).Several lines of evidence suggest that the structural lesions that characterize diabetic nephropathy include the expansion of mesangial volume (7).Several studies demonstrating that nephropathy is one of the most common complications of diabetes. Many studies showed that the diabetic disease is characterized by an increased glomerular basement membrane thickness and a decreased glomerular filtration rate (8-10). The above described studies suggest further work is needed to examine the complications of diabetic. No previous studies have assessed the effects of MNCs on kidney cells. We tested the hypothesis that MNCs from cord blood can provide a potential therapy for rat hyperglycemic.

## MATERIALS AND METHODS

### Isolation of Cord Blood MNCs

Isolation of cord blood MNCs was performed as described previously (11-13,21,22). In brief, human UCB cells were obtained from full term normal deliveries. Blood samples (n=10) from the umbilical cord were obtained from the Ayatollah Rouhani Hospital of Babol University of Medical Sciences , Babol, Iran. Informed consent was obtained from the mothers whose umbilical cord blood was collected. The approval of the Ethics Committee of Babol University was obtained (No: 91919-1671). Each cord blood sample was collected into a 50 ml sterile polypropylene test tube containing 5 ml of citrate phosphate dextrose (Sigma, USA) as an anticoagulant. The mean volume of cord blood varied from 32 to 51 mL. Immediately after collection of UCB samples, approximately 10 mL of collected cord blood was mixed with the same volume of phosphate buffered saline (PBS). Cord blood MNCs were isolated by a conventional centrifuge method through a Ficoll-density gradient, in brief, a whole –blood sample was layered onto the top of Ficoll( F5415,Sigma-Alderich ), which was followed by a centrifugation(Centrifuge, Behdad; Iran) at 1300 g for 30 min according to the manufactures manual. MNCs were recovered and washed twice with phosphate-buffered saline (PBS, PH 7.4),( 10010,Gibco ,invitrogen). Upon isolation, the cells of the mononuclear fraction were separated and counted (Hematology cell counter, Lc-10, Austria micros).

For characterization of MNCs in blood was determined using flow cytometry ( PAS , Germany ) .Seven days after alloxan injection , rats were randomly divided into a control group and MNCs treated group. The MNCs was centrifuged, re-suspended with PBS , and transplanted to the rats through the chest wall into the left cardiac ventricle or through the tail vein .

### Hyperglycemic rat model

Studies were conducted in adult male rats with initial body weight of about 170-230 g, obtained from Babol University Animal Center. The animals were housed with a light-dark cycle of 12 hours each, and with free access to food (standard chow) and water, during the study period. It was carried out under the control of the guidelines for animal experiments .All animals were carefully maintained under standard animal house conditions. Furthermore, all protocols involving animals were approved by Babol University Animal Care and Use Committee. Effort was made to minimize the number of animals. The approval of the Ethics Committee of Babol University was also obtained (NO: pj30-1671, 91/9/19). The rats were made hyperglycemic by intraperitoneal injection of alloxan monohydrate (Aldrich , A7413-25G ), 120 mg/kg body weight. Seven days later, induction of hyperglycemic was confirmed by measurements of tail blood glucose level using a spectrophotometer (Cecil, CE 1020, Cambridge,England).

### Experimental design

Hyperglycemic rats were then randomized to receive no cell treatment( n=6), or approximately $2\text{-}3\times10^6$of MNCs were infused into the hyperglycemic rats on day 10 and again on day 20 . , cells were counted (Sysmex x-1000i ). To avoid aggregation of the cells and to ensure reproducible deliver , MNCs were suspended in a large volume of phosphate buffer( 200 µl) at a concentration of 16000 cells per µl and injected through the chest wall into the left cardiac ventricle or through the tail vein . After 16 weeks of hyperglycemic, the rats were sacrificed, and the blood was collected for determinations of biochemical parameters such as blood glucose and urea levels.

### Isolation of kidney

At the end of experiments animals were sacrificed, and kidneys were rapidly removed, and weighed. The fixed kidneys were embedded in 7% agar and sliced into 1 mm slices using a macrotome perpendicular to the longitudinal axis . Approximately 8-10 slices were obtained from each kidney. The slices were arranged in a number of sequences on meshed tissue processing baskets and were then processed and embedded in paraffin. The blocks were

systematically sectioned at 5 micrometers, and every seventh section in order, with the first chosen randomly in the inteval. They were then mounted on glass slides and stained with periodic acid-Schiff (PAS). Every selected (1st and 7th) slice underwent a stereological analysis which involved the use of Olympus Optical BH2 microscopes. To estimate the volume of cortex, medulla and the whole kidney, every first section was viewed on a Motic loop microscope at a magnification of 20× with the image being projected on a computer monitor.

A fine grid of points distanced every 6 milimeters was superimposed over the visual field of the sampled sections. Point counting using Cavalieri principle was then used to estimate the volume of cortex, medulla and whole kidney using the following equation.

$$V = \frac{\Sigma P.a/p \ .t}{M^2}$$

V is the volume of kidney, Σ P is the sum of all points counted in sections, a/p is the area associated with each point, t is the distance between two consecutive sections in which points were counted, M is the linear magnification of the projector( 14-18).

**Measurement of biochemical parameters**

The body weights of rats were measured every 4 weeks. Also, blood glucose and urea levels were measured every 4 weeks. For measurement of blood biochemical parameters levels, blood was collected and was analyzed using standard kit according to the protocols of the manufacturer (Pars Azmoon, Tehran, IRAN) by a spectrophotometer (Cecil , CE1020, Cambridge, England). Serum glucose was measured based on glucose oxidase method and urea was determined by using indirect method based on preliminary hydrolysis of urea with urease followed by some process that described by the manufacturer.

**Statistical analysis**

All data expressed as Mean ± SD. A One-Way ANOVA followed by a Tuky test was used for analysis of differences in multiple comparisons between the groups. In each case, the null hypothesis was rejected if the probability of no differences was less than 5%.

## RESULTS

As shown in figure 1 hyperglycemic rats demonstrated increased blood urea compare to treated with MNC cells group (30.64 ±4.20, 15.20 ±4.96 mg/dl, $P<0.05$), respectively. As shown in figure 2 morphometric measurements indicate no significant differences in the mean kidney volume between hyperglycemic(445.76 ±60.57$mm^2$) and treated with MNC cells group (399.79 ±88.58 $mm^2$), $P>0.05$ . Also, as shown in figure 3 morphometric measurements revealed no significant differences in the medulla volume between hyperglycemic(67.86 ±8.56 mm) and treated with MNC cells group (78.65 ±19.13 mm ), $P>0.05$ . But in figure 4 increased in mean glomerular capillaries were observed in untreated rats (0.26 ±0.06 $mm^2$) compare to treated with MNC cells hyperglycemic rats( 0.16±0.03 $mm^2$, $P<0.05$ ). All groups of hyperglycemic rats demonstrated renal hypertrophy assessed both by kidney weight and kidney to body weight ratios.

The kidney-weight-to-body-weight ratio increased significantly in alloxan-induced hyperglycemic rats compared with treated with MNC cells . MNC cells treatment in alloxan-induced hyperglycemic rats resulted in a decrease in the ratio of kidney weight to body weight but failed to normalize it completely compared with non hyperglycemic rats controls.

In figure 5a light micrographs of kidney an untreated hyperglycemic rat was shown .

In figur 5b kidney morphology stained with PAS reagents from alloxan-hyperglycemic rats treated with MNC cell was shown. In 16-weeks-old alloxan-hyperglycemic rats treated with MNC cell, no visible change occurred in kidney morphology. In kidneys from MNC cells treated hyperglycemic rats that, kidney were more normal in appearance.

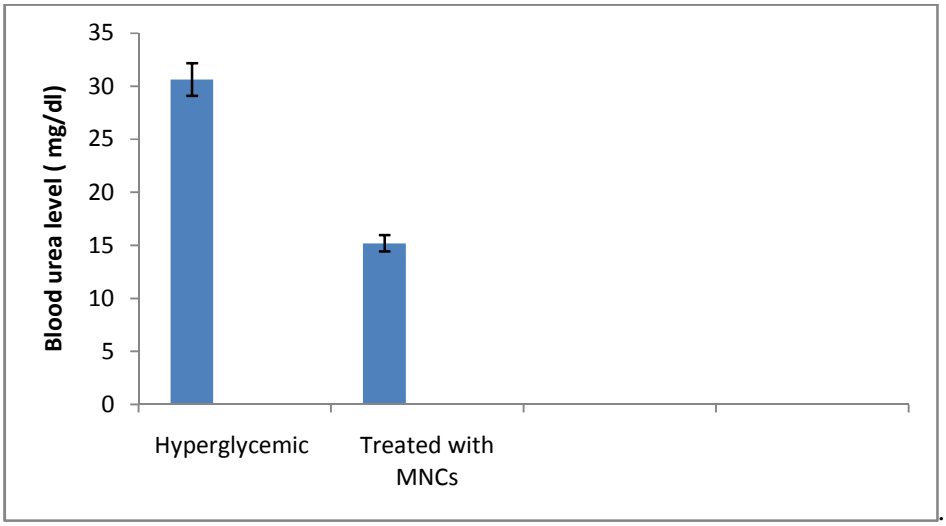


**Fig. 1 .Blood urea in hyperglycemic rats and in treated with MNC cells.**
*Values presented are mean ± SD of 6 rats for each group, P<0.05.*

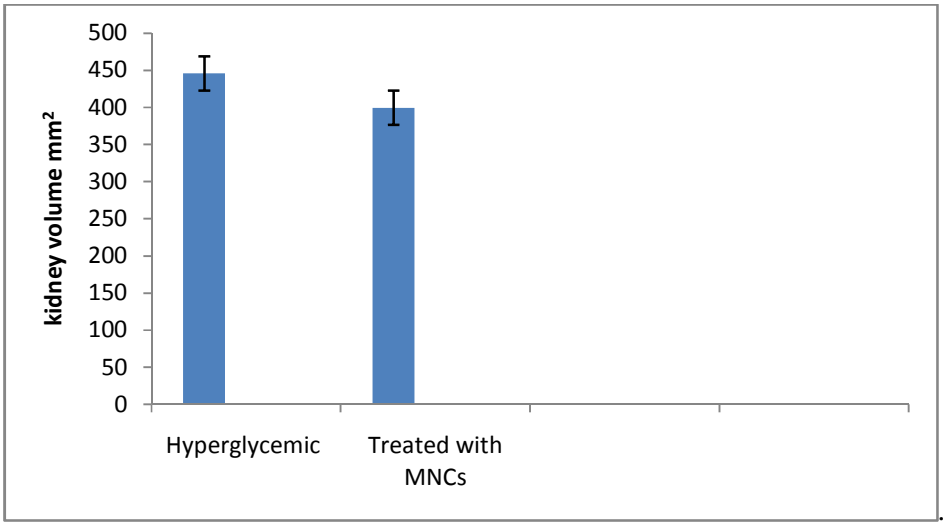


**Fig. 2 Kidney volume between hyperglycemic and treated with MNC cells .**
*Values presented are mean ± SD of 6 rats for each group, P>0.05.*

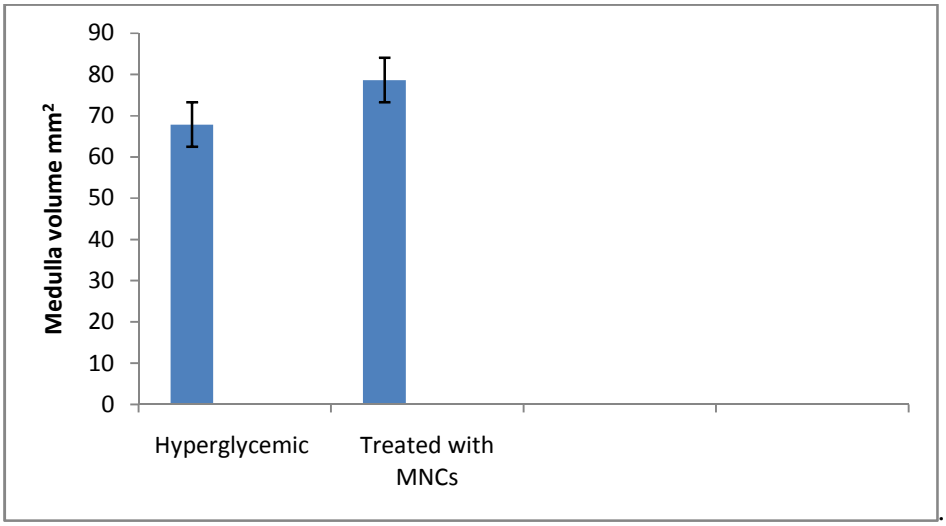


**Fig. 3 Medulla volume between hyperglycemic and treated with MNC cells .**
*Values presented are mean ± SD of 6 rats for each group, P>0.05.*

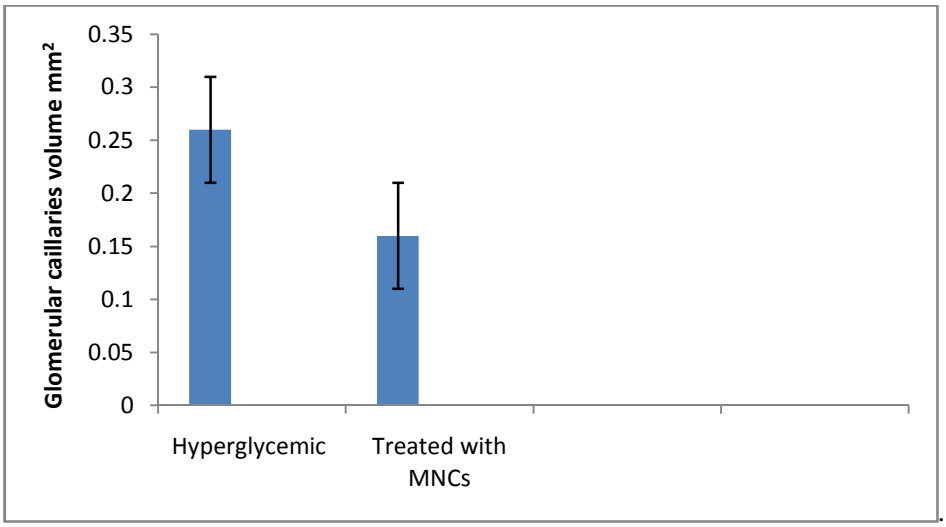


**Fig4. Glomerular capillaries volume between hyperglycemic and treated with MNC cells.**
*Values presented are mean ± SD of 6 rats for each group, P<0.05.*

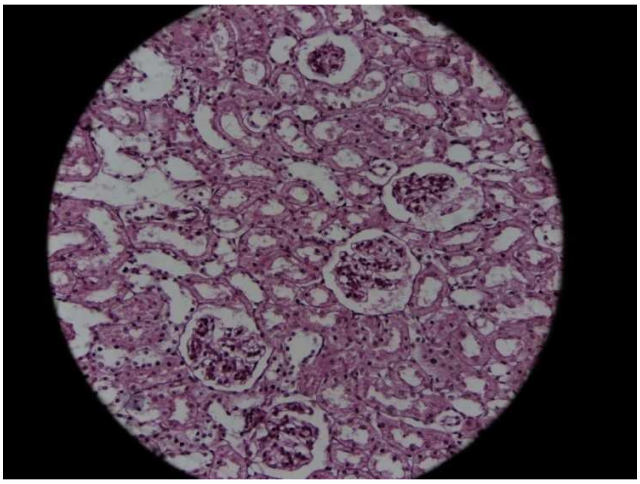

**Fig. 5a Light micrographs of kidney an untreated hyperglycemic rat. Hypertrophy glomerolus and urinary space are observed.**

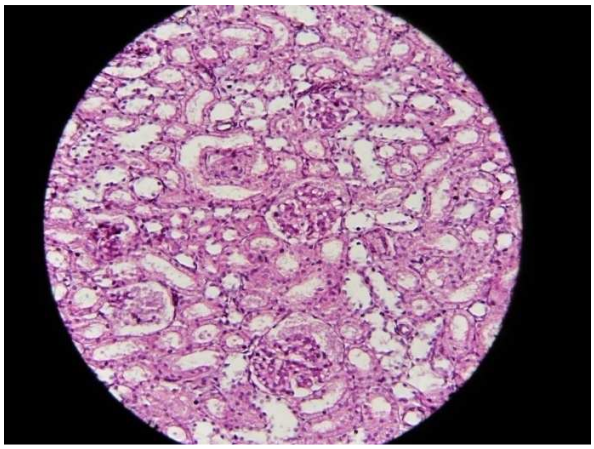

**Fig 5b Kidney morophology stained with PAS reagents from alloxan-hyperglycemic rats treated with MNC cell . In 16-weeks-old alloxan-hyperglycemic rats treated with MNC cell, no visible change occurred in kidney morophology.**

## DISCUSSION

Important findings of this study revealed that treated with MNC cells rats showed increased blood capillary and differences in the kidney volume between hyperglycemic and treated with MNC cells group. According to several studies hyperglycemia is the main cause of diabetic complication of the kidney (2, 4, and 5). In this study, we have demonstrated that urea level increased in hyperglycemic rats and decrease in treated group. Several theories have emerged regarding the hyperglycemia induces the biochemical changes leading to kidney lesions. It should be noted that urea is the major nitrogen –containing metabolic product of protein catabolism. Over the 90% of urea is excreted through the kidney. A wide variety of renal disease can cause an increase in serum urea concentration. One possibility is that the kidney lesion may cause change blood urea . On this regard, the increase of urea and histological changes in 16-weeks-old alloxan –hyperglycemic rats in our study was expected. It may be noted that our results indicated that this finding suggests the possibility that decreased urea was caused by the effect of MNC cells .In confirmation of previous results from our laboratory ( 11,21,22 ) MNC cells may modulate the stimulatory effect of hyperglycemia on the rat.

Despite similar degrees of hyperglycemia, the kidney had injured in the alloxan hyperglycemic rat but not in the MNC cells rat. Our findings suggest that the pathogenesis of kidney injury observed in the hyperglycemic rat is related to hyperglycemia-induced by alloxan. This study collectively support the conclusion that: (1) MNC cells exerts a protective effect against kidney injury.(2 )promotes kidney cells repair or regeneration.

It seems reasonable to conclude from our study that control of hyperglycemia may slow down the progress of diabetic complications. These findings are in agreement with observation by other investigators ( 19,20). However ,the exact mechanism influencing MNC cells are not to be fully determined . Further studies are needed to delimit the role of MNC cells on kidney cells.

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