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Autologous Adipose-Derived Mesenchymal Stem Cell Transplantation for Management of Chronic Kidney Disease Patients: A Clinical Trial in Human

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Abstract **Original Research Article**

The current clinical trial presents a review of CKD-patients enrolled for Autologous Adipose-Derived Mesenchymal Stem Cells (adMSCs) transplantation at Bangladesh Laser & Cell Surgery (BLCS) Institute & Hospital. Between April 2019 to June 2021, 52 CKD-patients were enrolled in this phase 1 & 2 trial receiving intravenous autologous adMSCs transplantation. Twenty-three CKD-patients who had received MSCs transplantation and had also followed-up with di-ethylene tri-amine Penta-acetic acid (DTPA) -based glomerular filtration rate (GFR) assessment, were considered for analysis. The mean (SD) DTPA-based GFR was significantly improved after adMSCs transplantation among study participants than the baseline mean (SD) DTPA-based GFR (19.79 [12.4] vs 15.07 [11.4], p=0.003). The DTPA-based GFR improved in stage-5 CKD patients with and without dialysis after MSCs transplantation, but a significant mean difference was observed only in CKD patients on dialysis (7.1 [6.9] vs 14.2 [12.6], p=0.011). Hence, pre-dialysis patients DTPA-GFR both at baseline and endline was higher than patients those on dialysis. The obtained results revealed that renal function improves in CKD patients through MSCs transplantation. No significant side effects or complication pertaining specifically to stem cell transplantation was observed during the study period.

Keywords: chronic kidney disease (CKD), adMSCs transplantation, glomerular filtration rate (GFR).

INTRODUCTION

The global prevalence of chronic kidney disease (CKD) is 13.4% (11.7-15.1%) and associated with higher mortality [1]. CKD can progress towards end-stage renal disease (ESRD) needing renal replacement therapy is estimated between 4.902 and 7.083 million [1]. ESRD currently accounts for 7.2% of the Medicare spending in the United States, and this economic burden is greater in less developed countries [2]. The overall CKD prevalence in Bangladesh was accounted for approximately 7-23% [3, 4], and it has a major impact on quality of life and life expectancy [5]. Current treatment modalities often fail to target the major underlying contributors for the progression of renal diseases [6]. The current CKD managements typically aim at control of the predisposing factors and supplementation of kidneys homeostatic functions, but not at the treatment of the diseased kidney itself [7]. On the other hand, for the reason of having inadequate facilities or financial constraints people of a developing country like Bangladesh are unable to continue long-term or lifelong dialysis [7]. Therefore, it is highly in need to develop therapeutic interventions to prevent, alleviate, or decelerate the progression of renal failure.

A stem cell is an unspecialized cell that is capable of long-term self-renewal through cell division but that can also be induced to differentiate into a specialized, functional cell. The multipotent stem cells can differentiate into some, or "multiple," but not all tissue types [8]. Stem cells that are harvested from

patient's own adipose tissue (the stromal vascular fraction [SVF]) with the intention of administering them back to the same patient are termed autologous adipose derived mesenchymal stem cells (adMSCs) or mesenchymal stromal cells. An important feature of MSCs is their capacity to induce proliferation of renal glomerular and tubular cells and also increasing cellular survival [9]. Advantages of adMSCs are, they are somatic stem cell population contained in fat tissue and have been shown to possess stem cell properties such as trans-differentiation and self-renewal [10]. Additionally, utilizing Adipose-derived stem cells (ADSCs) is advantageous in that large quantities of stem cells can easily be isolated using minimally invasive surgical procedures [11]. Some lines of evidence shown that SVF also contains progenitor cells that are able to differentiate into endothelial cells and participate in blood vessel formation [12, 13]. Additionally, a recent study demonstrated that SVF cells expressing both pericyte and mesenchymal markers reside in a peri-endothelial location and stabilize endothelial networks [13]. Another experimental study showed that ADSCs transplanted into an ischemic renal cortex preferentially migrate towards micro-vessels where they differentiate into vascular smooth muscle cells [14].

Considerable evidences accounted that among all the bone marrow cells, MSCs have shown the most promising results to regrow kidney cells, inhibit cell death, and encourage the kidney's own stem cells to repair kidney damage [11, 15]. A scarce of clinical trials have tested the safety and efficacy of MSCs for renal diseases to date. Reinders and colleagues studied safety and feasibility in six kidney allograft recipients who received two intravenous infusions of expanded autologous bone marrow-derived MSCs (bmMSCs) [16]. Importantly, delivery of autologous MSCs was not associated with adverse events, nor did it compromise graft survival. Recent clinical trials are currently underway to evaluate the therapeutic potential of autologous and allogeneic MSCs for the treatment of renal diseases [17]. Administration of both bmMSCs and adMSCs has demonstrated significant reno-protective effects including reduction of intrarenal inflammatory infiltrate, decreased fibrosis, and glomerulosclerosis [18]. In recent years, experimental studies have uncovered the potential of MSCs to improve renal function in several models of CKD, and several clinical studies have indicated their safety and efficacy in CKD [17]. Villanueva *et al.*, explored the effect of ADSCs on CKD by a single intravenous infusion of ADSCs on a nephrectomy induced CKD model of rats [19]. ADSC treatment was associated with reduced plasma creatinine, higher levels of epitheliogenic and angiogenic proteins, and improved renal function. Hyun *et al.*, illustrated the beneficial effects of ADSCs on improving renal function on a IgAN nephropathy mouse model [20]. Zhang *et al.*, found that repeated systemic administration of ADSCs attenuated proteinuria, glomerulus hypertrophy, and tubular interstitial injury in a diabetic nephropathy (DN) rat model [21]. A study conducted in Tehran, Islamic Republic of Iran, which was designed to provide confirmation of Mesenchymal stem cell therapy in CKD. A safety and efficacy study of autologous MSCs as a therapy for 10 CKD patients was conducted with an I/V injection of a high dose of 2x106/kg of autologous MSCs and reported the therapy was safe and effective. Where assessments were performed at 1, 3, 6, 12 and 18 months after cell injections [22].

MSCs have been shown to help avoid and reduce dialysis [20, 22]. It allows patients to work and continue life as productive citizens and also lessens dependency on family members by regaining independence. The financial benefits of eliminating dialysis and its consequences greatly outweigh the costs of stem cells. The long-term expense of dialysis is replaced by a short protocol of MSCs from SVF, which in all likelihood would eventually be a covered benefit of some insurance plans. An important feature of MSCs is their capacity to induce proliferation of renal glomerular and tubular cells, increasing cellular survival. By secreting proangiogenic and trophic factors, injected MSCs not only can enhance proliferation but also can decrease the apoptosis of tubular cells [23]. Several routes of administration (intra- parenchymal, sub-capsular, intra-venous) have been explored and all seem to be effective. Multiple, repeated injections of MSCs appear to be even more effective than single injections [24, 25].

STUDY OBJECTIVE

To observe the safety and efficacy of Autologous Adipose-Derived Mesenchymal Stem Cells transplantation in various stages of CKD patients.

METHODS AND MATERIALS

Study Population

The patients with CKD attending Bangladesh Laser & Cell Surgery Institute & Hospital considering the eligibility criteria who voluntarily agreed to participate in this study were recruited. A convenience sampling approach was used in this study. The recruitment continued until the desired sample was drawn.

Eligibility Criteria

A patient considered eligible if she/he was (i) aged 18-80 years, (ii) CKD stage 3 to 5 (estimated glomerular filtration rate [eGFR] 60 to 0 mL/min/1.73m^2). Patients with any of the following criteria excluded from the study, i) known hypersensitivity to any component used in the study, ii) inadequate hematologic function with: absolute neutrophil count (ANC) <1,500/µL OR platelets < 100,000/µL OR Hemoglobin < 8 g/dL; iii) impaired hepatic function with: serum bilirubin, aspartate

aminotransferase (AST), alanine aminotransferase (ALT) or alkaline phosphatase (AKP), prothrombin time above and normal reference and serum albumin below normal reference range; iv) hemoglobin A1c (HbA1c) > 8.0%; (v serious prior or ongoing medical conditions (e.g. concomitant illness such as Cardiovascular (e.g. New York Heart Association grade III or IV, Hepatitis e.g. Child-Pugh Class C, Psychiatric condition, alcoholism, drug abuse), medical history, physical findings, ECG findings, or laboratory abnormality that in the investigators' opinion could interfere with the results of the trial or adversely affect the safety of the patient; vi) pregnant or lactating women or premenopausal with childbearing potential but not taking reliable contraceptive method(s) during the study period; vii) history of human immunodeficiency virus (HIV) infection or any type of hepatitis; viii) judged to be not applicable to this study by investigator such as difficulty of follow-up observation; ix) any other serious diseases/medical history considered by the investigator not in the condition to enter the trial; x) known or suspected abuse of alcohol or narcotics; xi) known history of cancer within past 5 years; xii) any autoimmune disease; xiii) congenital kidney disease; xiv) precancerous condition or with raised tumor markers like Alpha feto protein, Carcino embryonic antigen (CEA), C.A 19.9, C.A 125, Serum PSA above normal reference range; xv) participants having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) less than 5 x 10^6.

Data Collection Procedure

The present study involved collection of both primary and secondary data. A semi-structured questionnaire was developed based on research objective for primary data collection. For acquiring secondary data a structured checklist was used. Primary data were collected by face to face interview of the patients or patient's attendant by the researcher at the health facility during the period of hospital stay, upon their consent and convenience. Socio demographic and personal information were recorded from patients through interviews, with a semi structured pre-tested questionnaire. Information regarding risk factors and risk behavior were inquired taking effort to minimize the recall bias. Secondary data about present state and diagnosis were collected from hospital records as well as from treatment sheets.

Sample Size Determination

The sample size for a phase-I trial generally requires less than 20 CKD subjects. In the present study, 23 CKD patients had received 1st dose of stem cell transplantation and completed at least 6 months of follow-up. CKD patients with a measurement of DTPA-based GFR before and after 6-12 months of transplantation targeted therapy were considered for analysis.

Treatment Allocation

All subjects were submitted for Stromal Vascular Fraction (Autologous Adipose Derived Mesenchymal Stem Cell) based treatment for CKD regardless of gender.

Study Equipment for Cell Therapies

The "UNISTATION^{TM}" is a specially designed medical device to satisfy diverse demands for various autologous cell therapies. It is the simplest & easiest way to various cellular therapies. Unique all-in-one solution. SVF isolation, PRP (Platelet-rich Plasma) isolation, fat purification, and incubation functions have been added to UNISTATION^{TM} (Sup. Pic 1). The CSUGOLD^{R} Ultraviolet irradiated closed cabinet with Hepa- filter and Laminar airflow was used as a work station (Sup. Pic 2). The LunaStem^{TM} (Sup. Pic 3) is a double fluorescent (A/O – Acridine Orange, P/I – Propidium Iodide) automated cell counter.

Procedures of Stem Cells Separation

Initially, 150 – 200 ml of tumescent fluid was injected subcutaneously into the anterior abdominal wall and left for half an hour to achieve local anesthesia, vasoconstriction, anti-inflammatory effect and to loosen up the subcutaneous fat. Then 40 – 50 ml of fat admixed with tumescent fluid was collected by liposuction. This fat was then processed in 4 steps to obtain the final SVF solution containing ADSC for intravenous injection, which is detailed below. A drop of SVF solution was collected as a sample for cell counting by LunaStem^{TM}. All the steps were carried out in an ultraviolet irradiated workstation under strict aseptic precautions. (Sup. Pic 2).

1st Step: Fat washing (A1)

(1) To collect aspirated fat and equal amount of water for balance in 2 UNIKITs.
(2) To put them into UNISTATION^{TM} Centrifuge.
(3) To touch SVF button once (Display shows A1)
(4) To touch the *Start button* once A1 is shown.
(5) After centrifugation, to remove RBC layer from the bottom.
(6) To collect 20ml pure fat after removing RBC layer in another UNIKIT.

2nd Step: Collagenase Digestion (A2)

(1) To prepare 20ml of 0.1% collagenase Solution.
(2) To transfer the Collagenase solution into the pure fat.
(3) To place shaking plate and put the pure fat with collagenase on if it and close the door
(4) To touch SVF button once again (Display shows A2)
(5) To touch the *Start button* once A2 is shown.
(6) To wait for 30 minutes for shaking and incubation and then take the fat out.

3rd Step: SVF Collection (A3)

(1) To put the collagen digested fat in UNISATION™ Centrifuge.
(2) To touch SVF button once again (Display shows A3)
(3) To touch the *Start button* once A3 is shown.
(4) After centrifugation, to take out UNIKIT very slowly so that layers should not be mixed. (Stem cell are at the bottom in 5ml SVF)
(5) Before removing cap to pull the hand piece slightly upwards so that stem cells should not be kept in the cap.
(6) To transfer 5ml SVF at the bottom into a 5ml or 10ml syringe and transfer it again into another UNIKIT.

4th Step: Neutralization and Washing of SVF (A4)

(1) To transfer 5ml PPP into the 5ml SVF.
(2) To transfer 30ml Normal saline into the 10ml of SVF and PPP.
(3) To put it in UNISTATION™ Centrifuge.
(4) To touch SVF button once again (Display shows A4)
(5) To touch the *Start button* if A4 is shown.
(6) After centrifugation, transfer 5ml SVF at the bottom into 5ml or 10ml syringe.

Preparation for Injection

The final 5 ml of SVF were transfused to the patient through the intravenous route. Before that, a drop of SVF sample is collected for cell counting by Luna Stem automated fluorescent cell counter.

Route of Delivery and Patient's Safety

Various routes for delivery of ADSCs, ADSC-induced cells, or ADSCs combined with compound materials have been developed for the treatment of different diseases or damaged tissues. These routes can be classified into two categories: systemic delivery through blood vessels (intravenous injection or intra-arterial injection) or local delivery directly into injured tissues or organs [26].

The route of MSC delivery may influence the cells' capacity to home and engraft the damaged tissue, and thereby their efficacy for renal repair. Commonly used experimental methods to deliver MSCs include systemic intravenous, intra-arterial, or intra-parenchymal delivery. The route of MSC delivery, intravenous, intra-arterial, or intra- parenchymal, might affect their efficiency for kidney repair. When labeled MSC intravenously infused into baboons were observed for 9-21 months, estimated levels of engraftment in the kidney, lung, liver, thymus, and skin ranged from 0.1-2.7% [27]. Indeed, the intravenous route lags in delivery efficiency, because MSC might initially be trapped in the lungs [28]. Intra-arterial infusion of MSC was the most effective route to achieve immunomodulation in rat kidney transplantation, possibly by avoiding lodging in the pulmonary circulation, allowing MSC to home to the injured kidney [29]. For the present study, we injected SVF to the patient through intravenous route. Because, to inject intra-arterially in Renal arteries, we need to visualize renal arteries using Iodine containing dye which is hazardous to already diseased kidneys [30].

Glomerular Filtration Rate (GFR) Assessments

The outcome measure of the present study was to assess changes in DTPA-based glomerular filtration rate (GFR) after 6-12 months of targeted therapy to determine the functional changes in the kidney. Patients were requested to perform the DTPA-based GFR test before 2/3 days of receiving MSCs transplantation. Patients were also requested to come with test results of different biomarkers such as serum creatinine, other renal markers plus CBC at the same time.

Statistical Methods

Descriptive statistics (mean, median, standard deviation, ranges for continuous data, and percentages for categorical data) were used to summarize patient characteristics and treatment administration. Response rates along with corresponding 95% confidence intervals were calculated, based on the exact binomial distribution. The normality of each continuous variable was checked. To estimate average pre- and post-treatment effect paired mean comparison were performed. The mean of discrete variables before and after stem-cells therapy were evaluated by the Wilcoxon Signed Ranked estimates. The relationship between clinical characteristics and changes in pre-and post-clinical outcomes over time was assessed using binary regression analysis to determine the predictors of outcome only among stage-5 CKD patients. We have selected only stage-5 CKD patients for binary regression analysis due to difference in disease severity of CKD patients by different stages. The outcome variable DTPA-based GFR was log transformed before putting into regression analysis. All analysis was performed using Stata 17 (StataCorp 2021, College Station, TX) and the p-values smaller than 0.05 will be considered significant.

RESULTS

Between April 2019– June 2021, 52 chronic kidney disease patients were enrolled in phase1 & 2 studies receiving targeted Intravenous Autologous Adipose-Derived Mesenchymal Stem Cell s Transplantation. Of these, 28 (54.9%) were males and 23 (45.1%) were females. Out of 52 patients, 23 patients have 2nd time assessment of DTPA- based GFR after 6 months to 12 months (mean±SD; 9.5±3.1 months) of 1st dose of stem cell therapy and were recruited all into the present study analysis. Of the remaining 29 patients, 12 patients were lost to follow-up due to Covid-19 lock down situation, 9 patients were died (1 due to CVD, 4 during dialysis and 4 due to CKD related complications) and 8 patients reported

improvement of kidney condition but still lacking the second DTPA assessment. Female CKD patients delayed more than male CKD patients for 2nd time assessment of DTPA-based GFR (mean±SD; 41.3 [4.9] vs 38.8 [2.9] weeks, p=0.667).

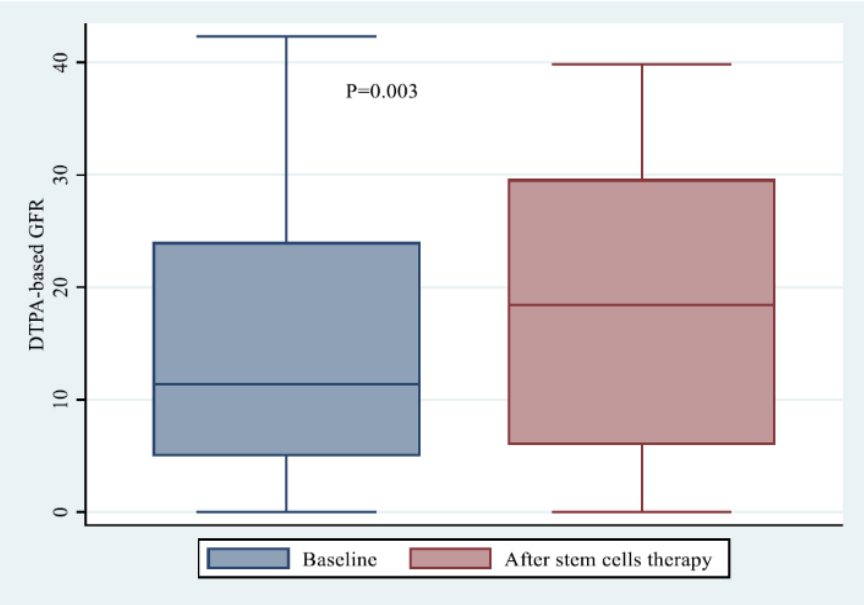

Figure 1: Mean distribution of DTPA-based total glomerular filtration rate (GFR) at the time of enrollment and after stem cells transplantation (N = 23)

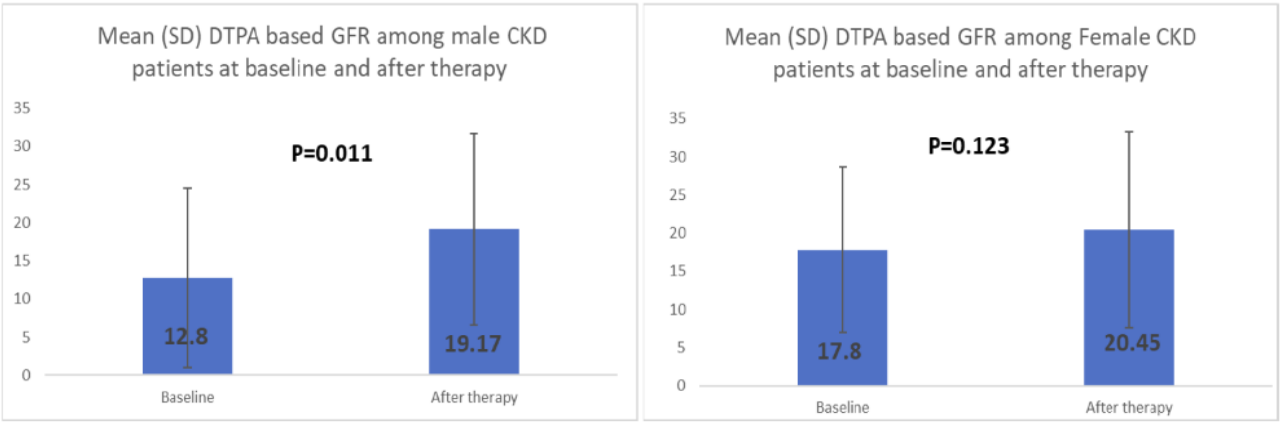

Figure 2: Mean (SD) DTPA-based GFR among CKD patients at baseline and after therapy by sex

The mean (SD) amount of DTPA-based GFR was significantly improved after stem cells therapy among study participants than the baseline mean (SD) DTPA-based GFR amount (15.07 [11.4] vs 19.79 [12.4], p=0.003) (Figure 1). The DTPA-based GFR improved in both males and females CKD patients after MSCs transplantation, but a significant mean difference was observed only in male CKD patients (12.8 [11.79] vs 19.17 [12.56], p=0.011) (Figure 2). The median age of all 23 patients was 54 years old, 12 (52.17%) males and 11 (47.83%) females. Overall 82.6% of patients had stage-5, 13.0% had stage-4 and 4.4% had stage-3B renal diseases. Of the CKD stage-5 patients, 42.1% patients had not initiated dialysis at baseline, 36.8% were taking dialysis twice a week and 21.1% were taking dialysis at 1 day interval (thrice a week). All CKD patients were reported to be hypertensive, followed by 52.2% had diabetes, 17.4% had hypothyroidism disorder, 7.8% had IHD < NyHA Gr III or IV and 5% had other co-morbidities. Around 74% (n=17) had multi co-morbidity (>1 morbidity). Nearly a third of the patients (28.6%) had pyuria or pus cells in urine. Over half of the CKD patients (56.5%) experienced reduced/scanty urine output. Of the CKD patients, weakness (43.5%), anorexia (30.4%), constipation (26.1%), weight loss (17.4%), swelling of the body (17.4%), muscle spasm (13.0%), and nausea (13.0%) were frequently reported symptoms (Table 1).

Table 1: Patient characteristics and baseline data N = 23

Indicators	**n (%), range**
Median age	54 (30-61)
Sex	
Male	12 (52.2)
Female	11 (47.8)
CKD stage based on eGFR	
Stage 3A	-
Stage 3B	1 (4.4)
Stage 4	3 (13.0)
Stage 5	19 (82.6)
Dialysis status	
No dialysis yet	8 (42.1)
Twice a week	7 (36.8)
At 1 day interval (Thrice a week)	4 (21.1)
Co-morbidities (multiple response)	
Hypertension	23 (100)
Diabetes Mellitus	12 (52.2)
Hypothyroidism	4 (17.4)
IHD < NyHA Gr III or IV	2 (8.7)
Others	5 (21.7)
Multi comorbidity (>1 morbidity)	17 (73.9)
Presence of pus cells in urine, n=21	
Pyuria (≥10 neutrophils)	6 (28.6)
No-pyuria (≤9 neutrophils)	15 (71.4)
Symptoms	
Urine output	
Normal	10 (43.5)
Reduced	7 (30.4)
Scanty	6 (26.1)
General Wellbeing, n=21	
Better	1 (4.8)
Good	3 (14.3)
As usual	17 (80.9)
Anorexia	7 (30.4)
Nausea	3 (13.0)
Weakness	10 (43.5)
Weight loss	4 (17.4)
Swelling of body	4 (17.4)
Vertigo	2 (8.7)
Constipation	6 (26.1)
Muscle spasm	3 (13.0)
Pain	2 (8.7)
Fever	1 (4.4)
Headache	2 (8.7)

The mean (SD) body weight of the study participants was 68.1 (16.6) kg at baseline and was 65.1 (15.9) kg after MSCs transplantation. Nearly one-fifth of CKD patients (17.4%) at baseline and over one-third of CKD patients (38.1%) after AdMSCs transplantation were presented with visible or reported mild-to-moderate oedema. Overall, one-third of the study participants had mild anemia at baseline and after stem-cells therapy (Table 2). The DTPA-based GFR improved in stage-5 CKD patients with and without on dialysis after MSCs transplantation, but a significant mean difference was observed only in CKD patients on dialysis (7.1 [6.9] vs 14.2 [12.6], p=0.011). Hence, pre-dialysis patients DTPA-GFR both at baseline and endline was higher than patients those on dialysis (Figure 3).

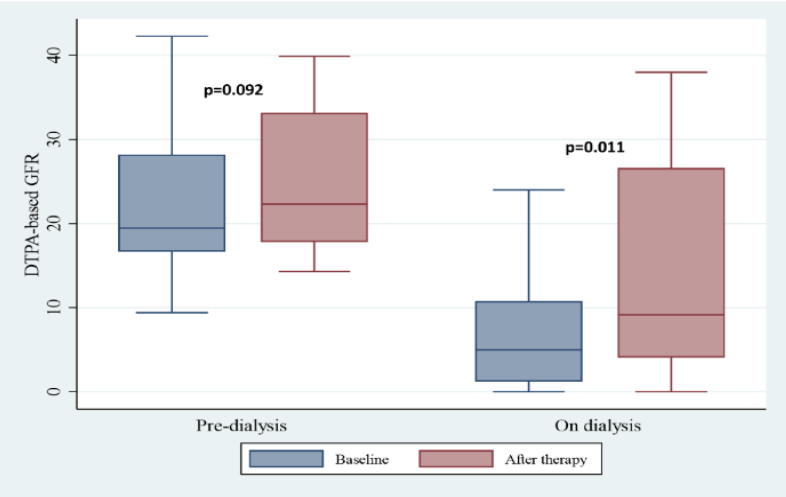

Figure 3: Mean (SD) DTPA-based GFR at baseline and after therapy among CKD patients with dialysis

Table 2: Clinical characteristics of patient at baseline and after 1st dose of stem cell transplantation N =23

Indicators	**Baseline**	**After therapy**
	N (%)	**N (%)**
Body weight, (Mean±SD) (n=21)	68.1±16.6	65.1±15.9
Systolic blood pressure (mmHg), (Mean±SD) (n=20)	141.5±24.5	133.4±25.2
Diastolic blood pressure (mmHg), (Mean±SD) (n=20)	85.8±13.5	80.8±14.4
Pulse rate/minute, (Mean±SD), n=18	73.1± 7.1	74.9± 5.4
Respiration (n1=19, n2=21)		
Cough	1 (5.3)	1 (5.3)
Normal	18 (94.7)	20 (95.2)
Temperature, (n1=18, n2=20)		
Mild fever	1 (5.6)	-
Normal	17 (94.4)	20 (100)
Oedema, (n1=23, n2=20)		
Mild	3 (13.0)	6 (28.6)
Moderate	1 (4.4)	2 (9.5)
Normal	19 (82.6)	13 (61.9)
Anemia, n1=23, n2=21		
Mild	8 (34.8)	7 (33.3)
Normal	15 (65.2)	14 (66.7)

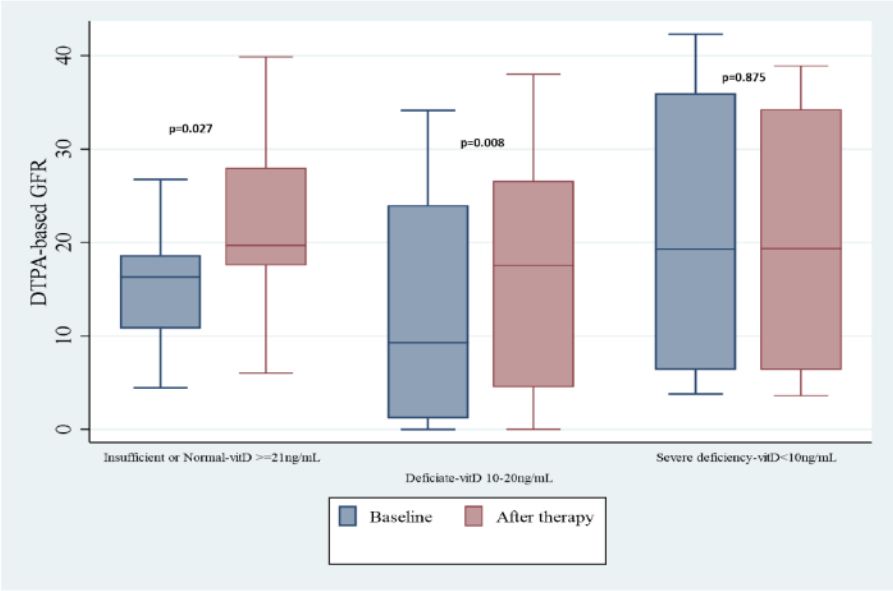

Figure 4: Mean (SD) DTPA-based GFR at baseline and after therapy among CKD patients by vitamin D level at baseline

Table 3: Patient biochemical characteristics at baseline and after 1st dose of stem cell transplantation N =23

	Baseline	**After therapy**	**p-value**
	Mean (SD)	**Mean (SD)**	
Total GFR by DTPA renogram	15.1 (11.4)	19.8 (12.4)	0.003
S. creatinine, n=23	6.8 (4.0)	5.8 (2.5)	0.665
eGFR based on S. creatinine (ml/min/1.73 m2), n=23	11.1 (6.9)	11.5 (5.6)	0.708
Blood urea (mg/dL), n=16	81.0 (36.8)	64.4 (27.3)	0.388
Urine Microalbumin-Creatinine Ratio (ACR), n=10	2622.1 (4395.9)	2495.6 (4321.9)	0.556
Urinary Protein-Creatinine ratio(PCR)	6.0 (10.2)	4.8 (2.5)	0.507
Random Blood Sugar (mmol/L), n=17	6.7 (2.4)	7.1 (3.2)	0.637
HbA1C (%), n=16	5.5 (1.0)	5.3 (1.0)	0.337
Serum Uric Acid (mg/dL), n=19	6.3 (1.9)	4.6 (1.8)	0.002
Serum Calcium (mg/dL), n=16	3.5 (2.3)	4.5 (3.0)	0.088
Serum Phosphate (mg/dL), n=20	3.7 (1.7)	3.9 (1.3)	0.509
Serum Vitamin D3 (ng/mL), n=9	17.6 (9.0)	36.3 (27.3)	0.179
Complete Blood Count (CBC)/ Erythrocyte Sedimentation Rate (*ESR*)			
Hb (gm/dl), n=22	10.4 (1.8)	10.7 (1.8)	0.311
ESR (mm in 1st hour), n=22	44.1 (23.7)	37.5 (22.6)	0.294
Platelet count (K/ul), n=21	242.9 (62.9)	202.7 (63.9)	0.005
WBC (103/μL), n=14	9.7 (2.2)	14.4 (2.1)	0.983
Neutrophil (%), n=21	68.9 (5.2)	67.9 (12.7)	0.671
Lymphocyte (%), n=21	22.6 (4.6)	22.1 (7.1)	0.732
Monocyte (%), n=21	5.7 (1.6)	4.2 (1.6)	0.007
Eosinophil (%), n=21	2.5 (1.0)	2.0 (1.0)	0.067
Basophil (%), n=19	0.09 (0.26)	0.05 (0.21)	0.125
Serum electrolytes			
Sodium (mEq/L), n=21	143.8 (5.3)	140.3 (2.8)	0.001
Potassium (mmol/L), n=21	4.7 (0.88)	4.5 (0.58)	0.344
Chloride (mEq/L), n=20	107.6 (6.4)	104.1 (4.4)	0.037
Tumor markers			
Serum PSA, n=12	1.8 (1.7)	1.5 (1.7)	0.850
Serum Alpha Feto Protein, n=20	11.1 (5.9)	10.5 (5.9)	0.729
Serum CEA, n=20	2.5 (1.7)	2.4 (1.2)	0.691
Serum C.A 19.9, n=21	11.8 (6.7)	23.9 (24.7)	0.016
Serum beta 2 micro globulin /Cell count, n=21	25.8 (17.1)	11.7 (9.4)	0.015
Serum LDH, n=19	285.3 (105.2)	314.6 (127.4)	0.347
Serum Albumin, n=12	4.2 (0.4)	4.1 (0.4)	0.752
Urine R/M/E (Pus cells)			
Pyuria (≥10 neutrophils)	6 (28.6)	3 (18.7)	0.006
No-pyuria (≤9 neutrophils)	15 (71.4)	13 (81.3)	

*Serum C.A 19.9, Serum carbohydrate antigen 19.9; RBC, Red blood cells; Serum PSA, Serum prostate specific antigen

The median concentration of serum creatinine was higher at baseline than in patients after MSCs transplantation, however the difference was not statistically significant (6.18 [4.05] vs 5.81 [2.53], p=0.665). The mean(SD) serum uric acid (mg/dL) concentration was significantly declined after MSCs transplantation among study participants than the baseline mean (SD) serum uric acid concentration (6.3 [1.9] vs 4.6 [1.8], p=0.002). A significant mean difference was observed for platelet count (K/ul) in patients at baseline than in patients after MSCs transplantation (242.9 [62.9] vs 202.7 [63.9], p=0.005), and a significant mean difference for monocyte (%) counts was observed among study participants after MSCs transplantation than in baseline monocyte (%) counts (5.7 [1.6] vs 4.2 [1.6], p=0.007). Both mean serum sodium and serum chloride concentrations were significantly reduced in patients after MSCs transplantation than in baseline concentrations, respectively. A significant lower mean serum beta-2microglobulin level was accounted for in patients after MSCs transplantation in comparison to mean serum beta-2microglobulin levels in patients at baseline (11.7 [9.4] vs 25.8 [17.1], p=0.015) (Table 3). Those patients baseline vitamin D level was <10ng/mL, showed no improvement at DTPA-based GFR after MSCs transplantation (Figure 4).

Table 4: Correlation co-efficient between DTPA-based glomerular filtration rate with co-variates among stage-5 CKD patients

Indicators	Unadjusted
	β-coefficient (95% CI) p-value
Assessment time (pre vs post)	
Baseline	-
After stem cells therapy	0.39 (0.14 – 0.65) 0.002
S. beta-2Microglobulin	-0.29 (-0.53 to -0.045) 0.020
Sex	
Male	-
Female	0.17 (-0.78 – 1.12) 0.731
Dialysis status	
No	-
Yes	1.21 (0.59 – 1.83) <0.001

Table 4 shows the correlation co-efficient between DTPA-based GFR with co-variates. A significant positive correlation coefficient was observed between baseline DTPA-based GFR and DTPA-based GFR after MSCs transplantation. The changes DTPA-based GFR after MSCs transplantation showed an inverse correlation with serum beta-2 microglobulin (β-coefficient =-0.38, [95% CI, -0.76 to -0.003]; p=0.048). Patient's current dialysis status showed an inverse correlation with changes in DTPA-based glomerular filtration rate after MSCs transplantation (Table 4).

Table 5: Frequency of adverse events reported by CKD patients after 1st dose of stem cell transplantation

Adverse events	N =23 (%)
Minor adverse events	
Pain from liposuction >7 days (early)	3 (13.04)
Fever >7 days (early)	1 (4.35)
Subcutaneous hematoma/abscess (early)	-
Allergic reaction (immediate)	-
Serious adverse events	
Anaphylactic reaction (immediate)	-
Pulmonary embolism / infarction (immediate)	-
Outset of new cardiovascular events (late)	-
Outset of neoplastic change (late)	-
Outset of new cerebrovascular or neurological events (late)	-

Table 5 shows the frequency of adverse events reported by CKD patients after 1st dose of stem cell transplantation. Only three patients (13.04%) complained of pain from liposuction for more than 7 days and one patient (4.35%) developed a subcutaneous hematoma. These minor adverse effects were managed conservatively. There was no anaphylactic reaction or pulmonary embolism/infarction immediately after stem cell transplantation. No serious adverse events like the outsets of any neoplastic change, new cardiovascular events, or new cerebrovascular or neurological events were observed in any of the patients during the study period.

DISCUSSION

The present phase-1&2 clinical trial conducted in Bangladesh has provided primary findings of AdMSCs transplantation on different stages of CKD patients. It has established a positive relationship between AdMSCs transplantation and improvement of renal function in CKD patients. To our knowledge, this is one of the few studies to reveal that AdMSCs transplantation improves renal function in CKD patients. As the kidneys have a limited regenerative capability on their own, eventually, CKD leads to end stage renal failure resulting in the need for dialysis or organ transplant to live. Therefore, the current study findings provide unique evidence to begin to explore the possibility of a new generation treatment of AdMSCs transplantation on renal function in humans. Former studies have reported the advantage of AdMSCs transplantation on renal function in animals, and a few studies showed the efficacy of AdMSCs transplantation on renal function in humans [15, 31]. One advantage of the present study was that it covered the largest number of CKD patients (n=23) so far and observed over a certain period of time (average 9 months). The current study also observed that dialysis status and a vitamin D level >10 ng/dL in CKD patients showed a better improvement in DTPA-based GFR after AdMSCs transplantation. It has been observed that all CKD stage-5 patients in Bangladesh do not receive hemodialysis, unless their clinical conditions deteriorate significantly; due mainly to economic constrain, fear of dialysis, and knowledge gap etc.

In the present study, the average DTPA-based GFR was significantly improved after AdMSCs transplantation (after 6 to 12 months of follow up) in CKD patients than the baseline average DTPA-based GFR. Nevertheless, due to COVID-19 pandemic, there is a variation of up to 6 months in the timing when the second assessment of DTPA-based GFR was performed. Therefore, future studies are required to all patients' assessment within a short time variation. Similar to our findings, a previous case study reported that a 13-year-old young patient with recurrent focal segmental glomerulosclerosis (after renal transplantation not responding to conventional therapy) showed a promising result of a stable renal function and an improvement of the proteinuria without plasmapheresis after receiving three MSC infusions without adverse events [32]. This study also found a lower serum uric acid in CKD patients after AdMSCs transplantation. Former experimental review study reported an improved renal function in several models of CKD after AdMSCs transplantation, and their safety and efficacy in CKD [17]. Experimental studies have shown promising results in bone marrow derived MSCs transplantation to regrow kidney cells, inhibit cell death, and encourage the kidney's own stem cells to repair kidney damage [11, 15]. Similarly, Our study showed an improved in renal function by adipose derived MSCs transplantation.

On the other hand, in the present study a number of CKD patient's (n=5) also showed a reduced DTPA-based GFR after AdMSCs transplantation. It has been observed that 4 out of these 5 CKD patients had vitamin D3 deficiency (<10 ng/mL) at baseline. Researchers found that hypovitaminosis D is prevalent in patients with renal diseases [33, 34]. In contrary, this deficiency also has been associated with albuminuria, faster progression of kidney disease and increased all-cause mortality for CKD patients [33].This aforementioned information supports our findings that stems cell therapy might not effective for CKD patients with severe hypovitaminosis D, because the autocrine role of vitamin D is a significant modulator of numerous systems including the immune, renal and cardiovascular systems [34]. A multi- country study conducted in Birmingham, Alabama, Rochester, Minnesota, Jackson, Mississippi, USA followed similar procedure (where stem cell product called "Mesenchymal stem cell" grown from person's own fat tissue infused back in to the patient's own kidney) and measured primary outcome after 3 months where renal tissue oxygenation increased and decrease in kidney inflammation, was seen as secondary outcome [35].

In general, DTPA-based GFR improved in both male and female CKD patients after AdMSCs transplantation, but male participants showed a prominent change in DTPA-based GFR after AdMSCs transplantation ($p<0.05$). The probable reason behind this difference in DTPA-based GFR by sex is due to a longer duration in follow up assessment of DTPA-based GFR after receiving AdMSCs transplantation. Female patient's delayed more than male patients for 2nd time assessment of DTPA-based GFR (41.3 [4.9] vs 38.8 [2.9], $p=0.667$). This indicates that CKD patients may take a second dose of MSCs transplantation sooner after 24 weeks for further clinical and functional improvement. Due to low statistical power, we are unable to conduct time varying rigorous analysis this time to check GFR of CKD patients by sex.

The median concentration of serum creatinine was also reduced in CKD patients after AdMSCs transplantation, however the difference with baseline concentration was not statistically significant. The main limitation of the present study is due to lack in number of cases in early stages of CKD (stage 3A, 3B & 4), we assume that AdMSCs transplantation therapy in early stage of CKD could have present significant changes in the outcome of future studies. Another limitation of the study is that we do have mixed before or after dialysis serum creatinine results. The mean(SD) serum uric acid (mg/dL) concentration was significantly declined after stem cells therapy (along with conventional drugs) among study participants than the baseline mean (SD) serum uric acid concentration ($p=0.002$). Serum uric acid is a known biomarker of impaired kidney function and a higher uric acid itself also increases inflammation in patients with CKD [36]. Therefore, a reduction in serum uric acid supports our findings of improvement in GFR in CKD patients after MSCs transplantation. A recent experimental study supports present study findings and revealed that treatment of the diabetic rats by MSCs reduces the serum creatinine and serum uric acid levels [37].

Moreover, in the present study it has been observed that after AdMSCs transplantation serum beta-2 microglobulin level significantly reduced than serum beta-2microglobulin levels at baseline ($p=0.015$). The changed DTPA-based GFR after AdMSCs transplantation showed an inverse correlation coefficient with serum beta-2 microglobulin. This is the first study reported any effect of AdMSCs transplantation on reduced serum beta- 2microglobulin in CKD patients, a known prognostic marker for renal diseases. Serum beta-2microglobulin is a better predictor than well-established factors associated with estimated glomerular filtration rate (eGFR) in pre-dialysis patients [38]. Therefore, present study data suggests that AdMSCs transplantation would be a beneficial treatment for all level CKD patients. In future studies, it suggests considering the following issues for better outcomes, i) to involve more subjects in earlier stages of CKD (stage 3A, stage 3B even stage 4), ii) to improve ADSC count, and iii) to include healthy comparison group / control group of CKD patients receiving conventional treatment. In this study, we were dependent only on the number of cells yielded from the patient's own fat, which is often limited. Expanding

patients' own MSCs by Culture or iPSCs (Induced Pluripotent Stem Cells) could have provided cell counts as high as 100 million per dose. But in that case, researchers need to address other issues like facility build-up, compliance, teratogenic effects, etc. which is quite expensive.

CONCLUSION

Currently, the obtained results revealed that renal function improves in CKD patients by AdMSCs transplantation. Pre-dialysis status and a vitamin D level of more than >10 ng/dL in CKD patients showed a better improvement in DTPA-based GFR after stem cell transplantation. Treatment of CKD patients by AdMSCs improved the test parameters (serum uric acid, beta-2 microglobulin) towards the stats of normal range. No significant side effects or complication pertaining specifically to stem cell transplantation was observed during the study period.

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