বাংলাদেশ চিকিৎসা গবেষণা পরিষদ
Bangladesh Medical Research Council
BMRC

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National Research Ethics Committee

Registration Number: 076(1) 25 10 2017.

Principal Investigator:
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Institute & Hospital, Aftabnagar, Dhaka.

Title of the Project:
"Evaluation of Therapeutic Potential of Stromal Vascular Fraction (Autologous Adipose Derived Mesenchymal Stem Cell) Based Treatment for Chronic Kidney Disease"

Duration of Project: 5 years

Budget: BDT- 8,00,000/-
In words: Eight Lacs Taka Only.

Subject: Ethical Clearance

With reference to your application on the above subject, this is to inform you that above mentioned Research Title has been registered and approved by the National Research Ethics Committee (NREC).

(Dr. Mahmood-uz-jahan)
Director

N.B: You are requested to follow the guidelines as mentioned at page two.

THE ETHICAL GUIDELINES TO BE FOLLOWED BY THE PRINCIPAL/ CO-INVESTIGATORS

- ☐ The rights and welfare of individual volunteers are adequately protected.
- ☐ The methods to secure informed consent are fully appropriate and adequately safeguard the rights of the subjects (in the case of minors, consent is obtained from parents or guardians).
- ☐ The Investigator(s) assume the responsibility of notifying the National Research Ethics Committee (NREC) if there is any change in the methodology of the protocol involving a risk to the individual volunteers.
- ☐ Data Safety Monitoring Board (DSMB) needs to be constituted according to NREC's advice.
- ☐ To report immediately to the DSMB / NREC if any evidence of unexpected or adverse reaction is noted in the subjects under study.
- ☐ Project will be supervised by BMRC authority.
- ☐ This approval is subject to Principal Investigator's reading and accepting the BMRC ethical principles and guidelines currently in operation.
- ☐ You are requested to submit a report to the BMRC half yearly and after completion of the research work.

Checked by: GV

RESEARCH PROTOCOL

EVALUATION OF THERAPEUTIC POTENTIAL OF STROMAL VASCULAR FRACTION (AUTOLOGOUS ADIPOSE DERIVED MESENCHYMAL STEM CELL) BASED TREATMENT FOR CHRONIC KIDNEY DISEASE

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STUDY DESCRIPTION

1. Title of the study

Evaluation of Therapeutic Potential of Stromal Vascular Fraction (Autologous Adipose Derived Mesenchymal Stem Cell) Based Treatment for Chronic Kidney Disease

2. Introduction

2.1 Chronic Kidney Disease (CKD)

CKD is a disease of alarmingly increasing prevalence (8 to 16%) associated with mortality [1]. CKD can progress towards end-stage renal disease (ESRD), requiring renal replacement therapy. ESRD currently accounts for 6.3% of the medicare spending in the United States, and is projected to increase by 85% by 2015 [2]. In a study conducted among rural population in Bangladesh overall CKD prevalence was found about 19% [3]. Furthermore, ESRD has a major impact on quality of life and life expectancy [4]. Therefore, it is very important to develop therapeutic interventions to prevent, alleviate, or decelerate progression of renal failure.

Diabetes mellitus and hypertension represent major causes of CKD and initiation of dialysis [5]. In addition, glomerular diseases, malnutrition, infectious diseases, and acute kidney injury can progress to ESRD, contributing to the increased global burden of death [6]. Current treatment modalities often fail to target the major underlying contributors for progression of renal disease [7]. Management of CKD at present mostly aims at control of the predisposing factors and supplementation of kidneys homeostatic functions but not at the treatment of the diseased kidney itself. Again due to lack of adequate facilities or financial constraints people of a developing country like Bangladesh are unable to continue long term or lifelong dialysis. Chronic glomerular and tubule-interstitial fibrosis is a common pathway to ESRD, often associated with apoptosis, oxidative damage, fibrosis and microvascular rarefaction. Unfortunately, the regenerative potential of kidney is limited under chronic conditions and inefficient to prevent progressive glomerulosclerosis and tubule-interstitial fibrosis [8]. Treatment strategies that boost cellular regeneration might therefore offer good alternatives for patients with CKD.

In the western world as well as in Bangladesh, high blood pressure and type II diabetes are on the rise and are contributing to higher rates of kidney disease. Demand for kidney transplants is increasing. But there are not enough donor organs to meet this growing need. So, cell based therapies may offer an alternative solution.

2.2 Stromal vascular fraction (SVF)

SVF of adipose tissue is a rich source of pre-adipocytes, mesenchymal stem cells (MSC), endothelial progenitor cell, T cells, B cells, mast cells as well as adipose tissue macrophages [9,10]. SVF is a component of the lipo-aspirate obtained from liposuction of subcutaneous tissue. Lipo-aspirate contains a large population of stem cells called adipose derived stem cells (ADSCs), which share a number of similarities with bone marrow stem cells, including the capacity for multilineage differentiation .

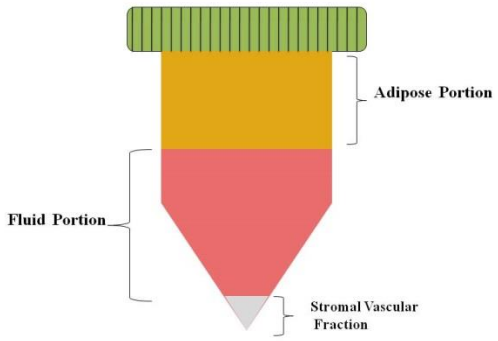


Figure 1: Stromal vascular fraction.

2.3 Stem Cells

A stem cell is a generic term referring to any unspecialized cell that is capable of long-term self-renewal through cell division but that can be induced to differentiate into a specialized, functional cell. Stem cells are generally two types, embryonic stem cells and adult stem cells. Adult stem cells can be obtained from many differentiated tissues including, but not limited to, bone marrow, bone, fat, and muscle. Obtaining adult stem cells also does not raise any ethical concerns. For most studies, the adult stem cell in question is actually a mesenchymal stem cell

(MSC) or mesenchymal stromal cell. They are multipotent but not pluripotent, which means they can differentiate into some, or "multiple," but not all tissue types [11]. Stem cells that are harvested from the patient with the intention of administering them back to the same patient are termed autologous MSCs. MSC can also be isolated from the bone marrow (bmMSC), peripheral blood, connective tissue, skeletal muscle, dental pulp (dpMSC), umbilical cord wall (ucMSC), umbilical cord blood (cbMSC), amniotic fluid (afMSC) and all have been used in experimental settings to treat various types of renal diseases. An important feature of MSCs is their capacity to induce proliferation of renal glomerular and tubular cells, increasing cellular survival [12].

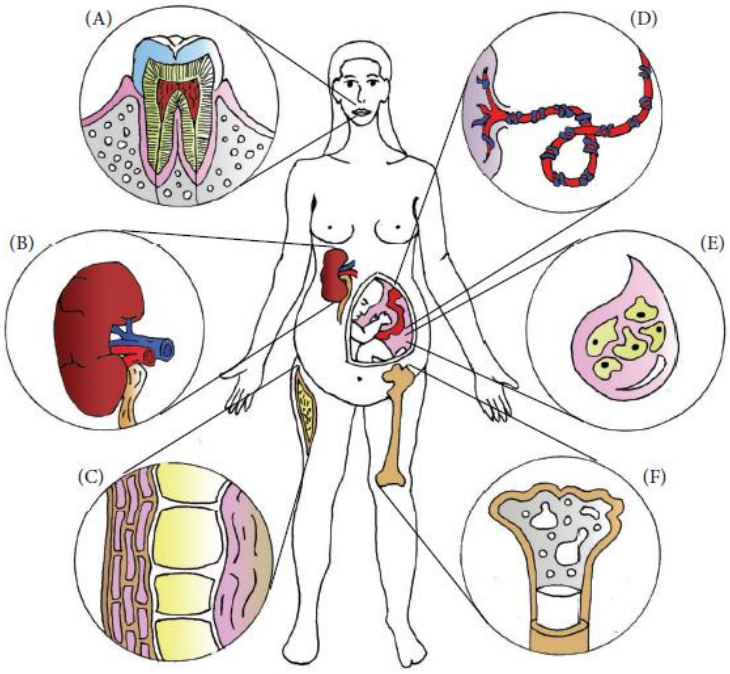


Figure 2: Sources of MSC used in experimental models of renal injury. Preclinical studies have shown that MSC used to treat renal diseases can be isolated from the following tissues: (A) tooth pulp, (B) kidney, (C) adipose tissue, (D) umbilical cord, (E) amniotic fluid, and (F) bone marrow.

2.4 Advantages of adipose tissue-derived stem cells (ADSCs)

ADSCs 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 [13]. Similar to other types of

MSCs, ADSCs express multiple CD marker antigens (CD73+CD90+CD105+ CD34+/- CD11b- CD104b- CD19- CD31- CD45- SMA-) [14,15]. Additionally, utilizing ADSCs is advantageous in that large quantities of stem cells are easily isolated using minimally invasive surgical procedures [16].

ADSCs are vascular precursor cells. Many studies have shown that SVF contains progenitor cells that are able to differentiate into endothelial cells and participate in blood vessel formation [17]. Additionally, a recent study demonstrated that SVF cells expressing both pericyte and mesenchymal markers reside in a peri-endothelial location and stabilize endothelial networks [17] Another study showed that ADSCs transplanted into an ischemic renal cortex preferentially migrate toward micro vessels where they differentiate into vascular smooth muscle cells [18]. Some trials on kidney transplant recipients as well as the one on FSGS and 2 on CKD patients include in their protocol the utilization of adMSC. Adipose tissue is an important source of MSC, with a frequency 100 to 1000 times higher than bmMSC. They also seem to possess a higher potential for angiogenesis or vasculogenesis [19].

2.5 Kidney disease and mesenchymal stem cells

A number of different types of cells from the bone marrow have been tested in animals and in clinical studies for potential use in kidney disease. Amongst all the cells under investigation, MSCs have shown the most promising results to date as they help kidney cells to grow, inhibit cell death and encouraging the kidney's own stem cells to repair kidney damage [20].

2.6 Clinical trials using mesenchymal stem cells for renal repair

Few clinical trials have tested safety and efficacy of MSCs for renal disease. Reinders and colleagues studied safety and feasibility in six kidney allograft recipients who received two intravenous infusions of expanded autologous bone marrow-derived MSCs [21]. Importantly, delivery of autologous MSCs was not associated with adverse events, nor did it compromise graft survival. Several clinical trials are currently underway to evaluate the therapeutic potential of autologous and allogeneic MSCs for treatment of renal diseases [22] Administration of both bmMSC and adMSC has demonstrated significant reno-protective effects including reduction of intrarenal inflammatory infiltrate, decreased fibrosis, and glomerulosclerosis [12]

2.7 How else could stromal vascular fraction (autologous adipose derived MSC) help tackle kidney disease?

MSCs possess unique immunomodulatory properties that ameliorate inflammation and immune responses, constituting a promising tool to facilitate renal repair. 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 [22].

ADSCs could be incorporated into damaged tissues or organs which could give rise to new functional components and also exert potent anti-inflammatory, anti-fibrotic, or immunomodulation effects through paracrine or autocrine routes (via vascular endothelial growth factor, granulocyte/macrophage colony stimulating factor, stromal-derived factor-1alpha and hepatocyte growth factor) [23,24]. Interestingly, it is proposed that even apoptotic or dying ADSCs exhibit distinctive immunosuppressive properties [25]. ADSCs have been shown to possess stronger anti-inflammatory and immuno-modulating functions than bone marrow derived MSCs [26].

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 [27]. ADSC treatment was associated with reduced plasma creatinine, higher levels of epitheliogenic and angiogenic proteins, and improved renal function. Work by Hyun et al[28] illustrated the beneficial effects of ADSCs on improving renal function on a IgAN mouse model. Zhang et al.[29] found that repeated systemic administration of ADSCs attenuated proteinuria, glomerulus hypertrophy, and tubular interstitial injury in a DN rat model.

Currently, several Clinical trials have been uploaded in the NIH database, all aim to test mainly the safety of using MSC and their efficacy in treating CKD. Two of them propose the use of autologous bmMSC and two adMSC. A study conducted in Tehran, Islamic Republic of Iran, which was designed to provide confirmation of Mesenchymal stem cell therapy in CKD. There 18 months safety and efficacy of autologous MSC as a therapy for CKD total of 10 patients were conducted with I/V injection of high dose of $2x10^6$ / kg of autologous MSC. Assessments were performed at 1,3,6,12 and 18 months after cell injections [30].

Another study conducted in Birmingham, Alabama, Rochester, Minnesota, Jackson, Mississippi, USA 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 primary outcome measured after 3 months where renal tissue oxygenation increased and decrease in kidney inflammation was seen as secondary outcome [31].

2.8 Route of delivery

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 tissue. 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 [32].

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. In nonhuman primates the cells distribute broadly into the kidneys, skin, lung, thymus, and liver with estimated levels of engraftment ranging from 0.1 to 2.7% [33].

The route of MSC delivery, intravenous, intra-arterial, or intra-parenchymal, may 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% [33] Indeed, the intravenous route lags in delivery efficiency, because MSC may initially be trapped in the lungs[34] 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 [35].

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 apoptosis of tubular cells [36]. Several routes of administration (intra-parenchymal, sub-capsular, intravenous) have

been explored and all seem to be effective. Multiple, repeated injections of MSCs appear to be even more effective than single injections [37,38].

2.9 Conclusion

Time has come to rethink about newer treatment approaches for increasing burden on haemodialysis and renal transplantation for CKD. MSCs have been shown to help avoid and reduce dialysis. 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 will eventually be a covered benefit of some insurance plans.

3. Objectives of the Study

3.1 General Objective

To assess the outcome of stromal vascular fraction (autologous adipose derived mesenchymal stem cell) based treatment as a new, safe and minimal invasive modality for the treatment of chronic kidney disease.

3.2 Specific Objectives:

(i) To assess the safety of stromal vascular fraction (autologous adipose derived mesenchymal stem cell) in the treatment of CKD
(ii) To determine the role of SVF in treating different stages of CKD
(iii) To see if SVF can reverse or limit the progression of CKD

4. Methodology

The study will be conducted considering following methodological aspects.

4.1 Study design

Study Type:	Interventional.
Primary Purpose:	Treatment
Study Phase:	Phase 1/Phase 2
Interventional Study Model:	Parallel Assignment
	Participants will be placed in either of the 2 groups, depending on the number of harvested total stem cell count.
	Those having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) between 1 x 10^6 to 2 x 10^6 will be placed in "Group A".

	Those having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) more than 2 x 10^6 will be placed in "Group B". Both groups will be studied for Phase I/ Phase II trial by the proposed outcome measures over the stipulated time frame. Those having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) less than 1 x 10^6 will be excluded from the study.
Number of Arms:	2
Masking:	Triple (Participant, Care Provider, Outcomes Assessor)
Allocation:	Non-Randomized
Enrollment:	31 [Anticipated]

4.2 Arms and Interventions

Arms: Participants having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) between 1 x 10^6 to 2 x 10^6 will be placed in "Group A".

Participants having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) more than 2 x 10^6 will be placed in "Group B".

Interventions : Both group A and Group B will be injected intravenously with harvested 5 ml of SVF containing Autologous Non Expanded ADSC and outcome will be observed over the period of 1(one) year.

4.3 Study duration

From April 2019 Onwards (Approximately Five years / Till completion of sample requirements with minimum one year of follow up).

4.4 Study place

Bangladesh Laser & Cell Surgery Institute & Hospital.

House No: 2, Road No: 2, Sector No: 2, Block : D

Aftabnagar Housing, Badda, Dhaka – 1212. Bangladesh.

4.5 Study population:

Patient of CKD who fulfill the selection criteria and admitted at the selected health facility for treatment.

4.6 Sample size determination:

α (two-tailed) = Threshold probability for rejecting the null hypothesis. Type I error rate.

β = Probability of failing to reject the null hypothesis under the alternative hypothesis. Type II error rate.

E = Effect size

$S(\Delta)$ = Standard Deviation of the change in the outcome.

Here, $\alpha = 0.05$

$\beta = 0.20$

E = 0.5

$S(\Delta) = 1.0$

The standard normal deviate for $\alpha = Z_\alpha = 1.960$

The standard normal deviate for $\beta = Z_\beta = 0.842$

A = 1.000

$B = (Z_\alpha + Z_\beta)^2 = 7.849$

$C = (E/S(\Delta))^2 = 0.250$

Sample size N = AB/C = 31 (approx.)

4.7 Sampling technique & Recruitment of study subjects

Participants of the study will be recruited from the patients with CKD attending the Bangladesh Laser & Cell Surgery Institute & Hospital considering the eligibility criteria. The recruitment will be continued till the desired sample is drawn.

4.8 Eligibility criteria

4.8.1 Inclusion criteria

A patient is eligible for the study if all of the followings apply:

a) Aged 18-80 years (inclusive)
b) With chronic kidney disease (CKD)stage 3 to 5 (eGFR 60 to 0 mL/min/1.73m2 (inclusive)) Note : eGFR = estimated glomerular filtration rate
c) Having provided informed written consent.

4.8.2 Exclusion criteria

Any patient meeting any of the exclusion criteria will be excluded from study participation.

1) Known hypersensitivity to any component used in the study.
2) With inadequate hematologic function with: absolute neutrophil count (ANC) <1,500/μL OR platelets < 100,000/μL OR Hemoglobin < 8 g/dL
3) With 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.
4) With hemoglobin A1c (HbA1c) > 8.0%
5) With serious prior or ongoing medical conditions (e.g. concomitant illness such as cardiovascular (e.g. New York Heart Association grade III or IV), hepatic 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 effect the safety of the patient

6) Pregnant or lactating women or premenopausal with childbearing potential but not taking reliable contraceptive method(s) during the study period
7) With known history of human immunodeficiency virus (HIV) infection or any type of hepatitis
8) Judged to be not applicable to this study by investigator such as difficulty of follow-up observation
9) With any other serious diseases/medical history considered by the investigator not in the condition to enter the trial
10) Known or suspected abuse of alcohol or narcotics
11) With known history of cancer within past 5 years
12) With any autoimmune disease
13) With congenital kidney disease
14) With precancerous condition or with raised tumour markers like Alpha feto protein, Carcino embryonic antigen (CEA), C.A 19.9, C.A 125, Serum PSA above normal reference range.
15) Participants having a harvested total "Adipose Derived Stem Cell (ADSC)" count (in 5 ml SVF solution) less than 1 x 10^6 will be excluded from the study.

4.9 Treatment allocation

All subjects would be submitted for Stromal Vascular Fraction (Autologous Adipose Derived Mesenchymal Stem Cell) Based Treatment for Chronic Kidney Disease

4.10 Pre-transplant and Post-Transplant Investigations

Investigations will be done according to the Annexure – D

4.11 Outcome measures

4.11.1 Primary Outcome Measure

a) Incidence of minor adverse events (MAEs) , serious adverse events (SAEs) which may be immediate, early or late - for Phase I [Time Frame: Week 48]

Minor adverse events (MAEs):

i) Pain from lipo-suction > 7 days (Early)

ii) Fever > 7 days (Early)
iii) Subcutaneous hematoma / abscess formation (Early)
iv) Allergic reaction (Immediate)

Serious adverse events (SAEs)

i) Anaphylaxis (Immediate)
ii) Pulmonary embolism or infarction (Immediate)
iii) Outset of any neoplastic change (Late)
iv) Outset of new Cardiovascular events (Late)
v) Outset of new Cerebrovascular or neurological events (Late)
vi) Reactivation of treated tuberculosis (Late)

b) Change from baseline to 24 week visit in estimated glomerular filtration rate (eGFR) and split renal function in all patients - for Phase II [Time Frame: Weeks 0, 24]

eGFR with split renal function will be evaluated using DTPA Renogram.

c) Change from baseline to 24 week visit in estimated glomerular filtration rate (eGFR) with serum creatinine level in patients with CKD 4 and below - for Phase II [Time Frame: Weeks 0, 2, 4, 12, 24]

eGFR will be calculated by Serum Creatinine level using MRDR formula during all visits.

d) Change from baseline to 24 week visit in need for dialysis in patients with CKD 5 - for phase II [Time Frame: Weeks 0, 2, 4, 12, 24]

Need for dialysis is described as

i) No dialysis needed - Score 0
ii) Randomly (more than 6 days interval) - Score 1
iii) At 6 (six) days interval / Once weekly - Score 2
iv) At 5 (five) days interval - Score 3
v) At 4 (four) days interval - Score 4

vi) At 3 (three) days interval / 2 times a week - Score 5

vii) At 2 (two) days interval - Score 6

viii) At 1 (one) day interval / every alternate day./ 3 times a week - Score 7

4.11.2 Secondary Outcome Measures

a) Change from baseline to all post-treatment visits in body weight [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

Weight in Kg will be recorded for each patient during each follow up

b) Change from baseline to all post-treatment visits in Blood-pressure [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

Blood pressure will be measured in each patient during each follow up

c) Change from baseline to all post-treatment visits in S.creatinine [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

   a. S. Creatinine level will be measured during each follow up.
   b. In case of patients having dialysis Pre and Post dialysis S. Creatinine levels will be measured at or near follow up dates.

d) Change from baseline to all post-treatment visits in blood urea nitrogen (BUN) [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

Blood Urea Nitrogen will be measured in all patients during each follow up.

e) Change from baseline to all post-treatment visits in Hemoglobin level [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

   a. Hemoglobin level will be measured in gm/dl and percentage
   b. Need for blood transfusion will be recorded
   c. Need for erythropoietin will be recorded

f) Change from baseline to all post-treatment visits in urine microalbumin-to-creatinine ratio (UMCR) [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

Urinary Microalbumin and creatinine will be measured in each patient during each follow up

g) Change from baseline to all post-treatment visits in hemoglobin A1c [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

HbA1C will be measured in each patient during each follow up

h) Change from baseline to all post-treatment visits in random blood sugar (RBS) [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

RBS will be measured in each patient during each follow up

i) Percentage of patients with hypoglycemia (defined as blood glucose < 55 mg/dL or 3.0 mmol/L) at all post-treatment visits [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

Patients will be asked if he or she had experienced any episode of hypoglycemia with clinical features of altered level of consciousness, sweating, nausea or vomiting with blood glucose < 55 mg/dL or 3.0 mmol/L

j) Change from baseline to all post-treatment visits in eGFR [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

eGFR will calculated using MDRD formula for each patient during each follow up

k) Change from baseline to all post-treatment visits in Anti-Hypertensive medication if there is any. [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

All anti hypertensive medicines with their doses will be recorded including any changes in each patient during each follow up

l) Change from baseline to all post-treatment visits in Hypoglycemic agent if there is any. [Time Frame: Weeks 0, 2, 4, 12, 24, 36, 48]

All hypoglycemic agents including their doses with any changes will be recorded for all diabetic patients during each follow up

m) Change from baseline to post-treatment visits in urine total protein-creatinine ratio (UPCR) [Time Frame: Weeks 0, 24, 48]

Urinary total protein and Creatinine ratio will be done in each patient during defined visit.

n) Change from baseline to post-treatment level of serum Alpha Feto Protein [Time Frame: Weeks 0, 24, 48]

Serum Alpha Feto protein will be measured as a tumour marker for Hepato-cellular carcinoma and also Tumour of Testis and Ovary.

o) Change from baseline to post-treatment level of serum CEA level [Time Frame: Weeks 0, 24, 48]

Serum CEA level will be measured as a tumour marker for Colo-rectal Carcinoma and also for Cancer of Stomach, pancreas, breast, lungs, thyroid and ovary.

p) Change from baseline to post-treatment level of serum CA 19.9 level [Time Frame: Weeks 0, 24, 48]

Serum C.A 19.9 level will be measured as a tumour marker for Pancreatic Carcinoma

q) Change from baseline to post-treatment level LDH level [Time Frame: Weeks 0, 24, 48]

Serum LDH level will be measured as tumour marker for Lymphoma

r) Change from baseline to post-treatment level of Beta 2 Microglobulin level [Time Frame: Weeks 0, 24,48]

Serum Beta 2 Microglobulin level will be measured as a prognostic tool, as CKD patients invariably has a raised serum level.

s) Change from baseline to post-treatment level of serum CA 125 level (in case of female patients) [Time Frame: Weeks 0, 24, 48]

Serum C.A 125 level will be measured as a tumour marker for Ovarian Cancer

t) Change from baseline to post-treatment level of PSA level (in case of male patients) [Time Frame: Weeks 0, 24,48]

Serum PSA level will be measured as a tumour marker for Prostatic Cancer.

4.12 Research instrument

For collection of primary data about patient a semi-structured questionnaire will be developed based on research objective. Pre-testing of the questionnaire will be done on other patients admitted at the same facility. After the pretesting, amendment of the items and question will be done based on study finding. In the final questionnaire both structured and open questions will be kept.

A check list will be prepared to compile the data from hospital records, treatment records, outcome of treatment and laboratory investigation reports.

4.13 Data collection procedure:

Current study involves collection of both primary and secondary data. Primary data will be collected by face to face interview of the patients or patient's attendant by the researcher at health facility during the period of hospital stay, upon their consent and convenience. Socio demographic and personal information will be recorded from patient through interview, with a semi structured pre-tested questionnaire. Information regarding risk factors and risk behavior will be inquired taking effort to minimize the recall bias.

Secondary data about present state and diagnosis will be collected from hospital record as well as from treatment sheet. For acquiring secondary data a structured checklist will be used.

4.14 Data processing

Data processing will include data cleaning and quality control check, editing of data, coding of data and data entry into computer. The edited data will then be entered on to the template of SPSS® 16 and STATA® 10/IC.

4.15 Data analysis

The edited data will then be entered on to the template of SPSS® 16 and STATA® 10/IC.

For Back ground variables and socio-demographic data descriptive statistics and relative frequency (percentage) will be generated.

Through univariate analysis the base line characteristics and treatment outcome will be compared. The effect of treatment will be identified through Multivariate analysis after adjusting for possible confounders. Relative risk with 95% CI will be generated through binary logistic regression adjusting for all possible confounders.

4.16 Data Presentation

Data will be presented in the form of table and graphs. Descriptive statistics will be presented with frequency table. Association will be illustrated with cross tables and test statistics will be added in the foot note of the table. Bar and pie charts will be generated to illustrate descriptive statistics.

5 Ethical considerations

Approval has already been taken from

National Research Ethics Committee,

Bangladesh Medical Research Council (BMRC)

Phone: +88029848396 Email: info@bmrcbd.org

BMRC Bhaban, Mohakhali, Dhaka 1212, Bangladesh.

Approval Number: 076 (1) 25 10 2017

Informed written consent (Annexure -) will be taken from the participant after explaining all the facts, potential dangers to the subjects in case of primary data collection. The study involves collection of non-sensitive Socio-demographic data (primary data), compilation of hospital and treatment records (secondary data) to be conducted researcher under supervision of relevant specialists along with few diagnostic test. The participants will be assured that the information acquired will be used for academic purpose. They will be assured of confidentiality, and for the purpose of data analysis no individual data were reported rather de identified data will be preceded for analysis.

6 Resources

6.1 Space and Budget

a) Principal and other investigators have their respective offices in the premises of the Bangladesh Laser & Cell Surgery Institute & Hospital, Dhaka, Bangladesh.

b) "Bangladesh Laser & Cell Surgery Institute & Hospital" Dhaka, Bangladesh has adequate facilities to carry out the procedure in its operation theater.

c) "Bangladesh Laser & Cell Surgery Institute & Hospital" Dhaka, Bangladesh has adequate facility to carry out post-operative management of the subjects of the current study.

d) "Bangladesh Laser & Cell Surgery Institute & Hospital" Dhaka, Bangladesh has proposed the budget for their operative and post- operative cost and the cost of the hospital stay.

e) "Bangladesh Laser & Cell Surgery Institute & Hospital, Dhaka, Bangladesh". has proposed the budget for data collection, data processing and rounding up the study over the expected time period.

6.2 Facilities

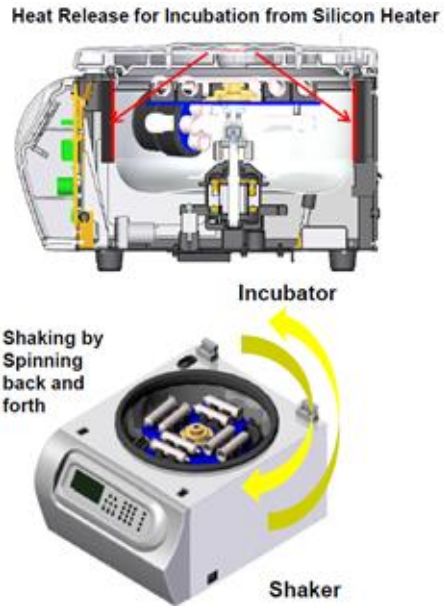
(a) Lab facilities (all modern equipments and Lab materials are available)

(b) Man power facilities (mentioned investigators, Co- investigators, Anesthesiologist, Medical Officer, Nurse, Ward Boy)

6.3 Materials

a) Study population According to sample size calculation 31 patients with established CKD will be recruited in the study over the estimated study period.

b) Equipment "UNISTATION™" a specially designed medical device to satisfy diverse demands for various autologous cell therapies.



UNISTATION is a specially designed medical device to satisfy diverse demands for various autologous cell therapies with only one device. PRP, PRF isolation, and fat purification functions have been added to UNISTATION™.

7 Procedure

After harvesting the abdominal adipose tissue via liposuction SVF (stromal vascular fluid) are collected. Subsequently these are centrifuged in UNISTATION™, a centrifuge machine specifically manufactured for this purpose. Later it is mixed with collagenase solution and fat is separated. Stem cells containing SVF is collected after centrifugation. SVF is neutralized and washed with normal saline. Then it is washed with patient's own serum and normal saline. A sample is collected for cell counting by **Luna automated cell counter**. The final SVF is ready for subcutaneous or IV injection if 80-90% of cell remain viable, active and mobile.

As it is a closed door automatic procedure within a same machine, so after collection of 40ml of sub-cutaneous fat, all procedures are automated. So there is no chance of contamination. Steps are mentioned below.

1st Step: Fat washing(A1) :

(1) To aspirate 40 ml fat and to take 40 ml water for balance in 2 UNIKITs.
(2) To put them is UNISTATION™ Centrifuge.
(3) To touch SVF button once (Display shows A1)
(4) To touch 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 in to the pure fat.

(3) To place shaking plate and put the pure fat with collagenase on if and close the door

(4) To touch SVF button once again (Display shows A2)

(5) To touch Start button once A2 is shown.

(6) To wait for 30 min for shaking incubation and take the fat out after finishing shaking incubation.

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 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 put the hand piece 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.

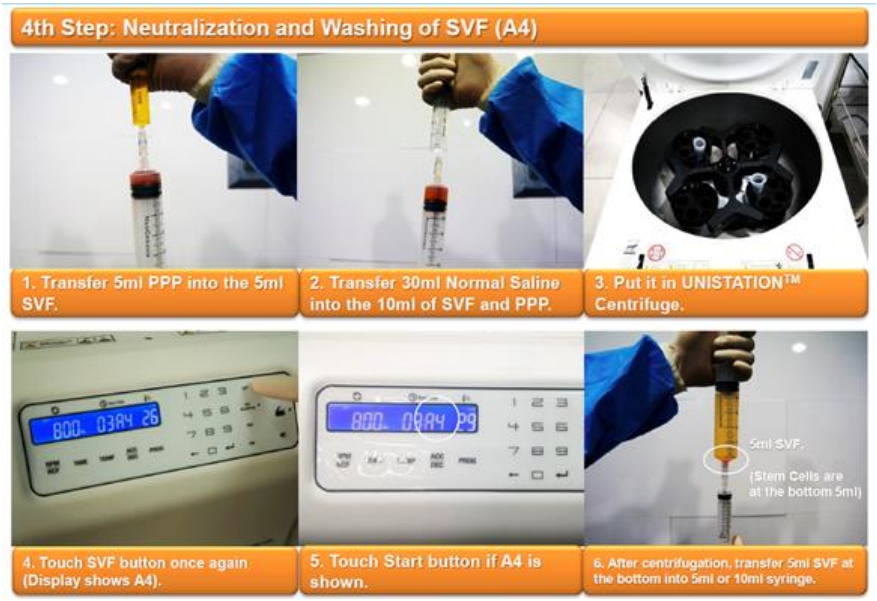
(2) To transfer 30ml Normal saline into the 10ml of SVF and PPP.

(3) To put it in UNISTATION™ Centrifuge.

(4) To touch SVF button once again (Display shows A4)

(5) To touch Start button if A4 is shown.

(6) After centrifugation. To transfer 5ml SVF at the bottom into 5ml or 10ml syringe.



Final Step: Preparation for Injection

To transfuse the final 5 ml of SVF to the patient through intravenous route. Before that to collect few drops of SVF sample for cell counting by Luna Stem automated fluorescent cell counter.

ANNEXURE - A

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ANNEXURE – B

CONSENT

Being fully conscious and oriented I, the undersigned willingly giving my consent

1) To enroll myself as a subject of this new research oriented treatment protocol. I have been explained the pros and cons of this study including the already existing alternative treatment modalities for my disease conditions, the complications that may occur, the possible outcome of this treatment etc. I will not hold anybody responsible for any complication that may arise during the course of treatment or if the procedure ends up in failure. Rather I will comply with the salvage procedure that may be taken to manage the complications or in case of failure.

2) To provide detail information regarding my disease condition and wellbeing without concealing anything particularly that of the co morbid disease conditions.

3) To participate in the follow up sessions as mentioned in the treatment protocol and extend my full co-operation during the follow up proceedings. I must not conceal or exaggerate any information that may actively or passively influence the result of the research.

Please tick as appropriate

1) Do you have complete idea about the type, ultimate goal and methodology of this treatment protocol?	Yes	No
2) Are you aware of the existing treatment facility for your disease?	Yes	No
3) Are you aware that you will not have any additional risk than that of the conventional treatment options ?	Yes	No
4) Have you decided intentionally to take this treatment, despite knowing the possibility of poor / no result of the treatment?	Yes	No
5) Do you think this research will violet your human rights?	Yes	No

6) Do you have confidence on the confidentiality of your records?	Yes	No
7) Do you consent for your medical records to be used for further medical Research?	Yes	No

_____	_____	_____
(Signature of the Physician)	(Signature of the Witness)	Signature of the Patient
Name:	Name:	Name:
Address:	Address:	Address:

ANNEXURE - C

QUESTIONNAIRE - 1

(CLINICAL ASSESSMENT - DURING ALL VISITS)

PARTICULARS OF THE PATIENT: **CODE NO:**

NAME: **AGE:** **SEX:** M / F

HOSPITAL REG. NO:

ADDRESS: **PHONE:**

CONTACT PERSON: **PHONE:**

DIAGNOSIS (Put Tick Mark Beside The Appropriate Selection):

1) CKD: Stage 3A Stage 3B Stage 4 Stage 5

a) GN	Need for dialysis	Score
b) IgA Nephropathy	a) No dialysis yet	0
c) FSGS	b) Occasional (> 6 days interval)	1
d) Interstitial Nephritis	c) At 6 (six) days interval / Once weekly	2
e) No Biopsy done	d) At 5 (five) days interval	3
	e) At 4 (four) days interval	4
	f) At 3 (three) days interval / 2 times a week	5
	g) At 2 (two) days interval	6
	h) At 1 (one) day interval / 3 times a week -	7

2) HYPERTENSION

3) DM

4) Hypothyroidism

5) HYPERPARATHYROIDISM

6) IHD < NyHA Gr III or IV

7) CLD < Child Pugh C

8) CVD

9) OTHERS (if any)

SYMPTOMS (Put Tick Mark Beside The Appropriate Selection):

Urine output:	Scanty / Reduced / Normal	**General Welbeing**	Better/Good/As usual/Bad/Worse
Anorexia:	Yes / No	**Convulsion:**	Yes / No
Nausea:	Yes / No	**Loss of Consciousness:**	Yes / No
Vomiting:	Yes / No	**Muscle spasm:**	Yes / No
Hiccough:	Yes / No	**Pain**	Yes / No
Weakness:	Yes / No	**Fever**	Yes / No
Weight loss:	Yes / No	**Headache:**	Yes / No
Swelling of body:	Yes / No	**Respiratory distress:**	Yes / No
Vertigo:	Yes / No	**Chest pain:**	Yes / No
Constipation:	Yes / No	**Abdominal Pain:**	Yes / No
Loose motion:	Yes / No		

Others (if any):

SIGNS:

Body weight: **Blood pressure:** **Oedema:**

Pulse: **Respiration:** **Dehydration:**

Temperature:

Others (if any):

ANNEXURE - C

QUESTIONNAIRE - 2

(CONCOMITANT TREATMENT - DURING ALL VISITS)

PARTICULARS OF THE PATIENT: **CODE NO:**

NAME: **AGE:** **SEX:**

REG. NO:

TYPE OF MEDICINE	NAME OF MEDICINE	DOSE	CHANGES IF ANY
ANTIHYPERTENSIVE			
HYPOGLYCAEMIC			
ANTIBIOTICS			
ANALGESICS			
ANTIULCERANT			
VITAMINS & MINERALS			
OTHERS			

ANNEXURE - D

(INVESTIGATIONS - 1ST VISIT - 0 WEEK)

PARTICULARS OF THE PATIENT: **CODE NO:**

NAME: **AGE:** **SEX:** **M / F**

REG. NO:

INVESTIGATIONS for Outcome Measures:

DTPA Renogram Total GFR
Right
Left

CBC/ESR

Hb (gm/dl)
ESR (mm in 1st hour)
PLATELET COUNT (K/ul)
WBC
Neutrophil (%)
Lymphocyte (%)
Monocyte (%)
Eosinophil (%)
Basophil (%)

BIOCHEMICAL:

RBS
HbA1C
S. Creatinine
e GFR (ml/min/1.73 m^2)
BUN
Urine Microalbumin-Creatinine Ratio (UMCR)
Urinary Total Protein-Creatinine ratio(UPCR)

TUMOUR MARKERS

S. PSA
S. ALPHA FETO PROTEIN
S. CEA
S. C.A 19.9
S. CA 125
S. Beta 2 Microglobulin
S. LDH

INVESTIGATIONS for Fitness & Maintainence:

BIOCHEMICAL:

S.URIC ACID
S. Vit D3
S. Calcium
S. Phosphate

S. ELECTROLYTES:

SODIUM
POTASSIUM
CHLORIDE
TCO2
ANION GAP

HORMONES

S. PTH
S. TSH
FT4

LIVER PROFILE

HBsAg
Anti HCV
ALT
S. Albumin
Prothrombin Time

Urine R/M/E

Selective Cases

USG of W/A
MRI of Brain
S. Protein Electrophoresis
CXR P/A View
ECG
ECHOCARDIOGRAM

OTHERS (If any)

ANNEXURE - D

(INVESTIGATIONS)

(2nd/3rd/4th/6th VISIT - 2/4/12/36 Weeks)

PARTICULARS OF THE PATIENT: **CODE NO:**

NAME: **AGE:** **SEX:** **M / F**

REG. NO:

INVESTIGATIONS for Outcome Measures:

CBC/ESR

Hb (gm/dl)
ESR (mm in 1st hour)
PLATELET COUNT (K/ul)
WBC
Neutrophil (%)
Lymphocyte (%)
Monocyte (%)
Eosinophil (%)
Basophil (%)

BIOCHEMICAL:

RBS
HbA1C
S. Creatinine
e GFR (ml/min/1.73 m^2)
BUN
Urine Microalbumin-Creatinine Ratio (UMCR)

INVESTIGATIONS for Fitness & Maintainence:

BIOCHEMICAL:

S.URIC ACID(umol/L)
S. Calcium
S. Phosphate

S. ELECTROLYTES:

SODIUM
POTASSIUM
CHLORIDE
TCO2
ANION GAP

Urine R/M/E

ANNEXURE - D

(INVESTIGATIONS)

(5th / 7th VISIT - 24, 48 WEEK)

PARTICULARS OF THE PATIENT: **CODE NO:**

NAME: **AGE:** **SEX:** **M / F**

REG. NO:

INVESTIGATIONS for Outcome Measures:

DTPA Renogram Total GFR
Right
Left

CBC/ESR

Hb (gm/dl)
ESR (mm in 1st hour)
PLATELET COUNT (K/ul)
WBC
Neutrophil (%)
Lymphocyte (%)
Monocyte (%)
Eosinophil (%)
Basophil (%)

BIOCHEMICAL:

RBS
HbA1C
S. Creatinine
e GFR (ml/min/1.73 m^2)
BUN
Urine Microalbumin-Creatinine Ratio (UMCR)
Urinary Total Protein-Creatinine ratio(UPCR)

TUMOUR MARKERS

S. PSA (ng/ml)
S. ALPHA FETO PROTEIN (ng/ml)
S. CEA (ng/ml)
S. C.A 19.9 (U/ml)
S. CA 125
S. Beta 2 Microglobulin (mg/L)
S. LDH

INVESTIGATIONS for Fitness & Maintainence:

BIOCHEMICAL:

S.URIC ACID(umol/L)
S. Vit D3 (ng/ml)
S. Calcium
S. Phosphate

S. ELECTROLYTES:

SODIUM
POTASSIUM
CHLORIDE
TCO2
ANION GAP