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# ORGAN DYSFUNCTION AND MANAGEMENT



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# ORGAN DYSFUNCTION & MANAGEMENT

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## Diabetes Mellitus: Current Understanding and Newer Insights into “Causation to Cure”

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Diabetes mellitus (DM) is taking the centre-stage of Medicine with its wings spreading across the globe to take the form of pandemic with India as the diabetic capital of the world. The current belief for causation of type-1 Diabetes mellitus (T1DM) (also called juvenile diabetes) is that it occurs in childhood due to the development of autoantibodies to insulin secreting  $\beta$ -islet cells causing their immune destruction. The process of  $\beta$ -cell destruction is initiated by various antibodies identified as  $\beta$ -islet cell autoantibodies, antibodies to insulin, glutamic acid decarboxylase (GAD) antibodies and antibodies to protein tyrosine phosphatase (IA2 or ICA512). HLA-DR3/4 and DQ8 genotype have been shown to be highly associated with beta-cell autoimmunity.<sup>1</sup> Type-2 DM on the other hand is believed to be due to inadequate synthesis of insulin, impaired action of insulin on the target organs, increased endogenous glucose output (EGO) and obesity, all of these mainly affecting adults above the age of 40 years and eventually causing end organ damage mainly affecting cardiovascular system, kidneys, central and peripheral nervous system and eyes.

While both types have impaired glucose tolerance and eventually lead to end organ failure, T1DM usually manifests with hyperglycemia, weight loss and diabetic ketoacidotic spells unlike T2DM manifesting as fatigue

and weight gain along with other nonspecific symptoms.

With this understanding about the causation of the disease, the therapeutic modalities were also different, T1DM being treated with insulin replacement which include the gamut of management ranging from synthetic insulin to islet cell/ stem cell transplantation. Sporadic attempts have even been made to address the antibodies. For T2DM, the only concept is limited to oral hypoglycaemic agents / exogenous insulin replacement. There is no path-breaking work and therefore no established therapy which can control or stop the progression of end organ damage resulting from insulin resistance in this group of patients.

We propose that both types of DM, T1DM and T2DM are caused due to immune dysregulation of T and B-cells leading to target destruction of insulin secreting  $\beta$ -islet cells. Winer brothers from Toronto, Canada have proposed that B-cells and T-cells are both responsible for production of pathogenic IgG antibodies in mice model of obesity associated DM.<sup>2</sup>

In the present issue, we have two articles related to DM, one by Kanodia et al where they have studied lesions in 124 diabetic patients referred to our institute for treatment from 2008 to 2013. They have described that non-diabetic renal diseases (NDRD) comprise a significant proportion of diabetic patients. They have found that 24.2 % diabetics had NDRD alone and another 28.2% had NDRD with diffuse glomerular sclerosis. These patients can be offered timely stem cell therapy (SCT) to prevent or arrest the progression of their diseases. Another article in this issue is by Thakkar et al, where they have described a prospective open-labeled 2-armed clinical trial with 10 T1DM patients in each arm. One group (Group-1) was offered autologous SCT and group-2 was offered allogenic SCT. Both the groups received stem cells, group-1 received about 103 ml with  $2.65 \times 10^2$  Insulin secreting cells (ISC) / $\mu$ L,

CD34+ 0.81% and CD45-/90+/73+ 81.5%, which are markers for Adipose Tissue Derived Mesenchymal Stem Cells (AD-MSC), 81.55%; and group-2 received about 95 ml SC with  $2.07 \times 10^2$  ISC/ $\mu$ L, CD34+ 0.32% and CD45-/90+/73+, 61.6%. No untoward effect was observed in this study. Sustained improvement in HbA1c and S.C-peptide was noted over a mean follow-up of 33.10 months in group-1 and 54.24 months in group-2 with decrease in GAD antibodies. Mean insulin requirement decreased from 63.9 to 38.6 IU/day in group-1 and 57.56 to 40.5 IU/day in group-2. This study has proved that we can generate stem cells including ISC *in vitro*, that T1DM does respond to SCT

and that autologous source is more beneficial than allogenic in terms of durability of sustained drop in insulin requirement and GAD antibodies. In addition this study has also encouraged us to try this therapy for T2DM patients who have not met with target organ damage.

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# Non-Diabetic Renal Disease in Patients with Type 2 Diabetes Mellitus

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## ABSTRACT

Diabetes mellitus (DM) is reaching the stage of global epidemic. Diabetic nephropathy is one of the commonest causes of end stage renal disease taking up major share of patients on waiting list of organ transplantation. Non-diabetic renal diseases (NDRD) form an important cause of morbidity and disturbance in the functioning of patients with DM. The current review discusses the prevalence and incidence of NDRD world over and has tried to cover different aspects of NDRD which include etiology, pathogenesis, clinical presentation, lab findings and histopathological lesions by which early prediction and prompt management can improve the outcome in this group of lesions.

**KEYWORDS:** Diabetes mellitus, non-diabetic renal diseases, chronic kidney disease, renal biopsy

## INTRODUCTION

The incidence and prevalence of diabetes mellitus (DM) is increasing worldwide. World Health Organization (WHO) has recognized DM as a global epidemic. Diabetic nephropathy (DN) is one of the major complications of DM and leading cause of chronic kidney disease (CKD) and end stage renal disease (ESRD).<sup>1,2</sup> About 20-40% of diabetic patients develop diabetic renal disease. Patients of type 2 DM can develop renal disease other than DN, pathologically unrelated to diabetes, known as non-diabetic renal disease (NDRD).<sup>3,4,5</sup> The precise diagnosis of these diseases has prognostic and therapeutic implications.<sup>6,7</sup> Clinical differentiation between NDRD and DN is not clear.

### NDRD in Type 1 DM

NDRD in Type 1 DM is rare and it accounts for hardly 2-3% in unselected cases with proteinuria.<sup>8</sup> Microhematuria, absence of diabetic retinopathy, uncharacteristic change in renal function and immunological abnormalities are the criteria for suspecting NDRD in cases of Type 1 DM.<sup>8,9</sup>

### NDRD in Type 2 DM

Prevalence of NDRD in Type 2 DM varies from 12-80% depending on selection criteria and the population being studied.<sup>10-16</sup> It is seen in 26.7% Asian and 22% European patients.<sup>16-19</sup> It is important to differentiate DN and NDRD because their therapy and prognosis are different.

### Indicators of NDRD

#### *Duration of diabetes*

NDRD patients have relatively shorter duration of diabetes than DN patients. In DN it often takes quite a long period of time to progress from microalbuminuria to macroalbuminuria and renal failure. The clinical abnormalities are detected 5-10 years after onset or diagnosis of DM. Lee et al concluded from their study that a shorter duration of diabetes was significantly associated with NDRD.<sup>4</sup> Similar results were reported in literature.<sup>3,10,18,20,21,22</sup> However, Mak et al and Bertani et al found no significant difference in the duration of diabetes among the different groups.<sup>23,24</sup>

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### *Hypertension (HTN)*

Hypertension is more prevalent and severe in DN than NDRD. Zhou et al have reported that the mean systolic blood pressure in DN group was higher than NDRD group.<sup>13</sup> The mechanisms that aggravates hypertension in DN are rennin angiotensin activity, sympathetic over-activity and endothelial cell dysfunction causing water- sodium retention. Hereditary relationship between hypertension and DN also play a role.<sup>25</sup> However Soni and Yaqub et al found no difference in NDRD and Diabetic glomerulosclerosis (DGS) patients.<sup>10,22</sup>

### *Hematuria*

The presence of microscopic hematuria is an important indicator of NDRD. Lee, Zhou, Mak and Matias et al found a strong correlation between NDRD and microscopic hematuria.<sup>4,13,23,26</sup> Many entities of NDRD, such as IgA nephropathy (IgAN) manifest as microscopic or gross hematuria. IgAN is more common in China, South Korea and Hong Kong.<sup>15</sup>

### *Diabetic retionopathy (DR)*

DR is more frequently seen in DN and absence of DR is predictor of NDRD. In Type 1 DM 90% patients develop DR but in Type 2 DM the incidence varies from 40 to 75 %.<sup>3</sup> Tone et al reported that absence of DR showed 87% sensitivity and 93% specificity for prediction of NDRD.<sup>20</sup> Several other studies also showed that absence of DR is an important predictor of NDRD.<sup>10,26,27</sup> However absence of DR cannot exclude the presence of diabetic nephropathy because 50-75% patients of DN do not have retinopathy.<sup>3</sup>

### *Proteinuria and serum creatinine*

Proteinuria in Type 2 DM is a manifestation of DN and NDRD. Lin, Yaqub and Mak et al reported that proteinuria was comparatively higher and favored DN than NDRD.<sup>11, 22,23</sup> Yaqub et al have also reported that serum creatinine levels were significantly higher in DN than NDRD.<sup>22</sup> Similar results were also reported by Matias et al.<sup>26</sup>

Wong et al reported that absence of retinopathy, with hematuria and/ or proteinuria  $\geq 2$  gm/day constitute the strongest indication for NDRD.<sup>18</sup> Thus combination of indicators constitute a more sensitive predictor of NDRD than any of them alone.

### *Renal biopsy in proteinuric Type 2 DM*

Renal biopsy is necessary in proteinuric Type 2 DM for accurate diagnosis, prompt initiation of disease specific treatment and ultimately better renal out come. However biopsy is still not popular due to its invasiveness and potential procedural risks like hematuria, arterial embolization, perirenal hematoma or contraindications like solitary kidney and cortical atrophy.<sup>10,15,22,27,28</sup> Several recent studies have revealed a heterogenous group of disease entities in Type 2 DM.<sup>3,10,15</sup> The indication of renal biopsy in Type 2 DM are sudden onset of proteinuria with normal renal function, sudden deterioration of renal function, absence of retinopathy, gross or microscopic hematuria, short duration of diabetes, low complement levels and active urinary sediments.<sup>3,4</sup>

### *Histological lesions of NDRD in Type 2 DM*

Three histological patterns are described, (1) Isolated NDRD, (2) Diabetic glomerulosclerosis (DGS), and (3) NDRD with DGS. In study of 233 patients from January 2005 to December 2005, Pham et al reported 53.2 % NDRD, 19.3 % NDRD with DGS and 27.5 % DGS.<sup>27</sup> Most common lesions with NDRD were FSGS (21%), minimal change disease (MCD) (15.3%), IgA nephropathy (15.3%) and membranous nephropathy (MN) (13.3%). Byun et al had reported isolated NDRD in 53.6 %, NDRD with DGS in 9.1 % and DGS alone in 37.3 % patients.<sup>15</sup> IgA nephropathy was the most common lesion in Korean population.<sup>15</sup> Indian studies revealed acute interstitial nephritis, membranous nephropathy and MCD as the common lesions of NDRD.<sup>10,29</sup>

### **Glomerular disease superimposed on DGS**

It is sometimes difficult to diagnose a combination of DGS with NDRD like, MN with DGS: GBM spikes,

granular IgG and C3 deposits in capillary walls on IF studies and subepithelial deposits on electron microscopy(EM) will help to diagnose MN. PIGN with DGS: history of infections, granular C3, IgG in GBM, subepithelial humps on EM will confirm PIGN. IgAN with DGS: Dominant IgA in mesangium and mesangial deposits in EM can confirm IgAN.

**Our study at IKDRC,Ahmedabad**

We analyzed renal biopsies of patients from January 2008 to September 2013. Post-transplant patients and inadequate renal biopsies were excluded. Out of 124 diabetic patients, 24.2 % (n=30) had isolated NDRD, 28.2% (n=35) had NDRD with DGS and 47.6% (n=59) had isolated DGS. The mean age was 55.25 ± 14.6 years, 52.5 ± 11.1 years and 60.8 ± 5.86 respectively in NDRD, NDRD with DGS and DGS group (Table 1). The indications of renal biopsy included nephrotic syndrome (NS) in 46.8% (n=55), acute kidney injury (AKI) in 17.4 % (n=22), chronic renal failure (CRF) in 12.1% (n=15), rapidly progressing renal failure (RPRF) in 10.5 % (n=13) and hypertensive nephropathy (HTN) in 12.9% (n=16) patients. The most common presentations in NDRD were NS in 48.3% (n=14) and AKI in 34.5% (n=10). The incidence

of NS, AKI and RPRF was in 24.3% patients (n=9) each, in the group having NDRD with DGS. In DN common presentations were NS in 60.3 % (n=30), HTN in 20.7% (n=12) and CRF in 13.8% (n=8) patients (Table 2).

The duration of DM was relatively short in NDRD (6.33 ± 5.33 years) than DN (10.6 ± 7.17 years) (p= 0.007). DR was absent in all NDRD patients. Systolic blood pressure was comparatively higher in DGS than NDRD patients. Hematuria was noted in 37.93 % in NDRD patients and in 31.03 % in DGS patients. Proteinuria was more in DGS patients. (Table 3)

The most common lesions of NDRD was MN (20.65%) followed by mesangial proliferative glomerulonephritis (MePGN) (17.24%) and IgA nephropathy (13.79%) (Figure 1). In combined disease NDRD with DGS, the most common lesion was acute tubulointerstitial nephritis (ATIN) (59%) followed by hypertension in 24% patients. The glomerular diseases include primary and secondary glomerular diseases. The primary glomerular diseases were MCD, post-infectious glomerulonephritis (PIGN), MN, crescentic glomerulonephritis, focal segmental glomerulosclerosis (FSGS), IgAN, diffuse proliferative

**Table 1 : Depicting the histological patterns of injury in diabetic patients with respect to age**

Variable	NDRD	NDRD+DN	DN	P value
Number (percentage)	30 (24.2%)	35 (28.2%)	59 (47.6%)	<0.01
Age in years (±SD)	55.25 ± 14.66	52.52 ± 11.12	60.8 ± 5.86	<0.01

**Table 2 : Renal syndromes at presentation**

Renal syndromes	NDRD (n=30)	NDRD +DN (n= 35)	DN (n= 59)	P value
NS (n=58) (46.77%)	14 (48.27%)	9 (24.32%)	35 (60.34%)	<0.01
AKI (n=22) (17.74%)	10 (34.48%)	9 (24.32%)	3 (5.17%)	<0.01
CRF (n=15) (12.09%)	2 (6.89%)	5 (13.51%)	8 (13.79%)	<0.57
RPRF (n=13) (10.48%)	3 (10.34%)	9 (24.32%)	1 (1.72%)	<0.01
HTN (n=16) (12.90%)	1(3.44%)	3 (8.10%)	12 (20.68%)	<0.05

**Table 3 : Clinical and biochemical parameters**

	NDRD (n=30)	NDRD +DN (n= 35)	DN (n= 59)	p-value
Diabetes duration (years)	6.33 ± 5.3	7.79 ± 4.14	10.6 ± 7.17	0.007
Retinopathy	NIL	5(13.5%)	7(12.6%)	<0.01
Proteinuria (gm/day)	2.68 ± 1.95	2.13 ± 1.4	4.53 ± 2.5	<0.01
Hematuria	11 (37.93 %)	13 (37.53%)	18 (31.03%)	0.75
S.Cr. (mg/dL)	3.85 ± 3.73	5.68 ± 3.22	3.57 ± 3.32	<0.01

glomerulonephritis (DPLN), MePGN and membrano-proliferative glomerulonephritis (MPGN). The secondary glomerular diseases were lupus nephritis and multiple myeloma. The tubulointerstitial diseases included ATIN and acute pyelonephritis (APN), and vascular diseases included HTN and thrombotic microangiopathy.

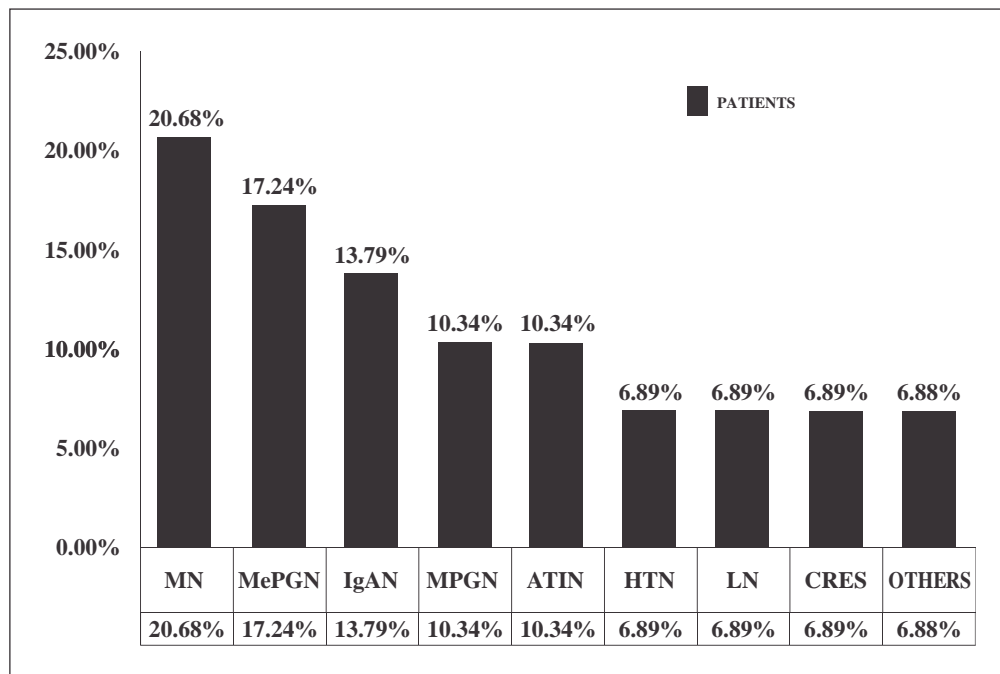
**Pathogenesis of NDRD in DM**

The pathogenesis of NDRD in DM with or without DGS is not well understood. The predisposition of DGS

to superimposed nephritis could be attributed to enhanced exposure of antigenic cellular components and triggering immune responses.<sup>30</sup> However, other authors found no difference in the prevalence of NDRD between patients with and without diabetes and that the coexistence of different GN in the diabetic kidney may be merely coincidental.<sup>18,31,32</sup>

**MANAGEMENT**

Renal biopsy should be performed in diabetics to determine the underlying cause of proteinuria and



**Figure 1: Histology of NDRD (MN : Membranous nephropathy; MePGN : Mesangial proliferative glomerulonephritis; IgAN :IgA nephropathy; ATIN : Acute tubulointerstitial nephritis; MPGN : Membranoproliferative glomerulonephritis; HTN: Hypertensive Nephropathy; LN: lupus nephritis; Cres: Crescentic glomerulonephritis and others.)**

differentiate DGS from NDRD. Corticosteroids with other immunosuppression are recommended for underlying NDRD.

## CONCLUSION

The incidence of NDRD (isolated/ combined with DSG) in Type 2 DM is very high. Shorter duration of diabetes, hematuria, absence of retinopathy, lower systolic blood pressure and nephrotic range proteinuria strongly predict NDRD. Renal biopsy is recommended for Type 2 DM patients with risk factors of NDRD for accurate diagnosis, prompt initiation of disease specific treatment and better renal outcome.

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# Role of Bispectral Index Monitoring on Propofol Requirement and Recovery Endpoints in Patients Undergoing Laparoscopic Donor Nephrectomy

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## ABSTRACT

**Background:** The Bi spectral index [BIS] is a electroencephalograph (EEG) derived parameter that correlates with level of sedation and hypnosis due to anesthetic drugs. This study was designed to evaluate the effect of BIS monitoring on requirement of propofol during Total Intravenous Anesthesia (TIVA) as well as the recovery status of the patient.

**Methods:** This was a prospective open-labeled two-armed study carried out in 120 adult patients with ASA physical status I and II undergoing laparoscopic donor nephrectomy under TIVA between January to December'11. Patients were randomly distributed in two demographically balanced groups. One was SP group (Standard clinical Practice group) without BIS monitoring and another was BIS group (Bi Spectral Index group). All patients received propofol, fentanyl and atracurium infusion during the procedure. Propofol dose was adjusted as per standard guidelines in SP group and in BIS group, it was adjusted to maintain BIS value between 40-60 during surgery, and 55-70 during last 15 minutes of surgery. Total amount of propofol used, hemodynamic parameters and recovery parameters were recorded in all patients.

**Results:** Hemodynamic changes were non-significant in both groups. Mean bolus propofol requirement was comparable in both groups ( $2.08 \pm 0.143$  mg/kg for BIS v/s  $2.14 \pm 0.203$  mg/kg for SP) while mean average infusion rate was significantly low in BIS group than SP group ( $93.55 \pm 7.13$  mcg/kg/min v/s  $112.91 \pm 7.42$  mcg/kg/min). There was faster emergence from anesthesia in BIS group compared to SP group. None of the patient in both the groups had intra-operative awareness.

**Conclusion:** During TIVA, use of BIS monitoring resulted in reduced propofol infusion consumption as well as faster recovery as compared to standard practice group.

**KEYWORDS:** Bi spectral index monitoring, fentanyl, atracurium, propofol infusion, recovery.

## INTRODUCTION

Laparoscopic donor nephrectomy has emerged as a standard of care all over the world due to advantages of minimally invasive surgery. The goal of anaesthetic management of these patients is to attenuate stress responses to CO<sub>2</sub> pneumoperitoneum as well as early and uneventful recovery. Monitoring the depth of anaesthesia can help in optimizing delivery of anaesthetic agents thereby resulting in fewer adverse events and improved recovery.

The bispectral index monitor (BIS) is a commercial device reflecting signal processed electroencephalograph (EEG) used to measure the level of hypnosis during anaesthesia. It estimates dimensionless number from 0 to 100 and decreasing values indicate more sedation and hypnosis. The value of BIS between 40 and 60 indicate surgical plane of anaesthesia. The aim of present study was to evaluate the effect of BIS monitoring on requirement of propofol during TIVA as well as the recovery status of the patients undergoing laparoscopic donor nephrectomy.

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## METHODS AND MATERIALS

This prospective controlled study was conducted in 120 ASA physical status I-II adult patients undergoing laparoscopic retroperitoneal donor nephrectomy during January '11 to December '11. Patients were randomly allocated in two groups, "Standard clinical Practice" (SP) group & "BIS monitoring" (BIS) group, by closed envelope method each having 60 patients. Institutional Review Board approval and written informed patient consent was obtained. Patients having baseline systolic blood pressure less than 100 mm of Hg, heart rate less than 55, BMI > 30, H/o allergy to propofol and patients taking sedative or hypnotic drugs were excluded from our study.

On the day of surgery, all vital parameters like electrocardiogram (ECG), non-invasive blood pressure (NIBP), SPO<sub>2</sub>, temperature, urine output and end tidal CO<sub>2</sub> were monitored and recorded in both groups. In BIS group, the EEG signal was acquired using four electrodes (Zipprep) applied to the forehead, with one on each outer malar bone, one at the center of the forehead, and one (ground) on the either side of the center electrode along with routine monitoring. The BIS (Rev 3.12 U) value was displayed on an Aspect EEG monitor (Model A1050, Aspect Medical Systems, Natick, MA) & initial BIS score was noted. Standard balanced general anaesthesia was given in both groups using intravenous fentanyl 2.0 µg/kg as a premedication and propofol 2-3 mg/kg for induction till the loss of consciousness and/or loss of eyelash reflex in SP group and BIS value around 40-50 in BIS group. Tracheal intubation was facilitated with intravenous succinylcholine 1.5 mg/kg. Maintenance of anaesthesia was done by continuous infusion of propofol 130 mcg/kg/min, fentanyl 1mcg/kg/hr and atracurium 0.5 mg/kg/hr after bolus dose of 0.5 mg/kg. No inhalation agent was used throughout the surgery. Intermittent positive pressure ventilation was adjusted to keep the ETCO<sub>2</sub> around 35-40 mm of Hg. Insufflation pressure was kept around 10-12 mm Hg during laparoscopic surgery.

In BIS group, the EEG was recorded continuously starting from pre-induction to extubation of the patient and infusion of propofol was adjusted to keep BIS value between 40 to 60 by increasing or decreasing the propofol concentration. In SP group, adjustment of propofol infusion was done on standard clinical signs. When there was hypertension (>20% rise from baseline), tachycardia (> 20% rise in pulse rate) or somatic movement, the dose of propofol was increased by 50 mg (5.0ml)/hr and if required antihypertensive (nitroglycerin infusion) was started. Bradycardia and hypotension i.e. >20% fall in pulse rate and blood pressure were managed with decreasing the dose of propofol by 50 mg (5.0ml)/hr, adjustment of fluid status or pharmacological agents if required. Dose of fentanyl was not changed. About 15 -20 minutes before the end of surgery i.e. after removal of the kidney, fentanyl and atracurium infusions were discontinued and propofol dose was gradually reduced. In BIS group, intravenous propofol dose was adjusted to keep BIS value 55 to 70 while in SP group; it was reduced 50% for first 5 minutes and then 25% for each 5 minutes. Propofol infusion and N<sub>2</sub>O were discontinued 5 minutes before the end of surgery. Patients were extubated after reversal of neuromuscular blockade when they had adequate muscle tone, respiration and were following verbal command. Intravenous tramadol 2.0 mg/kg was given for post-operative analgesia. They were shifted to recovery room for observation with routine monitoring.

Hemodynamic parameters like NIBP and heart rate were recorded every 5 minutes. Total duration of anesthesia and surgery, mean propofol requirement as bolus for induction and as infusion for maintenance were recorded. The time when propofol anesthesia discontinued was identified as the starting point (time = 0) of patient recovery. Time of initial wake up events (eye opening, response to verbal command, muscle tone, extubation) was also recorded.

Postoperatively, patients of both groups were asked for intraoperative awareness and recall of events like hearing vague sounds, feeling surgical instruments or dressing application, or dreaming on the first postoperative day.

### Statistical analysis

Sample size calculation was done to compare the effect of total amount of propofol infusion. Power analysis was performed. This analysis was based on two samples with statistical significance of 0.05 & 95% power. The sample size required to detect the standard difference of 0.78 are approximately 90 (45 in each group). Considering some dropouts during study, the power analysis indicated that the minimum number of patients in each group should be 60.

Statistical analysis was performed using SPSS (12.0). Continuous variables were presented as mean (SD) and compared using the t-test, but if the data were not normally distributed the Mann-Whitney U-test was used. Non-continuous numeric values are expressed as median (range) and were compared with the Mann-Whitney U-test. Categorical variables were represented as numbers (%) and compared using chi-square test.  $p < 0.05$  was considered to be statistically significant.

### OBSERVATION AND RESULTS

There were no significant differences in terms of patient's selection like age, height, weight, sex, BMI, duration of surgery and anaesthesia (Table-1). The hemodynamic changes during intubation and intra-operative period were comparable in both groups.

The average bolus dose of propofol requirement (Table-2) was  $2.08 \pm 0.143$  mg/kg in BIS versus  $2.14 \pm 0.203$  mg/kg in SP group.

Average propofol infusion rate was significantly less in the BIS group compared to SP group ( $93.55 \pm 7.13$  mcg/kg/min for BIS group v/s  $112.61 \pm 7.42$  mcg/kg/min for SP group) ( $p < 0.0001$ ). In BIS group, the emergence from anaesthesia was significantly faster compared with a standard practice group. After discontinuation of propofol, in BIS group, it took around  $21.60 \pm 1.81$  minutes for eye opening as compared to  $27.98 \pm 2.47$  minutes in SP group. Patients of SP group had significantly delayed response to

**Table 1: Demographic data**

Variables	BIS group (n=60)	SP Group (n=60)	P-value
Sex ( male/ female)	16/44	20/40	0.426
Age (yrs)	$43.6 \pm 10.39$	$44.9 \pm 11.9$	0.525
Height (cms)	$157.01 \pm 8.48$	$157.02 \pm 9.8$	0.995
Weight (kgs)	$61.7 \pm 13.6$	$59.5 \pm 11.9$	0.347
BMI (kg/ m <sup>2</sup> )	$25.2 \pm 4.63$	$24.3 \pm 4.42$	0.278

**Table 2: Propofol requirement**

Variables	BIS group (n=60)	SP Group (n=60)	P-value
Propofol bolus (mg/kg)	$2.08 \pm 0.143$	$2.14 \pm 0.203$	0.063
Mean Propofol infusion (mcg/kg/min)	$93.55 \pm 7.13$	$112.91 \pm 7.42$	$< 0.0001$
Total surgery time (min)	$123.93 \pm 17.33$	$118.18 \pm 15.44$	0.057
Total duration of Anesthesia (min)	$137.36 \pm 16.93$	$128.96 \pm 15.24$	0.055

**Table 3 : Recovery endpoints**

Recovery Endpoint	BIS group (n=60) (minutes)	SP Group (n=60) (minutes)	P-value
Opens eyes	$21.60 \pm 1.81$	$27.98 \pm 2.47$	$< 0.0001$
Respond to commands	$22.65 \pm 1.76$	$29.18 \pm 2.50$	$< 0.0001$
Muscle tone	$23.66 \pm 1.58$	$30.28 \pm 2.60$	$< 0.0001$
Extubation	$24.67 \pm 1.58$	$31.38 \pm 2.57$	$< 0.0001$

verbal commands than BIS group (29.18±2.50 v/s 22.65±1.76 minutes). Similarly, muscle tone and extubation were significantly faster in BIS group than SP group (P<0.0001). (Table 3) There was no incidence of intra-operative awareness, dream or recall of events in both groups.

## DISCUSSION

Anaesthetic depth is a simplified construct of hypnosis, antinociception and reflex suppression. Traditionally depth of anaesthesia is assessed by clinical signs such as hypertension, tachycardia, lacrimation and patient movement in response to noxious stimulation which are unreliable and suppressed by paralysis. Bispectral index has been the first device of EEG widely available to quantify the hypnotic effect of the anesthetic agents in clinical practice.<sup>1</sup> Aspect medical recommends maintaining BIS between 40 and 60 which ensures adequate hypnotic effect while improving recovery process.<sup>2</sup> Values<40 are consistent with deep anesthesia, values between 40 and 60 are the target range (surgical plane of anaesthesia) and values >60 are consistent with light anesthesia.<sup>3</sup> An upper limit of 60 has been validated by studies in which maintaining BIS below 60 decreased the incidence of intraoperative awareness.<sup>4</sup> Our target range was set at BIS between 40 and 60. Hypnotic drug titration to BIS value of 55-70 was allowed during wound closure to facilitate recovery.<sup>5</sup>

No significant differences between two groups were noted in intra-operative events. Hemodynamic changes during laparoscopic surgery usually result from combined effects of pneumoperitoneum, patient position, drugs and hypercapnia if present. We kept ETCO<sub>2</sub> in a defined range (35 - 40 mm Hg) in all patients to avoid hemodynamic changes related to retention of CO<sub>2</sub>.

Titration of propofol based on BIS resulted in reduced total amount of propofol used, reduced propofol infusion rate and improved recovery from anesthesia. Similar to the study of Payne et al, benefits of improved

recovery obtained using BIS monitoring were not obtained at the expense of an increase in events associated with inadequate anesthesia.<sup>6</sup> However, BIS monitoring may improve aspects of anesthetic administration but data about improving recovery process by BIS-titrated anesthetic agents have been inconsistent. Some studies suggest that BIS-controlled anesthesia may help to assess the hypnotic component of anesthesia as well as reduce drug consumption and shorten recovery times when compared with a standard practice protocol.<sup>7-12</sup> Present results support earlier data suggesting that BIS-controlled anesthesia leads to reduced propofol infusion, faster emergence and improves recovery.<sup>13-16</sup> Others were not able to show differences in the patients' recovery profiles after BIS monitored general anesthesia.<sup>17,18</sup>

Awareness is a potentially serious complication of anaesthesia which results from inadequate anaesthesia. It is unpleasant both for the patient and the anaesthetist and can have short and long term consequences to the patient. Role of BIS monitoring on reducing the incidence of awareness is controversial. During inhalational anesthesia, its superiority over End-tidal anesthetic gas (ETAG) monitoring is not proven however during TIVA it seems to be well correlated with blood concentrations of propofol as there is no effective method for routinely measuring the blood concentration of propofol.<sup>19</sup> Cochrane database of systemic review have concluded that BIS guided anaesthesia could reduce the risk of intraoperative recall in surgical patients with high risk of awareness and also could improve anaesthetic delivery and postoperative recovery from relatively deep anaesthesia.<sup>4</sup>

## CONCLUSION

During TIVA, BIS monitoring is a useful parameter of EEG which helps in titration of propofol for surgical plane of anesthesia. It reduces requirement of propofol and results in faster and improved recovery as compared to standard practice group.

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# Co-infusion of Insulin-secreting Adipose Tissue Derived Mesenchymal Stem Cells and Hematopoietic Stem Cells in type-1 diabetes: A Single Center Experience

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## ABSTRACT

**Introduction:** We report our experience of co-infusion of adipose-derived insulin-secreting mesenchymal stem cells (IS-AD-MS-C) + bone-marrow derived hematopoietic stem cells (BM-HSC) in type-1 diabetes mellitus (T1DM) from allogenic and autologous sources.

**Material and Methods :** This was a prospective open-labeled 2-armed trial with 10 T1DM patients in each arm. Group-1 received autologous stem cells (SC) and group-2 received allogenic SC. Group-1 had 9 males, 1 female with mean age, 20.2 years, disease duration (DD) 8.1 years, group-2 had 6 males and 4 females with mean age, 19.7 years, and DD 7.9 years. Glycosylated hemoglobin (HbA1c) was 10.99%; Serum(S.) C-peptide, 0.22 ng/ml and insulin requirement, 63.9 IU/day in group-1 and HbA1c was 11.93%, S.C-peptide 0.028 ng/ml and insulin requirement 57.55 IU/day in group-2. SC were infused into portal, thymic circulation and subcutaneous tissue under non-myeloablative conditioning. Patients were monitored for blood-sugar, S.C-peptide, GAD antibodies and HbA1c at 3-monthly interval.

**Results :** Group-1 received mean SC 103.14 ml with  $2.65 \times 10^2$  ISC/ $\mu$ L, CD34+ 0.81% and CD45-/90+/73+, 81.55%. Group-2 received mean SC volume 95.33 ml with  $2.07 \times 10^2$  ISC/ $\mu$ L, CD34+, 0.32% and CD45-/90+/73+, 61.6%. No untoward effect was observed. Sustained improvement in HbA1c and S.C-peptide was noted over mean follow-up, 33.10 months in group-1 and 54.24 months in group-2 with decrease in GAD-antibodies. Mean insulin requirement decreased from 63.9 to 38.6 IU/day in group-1 and 57.56 to 40.5 IU/day in group-2.

**Conclusion :** Co-infusion IS-AD-MS-C and BM-HSC is safe and viable option for T1DM. Autologous source offers better long term control of hyperglycemia as compared to allogenic source.

**KEY WORDS :** Type-1 diabetes mellitus, insulin requirement, C-peptide, glycosylated hemoglobin, autologous stem cell therapy, allogenic stem cell therapy, mesenchymal stem cells, hematopoietic stem cells

## INTRODUCTION

Type-1 Diabetes Mellitus (T1DM) is the second most common chronic disease of childhood believed to be autoimmune in nature, characterized by irreversible destruction of insulin-secreting pancreatic  $\beta$ -islet-cells, requiring life-long exogenous insulin therapy. Symptoms appear when insulin making cell mass gets

reduced by approximately 90% leading to severe insulin deficiency and hyperglycemia.<sup>1</sup> The incidence of diabetes mellitus (type 2 DM) has been increasing in an epidemic-like fashion in the last two decades globally. India is expected to become the world capital of DM by year 2030.<sup>2,3,4</sup> Sporadic cases of hematopoietic stem-cell transplantation (HSCT) have

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been reported with limited success.<sup>5</sup> Stem cell therapy (SCT) offers great promise for cure of many diseases including T1DM. Insulin secreting cells (ISC) generated from SC represent an attractive alternative.<sup>6</sup> Mesenchymal stem cells (MSC) have remarkable paracrine effects which can be divided into trophic ("nurturing"), immunomodulatory, anti-scarring and chemo attractant.<sup>7</sup> We have successfully generated Insulin secreting adipose tissue derived MSC (IS-AD-MSC) *in vitro*.<sup>8</sup> We present our experience of insulin replacement therapy (IRT) in T1DM patients by co-infusion of *in vitro* generated IS-AD-MSC and bone marrow (BM) derived HSC. In this study we compared the results of autologous SCT with allogeneic SCT. We assessed the safety & efficacy of SCT.

## MATERIAL AND METHODOLOGY

This study was approved by Institutional Review Board. Inclusion criteria were T1DM of >12 months duration with presence of glutamic acid decarboxylase (GAD) antibodies and age group of 8 to 45 years with low serum(S.) C-peptide level. Exclusion criteria were positive serology for hepatitis C/ B/ HIV infection, other systemic infections/ disorders, malignancy and pregnancy. Diabetic ketoacidosis (DKA) was not a contra-indication to SCT.

Key end-points of study were morbidity, mortality, untoward side effects from SCT, and changes in exogenous insulin requirements. Secondary end-points were GAD antibodies, S.C-peptide levels with mixed-meal tolerance test and glycosylated haemoglobin (HbA1c). Monitoring was done at 3 monthly intervals

Group-1 included autologous SCT in which patients' own abdominal fat and BM were used. Group-2 with allogeneic SCT included healthy non-diabetic volunteer donors from family of recipients with compatible blood group, who were willing to donate fat and BM.

### Patient Data

This was a prospective open-labeled 2- armed clinical trial. Group-1 had 9 males and 1 female with mean age of  $20.2 \pm 6.9$  years, mean disease duration  $8.1 \pm 3.4$  years, mean fasting blood sugar (FBS)  $269 \pm 93.04$

mg/dl, mean postprandial BS (PPBS)  $372 \pm 68.3$  mg/dl, mean HbA1c  $10.99 \pm 2.1\%$ , mean S.C-peptide  $0.22 \pm 0.2$  ng/ml and mean insulin requirement  $63.9 \pm 20.95$  IU/day.

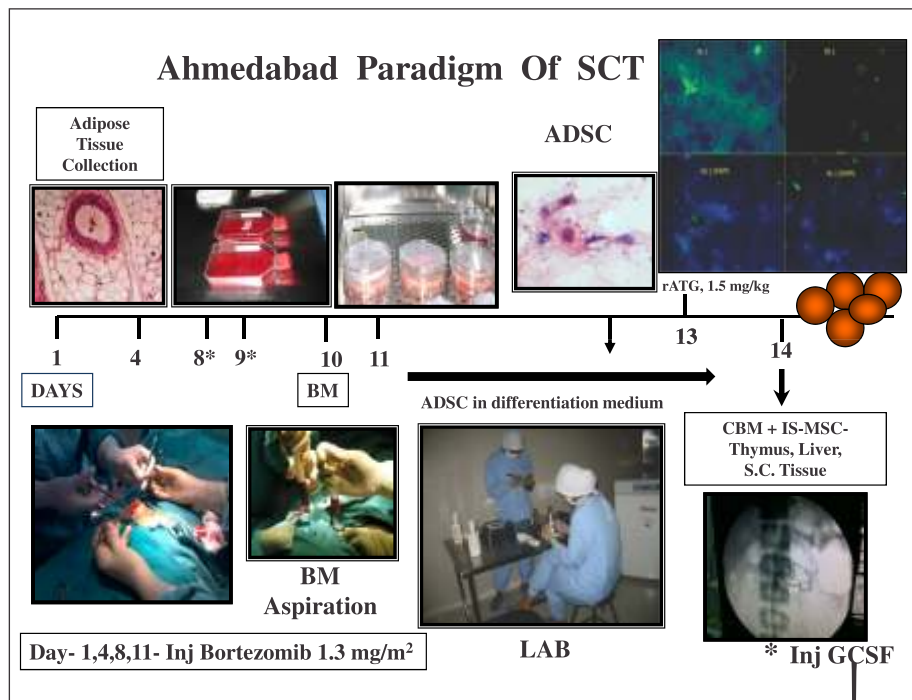
Group-2 had 6 males and 4 females with mean age of  $19.7 \pm 9.96$  years, mean disease duration  $9.9 \pm 7.1$  years, mean FBS  $309.5 \pm 67$  mg/dl, mean PPBS  $334.7 \pm 72.1$  mg/dl, mean HbA1c  $11.93 \pm 1.9\%$ , mean S.C-peptide  $0.028 \pm 0.01$  ng/ml and mean insulin requirement  $57.55 \pm 21.82$  IU/day.

### Study Design (Figure 1)

IS-AD-MSCs were generated as per our previously described technique<sup>1</sup> from 10-gram adipose tissue. On day 9, 100 ml BM was aspirated from posterior superior iliac crest under local anesthesia and sedation after stimulation with granulocyte colony stimulating factor, 300 µg subcutaneously 12 hourly for 2 days, and subjected to culture for generation of HSC. MSC were harvested on day 10, further differentiated into ISC on day 14, quantified and tested for sterility, viability and insulin-secreting markers PAX-6, IPF-1 and ISL-1 by immunofluorescence. C-peptide and insulin secretion were tested by chemiluminescence assay (Lumax, Lake Forest, CA, USA). The prepared inoculum was then mixed with HSC. Co-infusion of IS-AD-MSC and BM-HSC was carried out on day-14, into superior mesenteric artery to portal route and brachiocephalic artery to thymic circulation via femoral artery catheterization under local anesthesia, and into abdominal subcutaneous tissue. Conditioning was done with Bortezomib, 1.3 mg/m<sup>2</sup> body surface area along with methylprednisone, 125 mg, on days 1, 4, 8 and 11 followed by rabbit anti-thymocyte globulin, 1.5 mg/kg BW on day 13 in both the groups.

### Stem Cell Data

Generated ISCs expressed transcription factors ISL-1, PAX-6 and IPF-1 (Figure 1). In group-1, mean SC quantum infused was  $103.14 \pm 28.29$  ml with ISC  $2.65 \pm 0.8 \times 10^2$  /µL, CD34+  $0.81 \pm 0.61\%$ ; CD34+ 0.81% and CD45-/90+/73+,  $81.55 \pm 24.88\%$ . In group-2,



**Figure 1: Paradigm of stem cell therapy (SCT) and showing generated ISCs expressed transcription factors ISL-1, PAX-6 and IPF-1**

mean SC quantum infused was  $95.33 \pm 14.23$  ml with mean ISC  $2.07 \pm 0.67 \times 10^2/\mu\text{L}$ , CD34+  $0.32 \pm 0.33\%$ ; CD45-/90+/73+,  $61.6 \pm 24.99\%$ .

**Patient Monitoring**

Patients were monitored 4 hourly for blood sugar levels for first 2 days after infusion. FBS and PPBS levels after lunch and dinner were monitored for the next 5 days and patients were discharged at the end of 1 week. Subsequently patients were advised to monitor FBS and PPBS weekly for the first month, fortnightly for the next 2 months and monthly thereafter till the end of 1 year. Subsequently they were advised to check for FBS and PPBS every 3 months or as and when needed.

S.C-peptide and Hb1Ac were measured by chemiluminescence assay before infusion and 3 monthly after infusion. [Hb1Ac reference range: normal: 4.2-6.2%, good control: 5.5-6.8%, fair control: 6.8-7.6%, poor control: >8%) (Erba diagnostics, Germany)]. GAD antibodies were measured before infusion and 3 monthly post-infusion by ELISA technique (normal range: <10 IU/ ml) (Euroimmun -

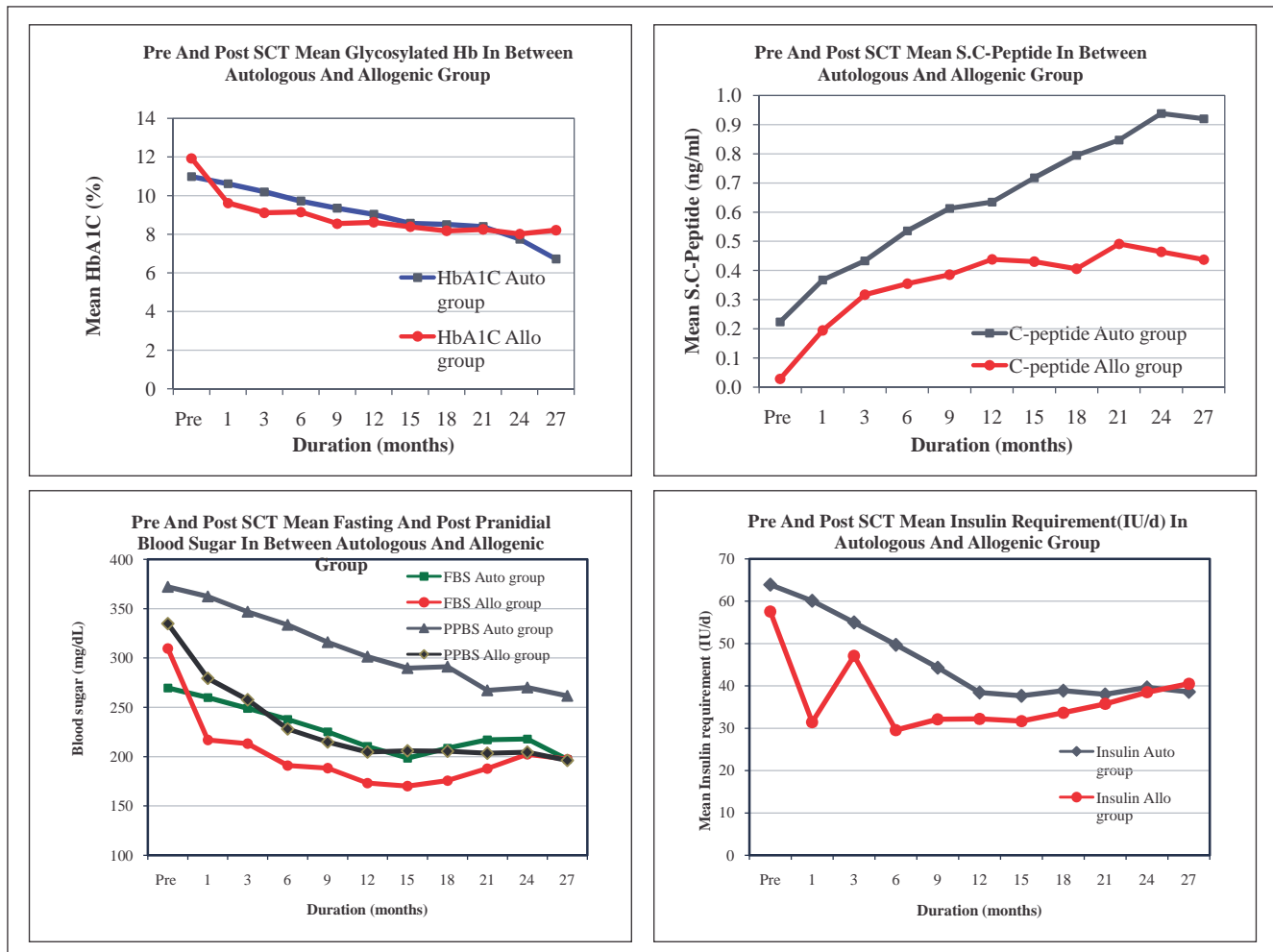
Medizinische Labordiagnostika AG, UK). Body weight and DKA episodes were also regularly monitored. Insulin administration was made on sliding scale with an objective of maintaining FBS 150 mg/dl (reference range: 70-110 mg/dl) and PPBS 200 mg/dl (reference range: 80-140 mg/dl).

**Statistical Analysis (Table 1)**

Statistical analysis was performed using SPSS version 12. Data are expressed as mean  $\pm$  SD (min-max) for continuous variables. Continuous variables were compared using Wilcoxon signed rank test.  $p < 0.05$  was considered to be statistically significant. Insulin requirement, Hb1Ac, serum C-peptide levels, FBS and PPBS were monitored.

**RESULTS**

No untoward effect, morbidity (pulmonary embolism, sepsis) or mortality due to SCT or conditioning was recorded in any patient. Variable and sustained improvement in mean FBS, PPBS, HbA1c and serum C-peptide was noted over a mean follow-up of  $33.10 \pm$



**Figure 2: Comparison of stem cell Therapy (SCT) response to glycosylated hemoglobin, serum C-peptide, daily insulin requirement and fasting - post prandial blood sugar status in autologous and allogenic stem cells infused patients.**

18.38 months in group-1 and  $54.24 \pm 15.74$  months in group-2. Mean GAD antibody decreased from  $327.8 \pm 652.0$  to  $107.2 \pm 228.0$  IU/ml in group-1 and  $722.5 \pm 686.6$  to  $133.1 \pm 216.9$  IU/ml in group-2. Mean insulin requirement decreased from  $63.9 \pm 20.9$  to  $38.6 \pm 8.5$  IU/day in group-1 and  $57.56 \pm 21.82$  to  $40.5 \pm 15.99$  IU/day in group-2. No functional correlation was observed between exogenous insulin requirement with C-peptide levels and GAD antibody levels. Group-1 showed better response to achieve insulinopenic stage than in group-2 (Figure 2). There was an impressive absence of DKA episodes in all of them with improved subjective energy levels.

## DISCUSSION

Potential therapy for T1DM needs to address insulin-replacement and immune dys-regulation arising in these patients. Islet cell transplantation is a well-known therapeutic option yet not feasible due to the shortage of available organs.<sup>9,10</sup> Optional cell therapy includes HSC and MSC, especially since MSC have the plasticity to adopt to pancreatic endocrine phenotype and migrate to the sites of tissue injury. They are also potent immunomodulators.<sup>11,12,13,14,15</sup>

In animal models of T1DM, MSC have shown beneficial effects in glycemic control, either isolated or combined with HSC.<sup>16,17</sup>

**Table 1: Follow - up for insulin requirement, Hb1Ac, serum C-peptide levels, FBS and PPBS over 27-months 10 patients from autologous and 10 patients from allogenic stem cell therapy.**

	Group, n=10	Pre SCT	1 month	3 month	6 month	9 month	12 month	15 month	18 month	21 month	24 month	27 month
<b>Insulin Requirement</b>	1	63.9±20.9	60.1±20.36	55±19.8	49.7±19.41	44.3±15.47	38.44±14.56	37.66±12.15	38.87±10.49	38±11.43	39.66±9.37	38.6±8.59
	2	57.5±21.8	31.44±9.09	31.0±10.22	29.55±11.52	32.11±9.64	32.22±10.88	31.66±9.13	33.66±7.24	35.75±8.71	38.5±13.34	40.5±15.99
	P-value	0.526	0.001	0.004	0.014	0.058	0.319	0.253	0.247	0.672	0.858	0.813
<b>HbA1c</b>	1	10.9±2.15	10.6±1.99	10.2±2.01	9.72±1.66	9.35±1.63	9.03±1.65	8.56±1.65	8.51±1.4	8.4±0.87	7.75±1.05	6.72±0.71
	2	11.93±1.9	9.11±1.76	9.62±1.54	9.15±1.61	8.55±1.56	8.62±1.38	8.38±1.48	8.17±1.1	8.25±1.01	8.01±1.04	8.21±1.4
	P-value	0.328	0.23	0.244	0.464	0.294	0.575	0.813	0.59	0.765	0.651	0.028
<b>C- peptide</b>	1	0.22±0.21	0.36±0.29	0.43±0.32	0.53±0.32	0.61±0.29	0.63±0.23	0.71±0.22	0.79±0.24	0.84±0.22	0.93±0.24	0.92±0.26
	2	0.02±0.01	0.19±0.27	0.31±0.37	0.35±0.4	0.38±0.39	0.43±0.36	0.43±0.30	0.4±0.31	0.49±0.29	0.46±0.29	0.43±0.29
	P-value	0.02	0.2	0.483	0.292	0.172	0.196	0.039	0.013	0.022	0.008	0.012
<b>Fasting Blood Sugar</b>	1	269.6±93.04	260±83.9	248.9±81.8	237.8±80.56	225.2±81.31	210.22±80.65	198.4±69.4	208.6±68.21	217.14±68.72	218.0±72.45	197.2±64.18
	2	309.5±67.01	216.8±59.26	213.11±56.8	191.11±44.36	188.3±49.2	173.33±48.53	170.11±43.6	175.77±47.17	188±41.53	202.5±51.34	197.5±40.5
	P-value	0.285	0.218	0.289	0.142	0.255	0.256	0.315	0.261	0.331	0.646	0.991
<b>Post-prandial Blood Sugar</b>	1	372±68.3	362.2±64.66	346.7±65.64	333.6±69.51	316.0±59.88	301.33±60.0	289.77±62.08	291.12±44.8	267.14±61.02	270.0±53.38	261.6±54.8
	2	334.77±72.13	279.44±74.09	257.55±72.46	228.11±50.8	214.88±39.67	204.77±36.98	206.00±35.32	205.4±26.38	203.5±28.03	204.37±27.5	196.25±32.8
	P-value	0.264	0.018	0.011	0.001	0.0005	0.001	0.003	0.0002	0.034	0.01	0.02

SCT, stem cell therapy; group-1, patients from autologous group; group-2, patients from allogenic group; HbA1c (glycosylated hemoglobin); FBS (fasting blood sugar); PPBS (post-prandial blood sugar); n=10, Ten patients in each group-1, 2

We have generated MSC in vitro from human adipose tissue which qualifies the definition standardized by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy.<sup>1</sup> We further differentiated them to ISCs under defined culture conditions phenotypically identical to pancreatic  $\beta$ -cells. These cells expressed transcription factors IPF-1, PAX-6, and ISL-1. All three are central controlling genes capable of reprogramming non-pancreatic cells to surrogate  $\beta$ -cell functions. Again our technique is a shortcut to reprogramming non-pancreatic cells as compared to vector-based gene transfer techniques.<sup>1</sup> Thymic infusion was carried out in our patients to achieve central tolerance and portal circulation was done to take the advantage of tolerogenicity of liver.<sup>18,19</sup> Subcutaneous tissue being an immunologically privileged site, we decided to inject part of the cells in abdominal subcutaneous tissue, so that it will serve as a “back-up reservoir” for insulin supply.<sup>20</sup>

In our previous experience of using allogenic SCT we did have sustained partial response of decreased insulin requirement along with sustained elevated levels of S.C peptide.<sup>21</sup> However we could not establish complete insulin-free status in our patients. One of the beliefs was that since the source of SC was allogenic, there was a possibility of these cells being rejected, since the major component of SC was HSC. We have never used any immunosuppression post-SCT. Hence we decided to carry out the present study to compare the effect of allogenic vs. autologous SCT.

In the present study, we have established that even if a patient has T1DM, IS-AD-MSC can be generated using the adipose tissue reservoir of SC. Secondly these cells have sustained effect of decreased exogenous insulin status in addition to sustained level of C-peptide. However we have still not been able to establish insulin-free status in this group of patients also.

## **CONCLUSION**

This is the first report of successfully treating T1DM with co-infusion of autologous vs. allogenic IS-AD-MSC and BM-HSC with relatively simple and easy

technique, offering a safe and viable approach. Autologous SC infusion shows better response in patients than allogenic SC infusion.

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## Original article

# Outcome of Living VS Deceased Donor Pediatric Renal Transplantation: A Single Center Experience

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## ABSTRACT

**Background:** Constraints in operating an effective maintenance dialysis program leaves renal transplantation (RT) as a more viable and better option for end-stage renal disease (ESRD) patients in our country. Data scarcity on long term outcome of living donor (LD) versus deceased donor (DD) pediatric RT in developing countries prompted us to review our experience.

**Patients and Methods:** This study was undertaken to evaluate patient and graft survival, function vis-a-vis serum creatinine (SCr), rejection episodes and mortality in 151 LD and 37 DD pediatric renal transplants (age < 18 years) performed in our center between 1998 and 2011. Commonest recipient disease leading to ESRD were congenital anomalies of kidney and urinary tract (n=60) and unexplained etiology (n=25). Demographics of both groups were comparable. Kaplan-Meier curves were used for survival analysis.

**Results:** Over a mean follow-up of  $4.2 \pm 3.61$  years, one-, five- and ten- year death-censored graft survival in LDRT was 87.4%, 72.1%, 72.1% and patient survival was 92.5%, 80.9%, 75.1% respectively; 19.8% (n=30) patients had biopsy proven acute rejection (BPAR) and 17.8% (n=27) patients died, mainly due to infections (n=12). In DDRT, over mean follow-up of  $3.93 \pm 3.5$  years, one-, five- and ten-year death-censored graft survival was 90.4%, 86.4%, 73.3% and patient survival was 83.4%, 67.9%, 67.9%, respectively; 21.6% (n=8) patients had BPAR and 27% (n=10) patients died, mainly owing to infections (n=6).

**Conclusion:** RT is viable option for children and achieves acceptable graft function with patient/graft survival over long-term follow-up, encouraging use of this approach.

**KEYWORDS :** Deceased donor, living donor, outcome, renal transplantation, pediatric

## INTRODUCTION

Renal transplantation (RT) is the best treatment for children with end-stage renal disease (ESRD).<sup>1-5</sup> Economic constraints in operating an effective maintenance dialysis program leaves RT as the preferred option for ESRD patients in our country.<sup>6-9</sup> There are many challenges in developing countries for RT in children due to resource limitations, low access to deceased donors(DD), non-adherence, and morbidity

and mortality due to infections.<sup>6-16</sup> Our pre-transplant patients are “more sick” than those in developed countries presumably related to resource limitations. Data scarcity on long term outcome of living donor (LD) versus deceased donor (DD) pediatric RT in developing countries prompted us to review our experience. Most RT in India take place from LD. This differs somewhat from transplant programs in developed countries where DDRT is more popular for

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children and numbers of LDRT are dropping. The present paper highlights important challenges in developing countries for successful transplantation and yet positive outcome for patients despite these restrictions. The rate of pediatric RT has been steadily rising over the past decade. However, because of lack of national or regional registries, information on the outcome of pediatric transplantation is limited to single-center reports.<sup>8, 17-22</sup> Here we report our long-term single center experience with LD vs DD pediatric RT.

## MATERIAL AND METHODS

This was a retrospective single center study of 151 LD and 37 DD pediatric RT (age 1-18 years) performed at our institute between June 1998 and December 2011. Demographics and post-transplant follow-up including investigations, immunosuppression requirement, rejection episodes, and survival were evaluated from clinic records.

**Pre-transplant evaluation:** The lower urinary tracts were evaluated using a voiding cystourethrogram to detect any abnormality that needed to be corrected prior to transplantation in patients with history of congenital abnormalities of the urinary tract or infection (CAKUT)<sup>23</sup>. Patients with high grade vesicoureteric reflux or recurrent urinary infection underwent nephroureterectomy to avoid the development of urosepsis.<sup>24</sup> Children with lower tract uropathies (eg. posterior urethral valves, neurogenic bladder) and abnormalities of bladder function were assessed carefully with urodynamic studies. The urinary tract, skin, teeth and sinuses were carefully examined for signs of infection or site of chronic infection. We encouraged offering LDRT to suitable transplant candidates if donors were readily available. If there was no available LD, patients were enrolled in DDRT waitlist. Year-wise numbers of LDRT is shown below; 1998=3, 1999=11, 2000=8, 2001=3, 2002=6, 2003=10, 2004=9, 2005=7, 2006=13, 2007=21, 2008=17, 2009=18, 2010=10, 2011=15. In DD group, kidneys were allocated according to waiting time. Since 2006, much effort has gone towards giving

children the priority for DD transplants. Year-wise number of DDRT is shown below; 1998=2, 1999=1, 2000=2, 2001 to 2003=0, 2004=2, 2005=3, 2006=10, 2007=6, 2008=2, 2009=2, 2010=3, 2011=4.

Contraindications for RT included sepsis, uncontrolled extra-renal malignancies, irreversible multi-organ failure, severe cardiac and pulmonary dysfunction not corrected by organ transplant, underlying life-threatening disorder not corrected by RT and a recent history of non-adherence to medical care. Factors related to the healthcare system, socio-economic and treatment-related factors to improve adherence to the immunosuppressive regimen were discussed in our previous report and other studies<sup>8, 25-30</sup>

Human leukocyte antigen (HLA) typing and lymphocyte crossmatch (LCM) were done by conventional serological technique (one lambda predot trays were used for HLA A, B, and DR typing). We routinely perform complement-dependent cytotoxicity crossmatches by serological method using auto dithiothreitol and standard cytotoxicity methods with T and B lymphocytes each, and flow cytometry crossmatch (FCM) was added since 2007. All patients were anti-human globulin-enhanced lymphocytotoxicity crossmatch assay (AHG-CDC) negative with a negative or acceptable FCM pre-RT. Chest radiography and clinical examination was performed in all to avoid donor-derived tuberculosis. Sputum examination, QuantiFERON-TB Gold assay, computed tomography scan, erythrocyte sedimentation rate and PPD test were done in patients with clinical suspicion of TB [unexplained fever, recurrent pleural effusion]. Thrombophilic screening studies for anti-phospholipid antibodies (anti-cardiolipin antibodies and lupus anticoagulants) were performed in all patients routinely in the pre-transplant preparation, and other tests such as protein C, protein S, antithrombin III deficiency; factor V Leiden mutation, prothrombin mutation gene were performed if there was any historical suggestion of a thrombotic tendency like an event of vascular access thrombosis.

**Immunosuppressive regimen**

All patients received induction with methylprednisolone (MP) (10 mg/kg intravenously (maximum dose of 500 mg); MP was continued for 3 days postoperatively and rabbit-anti-thymocyte globulin (r-ATG) (1.5 mg/kg, single dose) was added in high immunologic risk patients (n=20) and all DD. Maintenance immunosuppression consisted of prednisolone (2 mg/kg per day (60 mg/m<sup>2</sup> per day), with a gradual reduction to approximately 0.12 to 0.16 mg/kg per day within a 6 month period and continued thereafter), a calcineurin inhibitor (CNI) (cyclosporine (CsA) 3 to 5 mg/kg /day or tacrolimus(Tac), 0.05-0.8 mg/kg /day) administered in two divided doses) ± mycophenolate mofetil (MMF) or azathioprine (adjusted for signs of myelosuppression). CNI was replaced by sirolimus in event of toxicity (n=10). Doses of CNI were adjusted as per trough levels (C<sub>0</sub>) by Fluorescence Polarization Immunoassay (FPIA) technology for first 3 months and thereafter drug levels were assayed only in event of graft dysfunction due to financial constraints. Cyclosporine dosing was adjusted to achieve target C<sub>0</sub> concentrations of 150 and 300 µg/l for the first three to six months post-transplant. Long-term trough levels were targeted at 75 to 125 µg/l. Tacrolimus target trough whole blood levels were 5 to 10 ng/ml during the first 1-3 months post-transplantation and 4 to 8 ng/ml thereafter. Alternate-day steroid dosing were administered 6 to 12 months post-transplant to minimize the effect of corticosteroids on growth in event of steroid toxicity. None of the patients received steroid free regimens<sup>31-33</sup>. In this cohort tacrolimus was used starting from 2004<sup>34-35</sup>. All patients received prophylaxis against *Pneumocystis carinii* pneumonia, cytomegalovirus (CMV) and fungal infections.

**Post-transplantation follow-up**

All patients were followed at weekly intervals for the first 3 months, every 2 weeks for the next 3 months, monthly for the next 6 months, and at 1-3 monthly intervals thereafter. On every visit, complete blood counts, renal function tests and urine examination were

done and ultrasound Doppler studies and CNI levels were performed as per requirement. A team of transplant physicians, surgeons and anesthetics were involved in the peri-operative management and routine post transplant follow up including dose adjustment of immunosuppressant was mainly provided by transplant physicians.

**Diagnosis and treatment of rejection**

Graft biopsy was performed in cases of acute graft dysfunction, diagnosed by the modified Banff criteria, and treated accordingly<sup>32,33</sup>. Rejection was treated with standard anti-rejection therapy, as follows: T-cell rejections were treated with MP, 5 to 10 mg/kg for three to five days ± r-ATG 1.5 mg/kg, single dose, whereas antibody/ B-cell mediated rejections were treated with pulse MP ± plasmapheresis + intravenous immunoglobulins ± rituximab 375 mg/m<sup>2</sup> body surface area, single dose. In addition the maintenance immunosuppression therapy was optimized. Protocol biopsies were not performed. Graft loss was defined as requirement of maintenance dialysis. They were maintained on dialysis in the setting of allograft failure.

Statistical analysis- All statistical analysis was performed using Statistical Package for the Social Sciences (version 12.0; SPSS Inc., Chicago, IL). Continuous variables were summarized as mean and standard deviations (mean ± SD). Percentages were used to summarize categorical variables. Continuous variables were compared using Student T test. Chi square test of Fisher exact test were used to assess the effect of change in differences in categorical variables. Survivals were examined using Kaplan–Meier analysis and compared using the log-rank test. P values < 0.05 were considered significant.

**RESULTS****Recipient and donor characteristics**

Demographic data of LD and DD transplantation is shown in table 1. The relation to recipient was mother (n=100), father (n=27), brother (n=2), sister (n=2), grand-parent (n=5), extended family members (n=15)

**Table 1: Comparison of demographic data in live and deceased donor RTx in our center**

Parameter	LD (n=151)	DD(n=37)
Donor gender (male)	27.2%(n=41)	43.2%(n=16)
Recipient gender (male)	85.4%(n=129)	67.5%(n=25)
Donor age (yr) (range)	42.1 ± 8.31 (13 - 67)	38.8 ± 18.6 (4 - 72)
Recipient age (yr) (range)	14.7 ± 2.87 (6 - 18)	13.8 ± 3.1 (7 - 18)
6-12 yr	23.2 % (n=35)	35.1% (n=13)
12-18 yr	76.8 % (n=116)	64.9% (n=24)
Original disease - ESRD		
CAKUT	39.7%(n=60)	48.6%(n=18)
Chronic glomerulonephritis	16.5%(n=25)	18.9%(n=7)
Hereditary renal diseases	5.9% (n=9)	8.1%(n=3)
FSGS	2.6% (n=4)	10.8%(n=4)
Chronic interstitial nephritis	6.6%(n=10)	-
Chronic pyelonephritis	6.6%(n=10)	-
Crecentic glomerulonephritis	6.6%(n=10)	-
IgA nephropathy	5.9%(n=9)	-
Hypertension	3.9%(n=6)	-
Others	5.2%(n=8)	13.5%(n=5)
Mean dialysis duration		
pre-transplantation	9 months	15.5 months
HLA match ,(median ,range)	2.75±1.03, (3, 0 to 5)	-
ATG induction (1.5 mg/kg)	13.2%(n=20)	100%(n=37)
Pre-transplantation HCV ELIZA		
positive ,negative viral load	11	2
Preemptive transplantation	3.3%(n=5)	2.7%(n=1)

and DD (n=37). All the recipients/donors were ethnically homogeneous population (Asian). Monthly family income was < Rs 5,000 (50.3%), Rs 5,001-20,000(39.7%) and > Rs 20,000 (9.9%). Approximately 70 % were on hemodialysis and remaining on peritoneal dialysis. Pre-transplantation mean hemoglobin was  $8.9 \pm 1$  gm/dl, serum ionized calcium was  $0.8 \pm 0.09$  mg/dL (reference range 1.12–1.32 mg/dL) and serum phosphorus was  $5.5 \pm 1.9$  mg/dL (reference range 2.5-4.5 mg/dL); 69.5 % patients were anemic at the time of transplantation. Post-transplantation at one year, mean hemoglobin was  $12.1 \pm 2.1$  gm/dl, serum ionized calcium was  $1.05 \pm 0.09$  mg/dL and serum phosphorus was  $4.3 \pm 1.2$  mg/dL.

In LD group, mean distance from the transplant center was  $350 \pm 324$  kilometer (range 10-1500 kms) excluding 3 foreign national recipients. Regarding

residential status of patients, 69.5% (n=105) were from Gujarat, 13.2% (n=20) from Rajasthan, 7.9 % (n=12) from Madhya Pradesh, 7.2% (n=11) from other states of India and 3 patients were foreign nationals. In DD group, mean distance from the transplant center was  $268 \pm 378$  km (range 20-1500 kms). Regarding residential status of patients, 81.1% (n=30) were from Gujarat and 18.9% (n=7) from other states of India.

#### **Post-transplant outcome data**

Kaplan-Meier analysis for survival is shown in figure 1. Kaplan-Meier analysis for patient and graft survival in LD and DD transplantation is shown in figure 2 and 3, respectively. Comparison of outcome between LD and DD transplantation is shown in table 2. As shown in table 2, there was no significant difference in patient survival, graft survival and acute rejection in LD and DD transplantation. The number of patients who were at risk at each year is shown in table 3.

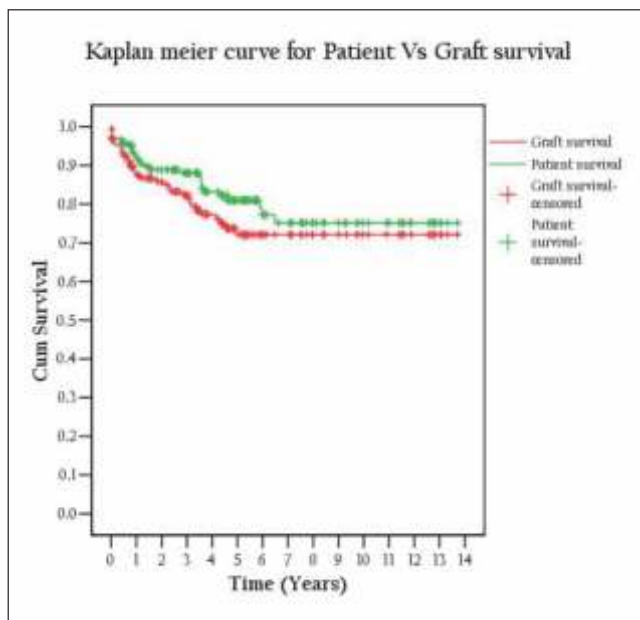


Figure 1: Kaplan-Meier analysis for patient and graft survival in LD transplantation

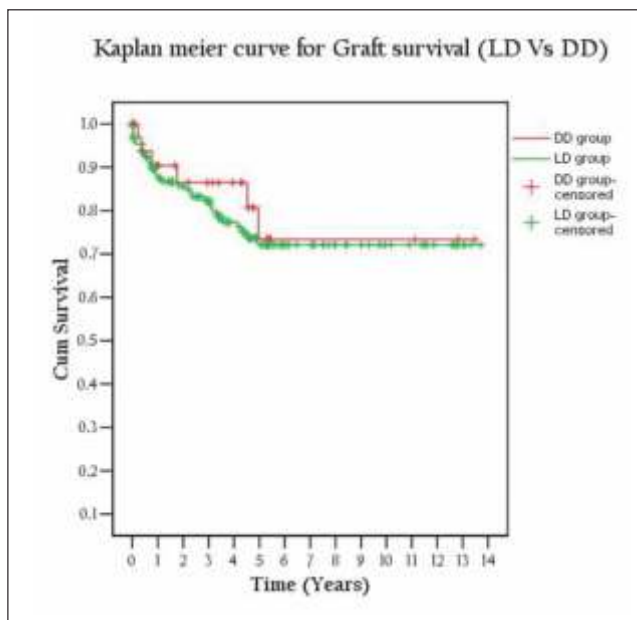


Figure 3: Kaplan-Meier analysis for graft survival in LD and DD transplantation

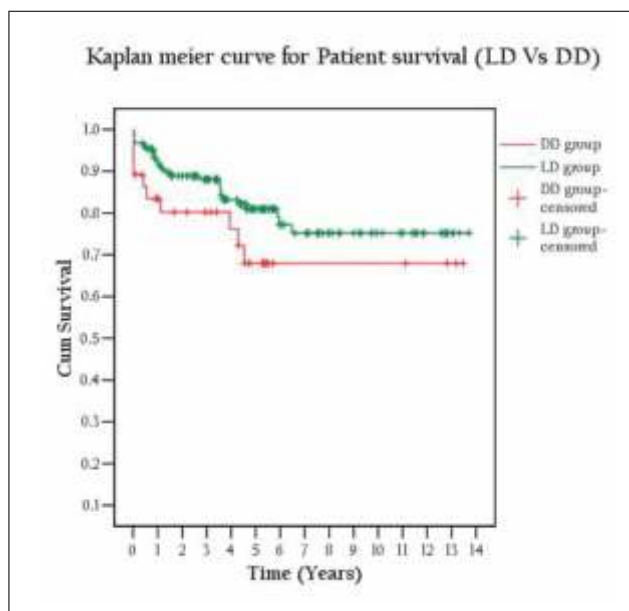


Figure 2: Kaplan-Meier analysis for patient survival in LD and DD transplantation

**Table 2: Comparison of outcome in live and deceased donor RTx at our center**

Parameter	LD(n=151)	DD(n=37)	P value
Patient survival (%)			0.11
1 yr	92.5	83.4	
5 yr	80.9	67.9	
10 yr	75.1	67.9	
Mean 95% CI , yr	10.1-11.9	7.8 - 11.6	
Graft survival (%)			0.61
1 yr	87.4	90.4	
5 yr	72.1	86.4	
10 yr	72.1	73.3	
Mean 95% CI , yr	9.5 - 11.4	8.6 - 12.7	
BPAR (%)	19.8(n=30)	21.6%(n=8)	0.812
Acute B cell	6.6%(n=10)	5.4% (n = 2),	
Acute T cell	4.63%(n=7)	10.8% (n = 4),	
Acute T+B Cell	8.6%(n=13)	5.4%(n = 2)	
Chronic rejection/IFTA	10.6%(n=16)	10.8% (n=4)	
Cortical necrosis with vascular graft thrombosis (n)	7	1	
CNI toxicity(n)	10	1	
BK virus nephropathy(n)	1	1	
SCr. (range) mg/dl (last follow-up)	1.2(0.6-3.5)	1.1 (0.5-2.3)	
eGFR (Bedside Schwartz Formula) mL/min/1.73 m <sup>2</sup>	56.8	55.07	
DGF	1.9%(n=3)	24.3% (n=9)	
Follow-up (years)	4.2 ± 3.61	3.93 ± 3.5	
75th Percentile of patients follow-up	4.32 yr	4.29 yr	

**Table 3: Number of patients who are at risk at each year**

At the end of	LD(n=151)	DD(n=37)
1 Year	129	27
2 Year	113	24
3 Year	101	22
4 Year	79	19
5 Year	59	13
6 Year	40	4
7 Year	36	4
8 Year	27	4
9 Year	23	4
10 Year	18	4
11 Year	15	4
12 Year	10	2
13 Year	4	2

**Post-transplant safety data in LD**

A total of 17.8% (n=27) of patients died, mainly due to post-transplant infections (n=12) [bacterial (n=5), tuberculosis (n=2), fungal (n=1), hepatitis C virus (n=3) and CMV (n=1)], post-transplantation graft failure (n=9), cerebrovascular accidents (n=3), cardiovascular diseases (CVD) (n=1) and other (n=2). Diagnosis of infection was done before dying, patients died in hospital; immunotherapy was reduced on admission /diagnosis of infection. Two patients who died from tuberculosis had reactivation of previous latent infection. Risk factors for post-transplant tuberculosis included allograft rejection occurring <6 months before the onset of TB, and rATG administration.

A total of 19.8% (n=30) patients had biopsy proven acute rejection (BPAR). Acute B-cell mediated rejections were noted in 6.6% (n=10), acute T-cell mediated rejections in 4.63% (n=7), and combined acute T- and B-cell mediated rejections in 8.6% (n=13). Majority of BPAR were observed in first year and patients responded to anti-rejection therapy. Totally 10.5% (n= 16) patients has chronic rejections which included chronic-B-cell mediated rejection in 3.31% (n=5), chronic T-cell mediated in 3.31% (n=5) and combined T+B-cell mediated in 3.97% (n=6). Acute tubular necrosis (ATN) (n=5), cyclosporine toxicity (n=6), tacrolimus toxicity (n=4), sirolimus toxicity (n=2), BK virus nephropathy (n=1), were other biopsy findings and kidney graft biopsy was unremarkable in 5 cases.

Etiologies of graft loss (n=33) in LD group were graft necrosis with thrombosis of graft vessel within 2 weeks after transplantation(n=4), graft necrosis with thrombosis of graft vessel and HCV positive at 9 months (n=1), severe acute T +B cell mediated rejection and ATN leading to graft necrosis with thrombosis of graft vessel at 2 months (n=1), acute hemolytic-uremic syndrome (HUS) leading to acute cortical necrosis and thrombosis of graft vessel at one month (n=1), CsA induced thrombotic microangiopathy with patchy cortical necrosis at 5 months (n=1) [thrombosis were associated with donor-specific antibody or prior sensitization (n=2) and thrombophilic state(n=2)], CsA toxicity (n=3), tacrolimus toxicity (n= 2), pyelonephritis (n=2), mucormycosis of graft with vascular invasion at 11 months (n=1), de novo anti-GBM at 5 months (n=1), recurrence of primary IgA nephropathy at 2 years, 2.2 years, 3 years (n=3), membranoproliferative glomerulonephritis (MPGN) recurrence at 3 years (n=1), de novo HUS at 1.7 years (n=1), and chronic rejection (n=11). There was no difference in risk for acute rejection (n =12 vs. n =15, p = 0.93), graft loss (n =17 vs. n=16, p=0.15), patient loss (n=15 vs. n=10, p=0.47) in the subset of 61 (40.5%) CsA-treated patients and 74 (49%) Tac treated patients in LD group.

### **Post-transplant safety data in DD group**

A total of 27% (n=10) of patients died, mainly due to infections (n=6) (bacterial (n= 3), tuberculosis (n=1), fungal (n=1) and CMV (n=1)), cardiovascular diseases (n=3) and other causes (n=1).The etiologies of graft loss (n=6) were vascular graft thrombosis within first 3 months (n=1), non-adherence to the immunosuppressive regimen (n=2) and rejection (n=3). There was no difference in risk for acute rejection (n = 5 vs. n = 3, p = 0.34), graft survival (n = 11 vs. n = 20, p = 0.15), patient survival (n = 10 vs. n = 17, p = 0.47), in the subset of 15 (40.5%) CsA-treated patients and 22 (59.5%) Tac treated patients in DD group.

Most children have improved statural and/or catch-up growth after successful renal transplantation. Non-adherence with immunosuppressive medications was noted in 10 patients (6.6%) in LD group and in two patients (5.4%) in DD group. Non-adherence was observed after 6 months and they had rejection episode leading to chronic rejection/graft loss. None of the patients had post-transplant malignancy in both groups and recurrence of primary disease in DD group.

### **DISCUSSION**

RT is best treatment for growth retardation in children with chronic kidney disease (CKD) and ESRD.<sup>36-37</sup> We have one of the largest centers in our country for RT. Our State Government provides free treatment for kidney disease under School health program to all children up to the age of 18 years. We have reported our long-term single-center experience with DDRT in pediatric recipients.<sup>8</sup> Table-4 shows outcome of pediatric RT from individual centers in India. Sinha et al<sup>18</sup> concluded that their results affirm that despite scarcity of resources and frequent infections, long-term outcome of pediatric RT is highly satisfactory. Gulati et al<sup>20</sup> reported that CsA discontinuation (n=12) due to financial constraints and/or non-compliance remain the most important reasons for suboptimal outcome in their study. The incidence of pediatric graft failure due to non-adherence is 10-15 %.<sup>25-30</sup> Several studies have shown a high incidence of non-adherence particularly

**Table 4: Outcomes of pediatric renal transplantation from individual centers in India**

	Our study		Sinha et al. <sup>18</sup>	Chacko et al. <sup>19</sup>	Gulati et al. <sup>20</sup>	Vasudevan et al. <sup>21</sup>	Phadke et al. <sup>22</sup>	
	Ahmadabad		Delhi	Vellore	Delhi	Bangalore	Bangalore	
Center	Ahmadabad		Delhi	Vellore	Delhi	Bangalore	Bangalore	
Total Recipients	151	37	45	90	39	33	47	
Duration, yr	1998 -2011		1995-2008		10	8	16	
Recipients age ,yr	14.7	13.8±3.1	13.3	15	15.6	12.5	10 (median)	
Live donor (%)	100	0	95.5	96	100	93.9	93.6	
Patient survival (%)								
1 yr	92.5	83.4	95.3	95	89	100	78.2	
5 yr	80.9	67.9	87.9	87	70(at 3years)	94	44.6	
10 yr	75.1	67.9	76.9	79	-	-	36.2	
Etiology of patient loss / morbidity	infection		infection	infection	infection	infection	infection	
Graft survival (%)								
1 yr	87.4	90.4	91.1	98	89	94	80	
5 yr	72.1	86.4	80.4	84	50	90	45.8	
10 yr	72.1	73.3	75.1	76	-	-	37.5	
AR (%)	19.8	21.6	31	46.7	45.8	18.2	23.4	
Follow-up (months)	51.2	3.9yr	15	42	31.5	42.39	30	

among adolescents and young adults.<sup>25-30</sup> In our study intervention to improve adherence to immunosuppressive regimen had positive impact (94.4%) leading to improved graft survival.<sup>30</sup> The limitations of our ability to detect post-transplant non-adherence was that it was determined by personal assessment of drug intake by transplant team and parents. It was very difficult to measure the non-adherence accurately and diagnosis was often selective (e.g. at the time of graft dysfunction/rejection/irregular follow-up).

Although initial results from steroid-avoidance protocols are encouraging, there is still a lack of long-term data from large, prospective randomized trials and there are not enough data to determine the optimal steroid-avoidance protocol for pediatric RT recipients.<sup>31-33</sup> We did not use steroid-avoidance protocols and we believe that major obstacles to either steroid avoidance or steroid withdrawal in our patients were economic constraints, inability to adequately determine optimal

immunosuppression prior to clinical manifestations; patients are not ready for routine use of protocol biopsies for detecting rejection prior to clinical consequences and high cost of such regimen.

The major causes of death after transplantation are CVD, infection and malignancy, variously reported as 30–36% for CVD, 24–56% for infection and 11–20% for malignancy.<sup>38-39</sup> Two other important factors that contribute to death are non-adherence to medications or treatment withdrawal and obesity.<sup>25-30</sup> The major causes of death were infections in our study, similar to experience by other studies in India.<sup>18-22</sup> Infections are a common cause of morbidity and mortality after transplantation in Asia and account for half the deaths in post-transplant patients in India.<sup>40</sup> The high infection rate despite judicious use of immunosuppressive agents, and infection prophylaxis is not specific to pediatric population but it is common in the transplantation setting in our country.<sup>40</sup> It is possible

that unhygienic living conditions, delayed presentation and diagnosis, tropical climate, presence of dormant endemic infections, in addition to economic constraints for treatment in majority of patients, may have contributed to high infection rate, similar to experience by other studies.<sup>18-22,41</sup>

Preemptive transplantation is the preferred modality in children, because it is associated with better long-term outcome in terms of growth, development, quality of life and mortality and stability in social life of patients and their families.<sup>42</sup> Despite these advantages, preemptive transplantation is still not achieved in majority of children with ESRD in most of the centres of the world because patients present in CKD stage-5 with insufficient preparatory time required for transplantation, small size, co-morbidity, non-adherence to medical therapy, family instability or other factors.<sup>6-15</sup> Growth hormone administration is an effective treatment to promote the growth velocity of children after RT. However patients with frequent rejection episodes cannot be considered for this option. We have not used GH treatment for growth retardation mainly due to economic constraints and fear of triggering rejection episodes.<sup>43-44</sup>

Majority of our LD were females (mothers) which is a cultural reality and not a program determinant. There are no ethical challenges to consider, given the scarcity of deceased donors regarding pressure to donate for their children. The reasons postulated include stronger child –mother relationships and a greater sense of obligation towards the child and family among women in the typical Indian culture. However, it should be emphasized that ethical concerns including coercion, privacy, confidentiality, and exploitation were carefully addressed before female donor selection. The attitude towards donation differs between mother and father and father tend to be ambivalent about the decision to donate.

To our knowledge this is the largest study of LDRT outcome in children from India with long-term follow-up and comparison with DDRT. The number of children undergoing RT has increased slowly over the past

decade. Our study information is of considerable importance to counsel the patients and families.

## CONCLUSIONS

RT is a viable option for children and achieves acceptable graft function with patient/graft survival over the long-term follow-up, encouraging use of this approach.

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## **Syndrome of De Novo Hemolytic Uremic Syndrome - Thrombotic Microangiopathy after Renal Transplantation: A Single Centre Experience**

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### **ABSTRACT**

**Background:** The syndrome of hemolytic uremic syndrome (HUS)-thrombotic microangiopathy (TMA) is a well-recognized serious complication of renal transplantation (RT), affecting patients on calcineurin inhibitors (CNI). We performed a retrospective study to evaluate HUS-TMA in our centre.

**Patients and Methods:** This study included 1538 RT biopsies performed between January,'08 and September,'13 in 1175 post-RT patients. Institute Transplant Registry records were reviewed for demographics and outcome. The offending drug was withheld in all and if required plasmapheresis was added as an adjuvant.

**Results:** De-novo TMA/HUS was observed in 34 (2.9%) patients with mean age of 32.9 years, and mean serum creatinine 3.4 mg/dl; 33 were drug-induced and 1 was due to CMV infection. TMA was observed in 25 and HUS in 9 patients. Syndrome developed within 3 months posttransplant in 23, within 1 year in 8 and after 1 year post-transplantation in 3 patients. Presenting features were graft dysfunction in all, anemia in 23.5%, thrombocytopenia in 41.2%, increased lactate dehydrogenase in 26.5% and schistocytes in 23.5%. Plasmapheresis was required in 11 patients yet 6 recovered poorly compared to complete recovery in 23 patients who did not require plasmapheresis. HUS group showed 55.6% recovery vs TMA group with 84% recovery. Over a mean follow-up of 2.7 years, patient survival was 82.3% and death-censored graft survival was 60.7%.

**Conclusion:** De novo TMA-HUS after RT is a rare but severe condition with poor graft outcome. Early allograft biopsy with prompt diagnosis and management helps in improving the outcome.

**KEY WORDS:** Immunosuppression, Tacrolimus, Cyclosporine

### **INTRODUCTION**

Hemolytic uremic syndrome (HUS) is the most severe form of calcineurin inhibitor (CNI) toxicity in renal transplant (RT) recipients affecting 3-14% of patients usually presenting with renal failure, hemolytic anemia and thrombocytopenia.<sup>1-6</sup> Tissue-limited HUS is termed as “thrombotic microangiopathy” (TMA). Post-transplant TMA/HUS can occur as recurrent or de novo disease. De novo TMA/HUS may also be associated with infections, mTOR inhibitor, antibody use (OKT3), or acute vascular rejection. TMA in kidney is characterized by sub-endothelial accumulation of

amorphous material in glomeruli, fibrinoid or mucoid change in the intima of small arteries, narrowing or occlusion of capillaries, glomerular and/or arterial fibrin thrombi, and fragmented red blood cells in the vascular wall, glomeruli, or interstitium.<sup>7,8</sup> The diagnosis of TMA is confirmed by renal allograft biopsy usually performed to exclude rejection as the cause of graft dysfunction. It is not known whether patients with TMA and HUS have different characteristics and clinical courses. The optimal management of post-transplantation TMA/HUS also remains controversial.

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We report 34 cases of HUS-TMA in RT patients and compare characteristics and outcome of patients with HUS and patients with TMA localized to the renal allograft.

## **MATERIAL AND METHODS**

We reviewed 1538 renal allograft biopsies performed between January 2008 and September 2013 in 1175 RT patients of our center. Histopathologic findings were reported as per the modified Banff criteria by performing hematoxylin and eosin, Periodic acid Schiff reagent, Jones's methanamine silver, Gomori trichrome, and C4d (polyclonal - Biomedica Gruppe, Germany) staining on 3µm-thick paraffin sections.<sup>9-10</sup> HUS-TMA were diagnosed on the basis of clinic-pathological findings. TMA was diagnosed on the basis of mesangiolytic, ectatically dilated bloodless glomerular capillaries or capillaries occluded with amorphous material/ hyaline/fibrin/platelet thrombi and mucointimal proliferation of small-caliber arteries with absence of C4d deposits along peritubular capillary membranes.<sup>11</sup> Patients with associated thrombocytopenia (platelet counts  $<1 \times 10^5/\mu\text{L}$ ) and elevated serum lactate dehydrogenase (LDH) levels ( $>480 \text{ U/L}$ ; normal range: 240 to 480 U/L) with or without hemolytic anemia were labeled as HUS. All patients with recurrence of HUS were excluded from the analysis. The offending drug (cyclosporine [CsA]/tacrolimus/sirolimus) was withheld in all cases. Plasmapheresis was performed only in patients who had rapid progressive disease and/or required dialysis, as an adjuvant therapy. Plasmapheresis (Cobe Spectra version 7, Gambro China) was performed on alternate days by exchanging 70-80% total plasma volume per sitting. Replacement of the removed plasma was done with 40% colloids (20% albumin) and 60% crystalloids (normal saline). Plasmapheresis was discontinued with clinical improvement in the form of improved urinary output, normalization of platelet counts and recovery of graft function in terms of serum creatinine (SCr). Outcome of these patients was compared with the other patients who did not have HUS-TMA.

## **Statistical Analysis**

Statistical analysis was performed using IBM SPSS statistics Software version 20 and `R` statistics Software version 2. Survival analysis was made by the Kaplan-Meier method with log-rank comparison which was also compared with 1141 RT patients without diagnosis of TMA/HUS. Statistical significance was assumed for p-value  $<0.05$ .

## **RESULTS**

Thirty-four (2.9%) RT patients (22 males, 12 females) showed findings of TMA/HUS in renal allograft biopsies. TMA/HUS was found in 20 (58.8%) patients on Tacrolimus, 12 (35.3%) on CsA, and 1 (2.9%) on sirolimus, while 1 (2.9%) had acute Cytomegalovirus (CMV) infection. Four out of 34 (11.8%) had deceased donor grafts and 30 (88.2%) had living related grafts. Mean age was  $32.9 \pm 9.8$  years and serum creatinine (SCr) was  $3.4 \pm 1.9 \text{ mg/dL}$ . Presenting features were graft dysfunction in all, thrombocytopenia in 14 (41.2%), anemia (hemoglobin  $<10 \text{ g/dl}$ ) in 8 (23.5%), elevated LDH in 9 (26.5%) and schistocytes in 23.5% patients. Nine (26.4%) patients experienced acute HUS with mean LDH of  $1256 \pm 541 \text{ U/L}$  (range: 654 to 2050 U/L) and platelet counts of  $0.67 \pm 0.27 \times 10^5/\mu\text{L}$  (range: 0.28 to  $0.96 \times 10^5/\mu\text{L}$ ). Twenty five patients displayed acute TMA with mean LDH levels of  $268.9 \pm 171 \text{ U/L}$  (range=156 to 360 U/L) and platelet counts of  $1.95 \pm 0.57 \times 10^5/\mu\text{L}$  (range=0.98 to  $2.74 \times 10^5/\mu\text{L}$ ). Average time for development of HUS/TMA post-transplant was  $156 \pm 163$  days (range: 5 to 978 days). HUS was observed earlier than TMA, with an average time of occurrence at  $104 \pm 118$  days (range: 5 to 266 days) in the former and  $175 \pm 192$  days (range: 14 to 978 days) in the latter. Out of 34 patients, 23 (67.7%) developed HUS/TMA within 3 months, eight (23.5%) developed it within 1 year and 3 (23.5%) developed it after 1 year post-transplantation.

At 3 months, out of 34 patients, two patients expired, graft function recovered in 26 (76.4%), while six (17.6%) developed end stage renal disease (ESRD). The HUS group showed 55.6% recovery as compared

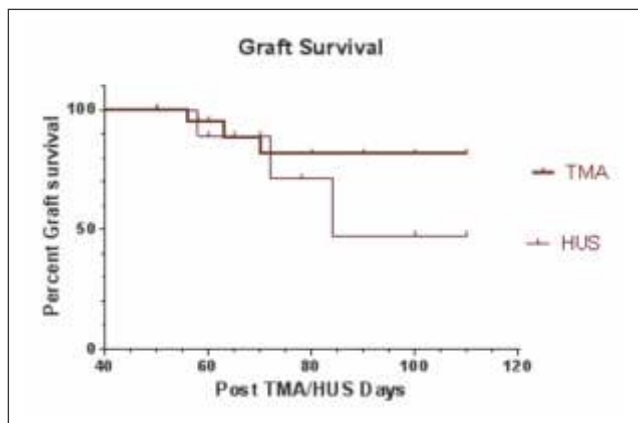


Figure 1: Kaplan-Meier graft survival in TMA versus HUS group (3 month post TMA/HUS)

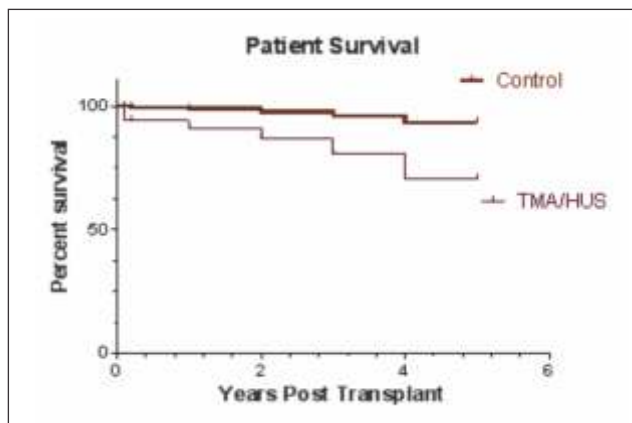


Figure 2: Kaplan-Meier patient survival in TMA/HUS versus control group (after mean follow up of 2.7 years)

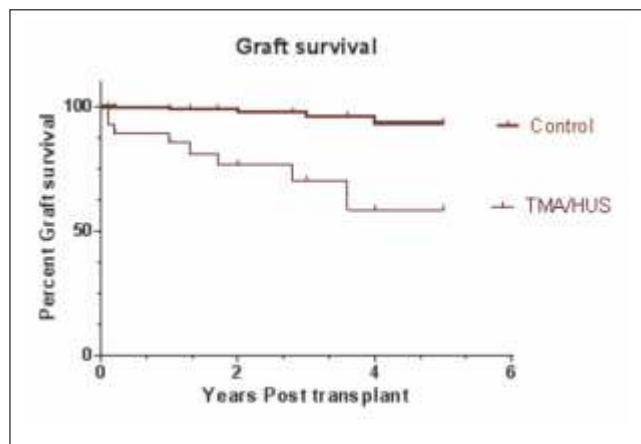


Figure 3: Kaplan-Meier death-censored graft survival in TMA/HUS versus control group (after mean follow up of 2.7 years)

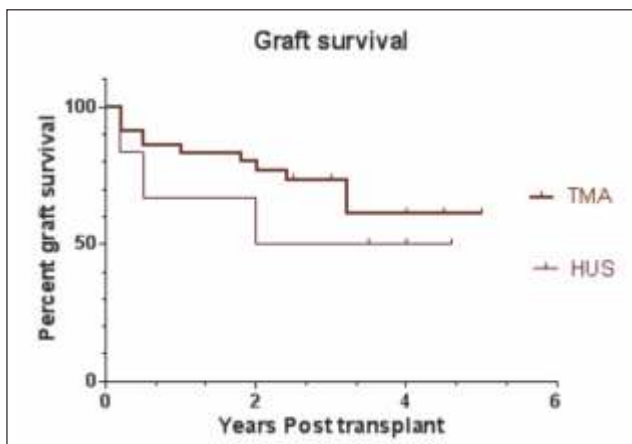


Figure 4: Kaplan-Meier death-censored graft survival in TMA versus HUS group (after mean follow up of 2.7 years)

to 84% recovery in the TMA group. (figure 1) Eight (88.9%) out of nine patients with HUS and 3 (12%) with TMA required dialysis and plasmapheresis support. However graft function recovered poorly (in 45.5%) in spite of dialysis and plasmapheresis compared to patients not requiring this support. Mean plasmapheresis procedures performed were  $6.4 \pm 1.7$  (range: 3-10). Patients with early TMA/HUS (< 3 months) and late TMA/HUS (> 3 months) showed similar graft function recovery, 82.6% and 81.8% respectively. Patient with CMV induced HUS presented with graft dysfunction and B/L pneumonitis. His immunosuppressants and antiviral agents were decreased. However he developed respiratory failure and was lost after 1 week of onset. One patient in TMA

group presented with graft dysfunction treated with dialysis and plasmapheresis, later developed bacterial pneumonitis with multi-organ failure and succumbed to it after 5 days.

We reintroduced CNI in 16 patients; however 1(6.2%) of them developed recurrent HUS after 2.5 months of re-introduction. CNI was stopped and substituted with sirolimus. In spite of plasmapheresis he progressed to ESRD later on.

Over a mean follow-up of 2.7 years, patient survival was 82.3% and 96.4% in TMA-HUS patients control group respectively (log-rank – 12.9; hazard ratio-0.18) (figure 2). Death-censored graft survival was 60.7% in these groups' vs 94.6% (log-rank – 25.3; hazard ratio-

0.12) in non-TMA/HUS group (figure 3). Intergroup analysis of death-censored graft survival was 66.7% in TMA group vs 50% in HUS group ( $p > 0.292$ ) (figure 4). Most common cause of death was cardiovascular complication in follow-up period.

## **DISCUSSION**

HUS is a serious post-transplant complication. The United States Renal Data System (USRDS) reported de novo TMA in 0.8% RT patients, whereas few studies have reported the incidence as high as 4% to 14%.<sup>2,12,13</sup> RT combines several factors that may act synergistically to injure the graft endothelium. Majority of de novo TMA appear to be related to the pro-thrombotic effects of CNI.<sup>14-17</sup> In our study most of patients were on CNI and 1 patient was on sirolimus which can rarely induce HUS.<sup>18</sup> Viral infections to which RT patients are susceptible, have been implicated in pathogenesis of de novo HUS. We found CMV infection associated HUS in 1 patient who responded poorly despite treatment as noted by other study also.<sup>19</sup>

Diagnosis of post-transplant TMA/HUS is made primarily on the basis of clinicopathological findings. Systemic manifestations are usually lacking, and many patients are characterized solely by rapidly progressive graft dysfunction. Classification of TMA according to clinical and pathological findings may be useful because the differential diagnosis between TMA and vascular rejection is difficult.<sup>20,21</sup> Thus, early allograft biopsy for any alteration in graft function is important for the diagnosis.<sup>21,22</sup> It is likely that vascular rejection could itself have contributory or causative role in the development of TMA/HUS.<sup>20,23</sup> None of our patients had combined TMA/HUS and acute rejection on histopathological evaluation as observed by others.

In the present study, the incidence of HUS was low compared to TMA. TMA has been reported to recur in 10% to 100% of patients with different series.<sup>2,10</sup> Reasons for this variability are not clear, but it is possible that proportion of TMA and HUS may vary at different centers or among physicians in relation to

threshold for performing graft biopsies. Additional cases of mild or subclinical localized TMA may be identified by more aggressive biopsy practices for renal allograft dysfunction.

The time of occurrence in our study was predominantly early after transplantation as opposed to reports showing variable onset.<sup>20,24</sup> HUS has been known to occur earlier than TMA and to be associated with a poorer prognosis.<sup>10</sup> It is unclear whether HUS occurs earlier after transplantation compared with TMA or whether it simply is associated with severe renal dysfunction leading to earlier diagnosis by renal biopsy. Our data indicate significant difference in the absolute increase in creatinine levels from nadir between TMA and HUS groups, making this likely reason for early biopsy, start of HD and plasmapheresis in HUS group.

There are no treatment guidelines for de novo posttransplant TMA. The main therapy for CNI-related TMA was withdrawal or reduction of the dose or substitution with other immunosuppressant. This strategy proved successful in another reports, being responsible for resolution of graft dysfunction.<sup>20,22,24</sup> Majority of patients who were subsequently reintroduced to CNI did not experience TMA recurrence,<sup>19,21,24</sup> as observed in our study. In a few cases, switching patients to another CNI resulted in recurrence of disease.<sup>25,26</sup> In these patients, substitution of sirolimus for CNI or mycophenolate - based immunosuppression may be alternative therapy.<sup>26</sup> In our study, seven patients were treated with sirolimus + mycophenolate and three with everolimus + mycophenolate with good results. In an attempt to limit the risk of CNI associated nephrotoxicity, use of mTOR inhibitors has been proposed. These drugs could substitute for CNI. However, de novo HUS has been reported with use of mTOR inhibitor also.<sup>27-29</sup> Combined administration of CNI and sirolimus confers pro-necrotic effect of CNI and pro-apoptotic effect of sirolimus, and is associated with a 16-fold higher risk of post-transplant TMA/HUS compared with CNI + mycophenolate regimen.<sup>28,29</sup>

Plasmapheresis has controversial role in severe de novo posttransplant TMA/HUS, achieving durable remission in up to 50% patients.<sup>21,22,24</sup> Plasmapheresis with CNI withdrawal achieved remission in 80% patients with de novo post-transplant HUS.<sup>13</sup> Given the retrospective and uncontrolled nature of our data, it is impossible to draw firm conclusions about the benefit of plasmapheresis in post-transplant TMA/HUS. Complication rate of plasmapheresis may be 30%; therefore, it would be useful to define a subset of patients with post-transplant TMA in whom plasmapheresis is indicated.<sup>30</sup> In our study requirement of plasmapheresis, dialysis, and graft loss was high in HUS group vs TMA group. Other investigators also reported low rates of TMA-related graft loss (0%-12%) despite infrequent use of plasmapheresis (0%-8%).<sup>2,4,7</sup> Our study suggests that patients with TMA respond to weaning/ discontinuing CNI and do not routinely require plasmapheresis for graft salvage.

The rate of graft loss is influenced by whether the disease is systemic or renal-limited, with localized TMA having a better short-term prognosis than HUS.<sup>10</sup> Patients with HUS were significantly more likely to have renal failure requiring dialysis therapy and early graft loss. Our findings are consistent with those of others in which high incidence of HUS was associated with increased and early graft loss.<sup>1, 5,6,31</sup> Studies with high proportion of TMA noted lower rates of graft loss (0% -12%).<sup>2,4,7</sup> Despite these differences, long-term survival of grafts with TMA and HUS was similarly poor (Fig 2B). In patients treated with cyclosporine, the incidence of de novo TMA was 4–15% with 43% graft survival and 1% in patients receiving FK506.<sup>32</sup> In our study, percentage of cases were maximum in tacrolimus group followed by CsA and sirolimus group.

There is no strong evidence regarding outcome in early and late onset TMA/HUS. In our study, patients with early TMA/HUS (< 3 months) and late TMA/HUS (> 3 months) showed similar graft function recovery. But other reports suggest that prognosis is favorable when TMA occurs later in post-transplant course or when it

affects recipients of allograft from living donors.<sup>5</sup> In our study, non-significant difference in early and late onset group, might be due to low threshold for allograft biopsy, which makes early diagnosis and treatment in early disease onset group.

The uncertainty of what constitutes TMA induced graft loss is a major unavoidable drawback of the present and other retrospective studies. Although early graft loss caused by TMA/HUS can be defined easily, given the temporal relationship of graft loss in the presence of an ongoing episode of TMA/HUS and the absence of other factors like rejection, no pathognomonic features distinguish graft loss occurring many months after successful treatment of TMA. Severe vascular damage is a hallmark of TMA and almost certainly contributes to accelerated graft loss in these cases. Main limitation of our study was retrospective study and non-screening for genetic susceptibility.

## CONCLUSION

De novo HUS-TMA after RT is a rare but severe condition with poor graft outcome. Early allograft biopsy and conversion/ temporary discontinuation of CNI can salvage the graft. Despite treatment HUS showed poor short term recovery than TMA. However long-term graft survival was poor in both TMA and HUS.

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## Case Report

# Successful Outcome of a Pregnancy in a Case of Systemic Lupus Erythematosus: A Case Report

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### ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disorder which can have devastating outcome in pregnant women with SLE, both in terms of maternal and infant mortality or intrauterine growth retardation. We report a 27 years old lady suffering from SLE for the last 10 years, who presented in emergency with pre-eclampsia at 30 weeks gestation. She was taken to successful termination of pregnancy and discharged with healthy baby.

**KEYWORDS:** Systemic lupus erythematosus, high risk pregnancy, nephritis

### INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder commonly diagnosed in women of child-bearing age. Women with SLE are at higher risk of antepartum and intra-partum risks like spontaneous abortion, intrauterine fetal death, preeclampsia, eclampsia, preterm delivery and intrauterine growth retardation (IUGR).<sup>1</sup> We report a case of 27 years old lady suffering from SLE for the last 10 years, who had a successful outcome of pregnancy.

### CASE REPORT

A 27 year old lady who was a known case of SLE presented at 32 weeks of gestation with preeclampsia. On admission she was pale, edematous, and had pulse 88/ minute and blood pressure of 160/90mmHg. On systemic examination cardio-respiratory and central nervous systems were unremarkable. Abdominal examination revealed 30 week pregnancy.

On investigation, her hemoglobin was 7.5 gm/dl, platelet count,  $1.12 \times 10^5/\mu\text{L}$ , serum (S.) creatinine, (SCr), 1.7 mg/dl, S. urea, 105 mg/dl, S.lactate dehydrogenase (LDH), 389 mg/dL, total protein, 5.5 gm/dl, S.anti-nuclear antibodies (ANA), 3.6

(positive), S. double stranded DNA (dsDNA), 17.00IU/ml, liver function tests were within normal limits and urine examination showed +4 proteinuria on qualitative test and active sediments on microscopy. Ultrasound examination revealed 30 week period of gestation with 1.2 Kg fetus, and remaining parameters were normal.

Patient was managed conservatively with steroids, anti-hypertensives and hematinics. On 9<sup>th</sup> day of admission, she had a flare up of SLE and developed difficulty in breathing. Hence she was taken for emergency lower section caesarian section (LSCS) to salvage the fetus. She delivered 1.3 kg baby which was shifted to neonatal intensive care unit (NICU) for further management. Echocardiography of baby was normal. Patient was shifted to ICU on ventilatory support. During postoperative period, patient had oliguria and raised SCr of 2.4 mg/dl. Chest X-ray showed bilateral pulmonary edema. Patient was started on supportive management and dialysis for acute renal failure and pulmonary edema. However her condition worsened on 3<sup>rd</sup> post operative day with SCr, 4.00 mg/dl and X-ray chest showed increased opacification. She was then subjected to maintenance hemodialysis. On 7<sup>th</sup> post

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operative day after 7 dialysis procedures, she started showing signs of improvement with increased urine output and pulmonary edema started resolving.

After 10 days of ventilatory support, she was reversed to spontaneous respiration. On 20<sup>th</sup> postoperative day, patient started maintaining oxygen saturation and renal function. She was on prednisolone 20 mg/day, diuretics, anti-hypertensives and diet restriction. Patient responded well to the treatment and was discharged on 60<sup>th</sup> postoperative day with a healthy baby.

Her hemoglobin was 10.5 gm/dl, SCr, 0.7 mg/dl at the time of discharge.

## DISCUSSION

There are two major issues regarding the risks and management of pregnancy in women with SLE and renal disease. First, pregnancy may accelerate the disease activity, and short and long term adverse effects on renal function potentially leading to accelerated progression of renal disease. Secondly, these pregnancies are at high risk for maternal and fetal complications, including spontaneous abortions, premature delivery, IUGR, and superimposed preeclampsia.<sup>2</sup>

Nephritis is known to be one of the most serious complications of SLE and is a strong predictor of poor outcome. Studies evaluating pregnancies for which conception occurred during active renal disease have reported flare rates of 48–62% in the pregnancies, whereas during remission of nephritis indicate flare rates between 7.4 % and 32 %.<sup>3</sup>

A review of current literature reveals that pregnancies in women with SLE are associated with increased rates of stillbirth, fetal death prior to 20 weeks' gestation, prematurity, IUGR and neonatal complications such as neonatal lupus erythematosus (LE).<sup>4</sup> Neonatal LE is a rare syndrome characterized by fetal and neonatal congenital heart block that may also occur with subacute LE skin lesions, thrombocytopenia, anemia,

hepatitis, glomerulonephritis, and neurological involvement.<sup>5</sup>

SLE is an autoimmune disease that affects primarily women, commonly in their reproductive years, but does not influence fertility. For these reasons, the clinician has often to face many problems related to pregnancy in patients with SLE including the influence of SLE on fetal outcome and that of pregnancy on SLE. Early reports emphasized a high fetal and maternal risk, in particular in patients with lupus nephritis. However, in the same period the prognosis of lupus nephritis was poor, and so it was difficult to know whether pregnancy actually influenced the prognosis of the disease. More recent prospective studies indicate that pregnancy is safe for majority of the mothers if it is planned when disease activity is quiescent. Instead, although fetal risk has been progressively reduced in the last 40 years, it continues to be higher than that occurring in pregnancies of healthy women. In particular, the presence of antiphospholipid antibodies considerably worsens the fetal outcome. Fortunately our patient with SLE and pregnancy which was complicated by preeclampsia, pulmonary edema and nephritis survived and was discharged with a healthy baby.

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## Case Report

# Primary Hydatid Cyst of Kidney: Case Report

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### ABSTRACT

We report a rare case of primary uretero-renal echinococcosis in a 21 year old male who presented with dysuria and left flank pain for 2 months. Ultrasonography showed enlarged left kidney (13.7 x 6.6 cm) with well defined multiple cystic lesions suggestive of hydatid cyst. His serum creatinine was 1.25 mg/dL with normal blood counts. Urine microscopy showed 40- 50 pus cells and 12-15 fresh RBC /high power field. CT-scan of abdomen and pelvis confirmed ultrasonography findings. Patient was subjected to laparoscopic left side nephrectomy. Histopathological examination revealed hydatid cyst in renal parenchyma and upper ureter; lined by outer chitinous and inner germinal layer surrounded by focal granulation tissue with calcification, daughter cysts and scolices. He received oral Albendazole 400 mg tablet twice a day for 3 months as a prophylaxis. Over a follow-up of 1 year he has no evidence of recurrence or involvement of other organs.

**KEYWORDS :** Hydatid cyst, Echinococcus granulosus, kidney

### INTRODUCTION

Hydatid disease (*Echinococcus granulosus*) is endemic in the Middle East and other parts of world including India, Africa, South America, New Zealand, Australia, Turkey and Southern Europe.<sup>1</sup> Primary renal involvement without that of liver and lungs is very rare.<sup>2</sup> Hydatiduria presents in 10-20 % of renal hydatidosis and is usually microscopic. We present a rare case of primary hydatid cyst in left kidney and upper ureter.

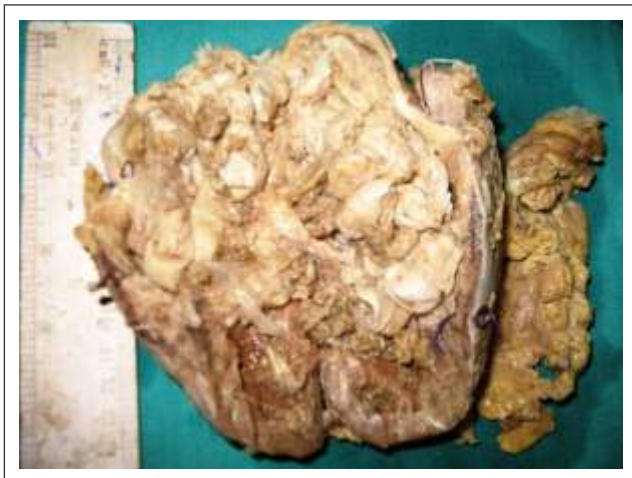
### CASE REPORT

A young man of 21 years presented with complaints of dysuria and left flank pain for 2 months. On investigations he was found to have enlarged left kidney (13.7 cm x 6.6 cm) with well-defined multiple cystic lesions with mother and daughter cysts, suggestive of hydatid cyst. Echogenicity was increased and cortico-medullary differentiation was altered. Right kidney was unremarkable, measuring 10.6 cm x 4.1 cm in size. His serum creatinine was 1.25 mg/dL and blood counts were within normal limits.

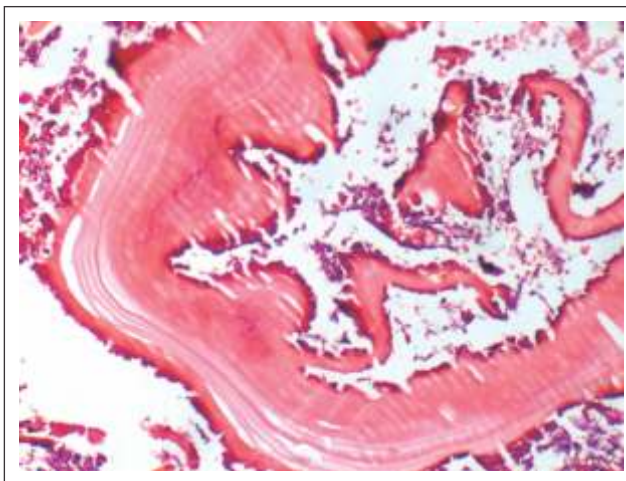
Urine routine examination was unremarkable except microscopy showing 40- 50 pus cells and 12-15 fresh RBCs /high power field. CT scan-abdomen and pelvis confirmed sonography findings and showed large multiseptate cystic lesions measuring 10 x 7 x 7 cm in upper pole of left kidney along with gross hydronephrosis, dilated renal pelvis and ureter. Lung, liver, pancreas, right kidney and urinary bladder were normal on CT scan. Patient were given oral Albendazole 400 mg tablet twice a day for a week before surgery. Patient was subjected to laparoscopic left sided nephrectomy with scolicedal therapy and measures to prevent rupture of cysts.

Gross specimen subjected to histopathology evaluation weighed 480 gms and measured 14 x 8.5 x 8 cm ( Figure 1). The cut surface had renal parenchyma replaced by multiple cysts of variable sizes with glistening white surface and thin walls, filled with straw colored fluid. Renal pelvis was dilated to 5 cm. Ureter was 6.0 cm long with 1.0 cm diameter. On microscopic examination, sections from cysts revealed cyst wall lined by outer chitinous layer and inner

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**Figure 1: Gross specimen showing multiple cystic walls replacing most of renal Parenchyma**



**Figure 2: cyst wall layers surrounded by focal granulation tissue with calcification (H & E, X 200)**

germinal layer surrounded by focal granulation tissue with calcification. Fair number of daughter cysts and scolices were visible. These cysts also plugged ureteric lumen with reactionary inflammatory changes. (Figure 2) Renal parenchyma revealed changes of moderately advanced hydronephrosis with chronic pyelonephritis. Final diagnosis was hydatid cyst (Echinococcosis) in kidney and ureter.

Patient was discharged on sixth post-operative day on oral Albendazole, 400 mg tablet twice a day for 3 months. On follow-up of six months and 1 year patient was healthy and had no recurrence/ involvement of any other organ.

## DISCUSSION

Etiological agent of Echinococcosis is larval stage of tapeworm Echinococcus (E), E. granulosus being the common species. Echinococcus belongs to the order Cestoda, family Taenia. It is about 5-6 mm long. The adult E. granulosus worm resides in the large bowel of foxes and dogs. Man is the intermediate host and infestation occurs by ingesting vegetables and water contaminated by the affected animal. Hydatid cyst may occur in any part of the body. Organs affected by E granulosus are liver (63 %), lungs (25 %), muscles (5 %), bones (3 %), kidneys (2-3 %), brain (1%), and spleen (1%).<sup>3,4,5</sup> Secondary involvement due to hematogenous dissemination may be seen in almost any anatomic location. Renal hydatid cysts usually remain asymptomatic for many years. It is not clear how the hydatid embryo reaches the kidney in cases of primary hydatid disease, it is postulated that it must pass through the portal system into the liver and retroperitoneal lymphatics.<sup>6</sup> The hydatid cyst of kidney is considered closed if all three layers of the cyst i.e. pericyst, ectocyst and endocyst are intact. When the cyst is no longer protected by the third layer i.e. pericyst or by the lining of collecting system it is considered to be an exposed cyst. If all the layers of the cyst have ruptured resulting in free communication with the calyces and pelvis, it is called an open or communicating cyst. Cystic rupture into the collecting system, causing pathognomonic hydatiduria, though seen in only 10-20 % of renal hydatidosis is usually microscopic.<sup>7</sup> Gross passage is rather uncommon, but has diagnostic utility. Eosinophilia is noted in about 50% cases. Serological tests in primary renal hydatidosis are usually negative. Advanced radiological techniques like CT scan and Magnetic resonance imaging (MRI) remain the mainstay of diagnosis. Plain X-rays are usually nonspecific and mostly non-revealing. A thin rim of calcification delineating a cyst is suggestive of an echinococcal cyst. Ultrasonography helps in the diagnosis of hydatid cysts when the daughter cysts and hydatids are evident. The CT scan with an accuracy of 98 % and higher sensitivity to demonstrate the daughter cysts and

usually shows an expansile, hypo-attenuating tumor with a well-defined wall and daughter cysts within the parent cyst. MRI usually reveals a solitary, high-signal-intensity mass consisting of multiple thin-walled lesions outlined by a thick, hypo-intense rim. High signal intensity is due to the characteristic high fluid content of the mass. Small peripheral cysts are usually hypo-intense relative to the central component.<sup>8</sup> In general, surgery is the treatment of choice in renal hydatid cyst. Nephron-sparing surgery is possible in most cases (75 %). Very few cases of laparoscopic removal of renal hydatid are reported. There is danger of cyst rupture and dissemination during dissection, entrapment and removal of the hydatid cyst during laparoscopy. Utmost care should be taken during the surgery to prevent spillage and resultant disseminated hydatidosis. Pre and postoperative courses of Albendazole should be considered in order to sterilize the cyst, decrease the chance of anaphylaxis and decrease the tension in the cyst wall (thus reducing the risk of spillage during surgery) and to reduce the recurrence rate postoperatively. During kidney-sparing surgery scolicidal solutions such as hypertonic saline should be used before opening the cavities to kill the daughter cysts and therefore prevent further spread or anaphylactic reaction.<sup>9</sup>

We conclude that *Echinococcus granulosus* can affect any organ in the body and a high suspicion of this disease is justified in any cystic neoplasm of any organ,

especially in endemic regions. However, medical treatment should precede and follow the surgical intervention to prevent recurrence and complete cure.

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## **Hemodialysis Arteriovenous Fistula Maturity Evaluation by Grey Scale Ultrasonography and Color Doppler**

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Dear Sir,

Grey scale ultrasound (US) and Color Doppler analysis of Hemodialysis Arteriovenous fistula (AVF) has helped in the objective vigilance of recently created fistulas and to act aptly at the earliest in case of any deviation from normal maturation process, hence reducing the waiting time of 4 to 6 months for routine assessment of maturity. We found little in the Indian literature about the objective evaluation of AVF maturity and we intended to study the same.

We studied 33 patients periodically, of which 43.9% were females and 56.1% males including 78.8% diabetics, assessed with pre procedure venous mapping and post-operative follow up at 1st day, 1st month, 3rd month to assess the value of the parameters Viz. flow volume in draining vein, Resistivity Index (RI) in draining vein, minimum caliber of draining vein and depth of draining vein from skin surface. These were then correlated with the established clinical assessment parameters for maturity assessment in use i.e. palpable thrill in draining vein for up to 10 cm distance by an experienced clinician. Most common type of fistula in our study was Left forearm radio cephalic (60%) fistula followed by left arm brachiocephalic fistula (18%). At the end of 12 weeks, 20 fistulas were found clinically mature and 13 not mature. Average minimum venous caliber was 5.89 mm $\pm$  1.7 and 3.42 mm  $\pm$ 1.56 at third month in mature and immature fistulas respectively; this was in agreement with previous study which found a mean diameter of 5.8 mm  $\pm$ 0.12 in the successful

radio cephalic fistula at 6 weeks.<sup>1</sup> Average depth from skin surface was 3.08 mm  $\pm$ 0.93 and 3.08 mm  $\pm$ 1.53 at third month in mature and immature fistulas respectively. Average flow volume in the draining vein was 1037mL/min  $\pm$  382 and 223.85mL/min  $\pm$  199.31 at third month in mature and immature fistulas respectively which was higher to that found in previous studies.<sup>1-3</sup> The relatively higher mean flow volume in our study can be attributed to inclusion of arm fistulas which are known to have higher flow volume as documented by Alamdaran et al.<sup>4</sup> Mean RI in draining vein of fistulas considered mature at 3rd month was 0.4 $\pm$  0.04; higher mean RI was noted among immature fistulas i.e. 0.52 $\pm$ 0.06 these values paralleled previous results.<sup>5</sup>

A potential problem with a single measurement of fistula diameter and blood flow rate is that they may increase with time. We obtained multiple measurements periodically; we found no significant difference in the means of flow volume measured at 1st and 3rd month. Thus, an initial US assessment at 1 month after surgery should help predict subsequent fistula maturity with a high degree of accuracy; this was in agreement with previous findings. (1) Objective US criteria may be used for adequacy assessment of the fistula if it does not appear mature clinically, or is questionable, and based on these findings appropriately managed in time. US evaluation is also useful when an experienced examiner is not available, which is a common experience at many places.

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## Image

# Pigment Nephropathy

*S. Raman, K.V. Kanodia, R.D. Patel, K.S. Suthar, L. Nigam, A.V. Vanikar*

From the Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad, India.

A 30 years old male presented with complaints of fever with chills on and off, cola colored urine in mornings for last 6 months, and decreased urine output for last 12 days. On examination he had pallor, pedal edema and icterus. Liver and spleen were palpable (3 fingers), other systemic examination was unremarkable. On investigations, his hemoglobin (Hb) was 3.2 gm/dL, urine albumin was trace with occasional pus cells and RBCs on microscopic examination, blood urea was 190 mg/dL, serum (S.) creatinine (SCr), 14.45 mg/dL, S. bilirubin (total/indirect), 3.3/1.6 mg/dL and S.uric acid was 11.8 mg/dl. Peripheral smear revealed acute hemolytic anemia with no malarial parasites, and reticulocyte production index 5.6% with positive direct Coomb's test. S. lactate dehydrogenase was 3216 IU/L (normal range: 90 to 190 IU/L). Urine hemosiderin, sucrose hemolysis test, osmotic fragility test, and unstable hemoglobin test were negative. S ceruloplasmin was low and KF ring was not seen. Prothrombin and activated prothrombin time were within normal range. Vasculitis markers and complement levels were also within normal range.

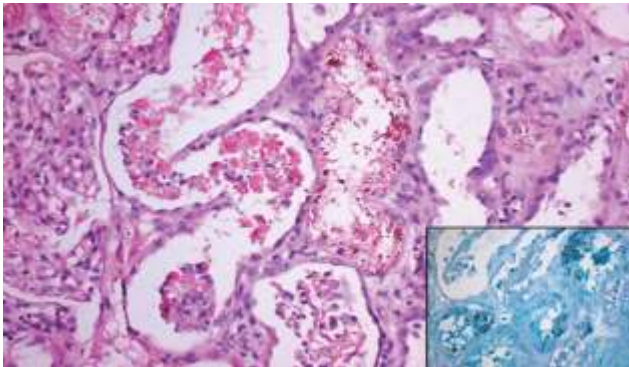
Renal biopsy performed consisted of a single core of renal tissue with 10 glomeruli, surrounding tubules and vessels. Glomeruli show mild mesangial prominence. Capillary lumina were fairly open, filled with RBCs/leucocytes and were lined by membranes of normal thickness. Bowman capsules were unremarkable. Tubules showed moderate injury with dilated lumina, sloughed epithelial cells, focal simplification and loss of nuclei in many tubular cells. Many tubular lumina contained brown pigment/ RBCs/ proteinaceous/ cellular casts staining positive with Pearl's Prussian blue stain for iron. Interstitium was moderately

prominent for edema and focal leucocytic infiltration. Blood vessels were unremarkable. There were no ischemic changes in the glomeruli / blood vessels. Immunofluorescence revealed absence of staining for immunoglobulins/ complements.

So the final diagnosis was Pigment nephropathy secondary to hemoglobinuria. CD55 and CD59 assay by flow cytometry for red blood cells were negative confirming the case to be Paroxysmal nocturnal hemoglobinuria (PNH) associated pigment nephropathy. Patient recovered completely with fluid replacement therapy, steroids, dialysis and transfusion of 8 units of packed cells. On follow up after 8 months, he appears healthy with SCr of 0.9 mg/dL.

PNH is a rare disorder estimated to affect 1-1.5 cases per million population. Heme pigment nephropathy is a common cause of acute kidney injury, usually secondary to rhabdomyolysis/ cardiopulmonary bypass surgery.<sup>1</sup> Less commonly heme pigment nephropathy results from massive intravascular hemolysis following spider-bite, malaria and leptospiral infection.<sup>2-5</sup> Heme has no nephrotoxic effects on renal tubules unless the urine is acidic. There are three main mechanisms that account for heme protein toxicity: (a) renal vasoconstriction with impaired renal circulation (b) intraluminal cast formation which induces intraluminal tubular obstruction and tubular cell necrosis and (c) direct heme protein-induced cytotoxicity since heme can oxidize lipid, denature proteins, and generate free radical oxygen species.<sup>2</sup> Early diagnosis and treatment are crucial to prevent disease progression and irreversible chronic kidney disease.

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**Figure 1: Coarse golden brown pigment embedded in renal tubular epithelium (arrow) along with acute tubular injury (H&E stain, X 200) with inset blue colored coarse hemosiderin granules in tubular epithelial cells (Pearl's Prussian blue stain, X 400)**

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## In Memorium: VIDHYA ACHARYA (1938-2014)



*Professor Vidhya Acharya, the first lady Nephrologist of India and Ex-Professor and Head of the Nephrology services at KEM hospital, Mumbai left her body in the early hours of 5<sup>th</sup> May, 2014. She was born on 8<sup>th</sup> January, 1938. Her entire education was in Mumbai. She took training in Nephrology at the University of Leeds, England. She returned to her alma mater in Seth G.S. Medical college to develop the department of Nephrology at King Edward VIII Memorial hospital (KEM) in Mumbai. After retirement, she served M.P.U. hospital in Nadiad. She was a tough fighter and evolved as a winner against many odds in the professional world of men in Mumbai. She belonged to the first generation Nephrologists of India and proudly trained hundreds of students in different ladders of education ranging from basic to*

*clinical sciences and MBBS to super-specialization. She was the first to start Dialysis and Transplantation facilities in Western India. She was a Founder Member and Past President of the Indian Society of Nephrology, Indian Society of Organ Transplantation, and Hypertension Society of India. Besides these, she was a member of several professional societies across the globe. She was a recipient of several awards including 'Life Time Achievement Award' in 1996 in recognition of her services for the development of Nephrology, dialysis and transplantation in India, Sandipani Rushi Award 2005 given by IKDRC-ITS, Ahmedabad, and World Medal of Freedom for Medicine and Nephrology – 2005. She has also been listed in several biographical manuals nationally and internationally like Rising personalities of India – 1998, Who's who in Science and Engineering – 2000/2002/ 2004, and many more. She published more than 250 publications, chapters in Medical Books, Advances, Updates etc., besides being co-editor of the very popular API textbook of Medicine. She has authored a book entitled 'Diet and Nutrition Guide for Patients with Renal Disease and Related Disorders,' which is extremely popular.*

*We have lost a dynamic friend who will be remembered by generations of Nephrologists, Transplanters and patients alike from this part of the world.*

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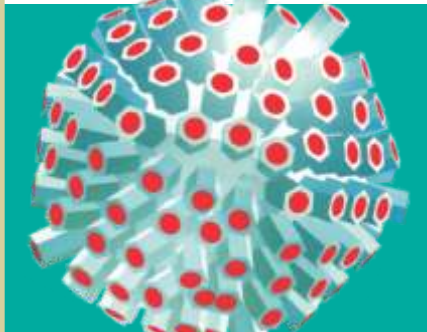
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