TANKAR Award Paper Submission

Title

"A Prospective Randomized Study Comparing The Impact Of Twice Divided Versus Once Daily Prednisolone Dosing On Hyperglycemia And Glycemic Variability Among Renal Transplant Patients"

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Abstract

Aim: Prednisolone exerts high glycemic variability leading to endothelial dysfunction and coronary events. This study compares glycemic excursions, hypothalamic–pituitary–adrenal axis suppression, sleep disturbance, lipid profile between divided twice daily (BD) and once daily (OD) prednisolone groups.

Materials and Methods: Thirty-two renal transplant recipients without diabetes were randomized to BD or OD prednisolone. Two hepatitis C positive patients under BD group and three serology negative patients from OD group developed NODAT and hence excluded. One BD group patient suffered graft nephrectomy and was excluded. Three weeks post-transplant, 4^{th} hourly venous glucose monitoring was performed for 3 days. Mean glucose, highest glucose and lowest glucose, exposure to hyperglycaemia, glycemic variability and HbA1C levels were assessed. 8AM serum cortisol, lipid profile, Athens Insomnia score, creatinine, sodium, potassium, urine culture and sensitivity were also assessed.

Results: Median age was 32yrs, 88.43% were males. Mean tacrolimus level [between 8-12 ng/ml (P< 0.512)] and mean glucose(P<0.68) among both groups were similar. BD group has less higher glucose value [206 versus 216 (P<0.007)] and exposure to hyperglycaemia ≥180mg/dl [4(30.8%) versus 11(84.6%) patients(P=0.034)]. Glycemic variability scores MAGE (P=0.0007), CONGA (P=0.0009), J-index (P=0.007) and Mean absolute glucose (P=0.0001), mean HbA1C (P=0.026), creatinine (P=0.016), cholesterol (P=0.665), triglycerides (P=0.112), LDL (P=0.243), VLDL (P=0.398), cholesterol/HDL ratio (P=0.717), urinary tract infections were less with BD group. HDL (P=0.605) was more with BD group. Fasting cortisol was suppressed in both groups (P=0.285). No difference noted in hemoglobin (P=0.379), sodium (P=0.942), potassium (P=0.166), Athens Insomnia score (P=0.19).

Conclusions: Divided prednisolone dosing reduces glycemic variability and hyperglycemia early post-transplant period. HbA1C was lower in the divided dose group. BD group patients have not developed NODAT except those with Hepatitis C infection. Fasting serum cortisol level appears suppressed in OD group also. Athens insomnia score showed no sleep disturbance among both groups.

DETAILED STUDY ARTICLE

Title

"A Prospective Randomized Study Comparing The Impact Of Twice Divided Versus Once Daily Prednisolone Dosing On Hyperglycemia And Glycemic Variability Among Renal Transplant Patients"

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Aims

1. To compare highest glucose, mean glucose and glycemic variability among subjects grouped into once a day and twice divided dose prednisolone.
2. To compare early morning serum cortisol level between the two groups to assess the suppression of hypothalamic–pituitary–adrenal axis.
3. To compare the degree of sleep disturbance between the two groups by means of Athens sleep score.
4. To compare HbA1C, serum fasting lipid profile between the two groups.

Materials and Methods

Study Design : Prospective Randomized

Study Place : Govt Kilpauk Medical College Hospital (KMC), Chennai

Study Population

Inclusion criteria:

1. Renal transplant recipients who are not on a steroid free regimen, admitted at Nephrology ward, KMC on the day of completion of 3rd post transplant week

2. Those patients who have given consent for the study.

Exclusion criteria:

1. Patients with known diabetes mellitus.

2. Patients with active infection.

3. Patients developing any complication in the 3 week post transplant period needing withdrawal of steroid.

4. Patients developing post transplant diabetes mellitus in the 3 week period needing to start anti-diabetic agents including insulin.

5. Patients not willing to participate in the study.

Sample size : 32

Methodology :

Thirty two renal transplant patients were included for the study at the end of their three weeks post transplant period. Of them twenty eight were live related transplants and remaining four were deceased donor transplants. All the live related transplant recipients received basiliximab induction 20 mg two doses (0 and 4^{th} days) and deceased donors received rabbit anti thymocyte globulin 1.5mg/kg single dose induction. Renal transplant patients uniformly received an initial dose of 1g methylprednisolone prior to kidney transplantation and 125mg methylprednisolone 6^{th} hourly on the first postoperative day. Maintenance immunosuppression comprised of tacrolimus, mycophenolate sodium and prednisolone, commencing 2–3 days prior to surgery. Tablet Prednisolone was started at 0.5 mg/kg/day with a minimum of 30 mg daily after the pulse steroids from post transplant day 2 onwards and slowly tapered after 2 weeks to reach 20mg a day in 4 weeks. Majority of the patients were taking 25 mg prednisolone during the study period. Target tacrolimus trough levels were adjusted for 8–12 ng/mL for the first and third weeks post transplantation.

Patients were divided under stratified randomization into OD (8:00 am) or BD (50% of total daily dose at 8:00 am and 50% at 8:00 pm) prednisolone dosing regimens. At the end of three weeks with routine post transplant follow-up, they were subjected to a 3 day study period. Since many studies analyzed the side effects after 3-4 weeks of starting steroids with a reasonably high dosage, 3 weeks interval was selected for this study. After the study period patients continued their respective dosing regimen up to the period when BD group dose falls below 10mg, during which they will be converted back to OD group. We have not dared to check the efficiency of twice divided low dose steroid maintenance in post transplant setting lower than 10 mg as long term careful monitoring is needed to prevent rejection and also on the insistence of Ethical committee to define an endpoint for this study.

Dietary Advice

Calorie intake was advised not to exceed 2500cal/day. Diet was split into breakfast, lunch and dinner with early morning beverage and evening snack – all were aimed to be within 2500 calories/day. No added extra meals entertained. Protein intake was aimed to be around 0.5mg/kg/day.

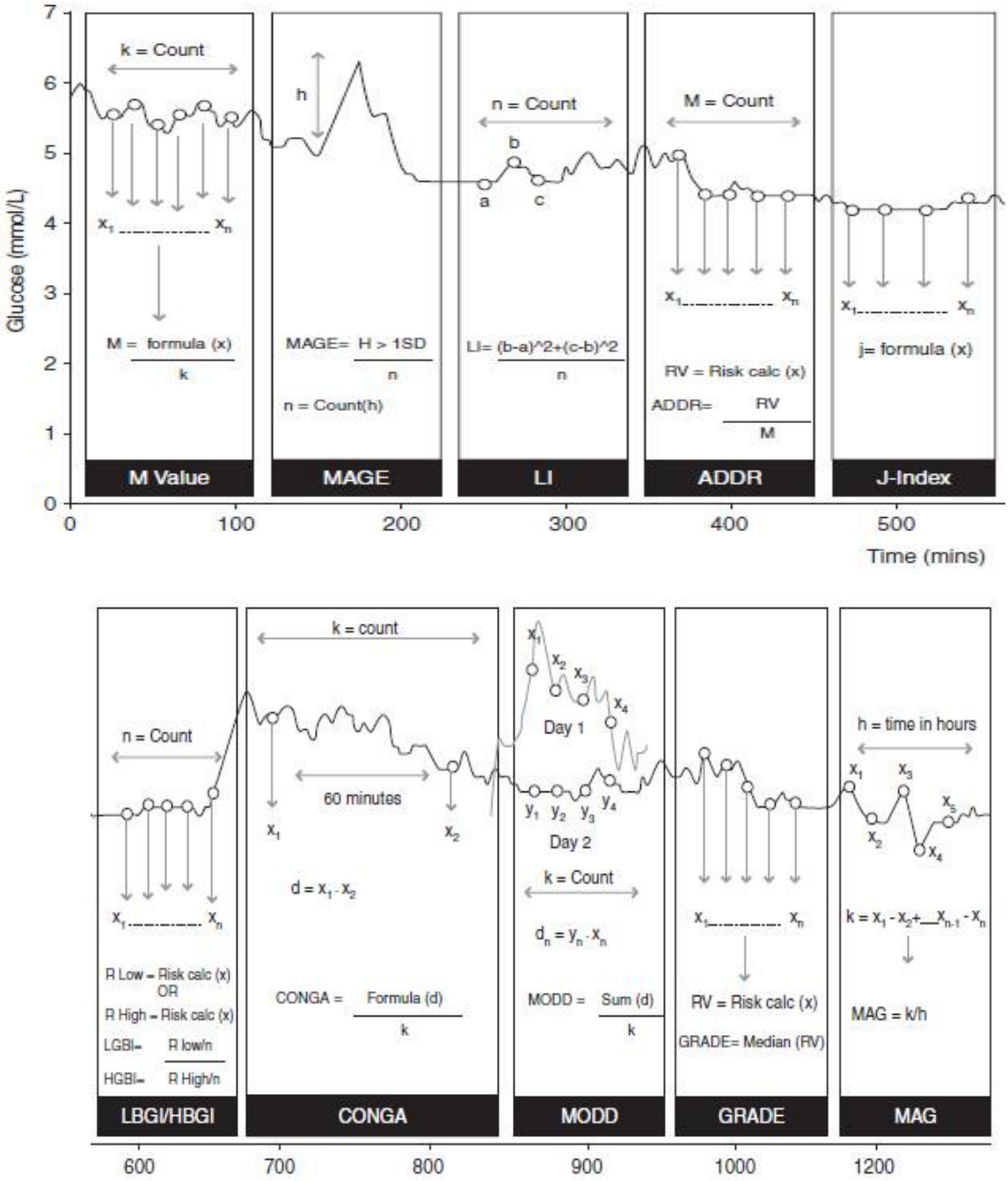
Fourth hourly venous blood glucose and blood pressure monitoring starting from 8AM on 1^{st} day to 4AM on the 4^{th} day, early morning cortisol level, fasting lipid profile, HbA1C, renal function tests, sodium, potassium, urine analysis, urine culture and sensitivity, haemoglobin, Athens sleep assessment score, bodyweight were recorded during the 3 day study period. Screening to rule out active infections with clinical examination and relevant investigations was carried out.

Conflict of interest: Nil

Financial grants: Nil

Statistical Analysis: Done using SPSS version 19.0

Graphical Illustration Of Different Glycemic Variability Assessment Methods



Glycemic variability was calculated using **EasyGV Software**. Normal reference values are set to estimate the magnitude of glycemic variability for diabetics, but not for subjects without diabetes.

Standard Deviation (SD)

SD is used for measuring variability of glycemic profiles. It shows the variation or dispersion extent from the average.

M-value

The M-value is calculated and when divided by the total number of values we get mean. Generally a default value of 120 was used for analysis.

MAGE

The MAGE is defined as the mean height of excursions which are greater than 1 SD that defines glycemic variability depending on the starting and ending od peaks and troughs. Modified MAGE-CGM is used where based on the direction of change, either upwards or downwards, of the previous and next data points a peak or trough is selected.

Average daily risk ratio

The average daily risk ratio (ADRR) is after transforming each glucose value, a risk value is attributed to the point of transformation.

Lability Index

The formula for Lability Index (LI) calculates for three glucose values and then picks up the next three, and repeats the same. The LI is calculated as the mean of these values whose default used is 60 min.

J-Index

J-Index is calculated as shown in the table using a simple formula.

Low Blood Glucose Index and High Blood Glucose Index

These are calculated by switching sugar values into risk scores. If the risk score lies below 0, then it is LBGI, and if it rises above 0, then it is HBGI.

Continuous overlapping net glycemic action (CONGA)

CONGA is computed by first estimating the difference between values intervals, and the difference is used for calculation. The default interval is 60 min or CONGA1 which can be changed.

Mean of daily differences (MODD)

MODD calculated as the mean of difference between values on many other days but at same time of the day.

Glycemic Risk Assessment in Diabetes Equation(GRADE)

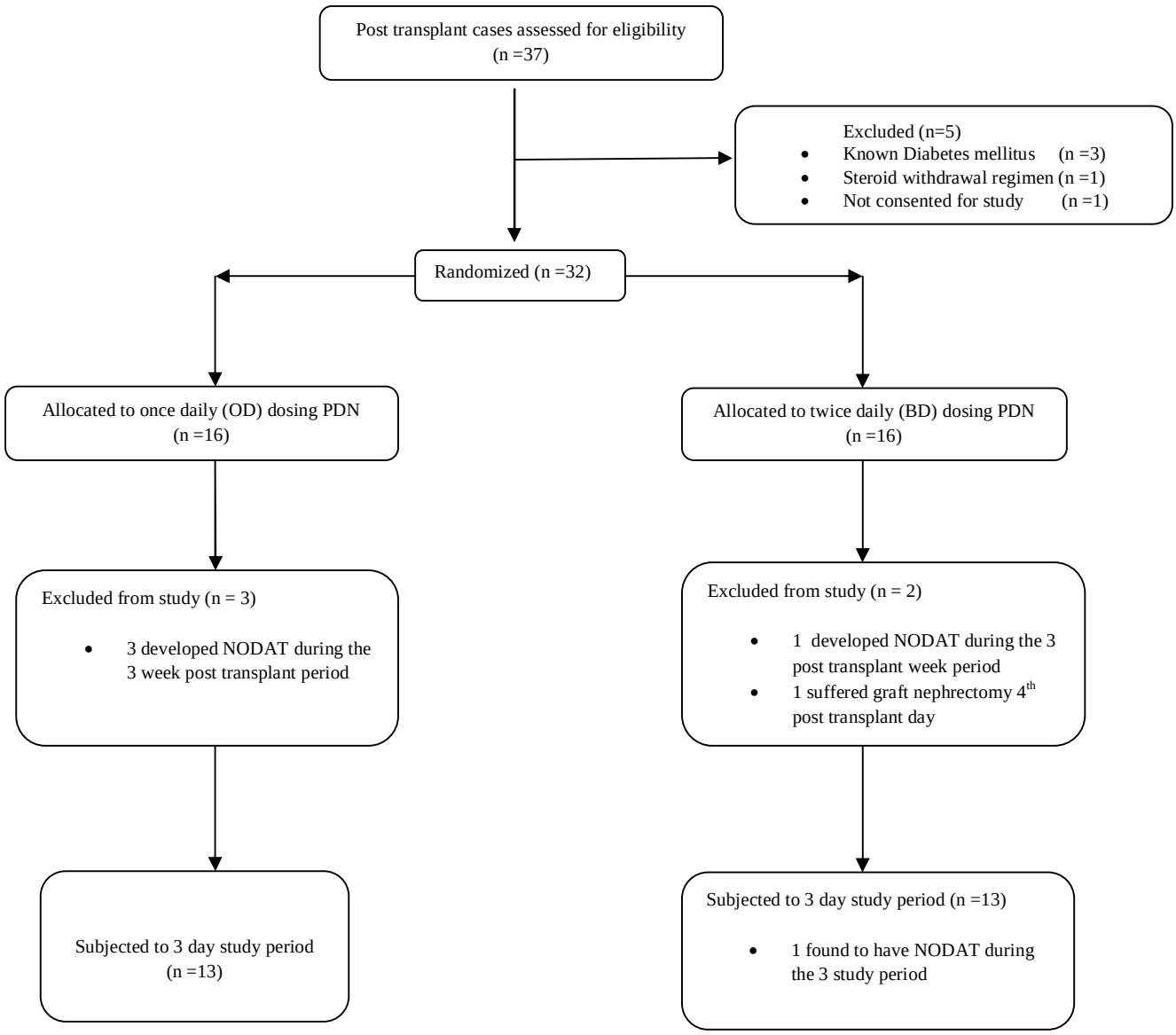
GRADE converts sugar values to a risk score, computes median, and also do the risk assessment linked to hypo and hyperglycemia.

Mean absolute glucose(MAG)

MAG is the addition of differences between successive values of sugar divided by the total duration in hours.

In our study among the formulas, MAGE, CONGA, J-index and MAG are used for assessing glycemic variations between the two groups.

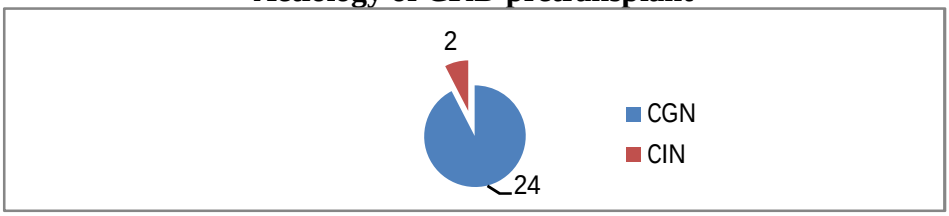
Results



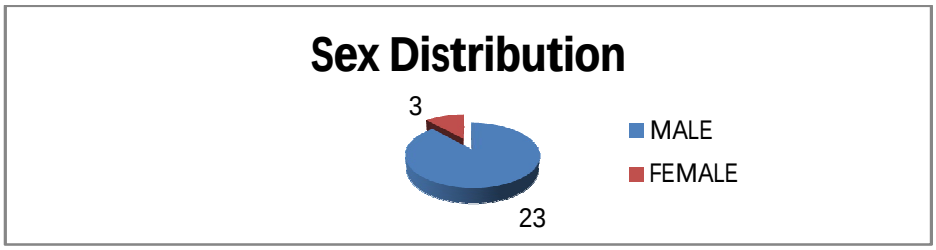
32 post renal transplantation patients were subjected to our study during the prescribed period, 16 patients under BD group and 16 patients under OD group. One patient from BD group and three from OD group developed NODAT around the second week after transplantation. One patient from BD group was found to have NODAT during the 3 days study period. So totally 5 patients (2 from BD and 3 from OD group) developed NODAT and got excluded from the study and all the 5 required insulin therapy for their glycemic control. One patient from BD group had failed transplant needing graft nephrectomy on the fourth day of post transplantation due to vascular thrombosis which was considered as a surgical complication. The remaining 26 patients were divided into BD or OD group by stratified randomization. The interesting

observation is that though patients from both groups developed NODAT as a complication, the two patients from BD group suffered from pre transplant Hepatitis C Genotype 1 infection. Two other hepatitis C patients from BD group were not complicated by NODAT. All the three NODAT patients from OD group had hepatitis negative serologies.

Aetiology of CKD pretransplant



The aetiology of renal failure among these subjects are chronic glomerulonephritis in 24 and chronic interstitial nephritis in 2 patients.



Gender	Number	Percentage
Male	23	88.46 %
Female	3	11.59 %

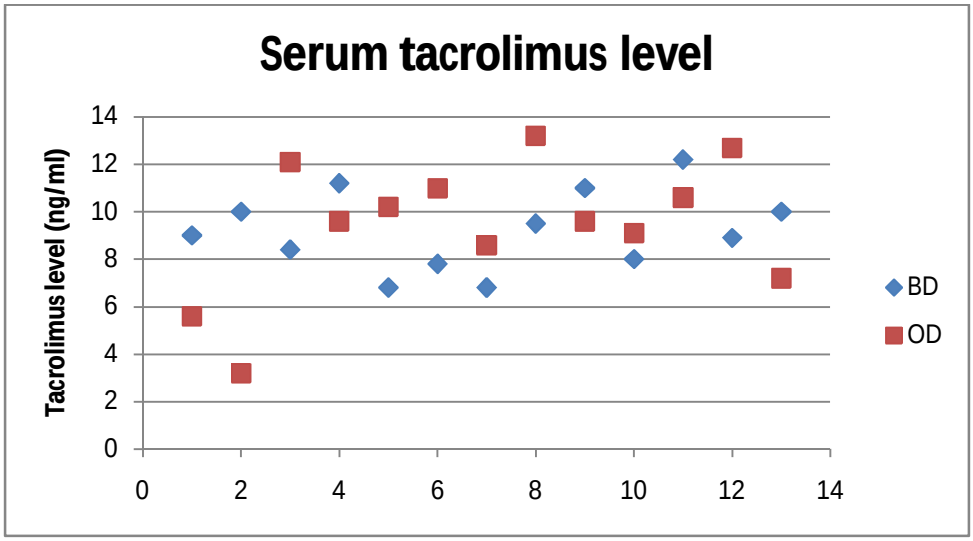
Among the 26 subjects 23 (88.46%) were males, the remaining 3 (11.59%) were females. The median age for the study group was 32 with the youngest being 17years and eldest 57years.

PDN dose	Mean	S.D	t	df	level of significance
BD (n=13)	23.8462	2.19265	.426	24	0.674 >0.05
OD (n=13)	23.4615	2.40192			Not Significant

The median prednisolone dosage for both the groups at the end of 3 weeks was 25mg/day.

Tacro level wk1	Mean	S.D	t	df	level of significance
BD (n=13)	9.9308	5.36724	-.666	24	0.512 >0.05
OD (n=13)	11.1308	3.66842			Not Significant

The median tacrolimus levels were 9 ng/ml for BD and 9.6 ng/ml for OD group (P value=0.79) which fell within the range of 8-12 ng/ml for a 3 week post transplant period.



Scatter diagram showing near equal distribution of tacrolimus levels among the two groups

Cr	Mean	S.D	t	df	level of significance
BD (n=13)	1.0308	.14936	-2.593	24	0.016 <0.05
OD (n=13)	1.4231	.52465			Significant

The serum creatinine levels were 1mg/dl in BD and 1.3mg/dl in OD group with a significant difference (p value -0.016).

Sr Cortisol (µg/dl)	Mean	S.D	t	df	level of significance
BD (n=13)	4.7815	3.84950	-1.095	24	0.285 >0.05
OD (n=13)	6.8077	5.45138			Not Significant

The cortisol levels were 4.78 and 6.8 in BD and OD group (p value-0.28) both falling under the category of adrenal suppressed state with a cut-off value of less than 6(µg/ml).

Hb	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	8.6769	.84079	.896	24	0.379 >0.05
OD (n=13)	8.3769	.86713			Not Significant

There is no statistically significant difference (p- 0.379) among the two groups with regard to haemoglobin levels.

HbA1C	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	5.1077	.33282	-2.370	24	0.026 <0.05
OD (n=13)	5.5231	.53721			Significant

The mean HbA1C was 5.1mg/dl and 5.5mg/dl among BD and OD groups respectively with a statistically significant difference having a p value of 0.026 was observed though HbA1C is not reliable in the immediate post transplant state up to 3 months.

Na	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	138.3846	3.90595	.074	24	0.942 >0.05
OD (n=13)	138.2308	6.43109			Not Significant
K	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	4.3923	.53145	1.428	24	0.166 >0.05
OD (n=13)	4.0154	.78935			Not Significant

There was no significant difference among the serum sodium and potassium among the groups.

Two patients from OD group had positive urine culture reports with E.coli and klebsiella, none from BD group.

SERUM LIPID PROFILE

Cholesterol	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	203.5385	46.68800	-.439	24	0.665 >0.05
OD (n=13)	210.2308	28.99182			Not Significant
TGL					
BD (n=13)	147.4615	54.56130	-1.648	24	0.112 >0.05
OD (n=13)	180.8462	48.53323			Not Significant
LDL					
BD (n=13)	126.6154	43.26187	-1.196	24	0.243 >0.05
OD (n=13)	144.7692	33.49168			Not Significant
HDL					
BD (n=13)	48.0769	24.04670	.524	24	0.605 >0.05
OD (n=13)	43.6154	19.05491			Not Significant
VLDL					
BD (n=13)	34.5846	18.62967	.861	24	0.398 >0.05
OD (n=13)	28.5846	16.86416			Not Significant
Cho/HDL	**Mean**	**S.D**	**t**	**df**	**level of significance**
BD (n=13)	5.4433	3.14024	-.367	24	0.717 >0.05
OD (n=13)	5.8804	2.92130			Not Significant

The mean cholesterol, TGL, LDL and VLDL were higher and HDL lower in OD group when compared with BD group though none are statistically significant. Also the cholesterol/HDL ratio represents high risk for both groups though with OD comparably higher than BD group.

Athens scale	**Median**	**S.D**	**t**	**Df**	**level of significance**
BD (n=13)	2	1.609	-1.347	24	0.190 >0.05
OD (n=13)	3	4.889			Not Significant

The median Athens sleep score was 2 in BD and 3 in OD group (p value-0.19) which showed no significant sleep disturbances in both the groups. Many of them complained of daytime somnolence but not as a major problem.

4th Hourly Glycemic Measurements

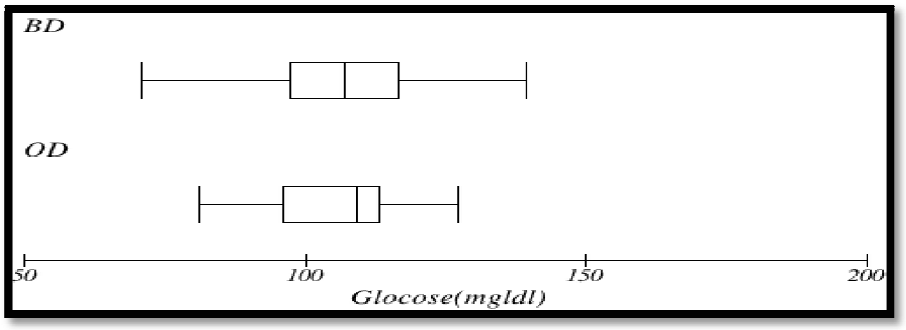
The average or mean glucose with BD dosing was almost the same with that of OD dosing, mean 129.2 versus 131 mg/dL (P <0.68). But when analyzed with that of 4th hourly sugar levels, we can observe drastic difference between the two groups. The mean glucose levels at the sampling timing as shown in the below table also indicates lower levels with BD group than the OD group that were statistically significant except the 8AM sample.

TIMING	MEAN SUGARS OD (mg/dl)	MEAN SUGARS BD (mg/dl)	P VALUE
8AM	105.5	106.4	0.08
12	168.2	139.2	**0.0001**
4PM	186.8	159.3	**0.0002**
8PM	104.6	122.8	**0.0022**
12	125.5	138.6	**0.04**
4AM	96.3	109.4	**0.018**
24 hours	131	129.2	0.68

Mean Sugars Between Two Groups

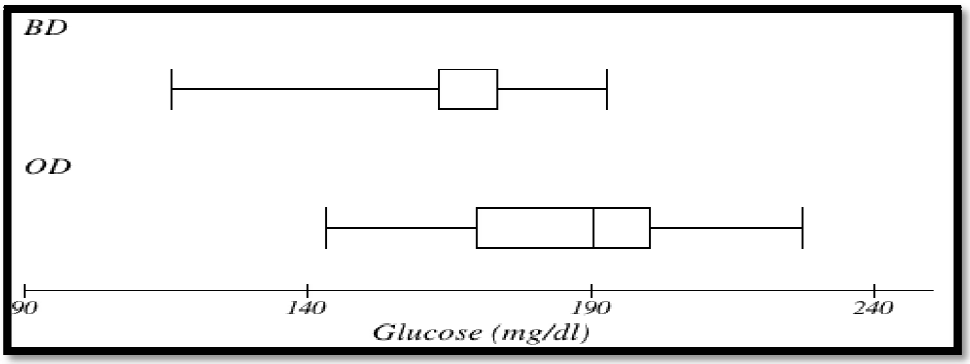
The average sugar values of each group at different sampling interval have been illustrated with the following charts. Boxes indicate the median sugars in that group. Error bars indicate the range for the group.

MEAN SUGARS AT 8AM

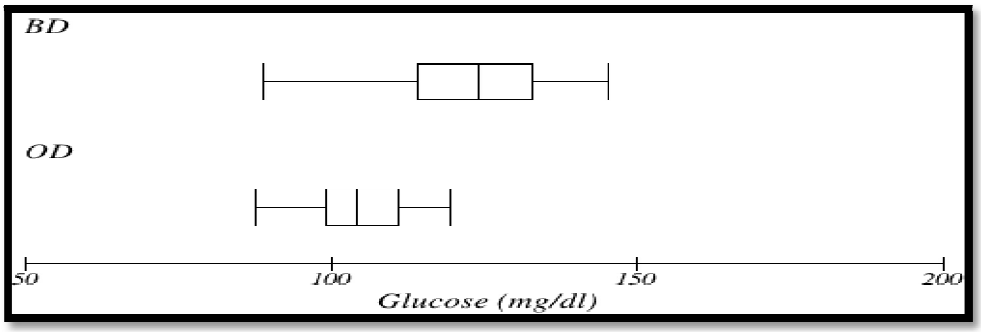


MEAN SUGARS AT 12 PM

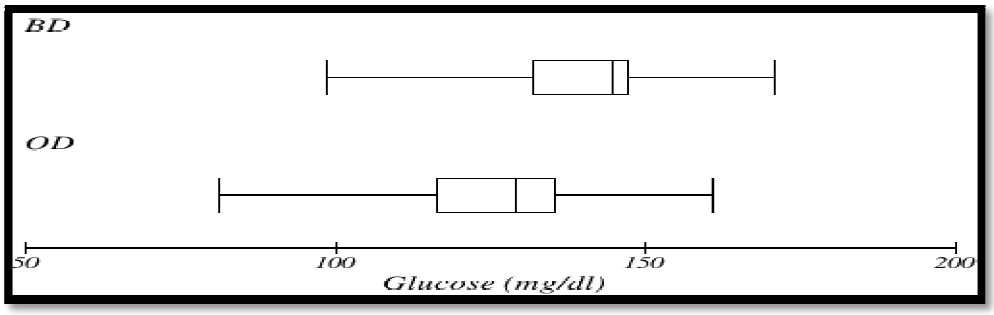
MEAN SUGARS 4 PM



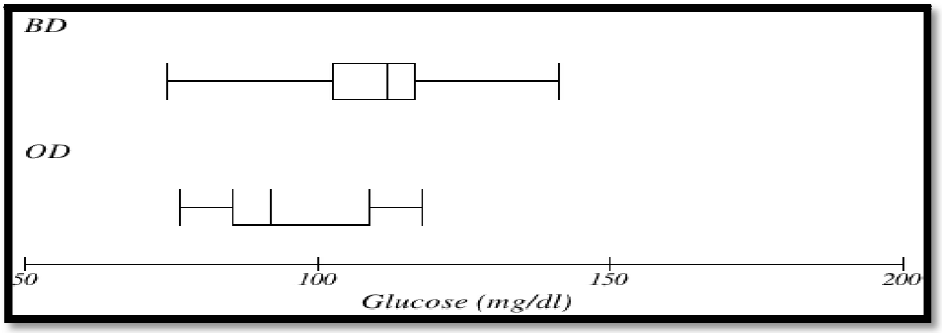
MEAN SUGARS 8 PM



MEAN SUGARS 12 AM



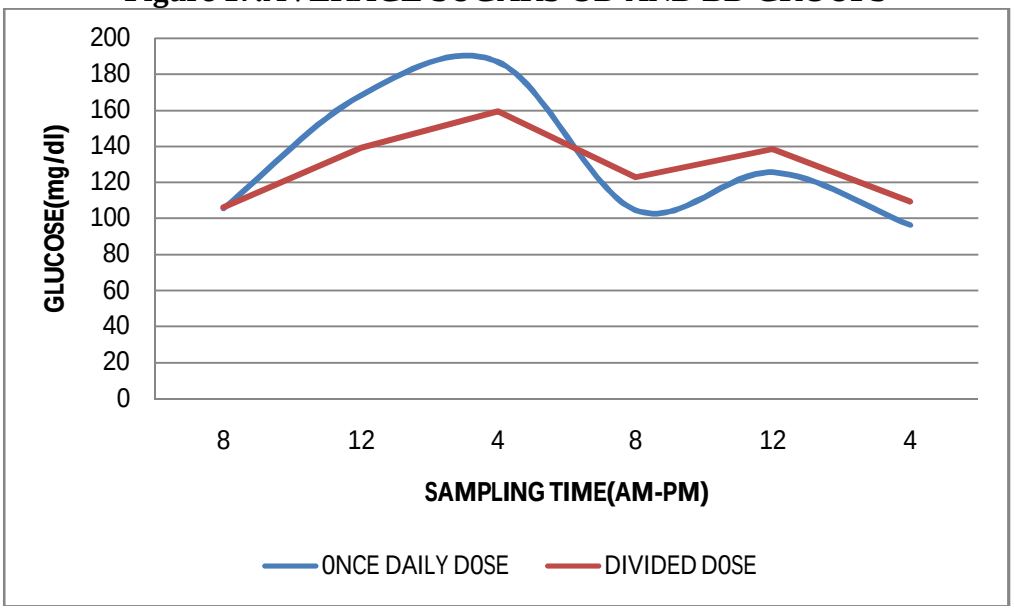
MEAN SUGARS 4 AM



Only with continuous glucose monitoring (CGM), the peak and trough levels can be exactly calculated, and the timing of occurrence of that events can be noted. So here with our 4th hourly sugar levels we can analyze the highest and lowest glucose levels of the day between the groups. The highest glucose was lower with BD than OD group, median 206 versus 216 mg/dL ($P < 0.007$). Glucose levels of $\geq$ 200 mg/dL occurred in 6 of 13 (46.1%) patients while taking OD, compared with 2 of 13 (15.3%) patients while taking BD dose of prednisolone ($P = 0.045$). Even when relaxing glucose level to $\geq$ 180 mg/dL, there were 11 of 13 (84.6%) OD patients versus 4 of 13 (30.8%) BD patients.($P=0.034$).

Sugars	>180mg/dl	>200mg/dl
Number of BD patients	4	2
Number of OD patients	11	6

Figure 17.AVERAGE SUGARS OD AND BD GROUPS



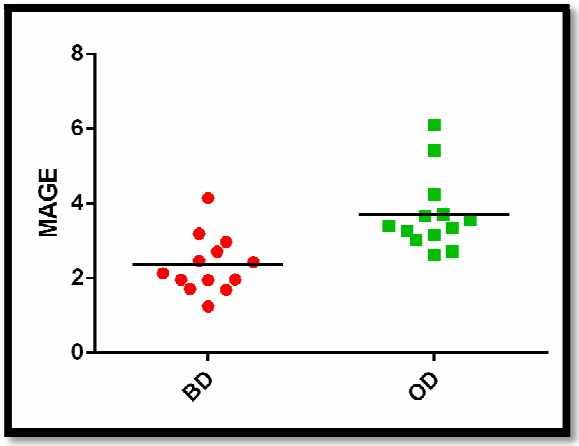
The mean 24 hour glycemic profiles at various sampling times for BD and OD dosages are shown as above. In that graph, following a single daily dose of prednisolone at 8AM, mean venous glucose was highest at 4PM in both groups with OD group sugar (186.8mg/dl) much more higher than the BD group(159.3mg/dl). Also the lowest sugar levels were noted at 4AM

with OD group sugar (96.3mg/dl) much more lower than the BD group(109.4mg/dl). At 8PM also lower values were observed with OD (104.6 mg/dl) much lower than BD group(122.8mg/dl).

Glycaemic variability indices namely MAGE, CONGA, J-index and MAG was low in BD group than in OD group as shown in tables and dot blot charts below.

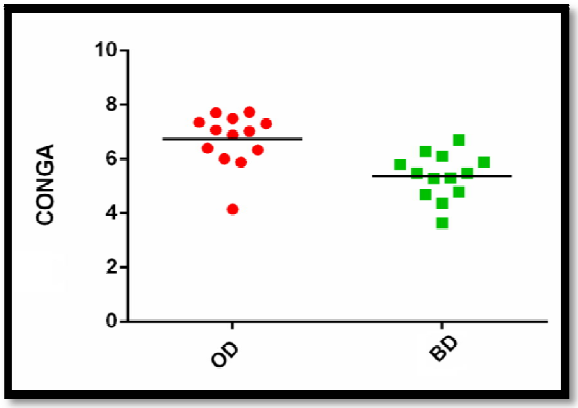
MAGE

MAGE	MEAN	MEDIAN	P value
OD	3.70	3.38	0.0007
BD	2.34	2.12	



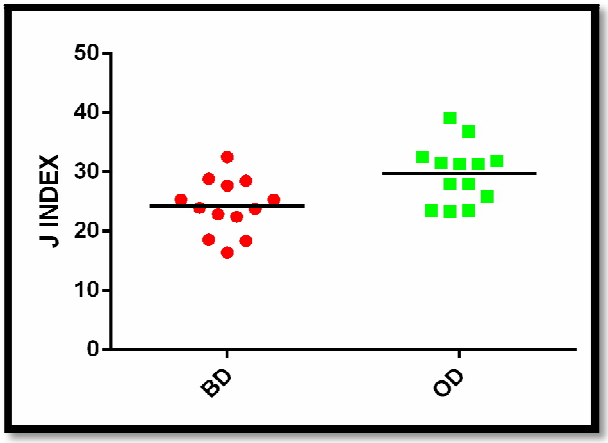
CONGA

CONGA	MEDIAN	MEAN	P value
OD	7.00	6.70	0.0009
BD	5.47	5.36	



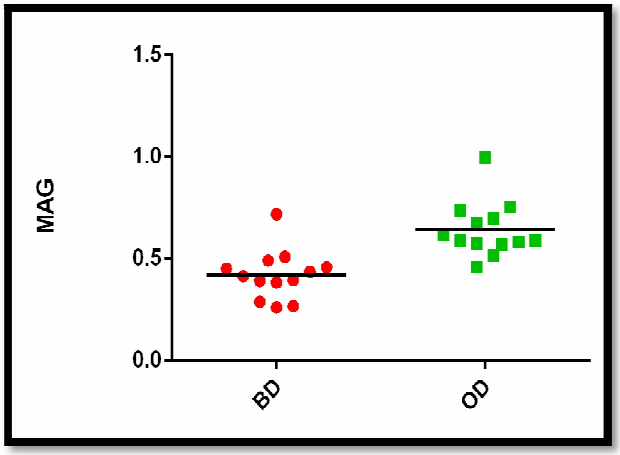
J INDEX

J INDEX	MEAN	MEDIAN	P VALUE
OD	29.6	31.3	0.007
BD	24.1	23.8	



MAG

MAG	MEAN	MEDIAN	P VALUE
OD	0.64	0.589	0.0001
BD	0.41	20.412	



All the above statistical formulas (detailed in methodology section) to assess the fluctuations of glucose, the culprit for oxidative stress injury, shows that BD group has a narrow range variation with that of mean glucose when compared with OD group.

Discussion

Prednisolone being the prime drug in renal transplantation, the dosage protocol remains unresolved, either once daily (OD) in view not to suppress the HPA axis or divided dose. Many studies state both are equally efficacious and some studies show higher efficacy when administered twice daily. Only few studies are available for post transplant setting. Particular emphasis is given to minimize glycemic variability due to its association with oxidative stress and endothelial dysfunction leading to more incidences of coronary events and 'Post-transplant diabetes mellitus(PTDM)' or NODAT. This adverse effect becomes multiplied in post transplant setting with numerous risk factors such as hypertension, impact of other immunosuppressive drugs like calcineurin inhibitors, dyslipidemia and rapidly induced erythropoiesis by the functioning kidney. Cardiovascular mortality is the leading cause of post transplant deaths ahead of infections. With this idea in mind, this study has been planned to compare the side effects of OD and BD prednisolone dosing with importance to dysregulated glucose metabolism.

A study demonstrated in the early postoperative period that those patients who suffered from acute rejection showed the clearance of methylprednisolone was much higher than those without rejection. The elimination half-life t1/2 of methylprednisolone after oral intake was about 2.5 hours which was shorter than 2.9 hours seen in patients with no rejection episodes. Hence, a low-dose steroid regimen divided twice daily might be efficacious in avoiding rejections post kidney transplantation.

Steroid elimination strategies like "Early steroid withdrawal" (within the first 7 days following transplantation) are highly preferred in recent practice to minimize its side effect. Some centers also follow very rapid taper to 5mg a day at the end of one week post transplant period itself. However no comparative studies exist to highlight the rejection rate and true benefits of each of those protocols. KDIGO transplantation guidelines 2012 suggest that, in patients who are at low immunological risk and who receive induction therapy, corticosteroids could be discontinued during the first week after transplantation though the level of evidence is just 2B.

In high risk transplants and in many centers where still steroid withdrawal protocols have not been considered, they continue to use them at a reasonable dosage even upto three months post transplant. Hence the timing of administering steroids should be considered to minimize glycemic excursions and oxidative injury.

In order to minimize hypothalamic–pituitary–adrenal axis suppression, once daily morning steroid administration is so far preferred. Also prednisolone clearance is lower in the morning compared to evening increasing its efficacy when given in the morning hours of the day. Also there are advantages of less sleep disturbances at night and less mood swings. Split or divided dose strategy of steroids can sustain steady state blood levels since the half life of prednisolone (just 3 hours) appears very short. But there are only limited studies regarding the efficacy of split steroid protocol in renal transplantation.

A study showed that a twice daily steroid dosage has more sustained immunosuppression when used in low-dose regimens. Another study involving 16 transplanted patients and 35 glomerulonephritis patients report twice divided dose was found to be more efficacious and less diabetogenic comparing to the standard once daily administration.

In our study, 32 patients were allocated into OD and BD groups equally under stratified randomization using lot method. However 3 from OD group and 2 from BD group got excluded due to NODAT during the 3 week post transplantation period. All the five were started on insulin treatment immediately. One female patient from BD group also got excluded from the study since she had a graft nephrectomy done due to vascular thrombosis which was attributed to surgery technical reasons. Hence 26 patients, 13 each from two groups underwent 3 day study period.

In our study, among the 26 patients, 23 were males (88.46%) and 3 (11.59%) were females. The median age for the study group was 32 with the youngest being 17years and eldest 57years.

We had four hepatitis C positive patients in our study, who underwent transplantation after achieving sustained virological response (SVR). Of them, two patients were excluded from the study due to NODAT. Unfortunately all the four hepatitis C patients got randomized to BD group. Even though both groups had NODATs (2 from BD and 3 from OD), the two patients from BD were hepatitis C positive, whereas all the three NODATs in OD group were with negative viral serology. We presume that the association of hepatitis C only dragged those two patients to NODAT or else the BD group would have been NODAT-free. Two other hepatitis C patients from BD group have not developed NODAT. Hepatitis C causes fourfold increased risk for NODAT through blocking Insulin-stimulated IRS-1 tyrosine phosphorylation leading to insulin resistance.

Dynamic tests like Insulin Tolerence Test and Corticotropin-releasing hormone test (CRH) are superior in providing information on the reserve capacity, function and the adrenal glands ability through HPA axis to respond to stress. Though serum cortisol level is having very low sensitivity, none of the dynamic tests have cleared the safety approval to be used in post transplant setting and hence not used in our study.The fasting cortisol levels were 4.78 and 6.8 in BD and OD group (p value-0.28) both falling under adrenal suppression state with a cut-off value of less than 10µg/ml . Here we have proven in both the groups, the HPA axis got suppressed.

Tacrolimus causes NODAT through the inhibition of FK binding protein-12 (FKBP12) which is found in abundance in pancreatic islet cells. The median tacrolimus levels were 9 ng/ml for BD and 9.6 ng/ml for OD group(P value=0.79) which fell within the range of 8-12 ng/ml for a 3 week post transplant period. Hence tacrolimus the main confounder for dysglycemia has been removed.

There was no significant difference (p- 0.379) in the hemoglobin levels among two groups. There was a significant difference noted in the glycosylated haemoglobin values (p value-0.026) among the two groups though HbA1c estimation is not reliable before 3 months post transplantation.There are studies showing even though HbA1C is in normal range, the high normal value cohort when compared with low normal values has got significant increase in cardiovascular morbidity and mortality due to endothelial dysfunction among non-diabetic hemodialysis patients. However similar studies have not been done so far in post transplant patients. In our study also, though both values are in normal range, a statistically significant decrease of nearly 0.4% (5.5 vs 5.1) HbA1C is observed among BD group patients.

The serum creatinine levels were 1mg/dl in BD and 1.3mg/dl in OD group with a significant difference (p value -0.016) (p value -1.9). There were three deceased donor recipients in our study out of which 2 had dialysis requiring delayed graft function and 1 had slow graft function. Unfortunately all the three got randomised to OD group. This can be one of the reasons for raised mean creatinine levels among the two groups however 2 other live donor recipients from OD group also had graft dysfunction. Long term follow-up is needed to come to a reasonable conclusion. This finding correlates with studies saying divided dose is more efficacious than OD dosing.

There was no significant difference among the serum sodium and potassium among the groups.

Two patients from OD group had positive urine culture reports with E.coli and klebsiella, and none from BD group. Though not significant, this could be the result of higher glucose exposure in OD group that favoured growth of organisms.

The mean cholesterol, TGL, LDL and VLDL were higher and HDL lower in OD group when compared with BD group though none are statistically significant.

Sleep disturbance with hypomaniac activities are troublesome for steroid users as well as caregivers. Night dose prednisolone may further aggravate sleep disturbance and can cause insomnia. Athens Insomnia Scale (AIS) is calculated by assessing 8 factors out of which first 5 are related to nocturnal sleep like sleep induction, awakenings during the night, final awakening, total sleep duration and quality of sleep. The last 3 factors are regarding daytime dysfunction like well-being during the day, functioning capacity during the day and sleepiness during the day. Rating is done on a 0–3 scale basis and the cumulative score of all factors determines an individual's sleep outcome. AIS is regarded as an effective sleep analysis tool and it gets validated in many countries. Insomnia is diagnosed with a cut-off score of ≥6 using AIS. Our study showed no sleep disturbance among OD patients when compared to BD patients. (BD 2 versus OD 3; p=0.190)

4th Hourly Glycemic Measurements

In our study, the mean glucose with BD dosing was almost the same with that of OD dosing, mean 129.2 versus 131 mg/dL (P <0.68).

But 4th hourly sugar levels of our study shows significantly lower mean levels with BD group than the OD group except for the 8AM sample. The highest glucose in BD group was lower than OD, median 206 versus 216 mg/dL (P < 0.007). Glucose levels of ≥ 200 mg/dl occurred in 6 of 13 (46.1%) patients while taking OD, compared with 2 of 13 (15.3%) patients while taking BD dose of prednisolone (P = 0.045). Even when relaxing glucose level to ≥ 180 mg/dl, there were 11 of 13 (84.6%) OD patients versus 4 of 13 (30.8%) BD patients.(P=0.034).

It was observed in our study that the mean venous glucose was highest at 4PM in both groups with OD group sugar (186.8mg/dl) much higher than the BD group (159.3mg/dl). Also the lowest sugar levels were noted at 4AM with OD group sugar (96.3mg/dl) much more lower than the BD group(109.4mg/dl). At 8PM also lower values were observed with OD (104.6 mg/dl) much lower than BD group(122.8mg/dl). The fall of sugar from a height of 186.8mg/dl at 4PM to a plateau of 104.6 mg/dl at 8PM is the bad event cautioned by many studies that might exaggerate glycemic variability injuring endothelium including coronaries. Also this fluctuation

is also proposed to cause hypoglycaemia too that can correlate with lower glucose levels of OD than BD dosage in our study.

Glycaemic variability scores namely CONGA, MAGE, J INDEX, MAG were significantly lower in BD group.

All the above statistical formulas (detailed in methodology section) to assess the fluctuations of glucose, the culprit for oxidative stress injury, shows that BD group has a narrow range variation with that of mean glucose when compared with OD group. Many studies conclude that the fluctuating glucose is much worse than persistently high glucose.

Follow-up report of patients

The efficacy comparison of the two groups within this short period is not reliable and long term follow-up is needed to have a conclusion. Three patients from OD group developed acute cell mediated rejection on follow-up beyond 3 months post transplant. To date 6 patients have successfully completed BD regimen to enter into low dose OD regimen. Five patients are still under the BD regimen. One male patient from BD group after 4 months of transplantation due to non compliance of immune suppressants, developed acute antibody mediated rejection leading to graft loss. Another male patient from BD group who persistently had low tacrolimus levels pulmonary and rhinocerebral mucormycosis and with amphotericin B, partial pneumonectomy and withdrawal of immunosuppressants we could save the patient with graft loss. All the other patients are now under regular follow-up with good graft function. Long follow-up is needed to compare the outcomes of the two groups.

Conclusions

1) The glycemic variability measures are significantly lower in divided dosing group than the single dosing group.
2) The highest and lowest sugar levels were noted in once daily regimen indicating wide glycemic variability.
3) HbA1C was significantly lower in the divided dose than the once daily dose group.
4) Patients from divided dose group has not developed NODAT except those with associated Hepatitis C infection.
5) Serum creatinine level was significantly higher in once daily regimen than divided dose regimen.
6) Fasting serum cortisol level at 8 AM appears suppressed even with once daily regimen.
7) Athens insomnia score among both groups showed no sleep disturbance.
8) Culture positive urinary tract infections were observed in once daily regimen.

Limitations Of The Study

1. The number of subjects in our study was small.
2. This study could not be blinded since every post transplant patient should clearly know the dose, duration and changes of their immunosuppressive medications.
3. Continuous glucose monitoring (CGM) was not used which can continuously record fluctuations of glucose so that peak and trough levels along with their exact timing of

occurrence and exact glucose variability could be assessed. But CGM is less sensitive than venous glucose oxidase method,

4. The observational period was very short so that long term efficacy and side effect profiles of both groups could not be analyzed.
5. The twice divided group got converted to once dose when the daily dose falls below 10 mg. So the effects of divided dose on long term maintenance regimen could not be followed up.

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We assure that this study has been done at Govt Kilpauk Medical College Hospital, Chennai, Tamilnadu, South India.

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