Recipient Pre-Operative Neutrophil Lymphocyte Ratio Better Predicts Delayed Graft Function Than Platelet Lymphocyte Ratio in Donation After Brain Death Kidney Transplantation.

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ABSTRACT

Background : Neutrophil lymphocyte Ratio (NLR) and Platelet lymphocyte Ratio (PLR) are an indicator of the status of inflammation. The objective of this study was to evaluate the relationship between recipient pre-operative Neutrophil lymphocyte Ratio (NLR) and Platelet lymphocyte Ratio (PLR) with delayed graft function in the kidney transplant patient. **Methods:** The preoperative full blood count, data regarding patient demographics and postoperative graft function was retrospectively evaluated from the database of our institution. All statistical calculations were carried out using SPSS 20.0 version(SPSS Inc., Chicago, IL, USA).A p-value<0.05 was considered statistically significant. **Results:** 289 patients were included in this study. DGF occurred in 33 cases. Elevated preoperative NLR had a sensitivity of 75.75% and specificity of 76.56% whereas elevated preoperative PLR had a sensitivity of 72.72% and specificity of 58.20% for predicting DGF. The area under the ROC curve was found to be 0.762 and 0.655 for NLR and PLR, respectively. Multivariate analysis showed NLR>3.5 and PLR>120 independently responsible for DGF.**Conclusion:**Recipient preoperative NLR and PLR can predict the occurrence of DGF following DBD renal transplantation. In addition, NLR is better than PLR in predicting DGF. DGF prolongs the total ICU and in-hospital stay.

Keywords: Neutrophil lymphocyte Ratio,Platelet lymphocyte Ratio, Delayed graft function, Kidney transplantation, Inflammation.

BACKGROUND

Kidney transplantation is the treatment of choice for the end-stage renal diseases (ESRD). Lots of advancement have been made in kidney transplantation since its commencement. Despite the advancements and ongoing research for the quest of a better outcome, various complications had hindered the graft survival and overall survival of the patients. Delayed graft function (DGF) is the common early complication of the kidney transplantation. Its incidence can vary from 2%-50% regarding different centers and their individual definitions[1]. There are various definitions of DGF, but most widely used isthe UK transplant definition "the requirement for dialysis within the first week after transplantation, unless this was performed for hyperkalemia"[2]. DGF results from immunologic and non-immunologic events that start during kidney preservation and progress after the time of reperfusion. Transplanted organs are subjected to ischemia-reperfusion injury, which is proportional to the length of cold (CIT) and warm ischemia times (WIT). Extended periods of CIT and WIT increase the damage caused by ischemia-reperfusion injury and subsequently can cause DGF. Neutrophils and platelets are thought to play an important role in the development of ischemia-reperfusion injury accumulating at sites of injury, adhering to the endothelium, releasing toxic metabolites, and increasing tubular and capillary leakage[2,3].The occurrence of DGF is associated with short-term problems like a longer hospital stay, higher incidence of acute rejection [4];andlong-term problems like graft loss and decreased patient survival[5].

The imbalance between the supply and demand of organs for transplantation has led to the increasing use of organs from extended criteria donors. Use of ECD organs significantly contributes to the occurrence of DGF. Preoperative prediction of probable occurrence of DGF in the recipients will help to carefully select the appropriate recipient. Since neutrophils and platelets contribute to the development of DGF, it will be beneficial to see the relation of NLR and PLR with the development of DGF in kidney transplant recipients. If a positive relation is found between them, then it will guide towards developing various intervention protocols and methods and hence help towards reducing the incidence of DGF in the future.

MATERIAL AND METHODS

Patients:

From 2013 to 2016, there were 303 kidney transplant recipients from donation after brain death (DBD) donors at our center. Recipients were excluded if the immediate preoperative full blood count was not available (within 24 hours from transplantation), if the recipients developed postoperative vascular complications requiring intervention that could result in DGF, or if they developed primary non-function. From the total number of patients, 14 patients were excluded from this study because 13 patients didn't have a complete blood count data done within 24hrs before the surgery and one patient died within seven days from the surgery. So, 289 patients were included in this study. Demographic, hematological and clinical data of these patients were collected from the patient's database of our center. NLR, PLR was calculated from the full blood count archived from the database of the institute. NLR was defined as the ratio of neutrophil count to lymphocyte count, value of both taken from a single complete blood count done in our center within 24 hr before the surgery. PLR was defined as the ratio of the platelet count to lymphocyte count, value of both taken from a single complete blood count done in our center within 24 hr. before the surgery. Receiver operating characteristic (ROC) curve analysis revealed NLR (>3.5) and PLR (>120) to be the most sensitive and specific determinant of DGF. So, NLR (>3.5) and PLR (>120) was considered elevated in this study.CIT was calculated from the start of abdomen perfusion during procurement until the end of hypothermic machine perfusion (HMP). WIT was calculated from the end of HMP until kidney reperfusion on recipient. We defined DGF as the requirement for dialysis within the first week after transplantation, unless this was performed for hyperkalemia, according to the UK transplant definition. Resistive Index (RI): RI was analyzed in the interlobar and segmental arteries and was calculated according to the following formula: RI = (peak systolic velocity) - (minimum diastolic velocity) / (peak systolic velocity).

A standardized immunosuppressive regimen was used for all kidney transplant recipients included in the study: 500mg of methylprednisolone and 20 mg

of Basiliximab at induction. Immunosuppression was maintained with Tacrolimus (0.1 mg/kg divided into 12 hourly doses) and Mycophenolate Mofetil (750 mg twice daily).

STATISTICAL ANALYSIS

Categorical values were evaluated using Chi-square test or Fisher's exact test. Continuous variables were expressed as Mean ± S.D. The normally distributed variables were compared using the Student t-test whereas the abnormally distributed variables were compared using the Mann-Whitney U test. Binary logistic regression analysis was used to investigate factors affecting the development of delayed graft function. A p-value<0.05 was considered statistically significant. All statistical calculations were carried out using SPSS 20.0 version(SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 289 patients were included in this study. 206 (71.3%) were male whereas 83 (28.7%) were female. The median age of the patients was 40 years with a mean and standard deviation of 41.16±10.61. The median body mass index (BMI) was 24kg/m^2 with a mean and standard deviation of 23.38±3.23. The median duration of dialysis was 13 months with a mean and standard deviation of 22.12±23.16. The median value for NLR in our patient was 3.22 with a mean and standard deviation of 3.11±0.96 whereas the median PLR was 115 with mean and standard deviation of 123.29±54.56. The median value for mean platelet volume was 9.2 with a mean and standard deviation of 9.44±2.70. Albumin had a mean of 44.23±6.56 with a median of 44.2. The median plasma fibrinogen was 3.41 with mean and standard deviation of 3.46±0.79. The median WIT was 17 with mean and standard deviation of 17.04±1.69 whereas median CIT was 9 with mean and standard deviation of 8.97±1.77. The resistive index in the Doppler ultrasonography done in the first post-operative day had a median value of 0.69 with a mean and standard deviation of 0.69±0.09. The median duration of total ICU stay was 5 days with a mean of 5.08±2.46 days while total hospital stay of the patients that underwent kidney transplantation had a mean of 26.41±13.91 days with a median of 24 days.

DGF occurred in 33 (11.4%) cases and 25 (75.8%) of these patients had NLR more than 3.5, hence providing elevated preoperative NLR a sensitivity of 75.75% for predicting DGF. While 8(24.2%) cases of DGF had NLR less than 3.5. Sixty patients with primary graft function (PGF) had elevated NLR while 196 patients with primary graft function had normal NLR, therefore, NLR >3.5 had a specificity of 76.56% for DGF. In the ROC curve analysis, the area under the ROC curve was found to be 0.762 **(fig: I).**

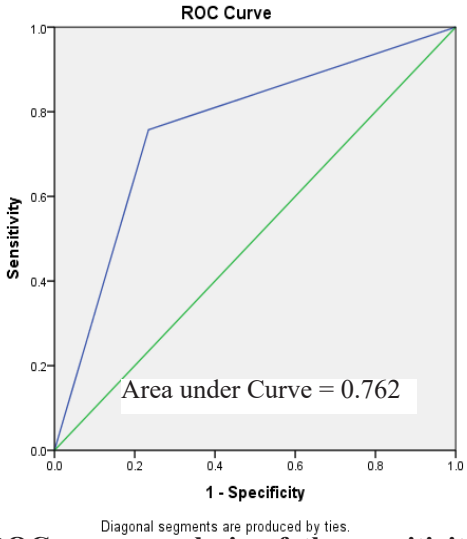

Fig I: ROC curve analysis of the sensitivity non-specificity of NLR for predicting DGF.

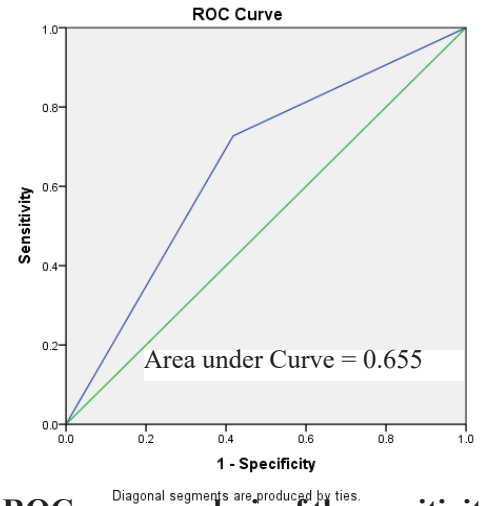

Fig II: ROC curve analysis of the sensitivity non-specificity of PLR for predicting DGF.

24(72.7%) cases with DGF had PLR more than 120 thus providing elevated PLR a sensitivity of 72.72% for predicting DGF meanwhile 9 (27.3%) cases of

DGF had PLR less than 120. Out of the total patients, 131(45.3%) had PLR more than 120 whereas 158 (54.7%) had PLR less than 120, therefore, giving PLR>120 a specificity for DGF of 58.20%. In ROC curve analysis **(fig: II)**, the area under the curve was found to be 0.655 for PLR. Comparison of clinico-demographic characteristics between patients with DGF and PGF is presented in **Table I**.

Table I: Comparison of Clinico-demographic characteristics in between DGF and PGF

Variables	DGF	PGF	P value
Age (in years)	40.12±8.82	41.30±10.83	0.548
>40 (n)	15	128	0.623
<40 (n)	18	128	
Gender (n)	Male:19	Male:187	0.064
	Female:14	Female:69	
BMI (kg/m²)	23.79±3.11	23.32±3.24	0.439
>25 (n)	10	65	0.545
<25 (n)	23	191	
Duration of Dialysis (in months)	29.36±28.99	21.18±22.20	0.056
Cause of Renal Failure			0.304
Diabetes (n)	2	6	
Hypertension (n)	1	20	
Others (n)	30	230	
Type of Dialysis			0.031*
Hemodialysis (n)	22	211	
Peritoneal Dialysis (n)	11	45	
NLR	4.12±1.11	2.98±0.86	0.000*
>3.5 (n)	25	60	0.000*
<3.5 (n)	8	196	
PLR	162±67.93	118.24±50.58	0.000*
>120 (n)	24	107	0.001*
<120 (n)	9	149	
Mean Platelet Volume (fL)	9.32 ± 1.59	9.46 ±2.81	0.782
Albumin (g/L)	44.75 ±7.91	44.17 ± 6.38	0.631
Plasma Fibrinogen (g/L)	3.74±0.92	3.42±0.77	0.045*
Warm Ischemia Time (in minutes)	16.94±1.76	16.85±1.69	0.705
Cold Ischemia Time (in hours)	9.24±1.60	8.93±1.80	0.349
Resistance in flow	0.72±0.096	0.69±0.092	0.080
Total ICU stay (in days)	6.61±2.12	4.48±2.44	0.000*
Total hospital stay (in days)	37.45±14.19	24.99±13.91	0.000*

DGF: Delayed Graft Function; BMI: Body Mass Index; NLR: Neutrophil Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio; WIT: Warm Ischemia time; CIT: Cold Ischemia time.

NLR>3.5 and PLR>120 were found to have an ample effect on the development of DGF on multivariate analysis. **(Table II)**.

Table II: Multivariate analysis of factors affecting DGF

Variables	P value	Odds Ratio	95% Confidence Interval
Age (in years) **<40 (n=146)** **>40 (n=143)**	0.822	0.909	0.397-2.083
Gender **Female (n=83)** **Male (n=206)**	0.478	1.379	0.567-3.354
BMI (kg/m²) **<25 (n=214)** **>25 (n=75)**	0.113	2.205	0.830-5.859

Type of Dialysis	0.311	2.205	0.830-5.859
Peritoneal Dialysis (n=56)			
Hemodialysis (n=233)			
NLR **<3.5 (n=204)** **>3.5 (n=85)**	0.000*	13.487	4.788-37.989
PLR **<120 (n=158)** **>120 (n=131)**	0.015*	3.163	1.256-7.963
Plasma Fibrinogen (fL)	0.431	1.244	0.722-2.142
Warm Ischemia Time (in minutes)	0.360	1.120	0.879-1.427
Cold Ischemia Time (in hours)	0.334	0.886	0.693-1.133
Resistive Index	0.387	0.121	0.001-14.418

DGF: Delayed Graft Function; BMI: Body Mass Index; NLR: Neutrophil Lymphocyte Ratio; PLR: Platelet Lymphocyte Ratio; WIT: Warm Ischemia time; CIT: Cold Ischemia time.

DISCUSSION

DGF is one of the most frequent and serious immediate postoperative complications of renal transplantation. Various risk factors related to donor, recipient, organ preservation and surgery have been implicated for the causation of DGF [6], however we have tried to evaluate NLR, PLR and frequently reported recipient related risk factors through this study.

Various previous studies have pointed out elevated NLR as the marker of systemic inflammation. Preoperative NLR value has been proposed as a prognostic predictor in many surgeries [7,8], medical conditions [9–15] and cancers [16–18]. Based on the previous reports and an increasing number of studies focusing on the predictive role of NLR on different medical conditions, we hypothesized that it might have some role in the prediction of DGF after kidney transplantation. This study shows that the patients who developed DGF had higher NLR value in comparison to those having PGF. Multivariate analysis showed that patients with elevated NLR (NLR>3.5) were thirteen times more prone to develop DGF than patients with normal NLR (NLR<3.5) (OR=13.487; 95% CI =4.788-37.989;P=0.000). In terms of the result, our study is quite similar to the previous study which showed an association of elevated NLR with the DGF in renal transplant patient [2], however, in their study, the prediction was significant for donation after cardiac death and living donors only. The present study shows the association of NLR with DGF in DBD kidney recipients.

PLR, as a marker of inflammation, has been studied in cardiovascular diseases [19,20]. More recently, PLR has been studied as a prognostic marker in end-stage renal diseases and in patients under dialysis [21–23]. Based on the previous studies, we tried to look for a relationship of preoperative recipient PLR with the DGF in kidney transplant patients. PLR when expressed as a continuous variable, it had a significant contribution on the development of DGF (P=0.000). After stratification of patients as having elevated PLR and normal PLR; elevated PLR on multivariate analysis also showed a significant prediction of DGF (HR=3.163; 95% CI=1.256-7.963; P=0.015). To the best of our knowledge, this is the first study showing the relationship of preoperative recipient PLR with the development of DGF. So, our study points out an area of research in kidney transplant which has a potential for further exploration.

DGF is the consequence of an ischemic injury to the graft aggravated by reperfusion syndrome. White blood cell differentiation, interplay between white blood cells and platelets is related with inflammatory changes, tissue repair /regeneration, and ischemia reperfusion injury. NLR and PLR are based primarily on the physiological link between neutrophilia, thrombocytosis and lymphopenia with systemic inflammation [24]. The possible explanation how upsurge of neutrophil and platelets could result in DGF might be that the higher amount of circulating neutrophils and platelets may lead to

excessive accumulation of these cells in the graft resulting in clogging of renal microvasculature during reperfusion or these cells might have been preoperatively primed to give essential inflammatory response during reperfusion period. Furthermore, previous studies have shown that neutrophils [25], and platelets [26] play imperative role in mediating the inflammatory response after ischemia reperfusion injury in kidney transplantation.

DGF is known to increase the total number of ICU and in-hospital stay. In the present study, the patients who developed DGF had increased duration of ICU and in-hospital stay compared to patients with PGF which was statistically significant (P=0.000). The association of DGF and longer postoperative hospital stay has been reported in many previous studies [27–32]. Furthermore, Salazar et al. also have reported the association of DGF with prolonged postoperative ICU stay [27]. In this aspect, the finding of our study fits along with the reported results of available previous literature. So, it is amenable to say that occurrence of DGF prolongs post-transplant ICU and hospital stay.

Through this study, we were able to show that NLR and PLR values can be used to predict the impending occurrence of DGF in renal transplant patients. However, there are some limitations of the study as it is a single centered retrospective study, even though data were obtained from our patient database which is rigorously maintained and has very few missing value, there is need of further prospective multi-center studies. Variables related to donor management and organ retrieval were not evaluated in this study, which might have some influence over our study. Our sample size is also relatively small compared to sample size obtained from the nationwide or international transplant registries. Despite these limitations, this study has strength with regard to the evaluation of NLR, PLR with the development of DGF in kidney transplant recipients. On the top of this, we believe this is the first study which has compared the efficacy of both NLR, PLR (preoperative) for prediction of development of the DGF.

CONCLUSION

This retrospective study shows that recipient preoperative NLR and PLR are associated with the development of DGF in patients who have undergone DBD renal transplantation. Furthermore, NLR was found to be superior to PLR in predicting the occurrence of DGF. DGF not only prolongs the post-transplant hospital stay but also prolongs the postoperative ICU stay, which might increase the cost of treatment. We do not recommend the instant application of our findings for decision making in clinical practice as it needs further multi-center confirmation before that; but this study definitely widens a new horizon towards preoperative prediction of DGF.

Conflict of interest statement:

None

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