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## A survey on lymphocyte T CD3, CD4 and CD8 in peripheral blood of kidney transplant recipients using mycophenolic acid

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### ABSTRACT

**Introduction:** Immunological monitoring could indirectly measure the suppressive effects of the drugs and provide early guidance on necessary preventive interventions in transplant recipients.

**Objectives:** Our goal was to determine whether mycophenolic acid (MPA) modulates peripheral blood lymphocyte T in kidney transplant recipients.

**Patients and Methods:** We assessed T lymphocytes CD3, CD4 and CD8 in peripheral blood in 30 donors and 35 recipients one day before and 10 days after transplantation using Becton Dickinson's direct immune fluorescent light.

**Results:** Comparisons showed that the number of T lymphocytes CD3+, CD4+, CD8+ in peripheral blood of transplant recipients were lower than donors (TCD3 was  $1690.31 \pm 503.45$  versus  $2280.73 \pm 522.48$ ; TCD4 was  $549.51 \pm 211.72$  cell/ $\mu$ L versus  $766.37 \pm 341.72$  cell/ $\mu$ L and CD8 was  $1134.37 \pm 431.07$  cell/ $\mu$ L versus  $1523.4 \pm 349.23$  cell/ $\mu$ L with  $P < 0.001$ ;  $P = 0.001$  and  $P = 0.0002$  respectively). Additionally, post-transplantation lymphocytes TCD4 decreased in 10/35 of recipients and increased in 22/35 of recipients ( $P = 0.036$ ).

**Conclusion:** The T lymphocytes CD3, CD4 and CD8 in peripheral blood should be monitored at multiple post-transplant times to make early predictions of transplant rejection during follow-up treatment.

### *Implication for health policy/practice/research/medical education:*

To determine whether mycophenolic acid (MPA) modulates peripheral blood lymphocyte T in kidney transplant recipients, we conducted a survey on lymphocyte T CD3, CD4 and CD8 in peripheral blood of 35 kidney transplanted recipients using mycophenolic acid. We assessed T lymphocytes CD3, CD4, CD8 in peripheral blood in 30 donors and 35 recipients one day before and 10 days after transplantation using Becton Dickinson's direct immune fluorescent light. The results showed that the T lymphocytes CD3, CD4 and CD8 in peripheral blood should be monitored at multiple post-transplant times to make early prediction of transplant rejection during follow-up treatment.

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### Introduction

The dual effects of immunosuppressant drugs in organ transplant patients in general and in kidney transplant patients in particular need to be strictly controlled. Currently, the quantitative determination of calcineurin inhibitors has been carried regularly and mycophenolic acid (MPA) has been conducted at major transplant centers around the world to make a correlation between pharmacokinetics and clinical dosage (1). Although

the biopsy provides a definitive diagnosis (2), the less interventional method offers an early diagnosis of transplant rejection, which is more beneficial for patients with kidney transplantation. Moreover, immunological control should be performed before clinical symptoms occurred. The performance of peripheral T lymphocyte assessment also contributes to the risk assessment (3, 4). Weimer et al mentioned the recipient's CD4 T lymphocytes associated with transplant acute rejection (5)

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and an increase in T lymphocytes in the one month post-transplantation of patients are predicted for acute graft rejection (6).

Clinically, post-transplant patients using mycophenolate mofetil or mycophenolate sodium, when ingested will be esterified into MPA, and participate in the process of inhibiting inosine monophosphate dehydrogenase (IMPDH), thereby inhibiting guanine nucleotide biosynthesis for recombinant DNA and cell division. Therefore, the reproduction process of lymphocytes is inhibited, including CD3, CD4 and CD8 T lymphocytes, which are cells involved in transplant rejection.

### Objectives

The objective of the study was to investigate the number of CD3, TCD4 and CD8 T lymphocytes in peripheral blood in patients with pre- and post-transplant kidney transplantation using MPA at Viet Duc hospital, Vietnam.

### Patients and Methods

The patients included two groups; i) control group (donor) of 30 healthy people who were screened including 20 people eligible for blood donation and 10 people eligible for organ donation; and ii) recipient group of 35 kidney transplant patients at Viet Duc hospital, Vietnam in the period February 2014 and November 2015 who was examined at the time of one day before transplantation and 10 days after transplantation.

The survey used cross-sectional descriptive method. To monitor T lymphocyte in peripheral blood on FACSCount machine, we used direct immunofluorescence kit of Becton Dickinson (USA) to determine the number of T-CD4 lymphocytes (cells/ $\mu$ L) and % T-CD4, therefore the percentage and number of cells CD8 and CD3 are calculated.

### Ethical issues

The protocol was approved by the Ethical Review Committee of Vietnam Military Medical University (Reference No.70/2017/VMMU-IRB). The study was also conducted using good clinical practice following the Declaration of Helsinki. Accordingly, written informed consent was obtained from all participants before any intervention.

### Statistical analysis

The data were analyzed by Microsoft Excel 2013 and SPSS16.0. Data were present as numbers of patients, mean  $\pm$  standard deviation or median, maximum and minimum for quantitative variables with normal and abnormal distribution. The results were considered to be significant at  $P < 0.05$ .

### Results

In this study, the kidney transplant patients were selected randomly among patients at Viet Duc hospital, Vietnam from February 2014 to November 2015. The proportion of male kidney transplant patients (62.9%) is higher than female ones (37.1%). The age of patients was between 19 to 61 years old. In this study group, the number of patients (16 patients) diagnosed within one year and received early kidney transplantation is quite high.

Table 1 shows 35 kidney transplant patients (22 male and 13 female) being taken for blood test one day before transplantation and 10 days after transplantation. Patients receive the anti-rejection medication according to the regimen treatment including; inhibiting calcineurin (cyclosporine or tacrolimus) + MPA (cellcept or myfortic) + corticoid

Table 2 shows that the group of pre-transplant patients had the number of TCD3 lymphocytes ranging from 1187 to 2193 cell/ $\mu$ L and TCD4 from 338 to 760 cell/ $\mu$ L. In the control group, the number of TCD3 lymphocytes ranged from 1758 to 2802 cell/ $\mu$ L and TCD4 from 425 to 1107 cell/ $\mu$ L. The differences between the two groups in terms of CD3, CD4 and CD8 cell numbers were statistically significant ( $P < 0.001$ ; 0.001 and 0.0002 respectively). However, different ratios of CD4/CD3 were not statistically significant ( $P = 0.557$ ).

Table 3 shows the results after 10 days of transplantation. At this time, patients had a TCD4 lymphocyte range from 214 to 1244 cell/ $\mu$ L and the number of TCD4 cells decrease in 10/35 patients and increased in 22/35 patients. The difference between pre- and post-transplant patients in TCD4 cell count was statistically significant ( $P = 0.036$ ). However, the remaining parameters were not different significantly.

There were 23/35 patients with AUC 30 to 60 mg/L/h and 3/35 patients with AUC (area under the curve) > 60 mg/L/h (Table 4). The difference between C0 and AUC concentrations at 10 days was not statistically significant ( $P = 0.574$ ).

### Discussion

The research results in Table 2 show that the control group has TCD3, TCD4, TCD8 lymphocytes data which

**Table 1.** Characteristics of kidney transplant patients

Characteristics	Number
Number of patients (male/female)	35 (22/ 13)
Age (y)	19-61
Weight (kg)	38 - 69
Time to detect kidney disease (month)	12-200
Organ donation (alive donor/brain dead donor)	29/ 6
Use MPA (cellcept/myfortic)	35 (24/ 11)

**Table 2.** Comparison of T lymphocyte numbers in pre-transplant patients with healthy people

Lymphocyte	Group of kidney transplant patients (n = 35)	Control group (Group of kidney donors)(n = 30)	P values
TCD3 (cell/ $\mu$ L)	1690.31 $\pm$ 503.45	2280.73 $\pm$ 522.48	<0.001 <sup>a</sup>
TCD4 (cell/ $\mu$ L)	549.51 $\pm$ 211.72	766.37 $\pm$ 341.72	0.001 <sup>b</sup>
Ratio TCD4/TCD3 (%)	33.36 $\pm$ 9.83	32.09 $\pm$ 6.96	0.557 <sup>a</sup>
TCD8 (cell/ $\mu$ L)	1134.37 $\pm$ 431.07	1523.4 $\pm$ 349.23	0.0002 <sup>b</sup>

<sup>a</sup> Student *t* test; <sup>b</sup> Wilcoxon sign rank test.

**Table 3.** T lymphocyte quantity in patients before and after transplant 10 days

Lymphocytes	Pre-transplantation	After transplantation	P values
TCD3 (cell/ $\mu$ L)	1690.31 $\pm$ 503.45	2069.14 $\pm$ 1374.4	0.222 <sup>a</sup>
TCD4 (cell/ $\mu$ L)	549.51 $\pm$ 211.72	729.46 $\pm$ 515.69	0.036 <sup>a</sup>
Ratio TCD4/TCD3 (%)	33.36 $\pm$ 9.83	35.49 $\pm$ 13.77	0.413 <sup>a</sup>
TCD8 (cell/ $\mu$ L)	1134.37 $\pm$ 431.07	1373.03 $\pm$ 1004.71	0.368 <sup>a</sup>

<sup>a</sup> Wilcoxon sign rank test.

**Table 4.** MPA levels of patients after 10 days of kidney transplant

C <sub>0</sub> (mg/L)	AUC <sub>0-12</sub> (mg/L/h)						Total	
	<30		30-60		>60		n	%
	n	%	n	%	n	%		
<1.5	7	20.0	12	34.3	1	2.9	20	57.1
1.5-2.5	2	5.7	10	28.6	2	5.7	14	40.0
>2.5	0	0.0	1	2.9	0	0.0	1	2.9
Total	9	25.7	23	65.7	3	8.6	35	100.0
P values							0.574	

are relatively consistent with the studies of Lien (7) and Thao (8).

Patients with chronic kidney disease often have diabetes, electrolyte disorders, hypertension and anemia. There are also changes in immune parameters – including T lymphocytes – and have been referred to dialysis patients. This is because of being a mismatch in the membrane of the filter and bacterial infection during dialysis that causes an inflammatory reaction in the patient. The exposure of mononuclear cells causes apoptosis (cell death) of lymphocytes (9, 10). The results from Table 2 show that pre-transplant patients had significantly reduced TCD3, CD4, and CD8 lymphocytes compared to the control group since the difference between the two groups in the number of CD3, CD4, and CD8 cells was statistically significant ( $P \leq 0.001$ ; 0.001; 0.0002). Our results are consistent with the research of Lisowska et al (11).

We studied the change of peripheral T lymphocytes in kidney transplant patients using MPA, in 10/35 (28.6%) of patients with TCD4 lymphocyte count reduction (Table 3) while 26/35 (73.3%) patients achieved MPA AUC concentration treatment (12). The research by Bravo Soto et al (1) also showed that the group of patients who used MPA had CD4 and CD8 T lymphocytes in peripheral blood decreased due to the cumulative effect, therefore the

number of T lymphocytes should be re-tested at different times and lasting after transplantation. In addition, the effectiveness of the drug depends not only on the dose of the drug, but also on the combination of immunosuppressive drugs as well as individual characteristics. The study by Mathieu et al (13) showed the effect of MPA on CD4 lymphocytes and CD8 lymphocytes in peripheral blood in the first month after heart transplant, while there was no difference between the cells under this group.

### Conclusion

In addition to quantifying anti-rejection drug concentrations, immunosuppression control in renal transplant patients including T lymphocytes count should be performed at multiple post-transplant times to make early predictions of transplant rejection during follow up treatment.

### Limitations of the study

The limitations of this study were the small sample size. There is a limitation on the assessment times as the measurement of T lymphocytes CD3, CD4, CD8 in peripheral blood in patients is conducted 1 day before and 10 days after transplantation.

### Authors' contribution

TMDD and QTH conducted the research and prepared the primary draft. PHAH and VDL revised and prepared the final manuscript. All authors read and approved the final paper.

### Conflicts of interest

The authors declare no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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