## RESULTS

### 1. Characteristics of renal transplant patients

The study examined the HA levels from 202 blood samples collected from stored samples obtained from the Drug Monitoring Center, Clinical Pharmacology Unit, Department of Pharmacology, Faculty of Medicine, Chiang Mai University. The blood samples were drawn from the renal transplant patients who were followed up at the Division of Nephrology, Department of Medicine, Faculty of Medicine, Chiang Mai University for analysis of cyclosporine or tacrolimus levels. Blood samples from 101 renal transplant patients were included in this study. Thirty-eight patients and 63 patients were treated with cyclosporine and tacrolimus, respectively. Seven patients were switched from cyclosporine to tacrolimus while only one tacrolimus-treated patient was switched to cyclosporine. The characteristics of these patients are summarized in Table 10. Sixty nine percent of renal transplant recipients were male and their average age were 46 years old. The first renal allograft was successful in 97 patients (96%) while 4 subjects (4%) required subsequent second renal transplantation. The most common cause of end-stage renal disease was glomerulonephritis.

**Table 10.** Characteristics of 101 renal transplant patients treated with cyclosporine or tacrolimus

| | Cyclosporine | Tacrolimus |
|---|---|---|
| Patients (male/female) (N) | 38 (29/16) | 63 (41/23) |
| Age (yr) | 47±9 | 45±11 |
| Transplantation status | | |
| First | 37 | 60 |
| Second | 1 | 3 |
| Cause of end-stage renal disease (N) | | |
| Glomerulonephritis | 19 | 30 |
| Chronic pyelonephritis | - | 1 |
| Obstructive uropathy | 3 | 5 |
| Diabetic nephropathy | 1 | 2 |
| Hypertensive nephropathy | 3 | 2 |
| Unknown | 12 | 21 |
| Other | - | 2 |

## 2. Correlation of HA level and immunosuppressive drug levels

The data obtained from the patients were divided into 6 groups according to the type of immunosuppressive drug and duration of treatment. Group 1, 2 and 3 referred to data from patients who received cyclosporine within the duration of < 60 days, 60-180 days and > 180 days, respectively. Similarly, group 4, 5 and 6 referred to data from patients who received tacrolimus < 60 days, 60-180 days and > 180 days, respectively. The average values of immunosuppressive drug levels and HA levels in all groups are shown in Table 11. The mean cyclosporine levels were respectively 300.89 ± 330.54, 193.06 ± 48.33 and 123.89 ± 52.73 ng/ml for patients in group 1-3. Likewise the mean tacrolimus levels were 12.76 ± 6.33, 11.50 ± 5.17 and 7.91 ± 3.67 ng/ml for patients in group 4-6, respectively. The average HA levels were 29.98 ± 6.37, 143.56 ± 90.41, 17.47 ± 4.59, 19.63 ± 3.55, 21.70 ± 16.44 and 20.98 ± 15.48 ng/ml for patients in group 1-6, respectively. The result showed that the mean HA levels for patients in group 2 (N=2) was higher than the values obtained from other groups. The patient in group 2 who had the highest HA levels of 207.49 ng/ml was previously being treated with tacrolimus and experienced adverse drug reactions, therefore, tacrolimus was switched to cyclosporine. During her first 2 months of cyclosporine therapy, she was classified to be in group 1 and her HA level was normal despite her lower cyclosporine level (< 250 ng/ml). The dose of cyclosporine was increased until her cyclosporine level reaching the therapeutic range when she was reclassified to be in group 2. Meanwhile, her HA level was found to rise significantly from 34.48 to 207.49 ng/ml. Her clinical symptom and graft status were retrospectively reviewed and was found to be unremarkable. Cyclosporine was continued until > 180 days and her data were also included in group 3. At this time, her cyclosporine level remained within the therapeutic range, however, her HA level declined to 19.00 ng/ml.

The association between immunosuppressive drug levels and HA levels were determined by using Spearman correlation coefficient (R) and the data were tested for difference by Kruskal-Wallis test. Group 1 and 2 had limited number of patients (N=2 in each group), therefore, statistical analysis could not be performed. The number of samples in group 3 was 45, however, correlation analysis showed no correlation between levels of cyclosporine and HA in patients chronically treated with cyclosporine (R = -0.05, $P$ = 0.76). The number of samples in group 4-6

(tacrolimus group) were 44, 44 and 65, respectively. Similar to cyclosporine, statistical analysis showed no correlation between tacrolimus levels and HA levels (R = 0.24, 0.08, -0.08 and $P = 0.11, 0.63, 0.50$ for group 4-6, respectively). The HA levels were quite low in blood samples obtained from patients who were recently or chronically treated with tacrolimus and these values were not significant difference within the group.

**Correlation of HA level and therapeutic drug levels**

Table 12 illustrates HA levels in each group of subjects based on the therapeutic drug levels of immunosuppressive drugs. Figure 7-10 depict scatterplots of HA levels and immunosuppressive drug levels as well as their correlation coefficient for group 3-6, respectively. Each of the two subjects in group 1 had their cyclosporine levels under and over the therapeutic range their corresponding HA levels were 34.48 and 25.47 ng/ml, respectively. The HA levels for 2 subjects in group 2 who had their cyclosporine levels within therapeutic range were 207.49 and 79.63 ng/ml (average 143.56 ng/ml). The average HA levels obtained from subjects in group 3 treated with subtherapeutic, therapeutic and toxic levels of cyclosporine were 17.04 $\pm$ 5.47, 18.55 $\pm$ 2.30 and 16.09 $\pm$ 6.14 ng/ml, respectively. Statistical analysis showed no correlation between the drug levels and HA levels (R = 0.32, -0.26, -0.01 for subjects whose cyclosporine levels were lower than, within, and higher than therapeutic range) (Figure 7) and their HA levels were not significant difference between groups ($P = 0.54$).

The HA levels of subjects in group 4-6 who were recently or chronically treated with subtherapeutic levels of tacrolimus were 19.72 $\pm$ 8.92, 25.06 $\pm$ 24.44 and 22.98 $\pm$ 19.49 ng/ml, respectively. High HA levels were noted in two subjects. Of one subject in group 5 whose HA level was 118.98 ng/ml and retrospective review of clinical data found that his immunosuppressive drug was recently switched from cyclosporine to tacrolimus. On the first 2 months, his tacrolimus level was within the therapeutic range and the HA level was low. However, during 60-180 days of tacrolimus therapy, his drug level fell below the therapeutic range and he had proteinuria, which was an early sign of renal allograft rejection. However, the renal biopsy was not done. The dose of tacrolimus was increased until the drug levels reached the satisfactory concentration when he was now in group 6 and his HA level returned to low level.

Another patients with high HA level was in group 6. Review of his clinical data was unremarkable except anemia.

The HA values for subjects whose tacrolimus levels were within and higher than the therapeutic range were 22.34 ± 8.18 and 20.55 ± 3.47, 18.82 ± 0.66 and 25.11± 11.56, 20.76 ± 10.11 and 21.22 ± 11.29 ng/ml for subjects in group 4-6, respectively. Similar to cyclosporine treatment, statistical analysis showed no significant difference between therapeutic tacrolimus levels (Figure 8-10) and HA levels with *P* values of 0.77, 0.12 and 0.60 for group 4-6, respectively.

**Table 11.** Correlation of hyaluronan level and immunosuppressive drug levels ($C_o$)

| | Immunosuppressive drug level (ng/ml) | Hyaluronan level (ng/ml) | Correlation (*P* value) |
|---|---|---|---|
| Patients taking cyclosporine | | | |
| - < 60 days (N =2) | 300.89±330.54 | 29.98±6.37 | - |
| (therapeutic level: 250 ng/ml) | | | |
| - 60-180 days (N =2) | 193.06±48.33 | 143.56±90.41 | - |
| (therapeutic level: 150-250 ng/ml) | | | |
| - > 180 days (N =45) | 123.89±52.73 | 17.47±4.59 | -0.05 (0.76) |
| (therapeutic level: 100-150 ng/ml) | | | |
| Patients taking tacrolimus | | | |
| - < 60 days (N =44) | 12.76±6.33 | 19.63±3.55 | 0.24 (0.11) |
| (therapeutic level: 15-20 ng/ml) | | | |
| - 60-180 days (N =44) | 11.50±5.17 | 21.70±16.44 | 0.08 (0.63) |
| (therapeutic level: 10-15 ng/ml) | | | |
| - > 180 days (N =65) | 7.91±3.67 | 20.98±15.48 | -0.08 (0.50) |
| (therapeutic level: 8-10 ng/ml) | | | |

**Table 12.** Correlation of hyaluronan level and therapeutic drug levels

| | Hyaluronan level (ng/ml) with respect to levels of drugs in | | | *P* value |
|---|---|---|---|---|
| | Subtherapeutic level | Therapeutic level | Toxic level | |
| Patients taking cyclosporine | | | | |
| - < 60 days (N=2) | 34.48 | - | 25.47 | - |
| (therapeutic level: 250 ng/ml) | | | | |
| - 60-180 days (N=2) | - | 143.56±90.41 | - | - |
| (therapeutic level: 150-250 ng/ml) | | | | |
| - > 180 days (N=45) | 17.04±5.47 | 18.55±2.30 | 16.09±6.14 | 0.54 |
| (therapeutic level: 100-150 ng/ml) | | | | |
| Patients taking tacrolimus | | | | |
| - < 60 days (N=44) | 19.72±8.92 | 22.34±8.18 | 20.55±3.47 | 0.77 |
| (therapeutic level: 15-20 ng/ml) | | | | |
| - 60-180 days (N=44) | 25.06±24.44 | 18.82±0.66 | 25.11±11.56 | 0.12 |
| (therapeutic level: 10-15 ng/ml) | | | | |
| - > 180 days (N=65) | 22.98±19.49 | 20.76±10.11 | 21.22±11.29 | 0.60 |
| (therapeutic level: 8-10 ng/ml) | | | | |

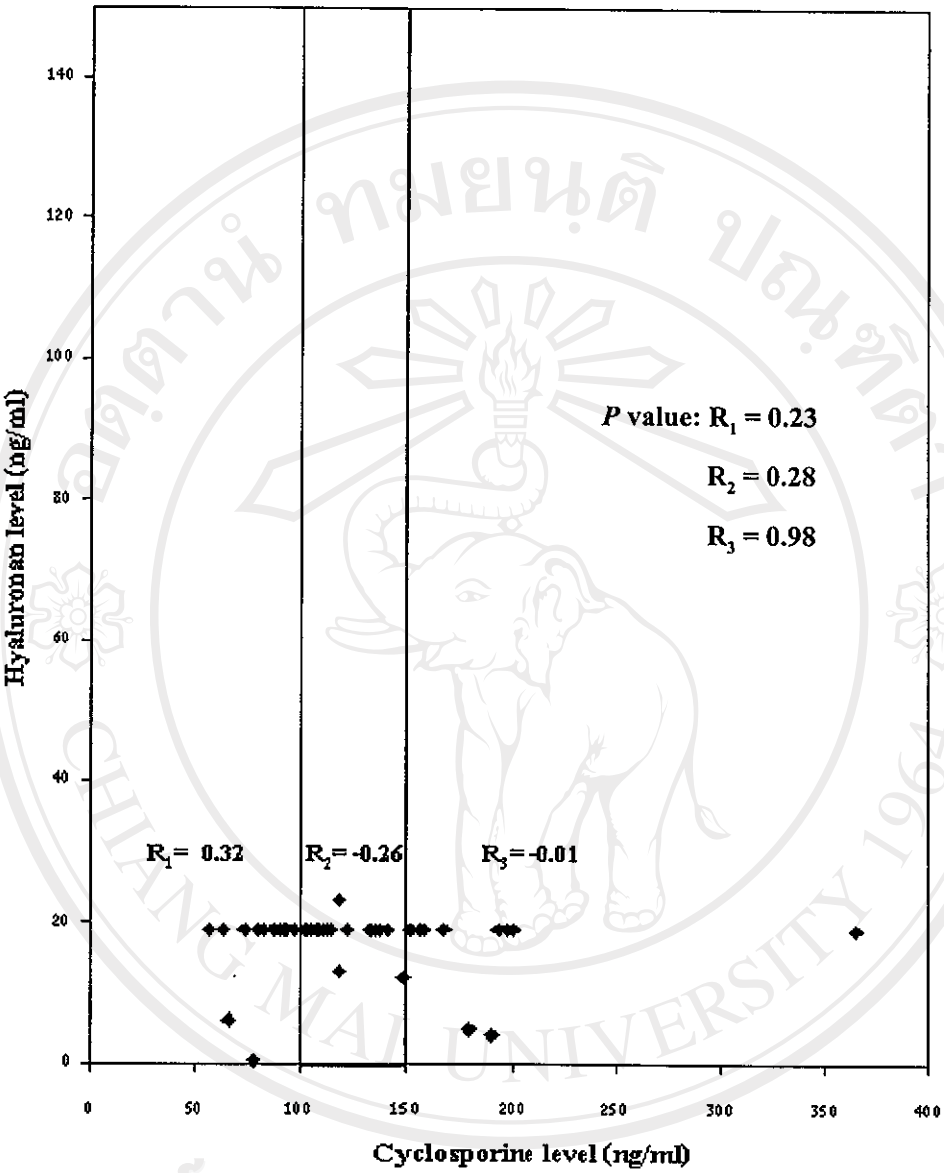


**Figure 7.** Correlation of HA level and cyclosporine level in group 3 (> 180 days). $R_1$, $R_2$ and $R_3$ represent the correlation coefficient of subtherapeutic, therapeutic and toxic levels of cyclosporine, respectively.

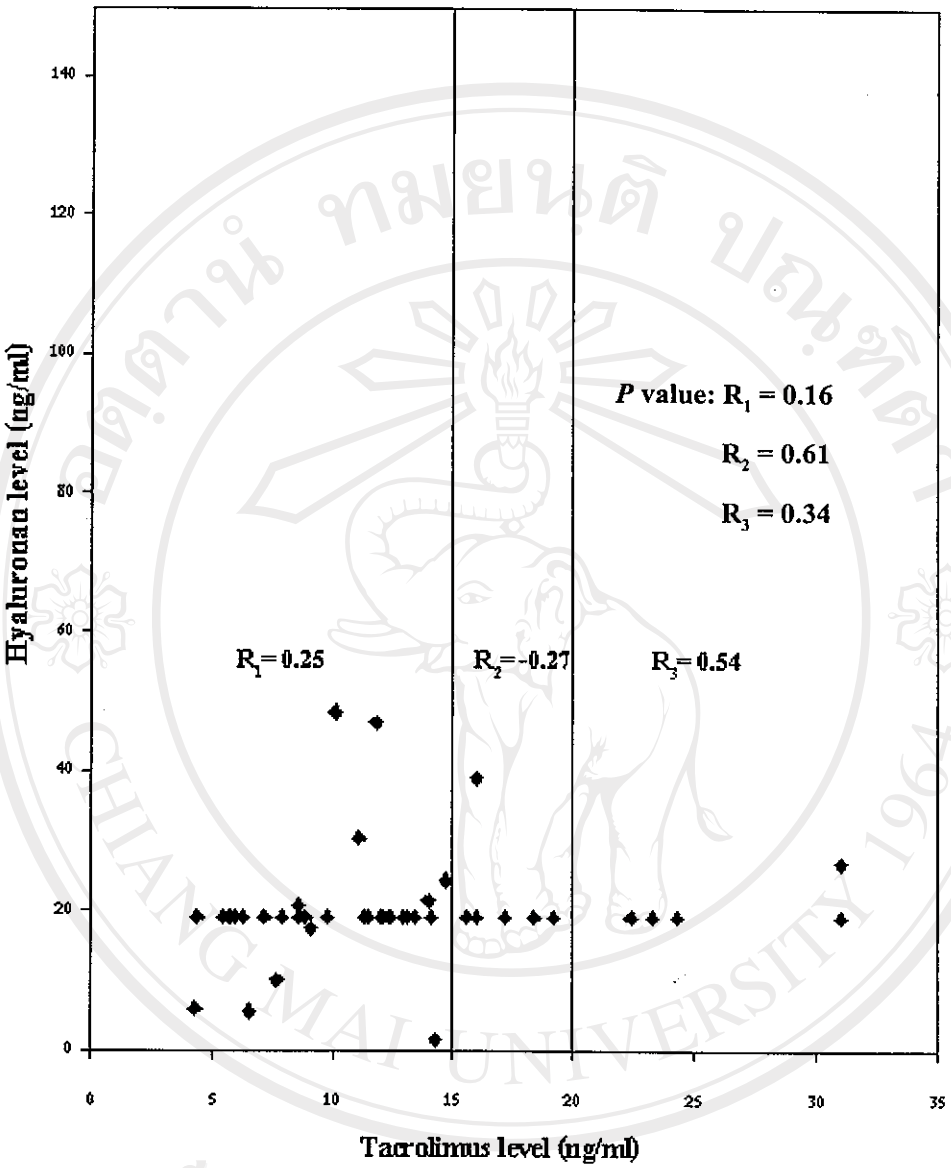


**Figure 8.** Correlation of HA level and tacrolimus level in group 4 (< 60 days). $R_1$, $R_2$ and $R_3$ represent the correlation coefficient of subtherapeutic, therapeutic and toxic levels of tacrolimus, respectively.

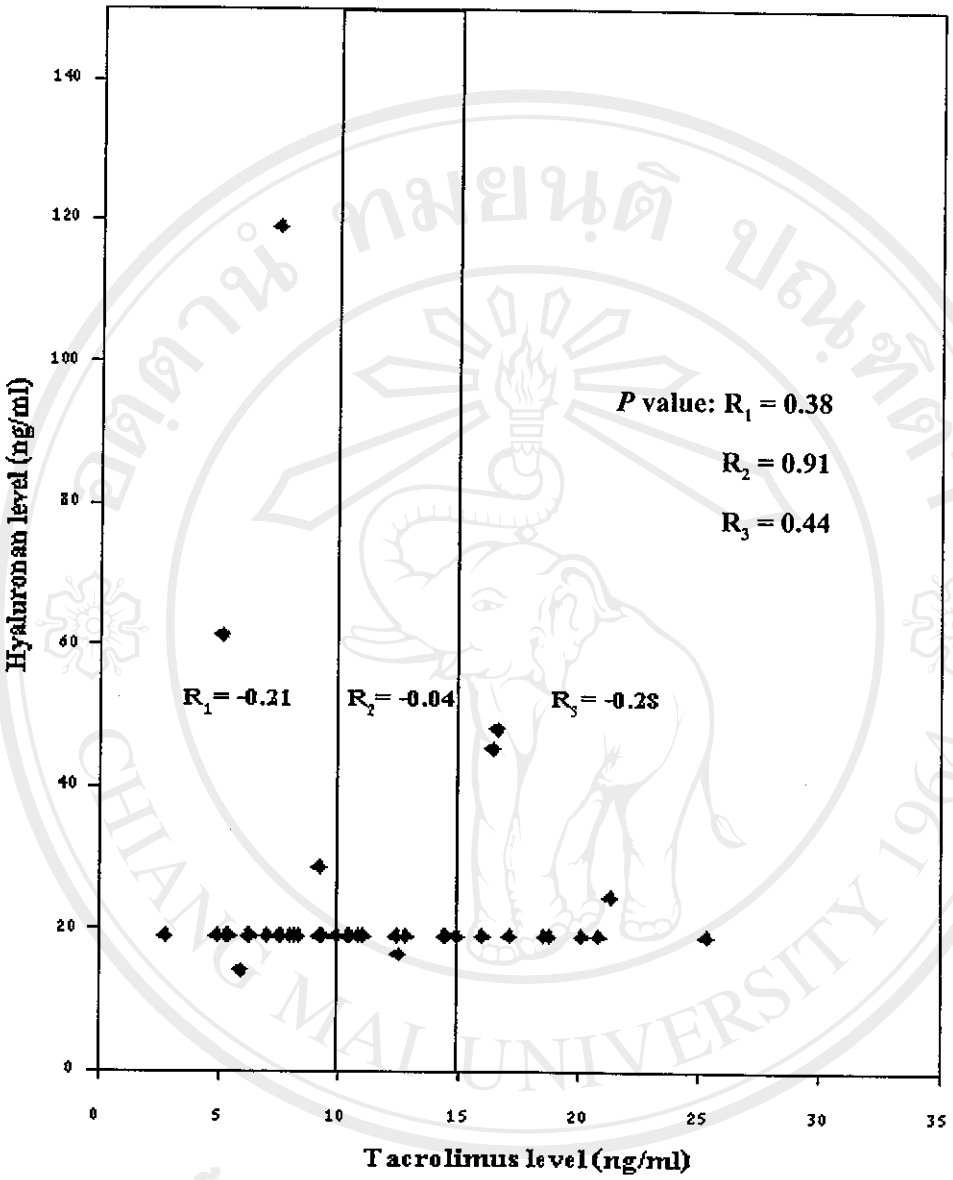


**Figure 9.** Correlation of HA level and tacrolimus level in group 5 (60-180 days). $R_1$, $R_2$ and $R_3$ represent the correlation coefficient of subtherapeutic, therapeutic and toxic levels of tacrolimus, respectively.

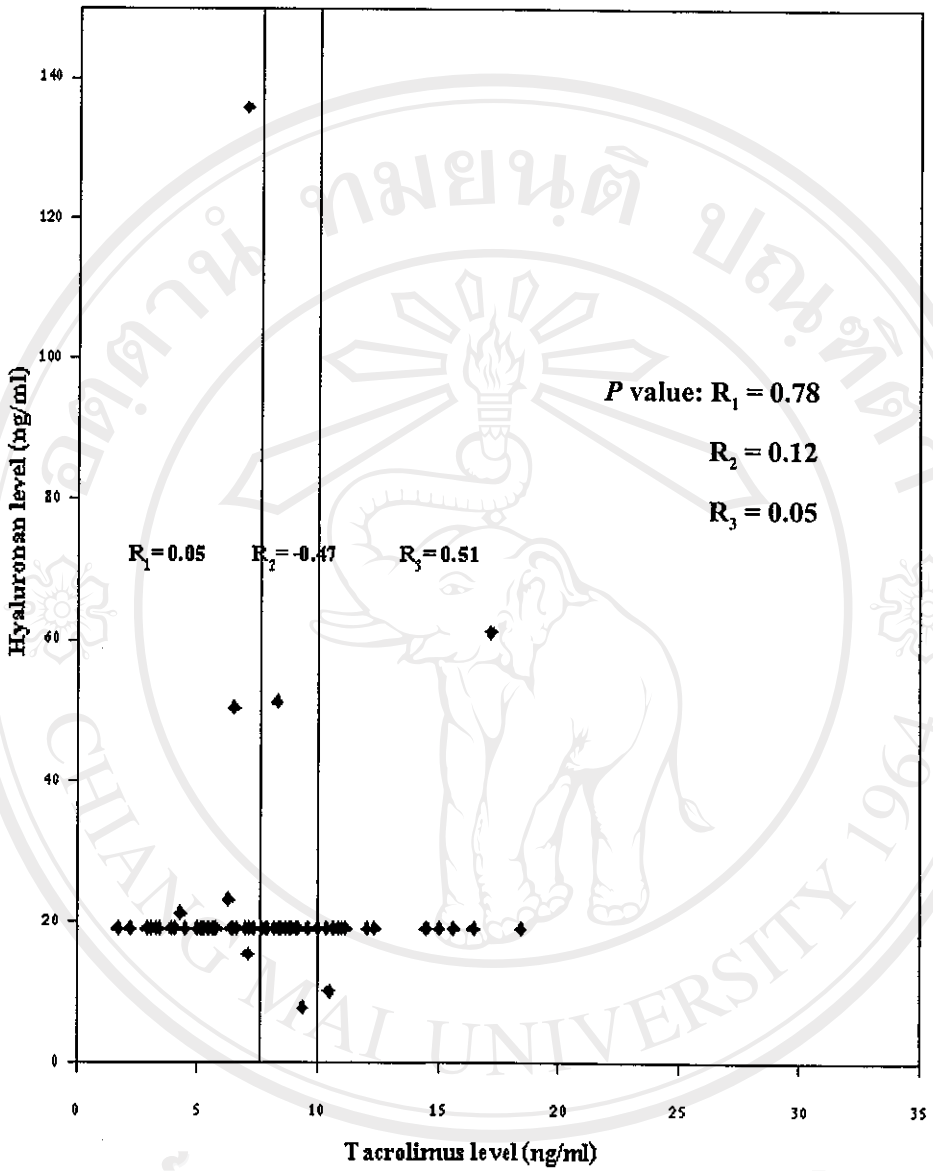


**Figure 10.** Correlation of HA level and tacrolimus level in group 6 (> 180 days). $R_1$, $R_2$ and $R_3$ represent the correlation coefficient of subtherapeutic, therapeutic and toxic levels of tacrolimus, respectively.

### 3. Correlation of HA level and graft status

The HA level of patients who recently underwent renal biopsy to assess their graft status is shown in Table 13. The result showed that the levels of HA were quite low in all groups and there were no correlation between HA levels and graft status ($P = 0.71$).

**Table 13.** Correlation of HA level and graft status[a]

| Graft Status | HA level (ng/ml) | *P* value |
|---|---|---|
| Acceptable graft (N=63) | 19.31±4.58 | |
| Threatened graft rejection (N=7) | ≤ 19.00 | |
| Post-rejection graft with successful therapy (N=31) | 21.84±21.39 | 0.71 |

[a]Data based on recent renal biopsy.