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Examination of Serum Amyloid A Protein in Kidney Transplant Patients – Comparison of Serum Amyloid A and C-Reactive Protein for Monitoring the Occurrence of Renal-allograft-related Complications –

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ABSTRACT

Serum amyloid A (SAA) is an inflammation -reactive protein, like C-reactive protein (CRP). In this study, we examined SAA levels in the sera of kidney transplant patients with acute rejection (N=12), chronic rejection (N=60) and cytomegalovirus (CMV) infection complications and compared them with serum CRP levels in terms of sensitivity and reactivity. The SAA and CRP showed almost similar kinetics in 10 patients within 2 months of kidney transplantation. However, in 2 patients SAA responded more sensitively to CMV infection and acute rejection. SAA increased significantly 10-fold relative to its baseline levels. The SAA levels also increased along with those of serum creatinine levels. Our experiments clearly showed that SAA and CRP responded sensitively to several stimuli with elevated serum levels including surgical trauma, acute allograft rejection and infection. However, the reactivity and sensitivity of SAA was clearly higher than those of CRP in patients with viral infections, on steroid therapy and undergoing chronic allograft rejection, suggesting that monitoring SAA levels provides more useful information than monitoring CRP.

Key words: *Serum amyloid A protein, C-reactive protein, Kidney transplantation*

Serum amyloid A (SAA) is an amyloid protein with a Mw. of 12000. It is an inflammation-reactive protein, like C-reactive protein (CRP)[8]. SAA is synthesized by the liver and secreted into the circulation to produce physiological concentrations below 10μg/ml. This increases several thousand times during inflammation[9]. The kinetics of SAA in response to various stimuli have been shown to be very similar to those of CRP[8,13,14,20]. However, it has been shown to respond more sensitively and quickly to inflammatory stimulus, even in patients on corticosteroid treatment, than CRP[8,13,14,20]. Therefore, it is anticipated that SAA will be a more useful indicator than CRP for monitoring infection and/or allograft rejection, following organ transplantation under steroid therapy. In this study, we examined SAA levels in the sera of kidney graft recipients with acute rejection, chronic rejection and cytomegalovirus infection (CMV) complications and compared them with serum CRP levels in terms of sensitivity and reactivity.

MATERIALS AND METHODS

The SAA and CRP levels in the sera of kidney transplant patients, normal healthy individuals and continuous ambulatory peritoneal dialysis (CAPD) patients were examined and compared. In an early phase study, 12 patients (10 living-related and 2 cadaveric kidney transplant recipients) with or without post-operative complications were studied within 2 months of kidney transplantation. Sixty patients who received a kidney graft more than 5 years before this study participated in a late phase study. None of these 60 patients had undergone regrafting or received dialysis treatment after transplantation. They were divided into 3 groups according to their serum creatinine(sCr) level as follows: group 1, sCr less than 1.0 mg/dl (n=14), group 2, sCr between 1.0 and 2.0 mg/dl (n=33), and group 3, sCr greater than 2.0

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mg/dl (n=13). The sCr and SAA levels were determined by calculating the average of 5 consecutive levels.

Serum samples were obtained from the patients either daily during their stay on the ward or every month when they attended the outpatient clinic. All kidney transplant patients had received methylprednisolone and cyclosporine in combination with either azathioprine, mizoribine or mycophenolate mofetil. As a control study, the SAA and CRP levels of 300 healthy individuals and 10 chronic renal failure patients on CAPD therapy were also analyzed. If a patient appeared to be suffering from an acute inflammatory condition, such as a common cold, urinary tract infection or trauma, his/her SAA data were not analyzed. Linear regression analyses were used to determine the association between β2m and sCr or SAA and CRP. Rejection was considered to have occurred if renal function deteriorated and the renal biopsy specimen showed positive cytological findings. CMV infection was defined as a positive antigenemia assay result[19].

SAA concentrations were measured using the latex agglutination nephelometric immunoassay system developed by Biochemical Laboratory Eiken Chemical Co. Ltd., Japan[4]. Briefly, SAA-enriched high density lipoprotein (HDL) was isolated[2], purified from the sera of rheumatoid arthritis patients, its SAA content was determined electrophoretically and it was used as the assay standard. An anti antiserum was produced by immunizing a rabbit against SAA, followed by purification of lgG. The SAA concentrations of serum samples was determined by a latex agglutination enzyme immunoassay[21]. Serum CRP concentrations were measured simultaneously by a standard latex agglutination method, and serum β 2microglobulin (β2m) concentrations were measured by radioimmunoassay, according to the manufacturer's instructions. The data were analyzed using the Mann-Whitney U- test, and differences of $p<0.05$ were considered significant.

RESULTS

Latex agglutination analysis of serum samples from 300 normal healthy individuals demonstrated that the cut-off values for SAA and CRP were 12 μg/ml and 1.0 μg/ml respectively (data not shown). Therefore, the basal SAA concentration was 10 times higher than that of CRP.

In the 10 living-related kidney transplant patients without any conspicuous complications, the SAA and CRP patterns showed almost identical kinetics and there was a strong positive correlation between them (Fig. 1).

The cases of the 2 renal transplant patients who developed complications within 2 months of transplantation were outlined (Fig. 2 and 3).

Figure 2 shows the post-operative SAA and CRP level fluctuations of Case 1. The patient was a 37-year-old male who had been on hemodialysis for chronic renal failure for 2 years. His underlying renal disease was chronic glomerulonephritis and he received a living related kidney graft from his mother. His immunosuppressive therapy com-

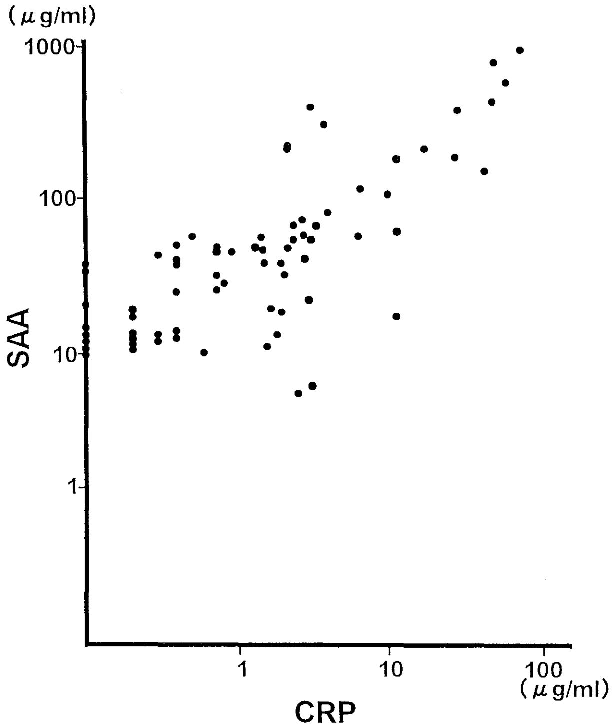

Fig. 1. Relationship between serum SAA and CRP of 10 patients within 2 months of kidney transplantation.
The SAA and CRP showed similar kinetics. $Y=24.2+11.X$, $r=0.88$, $p<0.0001$

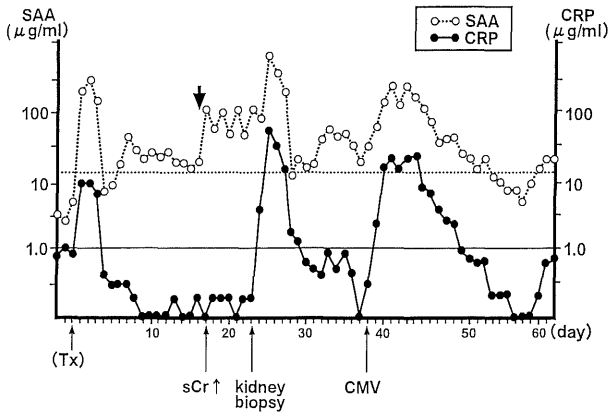

Fig. 2. Serial changes in serum SAA and CRP concentrations of a renal transplant patient experiencing reversible acute rejection.
Note the different responses of SAA and CRP (arrow). SAA increased significantly 10-fold relative to its beeline level and the sCr level rose simultaneously, whereas the CRP level retained unchanged throughout this episode.

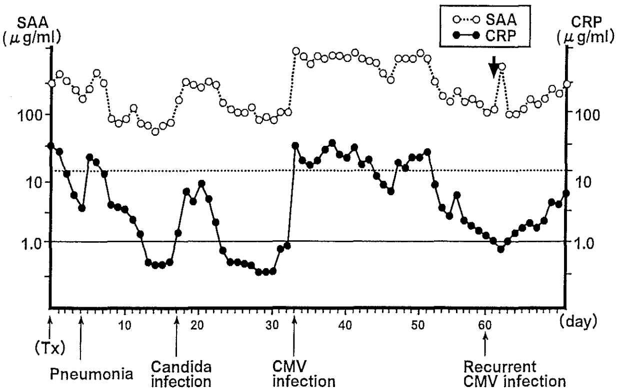

Fig. 3. Serial changes in serum SAA and CRP concentrations of a renal transplant patient experiencing 2 reversible CMV infections, diagnosed from the antigenemia assay results, on the 33rd and 60th days after the transplant.
SAA aided the detection of the CMV infection recurrence, whereas CRP remained unchanged during this episode.

prised metylprednisolone, micophenol mofetil and cyclosporine. On the 16th post operative day, his SAA level was elevated, but that of CRP did not change. One day later, his sCr increased suddenly from 1.5 to 4.1 mg/dl and acute rejection was suspected. Administration of pulse therapy using methylprednisolone for 3 days reduced his sCr to the level prior to this episode. Later examination of a renal biopsy specimen revealed acute rejection. Although he developed urethra obstruction due to hematuria following renal biopsy, this was resolved by inserting once single J catheter on the day it occurred. On the 38th day, he had a high-grade fever and diarrhea. The antigenemia assay revealed he was CMV antigen-positive and CMV infection was diagnosed, for which ganciclovir (10 mg/kg) was administered. His temperature was normal on the 5lst day and the ganciclovir was stopped. On the 60th day, he was discharged. Both SAA and CRP responded well to surgical trauma and CMV infection. However, the onset of acute rejection was only proceeded by an increase in SAA, whereas the CRP level did not alter during this episode.

Figure 3 shows the SAA and CRP changes of Case 2. The patient was a 39-year-old female who had been on hemodialysis for 5 years and 4 months. Her disease was chronic glomerulonephritis and she was determined to be anti CMV lgG-negative prior to the transplant operation. She received a cadaveric renal transplant from a CMV positive donor and there were 2 HLA antigen mismatches. Her immunosuppressive therapy comprised cyclosporine, azathioprine and methylprednisolone. She had pulmonary edema and infection on the 4th post-operative day. On the 32nd day she had a high-grade fever over 38°C and received ganciclovir and 7 globulin treatment from the 34th post-operative day for CMV infection. On the 60th day, she had a high-grade fever again, and was diagnosed with a CMV infection on the basis of the antigenemia assay results. At this time, only the SAA level responded to CMV infection by increasing, whereas CRP did not change. A full dose of ganciclovir therapy was started. Her temperature was normal on the 82nd day with no evidence of CMV antigen.

Figure 4 shows the relationships between the β2m and sCr levels of 60 renal transplant patients who had received their grafts more than 5 years prior to the study, and Table 1 shows the relationships among the β2m, sCr and SAA levels of 10 CAPD patients, respectively. The positive correlation between the β2m and sCr levels clearly shows that the former primarily reflect the glomerular filtration rate. In contrast, CAPD patients with sCr levels of 2.53–13.3 mg/dl (mean±SD=8.1 ± 3.7 mg/dl) consistently had low SAA concentrations (below 13.0 μg/ml, mean±SD=6.2± 3.9 μg/ml) and extraordinarily high β2m titer (21.1–71.2mg/liter, mean±SD=39.1± 15.3mg/liter). These results show that SAA is not influenced by renal function, unlike β2m, even though they have similar molecular weights.

The SAA levels of the 60 patients in the late phase study increased along with the sCr levels as follows: group 1 (sCr<1.0mg/dl), SAA=10.25±9.72 mg/dl; group 2 (sCr 1.0–2.mg/dl), SAA=13.34 ±7.73 mg/dl; group 3 (sCr≥2.0mg/dl), SAA=22.08± 14.95 mg/dl (Fig. 5). The differences between each combination of 2 groups were significant.

Table 1. Comparison of changes in SAA, CRP, β2m and sCr levels of 10 CAPD patients.

	sCr (mg/dl)	CRP (μg/ml)	SAA (μg/ml)	S2m (mg/liter)
Mean±SD	8.1±3.6	0.44±0.91	6.2±3.9	39.1±15.3
Normal ranges	(0.5–0.9)	(1.0>)	(12>)	(2.0>)

Values are means ±SD. All 10 patients showed abnormally high sCr and serum β2m concentrations. However, most SAA and CRP levels were within the normal ranges during the observation period, suggesting that neither parameter reflected the glomerular filtration rate.

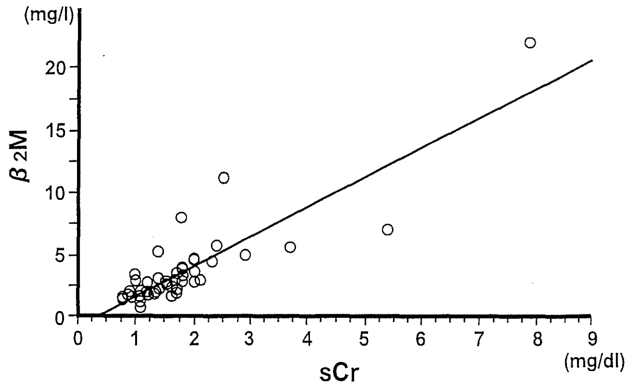

Fig. 4. Relationship between serum β2m and sCr levels of 60 kidney transplant patients.
There was a strong positive correlation between these 2 parameters. Y=2.4X-0.71, r=0.86, $p<0.0001$

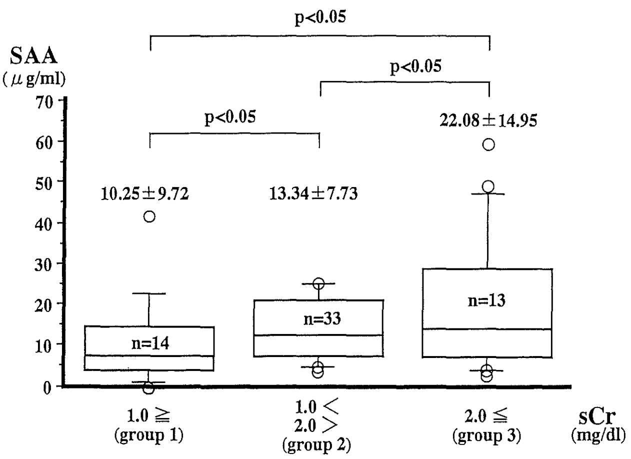

Fig. 5. Evaluation of SAA as a marker of chronic renal allograft rejection.
Sixty patients with renal allografts for more than 5 years were divided into 3 groups according to their sCr levels: group 1, sCr≦ 1.0 mg/dl;, group 2, 1.0 mg/dl<sCr<2.0 mg/dl; group 3, sCr≧2.0 mg/dl. The SAA levels increased along with those of sCr and there were significant differences between any 2 groups, whereas there were no differences among the CRP values of the 3 groups.

DISCUSSION

Our study on kidney transplant recipients in the acute phase clearly indicated that the kinetics of SAA were very similar to those of CRP in response to various stimuli following renal transplantation, although there were a few exceptions. In kidney transplant patients without any conspicuous complications, the SAA and CRP level returned to normal within a few days of the operation, reaching peak levels on day 2, presumably due to the influence of surgery.

In our two cases who developed complications in the acute phase, SAA responded more sensitively to CMV infection and acute rejection. SAA has been recommended as a sensitive acute rejection marker not only in kidney but also in liver and pancreas grafting[9,10,12] But the sole determination of the parameter SAA does not allow one to discriminate between acute rejection and infectious diseases.

It is of particular interest that there was a positive correlation between the sCr and SAA levels of the patient with chronic allograft rejection, as shown in Figure 5. In view of the fact that cytokine networks play roles in the mechanisms involved in chronic rejection reactions[5], these results suggest that SAA but not CRP is involved to some extent at least in chronic rejection, because CRP levels did not change during chronic rejection. Therefore, although SAA appears to resemble CRP in terms of its response to tissue damage[8,13,14,20], some pathological conditions actually exist under which only SAA responds. The biological characteristics of the 2 substances differ as follows: (1) SAA has a shorter half life than CRP[11,15]. (2) The physiological baseline SAA concentration is 10 times higher than that of CRP. Consequently, both its sensitivity and reactivity would be expected to be higher than those of CRP. (3) SAA may be influenced by biological reactions to viral infections[3,16] but is not influenced by treatment with steroid hormones[1,18].

Different physiological functions may also contribute to their reaction differences. Both SAA and CRP are known to be acute phase inflammatory proteins that are synthesized by the liver during inflammation. CRP attracts leukocytes to inflammatory sites to cope with nuclear destruction products associated with opsonization and complement activation. In contrast, SAA inhibits the oxidative burst response of N -formly peptid-stimulated neutrophils[7], inhibits IL-6- and TNF-induced fever and hypothalamic PGE2 in mice[6] and inhibits platelet activation[22]. Thus, SAA differs from CRP with respect to its functions and inhibition activities.

In conclusion, our experiments clearly showed that SAA and CRP responded sensitively to several stimuli with elevated serum levels, including surgical trauma, acute allograft rejection and infection. However, the reactivity and sensitivity of SAA was clearly higher than those of CRP in patients with viral infections, on steroid therapy and undergoing chronic allograft rejection, suggesting that monitoring SAA levels provides more useful information than monitoring CRP, particularly for evaluating several complications associated with kidney transplant patients on steroid treatment.

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