



# Serum alkaline phosphatase and $\gamma$ -glutamyl transferase in acute pyelonephritis

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**Background:** Elevated serum alkaline phosphatase (AP) and  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) are commonly observed in patients with acute pyelonephritis. The goal of this study was to examine the clinical significance of elevated serum AP and  $\gamma$ -GT levels and to explore the mechanisms underlying these changes.

**Methods:** We examined serum AP and  $\gamma$ -GT levels in 438 patients with acute pyelonephritis. Urine AP/creatinine (Cr), urine  $\gamma$ -GT/Cr, fractional excretion of AP, and fractional excretion of  $\gamma$ -GT ( $FE_{\gamma\text{-GT}}$ ) were evaluated in patients with elevated and normal serum levels. AP isoenzymes were also examined.

**Results:** We identified 77 patients (17.6%) with elevated serum AP and 134 patients (30.6%) with elevated serum  $\gamma$ -GT. Among them, both enzymes were elevated in 64 patients (14.6%). Older age, longer hospital stay, elevated baseline serum Cr, and complicated pyelonephritis were associated with increases in serum AP and  $\gamma$ -GT. Multivariate analysis showed that high serum AP levels were significantly correlated with renal impairment (odds ratio, 2.13; 95% confidence interval, 1.08–4.19;  $P = 0.029$ ).  $FE_{\gamma\text{-GT}}$  was significantly lower in patients with elevated serum enzyme levels. The liver fraction for AP isoenzyme profile did not increase in patients with elevated serum AP.

**Conclusion:** Our results demonstrated that elevated serum AP and  $\gamma$ -GT levels are associated with complicated pyelonephritis and renal impairment. Lower  $FE_{\gamma\text{-GT}}$  levels in patients with elevated serum enzymes may be the result of decreased urinary excretion of these enzymes.

**Keywords:** Alkaline phosphatase, gamma-Glutamyltransferase, Pyelonephritis

## Introduction

Routine analyses of alkaline phosphatase (AP) and

gamma-glutamyl transferase ( $\gamma$ -GT) levels are commonly performed in clinical settings because elevation of these enzymes is indicative of a wide range of diseases, including hepatobiliary and bone diseases. Serum AP levels alone are diagnostic for many renal diseases, including renal osteodystrophy, infarction, renal cell carcinoma, and renal allograft rejection [1–3]. Elevated serum AP levels may also be indicative of infection or inflammation involving the tubular interstitial cells in which these enzymes are abundant. Meanwhile, a significant increase in  $\gamma$ -GT activity was observed in cases of pyelonephritis, Alport's syndrome, Wilms' tumor, and glomerulonephritis [4]. However, despite the strong association between renal disease and elevated serum AP and  $\gamma$ -GT levels, a small subset of acute pyelonephritis patients exhibit ab-

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normally high AP and/or  $\gamma$ -GT levels without conspicuous cause. A few studies have investigated the elevated enzyme phenomenon [3–5], but none has offered a possible mechanism. Herein, we investigated the clinical significance of serum AP and  $\gamma$ -GT in adult patients with acute pyelonephritis, and we examined possible underlying mechanisms.

## Methods

### Study population

Using a retrospective design, we enrolled patients who were admitted to Hallym University Kangnam Sacred Heart Hospital (Seoul, Korea) after presenting with acute pyelonephritis between March 2002 and February 2005. All study-related protocols were approved by the Institutional Review Board of Kangnam Sacred Heart Hospital (IRB No 2008-8-44). Acute pyelonephritis was diagnosed when all three of the following symptoms were present: 1) fever  $\geq 38^\circ\text{C}$ , 2) flank pain or costovertebral angle tenderness, and 3) pyuria on urinalysis ( $> 5$  leukocytes per high-power field in spot urine specimens). Patients were excluded from the study if they met any of the following conditions:  $< 18$  years old, received antibiotic treatment prior to admission, presence of any other infectious disease that could interfere with pyelonephritis diagnosis, clinical or laboratory evidence of liver or bone disease (hepatitis B or C virus carriers, alcohol abusers), pregnancy or lactation in women, malignant tumor history, or chronic kidney disease history. A total of 438 patients were included in our study.

Complicated pyelonephritis was defined as acute pyelonephritis with any of the following conditions [6]: diabetes mellitus or immunocompromised status; underlying functional or structural urologic abnormalities (such as neurogenic bladder, urolithiasis, urinary tract obstruction, congenital abnormalities, or reflux); an indwelling urinary catheter; emphysematous pyelonephritis; or renal or perirenal abscess formation.

We prospectively performed functional evaluations of the mechanisms underlying elevated serum AP and  $\gamma$ -GT levels between March 2008 and May 2009. Thirty-seven subjects were selected for measurement of urinary AP and  $\gamma$ -GT excretion (urine AP/creatinine [Cr], urine  $\gamma$ -GT/Cr), fractional excretion of AP ( $FE_{AP}$ ),  $FE_{\gamma-GT}$  and AP isoenzyme levels.

### Laboratory analyses

Serum samples were collected and measured for complete blood counts and biochemical analyses, including liver and renal function tests. Urine samples were collected from all patients on initial presentation and prior to antibiotic infusion. In all cases, abdomen ultrasonography or computed tomography was performed during the acute infection phase. For hospitalized patients, we prescribed ceftriaxone empirically until culture results were available. Antibiotic therapy duration ranged from 10 to 14 days. Patients were discharged when their clinical parameters improved (fever, nausea, vomiting, poor oral intake, or flank pain) and when they were able to tolerate oral antibiotics.

The estimated glomerular filtration rate (eGFR) was estimated according to the Modification of Diet in Renal Disease (MDRD) study equation:  $eGFR (\text{mL}/\text{min}/1.73 \text{ m}^2) = 175 \times (\text{serum Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African-American})$ . Renal impairment was defined as baseline eGFR  $< 60 \text{ mL}/\text{min}/1.73 \text{ m}^2$ .

We measured AP activity using the *p*-nitrophenylphosphate method with diethanolamine buffers, and we evaluated  $\gamma$ -GT using the International Federation of Clinical Chemistry's (IFCC) method [7]. Both AP and  $\gamma$ -GT activities were quantified using a Hitachi 747 auto analyzer (Tokyo, Japan). Abnormal AP levels were defined as  $> 237 \text{ IU}/\text{L}$ . Abnormal  $\gamma$ -GT levels were defined as  $> 63 \text{ IU}/\text{L}$  for males and  $> 35 \text{ IU}/\text{L}$  for females. AP isoenzymes were separated by cellulose acetate membranes (Helena Laboratory, Beaumont, USA) and electrophoresed in a Tris-barbital-sodium buffer solution (ionic strength = 0.042).

### Statistical analyses

Continuous data with a normal distribution were expressed as means  $\pm$  standard deviations and compared by Student's *t* test. Continuous data without a normal distribution were expressed as medians and interquartile ranges and compared using the Mann–Whitney *U* test. Differences in proportions were assessed using the chi-squared test. Pearson's correlation coefficients were used to assess the linear relationship between enzymes and other measurements. The odds ratios (OR) and 95% confidence intervals (CI) of variables were estimated from multivariate logistic regression analyses. All statistical

analyses were performed using SPSS version 19.0 (IBM Co., Armonk, USA);  $P$  values  $< 0.05$  were considered statistically significant.

## Results

### Subject demographics and clinical characteristics

Of the 438 patients enrolled in this study, 414 were female and 24 were male. The mean age was  $45.9 \pm 18.6$

years (range, 18–93 years); basic characteristics and biochemical test results are summarized in Table 1. *Escherichia coli* was the most common pathogen (43.4% of all subjects; 86.4% of cases with positive microorganism culture) followed by *Klebsiella pneumoniae*. Ninety-one patients (20.8%) were diagnosed with complicated pyelonephritis (53 with diabetes, 29 with urolithiasis, 5 with neurogenic bladder, 3 with prostate disease, and 3 with congenital anomaly).

### Serum AP and $\gamma$ -GT in acute pyelonephritis

At the time of admission, the prevalences of high serum AP and  $\gamma$ -GT were 17.6% (77 cases) and 30.6% (134 cases), respectively. Both enzymes were elevated in 64 cases (14.6%).

Patients with high AP were significantly older than those with normal AP levels ( $54.4 \pm 16.4$  vs.  $44.0 \pm 18.5$  years,  $P < 0.001$ ) and they had longer hospital stays ( $9.7 \pm 7.0$  vs.  $6.7 \pm 5.1$  days,  $P < 0.001$ ; Table 2). The high AP group showed higher baseline serum Cr levels ( $1.25 \pm 0.74$  vs.  $0.97 \pm 0.68$  mg/dL,  $P = 0.003$ ), lower eGFR ( $62.2 \pm 26.1$  vs.  $79.9 \pm 24.2$  mL/min/1.73 m<sup>2</sup>,  $P < 0.001$ ), and exhibited a higher proportion of complicated pyelonephritis (31.2% vs. 18.6%,  $P = 0.020$ ). Antibiotic sensitivity analyses demonstrated that ciprofloxacin susceptibility was 75.9% (165/218). There were no differences in AP levels between the patients with ciprofloxacin-resistant *E. coli* and those with ciprofloxacin-sensitive *E. coli* ( $162.0 [135.0–242.5]$  vs.  $147.5 [121.3–209.25]$ ). However, in

**Table 1.** General patient characteristics (n = 438)

Variable	Value
Age (yr)	$45.9 \pm 18.6$ (18–93)
Female gender	414 (94.5)
Hospital days	$7.2 \pm 5.6$
WBC count (/ $\mu$ L)	$11,607 \pm 4,799$
SCr (mg/dL)	$1.02 \pm 0.70$
eGFR (mL/min/1.73 m <sup>2</sup> )	$76.8 \pm 25.4$
AST (IU/L)	$25.5 \pm 26.2$ (5–245)
ALT (IU/L)	$26.3 \pm 25.4$ (5–559)
AP (IU/L)	$185.3 \pm 123.4$ (62–1,423)
$\gamma$ -GT (IU/L)	$45.0 \pm 64.7$ (4–509)
Complicated APN	91 (20.8)
Positive urine culture	218 (49.8)

Data are presented mean  $\pm$  standard deviation (range) or number (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; APN, acute pyelonephritis; eGFR, estimated glomerular filtration rate;  $\gamma$ -GT, gamma-glutamyl transferase; SCr, serum creatinine; WBC, white blood cell.

**Table 2.** Comparison of the high and normal AP groups in acute pyelonephritis patients

Variable	High serum AP (n = 77)	Normal serum AP (n = 361)	$P$ value
Age (yr)	$54.4 \pm 16.4$	$44.0 \pm 18.5$	$< 0.001$
Sex, male	6 (7.8)	18 (5.0)	0.404
Hospital days	$9.7 \pm 7.0$	$6.7 \pm 5.1$	$< 0.001$
Complicated APN	24 (31.2)	67 (18.6)	0.020
WBC count (/ $\mu$ L)	$12,388 \pm 6,174$	$11,440 \pm 4,444$	0.204
SCr (mg/dL)	$1.25 \pm 0.74$	$0.97 \pm 0.68$	0.003
eGFR (mL/min/1.73 m <sup>2</sup> )	$62.2 \pm 26.1$	$79.9 \pm 24.2$	$< 0.001$
Serum AST (IU/L)	$30.0$ (17.0–59.0)	$17.0$ (13.0–23.3)	$< 0.001$
Serum ALT (IU/L)	$36.0$ (20.0–70.0)	$15.0$ (11.0–23.0)	$< 0.001$
Serum AP (IU/L)	$312.5$ (263.5–414.0)	$136.0$ (114.0–168.0)	$< 0.001$
Serum $\gamma$ -GT (IU/L)	$97.0$ (43.8–196.0)	$18.0$ (12.0–29.0)	$< 0.001$

Data are presented as mean  $\pm$  standard deviation, number (%), or median (range).

High serum AP levels were defined as  $> 237$  IU/L.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; APN, acute pyelonephritis; eGFR, estimated glomerular filtration rate;  $\gamma$ -GT, gamma-glutamyl transferase; SCr, serum creatinine; WBC, white blood cell.

most patients, AP values returned to normal levels within 2 weeks (75.9%) as patients responded to antibiotic therapy.

Patients in the high  $\gamma$ -GT group were significantly older than those in the normal  $\gamma$ -GT group ( $P < 0.001$ ) and had longer hospital stays ( $8.1 \pm 5.4$  vs.  $6.8 \pm 5.6$  days,  $P = 0.04$ ; Table 3). The high  $\gamma$ -GT group showed higher baseline serum Cr levels ( $1.19 \pm 1.05$  vs.  $0.94 \pm 0.45$  mg/dL,  $P = 0.009$ ) and lower eGFR ( $68.4 \pm 26.6$  vs.  $80.5 \pm 24.0$  mL/min/1.73 m<sup>2</sup>,  $P < 0.001$ ). There was no significant difference in the proportion of complicated pyelonephritis between the groups. There were no differences in  $\gamma$ -GT levels between the patients with ciprofloxacin-resistant *E. coli* and those with ciprofloxacin-sensitive *E. coli* ( $24.5$  [18.0–71.0] vs.  $21.0$  [13.0–21.5]). In 67.4% of the high  $\gamma$ -GT group, anti-

biotic treatment resulted in reduction of serum  $\gamma$ -GT to normal levels within 2 weeks of initiating treatment.

Hospital-stay duration had a significant positive correlation with both AP ( $r = 0.29$ ,  $P < 0.001$ ) and  $\gamma$ -GT ( $r = 0.14$ ,  $P = 0.003$ ) levels. Serum AP was also positively correlated with age ( $r = 0.26$ ,  $P < 0.001$ ), serum Cr ( $r = 0.14$ ,  $P = 0.005$ ), aspartate aminotransferase (AST;  $r = 0.40$ ,  $P < 0.001$ ), alanine aminotransferase (ALT;  $r = 0.43$ ,  $P < 0.001$ ), and  $\gamma$ -GT ( $r = 0.67$ ,  $P < 0.001$ ). Serum  $\gamma$ -GT was positively correlated with age ( $r = 0.14$ ,  $P = 0.003$ ), serum Cr ( $r = 0.16$ ,  $P = 0.001$ ), AST ( $r = 0.51$ ,  $P < 0.001$ ), and ALT ( $r = 0.54$ ,  $P < 0.001$ ). Multivariate regression analyses showed that high serum AP levels (OR, 2.47; 95% CI, 1.26–7.84;  $P = 0.008$ ) were significantly correlated with longer hospital stays (>

**Table 3.** Comparison of the high and normal  $\gamma$ -GT groups in acute pyelonephritis patients

Variable	High serum $\gamma$ -GT (n = 134)	Normal serum $\gamma$ -GT (n = 304)	P value
Age (yr)	51.1 ± 15.6	43.6 ± 19.3	< 0.001
Sex, male	9 (6.7)	15 (4.9)	0.496
Hospital days	8.1 ± 5.4	6.8 ± 5.6	0.029
Complicated APN	27 (20.1)	64 (21.1)	0.899
WBC count (/ $\mu$ L)	11,482 ± 4,863	11,662 ± 4,778	0.718
SCr (mg/dL)	1.19 ± 1.05	0.94 ± 0.45	0.009
eGFR (mL/min/1.73 m <sup>2</sup> )	68.4 ± 26.6	80.5 ± 24.0	< 0.001
Serum AST (IU/L)	28.0 (18.0–43.0)	16.0 (13.0–21.0)	< 0.001
Serum ALT (IU/L)	31.5 (20.0–52.3)	14.0 (10.8–19.3)	< 0.001
Serum AP (IU/L)	230.0 (159.0–325.8)	132.0 (112.8–165.3)	< 0.001
Serum $\gamma$ -GT (IU/L)	73.5 (50.8–129.8)	15.5 (11.0–21.0)	< 0.001

Data are presented as mean ± standard deviation, number (%), or median (range).

High serum  $\gamma$ -GT levels were defined as >63 IU/L for males, and >35 IU/L for females.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; APN, acute pyelonephritis; eGFR, estimated glomerular filtration rate;  $\gamma$ -GT, gamma-glutamyl transferase; SCr, serum creatinine; WBC, white blood cell.

**Table 4.** Independent factors associated with long hospital stays in acute pyelonephritis patients

Variable	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (yr)	1.032 (1.020–1.044)	< 0.001	1.016 (1.002–1.030)	0.029
Sex, male	2.981 (1.299–6.839)	0.010	2.193 (0.859–5.594)	0.100
Diabetes mellitus	4.139 (2.291–7.476)	< 0.001	2.399 (1.204–4.782)	0.013
SCr (mg/dL)	10.739 (4.609–25.022)	< 0.001	4.247 (1.860–9.695)	0.001
High serum AP	4.990 (2.972–8.375)	< 0.001	2.469 (1.260–4.838)	0.008
High serum $\gamma$ -GT	2.920 (1.872–4.554)	< 0.001	1.550 (0.856–2.808)	0.148
High serum AST	1.459 (0.792–2.687)	0.225		
High serum ALT	1.401 (0.740–2.654)	0.301		

High serum AP levels were defined as > 237 IU/L. High  $\gamma$ -GT levels were defined as > 63 IU/L for males, and > 35 IU/L for females. High AST/ALT levels were defined as > 45 IU/L.

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; OR, odds ratio;  $\gamma$ -GT, gamma-glutamyl transferase; SCr, serum creatinine.

7 days). The results also showed that serum Cr, diabetes mellitus, and age were independent risk factors for longer hospital stays (Table 4).

Our data also demonstrated a significant association of eGFR with both AP ( $r = -0.26$ ,  $P < 0.001$ ) and  $\gamma$ -GT ( $r = -0.214$ ,  $P < 0.001$ ). Multivariate analyses showed that high serum AP levels, not  $\gamma$ -GT, were significantly correlated with renal impairment (OR, 2.13; 95% CI, 1.08–4.19;  $P = 0.029$ ). The results also showed that age and diabetes mellitus were independent risk factors associated with renal impairment (Table 5).

Area under the curve (AUC) of the receiver operator characteristic (ROC) plot analysis was used to assess diagnostic and prognostic enzyme performance. ROC plot analysis showed that serum AP and  $\gamma$ -GT had significant discriminating power for long hospital stays (AUC, 0.635; 95% CI, 0.573–0.698;  $P < 0.001$  and AUC, 0.619; 95% CI, 0.557–0.680,  $P < 0.001$ ; respectively). Serum AP and  $\gamma$ -GT also had discriminating power for renal impairment (AUC, 0.611; 95% CI, 0.546–0.676;  $P = 0.001$  and AUC, 0.595; 95% CI, 0.532–0.659;  $P = 0.003$ ; respectively).

#### Fractional excretion and isoenzyme analysis

We evaluated urine AP/Cr, urine  $\gamma$ -GT/Cr,  $FE_{AP}$ , and  $FE_{\gamma-GT}$  in patients with elevated ( $n = 20$ , group A) and normal ( $n = 17$ , group B) serum AP and  $\gamma$ -GT levels. No significant differences were observed for urine AP/Cr,  $\gamma$ -GT/Cr, or  $FE_{AP}$  between the groups (Table 6). Urine  $\gamma$ -GT/Cr and  $FE_{AP}$  were marginally significantly lower in the elevated enzyme group compared with the normal enzyme group ( $P = 0.088$  and 0.082). Average  $FE_{\gamma-GT}$  was significantly lower in the elevated enzyme group compared with the normal group ( $P <$

0.001).

Serum AP isoenzyme analysis of 25 subjects was performed by electrophoresis using a cellulose acetate membrane. Normal isoenzyme patterns were observed in 6 of 15 patients (40.0%) in the normal AP group. Among patients with high AP levels ( $n = 10$ ), normal isoenzyme patterns were observed in five patients; elevated bone isoenzyme was evident in two of those patients. Among the remaining patients, no one had elevated liver fraction of AP isoenzyme. No significant differences in isoenzyme patterns were observed among the groups.

## Discussion

AP is present in most human tissues, including liver, bone, intestine, placenta, kidney, and white blood cells [8,9]. Within the kidney, AP activity is highest in the proximal convoluted tubules [8] with average activity levels 2.1-fold higher than in the distal convoluted tubules, and

**Table 6.** Urine levels and fractional excretion of AP ( $FE_{AP}$ ) and  $\gamma$ -GT ( $FE_{\gamma-GT}$ )

Variable	Group A ( $n = 20$ )	Group B ( $n = 17$ )	$P$ value
Urine AP/Cr (IU/mg)	0.28 (0.11–0.51)	0.19 (0.12–0.69)	0.446
Urine $\gamma$ -GT/Cr (IU/mg)	0.53 (0.24–0.62)	0.74 (0.49–0.89)	0.088
$FE_{AP}$	0.09 (0.04–0.13)	0.12 (0.07–0.36)	0.082
$FE_{\gamma-GT}$	0.43 (0.26–0.89)	3.40 (2.16–4.78)	< 0.001

Group A was defined as patients with elevated serum AP ( $> 237$  IU/L) and  $\gamma$ -GT ( $> 63$  IU/L for males and  $> 35$  IU/L for females). Group B was defined as normal serum AP ( $< 237$  IU/L) and  $\gamma$ -GT ( $< 63$  IU/L for males and  $< 35$  IU/L for females).

AP, alkaline phosphatase; Cr, creatinine; FE, fractional excretion;  $\gamma$ -GT, gamma-glutamyl transferase.

**Table 5.** Independent factors associated with renal impairment in acute pyelonephritis patients

Variable	Univariate		Multivariate	
	OR (95% CI)	$P$ value	OR (95% CI)	$P$ value
Age (yr)	1.068 (1.052–1.084)	< 0.001	1.064 (1.047–1.081)	< 0.001
Sex, male	0.604 (0.202–1.808)	0.367		
Diabetes mellitus	4.756 (2.623–8.624)	< 0.001	2.762 (1.411–5.406)	0.003
High serum AP	3.845 (2.292–6.453)	< 0.001	2.126 (1.080–4.185)	0.029
High serum $\gamma$ -GT	2.318 (1.473–3.647)	< 0.001	1.499 (0.818–2.746)	0.190
High serum AST	1.826 (0.995–3.351)	0.052		
High serum ALT	1.133 (0.577–2.227)	0.717		

High serum AP levels were defined as  $> 237$  IU/L. High  $\gamma$ -GT levels were defined as  $> 63$  IU/L for males, and  $> 35$  IU/L for females. High AST/ALT levels were defined as  $> 45$  IU/L.

AP, alkaline phosphatase;  $\gamma$ -GT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; OR, odds ratio.

4.7-fold higher than in the renal papilla. Because acute pyelonephritis usually involves both the renal pelvis and parenchyma, serum AP is likely to be significantly elevated in some acute pyelonephritis patients, making AP a promising indicator of severe renal parenchymal damage. A few studies have identified an association between elevated serum AP and acute pyelonephritis. Refaie et al [3] observed a 2.2-fold increase in serum AP levels in patients with acute pyelonephritis relative to controls, while another retrospective study identified a subset of acute pyelonephritis patients (14.7%) with significantly elevated serum AP [5].

Furthermore, a few studies have reported possible links between serum  $\gamma$ -GT and acute pyelonephritis. While  $\gamma$ -GT is present in a variety of tissues, including liver, pancreas, spleen, lung, small intestine, and placenta [10], the highest  $\gamma$ -GT concentrations are found in the brush border of the proximal tubules and the loop of Henle [11], suggesting an association with renal function. In our study, 17.6% of acute pyelonephritis patients exhibited elevated serum AP while 30.6% had elevated serum  $\gamma$ -GT; in 14.6%, both enzymes were elevated.

Our results showed a significant correlation between elevated serum AP and hospital-stay duration. Elevated serum AP was also modestly associated with age, serum Cr, and complicated pyelonephritis. These findings suggest a functional link between acute pyelonephritis severity and serum AP level. A previous study by Fotino [5] suggested that acute pyelonephritis may be attributable to elevated serum AP based on evidence of extensive parenchymal destruction in study patients' kidneys. Along with serum AP, we observed strong correlations between elevated serum  $\gamma$ -GT and hospital-stay duration, old age, and serum Cr in the subset of acute pyelonephritis patients. However, multivariate regression analyses showed that high serum  $\gamma$ -GT was not an independent risk factor for long hospital stays.

Likewise, serum AP, but not serum  $\gamma$ -GT, was useful for discriminating patients with renal impairment. A high degree of enzyme activity in the kidneys makes urinary tubular enzymes an excellent diagnostic tool for predicting acute kidney injury (AKI) [12,13] 12 hours to 4 days earlier than serum Cr [13,14]. There are four major categories of AKI biomarkers: functional markers (serum cystatin-C), up-regulated proteins (NGAL, KIM-1, L-FABP, and IL-18), low-molecular weight proteins (urine cys-

tatin-C), and enzymes (*N*-acetyl- $\beta$ -D-glucosaminidase, AP, and  $\gamma$ -GT) [15]. There are relatively few clinical studies of enzyme biomarkers, but Westhuyzen et al [14] reported that AP (AUC, 0.863) and  $\gamma$ -GT (AUC, 0.950) had excellent discriminating power for AKI. However, the AKI predictive power was low for either AP/urine Cr or  $\gamma$ -GT/urine Cr; both yielded AUCs < 0.7 [16]. Our study demonstrated an association between serum enzymes and concurrent renal impairment. However, we did not evaluate the effect of elevated enzymes upon sustained renal function decline or mortality, for which further research is warranted.

Next, we examined whether elevated serum enzymes were the result of increased enzyme release by inflammatory renal damage, decreased renal excretion, or nonspecific liver damage. Although  $FE_{\gamma\text{-GT}}$  was the only factor exhibiting a significant decrease in the high serum enzyme group, other factors, such as urine AP/Cr,  $\gamma$ -GT/Cr, and  $FE_{\text{AP}}$  were lower in the high serum enzyme group compared with normal controls. These results suggest that serum enzyme elevation can be attributed, in part, to decreased renal excretion, which likely stems from an increase in renal absorption of these enzymes.

An alternative explanation for elevated serum enzymes may be the release of enzymes originating from outside the kidneys, with the most likely source being the liver. Severe sepsis and hypotension result in nonspecific liver damage, which may result in the release of AP and  $\gamma$ -GT into circulation. However, liver fraction of AP isoenzyme was not elevated in acute pyelonephritis patients, suggesting that the liver is not the source of increased serum enzymes. Furthermore, complete normalization of AP and/or  $\gamma$ -GT levels in most acute pyelonephritis cases provides additional evidence that elevated AP and/or  $\gamma$ -GT levels are directly associated with acute pyelonephritis.

Our study has several limitations. First, we did not evaluate biomarkers such as cystatin C, NGAL, or KIM-1. Second, our sample size for fractional excretion and isoenzyme analyses was small. However, our data demonstrated that high AP and  $\gamma$ -GT levels were associated with complicated pyelonephritis and renal impairment. We also conclude that decreased renal enzyme excretion is a cause of high serum levels in acute pyelonephritis patients. A few studies have investigated the phenomenon of enzyme elevation [3–5], but no study has proposed an

underlying mechanism. Ours is the first study to suggest a possible mechanism for enzyme elevation in acute pyelonephritis patients.

In conclusion, our results demonstrated that serum AP and  $\gamma$ -GT levels are significantly elevated in a subset of acute pyelonephritis patients. Furthermore, lower urine AP/Cr, urine  $\gamma$ -GT/Cr,  $FE_{AP}$ , and  $FE_{\gamma-GT}$  levels combined with high serum enzymes in acute pyelonephritis patients suggest a causal link between diminished urinary excretion and elevated serum concentrations of these enzymes. A final analysis of AP isoenzyme profiles demonstrated that extra renal organs, including the liver, were unlikely to be the origin of serum AP elevation in acute pyelonephritis patients.

### Conflicts of interest

All authors have no conflicts of interest to declare.

### Authors' contributions

Chaehoon Han, Hayne Cho Park, and Sun Ryoung Choi participated in the data collection and wrote the manuscript. Ja Ryong Koo, Hyung Jik Kim, and Jung-Woo Noh participated in the study design and performed the statistical analysis. Min-Jeong Park participated in the conception, biochemical analysis, and interpretation of data. Jong-Woo Yoon and Ajin Cho provided intellectual content of critical importance to the work and technical support. Young-Ki Lee participated in the study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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