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Complete List of Authors:	Hwang, Kyungo ; Gyeongsang National University Hospital Chang, Ha Nee; Gyeongsang National University Hospital Lee, Tae Won; Gyeongsang National University Hospital, Internal Medicine Cho, Hyun Seop; Gyeongsang National University Hospital, Internal Medicine Bae, Eun Jin ; Changwon Gyeongsang National University Hospital, Internal Medicine Chang , Se-Ho ; Gyeongsang National University Graduate School of Medicine, Internal Medicine; Gyeongsang National University Hospital, Internal Medicine Park, Dong Jun; Gyeongsang National University Graduate School of Medicine, Internal Medicine; Changwon Gyeongsang National University Hospital, Internal Medicine
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The Incidence, Risk Factors, and Clinical Outcomes of Acute Kidney Injury Associated with Scrub Typhus

Kyungo Hwang[1], Ha Nee Chang[1], Tae Won Lee[1], Hyun Seop Cho[1,], Eun Jin Bae[2], Se-Ho Chang [1,3,4], Dong Jun Park[2,3,4]

[1]Gyeongsang National University Hospital, Internal Medicine,

[2]Changwon Gyeongsang National University Hospital, Internal Medicine,

[3]Gyeongsang National University School of Medicine, Internal Medicine,

[4]Institute of Health Science, Gyeongsang National University

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Address for corresponding author;

Dong Jun Park, MD, PhD.

Department of Internal Medicine, Changwon Gyeongsang National University Hospital, Internal Medicine,

11, Samjeongja-ro, Seongsan-gu, Changwon-si, Gyeongsangnam-do, Korea

Tel : 82-55-214-3735

Fax : 82-55-750-9255

E-mail : drpdj@naver.com

Abstract

Background Renal involvement in scrub typhus ranges from simple urinary abnormalities to acute kidney injury (AKI) leading to death. This study evaluated the incidence, predictors, and prognosis of AKI associated with scrub typhus according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria.

Study design We retrospectively evaluated the medical records of patients diagnosed with scrub typhus from January 2001 to November 2013 in Gyeongsang National University Hospital.

Results During the study period, 510 patients were diagnosed with scrub typhus and the incidence of AKI was 35.9%. There were 132 (73.4%) patients at risk, 37 (19.8%) with injury, and 14 (6.8%) with failure. In comparison with the non-AKI group, the AKI group was older (73.9 *vs*. 63.4 years, $P<0.001$) and had more co-morbidities such as hypertension, diabetes mellitus, and chronic kidney disease (CKD). AKI frequently occurs in hypertensive patients taking angiotensin receptor blockers or angiotensin converting enzyme inhibitors ($P=0.002$), and in diabetics with higher HbA1c levels ($P=0.033$). Hematuria and proteinuria were more frequent in the AKI group. There was no relationship between the severity of proteinuria and occurrence of AKI. Intensive care unit admission and death were more frequent in the AKI group. The renal function of most AKI patients recovered without sequelae, except for one patient who had underlying CKD. Multivariate analysis showed that age, presence of CKD, and serum albumin levels were independent predictors of AKI in patients with scrub typhus.

Conclusions According to the RIFLE criteria, AKI is relatively frequent in patients with scrub typhus and can lead to death, although the AKI in scrub typhus is usually mild

and renal recovery occurs in most patients. Physicians should bear in mind that scrub typhus is strongly associated with AKI and early intervention is needed for the prevention and treatment of AKI.

Strengths and limitations of this study

- This study has firstly demonstrated incidence, risk, and clinical outcomes of acute kidney injury (AKI) associated with scrub typhus according to RIFLE classification. Out study included many patients over many years
- Our findings suggest that a lot of AKI occurs in patients with scrub typhus. Older age, presence of underlying diabetes and hypertension, and lower serum albumin level were independent risk factors for AKI
- Our findings suggest that occurrence of AKI is directly associated with hospital duration and patient's prognosis, especially in patients with chronic kidney disease.
- A major limitation of this study was its retrospective design. In addition, because the research relied on medical records, the capacity to find other possible causes of AKI was limited and urine volume, an important factor in the RIFLE classification, could not be analyzed

INTRODUCTION

Scrub typhus is a mite-borne infectious disease caused by the intracellular Gram-negative bacteria *Orientia tsutsugamushi*. It is an acute disease characterized by a high fever, rash, and generalized symptoms such as myalgia and headache. The disease is common in Southeast Asia, Australia, Japan, China, and South Korea.[1-3] Renal involvement is common in scrub typhus, and ranges from simple hematuria or proteinuria to severe complications, including acute renal failure,[4-7] nephrotic syndrome,[8] and end-stage renal disease leading to long-term hemodialysis.[9] Although the incidence of acute kidney injury (AKI) in scrub typhus ranges from 8% to 40% according to the classification criteria used,[4 10-12] the risk factors and prognosis of AKI associated with scrub typhus are not clear. Therefore, we retrospectively studied the incidence, risk factors, and clinical outcomes of AKI in a large series of patients with scrub typhus.

PATIENTS AND METHODS

Registries

This study enrolled 510 scrub typhus patients who were admitted to Gyeongsang National University Hospital from January 2001 through November 2013. Their medical records were reviewed, including demographic data, clinical presentation, laboratory findings, and clinical outcomes to determine the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus.

Definitions

A diagnosis of scrub typhus was made when patients had a scab (eschar), acute febrile illness, skin rash, headache, muscle aches, lymph node swelling, hepatosplenomegaly, and a high initial indirect immunofluorescent antibody titer. If the initial titer was low, a four-fold increase in titer was considered significant. Proteinuria was categorized as trace, +, ++, or +++ using a urine dipstick test. Hematuria was defined as more than three red blood cells per high magnification field. In patients with underlying hypertension, the use of drugs that can affect renal function, such as angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and diuretics, was investigated. In patients with diabetes mellitus (DM), glycated hemoglobin was used to evaluate the degree of blood sugar control.

Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease study (MDRD) formula [1.86 × (PCr) – 1.154 × (age) – 0.203)] × (0.74 if female) × (1.210 if black). Shock was defined as a systolic blood pressure ≤ 90 mmHg. AKI was defined and categorized according to the risk, injury, failure, loss, end-stage kidney disease (RIFLE) classification.[13] We used the serum creatinine level and eGFR to establish the RIFLE category because we had no data on the 6- and 12-hour urine volumes. If the baseline creatinine values were unknown, the serum creatinine was estimated using the MDRD formula and assuming eGFR = 75 mL/min/1.73 m^2 as the normal value. To evaluate the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus, we divided the patients into AKI and non-AKI groups. Based on the occurrence of AKI, we also compared the clinical and laboratory characteristics of CKD patients among those with scrub typhus in the AKI and non-AKI

groups. We evaluated the severity of AKI categorized using RIFLE in CKD versus non-CKD groups.

The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023).

Statistical Analysis

All measurements are the mean ± standard deviation (SD). Pearson's chi-square and Fisher's exact test were used to analyze qualitative differences. The parametric Student's *t*-test was used to compare the means of samples with similar variances. Multivariate logistic regression analysis was used to identify significant risk factors for the occurrence of AKI from among the risk factors identified in univariate analyses. The statistical analysis was performed using SPSS for Windows software (ver. 21.0; SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was taken to indicate statistical significance.

RESULTS

Clinical and laboratory findings in all patients

Table 1 summarizes the demographic data, underlying diseases, clinical symptoms and signs, and laboratory findings of the 510 patients diagnosed with scrub typhus. The average patient age was 57.9 years and 48.0% were male. In total, 97 patients had underlying hypertension, of whom 22 took an ACEi or ARB (4.3%) and 12 took diuretics (2.4%). There were 61 patients with diabetes (12.0%) and 27 had CKD (5.3%). The average time to hospital presentation after symptom onset was 6.5 days.

Incidence of AKI and differences between the AKI and non-AKI groups

AKI occurred in 183 of the patients (35.9%). Of these, 132 patients were in the 'Risk' category (73.4%), 37 were in the 'Injury' category (19.8%), and 14 were in the 'Failure' category (6.8%). There was no 'Loss' or progression to end-stage renal disease (Figure 1). The AKI group was older (P<0.001). Hypertension, DM, and CKD were more frequent in the AKI group (P=0.002, 0.005, and <0.001, respectively) compared with the non-AKI group. ARB or ACEi were used more frequently in AKI patients with HT, whereas diuretics were not and glucose control was much poorer in the AKI group (P=0.033). The time to admission after symptom onset was longer in the AKI group (P=0.035). The serum albumin and hemoglobin levels were significantly lower in the AKI group (P<0.001 and P=0.000, respectively). Hematuria and proteinuria were more frequent in the AKI group (P=0.005 and 0.005, respectively), although the degree of proteinuria did not differ between the two group. The number of white blood cells, C-reactive protein, aspartate transaminase, and alanine transaminase levels, and *O. tsutsugamushi* antibody titer did not differ between the AKI and non-AKI groups. In total, 27 patients (5.3%) had underlying CKD, of which 19 (70.4%) developed AKI. This rate was significantly higher than that of non-CKD patients (Figure 2). Underlying renal function, measured using the MDRD formula, and the presence of hematuria were significant risk factors for AKI in these CKD patients (Table 2). The severity of AKI according to RIFLE category was significantly greater in CKD patients compared with non-CKD patients (Figure 3). In univariate analyses, older age, the presence of DM, HT, and CKD, and lower albumin and hemoglobin levels were significant predictors of AKI.

In the multivariate analysis, older age, presence of underlying DM and HT, and lower albumin level (<3.5 g/dL) remained independent risk factors for AKI (Table 3).

Outcomes of acute kidney injury

The hospital stay was much longer in the AKI group (P=0.039). The majority of surviving patients (99%) recovered from the AKI. Three patients underwent continuous renal replacement therapy (CRRT) in the AKI group, of which two had underlying CKD. Eight patients required intensive care unit (ICU) treatment for AKI. Four (0.8%) AKI patients died and all had underlying CKD: two underwent CRRT, while the other two did not. The death rate was also higher in CKD patients, compared with non-CKD patients (Figure 2). Renal function did not recover fully in only one (7.7%) of the surviving CKD patients, whereas it recovered in all of the non-CKD patients, including one patient who underwent CRRT. There was no information for five patients in the non-CKD group due to early discharge and follow-up loss. However, the renal function of these patients likely recovered because all initially had mild AKI (two 'Risk' patients and three 'Injury' patients). There was no case of end-stage renal disease requiring maintenance hemodialysis after the 3-month follow-up. The major cause of death was uncontrolled infection.

DISCUSSION

In our series, the incidence of AKI in scrub typhus was 35.9%. According to the RIFLE classification, AKI was associated with 'Risk' (73.4%), 'Injury' (19.8%), and 'Failure' (6.8%). AKI was more frequent in older patients with lower albumin levels and

underlying disease, such as uncontrolled DM or HT taking ACEi or ARB. In patients with underlying CKD, AKI was more frequent and had a poorer prognosis in terms of severity and survival.

There are few studies on the clinical outcome of AKI according to the RIFLE classification associated with scrub typhus.[4 12] Basu *et al*. first reported that the RIFLE classification is a valid indicator of AKI in acute febrile diseases, including scrub typhus, and can predict renal replacement therapy and the risk of mortality.[12] In their study, the incidence of AKI associated with scrub typhus was 42.6% and the severity of AKI was greater: 'Risk' (20.2%), 'Injury' (11.2%), and 'Failure' (11.2%). In addition, 5.9% of their patients underwent dialysis, the risk of mortality was associated with AKI severity, and mortality was significantly higher, at 13.3%, compared with our study. Our study also showed that the patients who died all had AKI. Basu *et al*. focused on the relationship between febrile infectious diseases and AKI according to the RIFLE classification, including other acute febrile diseases, and not the relationship between AKI and scrub typhus itself or predictors of AKI.

Attur *et al*. demonstrated that thrombocytopenia and ICU treatment were predictors of acute renal damage in patients with scrub typhus and AKI occurred in 23.2% of their patients.[6] The renal injury classifications were as follows: 'Risk' (38.4%), 'Injury' (21.7%), and 'Failure' (40%); and replacement therapy was given to 10% of their patients. The lower incidence of AKI (23.2% *vs*. 35.9%) compared with our study might be explained by the difference in the age of the enrolled patients (40 *vs*. 58 years). Age was an important predictor of AKI in our study. In addition, patients with underlying diseases such as CKD, DM, and hypertension were included in our

study. Underlying disease tends to increase in frequency with age. Easy access to a tertiary hospital might reduce the incidence and severity of AKI because of the regional characteristics of small areas. Our study also showed that the time from symptom onset to hospitalization was associated with AKI incidence.

The mechanism of AKI in scrub typhus is unclear. One plausible theory is that the invasion of rickettsia induces vasculitis, leading to direct renal involvement.[5 6] However, renal biopsies have not revealed evidence of renal vasculitis associated with scrub typhus, but showed inflammation and proliferation of the glomerular tubule-interstitium[4 5] and tubular necrosis due to direct involvement of tubules by rickettsia.[4-6] Membranous nephropathy has also been demonstrated,[8] although we cannot completely rule out renal vasculitis because very few renal biopsies have been done in scrub typhus.

Dumler *et al*. suggested that a decrease in renal blood flow accompanied by extravasation resulting from systemic vasculitis is the cause of pre-renal AKI.[14] Hypoalbuminemia caused by the leakage of serum albumin due to vasculitis is also postulated to be associated with AKI.[7] Although hypoalbuminemia was not a predictor of acute renal injury in previous studies,[4 12] the serum albumin level was significantly lower in our patients with AKI, and serves as an important predictor of AKI. Hypoalbuminemia is also an important marker of infection activity and CKD. Others have suggested different mechanisms, such as pan-vascular coagulation[4 6 7] and rhabdomyolysis,[4 6] but were not able to prove these hypotheses. The creatine phosphokinase level did not differ between the two groups in our study.

A close relationship between AKI and mortality has been reported. The mortality from scrub typhus in endemic areas in India is 2–12.2%.[15 16] The mortality

rates in two studies of AKI associated with scrub typhus were 13.3% and 0.8%.[4 12] All of the deaths occurred in the 'Failure' category of AKI. In our series of 510 patients, 4 died (0.8%) and all were in the 'Failure' category of AKI. Our patients had underlying CKD. Previous reports did not identify CKD as a risk factor for AKI or mortality.[4 12 15 16] The prognosis of AKI associated with scrub typhus has rarely been reported.[4 12] The renal prognosis after AKI is good if the patient survives. Permanent dialysis treatment was needed in one case study.[9] In our series, only one case did not recover to baseline renal function and there was no permanent renal loss requiring long-term dialysis.

A major limitation of this study was its retrospective design. In addition, because the research relied on medical records, the capacity to find other possible causes of AKI was limited and urine volume, an important factor in the RIFLE classification, could not be analyzed. Therefore, the incidence of AKI might have been underestimated. However, we believe that these limitations are overcome by the large subject pool and application of many variables to the statistical analysis. It was also a single-center study, so relatively similar lab values applied, most patients were followed at the same facility, and the same diagnostic criteria and treatment were used.

CONCLUSIONS

AKI is common in patients with scrub typhus. Predictors of AKI include underlying diseases such as DM, HT, and CKD, older age, delayed hospital presentation, and lower albumin level. Although renal function almost always recovers, AKI can be associated with loss of renal function and death, especially in patients with underlying CKD.

Unremitting efforts to diagnose and manage early scrub typhus should be made to decrease the incidence of AKI and improve the prognosis.

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Contributors KH conceptualized and drafted the manuscript; HNC participated in the design of the study. Both performed the statistical analyses and interpreting the results. TWL, HSC, EJB, and SC were involved in critically reviewing a draft of the manuscript and contributed to data collection. DJP further supervised the work. All authors approved the submission

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Competing interests None declared.

Ethics approval The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023)

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Table1. Clinical and laboratory data of AKI group and Non-AKI group

	Total (n=510)	AKI (n=183)	Non-AKI (n=327)	P value
Age (yr)	57.94 ± 18.85	64.47 ± 15.31	54.29 ± 19.67	<.001
Male	245 (48.0%)	97 (53.0%)	148 (45.3%)	.097
*HTN	97 (19.0%)	48 (26.2%)	49 (15.0%)	.002
*ARB use	22 (4.3%)	15 (8.2%)	7 (2.1%)	.002
Diuretics use	12 (2.4%)	6 (3.3%)	6 (1.8%)	.364
*DM	61 (12.0%)	32 (17.5%)	29 (8.9%)	.005
*CKD	27 (5.3%)	19 (10.4%)	8 (2.4%)	<.001
Time to admission from symptoms (days)	6.52 ± 6.76	9.70 ± 27.25	5.36 ± 9.18	.035
Symptoms and signs				
Fever	346 (85.0%)	128 (87.1%)	218 (83.8%)	.392
Myalgia	102 (25.1%)	32 (21.8%)	70 (26.9%)	.284
General weakness	48 (11.8%)	14 (9.5%)	34 (13.1%)	.338
Eschar	200 (39.2%)	80 (43.7%)	120 (36.7%)	.131
*WBC (x $10^3/u$L)	7.16 ± 3.54	7.56 ± 3.77	6.92 ± 3.39	.055
Hemoglobin (g/dL)	12.29 ± 1.75	11.91 ± 1.69	12.51 ± 1.76	.000
*CRP (mg/L)	49.91 ± 54.27	56.16 ± 62.53	46.04 ± 48.17	.072
*HbA1c (%)	7.59 ± 2.35	8.10 ± 2.42	6.87 ± 2.07	.033
Albumin (g/dL)	3.45 ± 0.60	3.19 ± 0.61	3.60 ± 0.55	<.001
*AST (U/L)	112.7 ± 400.6	150.9 ± 647.2	90.7 ± 104.0	.213
*ALT (U/L)	91.0 ± 167.2	104.5 ± 242.4	83.2 ± 100.7	.259
*CK (IU/L)	278.25 ± 673.77	371.05 ± 897.71	192.68 ± 345.53	.082
Creatinine (mg/dL)	0.75 ± 0.46	0.77 ± 0.72	0.74 ± 0.22	.571
Hematuria	179 (36.5%)	80 (44.4%)	99 (31.8%)	.005
Proteinuria	159 (32.3%)	73 (40.3%)	86 (27.7%)	.005
Trace	100 (62.9%)	44 (60.3%)	56 (65.1%)	.367
1+	43 (27.0%)	20 (27.4%)	23 (26.7%)	
2+	11 (6.9%)	6 (8.2%)	5 (5.8%)	
3+	5 (3.1%)	3 (4.1%)	2 (2.3%)	
Tsutsugamushi Ab titer	3234.04 ± 5785.24	3762.6 ± 3369.2	2862.2 ± 6046.5	.159
Hospital stay (days)	6.92 ± 18.01	9.70 ± 27.25	5.36 ± 9.18	.039
*CRRT	3 (0.6%)	3 (1.6%)	0 (0.0%)	.045
Shock	9 (1.8%)	5 (2.7%)	4 (1.2%)	.293
Admission to *ICU	8 (1.57%)	8 (4.4%)	0 (0.0%)	<.001
Death	4 (0.8%)	4 (2.2%)	0 (0.0%)	.016

HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hmeoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table2. Clinical and laboratory data of AKI and Non-AKI with CKD

	Total (n=27)	AKI (n=19)	Non-AKI (n=8)	P value
Age (yr)	72.59 ± 10.52	73.95 ± 9.03	69.38 ± 13.58	.311
Male	14 (51.9%)	11 (57.9%)	3 (37.5%)	.420
*HTN	11 (40.7%)	10 (52.6%)	1 (12.5%)	.090
*ARB use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Diuretics use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
*DM	9 (33.3%)	7 (36.8%)	2 (25.0%)	.676
Time to hospitalization from symptoms (days)	5.96 ± 4.57	6.11 ± 5.12	5.50 ± 2.35	.784
Symptoms and signs				
Fever	18 (75.0%)	12 (70.6%)	6 (85.7%)	.629
Myalgia	10 (41.7%)	7 (41.2%)	3 (42.9%)	1.000
General weakness	3 (12.5%)	2 (11.8%)	1 (14.3%)	1.000
Eschar	16 (59.3%)	12 (63.2%)	4 (50.0%)	.675
*WBC (x $10^3/uL$)	8.39 ± 4.20	8.16 ± 4.75	8.95 ± 2.64	.664
Hemoglobin (g/dL)	11.01 ± 1.51	10.74 ± 1.44	11.68 ± 1.56	.144
*CRP (mg/L)	83.18 ± 73.52	82.24 ± 80.14	85.19 ± 62.02	.928
*HbA1c (%)	7.99 ± 1.79	7.87 ± 1.90	8.80	.664
Albumin (g/dL)	3.10 ± 0.62	3.01 ± 0.67	3.31 ± 0.48	.258
*AST (U/L)	411.44 ± 1659.31	557.21 ± 1975.12	65.25 ± 23.43	.493
*ALT (U/L)	189.67 ± 567.68	244.47 ± 674.10	59.50 ± 31.46	.450
eGFR (mL/min/1.73m^2)	49.42 ± 8.02	47.15 ± 8.28	53.95 ± 5.42	.048
Hematuria	16 (59.3%)	14 (73.7%)	2 (25.0%)	.033
Proteinuria	15 (55.6%)	12 (63.2%)	3 (37.5%)	.398
Trace	4 (26.7%)	3 (25.0%)	1 (33.3%)	.891
1+	5 (33.3%)	4 (33.3%)	1 (33.3%)	
2+	4 (26.7%)	3 (25.0%)	1 (33.3%)	
3+	2 (13.3%)	2 (16.7%)	0 (0.0%)	
Tsutsugamushi Ab titer	2725.71 ± 3087.79	3080.00 ± 3463.41	1840.00 ± 1798.13	.420
Hospital stay (days)	6.59 ± 6.80	6.63 ± 7.67	6.50 ± 4.53	.964
*CRRT	2 (7.4%)	2 (10.5%)	0 (0.0%)	.567
Shock	3 (11.1%)	2 (10.5%)	1 (12.5%)	1.000
Admission to *ICU	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Death	4 (14.8%)	4 (21.1%)	0 (0.0%)	.285

* HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hemoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, eGFR estimated Glomerular Filtration Rate by MDRD, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table 3. Risk factors for the development of Scrub typhus associated AKI

Characteristics	Univariate analysis			Multivariate analysis		
	P value	*OR	95% *CI	*P* value	*OR	95% *CI
Age (>65 yr)	0.000	2.804	1.931-4.073	0.007	1.801	1.177-2.754
*HTN	0.002	2.017	1.289-3.157	0.273	1.331	0.798-2.221
*DM	0.005	2.178	1.270-3.734	0.146	1.564	0.856-2.859
*CKD	0.000	2.808	1.917-4.114	0.027	2.341	1.122-6.762
Albumin (<3.5 g/dL)	0.000	2.226	1.466-3.379	0.000	2.294	1.516-3.470
Hemoglobin (<12 g/dL)	0.032	1.495	1.035-2.160	0.787	1.598	0.625-1.427

* OR : Odds Ratio, CI : Confidence Interval, HTN : Hypertension, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease

Figure legend

Figure 1. Category of AKI according to RIFLE classification

Figure 2. Percentage of AKI occurrence and death according to presence of CKD

Figure 3. The comparison of AKI according to RIFLE category in patients with CKD and Non-CKD

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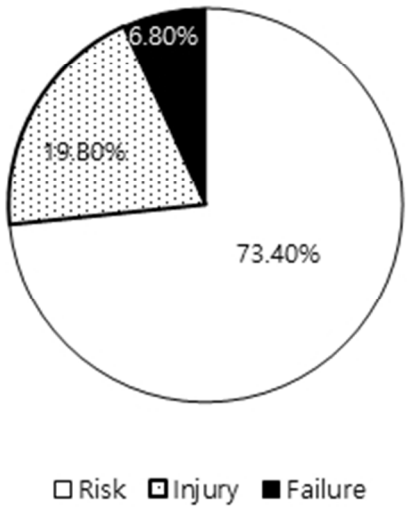
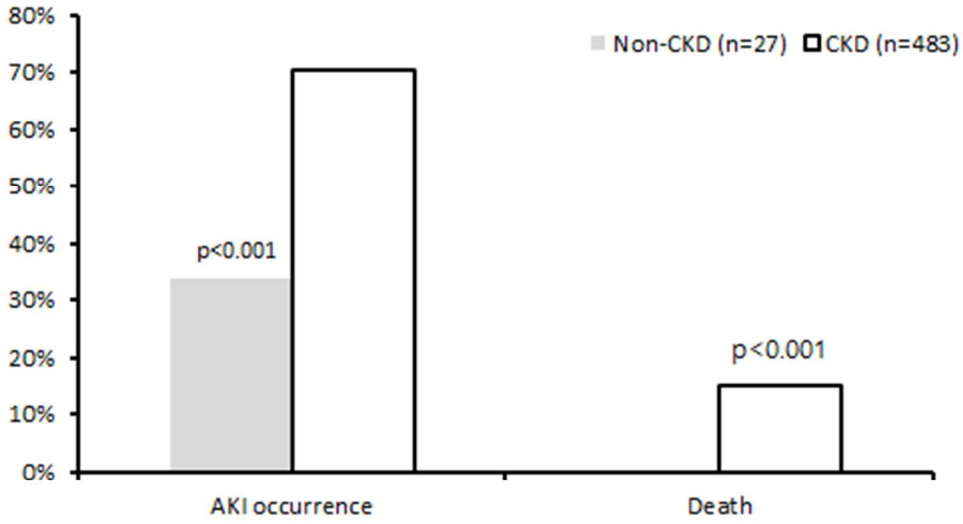


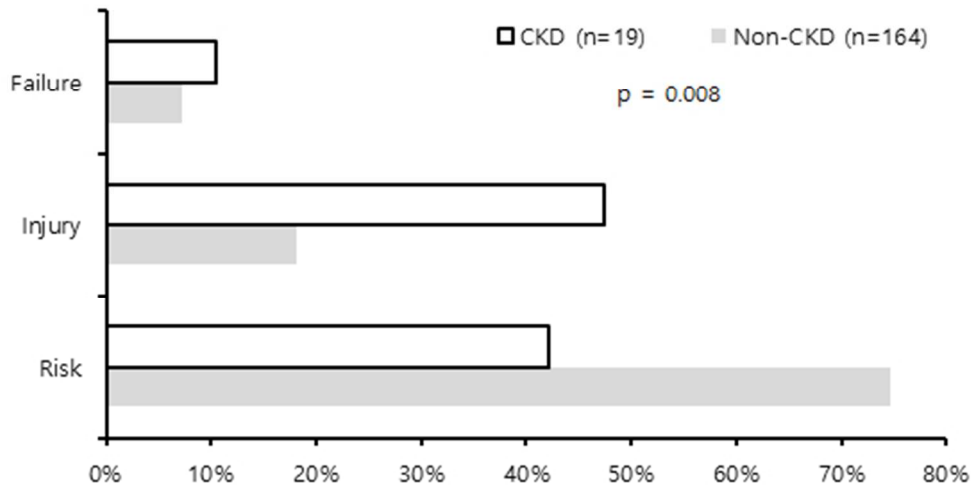
Category of AKI according to RIFLE classification

113x77mm (96 x 96 DPI)



Percentage of AKI occurrence and death according to presence of CKD

131x77mm (96 x 96 DPI)



The comparison of AKI according to RIFLE category in patients with CKD and Non-CKD

148x77mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	9,10,11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The incidence, risk factors, and clinical outcomes of acute kidney injury associated with scrub typhus: a retrospective study of 510 consecutive patients with scrub typhus

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The incidence, risk factors, and clinical outcomes of acute kidney injury associated with scrub typhus: a retrospective study of 510 consecutive patients with scrub typhus

Kyungo Hwang[1], Ha Nee Chang[1], Tae Won Lee[1], Hyun Seop Cho[1,], Eun Jin Bae[2], Se-Ho Chang [1,3,4], Dong Jun Park[2,3,4]

[1]Gyeongsang National University Hospital, Internal Medicine,

[2]Changwon Gyeongsang National University Hospital, Internal Medicine,

[3]Gyeongsang National University School of Medicine, Internal Medicine,

[4]Institute of Health Science, Gyeongsang National University

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Key Words: Scrub typhus, Acute Kidney Injury, RIFLE

Address for corresponding author;

Dong Jun Park, MD, PhD.
Department of Internal Medicine, Changwon Gyeongsang National University Hospital and Gyeongsang National University School of Medicine
11, Samjeongja-ro, Seongsan-gu, Changwon-si, Gyeongsangnam-do, Korea
Tel : 82-55-214-3735
Fax : 82-55-750-9255
E-mail : drpdj@naver.com

Abstract

Objectives: Renal involvement in scrub typhus ranges from simple urinary abnormalities to acute kidney injury (AKI) leading to death. This study evaluated the incidence, predictors, and prognosis of AKI associated with scrub typhus according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria.

Methods: We retrospectively evaluated the medical records of patients diagnosed with scrub typhus from January 2001 to November 2013 in Gyeongsang National University Hospital.

Results: During the study period, 510 patients were diagnosed with scrub typhus and the incidence of AKI was 35.9%. There were 132 (25.9%) patients at risk, 37 (7.3%) with injury, and 14 (2.7%) with failure. In comparison with the non-AKI group, the AKI group was older (73.9 *vs*. 63.4 years, $P<0.001$) and had more co-morbidities such as hypertension, diabetes mellitus, and chronic kidney disease (CKD). AKI frequently occurs in hypertensive patients taking angiotensin receptor blockers or angiotensin converting enzyme inhibitors ($P=0.002$), and in diabetics with higher HbA1c levels ($P=0.033$). Hematuria and proteinuria were more frequent in the AKI group. There was no relationship between the severity of proteinuria and occurrence of AKI. Intensive care unit admission and death were more frequent in the AKI group. The renal function of most AKI patients recovered without sequelae, except for one patient who had underlying CKD. Multivariate analysis showed that age, presence of CKD, and serum albumin levels were independent predictors of AKI in patients with scrub typhus.

Conclusions: AKI incidence associated with scrub typhus was 35.9%. Our current results suggest that underlying diseases such as DM, HT, older age, and lower albumin

level were important risk factors to determine occurrence of AKI. Whether earlier diagnosis and treatment in patients with above risk factors reduce the incidence and severity of AKI deserves to be investigated.

Strengths and limitations of this study

- This study is a large single-center study, including all patients with scrub typhus over about 12 year. Above all, same diagnostic criteria and identical laboratory conditions was applied to all patients.
- Despite large sample size, CKD patients was so small (5.3%) and this might exert an important effect in statistical analysis.
- A major limitation of this study was its retrospective design. Because the research relied on medical records, it might be insufficient to find other possible risk factor of AKI.
- Above all things, urine volume, important criteria of "RIFLE" criteria, could not be included. This might limit the power of our statistics in revealing the incidence of AKI associated with scrub typhus.

INTRODUCTION

Scrub typhus is a mite-borne infectious disease caused by the intracellular Gram-negative bacteria *Orientia tsutsugamushi*. It is an acute disease characterized by a high fever, rash, and generalized symptoms such as myalgia and headache. The disease is common in Southeast Asia, Australia, Japan, China, and South Korea.[1-3] Renal involvement is common in scrub typhus, and ranges from simple hematuria or proteinuria to severe complications, including acute renal failure,[4-7] nephrotic syndrome,[8] and end-stage renal disease leading to long-term hemodialysis.[9] Although the incidence of acute kidney injury (AKI) in scrub typhus ranges from 8% to 40% according to the classification criteria used,[4 10-12] the risk factors and prognosis of AKI associated with scrub typhus are not clear. Therefore, we retrospectively studied the incidence, risk factors, and clinical outcomes of AKI in a large series of patients with scrub typhus.

PATIENTS AND METHODS

Registries

This study enrolled 510 scrub typhus patients who were admitted to Gyeongsang National University Hospital from January 2001 through November 2013. Their medical records were reviewed, including demographic data, clinical presentation, laboratory findings, and clinical outcomes to determine the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus.

Definitions

A diagnosis of scrub typhus was made when patients had a scab (eschar), acute febrile illness, skin rash, headache, muscle aches, lymph node swelling, hepatosplenomegaly, and a high initial indirect immunofluorescent antibody (IFA) titer. If the initial titer was low, a four-fold increase in titer was considered significant. Proteinuria was categorized as trace, +, ++, or +++ using a urine dipstick test. Hematuria was defined as more than three red blood cells per high magnification field. In patients with underlying hypertension, the use of drugs that can affect renal function, such as angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and diuretics, was investigated. In patients with diabetes mellitus (DM), glycated hemoglobin was used to evaluate the degree of blood sugar control.

Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease study (MDRD) formula [1.86 × (PCr) – 1.154 × (age) – 0.203)] × (0.74 if female) × (1.210 if black). Shock was defined as a systolic blood pressure ≤ 90 mmHg. The method of creatinine formation was Jaffe one. RIFLE classification was firstly reported in 2004 whereas AKIN classification, later or modified version of "RIFLE" classification, in 2007. We have usually used RIFLE classification for AKI incidence in our institution because RIFLE classification can easily be applied when the baseline serum creatinine is known and has been largely validated in terms of determining the incidence of AKI. Furthermore, as we enrolled our patients from 2001 year, we used original version for classification of AKI. AKI was defined and categorized according to the risk, injury, failure, loss, end-stage kidney disease (RIFLE) classification.[13] We used the serum creatinine level and eGFR to establish the RIFLE category because we

had no data on the 6- and 12-hour urine volumes. If the baseline creatinine values were unknown, the serum creatinine was estimated using the MDRD formula and assuming eGFR = 75 mL/min/1.73 m^2 as the normal value. To evaluate the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus, we divided the patients into AKI and non-AKI groups. We also evaluated the distribution of incidence of AKI associated with scrub typhus as an interquartile. Based on the occurrence of AKI, we also compared the clinical and laboratory characteristics of CKD patients among those with scrub typhus in the AKI and non-AKI groups. We evaluated the severity of AKI categorized using RIFLE in CKD versus non-CKD groups.

The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023).

Statistical Analysis

All measurements are the mean ± standard deviation (SD). Pearson's chi-square and Fisher's exact test were used to analyze qualitative differences. The parametric Student's *t*-test was used to compare the means of samples with similar variances. Multivariate logistic regression analysis was used to identify significant risk factors for the occurrence of AKI from among the risk factors identified in univariate analyses. The statistical analysis was performed using SPSS for Windows software (ver. 21.0; SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was taken to indicate statistical significance.

RESULTS

Clinical and laboratory findings in all patients

Table 1 summarizes the demographic data, underlying diseases, clinical symptoms and signs, and laboratory findings of the 510 patients diagnosed with scrub typhus; 62, 108, 76, 264 patients in 1st, 2nd, 3rd, and 4th quartile, respectively. The average patient age was 57.9 years and 48.0% were male. In total, 97 patients had underlying hypertension, of whom 22 took an ACEi or ARB (4.3%) and 12 took diuretics (2.4%). There were 61 patients with diabetes (12.0%) and 27 had CKD (5.3%). The average time to hospital presentation after symptom onset was 6.5 days.

Incidence of AKI and differences between the AKI and non-AKI groups

AKI occurred in 183 of the patients (35.9%); 17 (27.4%), 38 (35.2%), 24 (31.6%), and 104 (39.4%) in 1st, 2nd, 3rd, and 4th quartile (p=0.264). Of these, 132 patients were in the 'Risk' category (25.9%), 37 were in the 'Injury' category (7.3%), and 14 were in the 'Failure' category (2.7%). There was no 'Loss' or progression to end-stage renal disease. The AKI group was older (P<0.001). Hypertension, DM, and CKD were more frequent in the AKI group (P=0.002, 0.005, and <0.001, respectively) compared with the non-AKI group. ARB or ACEi were used more frequently in AKI patients with HT, whereas diuretics were not and glucose control was much poorer in the AKI group (P=0.033). The time to admission after symptom onset was longer in the AKI group (P=0.035). The serum albumin and hemoglobin levels were significantly lower in the AKI group (P<0.001 and P=0.000, respectively). Hematuria and proteinuria were more frequent in the AKI group (P=0.005 and 0.005, respectively), although the degree of

proteinuria did not differ between the two group. The number of white blood cells, C-reactive protein, aspartate transaminase, and alanine transaminase levels, and *O. tsutsugamushi* antibody titer did not differ between the AKI and non-AKI groups. In total, 27 patients (5.3%) had underlying CKD, of which 19 (70.4%) developed AKI. This rate was significantly higher than that of non-CKD patients (Figure 1). Underlying renal function, measured using the MDRD formula, and the presence of hematuria were significant risk factors for AKI in these CKD patients (Table 2). The severity of AKI according to RIFLE category was significantly greater in CKD patients compared with non-CKD patients (Figure 2). In univariate analyses, older age, the presence of DM, HT, and CKD, and lower albumin and hemoglobin levels were significant predictors of AKI. In the multivariate analysis, older age, presence of underlying DM and HT, and lower albumin level (<3.5 g/dL) remained independent risk factors for AKI (Table 3).

Outcomes of acute kidney injury

The hospital stay was much longer in the AKI group (P=0.039). The majority of surviving patients (99%) recovered from the AKI. Three patients underwent continuous renal replacement therapy (CRRT) in the AKI group, of which two had underlying CKD. Eight patients required intensive care unit (ICU) treatment for AKI. Four (0.8%) AKI patients died and all had underlying CKD: two underwent CRRT, while the other two did not. The death rate was also higher in CKD patients, compared with non-CKD patients (Figure 1). Renal function did not recover fully in only one (7.7%) of the surviving CKD patients, whereas it recovered in all of the non-CKD patients, including one patient who underwent CRRT. There was no information for five patients in the

non-CKD group due to early discharge and follow-up loss. However, the renal function of these patients likely recovered because all initially had mild AKI (two 'Risk' patients and three 'Injury' patients). There was no case of end-stage renal disease requiring maintenance hemodialysis after the 3-month follow-up. The major cause of death was uncontrolled infection.

DISCUSSION

In our series, the incidence of AKI in scrub typhus was 35.9%. According to the RIFLE classification, AKI was associated with 'Risk' (25.9%), 'Injury' (7.3%), and 'Failure' (2.7%). AKI was more frequent in older patients with lower albumin levels and underlying disease, such as uncontrolled DM or HT taking ACEi or ARB. In patients with underlying CKD, AKI was more frequent and had a poorer prognosis in terms of severity and survival.

There are few studies on the clinical outcome of AKI according to the RIFLE classification associated with scrub typhus.[4 12] Basu *et al*. first reported that the RIFLE classification is a valid indicator of AKI in acute febrile diseases, including scrub typhus, and can predict renal replacement therapy and the risk of mortality.[12] Compared with our study, the incidence of AKI associated with scrub typhus was higher (42.6% vs 35.9%) and the severity of AKI was greater: 'Risk' (20.2% vs 25.9%), 'Injury' (11.2% vs 7.3%), and 'Failure' (11.2% vs 2.7%). In addition, 5.9% of their patients underwent dialysis, the risk of mortality was associated with AKI severity, and mortality was significantly higher, at 13.3%, compared with our study. Our study also showed that the patients who died all had AKI. Basu *et al*. focused on the relationship between febrile

infectious diseases and AKI according to the RIFLE classification, including other acute febrile diseases, and not the relationship between AKI and scrub typhus itself or predictors of AKI.

Attur *et al*. demonstrated that thrombocytopenia and ICU treatment were predictors of acute renal damage in patients with scrub typhus and AKI occurred in 23.2% of their patients.[4] The renal injury classifications were as follows: 'Risk' (8.9%), 'Injury' (5.0%), and 'Failure' (10.8%); and replacement therapy was given to 10% of their patients. The lower incidence of AKI (23.2% *vs*. 35.9%) compared with our study might be explained by the difference in the age of the enrolled patients (40 *vs*. 58 years). Age was an important predictor of AKI in our study. In addition, patients with underlying diseases such as CKD, DM, and hypertension were included in our study. Underlying disease tends to increase in frequency with age. Easy access to a tertiary hospital might reduce the incidence and severity of AKI because of the regional characteristics of small areas. Our study also showed that the time from symptom onset to hospitalization was associated with AKI incidence.

The mechanism of AKI in scrub typhus is unclear. One plausible theory is that the invasion of rickettsia induces vasculitis, leading to direct renal involvement.[5 6] However, renal biopsies have not revealed evidence of renal vasculitis associated with scrub typhus, but showed inflammation and proliferation of the glomerular tubule-interstitium[4 5] and tubular necrosis due to direct involvement of tubules by rickettsia.[4-6] Membranous nephropathy has also been demonstrated,[8] although we cannot completely rule out renal vasculitis because very few renal biopsies have been done in scrub typhus.

Dumler *et al*. suggested that a decrease in renal blood flow accompanied by extravasation resulting from systemic vasculitis is the cause of pre-renal AKI.[14] Hypoalbuminemia caused by the leakage of serum albumin due to vasculitis is also postulated to be associated with AKI.[7] Although hypoalbuminemia was not a predictor of acute renal injury in previous studies,[4 12] the serum albumin level was significantly lower in our patients with AKI, and serves as an important predictor of AKI. Hypoalbuminemia is also an important marker of severe infection and/or byproduct of chronic disease such as diabetes, hypertension and CKD. Others have suggested different mechanisms, such as pan-vascular coagulation[4 6 7] and rhabdomyolysis,[4 6] but were not able to prove these hypotheses. The creatine phosphokinase level did not differ between the two groups in our study.

A close relationship between AKI and mortality has been reported. The mortality from scrub typhus in endemic areas in India is 2–12.2%.[15 16] The mortality rates in two studies of AKI associated with scrub typhus were 13.3% and 0.8%.[4 12] All of the deaths occurred in the 'Failure' category of AKI. In our series of 510 patients, 4 died (0.8%) and all were in the 'Failure' category of AKI. Our patients had underlying CKD. Previous reports did not identify CKD as a risk factor for AKI or mortality.[4 12 15 16] The prognosis of AKI associated with scrub typhus has rarely been reported.[4 12] The renal prognosis after AKI is good if the patient survives. Permanent dialysis treatment was needed in one case study.[9] In our series, only one case did not recover to baseline renal function and there was no permanent renal loss requiring long-term dialysis.

The main methods in scrub typhus diagnostics remains serology, but currently available serological tests have limitations. Of that, the gold standard for scrub typhus is

IFA despite some limitations. Serological tests are most reliable when a fourfold rise in antibody titer is shown.[17] If the patient lives in non-endemic area, the diagnosis can be made from a single acute serum sample to require a cut-off antibody titer. This is impossible in patients living in endemic area because antibodies can be found in up to 18% of healthy populations.[18]

A major limitation of this study was its retrospective design. In addition, because the research relied on medical records, the capacity to find other possible causes of AKI was limited and urine volume, an important factor in the RIFLE classification, could not be analyzed. Therefore, the incidence of AKI might have been underestimated. However, we believe that these limitations are overcome by the large subject pool and application of many variables to the statistical analysis. It was also a single-center study, so relatively similar lab values applied, most patients were followed at the same facility, and the same diagnostic criteria and treatment were used.

CONCLUSIONS

AKI incidence associated with scrub typhus is 35.9%. Our current results suggest that underlying diseases such as DM, HT, older age, and lower albumin level were important risk factors to determine occurrence of AKI. Whether earlier diagnosis and treatment in patients with above risk factors reduce the incidence and severity of AKI deserves to be investigated.

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Contributors KH conceptualized and drafted the manuscript; HNC participated in the design of the study. Both performed the statistical analyses and interpreting the results.

TWL, HSC, EJB, and SC were involved in critically reviewing a draft of the manuscript and contributed to data collection. DJP further supervised the work. All authors approved the submission

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Competing interests None declared.

Ethics approval The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023)

Provenance and peer review Not commissioned; externally peer reviewed

Data sharing statement No additional data are available.

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Table1. Clinical and laboratory data of AKI group and Non-AKI group

	Total (n=510)	AKI (n=183)	Non-AKI (n=327)	P value
Age (yr)	57.94 ± 18.85	64.47 ± 15.31	54.29 ± 19.67	<.001
Male	245 (48.0%)	97 (53.0%)	148 (45.3%)	.097
*HTN	97 (19.0%)	48 (26.2%)	49 (15.0%)	.002
*ARB use	22 (4.3%)	15 (8.2%)	7 (2.1%)	.002
Diuretics use	12 (2.4%)	6 (3.3%)	6 (1.8%)	.364
*DM	61 (12.0%)	32 (17.5%)	29 (8.9%)	.005
*CKD	27 (5.3%)	19 (10.4%)	8 (2.4%)	<.001
Time to admission from symptoms (days)	6.52 ± 6.76	9.70 ± 27.25	5.36 ± 9.18	.035
Symptoms and signs				
Fever	346 (85.0%)	128 (87.1%)	218 (83.8%)	.392
Myalgia	102 (25.1%)	32 (21.8%)	70 (26.9%)	.284
General weakness	48 (11.8%)	14 (9.5%)	34 (13.1%)	.338
Eschar	200 (39.2%)	80 (43.7%)	120 (36.7%)	.131
*WBC (x $10^3/uL$)	7.16 ± 3.54	7.56 ± 3.77	6.92 ± 3.39	.055
Hemoglobin (g/dL)	12.29 ± 1.75	11.91 ± 1.69	12.51 ± 1.76	.000
*CRP (mg/L)	49.91 ± 54.27	56.16 ± 62.53	46.04 ± 48.17	.072
*HbA1c (%)	7.59 ± 2.35	8.10 ± 2.42	6.87 ± 2.07	.033
Albumin (g/dL)	3.45 ± 0.60	3.19 ± 0.61	3.60 ± 0.55	<.001
*AST (U/L)	112.7 ± 400.6	150.9 ± 647.2	90.7 ± 104.0	.213
*ALT (U/L)	91.0 ± 167.2	104.5 ± 242.4	83.2 ± 100.7	.259
*CK (IU/L)	278.25 ± 673.77	371.05 ± 897.71	192.68 ± 345.53	.082
Creatinine (mg/dL)	0.75 ± 0.46	0.77 ± 0.72	0.74 ± 0.22	.571
Hematuria	179 (36.5%)	80 (44.4%)	99 (31.8%)	.005
Proteinuria	159 (32.3%)	73 (40.3%)	86 (27.7%)	.005
Trace	100 (62.9%)	44 (60.3%)	56 (65.1%)	.367
1+	43 (27.0%)	20 (27.4%)	23 (26.7%)	
2+	11 (6.9%)	6 (8.2%)	5 (5.8%)	
3+	5 (3.1%)	3 (4.1%)	2 (2.3%)	
Tsutsugamushi Ab titer	3234.04 ± 5785.24	3762.6 ± 3369.2	2862.2 ± 6046.5	.159
Hospital stay (days)	6.92 ± 18.01	9.70 ± 27.25	5.36 ± 9.18	.039
*CRRT	3 (0.6%)	3 (1.6%)	0 (0.0%)	.045
Shock	9 (1.8%)	5 (2.7%)	4 (1.2%)	.293
Admission to *ICU	8 (1.57%)	8 (4.4%)	0 (0.0%)	<.001
Death	4 (0.8%)	4 (2.2%)	0 (0.0%)	.016

HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hmeoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table2. Clinical and laboratory data of AKI and Non-AKI with CKD

	Total (n=27)	AKI (n=19)	Non-AKI (n=8)	P value
Age (yr)	72.59 ± 10.52	73.95 ± 9.03	69.38 ± 13.58	.311
Male	14 (51.9%)	11 (57.9%)	3 (37.5%)	.420
*HTN	11 (40.7%)	10 (52.6%)	1 (12.5%)	.090
*ARB use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Diuretics use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
*DM	9 (33.3%)	7 (36.8%)	2 (25.0%)	.676
Time to hospitalization from symptoms (days)	5.96 ± 4.57	6.11 ± 5.12	5.50 ± 2.35	.784
Symptoms and signs				
Fever	18 (75.0%)	12 (70.6%)	6 (85.7%)	.629
Myalgia	10 (41.7%)	7 (41.2%)	3 (42.9%)	1.000
General weakness	3 (12.5%)	2 (11.8%)	1 (14.3%)	1.000
Eschar	16 (59.3%)	12 (63.2%)	4 (50.0%)	.675
*WBC (x $10^3/u$L)	8.39 ± 4.20	8.16 ± 4.75	8.95 ± 2.64	.664
Hemoglobin (g/dL)	11.01 ± 1.51	10.74 ± 1.44	11.68 ± 1.56	.144
*CRP (mg/L)	83.18 ± 73.52	82.24 ± 80.14	85.19 ± 62.02	.928
*HbA1c (%)	7.99 ± 1.79	7.87 ± 1.90	8.80	.664
Albumin (g/dL)	3.10 ± 0.62	3.01 ± 0.67	3.31 ± 0.48	.258
*AST (U/L)	411.44 ± 1659.31	557.21 ± 1975.12	65.25 ± 23.43	.493
*ALT (U/L)	189.67 ± 567.68	244.47 ± 674.10	59.50 ± 31.46	.450
eGFR (mL/min/1.73m^2)	49.42 ± 8.02	47.15 ± 8.28	53.95 ± 5.42	.048
Hematuria	16 (59.3%)	14 (73.7%)	2 (25.0%)	.033
Proteinuria	15 (55.6%)	12 (63.2%)	3 (37.5%)	.398
Trace	4 (26.7%)	3 (25.0%)	1 (33.3%)	.891
1+	5 (33.3%)	4 (33.3%)	1 (33.3%)	
2+	4 (26.7%)	3 (25.0%)	1 (33.3%)	
3+	2 (13.3%)	2 (16.7%)	0 (0.0%)	
Tsutsugamushi Ab titer	2725.71 ± 3087.79	3080.00 ± 3463.41	1840.00 ± 1798.13	.420
Hospital stay (days)	6.59 ± 6.80	6.63 ± 7.67	6.50 ± 4.53	.964
*CRRT	2 (7.4%)	2 (10.5%)	0 (0.0%)	.567
Shock	3 (11.1%)	2 (10.5%)	1 (12.5%)	1.000
Admission to *ICU	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Death	4 (14.8%)	4 (21.1%)	0 (0.0%)	.285

* HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hemoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, eGFR estimated Glomerular Filtration Rate by MDRD, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table 3. Risk factors for the development of Scrub typhus associated AKI

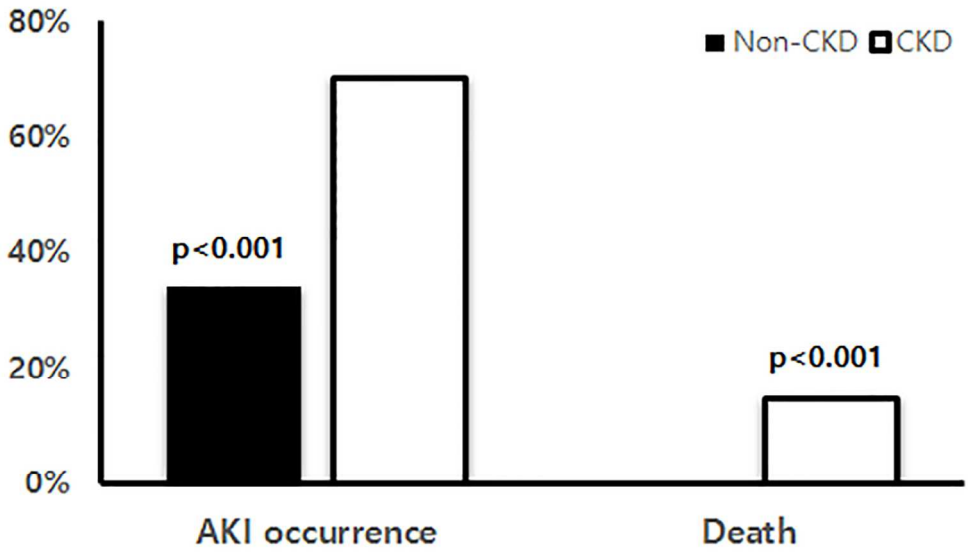
Characteristics	Univariate analysis			Multivariate analysis		
	P value	*OR	95% *CI	*P* value	*OR	95% *CI
Age (>65 yr)	0.000	2.804	1.931-4.073	0.007	1.801	1.177-2.754
*HTN	0.002	2.017	1.289-3.157	0.273	1.331	0.798-2.221
*DM	0.005	2.178	1.270-3.734	0.146	1.564	0.856-2.859
*CKD	0.000	2.808	1.917-4.114	0.027	2.341	1.122-6.762
Albumin (<3.5 g/dL)	0.000	2.226	1.466-3.379	0.000	2.294	1.516-3.470
Hemoglobin (<12 g/dL)	0.032	1.495	1.035-2.160	0.787	1.598	0.625-1.427

* OR : Odds Ratio, CI : Confidence Interval, HTN : Hypertension, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease

Figure legend

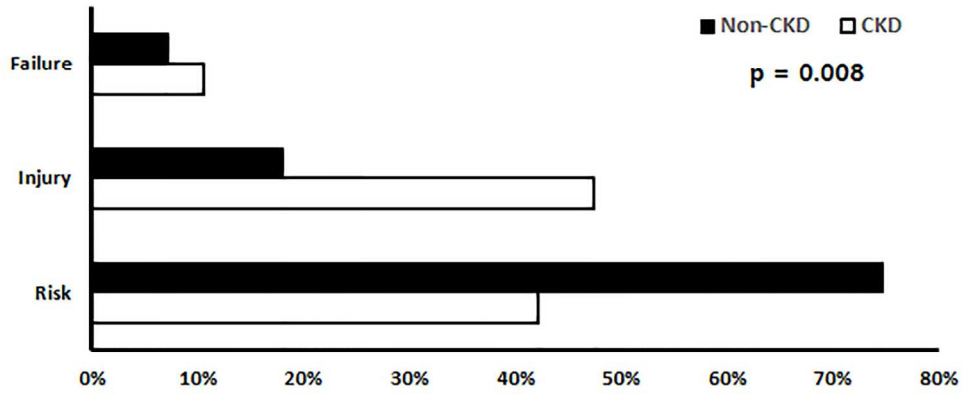
Figure 1. Percentage of AKI occurrence and death according to presence of CKD

Figure 2. The comparison of AKI according to RIFLE category in patients with CKD and Non-CKD



Percentage of AKI occurrence and death according to presence of CKD

126x74mm (300 x 300 DPI)



The comparison of AKI according to RIFLE category in patients with CKD and Non-CKD

161x76mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	9,10,11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Incidence, risk factors, and clinical outcomes of acute kidney injury associated with scrub typhus: a retrospective study of 510 consecutive patients in South Korea (2001~2013)

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Incidence, risk factors, and clinical outcomes of acute kidney injury associated with scrub typhus: a retrospective study of 510 consecutive patients in South Korea (2001~2013)

Kyungo Hwang[1], Ha Nee Chang[1], Tae Won Lee[1], Hyun Seop Cho[1,], Eunjin Bae[2], Se-Ho Chang[1,3,4], Dong Jun Park[2,3,4]

[1]Department of Internal Medicine, Gyeongsang National University Hospital

[2]Department of Internal Medicine, Changwon Gyeongsang National University Hospital

3Department of Internal Medicine, Gyeongsang National University School of Medicine

[4]Institute of Health Science, Gyeongsang National University

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Address for corresponding author;

Dong Jun Park, MD, PhD.

Department of Internal Medicine, Changwon Gyeongsang National University Hospital and Gyeongsang National University School of Medicine

11, Samjeongja-ro, Seongsan-gu, Changwon-si, Gyeongsangnam-do, Korea

Tel : 82-55-214-3735

Fax : 82-55-750-9255

E-mail : drpdj@naver.com

Abstract

Objectives: Renal involvement in scrub typhus ranges from simple urinary abnormalities to acute kidney injury (AKI) leading to death. This study evaluated the incidence, predictors, and prognosis of AKI associated with scrub typhus according to the RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria.

Methods: We retrospectively evaluated the medical records of patients diagnosed with scrub typhus from January 2001 to November 2013 in Gyeongsang National University Hospital.

Results: During the study period, 510 patients were diagnosed with scrub typhus and the incidence of AKI was 35.9%. There were 132 (25.9%) patients at risk, 37 (7.3%) with injury, and 14 (2.7%) with failure. In comparison with the non-AKI group, the AKI group was older (73.9 *vs*. 63.4 years, $P<0.001$) and had more co-morbidities such as hypertension, diabetes mellitus, and chronic kidney disease (CKD). AKI frequently occurs in hypertensive patients taking angiotensin receptor blockers or angiotensin converting enzyme inhibitors ($P=0.002$), and in diabetics with higher HbA1c levels ($P=0.033$). Hematuria and proteinuria were more frequent in the AKI group. There was no relationship between the severity of proteinuria and occurrence of AKI. Intensive care unit admission and death were more frequent in the AKI group. The renal function of most AKI patients recovered without sequelae, except for one patient who had underlying CKD. Multivariate analysis showed that age, presence of CKD, serum albumin level, and time to hospital presentation after symptom onset were independent predictors of AKI in patients with scrub typhus.

Conclusions: Our current results suggest that presence of underlying CKD, older age, lower serum albumin level, and time to hospital presentation after symptom onset were important risk factors to determine occurrence of AKI. Whether earlier diagnosis and treatment in patients with above risk factors reduce the incidence and severity of AKI deserves to be investigated.

Strengths and limitations of this study

- This study is a large single-center study, including all patients with scrub typhus over about 12 year. Above all, same diagnostic criteria and identical laboratory conditions was applied to all patients.
- Despite large sample size, CKD patients was so small (5.3%) and this might exert an important effect in statistical analysis.
- A major limitation of this study was its retrospective design. Because the research relied on medical records, it might be insufficient to find other possible risk factor of AKI.
- Above all things, urine volume, important criteria of "RIFLE" criteria, could not be included. This might limit the power of our statistics in revealing the incidence of AKI associated with scrub typhus.

INTRODUCTION

Scrub typhus is a mite-borne infectious disease caused by the intracellular Gram-negative bacteria *Orientia tsutsugamushi*. It is an acute febrile illness with the characteristic findings with by high fever, rash, and generalized symptoms such as myalgia and headache. The disease is common in Southeast Asia, Australia, Japan, China, and South Korea during autumn seasons.[1-3]The main reservoir is rodents, and their mites act as both the reservoir and the vector. Human infection occurs when the larvae of the thrombiculid mite infected with *O.tsutsugamushi* bite people and suck human tissue fluid.[4-6]The clinical prognosis of scrub typhus varies from mild to severe courses. Although most of the patients have a benign course, some patients suffer serious complications such as rickettsemia causing disseminated multi-organ vasculitis. According to organ involved, patients could present with pneumonitis, meningitis, encephalitis, myocarditis, acute pulmonary edema, pericarditis, hepatitis, and even multiple organ failure.[7-9] Respiratory distress and encephalitis are the principal cause of death in patients with severe disease.[10]

Acute kidney injury (AKI) is a major global health issue and its incidence is markedly arising[11] and affects an estimated 13-18% of hospitalized patients[12] resulting in increasing hospital stay, healthcare costs, poor short-term and long-term outcomes, [13] especially in chronic kidney disease (CKD) patients.[14] In patients with infectious disease, especially those with sepsis, the incidence of AKI is reported to range from 5% to 51%.[15, 16] Renal involvement is not uncommon in scrub typhus, and ranges from simple hematuria or proteinuria, 10% to 20% incidence of scrub typhus, to severe complications, including acute renal failure,[17-20] nephrotic syndrome,[21] and end-stage

renal disease leading to long-term hemodialysis.[22] It is known that the incidence of AKI in scrub typhus ranges from 8% to 40% according to the classification criteria used.[17, 23-26] The risk factors and prognosis of AKI associated with scrub typhus have been poorly studied.[26] We have encountered poor prognosis and long hospital stay in patients with scrub typhus if AKI was accompanied, especially in patients with comorbidities such as diabetes mellitus (DM), hypertension, and CKD. Therefore, we analyzed the clinical and laboratory data of AKI in patients with scrub typhus.

The aim of present study was to evaluate the incidence, risk factors, and clinical outcomes of AKI according to RIFLE classification in a large series of patients with scrub typhus.

PATIENTS AND METHODS

Registries

This study enrolled 510 scrub typhus patients who were admitted to Gyeongsang National University Hospital from January 2001 through November 2013. Their medical records were reviewed, including demographic data, clinical presentations, laboratory findings, and clinical outcomes to determine the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus.

Definitions

A diagnosis of scrub typhus was made when patients had a scab (eschar), acute febrile illness, skin rash, headache, muscle aches, lymph node swelling, hepatosplenomegaly, and a high initial indirect immunofluorescent antibody (IFA) titer. If the initial titer was low, a four-fold increase in titer was considered significant. Proteinuria was categorized

as trace, +, ++, or +++ using a urine dipstick test. Hematuria was defined as more than three red blood cells per high magnification field. In patients with underlying hypertension, the use of drugs that can affect renal function, such as angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and diuretics, was investigated. In patients with DM, glycated hemoglobin was used to evaluate the degree of blood sugar control.

CKD was defined as an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 using the Modification of Diet in Renal Disease study (MDRD) formula [1.86 × (PCr) – 1.154 × (age) – 0.203)] × (0.74 if female) × (1.210 if black). Shock was defined as a systolic blood pressure ≤ 90 mmHg. The method of creatinine formation was Jaffe one. RIFLE classification was firstly reported in 2004 whereas AKIN classification, later or modified version of "RIFLE" classification, in 2007. We have usually used RIFLE classification for AKI incidence in our institution because RIFLE classification can easily be applied when the baseline serum creatinine is known and has been largely validated in terms of determining the incidence of AKI. Furthermore, as we enrolled our patients from 2001 year, we used original version for classification of AKI. AKI was defined and categorized according to the risk, injury, failure, loss, end-stage kidney disease (RIFLE) classification.[27] We used the serum creatinine level and eGFR to establish the RIFLE category because we had no data on the 6- and 12-hour urine volumes. If the baseline creatinine values were unknown, the serum creatinine was estimated using the MDRD formula and assuming eGFR = 75 mL/min/1.73 m^2 as the normal value. To evaluate the incidence, risk factors, and clinical outcomes of AKI associated with scrub typhus, we divided the patients into

AKI and non-AKI groups. To show the distribution of AKI cases, we annually evaluated the incidence of AKI associated with scrub typhus and compared it as an interquartile. Based on the occurrence of AKI, we also compared the clinical and laboratory characteristics of CKD patients among those with scrub typhus in the AKI and non-AKI groups. We evaluated the severity of AKI categorized using RIFLE in CKD versus non-CKD groups.

The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023).

Statistical Analysis

All measurements are the mean ± standard deviation (SD). Pearson's chi-square and Fisher's exact test were used to analyze qualitative differences. The parametric Student's *t*-test was used to compare the means of samples with similar variances. Multivariate logistic regression analysis was used to identify significant risk factors for the occurrence of AKI from among the risk factors identified in univariate analyses. The statistical analysis was performed using SPSS for Windows software (ver. 21.0; SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was taken to indicate statistical significance.

RESULTS

Clinical and laboratory findings in all patients

Table 1 summarizes the demographic data, underlying diseases, clinical symptoms and signs, and laboratory findings of the 510 patients diagnosed with scrub typhus; 62, 108, 76, 264 patients in 1st, 2nd, 3rd, and 4th quartile, respectively. The average patient age was 57.9 years and 48.0% were male. In total, 97 patients had underlying hypertension, of

whom 22 took an ACEi or ARB (4.3%) and 12 took diuretics (2.4%). There were 61 patients with diabetes (12.0%) and 27 had CKD (5.3%). The average time to hospital presentation after symptom onset was 6.5 days.

Incidence of AKI and differences between the AKI and non-AKI groups

AKI occurred in 183 of the patients (35.9%); 17 (27.4%), 38 (35.2%), 24 (31.6%), and 104 (39.4%) in 1st, 2nd, 3rd, and 4th quartile (p=0.264). Of these, 132 patients were in the 'Risk' category (25.9%), 37 were in the 'Injury' category (7.3%), and 14 were in the 'Failure' category (2.7%). There was no 'Loss' or progression to end-stage renal disease. The AKI group was older (*P*<0.001). Hypertension, DM, and CKD were more frequent in the AKI group (*P*=0.002, 0.005, and <0.001, respectively) compared with the non-AKI group. ARB or ACEi were used more frequently in AKI patients with HT, whereas diuretics were not and glucose control was much poorer in the AKI group (*P*=0.033). The time to admission after symptom onset was longer in the AKI group (*P*=0.035). The serum albumin and hemoglobin levels were significantly lower in the AKI group (*P*<0.001 and *P*=0.000, respectively). Hematuria and proteinuria were more frequent in the AKI group (*P*=0.005 and 0.005, respectively), although the degree of proteinuria did not differ between the two group. The number of white blood cells, C-reactive protein, aspartate transaminase, and alanine transaminase levels, and *O. tsutsugamushi* antibody titer did not differ between the AKI and non-AKI groups. In total, 27 patients (5.3%) had underlying CKD, of which 19 (70.4%) developed AKI. This rate was significantly higher than that of non-CKD patients (Figure 1). Underlying renal function, measured using the MDRD formula, and the presence of hematuria were

significant risk factors for AKI in these CKD patients (Table 2). The severity of AKI according to RIFLE category was significantly greater in CKD patients compared with non-CKD patients (Figure 2). In univariate analysis, time to hospital presentation after symptom onset, older age, the presence of DM, HT, and CKD, and lower albumin and hemoglobin levels were significant predictors of AKI. In the multivariate analysis, time to hospital presentation after symptom onset, older age, presence of CKD, and lower albumin level (<3.5 g/dL) remained independent risk factors for AKI (Table 3).

Outcomes of acute kidney injury

The hospital stay was much longer in the AKI group (P=0.039). The majority of surviving patients (99%) recovered from the AKI. Three patients underwent continuous renal replacement therapy (CRRT) in the AKI group, of which two had underlying CKD. Eight patients required intensive care unit (ICU) treatment for AKI. Four (0.8%) AKI patients died and all had underlying CKD: two underwent CRRT, while the other two did not. The death rate was also higher in CKD patients, compared with non-CKD patients (Figure 1). Renal function did not recover fully in only one (7.7%) of the surviving CKD patients, whereas it recovered in all of the non-CKD patients, including one patient who underwent CRRT. There was no information for five patients in the non-CKD group due to early discharge and follow-up loss. However, the renal function of these patients likely recovered because all initially had mild AKI (two 'Risk' patients and three 'Injury' patients). There was no case of end-stage renal disease requiring maintenance hemodialysis after the 3-month follow-up. The major cause of death was uncontrolled infection.

DISCUSSION

In our series, the incidence of AKI in scrub typhus was 35.9%. According to the RIFLE classification, AKI was associated with 'Risk' (25.9%), 'Injury' (7.3%), and 'Failure' (2.7%). AKI was more frequent in older patients who had lower serum albumin level and visited hospital late after symptom onset and with underlying disease, such as uncontrolled DM or HT taking ACEi or ARB. In patients with underlying CKD, AKI was more frequent and had a poorer prognosis in terms of severity and survival.

There are few studies on the clinical outcome of AKI according to the RIFLE classification associated with scrub typhus.[17, 25] Basu *et al*. first reported that the RIFLE classification is a valid indicator of AKI in acute febrile diseases, including scrub typhus, and can predict renal replacement therapy and the risk of mortality.[25] Compared with our study, the incidence of AKI associated with scrub typhus was higher (42.6% vs 35.9%) and the severity of AKI was greater: 'Risk' (20.2% vs 25.9%), 'Injury' (11.2% vs 7.3%), and 'Failure' (11.2% vs 2.7%). In addition, 5.9% of their patients underwent dialysis, the risk of mortality was associated with AKI severity, and mortality was significantly higher, at 13.3%, compared with our study. Our study also showed that the patients who died all had AKI. Basu *et al*. focused on the relationship between febrile infectious diseases and AKI according to the RIFLE classification, including other acute febrile diseases, and not the relationship between AKI and scrub typhus itself or predictors of AKI.

Attur *et al*. demonstrated that thrombocytopenia and ICU treatment were predictors of acute renal damage in patients with scrub typhus and AKI occurred in 23.2% of their patients.[17] The renal injury classifications were as follows: 'Risk' (8.9%),

'Injury' (5.0%), and 'Failure' (10.8%); and replacement therapy was given to 10% of their patients. The lower incidence of AKI (23.2% *vs*. 35.9%) compared with our study might be explained by the difference in the age of the enrolled patients (40 *vs*. 58 years). Age was an important predictor of AKI in our study. In addition, patients with underlying diseases such as CKD, DM, and hypertension were included in our study. Underlying disease tends to increase in frequency with age. Easy access to a tertiary hospital might reduce the incidence and severity of AKI because of the regional characteristics of small areas. Our study also showed that the time from symptom onset to hospitalization was associated with AKI incidence.

The mechanism of AKI in scrub typhus is unclear. One plausible theory is that the invasion of rickettsia induces vasculitis, leading to direct renal involvement.[18, 19] However, renal biopsies have not revealed evidence of renal vasculitis associated with scrub typhus, but showed inflammation and proliferation of the glomerular tubule-interstitium[4 5] and tubular necrosis due to direct involvement of tubules by rickettsia.[17-19] Membranous nephropathy has also been demonstrated,[21] although we cannot completely rule out renal vasculitis because very few renal biopsies have been done in scrub typhus.

Dumler *et al*. suggested that a decrease in renal blood flow accompanied by extravasation resulting from systemic vasculitis is the cause of pre-renal AKI.[28] Hypoalbuminemia caused by the leakage of serum albumin due to vasculitis is also postulated to be associated with AKI.[20] Although hypoalbuminemia was not a predictor of acute renal injury in previous studies,[17, 25] the serum albumin level was significantly lower in our patients with AKI, and serves as an important predictor of AKI.

Hypoalbuminemia is also an important marker of severe infection and/or byproduct of chronic disease such as diabetes, hypertension and CKD. Others have suggested different mechanisms, such as pan-vascular coagulation[17, 19, 20] and rhabdomyolysis[17,19] but were not able to prove these hypotheses. The creatine phosphokinase level did not differ between the two groups in our study.

A close relationship between AKI and mortality has been reported. The mortality from scrub typhus in endemic areas in India is 2–12.2%.[29, 30] The mortality rates in two studies of AKI associated with scrub typhus were 13.3% and 0.8%.[17, 25] All of the deaths occurred in the 'Failure' category of AKI. In our series of 510 patients, 4 died (0.8%) and all were in the 'Failure' category of AKI. Our patients had underlying CKD. Previous reports did not identify CKD as a risk factor for AKI or mortality.[17, 25, 29, 30] The prognosis of AKI associated with scrub typhus has rarely been reported.[17, 25] The renal prognosis after AKI is good if the patient survives. Permanent dialysis treatment was needed in one case study.[22] In our series, only one case did not recover to baseline renal function and there was no permanent renal loss requiring long-term dialysis.

The main methods in scrub typhus diagnostics remain serology, but currently available serological tests have limitations. Of that, the gold standard for scrub typhus is IFA despite some limitations. Serological tests are most reliable when a fourfold rise in antibody titer is shown.[31] If the patient lives in non-endemic area, the diagnosis can be made from a single acute serum sample to require a cut-off antibody titer. This is impossible in patients living in endemic area because antibodies can be found in up to 18% of healthy populations.[32]

A major limitation of this study was its retrospective design. In addition, because the research relied on medical records, the capacity to find other possible causes of AKI was limited and urine volume, an important factor in the RIFLE classification, could not be analyzed. Therefore, the incidence of AKI might have been underestimated. However, we believe that these limitations are overcome by the large subject pool and application of many variables to the statistical analysis. It was also a single-center study, so relatively similar lab values applied, most patients were followed at the same facility, and the same diagnostic criteria and treatment were used.

Due to global warming and increase of travel to other countries, the incidence of contagious febrile diseases is on the rise in both developing and developed countries. Risk factors of AKI associated with both endemic and epidemic acute febrile illnesses such as malaria, leptospirosis, dengue fever, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and Severe Fever with Thrombocytopenia Syndrome (SFTS) remain to be established. The present study enrolls the large patients and shows the several clinical and biochemical risk factors of AKI associated with scrub typhus. Therefore, as independent risk factors, time to hospital presentation after symptom onset, older age, presence of CKD, and lower albumin level to predict AKI in our results can also be applied to predict AKI in above febrile diseases. High frequency and poor prognosis of AKI in CKD patients among our results should also be kept in mind to evaluate AKI of above acute febrile diseases.

CONCLUSIONS

AKI incidence associated with scrub typhus is 35.9%. Our current results suggest that presence of underlying CKD, older age, lower serum albumin level, and time to hospital presentation after symptom onset were important risk factors to determine occurrence of AKI. Whether earlier diagnosis and treatment in patients with above risk factors reduce the incidence and severity of AKI deserves to be investigated.

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Contributors KH conceptualized and drafted the manuscript; HNC participated in the design of the study. Both performed the statistical analyses and interpreting the results. TWL, HSC, EJB, and SC were involved in critically reviewing a draft of the manuscript and contributed to data collection. DJP further supervised the work. All authors approved the submission

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Competing interests None declared.

Ethics approval The study protocol was approved by the Institutional Review Board of Gyeongsang National University Hospital (IRB No: 2013-12-023)

Provenance and peer review Not commissioned; externally peer reviewed

Data sharing statement No additional data are available.

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Table1. Clinical and laboratory data of AKI group and Non-AKI group

	Total (n=510)	AKI (n=183)	Non-AKI (n=327)	P value
Age (yr)	57.94 ± 18.85	64.47 ± 15.31	54.29 ± 19.67	<.001
Male	245 (48.0%)	97 (53.0%)	148 (45.3%)	.097
*HTN	97 (19.0%)	48 (26.2%)	49 (15.0%)	.002
*ARB use	22 (4.3%)	15 (8.2%)	7 (2.1%)	.002
Diuretics use	12 (2.4%)	6 (3.3%)	6 (1.8%)	.364
*DM	61 (12.0%)	32 (17.5%)	29 (8.9%)	.005
*CKD	27 (5.3%)	19 (10.4%)	8 (2.4%)	<.001
Time to hospital presentation after symptom (day)	6.52 ± 6.76	9.70 ± 27.25	5.36 ± 9.18	.035
Symptoms and signs				
Fever	346 (85.0%)	128 (87.1%)	218 (83.8%)	.392
Myalgia	102 (25.1%)	32 (21.8%)	70 (26.9%)	.284
General weakness	48 (11.8%)	14 (9.5%)	34 (13.1%)	.338
Eschar	200 (39.2%)	80 (43.7%)	120 (36.7%)	.131
*WBC (x $10^3/u$L)	7.16 ± 3.54	7.56 ± 3.77	6.92 ± 3.39	.055
Hemoglobin (g/dL)	12.29 ± 1.75	11.91 ± 1.69	12.51 ± 1.76	.000
*CRP (mg/L)	49.91 ± 54.27	56.16 ± 62.53	46.04 ± 48.17	.072
*HbA1c (%)	7.59 ± 2.35	8.10 ± 2.42	6.87 ± 2.07	.033
Albumin (g/dL)	3.45 ± 0.60	3.19 ± 0.61	3.60 ± 0.55	<.001
*AST (U/L)	112.7 ± 400.6	150.9 ± 647.2	90.7 ± 104.0	.213
*ALT (U/L)	91.0 ± 167.2	104.5 ± 242.4	83.2 ± 100.7	.259
*CK (IU/L)	278.25 ± 673.77	371.05 ± 897.71	192.68 ± 345.53	.082
Creatinine (mg/dL)	0.75 ± 0.46	0.77 ± 0.72	0.74 ± 0.22	.571
Hematuria	179 (36.5%)	80 (44.4%)	99 (31.8%)	.005
Proteinuria	159 (32.3%)	73 (40.3%)	86 (27.7%)	.005
Trace	100 (62.9%)	44 (60.3%)	56 (65.1%)	.367
1+	43 (27.0%)	20 (27.4%)	23 (26.7%)	
2+	11 (6.9%)	6 (8.2%)	5 (5.8%)	
3+	5 (3.1%)	3 (4.1%)	2 (2.3%)	
Tsutsugamushi Ab titer	3234.04 ± 5785.24	3762.6 ± 3369.2	2862.2 ± 6046.5	.159
Hospital stay (days)	6.92 ± 18.01	9.70 ± 27.25	5.36 ± 9.18	.039
*CRRT	3 (0.6%)	3 (1.6%)	0 (0.0%)	.045
Shock	9 (1.8%)	5 (2.7%)	4 (1.2%)	.293
Admission to *ICU	8 (1.57%)	8 (4.4%)	0 (0.0%)	<.001
Death	4 (0.8%)	4 (2.2%)	0 (0.0%)	.016

HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hmeoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table2. Clinical and laboratory data of AKI and Non-AKI with CKD

	Total (n=27)	AKI (n=19)	Non-AKI (n=8)	P value
Age (yr)	72.59 ± 10.52	73.95 ± 9.03	69.38 ± 13.58	.311
Male	14 (51.9%)	11 (57.9%)	3 (37.5%)	.420
*HTN	11 (40.7%)	10 (52.6%)	1 (12.5%)	.090
*ARB use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Diuretics use	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
*DM	9 (33.3%)	7 (36.8%)	2 (25.0%)	.676
Time to hospital presentation after symptom (day)	5.96 ± 4.57	6.11 ± 5.12	5.50 ± 2.35	.784
Symptoms and signs				
Fever	18 (75.0%)	12 (70.6%)	6 (85.7%)	.629
Myalgia	10 (41.7%)	7 (41.2%)	3 (42.9%)	1.000
General weakness	3 (12.5%)	2 (11.8%)	1 (14.3%)	1.000
Eschar	16 (59.3%)	12 (63.2%)	4 (50.0%)	.675
*WBC (x $10^3/uL$)	8.39 ± 4.20	8.16 ± 4.75	8.95 ± 2.64	.664
Hemoglobin (g/dL)	11.01 ± 1.51	10.74 ± 1.44	11.68 ± 1.56	.144
*CRP (mg/L)	83.18 ± 73.52	82.24 ± 80.14	85.19 ± 62.02	.928
*HbA1c (%)	7.99 ± 1.79	7.87 ± 1.90	8.80	.664
Albumin (g/dL)	3.10 ± 0.62	3.01 ± 0.67	3.31 ± 0.48	.258
*AST (U/L)	411.44 ± 1659.31	557.21 ± 1975.12	65.25 ± 23.43	.493
*ALT (U/L)	189.67 ± 567.68	244.47 ± 674.10	59.50 ± 31.46	.450
eGFR (mL/min/1.73m^2)	49.42 ± 8.02	47.15 ± 8.28	53.95 ± 5.42	.048
Hematuria	16 (59.3%)	14 (73.7%)	2 (25.0%)	.033
Proteinuria	15 (55.6%)	12 (63.2%)	3 (37.5%)	.398
Trace	4 (26.7%)	3 (25.0%)	1 (33.3%)	.891
1+	5 (33.3%)	4 (33.3%)	1 (33.3%)	
2+	4 (26.7%)	3 (25.0%)	1 (33.3%)	
3+	2 (13.3%)	2 (16.7%)	0 (0.0%)	
Tsutsugamushi Ab titer	2725.71 ± 3087.79	3080.00 ± 3463.41	1840.00 ± 1798.13	.420
Hospital stay (days)	6.59 ± 6.80	6.63 ± 7.67	6.50 ± 4.53	.964
*CRRT	2 (7.4%)	2 (10.5%)	0 (0.0%)	.567
Shock	3 (11.1%)	2 (10.5%)	1 (12.5%)	1.000
Admission to *ICU	3 (11.1%)	3 (15.8%)	0 (0.0%)	.532
Death	4 (14.8%)	4 (21.1%)	0 (0.0%)	.285

* HTN : Hypertension, ARB : Angiotensin Receptor Blocker, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease, WBC : White Blood Cell, CRP : C-Reactive Protein, ESR : Erythrocyte Sedimentation Rate, HbA1c : Hemoglobin A1c, AST : Aspartate Aminotransferase, ALT : Alanine Aminotransferase, CK : Creatine Kinase, eGFR estimated Glomerular Filtration Rate by MDRD, CRRT : Continuous Renal Replacement Therapy, ICU : Intensive Care Unit

Table 3. Risk factors for the development of Scrub typhus associated AKI

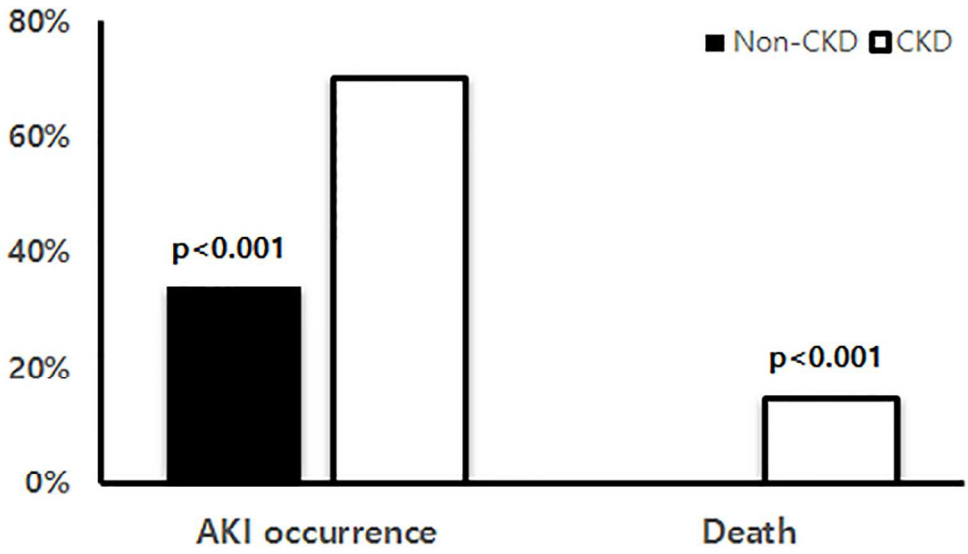
Characteristics	Univariate analysis			Multivariate analysis		
	P value	*OR	95% *CI	*P* value	*OR	95% *CI
Age (>65 yr)	0.000	2.804	1.931-4.073	0.002	1.965	1.270-3.040
*HTN	0.002	2.017	1.289-3.157	0.211	1.403	0.825-2.386
*DM	0.005	2.178	1.270-3.734	0.188	1.516	0.816-2.816
*CKD	0.000	2.808	1.917-4.114	0.013	3.526	1.305-9.525
Albumin (<3.5 g/dL)	0.000	2.226	1.466-3.379	0.001	2.095	1.367-3.211
Hemoglobin (<12 g/dL)	0.032	1.495	1.035-2.160	0.769	0.938	0.613-1.437
Time to hospital presentation after symptom onset (>7 day)	0.034	1.601	1.039-2.465	0.042	1.625	1.017-2.597

* OR : Odds Ratio, CI : Confidence Interval, HTN : Hypertension, DM : Diabetes Mellitus, CKD : Chronic Kidney Disease

Figure legend

Figure 1. Percentage of AKI occurrence and death according to presence of CKD

Figure 2. The comparison of AKI according to RIFLE category in patients with CKD and Non-CKD



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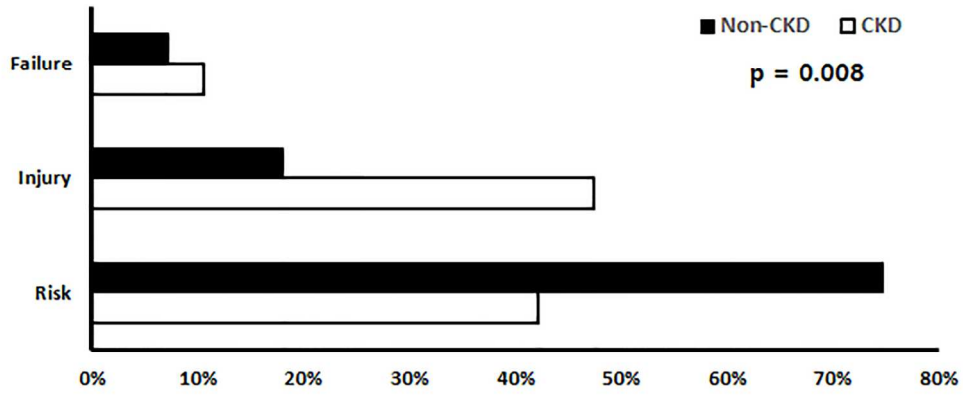

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1, line 1 Page 2, line 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2, line 1 ~ Page 3, line 18
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4, line 1~ Page 5, line 8
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 5, line 9 ~ Page 5, line 11
Methods			
Study design	4	Present key elements of study design early in the paper	Page 5, line 14 ~ Page 5, line 15
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5, line 14 ~ Page 5, line 18
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Page 5, line 14 ~ Page 6, line 17
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 5, line 20 ~ Page 7, line 6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5, line 20 ~ Page 7, line 6
Bias	9	Describe any efforts to address potential sources of bias	Page 5, line 20 ~ Page 5, line 23
Study size	10	Explain how the study size was arrived at	Page 5, line 14 ~ Page 5, line 15
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 7, line 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 7, line 10 ~ Page 7, line 17
		(*b*) Describe any methods used to examine subgroups and interactions	Page 7, line 3 ~ Page 7, line 6
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	NA

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 7, line 19 ~ Page 8, line 4
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 7, line 19 ~ Page 8, line 4 Page 19, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Page 7, line 19 ~ Page 8, line 4
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 8, line 6 ~ Page 10, line 2
		(*b*) Report category boundaries when continuous variables were categorized	Page 19, Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 9, line 3 ~ Page 9, line 7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 8, line 6 ~ Page 8, line 7
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 10, line 5 ~ Page 10, line 10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 13, line 3 ~ Page 13, line 10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 10, line 11 ~ Page 13, line 2
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 13, line 9 ~ Page 13, line 19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 14, line 11 ~

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.