HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.06.011

Prognostic value of plasma C-reactive protein in the evaluation of paraquat poisoning patients

Zong Ning[Δ], Yu-Long Bai[Δ], Hua Lu, Kang-Lin Mo[*]

Department of Emergency, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, PR China

ARTICLE INFO

Article history:
Received 2 Jun 2015
Received in revised form 15 Jun 2015
Accepted 27 Jun 2015
Available online 29 Jul 2015

Keywords:
Paraquat poisoning
C-reactive protein
Prognosis

ABSTRACT

Objective: To investigate the prognostic value of plasma C-reactive protein (CRP) level in patients with paraquat poisoning.
Methods: This study included 162 patients with paraquat poisoning. The data of plasma paraquat, CRP level and arterial blood gas were analyzed. Cox regression analysis was applied to evaluate the risk factors of prognosis. Receiver operating characteristics curve analysis and area under curve were used to calculate the predictive power of significant variable. Differences in patient survival were determined using the Kaplan–Meier method and a log-rank test.
Results: Plasma CRP level was significantly increased in non-survival patients compared with survival patients ($P < 0.05$), and positively correlated with plasma paraquat level ($P < 0.05$). Cox regression analysis revealed that plasma CRP level was an independent prognostic marker of mortality within 30 days. The receiver operating characteristics curve analysis indicated that area under curve of plasma CRP level was 0.867 (95% *CI*: 0.81–0.93), and the cut-off value was 18 mg/L, and patients with CRP level over this value had a poor survival time compared with those with less than this value.
Conclusions: These results suggest that plasma CRP level is distinct increased in patients with paraquat poisoning, and the plasma CRP level may be useful for the prediction of prognosis in paraquat poisoning.

1. Introduction

Paraquat is one of the most widely used potent herbicides in the world, especially in the developing countries, such as China. However, due to its easily accessible, paraquat is frequently ingested by some people, both intentionally and accidentally, and these people often die within a few days. Although distinct advancement has been made in the treatment of paraquat poisoning, the clinical efficacy of these approaches remains indeterminate [1]. A reliable predictor of prognosis of patients with paraquat poisoning may guide treatment and future research. Several prognostic markers have been found to have prognostic significance in the evaluation of patients with paraquat poisoning at present, such as plasma paraquat concentration [2,3], arterial lactate level [4], severity scoring system [5], and arterial blood gas analysis [6]. However, these prognostic markers cannot be applied widely in numerous hospitals in the developing countries, due to the higher requirement of assay or complicated calculation [7].

Several mechanisms have been reported to be involved in the tissue injury caused by paraquat poisoning, such as redox reaction by reactive oxygen species and lipid peroxidation of cellular membranes [8,9]. In addition, inflammatory response is the initial and main mechanism of tissue injury after paraquat poisoning [10]. C-reactive protein (CRP) is an acute-phase protein that has been evaluated extensively in the critical illness [11]. CRP level is positively correlated with the degree of inflammation during the early course of illness [12]. Currently, studies have indicated that CRP is able to predict prognosis and mortality of several

*Corresponding author: Kang-Lin Mo, Department of Emergency, The First Affiliated Hospital of Guangxi Medical University, Shuang-Yong Road 6, Nanning 530021, Guangxi, PR China.
Tel/Fax:+86 0771 5356758
E-mail: gxmokanglin@163.com
Peer review under responsibility of Hainan Medical University.
[Δ] These authors contributed equally to this work.
Foundation Project: Supported by grants from the Guangxi Project Of Scientific Research And Technology (201501-2-10) and Guangxi Project Of Health Suitable Technology (S201302-04).

diseases, such as bacterial infection in cirrhosis [13], H7N9 avian influenza [14] and organophosphate poisoned patients [15]. However, the association of plasma CRP level with the outcome of patients with paraquat poisoning has not been evaluated. Therefore, the aim of the present study was to investigate the potential role of the plasma CRP level as a prognostic marker in patients with paraquat poisoning.

2. Materials and methods

2.1. Patients

This study was carried out in the First Affiliated Hospital of Guangxi Medical University between June 2010 and March 2014. Patients with acute oral paraquat poisoning were enrolled in this study. The duration of follow-up was 30 days. Patients of non-oral ingestion poisoning or paraquat exposure of > 24 h previous to presentation were excluded. We also excluded patients who have inflammatory arthritis and connective tissue disease, malignancies, and recent (< 2 months) surgery or major trauma. All primary data were collected according to procedures outlined in epidemiology guidelines that strengthen the reporting of observational studies. The mortality occurred during the stay at hospital was recorded. The following variables were also collected, including patient's age, gender, admission time, oral paraquat amount. The study protocol was approved by Review Boards Guangxi Medical University for human studies. The requirement for written informed consent was waived because there was no intervention.

2.2. Laboratory analysis

Blood samples were collected immediately following admission. Plasma paraquat was measured quantitatively by high performance liquid chromatography. Arterial blood gas, including PaO_2, $PaCO_2$, HCO_3-, base excess (BE) were determined by blood gas analyzer (GEM premier 3000; Instrumentation Laboratory, Bedford, IL, USA). Liver function variables [alanine transaminase (ALT), aspartate aminotransferase (AST)], renal function [blood urea nitrogen (BUN), creatinine (Cr)] were analyzed by the auto biochemistry analyzer (Automatic Analyzer 7600-120, Hitachi, Tokyo, Japan). CRP level measurements were performed using an immunoturbidimetric method that employed a commercially available test (turbidimetric test, Boehringer Mannheim, Germany).

2.3. Treatment protocol

Patients were given treatment immediately following admission. All the patients were treated under local protocol by gastric lavage, catharsis and fluid diuresis. Briefly, gastric lavage with a large amount of saline was performed every 4 h. Charcoal haemoperfusion with a charcoal-containing (Adsorba, Gambro, Germany) dialysis machine (Surdial, Nipro, Japan) was performed if the urine paraquat value > 5 mg/L. A second session of haemoperfusion was arranged when the urine paraquat value > 5 mg/L at 4 h after the first haemoperfusion administration. Intravenous dexamethasone (20 mg/day) was administrated for another 11 days after methylprednisolone therapy. Cyclophosphamide and methylprednisolone were repeated if the PaO_2 was < 60 mmHg and the duration was > 2 weeks after the initial treatment. Acetylcysteine for injection sodium and Edaravone injection were also administered to the patients.

2.4. Statistical analysis

Data are presented as mean ± SD. Statistically significant differences between the two groups were analyzed using the independent two-sample *t*-test or Mann–Whitney *U* test depending on data distribution. Categorical data were analyzed using the *Chi*-square test. The Pearson correlation test was used for correlation analysis. Cox regression analysis based on forward elimination of data was applied to evaluate the risk factors of prognosis. Receiver operating characteristics (ROC) curve analysis was used to calculate cut-off values, sensitivity, specificity, and overall correctness of designated variable. The best cut-off values were determined by analyzing the Youden's index (sensitivity + specificity−1) and the maximized area under curve (AUC) of the ROC. Kaplan–Meier survival test and log-rank test were used to compare the survival rate between two groups. SPSS statistical software package 16.0 was used to perform statistical analysis. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Subject characteristics at admission

There were 162 patients with paraquat poisoning enrolled in this study. Among these patients, 75 died and 87 survived after the treatment. We divided these patients into survival group and non-survival group, respectively. The clinical characteristics at admission of the patients are summarized in Table 1. We found that the plasma CRP levels, amount of paraquat intake, ALT, AST, PaO_2, $PaCO_2$, BE, HCO_3-, BUN, Cr, and plasma paraquat level were significantly increased in non-survival group compared with survival group ($P < 0.05$); while age, gender, AST and white blood count had no distinct difference between two groups ($P > 0.05$).

Table 1
Characteristics of 162 paraquat patients at admission.

Parameters	Non-survival (n = 75)	Survival (n = 87)
PPL (μg/mL)	45.17 ± 2.98	25.61 ± 3.02**
Age (years)	29.74 ± 9.50	28.47 ± 10.69
Gender (male/female)	48/27	52/35
Admission time (h)	5.06 ± 1.80	4.44 ± 1.57*
Intake amount (mL)	28.09 ± 1.13	25.89 ± 1.11**
ALT (IU/L)	39.61 ± 5.81	37.80 ± 5.36*
AST (IU/L)	40.51 ± 5.14	39.33 ± 5.50
PaO_2 (mmHg)	7.71 ± 1.07	9.52 ± 0.83**
$PaCO_2$ (mmHg)	5.18 ± 0.44	5.00 ± 0.55*
BE (mmol/L)	4.49 ± 0.28	2.05 ± 0.57**
HCO_3- (mmol/L)	2.25 ± 0.13	2.15 ± 0.09**
BUN (mmol/L)	9.04 ± 0.55	9.21 ± 0.50*
Cr (mmol/L)	99.96 ± 12.00	95.91 ± 11.85*
WBC ($\times 10^9$)	20.55 ± 2.30	19.88 ± 12.25
CRP (mg/L)	23.12 ± 9.14	13.75 ± 6.48**

PPL: Plasma paraquat level; WBC: White blood count. *: $P < 0.05$, **: $P < 0.01$.

Table 2
Risk factors for mortality within 30 days in Cox regression analysis (n = 162).

Parameters	B	SE	Wald	*df*	*P*	HR	95% *CI*
CRP	0.515	0.168	9.375	1	0.002	1.673	1.203–2.326
PPL	0.100	0.025	15.863	1	<0.001	1.105	1.052–1.161
BE	0.006	0.002	6.488	1	0.011	1.006	1.001–1.010

PPL: Plasma paraquat level; B: Regression coefficient; Wald: Test statistic; HR: Hazard ratio.

3.2. Clinical predictors of mortality within 30 days

Positive correlations were observed between plasma paraquat level and CRP level (r = 0.625, P = 0.013). Multivariate Cox regression analyses indicated that plasma paraquat level (P < 0.001), CRP level (P = 0.002), and BE (P = 0.011) were independent predictors of mortality within 30 days (Table 2).

3.3. ROC curve analysis

By using the ROC curve analysis, we observed that the AUC of CRP was higher than that of BE. The CRP level had an AUC of 0.867 (95% *CI*: 0.81–0.93) and the best cut-off value was 18.05 mg/L (sensitivity: 80.0%; specificity: 83.9%; Youden's index: 0.693). While the AUC of BE was 0.759 (95% *CI*: 0.68–0.84), and the sensitivity and specificity was 79.8% and 84.5%, respectively (Figure 1).

3.4. Kaplan–Meier survival test

We compared the survival time of patients with 18.0 mg/L of CRP as cut-off value, and we found that patients with CRP level over 18.0 mg/L had a poor survival time compared with those with less 18.0 mg/L during the follow-up period (log-rank χ^2 = 139.4, P < 0.001) (Figure 2).

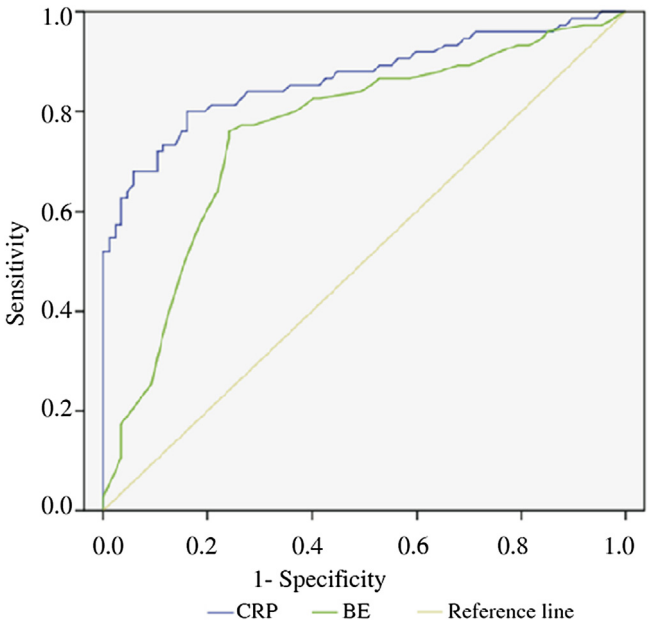

Figure 1. ROC curves for CRP and BE.
Diagonal segments are produced by ties.

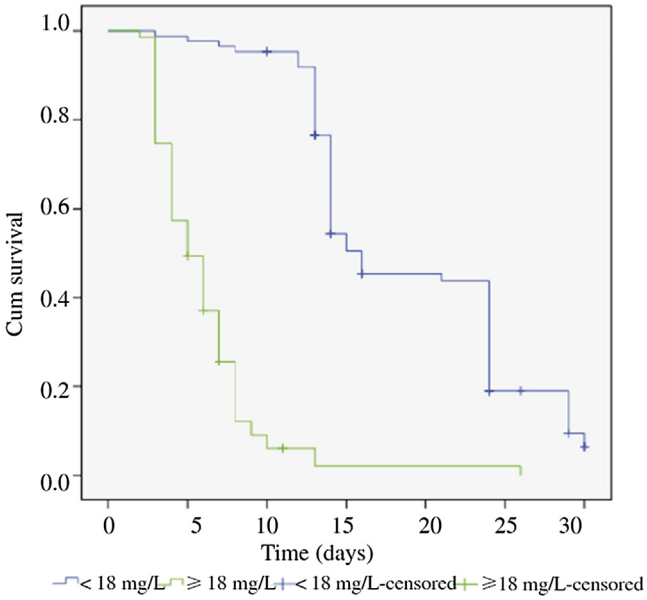

Figure 2. Kaplan–Meier survival curve of patients with paraquat poisoning.

4. Discussion

Many clinical parameters have been proposed as mortality predictors for patients with paraquat intoxication. Plasma paraquat level has been considered as a marker of severity and prognosis. Patient with high plasma paraquat level on admission often has a higher incidence of mortality and morbidity [2,3]. In line with previous studies, we also observed that patients in survival group have lower plasma paraquat level compared with those in non-survival group. The Cox regression analysis further confirmed that plasma paraquat was an independent predictor for the mortality of patients with paraquat poisoning. However, the detection of plasma paraquat is not available to numerous hospitals in the developing countries, which limited the application of this prognostic marker. Recently, serum lactate level was reported to be a good prognostic marker, and patients with higher lactate level were risked higher mortality [4]. However, initial arterial lactate level may vary greatly as blood samples are collected at different times. Thus, the reliability of this prognostic marker is undermined [16].

CRP is a marker of inflammation and is usually increased in the pathological conditions of inflammatory response [17]. Since inflammatory response is one of the important mechanisms of tissue injury cause by paraquat, the current treatment modalities, such as methylprednisolone and cyclophosphamide, are aimed to reduce the inflammatory response in patients with paraquat poisoning [18,19]. In the present study, we observed that plasma CRP level was increased in patients with paraquat poisoning, and the level was significantly higher in non-survival patients compared with those survivals, suggesting that much greater inflammatory response was happened in those non-survival patients compared with those survivals. We also observed that the plasma CRP level was positively correlated with the plasma paraquat levels, indicating that CRP may be a good marker reflecting the severity of paraquat poisoning, and potentially be a prognostic marker of patients with paraquat poisoning.

In the Cox regression analysis, we found that together with plasma paraquat level and BE, plasma CRP level was

association with 30-day mortality and was an independent prognostic factor in patients with paraquat poisoning. Therefore, we further explored the prognostic value of plasma CRP level by using ROC curve analysis. We found that the AUC of plasma CRP level reach to 0.867, suggesting a higher prognostic value. We go further to validate the prognostic value by using the best cut-off value from ROC curve analysis, then we found that like the plasma paraquat levels, patients whose plasma CRP level over 18.0 mg/L have a more poor prognosis. These results indicating plasma CRP level at admission have a discriminative power as a practical tool in predicting the prognosis of patients with paraquat poisoned.

In the present study, we also observed that BE was an independent predictor of patients with paraquat poisoning, which was similar to the report of Huang *et al.* [6]. However, in the ROC analysis, we found that the AUC of BE is less than that of CRP, suggesting that the overall prognostic value of CRP is superior to that of BE. Compared with other prognostic tools, such as arterial lactate concentration-time data [16], acute physiology and chronic health evaluation (APACHE II) scores [20], which are reported to have a good prognostic value of paraquat poisoning, the using of plasma CRP to predict the outcome of these patients is more simple. When consider the limitations of current prognostic markers or tools, our results on the prognostic value plasma CRP level have special clinical significance.

To the best of our knowledge, the present study is the first analysis of the association of plasma CRP level with outcomes of patients with paraquat poisoning. However, our study also has several limitations. First, this study was a retrospective and observational study, and was conducted in a single institution, thus, the results of the analysis may not be generally applicable. Second, the sample size of this study was relatively small, some associations may be detected by chance, and some other potential associations could not be detected. Therefore, prospective studies with large patient cohorts should be performed to verify our findings. Third, the follow-up in this study was only 30 days, although most paraquat poisoned patients die within this period, some later death could not be analyzed in this study.

In summary, the results of present study suggest that plasma CRP level is distinct increased in patients with paraquat poisoning, and the plasma CRP level may be useful for the prediction of prognosis in paraquat poisoning. However, due to the limitations of this study, larger prospective studies are warranted to further confirm these findings.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by grants from the Guangxi Project of Scientific Research and Technology (201501-2-10) and Guangxi Project of Health Suitable Technology (S201302-04).

References

[1] Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. *Br J Clin Pharmacol* 2011; **72**(5): 745-57.

[2] Liu P, He YZ, Wang HC, Li G, Zhang CG, Zhang XG, et al. [Study on the prognosis of patients with acute paraquat intoxication]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2011; **29**(3): 212-5. Chinese.

[3] Shi Y, Bai Y, Zou Y, Cai B, Liu F, Fu P, et al. The value of plasma paraquat concentration in predicting therapeutic effects of haemoperfusion in patients with acute paraquat poisoning. *PLoS One* 2012; **7**(7): e40911.

[4] Liu XW, Ma T, Qu B, Ji Y, Liu Z. Prognostic value of initial arterial lactate level and lactate metabolic clearance rate in patients with acute paraquat poisoning. *Am J Emerg Med* 2013; **31**(8): 1230-5.

[5] Min YG, Ahn JH, Chan YC, Ng SH, Tse ML, Lau FL, et al. Prediction of prognosis in acute paraquat poisoning using severity scoring system in emergency department. *Clin Toxicol (Phila)* 2011; **49**(9): 840-5.

[6] Huang C, Zhang X. Prognostic significance of arterial blood gas analysis in the early evaluation of paraquat poisoning patients. *Clin Toxicol (Phila)* 2011; **49**(8): 734-8.

[7] Chang SS, Lu TH, Eddleston M, Konradsen F, Sterne JA, Lin JJ, et al. Factors associated with the decline in suicide by pesticide poisoning in Taiwan: a time trend analysis, 1987–2010. *Clin Toxicol (Phila)* 2012; **50**(6): 471-80.

[8] Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Crit Rev Toxicol* 2008; **38**(1): 13-71.

[9] Morán JM, Ortiz-Ortiz MA, Ruiz-Mesa LM, Fuentes JM. Nitric oxide in paraquat-mediated toxicity: a review. *J Biochem Mol Toxicol* 2010; **24**(6): 402-9.

[10] He X, Wang L, Szklarz G, Bi Y, Ma Q. Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J Pharmacol Exp Ther* 2012; **342**(1): 81-90.

[11] Póvoa P. C-reactive protein: a valuable marker of sepsis. *Intensive Care Med* 2002; **28**(3): 235-43.

[12] Rey C, Los Arcos M, Concha A, Medina A, Prieto S, Martinez P, et al. Procalcitonin and C-reactive protein as markers of systemic inflammatory response syndrome severity in critically ill children. *Intensive Care Med* 2007; **33**(3): 477-84.

[13] Pieri G, Agarwal B, Burroughs AK. C-reactive protein and bacterial infection in cirrhosis. *Ann Gastroenterol* 2014; **27**(2): 113-20.

[14] Lu S, Li T, Xi X, Chen Q, Liu X, Zhang B, et al. Prognosis of 18 H7N9 avian influenza patients in Shanghai. *PLoS One* 2014; **9**(4): e88728.

[15] Lee JH, Lee YH, Park YH, Kim YH, Hong CK, Cho KW, et al. The difference in C-reactive protein value between initial and 24 hours follow-up (D-CRP) data as a predictor of mortality in organophosphate poisoned patients. *Clin Toxicol (Phila)* 2013; **51**(1): 29-34.

[16] Sun L, Li GQ, Yan PB, Liu Y, Li GF, Wei LQ. Prediction of outcome following paraquat poisoning by arterial lactate concentration-time data. *Exp Ther Med* 2014; **8**(2): 652-6.

[17] Sierra R, Rello J, Bailén MA, Benítez E, Gordillo A, León C, et al. C-reactive protein used as an early indicator of infection in patients with systemic inflammatory response syndrome. *Intensive Care Med* 2004; **30**(11): 2038-45.

[18] Lin JL, Lin-Tan DT, Chen KH, Huang WH, Hsu CW, Hsu HH, et al. Improved survival in severe paraquat poisoning with repeated pulse therapy of cyclophosphamide and steroids. *Intensive Care Med* 2011; **37**(6): 1006-13.

[19] Zerin T, Kim YS, Hong SY, Song HY. Protective effect of methylprednisolone on paraquat-induced A549 cell cytotoxicity via induction of efflux transporter, P-glycoprotein expression. *Toxicol Lett* 2012; **208**(2): 101-7.

[20] Huang NC, Hung YM, Lin SL, Wann SR, Hsu CW, Ger LP, et al. Further evidence of the usefulness of Acute Physiology and Chronic Health Evaluation II scoring system in acute paraquat poisoning. *Clin Toxicol (Phila)* 2006; **44**(2): 99-102.