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Original Research Article

Urinary paraquat concentration and white blood cell count as prognostic factors in paraquat poisoning

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Abstract

Purpose: *To investigate the effect of white blood cell (WBC) and urinary paraquat (PQ) levels on prognostic factors in patients exposed to PQ intoxication using multivariate logistic regression analysis.*
Methods: *A total of 104 subjects intoxicated with PQ between December 2015 and July 2016 were used in this retrospective study. They comprised patients who survived (n = 78), and patients who died (n = 26). Clinical features and prognostic parameters were analyzed in both groups. Multivariate logistic regression analysis was used to establish a prognostic correlation model based on results from single factor variables.*
Results: *Comparison of demographic and clinical attributes between the two groups, survivors (n = 78) and non-survivors (n = 26), revealed that those who survived were not as old (33.3 ± 9.9 years) as non-survivors (41.5 ± 12.9 years). In addition, on admission, it was found that the survivors ingested lower amounts of PQ (31.6 ± 13.8 ml) than non-survivors (67.88 ± 31.2 ml). There were significant differences between the two groups with respect to WBC, neutrophils, lymphocytes, lactate dehydrogenase (LDH), creatine kinase (CK), amylase, uric acid (UA), pH, partial pressure of oxygen (PaO2), base excess (BE), lactic acid, and D-dimer levels ($p < 0.05$).*
Conclusion: *WBC and urine PQ concentration have strong correlation with prognostic factors in PQ poisoning.*

Keywords: *Paraquat intoxication, Dithionite test, Multivariate logistic analysis, Prognosis, Predictors*

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INTRODUCTION

Paraquat (PQ) is an active, rapid-action herbicidal agent used all over the world. Humans may ingest PQ either by accident or deliberately through attempts at suicide, which may result in death [1]. This is so because ingested PQ is extremely toxic.

Paraquat is environmentally harmless due to the fact that once it is in contact with the soil, it is rapidly decomposed to non-toxic compounds [2]. Unfortunately, it exerts extreme toxicity in man, with mortalities in the range of 50 to 90 % [3]. In view of the high toxicity of this herbicide to humans, it is of concern that no effective

treatments for PQ poisoning have so far been developed [4]. Paraquat toxicity is a free radical-mediated process which results in oxidative stress and cellular apoptosis [5-9].

Studies on the immune-stimulatory potential of PQ revealed that it enhances the expressions of CXL10, CXL11, and IL-10 (genes associated with inflammation) [10]. Since effective treatments for PQ poisoning are not yet available, it is necessary to develop a method for predicting patient mortality. This is important because when unavoidable mortality is predicted timely, wrong treatment strategies can be discontinued in favor of more drastic remedies, especially when the PQ intoxication is acute.

A number of prognostic factors have been proposed for predicting risks to patients with acute PQ poisoning. Single laboratory analyses, such as serum PQ concentration [11-13], arterial lactate [14], uric acid [15], lymphocyte and neutrophil counts, and creatinine [16] have been used in risk stratification. However, the level of PQ in plasma, and the level of ingested PQ are the most valid factors for predicting PQ-related fatalities [1,17]. Aside from the difficulty often encountered with accurate determination of ingested PQ, the assay facilities for serum PQ are lacking in rural hospitals. Moreover, results from analysis of plasma PQ concentration are not readily available in time in the hospital Emergency Departments (EDs). Plasma PQ level does not necessarily represent the ingested amount or the body burden of PQ, particularly when measured during the first few hours, because it peaks 1 h following ingestion, prior to a fast fall as it enters other body compartments [18].

Another potential indicator is the ingestion volume of PQ, which is difficult to calculate accurately, and even more difficult if post-ingestion vomiting occurred [19]. Therefore, prognostic factors that affect survival of patients with PQ poisoning were investigated in the present study with a view to predicting the probability of survival through the initial laboratory findings at the point of hospital admission.

METHODS

This study is a retrospective cohort investigation based on observation of subjects admitted at ED ward of Qilu Hospital, Shandong Province between December 2015 and July 2016. A total of 104 patients who ingested PQ were enrolled initially for further selection. The exclusion criteria were: (a) weak or negative urine dithionite test (23 patients); (b) evidence of hemo-perfusion prior to hospital admission more than 24 h after PQ ingestion (103 patients); (c) PQ poisoning by routes other than ingestion (15 patients); (d) pregnant or lactating patients; (e) patients with cardiac arrest after PQ poisoning (n = 9); (f) medical history of pancreatic, heart, liver, kidney, or central nervous system disease, and/or refusal of consent (7 patients); and inability to obtain APACHE2 score after admission. Included subjects were assigned to 2 groups i.e. survivors and non-survivors), and their initial laboratory data were compared and analyzed. This work was approved by the Ethical Committee of Qilu Hospital (approval no. 201707832) and complied with the guidelines of Declaration of Helsinki promulgated in 1964 as amended in 1996 [20].

On admission, all patients received standardized medical emergency treatments. These included methylprednisolone administration at decreasing doses in response to improvements in patients' status; myocardial nutrition, complete lavage of the gastrointestinal tract, hemo-perfusion, protection of the liver, gastrointestinal mucosa and the kidneys; ROS neutralization, and ensuring water and electrolyte homeostasis by administering the *Qilu scheme* of the Department of Poisoning and Occupational Diseases [21].

On arrival, each patient's urinary PQ was checked semi-quantitatively by the doctor on duty, using the dithionite method. The results of urinary PQ test were recorded as Grades 1 - 4 by comparison with a standard color card viz: < 10 µg/mL = black; 10 - 30 µg/mL = deep blue; 30 – 100 µg/mL = light blue, and > 100 µg/mL = barely discernable blue. The genders and ages of the subjects were recorded, as well as the lag in time between admission and exposure to PQ, in addition to vital signs. Laboratory results on hematological parameters such as WBC count, lymphocyte count and neutrophil count were compiled, in addition to patient data on arterial blood pH, base excess (BE), $Pa_{O2,}$ Pa_{CO2}, base excess (BE), level of PQ in plasma, and BUN. Other records obtained included plasma potassium and sodium levels, total bilirubin (TBil), lactate dehydrogenase (LDH), creatine kinase (CK), glutamate pyruvate transaminase (AST), glutamate pyruvate transaminase (ALT), D-dimer, blood glucose, and uric acid (UA). This investigation was hinged on mortality within thirty days of hospital presentation. Thus, if a subject got discharged during this time frame, efforts were made to determine whether they took part in follow up as outpatients, and regular contact was made with them through telephone interview.

Statistical analysis

Continuous variable data are presented as mean ± standard deviation (SD), and compared between survivors and non-survivors using Mann-Whitney test. Categorical variable data are presented as frequency (%), and compared between the two groups using Fisher's exact test or chi square test. Mortality determinants were identified using multivariate logistic stepwise regression analysis, and expressed in terms of odds ratios (ORs) with 95 % CI. All analyses were carried out with SPSS 13.0. Statistical significance was fixed at $p < 0.05$.

RESULTS

Baseline features of subjects

Sixty (60) of the study participants were males (57.7 %). On the average, the time lag from PQ intake to hospital admission was 6.4 h. Comparison of demographic and clinical features between the two groups showed that those who survived were significantly younger in age (33.3 ± 9.9 years, in contrast to 41.5 ± 12.9 years for non-survivors, $p = 0.049$), and ingested significantly lower PQ as seen on admission (31.6 ± 13.8 mL in contrast to 67.88 ± 31.2 mL in non-survivors, $p = 0.001$). However, there were no differences in gender and time lag before PQ ingestion and hospitalization between the 2 groups ($p = 0.670$). These results are shown in Table 1.

Table 1: Clinical and demographic features of subjects

Parameter	Subjects alive (n = 78)	Dead subjects (n = 26)	*p* value
Male/Female	52/26	18/8	0.073
Age (years)	33.3 ± 9.9	41.5 ± 12.9	0.049
Amount of PQ ingested (mL)	31.6 ± 13.8	67.8 ± 31.2	0.001
Time lag before hospital admission (h)	6.1 ± 5.0	7.2 ± 3.9	0.670

Clinical features of dead and live subjects

The results in Table 2 show that the initial laboratory data on WBC, neutrophils, lymphocytes, LDH, CK, UA, pH, Pa_{CO2}, BE, lactic acid, and D-dimer differed significantly ($p < 0.05$) between survivors and non-survivors. The proportion of +ve or strongly +ve urine dithionite test results was larger in non-survivors than in survivors.

Table 2: Initial laboratory data at point of admission

Variable	Survivors (n = 78)	Non-survivors (n = 26)	*P*-value
Urine PQ	n (%)	n (%)	-
< 10 µg/ml	20 (25.6)	0	-
10 - 30 µg/ml	18 (23.1)	0	-
30 - 100 µg/ml	34 (43.6)	2 (7.7)	-
> 100 µg/ml	6 (7.7)	24 (92.3)	< 0.001
WBC (10^9/L)	9.71 (4.2)	22.9 ± 7.7	< 0.001
Neutrophils (10^9/L)	7.63 (3.7)	20.6 ± 7.6	< 0.001
Lymphocytes (10^9/L)	1.3 ± 0.6	0.47 (0.7)	0.038
ALT (U/L)	27 (21)	18 (16)	0.305
AST (U/L)	29 (14)	46(83)	0.060
TBil	14 (12)	17 (8)	0.156
BUN	5.2 ± 1.5	4.9 (5.0)	0.336
Cr	66 (23)	121.0 ± 67.3	0.063
LDH (U/L)	201.8 ± 56.1	233.0 ± 43.2	0.006
CK (U/L)	122.3 ± 51.2	140.0 ± 62.4	0.023
K (mEq/L)	3.7 ± 0.3	2.9 ± 0.4	<0.001
Amylase (IU/L)	74(92)	176 (370)	<0.001
UA (µmol/L)	320.0 ± 81.0	390 (104)	0.049
PH	7.42 ± 0.03	7.35 ± 0.1	0.04
Pa_{CO2} (mmHg)	35.2 ± 3.7	25.2 ± 4.2	< 0.001
BE (mEq/L)	0.8 (1.9)	-8.9 ± 5.6	< 0.001
Lactic acid (mEq/L)	1.6 (1.0)	7.5 (10.2)	< 0.001
Blood glucose	6.5 (1.9)	7.5 ± 1.2	0.074
D-dimer	0.29 (0.36)	0.48 (0.35)	0.047

Univariate logistic regression

Results of univariate logistic regression analysis carried out to select the predictors of death from PQ poisoning showed that 13 predictors had *p* values lower than 0.05 (Table 3).

Multivariate logistic regression analysis

Arising from the results of univariate logistic analysis, multivariate logistic stepwise regression analysis was carried out. The results showed that WBC and urine PQ concentration had strong correlations with prognosis-related factors in PQ intoxication in accordance with the equation:

Logit (P/1 - P) = 0.088 [urine PQ] + 0.267[WBC] - 11.742 …… (1)

This relationship is useful in predicting survival of persons exposed to acute PQ intoxication.

Table 3: Univariate logistic regression data

Variable	*p* value	OR	95 % CI for OR
WBC (10^9/L)	0.001	1.341	1.133-1.588
Neutrophils (10^9/L)	< 0.001	1.322	1.125 1.554
Lymphocytes (10^9/L)	0.656	0.868	0.466 1.618
ALT (U/L)	0.719	0.994	0.959 1.029
AST (U/L)	0.011	1.037	1.009 1.066
TBil	0.191	1.036	0.983 1.093
BUN	0.067	1.191	0.988 1.437
Cr	0.014	1.019	1.004 1.034
LDH (U/L)	0.001	1.003	1.002 1.004
CK (U/L)	0.002	1.000	1.000 1.001
Potassium (mEq/L)	0.001	0.024	0.003 0.230
Amylase (IU/L)	0.003	1.009	1.003 1.016
UA (μmol/L)	0.035	1.008	1.001 1.016
pH	0.007	< 0.001	0.017
pCO2 (mmHg)	0.001	0.687	0.553 0.852
BE (mEq/L)	0.001	0.680	0.545 0.850
Lactic acid (mEq/L)	0.002	1.459	1.152 1.847
Blood glucose	0.403	1.124	0.854 1.480
D-dimer	0.185	2.982	0.593 15.005

DISCUSSION

The plasma levels of PQ are of prognostic value in patients exposed to acute PQ intoxication. Indeed, data on changes in plasma PQ levels with time have been applied in the prediction of prognosis in PQ-poisoned patients for several years [3]. Recently, the bio-markers lipocalin and pentraxin have been used for predicting survival in patients poisoned with PQ [22,23]. However, these predictors were derived from small-population studies, and they predict mortality rather than revival [24]. The present study has revealed that WBC is an independent prognostic factor in PQ intoxication. The link between PQ exposure, WBC levels, and 30-day mortality is unclear. It is possible that PQ poisoning induces immune-stimulation which results in increased levels of WBC. This may explain the high prognostic potential of WBC with respect to prediction of 30-day mortality after PQ ingestion, which is considered a very important finding in the present study.

The volume of ingested PQ was determined on the basis of adult mouthful swallow, with a mean volume of approximately 20 mL, which is in agreement with previous reports [25-27]. However, in some instances, the precise amount of PQ ingested could not be confirmed especially for subjects who got exposed to PQ by drinking from a bowl, cup or glass. Moreover, due to alcohol bemusement or an upset state of mind at the point of exposure, some subjects were unable to recall the volume of PQ consumed or the exact time of exposure to PQ [28-30]. However, these lapses are less significant than problems associated with interpreting data from blood PQ profiles which change appreciably with time lag after PQ intake [31,32]. Therefore, a more correct and authentic index for prediction of outcomes of PQ poisoning is urinary dithionite test. Moreover, it is easy to carry out, and the reagents are readily available, especially in the grass-root hospitals.

Study limitations

This study has some limitations. Being retrospective in nature, and due to the use of a semi-quantitative method for determination of urinary PQ concentration, it was not possible to draw ROC curve. Thus, the results of this study can only support the theory of conclusions on correlation through the multifactor analysis. Moreover, the study focused only on laboratory examination, which limits its significance. The clinical significance of the findings would have been enhanced by analyzing them with APACHE II scores.

CONCLUSION

The results obtained in this study demonstrate that initial clinical laboratory data are very crucial for assessing the outcome of PQ poisoning. In particular, WBC and urine PQ concentration have strong correlation with prognostic factors in PQ intoxication, and are useful for predicting survival in acute PQ-intoxicated patients.

Table 4: Multivariate logistic regression data

Index	Coefficient	Standard error	Wald	*p* value	OR	95 % CI	
						Lower	Upper
Urine PQ	0.088	0.040	4.909	0.027	1.092	1.010	1.181
WBC	0.267	0.108	6.143	0.013	1.307	1.058	1.614
Constant term	- 11.742	4.779	6.037	0.014	0.000		

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Xiangdong Jian conceived and designed the study. Xinli Wang, Qiang Wu, Baotian Kan, Beijun Gao and Ke Wang collected and analysed the data, while Qinliang Xu wrote the manuscript.

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