Original Article

Histo-pathological findings and their relationship with age, gender and toxin amounts in paraquat intoxication

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ABSTRACT

Objective: Acute paraquat (PQ) poisoning which is taken either to commit suicide or by mistake has become a social concern worldwide. The present study was conducted to determine the histo-pathological findings in deceased PQ poisoning patients and evaluate their relationship with age, gender, amounts of ingested PQ, time from ingestion to admission.

Methodology: Forty-two patients of PQ poisoning with positive urinary dithionate test were included in the study. Deceased patients underwent autopsy and their histo-pathological findings were examined.

Results: The mean age of men and women were 29.06 ± 10.6 and 16.66 ± 4.6 years respectively. Hepatocellular damage (60%), Tubular nephritis (60%) and pulmonary edema (50%) were the main pathological lesions. Logistic regression analyses identified the following significant predictors for poisoning-related liver lesions: age more than 20 years [odds ratio (OR), 13.75; 95% Confidence Interval (CI), 1.48 – 127.47], and ingested paraquat amounts more than 30 mg /kg (OR, 16; 95% CI, 1.32 – 194.62). There was also a significant correlation between liver lesions and age (Correlation Coefficient, 0.50; P value, 0.024); gender (Correlation Coefficient, 0.57; P value, 0.008); amount of ingested PQ (Correlation Coefficient, 0.55; P value, 0.012). There were no correlations between lung and kidney histopathological lesions with different variables.

Conclusion: Age, amount of ingested PQ, and gender are important parameters which may correlate with histopathological liver lesions in deceased PQ poisoning patients.

KEY WORDS: Paraquat, Toxicity, Histopathology, Mortality, Poisoning, Autopsy.

INTRODUCTION

Paraquat (PQ) is a bipyridilium herbicide which is used worldwide in many countries. The non-control availability of PQ results in its ingestion as a suicide or by mistake can lead to death. Death is usually associated with respiratory insufficiency injury due to an oxidative insult to the alveolar epithelium with subsequent obliterating fibrosis.[1,2]

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Morphologically, alveolitis, pulmonary edema and infiltration of inflammatory cells are the major findings in PQ-induced lung injury.[3,4] Impaired renal functions play an important role in determining the outcome of PQ intoxication. Since PQ is eliminated mainly by the kidney, oliguric and non-oliguric acute renal failure is a frequent complication of PQ poisoning.[5,6] Damage to the proximal renal tubule is often more reversible than the destruction to lung tissue. Beebeejaun et al found proximal renal tubular necrosis by histopathological examination of a fatal case of PQ poisoning.[7] Liver damage following exposure to high doses of PQ has been demonstrated in several reports.[8,9] Centrilobular

necroses of hepatocytes with proliferation of the kupffer cells and bile canals have been described.

Previous human *postmortem* studies related to fatal PQ poisonings did not show any correlation between the toxicokinetic of PQ and the histophatological lesions.[2,10] In addition, *postmortem* analyses showed the poor efficacy of decontamination and anti-Inflammatory and immunosuppressive medications in PQ poisoning.[10] Some studies evaluated the PQ organ damaged in autopsy findings. However, a few studies have described their relationships with other components. In a previous study in paraquat poisoning a large amount of ingested PQ and age has been considered important variables for prediction of fatality rate of PQ poisoning. For the prediction of fatality, the best cut-off point for age was 17 years (area under curve 0.937 ± 0.059, 95% confidence interval [CI], 0.73–0.99; $p < 0.0001$), sensitivity 93.75 (95% CI, 69.7–99) and specificity 100 (95% CI, 30.5–100).[11]

Our objective was to determine the frequency of histo-pathological findings in deceased PQ poisoning patients and to evaluate their relationship with age, gender, amount of ingestion, time from ingestion to admission and time of death after ingestion.

METHODOLOGY

This cross-sectional study was conducted in the Poisoning Emergency Department (PED) of our university teaching hospital and Forensic Medical Center. PED is a main referral center of the second largest province in our country, and is specifically staffed and designed exclusively for the management of poisoned patients. Approximately 400 patients are admitted monthly.

Forty-two patients with confirmed paraquat (PQ) toxicity with positive urinary dithionate test[12] were included in the study. Medical emergency procedures were conducted according to our local guideline for PQ poisoning. Briefly, gastric evacuation and if needed gastric lavage was performed on all subjects seen within 6 hours after ingestion, and 1 g / kg activated charcoal was given. Haemodialysis was performed if a urinary PQ test was positive. Informed consent was obtained for haemodialysis. All patients also received vitamine E and C, N-acethyl-cysteine, corticosteroid and hemodialysis during hospitalization.[13]

Serial hematological and biochemical tests, chest X-ray and Arterial Blood Gas (ABG) analysis were tested if needed. Patients who died underwent a standard medicolegal autopsy in Forensic Medical Center. Data including age, gender, amount of ingested PQ, time interval between PQ ingestion and admision, time of death and histo-pathological findings were recorded in a check list. Chi square or fisher exact tests were used to compare differences. Spearman analysis was used to determine correlation of histopathological findings with different variables.

Logistic regression was applied to calculate odds ratio (OR) with 95% Confidence Interval (CI) to show how predictive is the age, gender, amount of ingested PQ, time interval between PQ ingestion and admision, for histopathologicl lesions. We also calculated the positive predictive value (proportion of patients predicted to show organs histopathological lesions to those who actually developed) and the negative predictive value (proportion of patients predicted not showing organs histopathological lesions to those who actually showed histopathological lesions). For each measure we identified the optimal cut-off point. The area under the curve (AUC) and its standard error were calculated to measure the prognostic information provided by each variables. AUCs between 0.7 and 0.8 were classified as "acceptable" and between 0.8 and 0.9 as "excellent" discrimination.[14] The data was analyzed using the Statistical Package for the Social Sciences version 17.0 (SPSS Inc, Chicago, IL, USA) and MedCalc (MedCalc Software Inc, Mariakerke, Belgium) statistical software. P value less than 0.05 was considered as significant results.

RESULTS

With the mortality rate of 47%, patients underwent a standard medicolegal autopsy. 85% were men with the mean age of 29.06 (SD: 10.6) and 15% were women with the mean age of 16.66 (SD: 4.6) years old. The frequency of histo-pathological lesions in autopsy findings is shown in Table-I. The combined

Table-I: Frequencies of histo-pathological lesions in autopsy of paraquat poisoning patients.

Organ	Histo-pathological findings	No. (%)
Liver	Hepatocyte degeneration	12 (60)
	Cholestasis	1 (5)
	None	7 (35)
Kidney	Tubular nephritis	12 (60)
	None	8 (40)
Lung	Pulmonary edema	10 (50)
	Fibrosis	2 (10)
	None	8 (40)

Table-II: Distribution of histo-pathological lesions with respect to different variables.

Variables		Liver Lesions		Kidney lesions		Lung lesions	
		+	-	+	-	+	-
Gender							
	Male	13	4	11	6	10	7
	Female	0	3	1	2	2	1
Age (years)	≤ 20	2	5	4	3	6	1
	> 20	11	2	8	5	6	7
Toxin Amount (mg / kg)	≤ 30	2	0	2	0	0	2
	> 30	8	1	5	4	4	5
	Unknown	3	6	5	4	8	1
Time from ingestion to admission (hours)	≤ 24	11	7	11	7	10	8
	>24	2	0	1	1	2	0
Time of Death (hours)	> 24	7	4	6	5	7	4
	> 2 4	6	3	6	3	5	4

Results are expressed as number of patients.

organ injuries had more frequencies in PQ toxicity in our results. Liver with kidney; liver with lung; and kidney with lung lesions were observed with the same frequency (20%) in deceased patients. Hepatocellular damage (60%), tubular nephritis (60%) and pulmonary edema (50%) were the main pathological findings in their organs autopsies.

The distributions of histopathological organ damages with respect to different variables have been shown in Table-II. Among 18 patients who were admitted during the first 24 hours after ingestion, hepatocytes degeneration and tubular nephritis were detected in 11 patients. The time interval between admission and PQ ingestion had no significant correlation with the frequency of organ damages. In patients who died during the first 24 after ingestion, tubular nephritis (eight patients), hepatocyte degenerations (six patients), pulmonary edema (six patients), cholestasis (one patient), and fibrosis (one patient) were found in their autopsies. Four patients had no liver and lung damages and five patients were without kidney damages.

Age more than 20 years was associated with a significantly higher risk of liver lesions (odds ratio 13.75; 95% Confidence Interval (CI), 1.48- 127.4); P value, 0.022). Spearman analysis also showed there was a significant correlation between liver lesions and age [Correlation Coefficient, 0.50; P value, 0.024], gender (Correlation Coefficient, 0.57; P value, 0.008), amount of ingested PQ (Correlation Coefficient, 0.55, P value, 0.012). There were no correlations between lung and kidney histopathological lesions with different variables.

Logistic regression results indicated that the chance of liver histopathological lesions is 13.75 times for patients with age more than 20 years old than in comparison with age equal or less than 20. It also showed that patients with ingestion of more than 30 mg /kg of PQ had 16 times the risk of liver histopathological lesion in comparison with those of less than 30 mg /kg PQ ingestion (Table-III). None of the variables were predictive for kidney or lung lesions.

For all studied variables the area under ROC curve, sensitivity, and specificity at the best cutoff point was determined and compared. Discrimination was excellent for age as well as toxin amounts. Age older than 20 years was associated with a significantly higher risk of liver lesions and had a sensitivity of 84.62% and specificity of 71.43% in predicting histopathological liver lesions (Table-IV).

DISCUSSION

In this study, the mortality rate was 47% which was comparable with other studies.[11,13] Sandhu JS, et al showed 35% mortality in paraquat poisoning within a five years follow-up study.[15]

Table-III: Predictive variables of Liver lesions in autopsy of patients with paraquat poisoning.

Variable	B	SE	P value	Unadjusted OR (95% CI)
Age (Years)	2.62	1.14	0.02	13.75 (1.48 – 127.47)
Toxin amount (mg /kg)	2.77	1.27	0.03	16 (1.32 – 194.62)

B, estimated coefficient; SE, standard error of mean; OR, odds ratio; CI, Confidence Interval.

Table-IV: Areas under the ROC curves of each variable for liver lesions.

Variable	AUC ± SE(95% CI)	Cutoff point	Sensitivity(95% CI)	Specificity(95% CI)	+LR	-LR	PValue
Age (years)	0.80 ± 0.10 (0.57-0.94)	20	84.62(54.5-97.6)	71.43(29.3 – 95.5)	2.96	0.22	0.002
Gender	0.71± 0.12(0.47– 0.89)	*	100(75.1-100)	42.86(10.4 – 81.2)	1.75	0.00	0.06
Toxin amount (mg/ kg)	0.80± 0.09 (0.57-0.94)	30	76.92(46.2- 94.7)	85.71(42.2 - 97.6)	5.83	0.27	0.002

ROC, Receiver under Operating Curve; AUC, area under the curve; SE, Standard error of mean; CI, Confidence Interval; +LR, Positive likelihood ratio; -LR, Negative likelihood ratio;   * refers to male patients.

The incidence of PQ toxicity was more prevalent in men and third decade of life. This finding was consistent with Agarwal study which showed that PQ toxicity was more prevalent in 30 years old patients.[16] It might be due to psychological problems and availability of this poison for men agricultural workers. Seok and et al also showed higher incidence of poisoning in men compared with women.[17]

Hepatocellular damage (60%), Tubular nephritis (60%) and pulmonary edema (50%) were the main pathologies in their organs which were in accordance with the study by Sandhu and et al.[15] This property is due to superoxide demolish effects on cellular membranes. PQ is a highly toxic nonselective herbicide for humans and animals.[1,18] PQ is known to cause oxidative damage to the liver, kidney and lung by generating superoxide anion.[19,20] Circulatory collapses is the main cause of death in severe PQ poisoned patients; however, if the clinical course is prolonged, pulmonary fibrosis would be developed.

In the liver, hepatocyte degeneration (12 patients) and Cholestasis (one patient) were observed in the autopsy analysis. Hepatocellular necrosis in acute PQ poisoning has been demonstrated in the study by Takegoshi et al.[21] The analysis of 13 patients with hepatic injury associated with PQ poisoning, damage of the intrahepatic bile excretory pathways in ten of these patients were observed.[22] Two phases in PQ hepatotoxicity have been described by Mullick et al, first accumulation of PQ induces hepatocellular injury and then excretion of PQ into the bile or absorption via enterohepatic circulation, with subsequent elimination into bile causes cholangiocellular and cholestatic damage.[22] Intrahepatic cholestasis in PQ poisoning in humans secondary to bile duct injury has been supported in other studies as well.[21-25] Apart from the excretion role of biliary tract, the enterohepatic recirculation has been considered to occur in humans.[26-28]

Tubular nephritis was observed in our study. Histopathological examination of a fatal case of PQ poisoning has shown proximal renal tubular necrosis in study by Beebeejaun and colleagues.[7] Tubular filtration and active tubular secretion is the main route of PQ elimination in human.[29] Tubular necrosis with a decrease in the glomerular filtration and tubular secretion increase the elimination half-life of the PQ after ingestion of large doses of PQ.[30]

Lungs were abnormally affected in deceased patients in our study. Pulmonary edema and fibrosis were the main pathological findings in autopsy. Fibrosis was observed in the two cases. Lung fibrosis was observed in one of the patient who died during the first 24 after PQ ingestion. The proliferation of fibroblasts in the alveoli and inflammation is usually correlated with the longer survival time.[10,31] This well known pattern was not evident in our case since his death were observed during the first 24 hours, not enough time was available for synthesis and deposition of collagen.

The correlation with clinical data is often fundamental for the pathological diagnosis in cases of postmortem investigation, as tubular changes rapidly occurs postmortem thus making the diagnosis very tricky. Similar observations are also valid for the hepatocellular damage. However we did not consider the influence of each clinical manifestation in this regard.

Logistic regression analyses identified the following significant predictors for poisoning-related liver lesions: age older than 20 years, and toxin amounts more than 30 mg /kg. There are some reports of the patients younger than 20 years old who survived after ingestion of potential lethal dose of PQ.[32] Ingestion of 30 mg /kg of PQ is considered to be fatal.[33] It is also a direct hepatotoxic agent with high dose exposure.[34] The inverse relationship between the amounts of PQ ingested with the survival period has been shown in some studies.[10,11,13]

Although the ingested dose is an important issue in fatality, the real amounts absorbed are highly dependent on emesis and/or gastric lavage. However even absorption of small amount of PQ can cause organ damages. The spearman analysis also showed that gender correlated with liver

damages. This might attributed to differences of enzyme activity between women and men.

The survival period of most patients were less than 24 hours representing fatal acute intoxications. Kim et al demonstrated that age, time interval between PQ ingestion and the arrival time at the hospital, plasma PQ level, and estimated amount of PQ ingestion were significantly lower in the patients with high survival rate.[35] They reported that the estimated amount of PQ ingestion and the time interval between PQ ingestion and the arrival time at the hospital were predictive factors of acute kidney injury.[35] However, we found no correlation between amounts of ingestion with kidney damages which was not consistent with Kim et al study. The studied patients in Kim et al study were different from ours. They excluded patients who were in a very poor physical condition within one day of ingestion. However all PQ poisoning cases were included in our study.

There were no correlations between lung, and kidney histopathological lesions with different variables in our study. We have no explanation for that. The absence of correlation may be due to small sample size.

Since PQ poisoning is still a problem in developing countries, it is important to decrease the accessibility of PQ to improve suicide prevention. Therefore control of the PQ storage on farms and control of its purchase by farmers would be helpful.

Limitations of the study:

1) Our results may not be extrapolated to other institutions. It is a single-centre study, which may not be representative of all patients.
2) The overall number of our patients with complications or death was relatively small.
3) The toxicological screening was not carried out to be able predict correlation of histopathological findings with outcome of paraquat poisoning by measuring the plasma paraquat concentration. Outcome prediction of paraquat poisoning by measurement of the plasma paraquat concentration has been studied previously.[36]
4) Lack of measuring intensity of treatment may affect the rate of complications.
5) One parameter of paramount importance in histopathology studies on forensic material is the postmortem interval, time of death and the collection of histology specimens which has not been evaluated in our study since the histology specimens was taken in the Forensic Medical Center not located in our hospital.

In conclusion, age, amount of paraquat ingested, and gender are important parameters which may correlate with histopathological lesions in PQ deceased patients. Identifying risk factors may allow better identification of those at greater mortality risk, looking for risk factor in future studies. A comparison study of different treatments in patients younger and older than 20 years old is recommended.

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Authors Contribution:

All authors carried out the design and coordinated the study and run all modeling studies and prepared the manuscript. All authors have read and approved the content of the manuscript.

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