

Original Article

Cytokine changes in fatal cases of paraquat poisoning

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Abstract: Cytokine-mediated inflammation is involved in the pathophysiology of paraquat toxicity. Nevertheless, few human studies have examined fluctuations in circulating cytokine levels. Blood samples were obtained from 21 patients with paraquat poisoning and compared to those of 18 healthy controls. All paraquat patients received a standard detoxification protocol composed of hemoperfusion, pulse therapies of methylprednisolone and cyclophosphamide, followed by dexamethasone therapy. Nonsurvivors not only had higher scores for the severity index of paraquat poisoning ($P=0.004$) but also presented with higher white blood cell counts ($P=0.046$) than survivors. Multiplex immunoassays revealed higher circulating levels of interleukin 2 (IL-2), interleukin 9 (IL-9), interleukin 10 (IL-10) and macrophage inflammatory protein-1 beta (MIP-1 β) in survivors than in healthy controls. Furthermore, the circulating levels of interleukin 1 beta (IL-1 β), IL-2, interleukin 5 (IL-5), interleukin 8 (IL-8), IL-9, IL-10, interleukin 12 (IL-12 p70), interleukin 17A (IL-17A), eotaxin, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP-1), interferon gamma-induced protein 10 (IP-10) and MIP-1 β were higher in nonsurvivors than in healthy controls. Finally, the circulating levels of IL-1 β and MCP-1 were higher in nonsurvivors than in survivors. Therefore, the observation of cytokine-mediated inflammation is in line with the detoxification protocol because glucocorticoids and cyclophosphamide are potent anti-inflammatory agents. Additionally, circulating levels of IL-1 β and MCP-1 could serve as promising prognostic markers for patients with paraquat poisoning.

Keywords: Paraquat poisoning, severity index of paraquat poisoning, mortality, cytokine, inflammation, multiplex immunoassays

Introduction

Paraquat is a bipyridyl pesticide with extreme toxicity that is frequently used in Asian countries [1]. Because of its easy access, this pesticide is commonly used in cases of attempted suicide by self-poisoning in Taiwan [2]. Paraquat is the second most common agent responsible for pesticide poisoning in Taiwan and the most lethal agent, resulting in 18.9% pesticide poisoning and 46.3% poison-associated deaths [3].

Current hypotheses on the toxicity mechanisms of paraquat [4] focus on the formation of

superoxide anions, resulting in the development of reactive oxygen species and oxidation of cellular nicotinamide adenine dinucleotide phosphate (NADPH), leading to interference with the NADPH-associated pathway. The main reason for death in paraquat intoxication is respiratory failure following oxidative stress to alveolar epithelial cells with ensuing obliterating fibrosis [4]. Apart from the lung, paraquat ingestion has been proven to damage other organ systems [5]. Theoretically, there are three grades of severity for paraquat poisoning [6]. Mild poisoning provokes oral mucosa ulceration and gastrointestinal discomfort but ultimate recovery. Moderate to severe poisoning

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normally causes toxic hepatitis, kidney injury, and pneumonitis, followed by lung fibrosis, which frequently results in mortality in two to three weeks. Fulminant poisoning leads to mortality in one week due to multiorgan failure and cardiovascular collapse.

There is a paucity of human studies on cytokine changes after paraquat exposure (**Table 1**). In a study of 36 patients with paraquat poisoning allocated to either conventional therapy or Xuebijing plus conventional therapy, Zheng et al. [7] showed that circulating transforming growth factor beta 1 (TGF- β 1) concentrations were greater in patients poisoned with paraquat, and treatment with Xuebijing plus conventional therapy reduced TGF- β 1 concentrations. In a study of 26 patients with pulmonary damage caused by paraquat poisoning, Wu et al. [8] demonstrated that the patients exhibited higher circulating interleukin 6 (IL-6) levels than healthy subjects. In a study of 174 cases with paraquat poisoning, Meng et al. [9] revealed that parenteral nutrition with additional omega-3 fish oil emulsion treatment attenuated circulating tumor necrosis factor alpha (TNF- α) levels, altered the nutritional state, and was associated with improved 90-day survival compared with therapy without omega-3 fish oil. In a study of 44 cases with paraquat poisoning, Yi et al. [10] showed that edaravone reduced circulating concentrations of IL-6, interleukin 10 (IL-10) and TNF- α in patients with paraquat poisoning. In a study of 75 patients with multiple organ dysfunction syndrome caused by paraquat poisoning, Dai et al. [11] reported that circulating Toll-like receptor 4 (TLR4) levels were increased in patients with multiple organ dysfunction syndrome caused by paraquat poisoning compared with healthy controls, and a positive correlation of TLR4 with inflammatory cytokines was found. In a study of 82 patients with multiple organ dysfunction syndrome caused by paraquat poisoning, Dai et al. [12] also indicated that circulating IL-10 levels were higher in patients with multiple organ dysfunction syndrome caused by paraquat poisoning than in healthy controls.

The rationale for this study was based on an important, but as yet unanswered, question that arose for many paraquat patients treated at our hospital. Patients with paraquat poisoning are routinely treated with a standard detoxification method that includes hemoperfusion, pulse therapies with methylprednisolone and

cyclophosphamide, and prolonged dexamethasone therapy. With this approach, pulmonary function and arterial oxygen concentrations could return to near normal within 3 to 6 months [13]. The central dogma for this detoxification protocol is cytokine-mediated inflammation, which is involved in the pathophysiology of paraquat toxicity. Methylprednisolone and dexamethasone are glucocorticoid steroids that exert anti-inflammatory action through the inhibition of phospholipase A₂ [14], whereas cyclophosphamide is an alkylating agent that inhibits immune reactions mediated by T cells [15]. Nevertheless, few human studies have examined changes in circulating cytokines, which inspired our interest in this research. In this study, we hypothesized that increased concentrations of circulating cytokines and immune cell hyperactivation could be initiated by paraquat poisoning. Hence, this study aimed to examine the changes in circulating cytokine concentrations after paraquat ingestion.

Material and methods

Ethical statement

This study was approved by the Medical Ethics Committee of Chang Gung Memorial Hospital. This research adhered to the Declaration of Helsinki, and each patient signed an informed consent form before participation. The institutional review board numbers were 201601687B0 and 201801259B0.

Patients

Between 2016 and 2018, 21 patients with paraquat poisoning were treated at Chang Gung Memorial Hospital. All pesticide exposures were oral, and none of the patients had coingestion. Baseline demographic, clinical, laboratory and mortality data were recorded for analysis. Blood samples were collected in the hospital upon arrival.

Patient group

Patients were stratified into two subgroups according to survival status: survivors (n=7) or nonsurvivors (n=14).

Clinical diagnosis

A presumptive diagnosis of paraquat poisoning was dependent on pesticide exposure history, clinical and laboratory findings, particularly the

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Table 1. Published human studies of cytokine changes in paraquat poisoning

Study	Year	Sample size	Excluded disease	Age	Time to the hospital, hour	Blood paraquat level, mg/L	Severity index of paraquat poisoning score, mg hour/L	Cytokine changes	Mortality rate, %
Present study	2021	21	None	48.9±18.4	11.3±24.1	4.0±3.8	9.7±10.0	IL-1β, IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70, IL-17A, eotaxin, G-CSF MCP-1, IP-10, and MIP-1β	66.7
Dai et al. [12]	2020	82	Immune or immune-related diseases	Survivors: 32.0±7.4; Non-survivors: 29.8±6.6	Survivors: 11.5±4.7; Non-survivors: 10.9±5.2	Not mentioned	Not mentioned	IL-10	34.1
Dai et al. [11]	2020	75	Immune or immune-related diseases	Survivors: 30.1±9.8; Non-survivors: 30.8±8.9	Survivors: 10.1±6.0 Non-survivors: 10.7±4.9	Not mentioned	Not mentioned	TLR4, IL-1 and TNF-α	65.3
Yi et al. [10]	2019	44	Pulmonary or organ dysfunction and patients who died or were discharged in 24 hours	Control group: 35.6±6.1; Edaravone group: 37.2±5.8	Control group: 4.9±3.1; Edaravone group: 5.0±2.8	Control group: 0.8±3.6; Edaravone group: 1.2±2.9	Control group: 4.5±6.4; Edaravone group: 5.1±5.9	IL-6, IL-10, and TNF-α	63.6
Meng et al. [9]	2019	174	Chronic obstructive pulmonary disease, psychosis, and diabetes with severely impaired liver or renal function	Survivors: 31.2±11.5; Non-survivors: 34.3±12.5	Survivors: 2.8±2.7; Non-survivors: 3.7±6.3	Survivors: 6.4±4.0; Non-survivors: 6.8±4.0	Not mentioned	TNF-α	45.4
Wu et al. [8]	2018	26	Complications or infection of the heart, liver or kidney and immune or immune-related diseases	Median: 39	Not mentioned	Not mentioned	Not mentioned	IL-6	Not mentioned
Zheng et al. [7]	2012	36	Not mentioned	Control group: 39.5±12.6; Xuebijing group: 37.3±12.2	Control group: 3.1±0.6; Xuebijing group: 3.2±0.7	Not mentioned	Not mentioned	TGF-β1	66.7

Note: IL-1β, interleukin 1 beta; IL-2, interleukin 2; IL-5, interleukin 5; IL-6, interleukin 6; IL-8, interleukin 8; IL-9, interleukin 9; IL-10, interleukin 10; IL-12 p70, interleukin 12 p70; IL-17A, interleukin 17A; IP-10, interferon gamma-induced protein 10; G-CSF, granulocyte colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1 beta; TNF-α, tumor necrosis factor alpha; TGF-β1, transforming growth factor beta 1; TLR4, toll-like receptor 4.

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urine sodium dithionite test [16]. Nevertheless, urine test was merely a screening test. A definite diagnosis of paraquat intoxication was obtained by examination of blood paraquat concentration (spectrophotometry, Hitachi, Tokyo, Japan).

Inclusion and exclusion criteria

Only patients aged 18 years and above who had positive urine and blood tests for paraquat were enrolled in the study. Patients were excluded if they were under 18 years old or if they did not have positive urine and blood paraquat tests despite suspicions of paraquat ingestion.

Severity index of paraquat poisoning

A severity index of paraquat poisoning was defined as the product of blood paraquat level (ppm) and time elapsed since exposure to arrival at hospital (hour) [17].

Medical detoxification

The detoxification procedures [18] included gastric lavage with 2000 mL of 0.9% saline or distilled water, after which activated charcoal (1 g/kg) and magnesium citrate (250 mL) were administered via a nasogastric tube. Hemoperfusion with a charcoal-containing hollow fiber (Adsorba, Gambro, Germany) was performed for eight hours if the urine paraquat level was more than 5 ppm [19]. A second round of hemoperfusion was performed if the urine paraquat level was more than 5 ppm at 4 hours after previous hemoperfusion. The methods also incorporated cyclophosphamide (15 mg per kg per day) for 2 days and methylprednisolone (1 g per day) for 3 days [13]. Dexamethasone (20 mg per day) was prescribed for another 11 days after methylprednisolone therapy. Notably, cyclophosphamide and methylprednisolone pulse therapies were prescribed again if the arterial oxygen partial pressure was less than 60 mm Hg at two weeks after the first round of therapy. Last, patients refrained from having oxygen therapy during the period of hospitalization. The reason for normal inspired oxygen therapy (FiO₂ 21%) was that an increase in the fraction of inspired oxygen could bring more oxidative insult, and the generation of free radicals and superoxide could exacerbate paraquat toxicity [20].

Cytokine measurements using multiplex immunoassay

Blood samples were collected in the hospital upon arrival. Since there were no normal laboratory reference values for serum cytokines in humans, blood samples from 18 healthy controls were included for comparison. Blood specimens were obtained, centrifuged, and stored at -80°C. Blood cytokine levels were analyzed simultaneously with a Bio-Plex Human Cytokine Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA), namely, eotaxin, granulocyte colony-stimulating factor (G-CSF), interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin 5 (IL-5), interleukin 8 (IL-8), interleukin 9 (IL-9), IL-10, interleukin 12 (IL-12 p70), interleukin 17A (IL-17A), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 beta (MIP-1β). Briefly, 50 μl antibody-coupled beads per well were added to flat bottom plates and washed twice. Next, 50-μl serum/plasma samples were incubated with antibody-coupled beads for 30 minutes at room temperature. After washing 3 times to eliminate unbound substances, the beads were incubated with 25 μl biotinylated detection antibodies for 30 minutes at room temperature. After washing the unbound biotinylated antibodies three times, the beads were incubated with 50 μl streptavidin-phycoerythrin for 10 minutes at room temperature. After cleaning of extra streptavidin-phycoerythrin by washing three times, the beads were resuspended in 125 μl assay buffer. Beads were read on the Bio-Plex suspension array system, and the results were analyzed with Bio-Plex Manager software version 6.0. The limits of detection were as follows, eotaxin 0.01 pg/ml, G-CSF 0.42 pg/ml, IL-1β 0 pg/ml, IL-2 0.07 pg/ml, IL-5 0.23 pg/ml, IL-8 0.07 pg/ml, IL-9 0.15 pg/ml, IL-10 0.1 pg/ml, IL-12 p70 0.08 pg/ml, IL-17A 0.11 pg/ml, IP-10 0.19 pg/ml, MCP-1 0.03 pg/ml, and MIP-1β 0.03 pg/ml.

Statistical analysis

Continuous variables were presented as the means with standard deviations, while categorical variables were presented as numbers with percentages in parentheses. Comparisons between survivors and non-survivors were conducted using Student's t-test for quantitative

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variables and chi-square tests for qualitative variables. A *P* value of less than 0.05 was considered statistically significant. All analyses were accomplished with IBM SPSS Statistics Version 20.0.

Results

As shown in **Table 2**, the patients were 48.9 ± 18.4 years old, and most were male (76.2%). The majority of paraquat poisonings were due to suicide attempts (81.0%). Following paraquat ingestion, the patients were sent to the hospital in 11.3 ± 24.1 hours. There were no differences in demographic characteristics between nonsurvivors and survivors.

Paraquat ingestion was related to extreme morbidity and often led to medical complications, including acute kidney injury (81.0%), acute respiratory failure (66.7%), shock (61.9%), gastrointestinal tract bleeding (33.3%) and acute respiratory distress syndrome (23.8%). Notably, nonsurvivors suffered higher incidence rates of shock (92.9 versus 0%, $P < 0.001$) and acute respiratory failure (14.0 versus 0%, $P < 0.001$) than survivors.

Nonsurvivors not only had higher blood paraquat concentrations (5.6 ± 3.8 versus 0.8 ± 0.8 mg/L, $P = 0.004$) and severity indexes of paraquat poisoning scores (14.1 ± 9.9 versus 1.5 ± 1.0 mg hour/L, $P = 0.004$) but also suffered higher peak alveolar-arterial oxygen gradients (126.7 ± 118.4 versus 34.7 ± 9.1 mm Hg, $P = 0.028$) and white blood cell counts (15878.6 ± 5938.0 versus $10600 \pm 4831.5/\mu\text{L}$, $P = 0.046$) than survivors. There were no differences in other laboratory variables between nonsurvivors and survivors.

All patients received a standard detoxification protocol comprising gastric lavage, active charcoal, hemoperfusion, and cyclophosphamide and glucocorticoid pulse therapies. Nonsurvivors received fewer glucocorticoid and cyclophosphamide pulse therapies than survivors (42.9 versus 100.0%, $P = 0.018$).

Multiplexing immunoassays for cytokine detection in the serum of paraquat patients are presented in **Figures 1 and 2**.

As shown in **Figure 1**, circulating levels of IL-2, IL-9 and IL-10 were significantly higher in survi-

vors of paraquat poisoning than in healthy controls. Circulating levels of IL-1 β , IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70 and IL-17A were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Circulating levels of IL-1 β were significantly higher in nonsurvivors of paraquat poisoning than in survivors.

Circulating levels of MIP-1 β were significantly higher in survivors of paraquat poisoning than in healthy controls (**Figure 2**). Circulating levels of eotaxin, G-CSF, MCP-1, IP-10 and MIP-1 β were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Circulating levels of MCP-1 were significantly higher in nonsurvivors of paraquat poisoning than in survivors.

Discussion

These data are important because there are few human studies available on cytokine changes after paraquat poisoning. Our analysis found that paraquat poisoning is characterized by a systemic inflammatory response that involves elevated levels of circulating inflammatory cytokines and hyperactivation of immune cells (**Figure 3**). There were higher circulating levels of IL-2, IL-9, IL-10 and MIP-1 β in survivors than in healthy controls. Furthermore, circulating levels of IL-1 β , IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70, IL-17A, eotaxin, G-CSF, MCP-1, IP-10 and MIP-1 β were higher in nonsurvivors than in healthy controls. Finally, circulating levels of IL-1 β and MCP-1 were higher in nonsurvivors than in survivors. This study first demonstrated that various types of cytokines were involved in systemic inflammation following paraquat poisoning in humans, such as type 1 T helper cytokines, type 2 T helper cytokines, type 17 T helper cytokines, and regulatory cytokines. After receiving signals from the T-cell receptor and CD28 costimulator, naive T helper cells are stimulated to produce IL-2 and enter the cell cycle. After rapid cell division, these naive T helper cells differentiate into effector cells. Various subsets of effector cells have been defined based on their specific cytokine profiles, such as type 1 T helper cells, type 2 T helper cells and type 17 T helper cells. Cytokines produced by these subsets influence and modify the immune response [21]. In brief, IL-1 β , IL-2, IL-12 p70, IP-10 and G-CSF fall into the category of type 1 T helper cytokines. IL-5

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Table 2. Clinical characteristics and outcomes in paraquat poisoning (n=21)

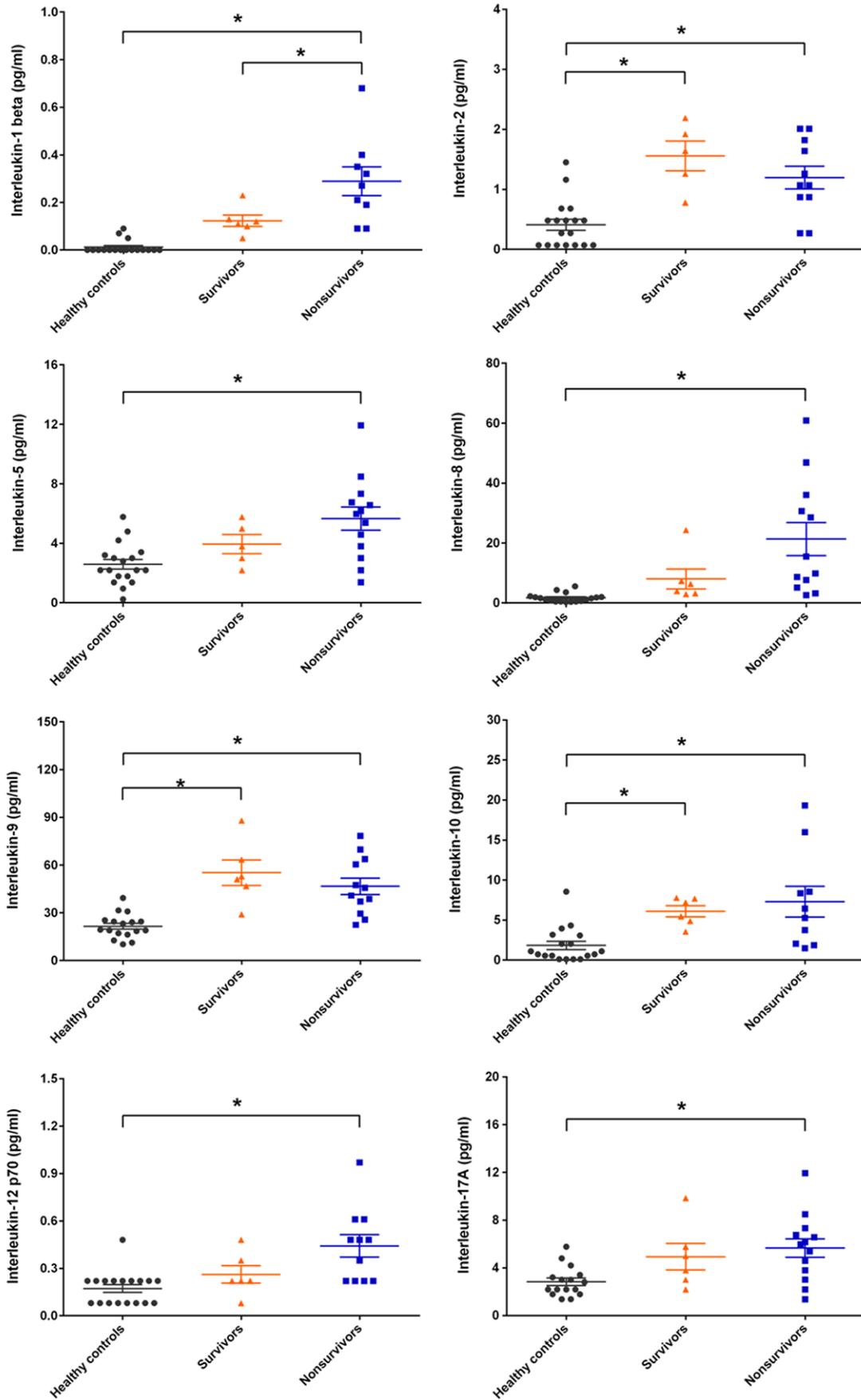
Variable	Total (n=21)	Survivors (n=7)	Nonsurvivors (n=14)	P value
Baseline demographics				
Male, n (%)	16 (76.2)	5 (71.4)	11 (78.6)	0.717
Age, year	48.9±18.4	44.3±19.1	51.1±18.4	0.435
Diabetes mellitus, n (%)	3 (14.3)	2 (28.6)	1 (7.1)	0.247
Hypertension, n (%)	7 (33.3)	3 (42.9)	4 (28.6)	0.638
Time to hospital, hours	11.3±24.1	4.9±6.8	14.7±29.4	0.399
Suicide attempt, n (%)	17 (81.0)	6 (85.7)	11 (78.6)	0.767
Medical complications				
Cardiovascular				
QTc, msec	457.3±40.8	445.8±49	465.6±35.4	0.434
Shock, n (%)	13 (61.9)	0 (0)	13 (92.9)	<0.001***
Respiratory				
Acute respiratory distress syndrome, n (%)	5 (23.8)	0 (0)	5 (35.7)	0.123
Pneumomediastinum, n (%)	0 (0)	0 (0)	0 (0)	1.000
Acute respiratory failure, n (%)	14 (66.7)	0 (0)	14 (100)	<0.001***
Gastrointestinal				
Gastrointestinal tract bleeding, n (%)	7 (33.3)	2 (28.6)	5 (35.7)	1.000
Acute pancreatitis, n (%)	1 (4.8)	0 (0)	1 (7.1)	1.000
Renal				
Acute kidney injury, n (%)	17 (81.0)	5 (71.4)	12 (85.7)	0.574
Neurological				
Glasgow coma scale score	14.6±1.2	15±0	14.4±1.4	0.108
Seizure, n (%)	1 (4.8)	0 (0)	1 (7.1)	1.000
Infection				
Aspiration pneumonia, n (%)	2 (9.5)	0 (0)	2 (14.3)	0.533
Urinary tract infection, n (%)	4 (19.0)	2 (28.6)	2 (14.3)	0.574
Laboratory				
Blood paraquat, mg/L	4.0±3.8	0.8±0.8	5.6±3.8	0.004**
Severity index of paraquat poisoning score, mg hour/L	9.7±10.0	1.5±1.0	14.1±9.9	0.004**
Blood gas analysis				
Nadir partial pressure of oxygen, mm Hg	60.5±36.1	70.7±13.1	54.1±44.7	0.359
Peak partial pressure of carbon dioxide, mm Hg	33.1±6.0	35.5±3.9	31.6±6.7	0.190
Peak alveolar-arterial oxygen gradient, mm Hg	90.9±102.0	34.7±9.1	126.7±118.4	0.028*
Hemogram				
Hemoglobin, g/dL	15.6±1.7	15.5±2.1	15.6±1.6	0.924
White blood cell count/uL	14119.1±6035.2	10600±4831.5	15878.6±5938.0	0.046*
Platelet count, 10 ³ /mL	244.6±57.9	259.4±58.2	237.2±58.4	0.421
Biochemistry				
Peak urea nitrogen, mg/dL	47.8±24.5	42.3±23.7	51.6±25.4	0.456
Peak creatinine, mg/dL	2.5±1.7	2.1±1.4	2.7±1.9	0.506
Peak aspartate transaminase, U/L	153±290.2	60.5±29.3	245.5±404.9	0.290
Peak alanine aminotransferase, U/L	151.8±451.3	79.4±58.0	187.9±554.6	0.616
Peak total bilirubin, mg/dL	1.9±1.6	1.0±0.6	2.6±1.8	0.138
Treatment				
Gastric lavage, n (%)	17 (81.0)	6 (85.7)	11 (78.6)	1.000
Active charcoal and magnesium citrate, n (%)	17 (81.0)	6 (85.7)	11 (78.6)	1.000
Charcoal hemoperfusion, n (%)	20 (95.2)	7 (100.0)	13 (92.9)	1.000
Glucocorticoid and cyclophosphamide pulse therapy, n (%)	13 (61.9)	7 (100.0)	6 (42.9)	0.018*
Duration of hospitalization, day	13.1±11.8	24.0±4.2	7.7±10.5	0.001**

Note: QTc corrected QT interval; *P<0.05, **P<0.01, and ***P<0.001.

belongs to the category of type 2 T helper cytokines. IL-9 comes under the category of type 9

T helper cytokines, and IL-17A comes under type 17 T helper cytokines. IL-10 belongs to the

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Figure 1. Multiplex cytokine immunoassay. Circulating levels of IL-2, IL-9 and IL-10 were significantly higher in survivors of paraquat poisoning (n=7) than in healthy controls (n=18). Circulating levels of IL-1 β , IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70 and IL-17A were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in healthy controls (n=18). Circulating levels of IL-1 β were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in survivors (n=7). Abbreviations: IL-1 β , interleukin 1 beta; IL-2, interleukin 2; IL-5, interleukin 5; IL-8, interleukin 8; IL-9, interleukin 9; IL-10, interleukin 10; IL-12 p70, interleukin 12 p70; IL-17A, interleukin 17A.

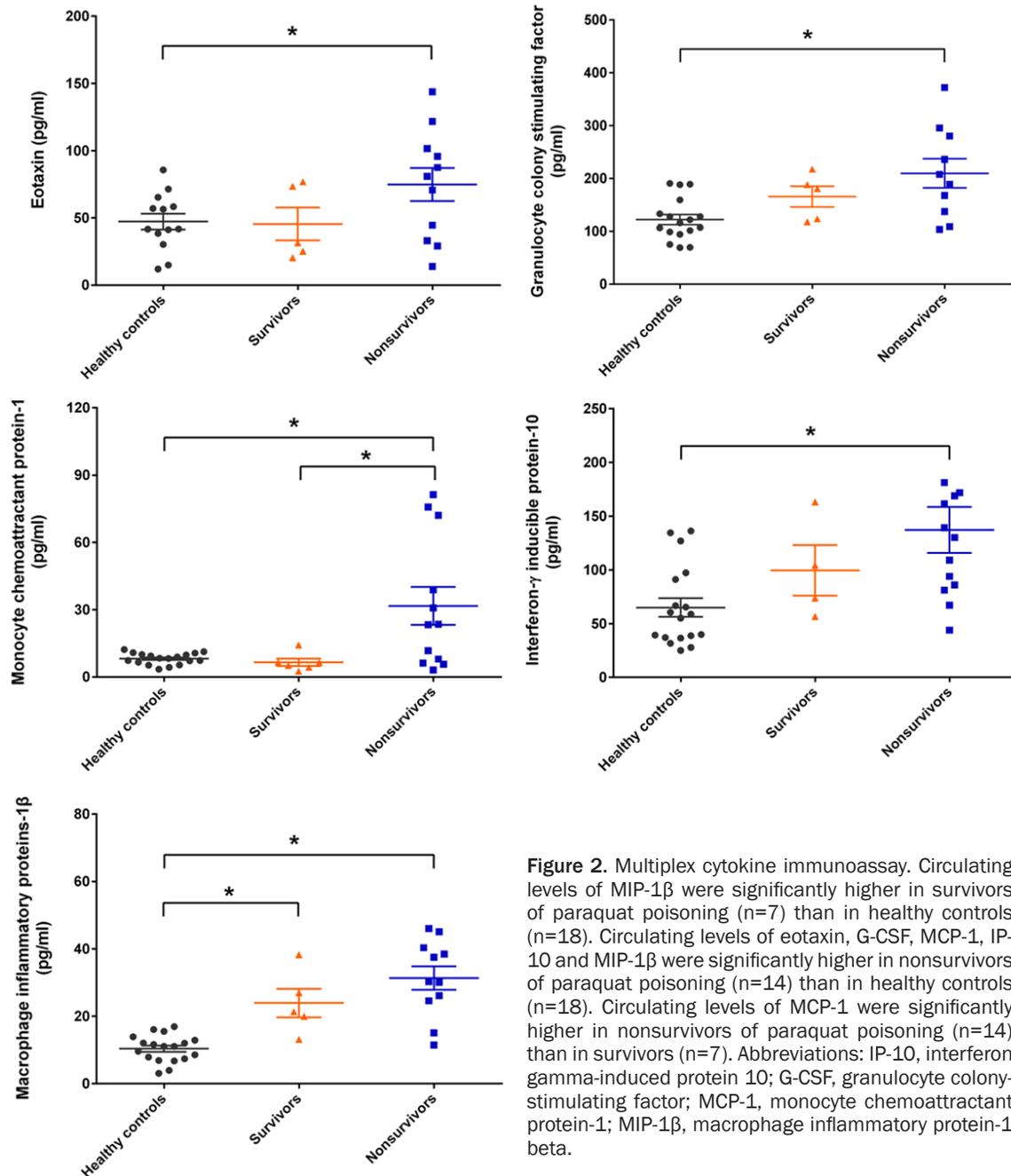


Figure 2. Multiplex cytokine immunoassay. Circulating levels of MIP-1 β were significantly higher in survivors of paraquat poisoning (n=7) than in healthy controls (n=18). Circulating levels of eotaxin, G-CSF, MCP-1, IP-10 and MIP-1 β were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in healthy controls (n=18). Circulating levels of MCP-1 were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in survivors (n=7). Abbreviations: IP-10, interferon gamma-induced protein 10; G-CSF, granulocyte colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1 β , macrophage inflammatory protein-1 beta.

category of regulatory cytokines, and IL-8 belongs to innate cytokines. Nonetheless, these cytokines may have overlapping roles.

Previous studies (**Table 1**) have demonstrated cytokine changes in patients with paraquat poisoning. However, the cytokine changes in our

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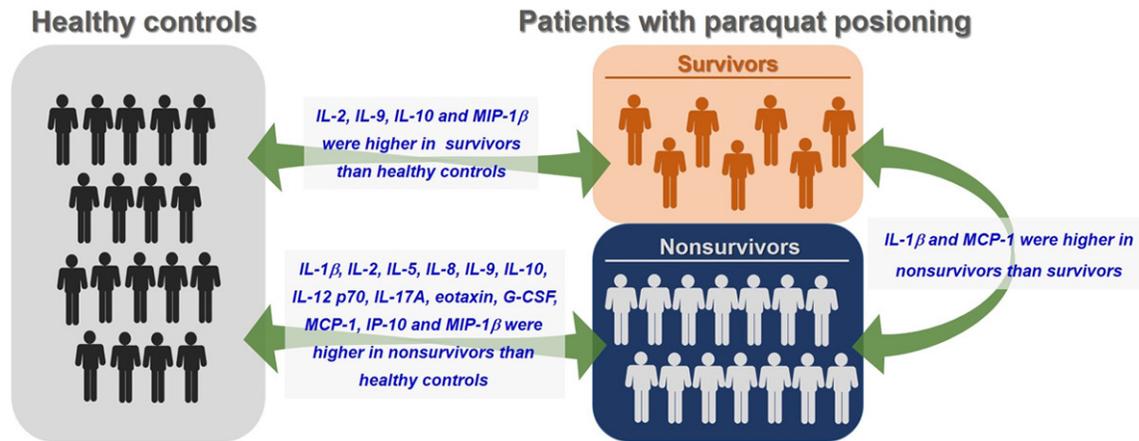


Figure 3. Multiplex cytokine immunoassay. Circulating levels of IL-2, IL-9, IL-10 and MIP-1 β were significantly higher in survivors of paraquat poisoning (n=7) than in healthy controls (n=18). Circulating levels of IL-1 β , IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70, IL-17A, eotaxin, G-CSF, MCP-1, IP-10 and MIP-1 β were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in healthy controls (n=18). Circulating levels of IL-1 β and MCP-1 were significantly higher in nonsurvivors of paraquat poisoning (n=14) than in survivors (n=7). Abbreviations: IL-1 β , interleukin 1 beta; IL-2, interleukin 2; IL-5, interleukin 5; IL-8, interleukin 8; IL-9, interleukin 9; IL-10, interleukin 10; IL-12 p70, interleukin 12 p70; IL-17A, interleukin 17A; IP-10, interferon gamma-induced protein 10; G-CSF, granulocyte colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MIP-1 β , macrophage inflammatory protein-1 beta.

analysis were slightly different from those reported in previous studies. The results of this study are different from prior studies in humans and may have been influenced by various factors. First, previous studies excluded patients with systemic diseases. Second, the age of the patients in the present research was older than the ages of the patients in previous studies. Third, the severity index of paraquat poisoning scores was not recorded in some of the previous studies. Finally, it is noteworthy that the technique used to measure cytokines in the present study is multiplex immunoassays. Multiplex immunoassays allow the quick profiling of biomarker proteins in body fluids, using less sample and labor than single traditional immunoassays. Another advantage of multiplex immunoassays is their ability to concurrently monitor the roles of various biomarkers during inspections of complex biological systems.

Circulating levels of IL-1 β were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Moreover, circulating levels of IL-1 β were significantly higher in nonsurvivors of paraquat poisoning than in survivors. Animal studies have revealed that IL-1 is an inflammatory cytokine that can induce a shock-like state [22] and lung edema associated with pulmonary vascular injury [23]. Previous studies demonstrated that IL-1 is implicated in

the pathophysiology of paraquat-associated lung damage [24-26]. One animal study demonstrated that paraquat increased the IL-1 β concentration in the hippocampal dentate gyrus, leading to neural stem cell impairments and memory dysfunction in adult mice, indicating the neurotoxic effect of paraquat [27].

Circulating levels of IL-2 were significantly higher in both nonsurvivors and survivors of paraquat poisoning than in healthy controls. IL-2, a cytokine secreted by T cells, plays a vital role in the growth and expansion of lymphocytes and helps to enhance the immune system [28]. One team [29] found an increase in serum IL-2 concentration in a rat model of paraquat-induced renal damage, which indicated that IL-2 was an inflammatory factor involved in paraquat-associated acute kidney injury.

Circulating levels of IL-12 p70 were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Bioactive IL-12 p70 constitutes the IL-12 p35 and p40 subunits. IL-12 is an inflammatory cytokine generated by macrophages, dendritic cells and B cells. The cytokine is fundamental in the induction of interferon-gamma production by T cells, which facilitates type 1 T helper cell differentiation [30]. No previous laboratory study has investigated the involvement of IL-12 p70 in paraquat poisoning.

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Circulating levels of IP-10 were higher in non-survivors of paraquat poisoning than in healthy controls. Type 1 T helper cells produce interferon-gamma, which induces the production of IP-10 by different cell types. IP-10 in turn attracts and recruits type 1 T helper cells [31]. No previous laboratory study was found discussing the impact of paraquat on IP-10. Nonetheless, as mentioned earlier, interferon-gamma was found to be involved in the systemic inflammation and oxidative stress caused by paraquat [32-35]. Various studies have demonstrated that interferon-gamma is involved in the systemic inflammation and oxidative stress caused by paraquat poisoning. In one animal study, a significant decrease in blood interferon-gamma levels was noted in paraquat-exposed rats compared to controls, but a significant increase in interferon-gamma was observed after treatment with *Zataria multiflora* extract and carvacrol [32]. Similarly, in another animal study, there was a significant reduction in serum interferon-gamma levels in paraquat-exposed rats compared to controls, but a significant increase in interferon-gamma was observed after treatment with curcumin [33]. Interestingly, in another animal study, exposure to paraquat in rats significantly increased interferon-gamma levels in bronchoalveolar lavage fluid and the escape latency and traveled distance in the Morris water maze test. Treatment with *Zataria multiflora* extract and dexamethasone improved both the behavioral and lung changes induced by paraquat exposure [34]. In another study, paraquat poisoning in common carp also caused an increase in serum proinflammatory cytokines, such as interferon-gamma and IL-1 β [35].

Circulating levels of G-CSF were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. G-CSF is a hematopoietic growth factor that mediates neutrophil production. The clinical application of G-CSF has been explored in various disease states, such as to promote the production of neutrophils in chemotherapy-related neutropenia and to mobilize hematopoietic stem cells from the bone marrow into the blood to increase the safety and efficiency of hematopoietic stem cell transplantation [36]. Another common type of hematopoietic growth factor is granulocyte-macrophage colony-stimulating factor (GM-CSF). The receptors of GM-CSF are more

widely expressed than the receptors of G-CSF [37], therefore resulting in biological differences. Although no previous animal or in vitro studies illustrated the impact of paraquat on G-CSF after a literature review, one study showed that the concentrations of GM-CSF increased after paraquat treatment in mice [38]. Interestingly, another study showed that systemic administration of GM-CSF in mice exposed to paraquat revealed a neuroprotective effect by modulating neuroinflammatory glial responses [39].

Circulating levels of IL-5 were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. IL-5 was initially identified as a T-cell-producing cytokine that triggered the differentiation of activated B cells into antibody-secreting plasma cells [40]. IL-5 was later recognized to regulate the expression of genes associated with the proliferation, cell survival and effector functions of B cells and eosinophils, indicating its critical role in innate and acquired immunity [41]. No previous laboratory study has investigated the involvement of IL-5 in paraquat poisoning.

Circulating levels of IL-9 were significantly higher in both nonsurvivors and survivors of paraquat poisoning than in healthy controls. IL-9 is a cytokine mainly generated by T lymphocytes and has been commonly linked with allergic inflammation [42] and immunity to parasites [43-45]. Emerging research has revealed its participation in type 2 T helper cell/type 17 T helper cell-mediated inflammation [42]. An in vitro study revealed that paraquat upregulated the mRNA expression of type 17 T helper cell-promoting cytokines, including IL-9 and IL-21, demonstrating the immunotoxicity of paraquat [46].

Circulating levels of IL-17A were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. IL-17 is a cytokine that functions as an inflammation mediator. It plays a critical role not only in protective immunity against extracellular and intracellular pathogens but also in the pathogenesis of numerous autoimmune inflammatory diseases [47]. One study demonstrated that the signaling cascade from high-mobility group box 1 to Toll-like receptor 4, interleukin-23, and finally to IL-17A is important during paraquat-associated pulmonary damage mediated by neutrophil infiltration

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in mice [48]. Another study revealed that IL-17A participated in the pathological process of lung injury induced by paraquat by activating NF-kappaB p65 and recruiting neutrophils in mice [49]. The crucial role of NF-kappaB signaling and IL-17 signaling in the pathophysiology of paraquat-associated pulmonary damage was demonstrated in another study [50].

Circulating levels of IL-10 were significantly higher in both survivors and nonsurvivors of paraquat poisoning than in healthy controls. IL-10 is an anti-inflammatory cytokine that maintains the balance of the immune reaction, allowing the clearance of infection with minimal host injury [51]. In one human study of 82 subjects, Dai et al. [12] demonstrated that the augmented concentration of IL-10 and reduced concentration of microRNA-27a in serum probably reflected the inflammatory reaction in patients with multiple organ damage syndrome after paraquat poisoning. In another human study of 44 subjects, Yi et al. [10] revealed that edaravone, a free radical scavenging drug, could increase the serum levels of superoxide dismutase and reduce the levels of IL-10 and malondialdehyde in acute paraquat poisoning.

Circulating levels of IL-8 were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. IL-8 is a chemoattractant cytokine generated by a diversity of tissues and blood cells, participates in the process of leukocyte transmigration into tissues and triggers numerous functions of neutrophils [52]. One animal study showed that the level of IL-8 in bronchoalveolar lavage fluid increased in rats following paraquat poisoning and decreased after treatment with human umbilical cord cells overexpressing antioxidant genes [53]. Another study demonstrated that the expression levels of genes involved in the inflammatory process, including IL-8, were increased in zebrafish following paraquat poisoning. Furthermore, another study revealed that paraquat promoted the expression of inflammatory cytokines, such as IL-8, and inhibited the expression of anti-inflammatory cytokines in common carp [35].

Circulating levels of eotaxin were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Eotaxin is a potent eosinophil chemoattractant that has been described in several diseases ranging from air-

way inflammation [54] to neurodegenerative diseases [55] and intestinal inflammation [56]. No previous laboratory study has investigated the involvement of eotaxin in paraquat poisoning.

Circulating levels of MCP-1 were significantly higher in nonsurvivors of paraquat poisoning than in healthy controls. Moreover, circulating levels of MCP-1 were significantly higher in nonsurvivors of paraquat poisoning than in survivors. MCP-1 is a potent chemotactic factor for monocytes that are involved in a variety of diseases, ranging from allergic asthma to multiple sclerosis, HIV neurological complications and tumor neovascularity [57]. In one study, the messenger RNA expression of MCP-1 was upregulated in the lung tissue of paraquat-treated rats [58]. Similarly, Ishida et al. [59] showed that the expression of MCP-1 was significantly intensified in the lungs of paraquat-treated mice. In another study, the levels of various inflammatory cytokines, including MCP-1, were elevated in the lung tissue of mice following paraquat treatment [60]. In another *in vitro* study, p38 MAPK-dependent proinflammatory cytokine secretion, including MCP-1 secretion, was detected in mature retinal astrocytes exposed to paraquat [61].

Circulating levels of MIP-1 β were significantly higher in both survivors and nonsurvivors of paraquat poisoning than in healthy controls. MIP-1 [62] plays a valuable role in the induction and modulation of inflammatory reactions. Additionally, MIP-1 proteins mediate several aspects of tissue homeostasis. In the aforementioned *in vitro* study, p38 MAPK-dependent MIP-2 α secretion was also detected in mature retinal astrocytes exposed to paraquat [61]. In another aforementioned study, the intrapulmonary expression of MIP-1 α and MIP-2 was significantly enhanced in paraquat-treated mice [59].

This study is limited by the small sample size and short follow-up duration. More detailed research is needed.

Conclusions

The findings of this translational study provide clinical significance. Our analysis found that paraquat poisoning is characterized by a systemic inflammatory response that involves ele-

vated levels of circulating inflammatory cytokines and hyperactivation of immune cells. There were higher circulating levels of IL-2, IL-9, IL-10 and MIP-1 β in survivors than in healthy controls. Furthermore, circulating levels of IL-1 β , IL-2, IL-5, IL-8, IL-9, IL-10, IL-12 p70, IL-17A, eotaxin, G-CSF, MCP-1, IP-10 and MIP-1 β were higher in nonsurvivors than in healthy controls. Finally, circulating levels of IL-1 β and MCP-1 were higher in nonsurvivors than in survivors. Cytokines that have not been previously reported in human cases of paraquat poisoning included IL-2, IL-5, IL-8, IL-9, IL-12 p70, IL-17A, eotaxin, G-CSF, MCP-1, IP-10, and MIP-1 β . The observation of cytokine-mediated inflammation is in line with the detoxification protocol because glucocorticoids and cyclophosphamide are potent anti-inflammatory agents. Additionally, since circulating levels of IL-1 β and MCP-1 were higher in nonsurvivors than in survivors, these two cytokines might have clinical potential as promising prognostic markers for paraquat poisoning. In this context, clinical determination of the circulating inflammatory response, particularly IL-1 β and MCP-1 levels, could serve as a valuable adjunct to physiological predictors and the severity index of paraquat poisoning scores for the prediction of mortality.

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Disclosure of conflict of interest

None.

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