Elevated levels of IL-8 in fatal leptospirosis

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ABSTRACT

Leptospirosis causes a wide range of clinical outcomes, including organ failure and death. Early treatment significantly increases the chances of cure. Interleukin-8 (IL-8) is a chemoattractant cytokine for neutrophil and is associated with multiple organ failure. Research has indicated IL-8 to be raised in severe and fatal cases of leptospirosis, but its suitability as a prognostic biomarker has yet to be confirmed. This study aimed to evaluate the significance of IL-8 with the clinical outcomes of leptospirosis patients. Plasma IL-8 was measured in fifty-two samples from hospitalized patients and nineteen healthy controls. The comparisons were made between mild, severe-survived and fatal groups identified by clinical or laboratory findings. IL-8 was significantly higher in fatal ($p = 0.01$) compared to mild cases. IL-8 was also significantly higher in fatal ($p = 0.02$) when compared to survived cases of leptospirosis. IL-8 levels in the plasma of fatal leptospirosis cases were significantly elevated compared to survived cases and may serve as a potential prognostic biomarker in determining the possible outcome of leptospirosis patients.

KEYWORDS

IL-8; cytokines; leptospirosis; clinical Outcomes; malaysia

Introduction

Leptospirosis is a significant zoonotic disease with over one million incidences and 60,000 estimated deaths reported annually, specifically in tropical and subtropical countries such as Malaysia [1,2]. The actual prevalence is expected to be higher as leptospirosis is often under-reported and misdiagnosed due to its similarities with other infectious diseases such as dengue fever, influenza, hemorrhagic fever, and malaria [3,4].

The clinical outcome of leptospirosis is highly variable. Most cases are mild, however, there are a significant number of patients with severe medical complications and even death. Mild leptospirosis symptoms include fever, headaches, and myalgia but a small percentage can manifest as conjunctival suffusion, meningitis, rash, jaundice, and renal insufficiency [5,6]. Leptospirosis may progress to Weil's disease (severe form) exhibiting jaundice, acute renal failure and bleeding with a mortality rate exceeding 10%, or severe pulmonary hemorrhage syndrome (SPHS) with a mortality rate of more than 50% [7–9]. Variation in clinical outcomes has the potential to impede the diagnosis and management of leptospirosis cases. Leptospirosis disease screening uses rapid immunological tests and is confirmed either through a microscopic agglutination

test (MAT) or *Leptospira* antigen detection by polymerase chain reaction (PCR) [10].

However, none of these tests can determine disease severity. It is hypothesized that variation in patients' outcome may be immune-related and is elicited via cytokine storms, leading to tissue injury and organ damage during a *Leptospira* infection [11]. Comparative models of infection between resistant and susceptible animal hosts also indicated the involvement of inflammatory response in predicting disease outcome for leptospirosis [12,13].

Clinical studies have proposed using leptospirosis immune response evaluations through cytokine production as a means to discriminate mild, severe, and possibly fatal cases [7,14–19]. Significantly-elevated levels of pro-inflammatory cytokines are associated with tissue injury preceding organ failure [20]. In a previous study, we found that IL-6, IL-17A, and IL-22 were significantly higher in fatal leptospirosis [21]. In addition to these cytokines, significantly higher expressions of IL-8 were also reported in severe cases of leptospirosis [14,15].

IL-8, also known as CXCL-8, is one of the significant chemokines for leukocyte recruitment expressed by various cells such as macrophages/monocytes, endothelial cells, fibroblasts, epithelial cells, and neutrophils. Several

studies found increased IL-8 levels in fatal compared to survived leptospirosis cases [19,22,23]. Information on IL-8 in fatal leptospirosis patients in the Asian region is still limited. Identification of biomarkers may be useful to assist clinicians in early detection of severe leptospirosis cases. Therefore, in this present study, we examined the association of IL-8 cytokine with different leptospirosis clinical outcomes.

Materials and methods

Study participants

This study was conducted from January 2016 to December 2017. Samples were collected from hospitalized patients suspected of leptospirosis in the states of Selangor and Perak, Malaysia. Ethical approvals were obtained from the Institutional Review Board of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-15-2148-27536 and NMRR-15-756-25320). Written informed consent was obtained from participants for the study. Leptospirosis cases were confirmed by PCR and MAT according to the World Health Organization (WHO) definition of laboratory-confirmed cases of leptospirosis [10]. Patients under the age of eighteen years, and those diagnosed with infections other than leptospirosis were excluded. Healthy controls were recruited from nineteen healthy volunteers. The exclusion criteria for healthy controls included comorbid/underlying disease, genetic/auto-immune disease, fever/flu within two weeks prior to sample collection, smoking, and pregnancy.

Classification of leptospirosis

Patients were divided into three groups; (a) patients with mild infection (leptospirosis patients without complications or organ involvement) [$n = 12$]; (b) severe infection but survived (leptospirosis patients with renal, liver, and/or lung involvement based on clinical or laboratory findings) [$n = 28$]; (c) and fatal outcome (deceased patients) [$n = 12$]. Severe organ involvement was defined as liver dysfunction with jaundice or total bilirubin of $>20.5 \mu\text{mol/L}$, blood alanine aminotransferase (ALT) of $>55 \text{ U/L}$, blood aspartate aminotransferase (AST) of $>34 \text{ U/L}$; renal dysfunction with oliguria (passing urine less than 0.3 ml/kg/day) and/or abnormal creatinine level of $>115 \mu\text{mol/L}$, blood urea nitrogen (BUN) of $>9.2 \text{ mmol/L}$; pulmonary dysfunction includes pneumonia, hemoptysis and/or abnormal chest radiography or requiring mechanical ventilation support; and hemorrhagic manifestations including bleeding or abnormal coagulation studies.

Plasma sample collection

Ethylenediaminetetraacetic acid (EDTA)-preserved blood was centrifuged at $200 g$ for 10 minutes. The separated plasma was aliquoted and stored at 70°C on the same day of collection. All procedures were performed according to standardized protocols of samples collection, processing, and storage conditions.

Simple plex™ for cytokines

Concentrations of plasma IL-8 cytokines were determined in triplicates using Simple Plex™ assays from Protein Simple (San Jose, CA, USA) and expressed in 'pg/mL' concentration. Samples were loaded into corresponding inlets on the test cartridge following the manufacturer's instructions. The loaded test cartridges were analyzed using Ella analyzer (San Jose, CA, USA).

Statistical analysis

Graphpad® Prism v6 software (San Diego, CA, USA) was employed for statistical analyses. The Mann-Whitney U test was used to evaluate the significant difference in cytokine levels between leptospirosis-positive and healthy control groups. Kruskal-Wallis with Dunn's multiple comparison tests for post-hoc analysis was performed to determine the statistical significance between cytokine expressions in more than two groups: healthy control, mild, severe and survived, and fatal outcome in leptospirosis patients.

Results

Fifty-two hospitalized leptospirosis patients and nineteen healthy individuals were selected for plasma IL-8 measurement. The patient group included thirty-five males and seventeen females, with a median age of thirty-seven years, while the healthy control group comprised of eight males and eleven females, with a median age of thirty years.

Amongst these fifty-two patients, twelve (23%) were associated with fatal outcomes, twenty-eight (54%) suffered severe disease but survived, and twelve (23%) presented with mild symptoms without organ involvement (Table 1). All the leptospirosis patients presented with fever ($\geq 38^\circ\text{C}$).

IL-8 levels were significantly higher in leptospirosis patients compared to healthy controls ($p = 0.001$) (Table 2) with mild, severe and fatal cases at $p = 0.04$, $p < 0.0001$ and $p < 0.0001$, respectively (Figure 1). IL-8 level was also significantly elevated in fatal compared to survived cases (mild and severe-survived combined) ($p < 0.02$).

Table 1. Characteristics of demographic, clinical and laboratory presentation of healthy controls and leptospirosis patients.

Characteristics	Healthy Control (n = 19)	Mild (n = 12)	Survived: Severe (n = 28)	Fatal outcome (n = 12)
Demographic				
Age year, median (range)	30 (20–38)	30 (18–62)	35 (18–77)	63.5 (33–83)
Male sex, n (%)	8 (42)	9 (75)	20 (71)	6 (50)
Clinical and laboratory diagnostic findings				
Lung involvement (%)	NA	NA	4 (14)	1 (8)
Renal involvement (%)	NA	NA	11 (39)	2 (17)
Liver involvement (%)	NA	NA	12 (43)	3 (25)
Renal and liver involvement (%)	NA	NA	6 (5)	5 (42)
Bleeding (%)	NA	NA	NA	1 (8)
BUN (RR 3.2–9.2) mmol/L, median (range)	ND	3.4 (3.2–5.5)	7.8 (4.4–13.4)	15.8 (8.0–22.2)
Serum creatinine (RR 62–115) umol/L	ND	70 (64–81)	90 (68–190)	229 (118–396)
AST (RR 5–34) U/L	ND	31.5 (24.8–35.8)	61.0 (35.0–118.0)	61.0 (50.3–124.0)
ALT (RR 0–55) U/L	ND	24.5 (14.0–30.1)	51.0 (28.1–88.1)	38.0 (30.5–70.5)
Bilirubin (RR 3.4–20.5) umol/L	ND	8.9 (6.6–12.9)	13.65 (9.8–37.8)	16.3 (9.5–127.5)

BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NA, Not available; ND, not determined

Table 2. Plasma cytokine levels, in healthy controls and leptospirosis patients (Mann Whitney U test).

	Median (range)		p value
	Healthy (n = 19)	Leptospirosis (n = 52)	
IL-8 (pg/mL)	5.8 (3.92–12.13)	12.81 (2.58–111.5)	0.001**

Figure 1. Comparison of plasma cytokines concentrations in healthy, mild, severe but survived, and fatal leptospirosis.

Scatter plot of plasma cytokine concentrations among samples of patients with mild and survived, severe but survived, and fatal outcome of leptospirosis. Statistical analysis among groups was performed using Kruskal-Wallis test. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Discussion

Malaysia is one of the endemic countries for leptospirosis, with an incidence rate of 7.8–12.5 cases per 100,000 populations [3,24]. A recent study by Jumat et al. on the cytokine profile of leptospirosis patients in Sabah, Malaysia did not include comparison between the different severities of leptospirosis [25]. Our study demonstrated the association of elevated plasma IL-8 levels with fatal outcomes of leptospirosis. To the best of our knowledge, this is the first study to evaluate IL-8 levels in the different clinical outcomes of hospitalized leptospirosis patients in Malaysia.

In agreement with other studies, all clinical stages of leptospirosis have significantly increased IL-8 levels compared to those who are healthy [14,22,26]. The significantly higher concentration of IL-8 in fatal cases is consistent with Reis et al. [19] and Wageneer et al. [22] on leptospirosis-positive patients with fatal outcomes. Reis et al. also demonstrated elevated IL-8 expression in the majority of patients with SPHS compared to those without [19]. Contradictory findings were observed in other reports on fatal leptospirosis cases, however, these studies had a much lower number of fatal cases [15,26,27].

Papa et al. reported that IL-8 levels for severe leptospirosis cases peak on days 6–10 of disease onset [26] which may not correspond with the patients' initial presentation. This could explain the inconstant finding by Raffray et al., which showed no significant differences between mild and severe leptospirosis IL-8 levels as their samples were collected within five days of post-leptospirosis infection [28].

Our study found no significant difference between IL-8 levels in mild and severe-survived cases. However, a study by Chirathaworn et al. [14] with similar patient classifications reported a significant IL-8 increase in cases with organ dysfunction compared to mild cases with no organ involvement. It was not mentioned in their study whether these cases with organ dysfunction included fatal cases which may be a factor of the differences in results.

Our study suggests that IL-8, a primary pro-inflammatory mediator might contribute to patient severity. This is similar to other studies which indicated that IL-8 in sepsis promotes tissue injury [29] and sepsis, severity, and mortality in burn patients were associated with high levels of IL-8 [30].

The main limitation of this study is the lack of power due to small sample size. Further studies involving a more significant number of patients and a prospective study on the difference in days between disease onset and sample collection may prove vital toward understanding the phases of cytokine expression in response to leptospirosis infections against

time and in determining the potential clinical value of this study. Elucidating the mechanism of IL-8 involvement in disease severity should also be conducted.

Conclusions

The findings of this study suggested that IL-8 may serve as a potential prognostic biomarker in the management of leptospirosis patients. Collectively, these findings could lead toward a better understanding of the significant roles played by cytokines through immune reactions in the clinical outcome of leptospirosis infection. These are potential data in developing a prognostic screening method for patients suffering from leptospirosis.

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Availability of data and materials

All the data supporting the conclusions of this article are included in the report.

Ethical approval and consent to participate

Samples were collected from study participants after they have given their written informed consent. Ethical approval for the study was obtained from the Medical Research and Ethics Committee, Ministry of Health Malaysia. (NMRR (National Medical Research Register)-15-2148-27536 and NMRR-15-756-25320).

Informed consent

Written informed consent was obtained from the study participants for publication of their details in this manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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