Evaluation of the efficiency of ciprofloxacin against *S.* Typhi by altering the production of cytokines in acute typhoid fever in patients at Al-Diwaniyah Hospitals, Iraq

Baleegh A Kadhim[1]*, Zeayd Fadhil Saeed[2] and Sura A Al-Ganahi[1]

[1]Department of Biology, College of Science, University of Al-Qadisiyah, Iraq.
[2]Department of Nursing, Technical Institute of Samawa, Al-Furat Al-Awsat Technical University, Iraq.
Email: baleegh.kadhim@qu.edu.iq

ABSTRACT

Aims: Typhoid fever is a life-threatening disease in the developing world that claims >600,000 deaths per year. Its causative agent *Salmonella* Typhimurium (*S.* Typhi) can be treated with ciprofloxacin, an effective broad-spectrum antibiotic that enhances the natural host defenses. However, the emergence of resistant bacterial strains may be a warning alarm against the clinical use of this antibiotic. This study was aimed to investigate the efficiency of ciprofloxacin treatment (250 mg/mL) against *S.* Typhi by altering the production of serum cytokines IL-10, 1L-6 and TNF-α in acute typhoid fever patients in Diwanyah Hospitals.
Methodology and results: ELISA and Western Blot methods were used to investigate cytokine levels in patients and healthy controls sera. Our results showed that all cytokines' levels before treatment with ciprofloxacin were significantly higher than the control (healthy group). However, treated patients with ciprofloxacin revealed a significantly reduced concentration of IL-10 and TNF-α compared to untreated control samples. However, the level of IL-6 was higher even with ciprofloxacin treatment.
Conclusion, significance and impact of study: The study concluded that ciprofloxacin (250 mg/mL) might significantly alter serum cytokines IL-6, IL-10 and TNF-α levels in acute typhoid fever patients. Therefore, further molecular studies are essential to understand the effect of ciprofloxacin on the production of cytokines.

Keywords: Ciprofloxacin, IL-10, 1L-6, TNF-α, *Salmonella* Typhi

INTRODUCTION

Typhoid fever remains a significant public health threat, particularly in developing countries causing high morbidity and mortality, resulting in 20.6 million cases and 223000 deaths per year (Sur *et al.*, 2009; Amicizia *et al.*, 2017). It is the most commonly diagnosed fever among patients in most hospitals in Iraq (Allu *et al.*, 2019; Zubair and Mohammad, 2020). Risk factors are mainly due to poor hygiene, adequate sewage disposal and devoid clean water supply (Mogasale *et al.*, 2018). Moreover, the emergence of antibiotic-resistant bacterial strains that have shown resistance to one or more antibiotics used in treatment (Nair *et al.*, 2018; Shahid *et al.*, 2021). The host immune system recognises the causative agent *Salmonella* via recognition receptors, importantly Toll-like receptors (TLR) (Kurtz *et al.*, 2017). This could promote downstream signalling pathways and activate an inflammatory response by releasing cytokines such as TNF-a, IL-1, IL-6 and IL-8 (Andrade and Andrade Júnior, 2003; Febriza *et al.*, 2020). These crucial mediators including cytokines play a key role in attracting phagocytosis cells like macrophages, polymorphonuclear neutrophils (PMNs) and monocytes, which are involved in bacterial inhibition (Febriza *et al.*, 2020).

Early diagnosis with effective treatment is essential in eliminating *S.* Typhi and promoting a quick recovery. Ciprofloxacin belongs to the fluoroquinolone class of antibiotics that are commonly used for the treatment of salmonellosis (Sharma *et al.*, 2010; Thai *et al.*, 2021). However, the potential effect of antimicrobial agents on inflammatory cytokine responses in acute typhoid fever is still unclear. Therefore, we sought to evaluate the effectiveness of ciprofloxacin in eliminating *S.* Typhi bacterium by altering cytokine expression levels in response to antibiotic therapy during acute typhoid fever.

MATERIALS AND METHODS

Regents

All reagents used in this study are listed in Table 1.

---

*Corresponding author

Table 1: Reagents used in this study.

Reagent	Source	Application	Dilution
Antibodies			
Polyclonal anti-TNF alpha antibody	Antibodies.com	Detection of TNF-α levels	1:5000
Polyclonal anti-IL-6 antibody	Abcam	Detection of IL-6 levels	1:5000
Polyclonal anti-IL-10 antibody	Abcam	Detection of IL-10 levels	1:5000
Polyclonal anti-Transferrin antibody	Abcam	Load control	1:2000
Alkaline-phosphatase conjugate anti-mouse IgG	Aviva Systems Biology	Secondary antibodies	1:2000
Alkaline-phosphatase conjugated anti-rabbit IgG	Sigma-Aldrich	Secondary antibodies	1:5000
Chemicals			
BCIP®/NBT Liquid Substrate System	Sigma-Aldrich	Visualise protein bands on a nitrocellulose membrane	
SERVA Gel™TG Prime™ 4-20% 10 samples wells	SERVA	SDS-PAGE electrophoresis	
Widal agglutination kit	TYDAL® kit	Laboratory diagnosis	
ELISA-IgG kit	SIGMA-ALDRICH® kit	Laboratory diagnosis (chronic infection)	
ELISA-IgM kit	SIGMA-ALDRICH® kit	Laboratory diagnosis (acute infection)	
ELISA-IL-6 kit	abcam, ab46027	Measurement IL-6 level	
ELISA-IL-10 kit	RayBio®	Measurement IL-10 level	
ELISA-TNF-alpha kit	DRG International, Inc.	Measurement TNF-α level	

Serum samples

Serum samples were collected during April 2020-July 2021 from patients who attended Al-Diwaniyah Teaching Hospital, Iraq. In total, 76 patient serum samples were collected, including 63 from males and 13 from females aged 16-50 years. A laboratory diagnosis confirmed positive cases of salmonellosis using serological tests (Widal and ELISA-IgG-IgM). Other serum samples were obtained from patients diagnosed with acute typhoid fever (IgM) to investigate the effect of ciprofloxacin on cytokines levels before and during treatment. Control sera were from 10 healthy individuals aged 18-45 years. The serum of patients and the healthy control group was prepared by drawing 5 mL of blood from the vein and collected into a redtop tube without an anticoagulant reagent (BD Vacutainer® Plus, 6 mL). To separate sera, tubes were centrifuged at 5000× g for 15 min. All samples were stored at -20 °C or immediately used in the experiments.

Laboratory confirmation and cytokine measurement

Salmonellosis diagnosis was carried out using serological tests. Widal test (TYDAL® kit), an agglutination test was preliminary used to confirm a typhoid fever infection. ELISA IgG-IgM assays (SIGMA-ALDRICH® kit) were performed to diagnose acute and chronic infections by detecting IgM and IgG antibodies against *S.* Typhi.

The serum levels of cytokines were investigated by using an ELISA assay, TNF-alpha (DRG International, Inc. kit), IL-6 (abcam, ab46027) and IL-10 (RayBio® Human IL-10 ELISA Kit). All ELISA methods were performed according to the manufacturer's instructions.

SDS-PAGE and Western Blot

Proteins were investigated and separated on gradient polyacrylamide gels (4-20% SERVA) according to their molecular weight. A desirable amount of cytokine protein 5 µg/mL was mixed with sample buffer and heated at 95 °C for 10 min. All protein samples and protein ladder (Geneflow) were loaded onto a gel and then run at 200 V for 55 min using SDS-PAGE running buffer. Gels were either used directly for Western Blot assay or stained overnight with Colloidal Coomassie blue stain (BIO-RAD) to determine the protein bands on GS 710 BIO-RAD scanner. Western Blot was performed to detect separated proteins using specific antibodies (Table 1). A protein-membrane transferring was performed using the Western Blot Transfer System (BIO-RAD). First antibodies (abcam) were added to the membrane and incubated 2 h with shaking at room temperature. Second antibodies (Sigma-Aldrich) were added after washing three times with TBST buffer and incubated with shaking at 4 °C. BCIP®/NBT Liquid Substrate was added onto membranes and left in the dark until the appearance of protein bands. Membranes were then washed in water to end the reaction and scanned on GS 710 BIO-RAD scanner system for further analysis.

Statistics analysis

Data analysis of at least three independent experiments was performed using Prism 8 software. The comparison among groups of the current study was statistically analysed using Ordinary one-way ANOVA and an unpaired t-test with a probability value of $P<0.05$. All
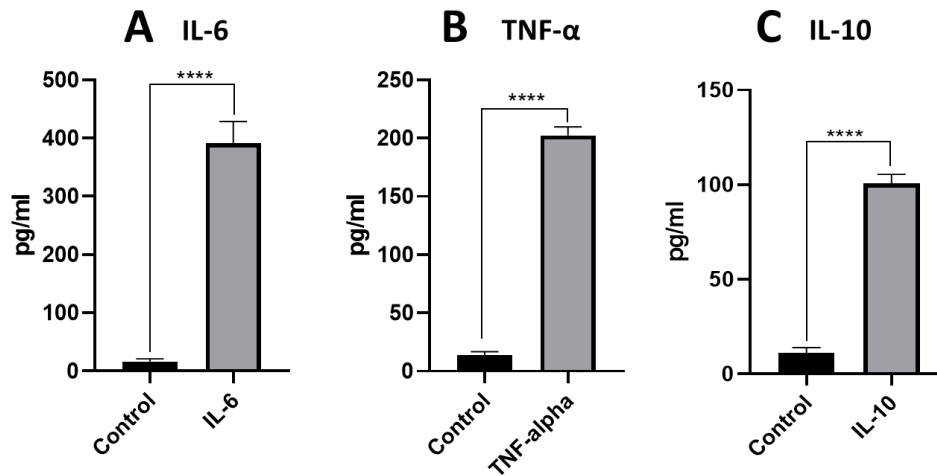
Figure 1: Cytokines levels in patients' sera with untreated ciprofloxacin. All cytokines' levels were measured using ELISA assays with significantly different (P<0.05; means ± SEM). A: IL-6 (375.0 ± 21.45 pg/mL), B: TNF-α (188.6 ± 4.635 pg/mL), C: IL-10 (89.76 ± 3.258 pg/mL).

Table 2: Number of positive tested samples and percentages using Widal test and ELISA IgM and IgG antibodies in patient sera with typhoid fever.

Serological tests	Total no. samples 76		Total positive cases	Percentage %
	Male	Female		
Widal	63	13	31	40.78
ELISA-IgG	63	13	44	57.89
ELISA-IgM	63	13	38	50

cytokine levels (pg/mL) used in this study were expressed as means ± SEM.

RESULTS AND DISCUSSION

Patient characteristics and epidemiological study

The serological diagnosis results from 76 serum samples are described in Table 2. This study excluded children under 15 and pregnant women. Moreover, it selected only patients who did not take any treatment before hospital admission. The preliminary diagnosis included clinical presentations such as fever, diarrhea, vomiting, nausea and abdominal pains. Additionally, laboratory analyses based on a Widal test were also performed to confirm the infection with a *S.* Typhi bacterium. ELISA IgG and IgM assays were used for reliable laboratory confirmation and for determining acute and chronic infections.

The number of patients hospitalized with typhoid fever was higher in males (63) than in females (13). The total positive cases by using a Widal test was 31 with a percentage (40.78%), while by using ELISA-IgM and IgG were 38, 44 with percentages of 50% and 57.89%, respectively (Table 2).

The epidemiological study included the prevalence and incidence of infection with *S.* Typhi in Diwaniya city, Iraq. The two criteria were calculated depending on the formulas: Prevalence of infection = total number (old and new) of recorded cases/number of populations at risk of infection. Incidence rate = a number of newly registered cases/number of people at risk. The total incidence of *S.* Typhi infection by using the Widal test was I = (31/1158000)*100 = 0.0026770293609. With the use of ELISA-IgG and IgM, the total prevalence and incidence of *S.* Typhi infection were also calculated as follows: I = (38/1158000)*100 = 0.0328151986183074; P = (44/1158000)*100 = 0.0037996545768566. The population of Al-Diwaniyah Governorate is 1158000 people. ELISA-IgM assay indicates acute (new) infections and represents the incidence rate, whereas ELISA-IgG assay suggests chronic (old) infections and refers to the prevalence rate.

Cytokines levels in acute typhoid fever sera before treatment with ciprofloxacin

Cytokine levels of IL-6, TNF-alpha and IL-10 in untreated patients with ciprofloxacin who were infected with *S.* Typhi were investigated using ELISA assays. The results were compared with the control group of healthy individuals at the probability level (P<0.05). Patients (n =38) had significantly increased cytokines levels of IL-6 (375.0 ± 21.45 pg/mL), TNF-α (202.3 ± 4.635 pg/mL) and IL-10 (89.76 ± 3.258 pg/mL) comparing to healthy controls (n=15) (16.429 ± 1.481, 13.735 ± 5.987 and 10.500 ± 2.912 pg/mL, respectively; means ± SEM) (Figure 1).

Ciprofloxacin could alter cytokines levels in patients with acute typhoid fever

The cytokine levels of IL-6, TNF-α and IL-10 post-treatments with 250 mg/mL ciprofloxacin were investigated using ELISA and Western Blot assays (Figure 2). Interestingly, the results show a significant increase of IL-6 in patients' sera after 3 and 6 doses (575.0 ± 21.45 pg/mL; 675.0 ± 21.45 pg/mL) respectively compared with control samples before treatment (375.0 ± 21.45 pg/mL) and healthy people (16.429 ± 1.481 pg/mL) (Figure 2A). In Western blot assays, the expression of IL-6 after treatment was also examined using anti-IL-6 antibodies. A significant increase in IL-6 levels after treatment was detected by observing protein bands on the predicted size (~23 kDa) (Figure 2B).

On the contrary, we importantly noticed that TNF-α and IL-10 levels were decreased post-treatments with 250 mg/mL ciprofloxacin (Figure 2). After three and six doses, the ELISA results showed a decrease in the levels of TNF-α (185.303 ± 4.635 pg/mL; 136.9703333 ± 4.635 pg/mL), respectively. In a similar way, the concentrations of IL-10 also decreased significantly to (64.663 ± 3.258 pg/mL; 52.996 ± 3.258 pg/mL) compared with control samples (Figure 2A). In Western Blot assays, the concentrations of TNF-α and IL-10 were detected using anti-TNF-α and IL-10 antibodies. The results showed a significant decrease in the expression levels of TNF-α and IL-10 post-treatments with 250 mg/mL ciprofloxacin. The predicted band size of TNF-α is 25.6 kDa; however, we detected it at 17 kDa as a possible cleavage fragment.

On the other hand, the expression of IL-10 was identified at 21 kDa (Figure 2B). Approximately 3 μg of Transferrin-Serum protein was used as a loading control in all experiments.

Typhoid fever is a crucial health problem worldwide, particularly in developing countries (Crump et al., 2004). This study was carried out in Diwaniya city, Iraq, which suffers mainly from a lack of clean drinking water and wastewater disposal (Tisti and Ghawi, 2020). The epidemiological study results showed that the number of people infected with typhoid fever is higher in males than in females. The reason for that is due to the behaviours and habits of males in eating fast food in unclean restaurants, unlike females who prefer to eat healthy food at home (Driskell et al., 2006; Herman and Polivy, 2010; Majabadi et al., 2016). Serological diagnosis results indicated high levels of IgG than IgM, which refers to the presence of a long-term (chronic) infection, whereas IgM levels represent a recent (acute) infection. Patients with acute typhoid fever (positive IgM) were selected to investigate the potential effect of ciprofloxacin on cytokine levels.

In this study, cytokines levels of IL-6, TNF-α and IL-10 were investigated in acute typhoid fever patients prior to treatment with 250 mg/mL ciprofloxacin. The current results showed that there were significant differences between the concentrations of IL-6, TNF-α and IL-10 in the sera of patients and the sera of the healthy control group at the probability level (P<0.05) (Figure 1). The

increased level of these cytokines is due to their main role in controlling the inflammatory response (Mourtzikou et al., 2014). Following S. Typhi infection, signalling pathways within cells trigger the release of cytokines (Keuter et al., 1994). Importantly, IL-6 is an essential multifunctional cytokine that exerts its proinflammatory and anti-inflammatory properties (Febriza et al., 2020). This cytokine derives from different cells of the human body, such as endothelial cells, lymphocytes, macrophages, astrocytes and ischemic myocytes; thus, it can regulate many pathological and physiological activities (Niculet et al., 2021). Furthermore, IL-6 can promote differentiation of T and B lymphocytes and induce the acute phase response (Tanaka et al., 2014). As shown in (Figure 1A), the elevated level of IL-6 in the current study is in agreement with previous studies that suggested a noticeable increase in this cytokine in the early stage of inflammation by S. Typhi infection (Butler et al., 1993; Febriza et al., 2020). The present finding is also supported by Eman et al., (2021), who indicated that the levels of IL-6 in the serum of patients are significantly higher than in the comparison group (Eissa et al., 2018; Eman et al., 2021). Moreover, this result is similar to a recent study that showed a high concentration of IL-6 in patients with acute typhoid fever (Al-Dahhan et al., 2020). Similarly, the results of this study are consistent with recently published studies that suggested elevated levels of IL-6 in the early infection of S. Typhi could play essential roles in the inflammatory process and control the development of the disease (Abdul-Hassan et al., 2020; Tang et al., 2020; Mohammed et al., 2021; waad Hadi Tokan et al., 2021).

Another important result in the present study is significantly high levels of TNF-α in patients with acute typhoid fever than in healthy controls (Figure 1B). TNF-α is a proinflammatory cytokine that is mainly produced upon activation of macrophages, monocytes and T lymphocytes (Parameswaran and Patial, 2010). The elevated concentration of TNF-α in sera of patients is due to its essential role in inflammatory responses against enteric bacterial infection (Ma et al., 2010). This cytokine, along with other interleukins such as IL-6, stimulates the proliferation and differentiation of B-cell lymphocytes (Ali and Abdulaziz, 2010). It can also participate in recruiting phagocytosis cells to sites of infection to inhibit bacterial division in the acute infection (Tanaka et al., 2014). This finding supports other recent research which found that TNF-α levels increased in the acute inflammatory response to Gram-negative bacteria during the acute phase of infection (Eissa et al., 2018; Abdul-Hassan et al., 2020; Masriadi et al., 2020; Syarif et al., 2020).

In this study, we also describe the importance of the anti-inflammatory cytokine IL-10 to the acute infection caused by S. Typhi (Figure 1C). The current data confirm that the elevated level of IL-10 in patients' sera is because of its essential role in hindering inflammation and preventing tissue damage (Iyer and Cheng, 2012). Upon salmonellosis infection, other proinflammatory cytokines are also produced by immune cells to promote an acute inflammatory response and control pathogen infection.
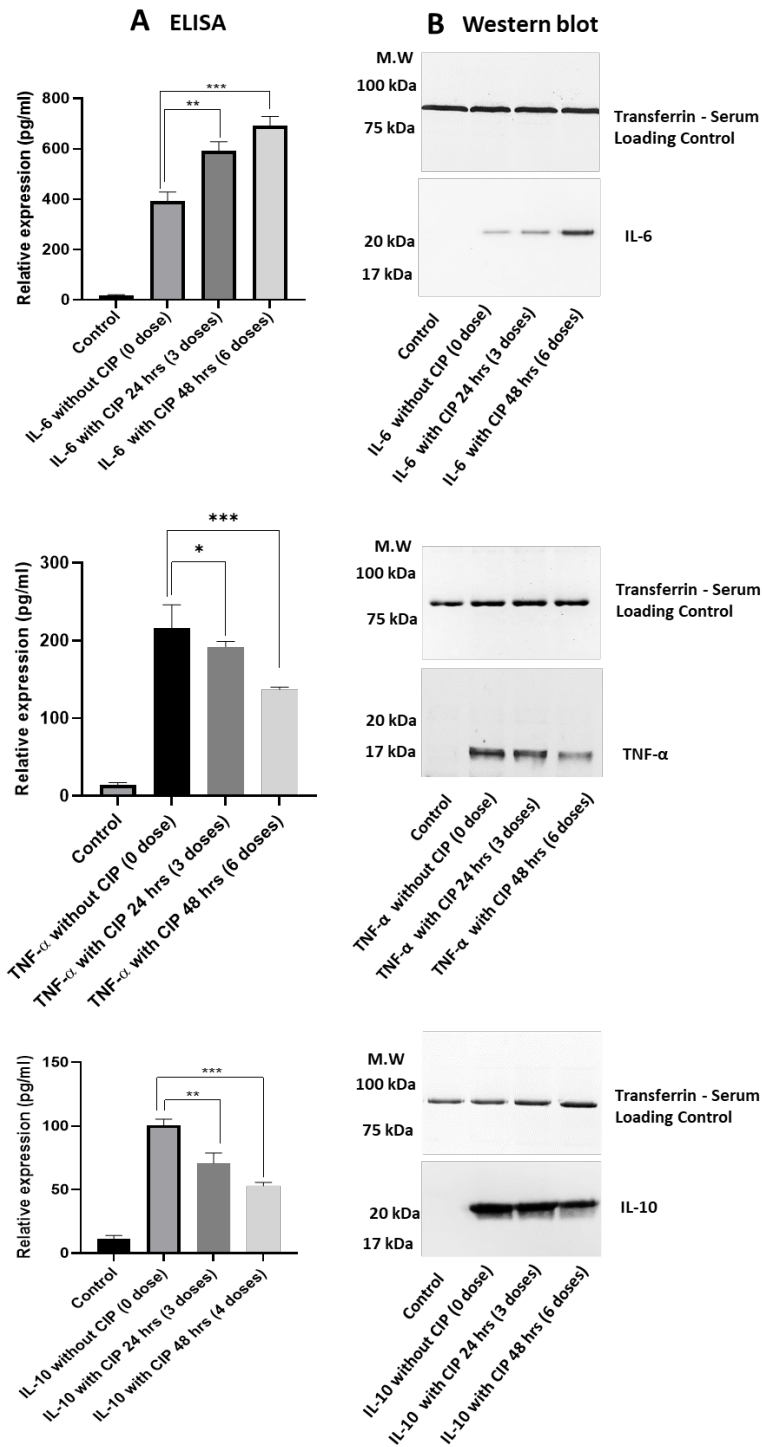
Figure 2: IL-6, TNF-α, IL-10 expression levels in acute typhoid sera post-treatments with CIP. A: ELISA assays show a high IL-6 concentration after 3 (575.0 ± 21.45 pg/mL) and 6 (675.0 ± 21.45 pg/mL) doses of 250 mg/mL CIP comparing with control samples. However, TNF-α (185.303 ± 4.635 pg/mL; 136.9703333 ± 4.635 pg/ml) and IL-10 (64.663 ± 3.258 pg/ml; 52.996 ± 3.258 pg/mL) were decreased after treatments with CIP. B: Western Blot using anti-IL-6 polyclonal antibodies shows that IL-6 was expressed at the predicted size (~23 kDa). TNF-α was detected at 17 kDa while IL-10 bands were identified at 21 kDa using anti-TNF-α and IL-10 antibodies, respectively. Transferrin-Serum protein was used as a loading control in all experiments.

However, this interleukin is mainly produced from macrophages that can inhibit the production of proinflammatory cytokines such as IL-12, TNF-α, INF-γ and IL-2 from Th1 lymphocytes and reduce extreme inflammation of the affected tissues (Couper *et al.*, 2008; Gabryšová *et al.*, 2014). In accordance with the current results, recent previous studies have also claimed that the high concentration of IL-10 in patients with acute typhoid fever is significant in promoting systemic *S.* Typhi infection and regulating inflammatory response (Rutz and Ouyang, 2016; Salazar *et al.*, 2017; Bakiri and Mingomataj, 2019; Peñaloza *et al.*, 2019). The well-balanced functions of proinflammatory and anti-inflammatory cytokines produced by inducing Th1 and Th2 cells could regulate excessive inflammation and prevent host damage (Zhang and An, 2007).

It is well known that antibiotics play vital roles in bacteriostatic and bactericidal activities. However, the emergence of antibiotic-resistant strains of *S.* Typhi has become the main factor in determining the efficacy of antibiotics used in the treatment of typhoid and other infectious diseases (Azhar *et al.*, 2019; Dyson *et al.*, 2019). Furthermore, recent studies claim that some antibiotics could interact with pro or anti-inflammatory cytokines and modulate immune response functions, which can be necessary for eliminating bacterial infections (Anuforom *et al.*, 2016, Abdul-Hassan *et al.*, 2020). Therefore, the purpose of this study is to investigate the efficacy of ciprofloxacin (250 mg/mL) against *S.* Typhi by detecting the concentration of inflammatory or proinflammatory cytokines during acute typhoid fever infection in human sera.

Ciprofloxacin is a fluoroquinolone antibiotic that has antibacterial activity and is widely used to prevent various bacterial infections including *S.* Typhi (Jin *et al.*, 2019). The main target of this antibiotic is bacterial DNA gyrase (alpha subunits domain); therefore, it can inhibit the replication of prokaryotic DNA and kill bacteria (Dighe and Collet, 2020). It has been recently reported that ciprofloxacin can alter the production of cytokines in response to typhoid fever *in vitro*. The authors suggested that IL-6 and TNF-α levels were significantly elevated in macrophages infected with *Salmonella* Typhimurium (strain SL1344) and treated with ciprofloxacin (Anuforom *et al.*, 2016). In our study, the results further supported the idea of increasing the level of IL-6 in the serum of patients in response to treatment with ciprofloxacin (Figure 2). One possible explanation for the overexpression of IL-6 is that IL-6 is a dominant inflammatory cytokine that can be highly produced in blood circulation during acute typhoid fever (Scheller *et al.*, 2011; Febriza *et al.*, 2020). Additionally, IL-6 can be mainly elevated in any inflammatory condition, even during exercise, stress and muscle injury (Pedersen *et al.*, 2001; Gómez-Rubio and Trapero, 2019).

In contrast to previous work (Anuforom *et al.*, 2016), the current study's findings showed that TNF-α concentration was lower in the serum of patients after treatment with ciprofloxacin (Figure 2). TNF-alpha is not identified in the serum of healthy individuals but is often found in the serum of patients during infection and inflammation (Cigana *et al.*, 2007). To explain this result, giving ciprofloxacin to patients eliminated many *S.* Typhi bacterium and therefore lowered the production of proinflammatory cytokines by macrophages (Gasem *et al.*, 2003). Other studies that used different antibiotics also claimed that TNF-α levels declined after treatment. Araujo *et al.* (2002) showed that the antibiotic telithromycin could reduce TNF-α secretion by monocytes after treatment (Araujo *et al.*, 2002). Previous research by Lankelma *et al.* (2016) demonstrated that using ciprofloxacin and vancomycin metronidazole disrupted the secretion of TNF-α by mononuclear cells (Lankelma *et al.*, 2016). Another study showed that the treatment with azithromycin decreased TNF-α levels in cystic fibrosis patients (Cigana *et al.*, 2007).

This is the first study to investigate the effect of ciprofloxacin on the secretion of IL-10 in human sera. The present findings indicated that the level of IL-10 was suppressed after treatment with 250 mg/mL ciprofloxacin (Figure 2). Previous work revealed that both Fosfomycin and clarithromycin elevated the secretion of IL-10 and IL-6 by monocytes stimulated with LPS (Morikawa *et al.*, 1996). On the other hand, they also showed dexamethasone was able to reduce the concentrations of IL-10 and TNF-α, which is consistent with our current study (Morikawa *et al.*, 1996). Many mechanisms could influence the plasma levels of IL-10 in patients. *Salmonella* Typhi is among microbes that induce IL-10 production by monocyte in humans and therefore, the decrease in the concentration of IL-10 is due to the efficiency of ciprofloxacin in clearing bacteria from the host (Duell *et al.*, 2012). Additionally, the authors reported that TNF-α regulates the synthesis of IL-10 during bacterial infection (van Der Poll *et al.*, 1997). Others found that inhibition of TNF-α is also accompanied by a decrease in the concentration of IL-10 (van der Poll *et al.*, 1996a). Furthermore, in whole human blood, IL-10 production induced by endotoxin is partially TNF-dependent (Van der Poll *et al.*, 1996b).

CONCLUSION

In conclusion, the current study reveals that ciprofloxacin is a unique fluoroquinolone antibiotic that is still successfully useful in treating *S.* Typhi infection by altering the levels of cytokines. Moreover, this antibiotic might modulate the immune response during monocytes and macrophages' pathogen recognition and cytokine production. Therefore, it might have possible clinical implications. However, the exact molecular actions that regulate the secretion of inflammatory cytokines based on the effect of ciprofloxacin remain unclear. Further molecular studies are therefore needed to evaluate whether ciprofloxacin has a direct effect on bacterial growth or could boost the production of inflammatory cytokines.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

There are no conflicts of interest associated with this publication.

REFERENCES

Abdul-Hassan, L. S., Hadi, O. M. and Njum, A. A. (2020). Cytokine responses to multidrug-resistance – *Salmonella* Typhi in patients with typhoid fever in Najaf province. *Drug Invention Today* **13(2),** 269-272.

Al-Dahhan, N. A. A., Hussein, B. J. and Issa, I. H. (2020). Assessment of liver enzymes and cytokines in typhoid fever. *Systematic Reviews in Pharmacy* **11(3),** 297-300.

Ali, Y. F. and Abdulaziz, A. M. (2010). Circulating interleukin-6 and tumor necrosis factor receptor-1 predict resistance to therapy of typhoidal salmonellosis. *Egyptian Journal of Pediatric Allergy and Immunology* **8(2),** 55-59.

Allu, M. A., Assafi, M. S., Polse, R. F. and Al-Berfkani, M. I. (2019). Present status of *Salmonella* Typhi in different age groups hospitalized patients in Duhok City, Iraq. *ZANCO Journal of Pure and Applied Sciences* **31(6),** 123-129.

Amicizia, D., Arata, L., Zangrillo, F., Panatto, D. and Gasparini, R. (2017). Overview of the impact of typhoid and paratyphoid fever. Utility of Ty21a vaccine (Vivotif®). *Journal of Preventive Medicine and Hygiene* **58(1),** E1-E8.

Andrade, D. R. D. and Andrade Júnior, D. R. D. (2003). Typhoid fever as cellular microbiological model. *Revista do Instituto de Medicina Tropical de São Paulo* **45(4),** 185-191.

Anuforom, O., Wallace, G. R., Buckner, M. M. and Piddock, L. J. (2016). Ciprofloxacin and ceftriaxone alter cytokine responses, but not Toll-like receptors, to *Salmonella* infection *in vitro*. *Journal of Antimicrobial Chemotherapy* **71(7),** 1826-1833.

Araujo, F. G., Slifer, T. L. and Remington, J. S. (2002). Inhibition of secretion of interleukin-1α and tumor necrosis factor alpha by the ketolide antibiotic telithromycin. *Antimicrobial Agents and Chemotherapy* **46(10),** 3327-3330.

Azhar, A. B., Khalid, A. and Shah, S. (2019). The implications of extensive drug-resistant typhoid fever: A case report. *Cureus* **11(6),** e5032.

Bakiri, A. H. and Mingomataj, E. Ç. (2019). Novel insights on interleukin-10 functions: A manipulative tool for the deviation of immune response and disease outcome. *EMJ Allergy and Immunology* **4,** 88-94.

Butler, T., Ho, M., Acharya, G., Tiwari, M. and Gallati, H. (1993). Interleukin-6, gamma interferon, and tumor necrosis factor receptors in typhoid fever related to outcome of antimicrobial therapy. *Antimicrobial Agents and Chemotherapy* **37(11),** 2418-2421.

Cigana, C., Assael, B. M. and Melotti, P. (2007). Azithromycin selectively reduces tumor necrosis factor alpha levels in cystic fibrosis airway epithelial cells. *Antimicrobial Agents and Chemotherapy* **51,** 975-981.

Couper, K. N., Blount, D. G. and Riley, E. M. (2008). IL-10: The master regulator of immunity to infection. *Journal of Immunology* **180(9),** 5771-5777.

Crump, J. A., Luby, S. P. and Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization* **82(5),** 346-353.

Dighe, S. N. and Collet, T. A. (2020). Recent advances in DNA gyrase-targeted antimicrobial agents. *European Journal of Medicinal Chemistry* **199,** 112326.

Driskell, J. A., Meckna, B. R. and Scales, N. E. (2006). Differences exist in the eating habits of university men and women at fast-food restaurants. *Nutrition Research* **26(10),** 524-530.

Duell, B. L., Tan, C. K., Carey, A. J., Wu, F., Cripps, A. W. and Ulett, G. C. (2012). Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis. *FEMS Immunology and Medical Microbiology* **64(3),** 295-313.

Dyson, Z. A., Klemm, E. J., Palmer, S. and Dougan, G. (2019). Antibiotic resistance and typhoid. *Clinical Infectious Diseases* **68,** S165-S170.

Eissa, E. A., El-Sayed, T. I., Attia, A. A., Rashed, M. E. and Refaat, H. M. (2018). Proinflammatory cytokines in plasma of patients with typhoid fever and resistance to therapy. *Egyptian Journal of Microbiology* **53(1),** 141-149.

Eman, W. H., Sundus, J. M. and Mohanad, H. M. (2021). Study of Some Proinflammatory Cytokines and Biochemical Parameters in Patients with Typhoid Fever in Salah Al-Din Governorate. *Annals of RSCB*, **25 (6),** 15995-16003.

Febriza, A., Natzir, R., Hatta, M., As'ad, S., Budu, Kaelan, C., Kasim, V. N. and Idrus, H. H. (2020). The role of IL-6, TNF-α, and VDR in inhibiting the growth of *Salmonella* Typhi: *In vivo* study. *The Open Microbiology Journal* **14,** 65-71.

Gabryšová, L., Howes, A., Saraiva, M. and O'Garra, A. (2014). The regulation of IL-10 expression. *In*: Interleukin-10 in Health and Disease. Fillatreau, S. and O'Garra, A. (eds.). Springer Berlin, Heidelberg. **pp. 157-190.**

Gasem, M. H., Keuter, M., Dolmans, W. M., van der Ven-Jongekrijg, J., Djokomoeljanto, R. and van der Meer, J. W. (2003). Persistence of Salmonellae in blood and bone marrow: Randomized controlled trial comparing ciprofloxacin and chloramphenicol treatments against enteric fever. *Antimicrobial Agents and Chemotherapy* **47(5),** 1727-1731.

Gómez-Rubio, P. and Trapero, I. (2019). The effects of exercise on IL-6 levels and cognitive performance in patients with schizophrenia. *Diseases* **7(1),** 11.

Herman, C. P. and Polivy, J. (2010). Sex and gender differences in eating behavior. *In*: Handbook of Gender Research in Psychology. Chrisler, J. C. and McCreary, D. R. (eds.). Springer, New York. **pp. 455-469.**

Iyer, S. S. and Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critical Reviews™ in Immunology* **32(1), 23-63**

Jin, C., Gibani, M. M., Pennington, S. H., Liu, X., Ardrey, A., Aljayyoussi, G., Moore, M., Angus, B., Parry, C. M., Biagini, G. A., Feasey, N. A. and Pollard, A. J. (2019). Treatment responses to azithromycin and ciprofloxacin in uncomplicated *Salmonella* Typhi infection: A comparison of clinical and microbiological data from a controlled human infection model. *PLoS Neglected Tropical Diseases* **13(12), e0007955.**

Keuter, M., Dharmana, E., Gasem, M. H., van der Ven-Jongekrijg, J., Djokomoeljanto, R., Dolmans, W. M., Demacker, P., Sauerwein, R., Gallati, H. and van der Meer, J. W. (1994). Patterns of proinflammatory cytokines and inhibitors during typhoid fever. *Journal of Infectious Diseases* **169(6), 1306-1311.**

Kurtz, J. R., Goggins, J. A. and McLachlan, J. B. (2017). *Salmonella* infection: Interplay between the bacteria and host immune system. *Immunology Letters* **190, 42-50.**

Lankelma, J. M., Belzer, C., Hoogendijk, A. J., de Vos, A. F., de Vos, W. M., van der Poll, T. and Wiersinga, W. J. (2016). Antibiotic-induced gut microbiota disruption decreases TNF-α release by mononuclear cells in healthy adults. *Clinical and Translational Gastroenterology* **7(8), e186.**

Ma, J., Zhang, Y. G., Xia, Y. and Sun, J. (2010). The inflammatory cytokine tumor necrosis factor modulates the expression of *Salmonella* typhimurium effector proteins. *Journal of Inflammation* **7, 42.**

Majabadi, H. A., Solhi, M., Montazeri, A., Shojaeizadeh, D., Nejat, S., Farahani, F. K. and Djazayeri, A. (2016). Factors influencing fast-food consumption among adolescents in Tehran: A qualitative study. *Iranian Red Crescent Medical Journal* **18(3), e23890.**

Masriadi, Idrus, H. H. and Sukmawati. (2020). Biological effects of tumor necrosis factor alpha (TNF-α) in systemic inflammation. Running title: TNF-α for systemic inflammation. *Indian Journal of Forensic Medicine and Toxicology* **14(4), 4361-4367.**

Mogasale, V. V., Ramani, E., Mogasale, V., Park, J. Y. and Wierzba, T. F. (2018). Estimating typhoid fever risk associated with lack of access to safe water: A systematic literature review. *Journal of Environmental and Public Health* **2018, Article ID 9589208.**

Mohammed, K. I. A., Al-Wattar, W. M. A., Al-Fukhar, S. A., Ali, S. H. M. and Mousa, J. M. (2021). Assessment of IL-6, IL-23 and serum amylase levels in patients with enteric fevers. *Indian Journal of Forensic Medicine and Toxicology* **15(3), 3359-3363.**

Morikawa, K., Watabe, H., Araake, M. and Morikawa, S. (1996). Modulatory effect of antibiotics on cytokine production by human monocytes *in vitro*. *Antimicrobial Agents and Chemotherapy* **40(6), 1366-1370.**

Mourtzikou, A., Alepaki, M., Stamouli, M., Pouliakis, A., Skliris, A. and Karakitsos, P. (2014). Evaluation of serum levels of IL-6, TNF-α, IL-10, IL-2 and IL-4 in patients with chronic hepatitis. *Inmunología* **33(2), 41-50.**

Nair, D. V. T., Venkitanarayanan, K. and Johny, A. K. (2018). Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Foods* **7(10), 167.**

Niculet, E., Chioncel, V., Elisei, A. M., Miulescu, M., Buzia, O. D., Nwabudike, L. C., Craescu, M., Draganescu, M., Bujoreanu, F., Marinescu, E., Arbune, M., Radaschin, D. S., Bobeica, C., Nechita, A. and Tatu, A. L. (2021). Multifactorial expression of IL-6 with update on COVID-19 and the therapeutic strategies of its blockade. *Experimental and Therapeutic Medicine* **21, 263.**

Parameswaran, N. and Patial, S. (2010). Tumor necrosis factor-α signaling in macrophages. *Critical Reviews™ in Eukaryotic Gene Expression* **20, 87-103.**

Pedersen, B. K., Steensberg, A. and Schjerling, P. (2001). Muscle-derived interleukin-6: Possible biological effects. *Journal of Physiology* **536, 329-337.**

Peñaloza, H. F., Noguera, L. P., Ahn, D., Vallejos, O. P., Castellanos, R. M., Vazquez, Y., Salazar-Echegarai, F. J., González, L., Suazo, I., Pardo-Roa, C., Salazar, G. A., Prince, A. and Bueno, S. M. (2019). Interleukin-10 produced by myeloid-derived suppressor cells provides protection to carbapenem-resistant *Klebsiella pneumoniae* sequence type 258 by enhancing its clearance in the airways. *Infection and Immunity* **87(5), e00665-18.**

Rutz, S. and Ouyang, W. (2016). Regulation of interleukin-10 expression. *In*: Regulation of Cytokine Gene Expression in Immunity and Diseases. Ma, X. (ed.). Springer, Dordrecht. **pp. 89-116.**

Salazar, G. A., Peñaloza, H. F., Pardo-Roa, C., Schultz, B. M., Muñoz-Durango, N., Gómez, R. S., Salazar, F. J., Pizarro, D. P., Riedel, C. A., González, P. A. and Alvarez-Lobos, M. (2017). Interleukin-10 production by T and B cells is a key factor to promote systemic Salmonella enterica serovar Typhimurium infection in mice. *Frontiers in immunology*, *8*, 889.

Scheller, J., Chalaris, A., Schmidt-Arras, D. and Rose-John, S. (2011). The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1813(5), 878-888.**

Shahid, S., Mahesar, M., Ghouri, N. and Noreen, S. (2021). A review of clinical profile, complications and antibiotic susceptibility pattern of extensively drug-resistant (XDR) *Salmonella* Typhi isolates in children in Karachi. *BMC Infectious Diseases* **21, 900.**

Sharma, P. C., Jain, A., Jain, S., Pahwa, R. and Yar, M. S. (2010). Ciprofloxacin: Review on developments in synthetic, analytical, and medicinal aspects. *Journal of*

Enzyme Inhibition and Medicinal Chemistry **25(4), 577-589.**

Sur, D., Chatterjee, S., Riewpaiboon, A., Manna, B., Kanungo, S. and Bhattacharya, S. K. (2009). Treatment cost for typhoid fever at two hospitals in Kolkata, India. *Journal of Health, Population, and Nutrition* **27(6), 725-732.**

Syarif, L. I., Junita, A. R., Hatta, M., Dwiyanti R., Kaelan, C., Sabir, M., Noviyanthi, R. A., Primaguna, M. R. and Purnamasari, N. I. (2020). A mini review: Medicinal plants for typhoid fever in Indonesia. *Systematic Reviews in Pharmacy* **11(6), 1171-1180.**

Tanaka, T., Narazaki, M. and Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology* **6(10), a016295.**

Tang, Y., Jones, M., Barrow, P. A. and Foster, N. (2020). Immune modulation and the development of fowl typhoid: A model of human disease? *Pathogens* **9(10), 843.**

Thai, T. T., Le, P. T. V., Huynh, Q. H. N., Pham, P. T. T. and Bui, H. T. H. (2021). Perceived stress and coping strategies during the COVID-19 pandemic among public health and preventive medicine students in Vietnam. *Psychology Research and Behavior Management*, **14, 795.**

Tisti, H. J. M. and Ghawi, A. H. (2020). Performance improvement of package water treatment plant by using static mixer and natural Coagulant. *Journal of Green Engineering* **10, 3717-3744.**

van der Poll, T., Barber, A. E., Coyle, S. M. and Lowry, S. F. (1996a). Hypercortisolemia increases plasma interleukin-10 concentrations during human endotoxemia – A clinical research center study. *Journal of Clinical Endocrinology and Metabolism* **81(10), 3604-3606.**

van der Poll, T., Coyle, S. M., Barbosa, K., Braxton, C. C. and Lowry, S. F. (1996b). Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin 10 production during human endotoxemia. *The Journal of Clinical Investigation* **97(3), 713-719.**

van Der Poll, T., Marchant, A. and van Deventer, S. J. (1997). The role of interleukin-10 in the pathogenesis of bacterial infection. *Clinical Microbiology and Infection* **3(6), 605-607.**

waad Hadi Tokan, E., Muhammed, S. J. and Mahmood, M. H. (2021). Study of some proinflammatory cytokines and biochemical parameters in patients with typhoid fever in Salah Al-Din Governorate. *Annals of the Romanian Society for Cell Biology* **25(6), 15995-16003.**

Zhang, J. M. and An, J. (2007). Cytokines, inflammation and pain. *International Anesthesiology Clinics* **45(2), 27-37.**

Zubair, O. A. R. and Mohammad, M. Y. (2020). Prevalent sources of enteric fevers among women in Mosul city. *Annals of Tropical Medicine and Public Health* **23(13B).**