Egyptian Journal of Medical Microbiology Volume 31 / No.3 / July 2022 57-61 Online ISSN: 2537-0979

ORIGINAL ARTICLE

# Macrophage Secretory Function among Urinary Tract Infection Patients before and after Ciprofloxacin Treatment: A Prospective Study

**Mahmoud R. M. El-Ansary**
Department of Immunology and Medical Microbiology, Faculty of Medicine, Misr University for Science and Technology, Giza, Egypt

***Key words:***
***Proinflammatory cytokines; Urinary tract infection; Ciprofloxacin; Prospective Study***

***Corresponding Author:***
Mahmoud R. M. El-Ansary
6th October City, Giza Governorate
Tel.: : +201143666640
melansary2022@gmail.com

## ABSTRACT

***Background****: Attacks of Urinary tract infections (UTIs) reached about 150 million patients every year worldwide. They are considered a major reason for morbidity in females in all age groups, older men and young boys.* ***Objectives:*** *To investigate the effect of Ciprofloxacin treatment during urinary tract infection (UTI) infection on the proinflammatory cytokines secretion including IL-1, IL-6, IL-10, IL-12, and TNF in the adult patients.* ***Methodology****: This prospective, observational cohort study involved 50 patients diagnosed with UTI over a period of 3 months from January to March 2019. On the day of admission (before starting ciprofloxacin treatment), all the UTI patients were submitted to an estimation of urine levels of proinflammatory cytokines including IL-1, IL-6, IL-12, IL-10, and TNF during the first 48 hours after hospital admission. Cytokine levels were estimated by enzyme-linked immunoassay (ELISA) kits (R&D Systems, Inc., Minneapolis, Minnesota, USA). The patients were treated with ciprofloxacin 500mg for 3 days. After 5 days of ciprofloxacin treatment course, the patient underwent remeasurement of urine level of cytokines.* ***Results****: After the fifth day of ciprofloxacin treatment course, it has been observed that there were significant decreases regarding proinflammatory cytokine TNF, IL1, and IL6. On the other hand, there was a significant increase in IL10 levels in the study cohort. Besides, there was no significant difference between before and after ciprofloxacin treatment regarding IL12 levels (P-value = 0.06).* ***Conclusion****: Ciprofloxacin treatment significantly reduces the levels of the proinflammatory cytokine in patients with UTIs.*

## INTRODUCTION

Urinary tract infections (UTIs) are some of the most bacterial infections. Globally, UTIs attack about 150 million patients every year. In Egypt, the prevalence of urinary tract infection during pregnancy was 30.29% in Suez governorate and ranged between 22 to 35% in Zagazig governorate in 2021.[1] In the united states, in one year (2007) only, the national registry recorded around 10.5 million office visits for UTI complaints and about three million emergency visits.[2] Besides, UTIs are a major reason for morbidity in females in all age groups, older men and young boys. Inflammation can affect the lower urinary tract and, also, it can extend to the renal parenchyma.[3]

Many organisms are associated with UTIs inflammations encompassing gram-negative, gram-positive bacteria, and candida infections.[3] Gram-negative *Escherichia coli (E. coli)* and *Staphylococcus saprophyticus* together give rise to around 95% of UTIs. These pathogens usually have high recurrence and chronicity rates that require prophylaxis treatment in a long-term manner.[4] Essentially, UTIs are managed using antibiotics in the first line. Several types of antibiotics were used in the past for such cases including Nitrofurantoin, β Lactams, Fosfomycin, Fluoroquinolones, etc. But this empirical approach was associated with higher recurrence and resistance rates. Currently, the cornerstone of UTI management is Ciprofloxacin (fluoroquinolone). It blocks the Topoisomerase type II subtype β (TOP2β) enzyme. This enzyme regulates the supercoiling phenomenon of the mitochondrial DNA (mtDNA). Absence of this enzyme subsequently leads to mtDNA accumulation and encumbrance of the replication pursuit of the organism.[5]

Cytokines are soluble proteins generated by several cells. Infections and inflammations are the main stimulators for their production. For instance, about 40 interleukins were recognized and several ones were divided into subtypes. Interleukin (IL)-1 is a proinflammatory cytokine produced by macrophages in the early stage of inflammation. Its importance encounters the initiation and the regulation of the inflammatory process.[6] Furthermore, IL-6 plays a substantial role in the regenerative activity during the early stages of bacterial infection. Absence of this

essential cytokine exasperate the inflammatory activities. It has been found that this anti-inflammatory pursuit was arbitrated by trans-signaling.[7] Also, IL-10 has multiple, pleiotropic impacts on the array of the inflammatory and hemostatic activities. It mediates the B cells survival, division, and antibody secretion. Besides, the down regulation of T helper (Th)1 cytokines and MHC class II antigen expressions are achieved by IL-10.[8] The current study aims to investigate the effect of Ciprofloxacin treatment during UTI infection on the proinflammatory cytokines secretion including IL-1, IL-6, IL-10, IL-12, TNF in the adult patients.

## METHODOLOGY

**Study setting and study design:**

This study was conducted in the Internal Medicine Department of Misr University for Science and Technology Medical Center. This prospective, observational cohort study involved 50 patients diagnosed with urinary tract infection (UTI) over a period of 3 months from January to March 2019. Data collection was initiated after getting the ethical approval _in January 2019_ from the institutional review board of Misr University for Science and Technology. All patients were diagnosed with UTIs due to *E. coli.* Urine samples of those patients confirmed significant *E. coli* growth (a single type of bacterial growing > $10^5$ colony-forming units (CFU/mL)). Samples were collected properly from midstream "clean catch". Patients with UTI combined with any bacteria other than *E. coli* were excluded.

**Macrophages secretory function assessment:**

On the day of admission (before starting ciprofloxacin treatment), all the UTI patients were submitted to an estimation of urine levels of proinflammatory cytokines including IL-1, IL-6, IL-12, IL-10, and TNF during the first 48 hours after hospital admission. Urine samples were centrifuged, washed, buffered, and stored for 2 to 3 hours. Cytokine levels were estimated by enzyme-linked immunoassay (ELISA) kits (R&D Systems, Inc., Minneapolis, Minnesota, USA). The patients were treated with ciprofloxacin 500mg for 3 days. After 5 days of ciprofloxacin treatment course, the patient underwent remeasurement of urine levels of IL-1, IL-6, IL-12, IL-10, and TNF. Both levels for all proinflammatory cytokines at the two different time points were compared.

**Data analysis:**

Data were analyzed using the statistical package for the social sciences (SPSS version 22.0). Categorical data were presented in numbers and percentages. Continuous data were expressed in mean and standard deviation. A comparison between interleukins levels before and after ciprofloxacin treatment was done using paired sample t-test. A *P*-value less than 0.05 was considered significant and the null hypothesis was rejected.

## RESULTS

The current study included 50 patients diagnosed with UTIs due to *E. coli*. The average age of the study cohort was 31±5 years. 23 from 50 (46.0%) of the patients were males. There was no significant difference between males and females considering their age (*p-value* = 0.092).

**Macrophage secretory function:**

After the fifth day of ciprofloxacin treatment course, it has been observed that there were significant decreases regarding proinflammatory cytokine TNF, IL-1, and IL-6 with P-values of <0.001, <0.001, and <0.001 respectively.

On the other hand, there was a significant increase in IL-10 levels between the studied participants with P-value of 0.025. Besides, there was no significant difference between before and after ciprofloxacin treatment regarding IL-12 levels (P-value = 0.06). (**Table 1**)

The baseline value of IL-1 was 6.20 pg/ml ± 1.31 and it showed a significant decrease after treatment (3.80 pg/ml ± 1.48) showing the effectiveness of ciprofloxacin treatment for UTI infection and its relation with proinflammatory cytokines secretion after fifth day treatment course. Similary, ciprofloxacin treatment showed a significant decrease of IL-6 from 16.95 pg/ml ± 3.67 to 11.18 pg/ml ± 3.71. TNF decreases significantly from 15.0 pg/ml ± 6.0 pretreament to 9.02 pg/ml ± 4.68 post-treatment. IL-12 values were also decrease for UTI patietns but not significantly deacrease. IL-12 values decreased from 8.61 pg/ml ± 2.23 pretreatment to 4.44 pg/ml ± 2.34 post-treatment. **(Figure 1)**

Interestingly, IL-10 showed a significant increase after treatment with ciprofloxacin treatment for UTI patietns. It increase from 4.74 ± 11.98 to 8.89 ± 3.33 at the fifth day of ciprofloxacin treatment. **(Figure 1)**

**Table 1:** Comparison between the study cohort before and after ciprofloxacin treatment.

| Parameters | Baseline | Post treatment | P value |
|---|---|---|---|
| **IL-1, *pg/ml*** | 6.20 ± 1.31 | 3.80 ± 1.48 | <0.001 |
| **IL-6, *pg/ml*** | 16.95±3.67 | 11.18±3.71 | < 0.001 |
| **IL-12, *pg/ml*** | 8.61 ± 2.23 | 4.44 ± 2.34 | 0.06 |
| **TNF, *pg/ml*** | 15.0 ± 6.0 | 9.02 ± 4.68 | < 0.001 |
| **IL-10, *pg/ml*** | 4.74±11.98 | 8.89 ± 3.33 | 0.025 |

Data are presented as mean ± SD; TNFα: tumor necrosis factor alpha; IL: interleukin.

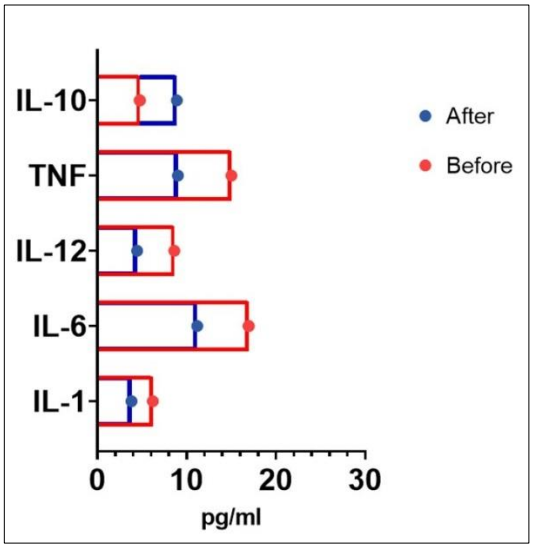


**Figure 1.** Paired sample t-test for macrophage secretory function before and after ciprofloxacin evaluation

## DISCUSSION

In the present study, we highlighted the impact of Ciprofloxacin on the secretion of the proinflammatory cytokine during the management of urinary tract infection (UTI). Our findings showed a significant reduction in the levels of TNF, IL1, and IL6. Similarly, the level of IL-12 was decreased after the administration of Ciprofloxacin; however, the difference was not significant. On the other hand, there was a significant increase in IL10 levels in the study cohort.

Many animal studies showed that some fluoroquinolones, including Ciprofloxacin and trovafloxacin, have a protective effect in lipopolysaccharide (LPS)-stimulated mouse by reducing the levels of TNF-α and IL-1 and IL-6 production.[9–12] This effect was observed with both low (50 mg/kg) and high (250 mg/kg) doses of Ciprofloxacin. Regarding in vitro studies, Bailly et al.[13], demonstrated that 100 µg/ml of Ciprofloxacin was enough to induce a significant reduction in the TNF production in LPS-stimulated human monocytes. In patients with severe sepsis, Gogos et al.[14], showed that these patients responded with a marked reduction in the TNF-α after the administration of Ciprofloxacin (400 mg/twice a day). Some investigators proposed that all fluoroquinolones with a cyclopropyl group at the N1 position and a piperazinyl group at the C7 position, have the same inhibitory effect on TNF-α and IL-1β production.[10,15]

The downregulation of IL-10, an essential anti-inflammatory cytokine, was reported to be associated with an exaggerated inflammatory response, as it works to enhance the production of anti-inflammatory cytokines and the suppression of the proinflammatory cytokines.[16,17] These both actions are key players in both the innate and adaptive immune responses. Furthermore, it demonstrated substantial inhibition of cytokine synthesis by CD41 T cells, and antigen presentation capacity, attenuating the development of Th1 immunity.[18] In terms of UTIs, IL-10 has a potent role in defending the urinary tract from UroPathogenic *Escherichia coli* infection.[19] In addition, the high concentration of IL-10 in the bladder allows the prompt regeneration of damaged epithelia.[20]

In our study, Ciprofloxacin resulted in a significant upregulation of IL-10, which confirms its protective role. These findings were also confirmed by the study of Tan and his colleagues,[21] who reported a significant elevation in the levels of IL-10 after the administration of 1000 mg/mL of Ciprofloxacin. Similarly, the upregulation of IL-10 was observed by many in vivo and in vitro studies.[11] It was reported that pre-treatment with a dose of Ciprofloxacin decreased the level of IL-12 and TNF, and increased the concentration of IL-10, which results in increasing the survival rate of LPS-stimulated mice.[12] Despite the aforementioned evidence, almost all previous studies agreed that low doses of Ciprofloxacin enhanced the production of IL-1, IL-6, and TNF-α by human monocytes. Therefore, the pre-calculated dose is essential to reduce any potential reverse effect.

Regarding the IL-6, it was known as a potential biomarker of infection in older patients with UTI.[22] Elevation of IL-6 was associated with bacteriuria or acute cystitis.[23] Azab et al.[24] showed that the concentration of IL-6 in urine could be used in the differentiation between acute pyelonephritis and lower tract infection. Moreover, it can be used as a marker of therapeutic response; normalization of urinary IL-6 levels is associated with the process of recovery from the infection. Therefore, the reduction of IL-6 level in this study indicates the impact of the Ciprofloxacin on UTIs. In the human endothelial cell line, Galley et al.[25], showed that application of Ciprofloxacin associated with a significant reduction in the IL-6 accumulation ($p=0.001$). Also, Gogos et al.[26], reported a significant reduction in the levels of TNF-α and IL-6 in patients with severe sepsis caused by gram-negative bacteria assigned to Ciprofloxacin compared with ceftazidime.

The administration of Ciprofloxacin was associated with a significant accumulation of cAMP and the cAMP-protein kinase A, resulting in activating the intracellular protein phosphorylation and suppressing the TNF production.[27] Furthermore, Ciprofloxacin directly induces the production of PGE2, which enhance the expression of cyclooxygenase (COX-2) protein, resulting in increasing the level of IL-10.[28,29] These mechanisms can illustrate the protective role of Ciprofloxacin against the proinflammatory cytokines.

This study showed some limitations, including the relatively small sample size and the single endpoint (after five days of ciprofloxacin administration). The

majority of studies assessed the proinflammatory cytokines concentrations 24h, 48, and 72h after treatment.

## CONCLUSION

In conclusion, we showed a significant immunomodulatory effect for the Ciprofloxacin in patients with UTIs. IL-6 can be used as a biomarker of treatment response. IL-10 upregulation as a result of ciprofloxacin administration can help in suppressing the proinflammatory cytokines. Further studies with larger sample sizes are required to confirm the current findings.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

**Declarations**

The author confirms no financial or personal relationship with a third party whose interests could be positively or negatively influenced by the article's content. Regarding the funding resources, this work did not received any kind of grants from other institutions including universities or third-party companies. No acknowledgment relared to this paper.

## REFERENCES

1. Mohamed NR, Omar HHH, Abd-Allah IM. Prevalence and Risk Factors of Urinary Tract Infection among Pregnant Women in Ismailia City, Egypt. *IOSR Journal of Nursing and Health Science*. 2017;06(03):62-72. doi:10.9790/1959-0603076272
2. Ambulatory Medical Care Utilization Estimates for 2007 - PubMed.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 2015;13(5):269-284. doi:10.1038/nrmicro3432
4. Shapouri Moghaddam A, Arfaatabar M, Tavakol Afshari J, Shakerimoghaddam A, Mohammadzamani Z, Khaledi A. Prevalence and Antimicrobial Resistance of Bacterial Uropathogens Isolated from Iranian Kidney Transplant Recipients: A Systematic Review and Meta-Analysis. *Iranian journal of public health*. 2019;48(12):2165-2176.
5. Stefan CP, Koehler JW, Minogue TD. Targeted next-generation sequencing for the detection of ciprofloxacin resistance markers using molecular inversion probes. *Scientific Reports*. 2016;6. doi:10.1038/srep25904
6. Sims JE, Smith DE. The IL-1 family: Regulators of immunity. *Nature Reviews Immunology*. 2010;10(2):89-102. doi:10.1038/nri2691
7. Gokce I, Alpay H, Biyikli N, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. *Pediatric Nephrology*. 2010;25(5):905-912. doi:10.1007/s00467-009-1396-2
8. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nature Reviews Immunology*. 2010;10(3):170-181. doi:10.1038/nri2711
9. Dalhoff A. Immunomodulatory Activities of Fluoroquinolones. *Infection*. 2005;33(S2):55-70. doi:10.1007/s15010-005-8209-8
10. Zapater P. Immunomodulating effects of antibiotics used in the prophylaxis of bacterial infections in advanced cirrhosis. *World Journal of Gastroenterology*. 2015;21(41):11493. doi:10.3748/wjg.v21.i41.11493
11. Dalhoff A, Shalit I. Immunomodulatory effects of quinolones. *The Lancet Infectious Diseases*. 2003;3(6):359-371. doi:10.1016/S1473-3099(03)00658-3
12. Ogino H, Fujii M, Ono M, Maezawa K, Kizu J, Hori S. In vivo and in vitro effects of fluoroquinolones on lipopolysaccharide-induced pro-inflammatory cytokine production. *Journal of Infection and Chemotherapy*. 2009;15(3):168-173. doi:10.1007/s10156-009-0680-1
13. BAILLY S, FAY M, FERRUA B, GOUGEROT-POCIDALO MA. Ciprofloxacin treatment in vivo increases the ex vivo capacity of lipopolysaccharide-stimulated human monocytes to produce IL-1, IL-6 and tumour necrosis factor-alpha. *Clinical & Experimental Immunology*. 1991. doi:10.1111/j.1365-2249.1991.tb05728.x
14. Gogos CA, Skoutelis A, Lekkou A, et al. Comparative effects of ciprofloxacin and ceftazidime on cytokine production in patients with severe sepsis caused by gram-negative bacteria. *Antimicrobial Agents and Chemotherapy*. 2004. doi:10.1128/AAC.48.8.2793-2798.2004
15. Abdel-Daim MM, Abushouk AI, Bahbah EI, et al. Fucoidan protects against subacute diazinon-induced oxidative damage in cardiac, hepatic, and renal tissues. *Environmental science and pollution*

*research international*. January 2020. doi:10.1007/s11356-020-07711-w

16. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critical Reviews in Immunology*. 2012. doi:10.1615/critrevimmunol.v32.i1.30
17. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *Journal of immunology (Baltimore, Md : 1950)*. 1991;147(11):3815-3822. doi:10.4049/jimmunol.177.3.1628
18. Abrams J, Figdor CG, De Waal Malefyt R, Bennett B, De Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. *Journal of Experimental Medicine*. 1991. doi:10.1084/jem.174.5.1209
19. Duell BL, Tan CK, Carey AJ, Wu F, Cripps AW, Ulett GC. Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis: Table 1. *FEMS Immunology & Medical Microbiology*. 2012;64(3):295-313. doi:10.1111/j.1574-695X.2012.00931.x
20. Choi HW, Bowen SE, Miao Y, et al. Loss of Bladder Epithelium Induced by Cytolytic Mast Cell Granules. *Immunity*. 2016;45(6):1258-1269. doi:10.1016/j.immuni.2016.11.003
21. Tan EE, Quah SY, Bergenholtz G, Rosa V, Hoon Yu VS, Tan KS. Antibiotics Used in Regenerative Endodontics Modify Immune Response of Macrophages to Bacterial Infection. *Journal of Endodontics*. 2019;45(11):1349-1356. doi:10.1016/j.joen.2019.08.001
22. Jensen D, Sherman KE, Hézode C, et al. Daclatasvir and asunaprevir plus peginterferon alfa and ribavirin in HCV genotype 1 or 4 non-responders. *Journal Of Hepatology*. 2015;63(1):30-37. doi:10.1016/j.jhep.2015.02.018
23. Rodhe N, Löfgren S, Strindhall J, Matussek A, Mölstad S. Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria. *Scandinavian Journal of Primary Health Care*. 2009;27(2):74-79. doi:10.1080/02813430902757634
24. Azab S, Zakaria M, Raafat M, Seief H. The combination of urinary IL - 6 and renal biometry as useful diagnostic tools to differentiate acute pyelonephritis from lower urinary tract infection. *International braz j urol*. 2016;42(4):810-816. doi:10.1590/S1677-5538.IBJU.2016.0049
25. Galley HF, Nelson SJ, Dubbels AM, Webster NR. Effect of ciprofloxacin on the accumulation of interleukin-6, interleukin-8, and nitrite from a human endothelial cell model of sepsis. *Critical Care Medicine*. 1997;25(8):1392-1395. doi:10.1097/00003246-199708000-00029
26. Gogos CA, Skoutelis A, Lekkou A, et al. Comparative Effects of Ciprofloxacin and Ceftazidime on Cytokine Production in Patients with Severe Sepsis Caused by Gram-Negative Bacteria. *Antimicrobial Agents and Chemotherapy*. 2004;48(8):2793-2798. doi:10.1128/AAC.48.8.2793-2798.2004
27. Mori S, Takahashi H, Liu K, et al. Ciprofloxacin inhibits advanced glycation end products-induced adhesion molecule expression on human monocytes. *British Journal of Pharmacology*. 2010;161(1):229-240. doi:10.1111/j.1476-5381.2010.00880.x
28. Gómez-Hurtado I, Zapater P, Bellot P, et al. Interleukin-10-mediated heme oxygenase 1-induced underlying mechanism in inflammatory down-regulation by norfloxacin in cirrhosis. *Hepatology*. 2011;53(3):935-944. doi:10.1002/hep.24102
29. Elhelaly AE, AlBasher G, Alfarraj S, et al. Protective effects of hesperidin and diosmin against acrylamide-induced liver, kidney, and brain oxidative damage in rats. *Environmental Science and Pollution Research*. November 2019. doi:10.1007/s11356-019-06660-3