Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

# Interleukin-6 Dynamics in Host Immune Response: A Cross-Species Study in Humans and Camels During Coronavirus Infections

**Ibrahim Hasan Madhloom[1]*, Hussein Ali Mohammed Al-Bayati[2], Rasha Munther Othman[1]**

[1]Department of Microbiology, College of Veterinary Medicine, University of Basrah, IRAQ
[2]Department of Pathological Analysis, College of Science, University of Wasit, IRAQ

*Corresponding Author: I. H. Madhloom

DOI: https://doi.org/10.31185/wjps.825
Received 24 March 2025; Accepted 22 June 2025; Available online 30 March 2026

**ABSTRACT:** This study investigates the role of interleukin-6 (IL-6) as a key pro-inflammatory cytokine in coronavirus-infected humans and camels. A total of 75 serum samples from each species were analyzed using ELISA. The results revealed a significant elevation of IL-6 levels in infected individuals compared to controls ($P<0.0001$), indicating a strong inflammatory response. Notably, this is the first study to quantify IL-6 protein levels in dromedary camels during natural coronavirus infection, addressing a gap in camel immunology. The findings underscore IL-6 as a critical biomarker of inflammation across species. Further research is warranted to explore its mechanistic role in disease severity.

**Keywords:** Cytokines, Interleukin-6 (IL-6), Inflammation, Immune response, Pro-inflammatory cytokines



## 1. INTRODUCTION

Long COVID has emerged as a significant challenge since the COVID-19 pandemic, which was declared as an outbreak in March 2020, marked by diverse symptoms and prolonged duration of disease [1]. The immune system is divided into 2 parts: the innate immune system and the adaptive immune system, and they work in essential and powerful ways for SARS-CoV-2 managing and reducing COVID-19 infections [2]. Severe morbidity and mortality in COVID-19 are linked to an inflammatory 'cytokine storm' driven by hyperactive macrophages and altered monocyte function [3]. Cytokines are a diverse family of intercellular signaling molecules that regulate inflammation and immune responses [4]. A variety of cells produce cytokines and usually act in an autocrine or paracrine manner at very low concentrations in tissues. Localized inflammation is usually a physiological protective response to initial tissue injury [5]. Two broad classes of cytokines are generally recognized: pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines are involved in the up-regulation of inflammatory reactions, while anti-inflammatory cytokines counteract these effects to downregulate the pro-inflammatory cytokine response [6]. Interleukin 6 (IL-6) is a cytokine critical to proinflammatory and immune regulatory cascades. Emerging data have identified important roles for IL-6 in innate immune responses and adaptive immunity [7]. IL-6, promptly and transiently produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions [8]. It was first described in 1973 as a protein secreted by T lymphocytes that aids B cell differentiation into antibody-producing cells; thus, it was initially known as B cell stimulatory factor 2 (BSF2) [9].

IL-6 is a small polypeptide (molecular weight of 19–28 kDa), comprised of four α helices. Usually existing in a monomer form, it consists of 184 amino acid residues, glycosylation sites, and two disulfide bonds [10]. It is produced by B lymphocytes, T lymphocytes, macrophages, including microglia, as well as fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, and dendritic cells [11]. IL-6 secretion is stimulated during the inflammatory response secondary to tissue injury or infection. After it is produced, it moves through the bloodstream to the liver,

*Corresponding author: ibrahim1987hasan@gmail.com

triggering production of acute phase reactants such as C-reactive protein (CRP), serum amyloid A (SAA), α1-antichymotrypsin, fibrinogen, and haptoglobin [12]. IL-6 is a growth factor for B cells, inducing their maturation and differentiation into plasma cells and increasing their survival [13], [14].

This study aims to investigate the dynamics of Interleukin-6 as a key pro-inflammatory cytokine involved in the host immune response during coronavirus infections, through a comparative analysis between humans and camels.

## 2. MATERIALS AND METHODS

### 2.1 SAMPLE COLLECTION

Between November 2022 and April 2023, a total of 75 blood samples were aseptically collected from humans and another 75 from camels, all of which were confirmed positive for coronavirus infection by real-time PCR. In camels, the detection was based on amplification of a 113 bp fragment of the nucleocapsid (N) gene of camel coronavirus using a validated RT-qPCR protocol [15]. In humans, confirmation was performed using the Molaccu Fast SARS-CoV-2 RT-qPCR Detection Kit, which targets the ORF1ab and N genes through RT-qPCR technology, and includes reagents for both amplification and detection. The inclusion criteria for sample selection were based on the presence of clinical signs consistent with coronavirus infection, the age group of the individuals, and the type of sample collected. Each sample consisted of 5 mL of blood, collected using sterile needles and serum separation tubes (SST). For humans, venipuncture was performed using a tourniquet and alcohol disinfection. In camels, the jugular vein area was cleaned with alcohol before collection. Tubes were labeled with sample ID, date, and species. Samples were immediately placed in a cooler box containing ice packs and transported to the Microbiology Laboratory at the Department of Pathological Analysis, College of Science, University of Wasit. All samples were left to clot at room temperature for 30 minutes and subsequently centrifuged at 1500 × g for 10 minutes to separate the serum, and stored at −20°C for long-term storage.

### 2.2 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) PROCEDURE

Two commercial ELISA kits were used for the quantification of IL-6 levels in serum samples. The Human Interleukin 6 (IL-6) ELISA Kit (Sunlong Biotech, China; Generic Name: Human Interleukin 6 ELISA Kit) was used for human samples, while the Camel Interleukin 6 (IL-6) ELISA Kit (Sunlong Biotech, China) was employed for camel samples. Both kits utilize the sandwich ELISA principle, wherein microplate wells pre-coated with anti-IL-6 antibodies bind to the IL-6 present in the sample. After binding, an HRP-conjugated detection antibody is added, followed by the TMB substrate. The resulting color intensity, measured at 450 nm, is proportional to IL-6 concentration. Quantification was achieved by comparing the optical density (OD) of the samples with a standard curve provided in each kit.

The ELISA assay was performed according to the manufacturer's instructions. For comparison purposes, a control group was included comprising 20 clinically healthy humans and 20 clinically healthy camels, with no reported symptoms of coronavirus infection. All control subjects tested negative for coronavirus by real-time PCR using the same respective diagnostic protocols described above. For sample wells, 40 µL of sample dilution buffer and 10 µL of each sample (1:5 dilution) were carefully added to the bottom of the wells without touching the side walls, followed by gentle mixing. The plate was sealed with a closure membrane and incubated at 37°C for 30 minutes. After incubation, the membrane was removed, and the wells were washed five times by aspirating the solution, refilling with wash buffer, and allowing it to rest for 30 seconds before discarding. Next, 50 µL of HRP-conjugated reagent was added to each well except the blank, followed by another incubation at 37°C for 30 minutes. The washing step was then repeated as previously described. Subsequently, 50 µL each of chromogen solutions A and B were added to all wells. The plate was gently mixed and incubated in the dark at 37°C for 15 minutes. Then, 50 µL of stop solution was added to each well, resulting in a color change from blue to yellow. Finally, the OD was measured at 450 nm using a microplate reader. The OD of the blank well was used as the reference (zero), and readings were completed within 15 minutes of adding the stop solution.

Statistical analysis was performed using GraphPad Prism version 10 (GraphPad Software, USA). Since unequal variances were expected between the groups, Welch's t-test was applied for all pairwise comparisons between case and control groups. Data were expressed as mean ± standard error of the mean (SEM), and p-values $\leq 0.05$ were considered statistically significant.

## 3. RESULTS

Quantitative analysis revealed a significant elevation in the serum concentrations of IL-6 in the case groups compared to the control groups across both species studied. As illustrated in Figure 1, in humans, the mean concentration of IL-6 was markedly elevated in the case group (26.51 ± 3.16 pg/ml) relative to the control group (4.85 ± 0.55 pg/ml), and this difference was statistically highly significant ($P < 0.0001$). This marked increase reflects a pronounced pro-inflammatory cytokine response among infected individuals.

In camels, the mean concentration of IL-6 in the case group (31.03± 2.19 pg/ml) was moderately higher than that observed in the control group (4.85 ± 0.55 pg/ml), with the difference also reaching statistical significance. These findings indicate a species-specific variation in the magnitude of the inflammatory response. The detailed statistical comparisons presented in Table 1 further support these observations, with both comparisons demonstrating highly significant differences ($P < 0.0001$) between the case and control groups. These results reinforce the reliability and reproducibility of IL-6 as a measurable biomarker of inflammation during infection.

**Table 1: Summary of Welch's t-test results: IL-6 comparison between case and control groups.**

| IL-6 | Human | Camel |
|---|---|---|
| | **Mean±SEM** | **Mean±SEM** |
| **Case group** | 26.51±3.16 | 31.03±2.19 |
| **Control** | 4.85±0.55 | 4.85±0.38 |
| ***P* Value** | <0.0001 | <0.0001 |
| **Summary** | **** | **** |

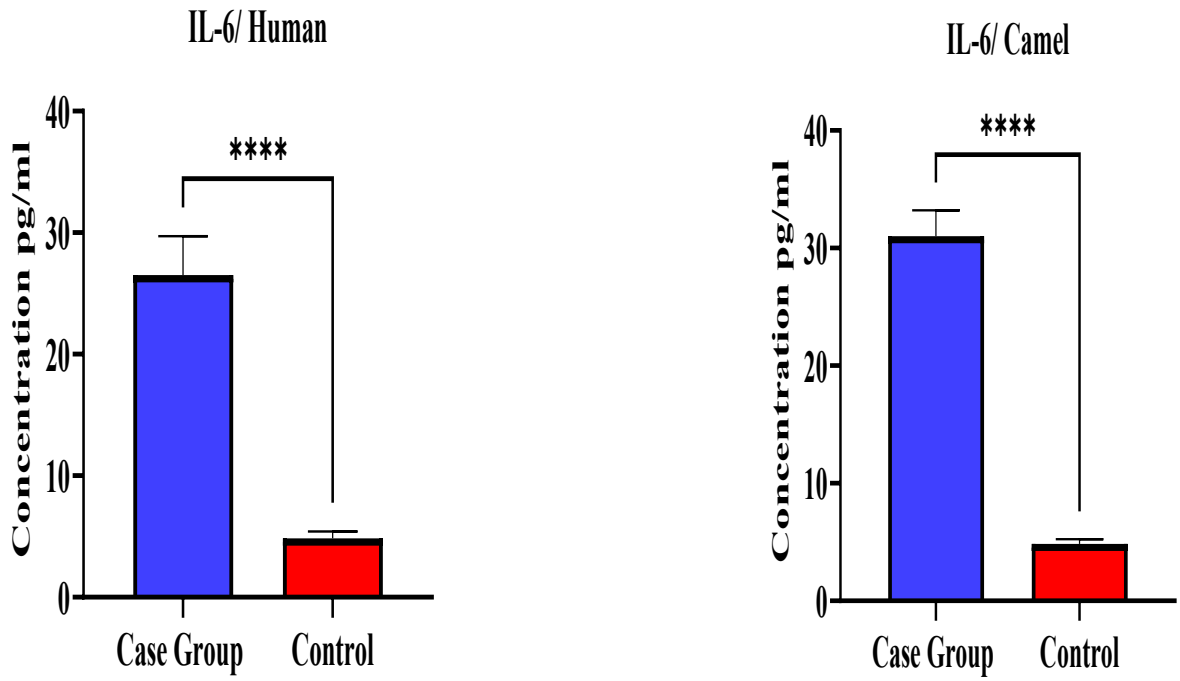


**Figure 1: Concentrations of serum IL-6 between the case group and the control group using the Welch's t-test in humans and camels.**

## 4. DISCUSSION

Cytokine release syndrome or cytokine storm is a potentially life-threatening condition that results from the pathologic overactivation of T cells, leading to hypersecretion of cytokines. COVID-19 patients showed increased serum levels of proinflammatory cytokines such as IL-6, and the evidence shows that cytokine storm is directly related to the severity of the disease process [16]. Cytokines play a critical role in regulating the immune response during viral infections, including those caused by coronaviruses. As key mediators of inflammation, cytokines such as IL-6 help coordinate the balance between protective immunity and harmful immune overactivation. The findings, as detailed in Table 1 and visually represented in Figure 1, demonstrated significantly elevated IL-6 levels in infected groups compared to controls across humans, camels, and bovines ($P<0.0001$). In humans, infected cases showed IL-6 concentrations of 26.51 ± 3.16 pg/mL, whereas control samples showed 4.85 ± 0.55 pg/mL. These findings align with those of Hafez *et al.,* who highlighted IL-6 as a key marker of inflammatory severity in COVID-19, where severe cases exhibited median levels of 48.8 pg/mL ($P=0.001$), and long COVID-19 patients demonstrated elevated IL-6 with a mean difference of 9.75 pg/mL, $P<0.00001$ [17], [18].

Moreover, previous studies have shown that IL-6 levels above 43.5 pg/mL may indicate a higher risk of severe illness in COVID-19 patients[17] . Notably, the control values in this study, 4.85 pg/mL, are closely aligned with the normal IL-6 thresholds previously reported–5.186 pg/mL by Said *et al.* and 8 pg/mL by Hara *et al.* [19], [20]. In contrast, these values remain substantially lower than the critical IL-6 levels observed in long COVID-19 cases, which reached 20.92 pg/mL [18]. The observed elevation of IL-6 levels in the human cohort mirrors previously reported trends in gender-specific immune responses, where concentrations were notably higher in men, ranging between 100–1000 pg/mL, with statistical significance at $P<0.001$ [21]. Furthermore, these findings align with the established role of IL-6 in differentiating between varying severities of COVID-19, particularly between mild and moderate cases, where $P = 0.001$, as well as between mild and severe cases, where $P= 0.009$ [22]. Furthermore, the role of IL-6 as a mediator of hyperinflammation in viral infections is well-documented in humans [18], [23].

On the other hand, dromedary camels, as a relatively neglected host in coronavirus research, have received limited attention concerning cytokine-mediated immune responses. Within the broader scope of this study, which aims to characterize host-specific immune dynamics, camels were included to address this notable gap in understanding their inflammatory profile during coronavirus infection. Despite the growing scientific interest in the zoonotic potential of MERS-CoV, studies focusing on cytokine responses in dromedary camels remain remarkably scarce. Most existing research has concentrated on seroprevalence surveys and viral detection, with limited insights into the immunological mechanisms involved, particularly at the cytokine level. For instance, a recent seroepidemiological study conducted in Iraq revealed a high prevalence of MERS-CoV antibodies in dromedary camels [24].

This study provides novel insight into IL-6 protein levels in dromedaries, marking the first assessment of this cytokine during natural MERS-CoV infection**.** As shown in Table 1 and Figure 1, infected camels exhibited a mean IL-6 concentration of $31.03 \pm 2.19$ pg/mL**,** whereas control animals recorded $4.85 \pm 0.38$ pg/mL. This approximately tenfold elevation indicates a pronounced inflammatory response following infection. Historically, camel immunological studies have largely focused on cytokine mRNA expression, such as IFN-γ and IL-4, with limited exploration of protein-level markers [25], [26]. The lack of species-specific immunological reagents, particularly monoclonal antibodies against camel IL-6, has hindered progress in functional cytokine analysis [27]. Therefore, the current findings fill a critical knowledge gap in camel immunology.

The observed IL-6 elevation suggests that dromedaries can mount a measurable pro-inflammatory response, potentially resembling patterns observed in human coronavirus infections. This upregulation is likely driven by innate immune activation in response to viral replication and tissue damage, given IL-6's established role in acute-phase inflammation. Nonetheless, further studies are essential to validate these findings and to elucidate the mechanistic role of IL-6 in camelid viral pathogenesis. In conclusion, IL-6 exhibited a marked elevation in all investigated species, including humans and camels, underscoring its pivotal role in the host immune response to infection and highlighting its potential as a key biomarker of inflammation.

## 5. ACKNOWLEDGEMENT

We express our sincere gratitude to Captain Haider Al-Badiri from the Iraqi Camel Federation and extend our heartfelt thanks to Dr Khalid Sajit from the Veterinary Hospital in Wasit, Veterinary Directorate, Ministry of Agriculture, Iraq, for his invaluable assistance in collecting camel samples. Their contributions are crucial to the success of this research.

## 6. ETHICAL APPROVED

This study was conducted according to ethical guidelines and was approved by the Scientific Committee of the College of Veterinary Medicine, University of Basrah (Approval Number: 39/2024). All procedures involving animals complied with institutional and international standards for animal welfare. Efforts were made to minimize animal suffering and ensure proper care during the study.

## REFERENCES

[1] A. H. Abbas *et al.*, "A multidisciplinary review of long COVID to address the challenges in diagnosis and updated management guidelines," *Annals of Medicine & Surgery*, vol. 87, no. 4, pp. 2105–2117, Apr. 2025, doi: 10.1097/MS9.0000000000003066.

[2] A. M. Ashraf, M. Y. Al-Maqtoofi, and A. A. Burghal, "COVID-19 story: Entry and immune response," *Vacunas (English Edition)*, vol. 26, no. 1, p. 100380, Jan. 2025, doi: 10.1016/j.vacune.2025.100380.

[3] M. Subkhan *et al.*, "Low platelet counts and monocytosis were associated with reduced risk of severe COVID-19 manifestation: a single-center study from Indonesia," *Immunopathologia Persa*, vol. 11, p. e40595, Jan. 2025, doi: 10.34172/ipp.2025.40595.

[4] H. Hasegawa, I. Mizoguchi, Y. Chiba, M. Ohashi, M. Xu, and T. Yoshimoto, "Expanding Diversity in Molecular Structures and Functions of the IL-6/IL-12 Heterodimeric Cytokine Family," *Front Immunol*, vol. 7, Nov. 2016, doi: 10.3389/fimmu.2016.00479.

[5] K. Suzuki, "Cytokine Response to Exercise and Its Modulation," *Antioxidants*, vol. 7, no. 1, p. 17, Jan. 2018, doi: 10.3390/antiox7010017.

[6] A. Y. Majeed, N. E. S. Zulkafli, and A. H. Ad'hiah, "Serum profiles of pro-inflammatory and anti-inflammatory cytokines in non-hospitalized patients with mild/moderate COVID-19 infection.," *Immunol Lett*, vol. 260, pp. 24–34, Aug. 2023, doi: 10.1016/j.imlet.2023.06.008.

[7] S. C. Jordan *et al.*, "Interleukin-6, A Cytokine Critical to Mediation of Inflammation, Autoimmunity and Allograft Rejection," *Transplantation*, vol. 101, no. 1, pp. 32–44, Jan. 2017, doi: 10.1097/TP.0000000000001452.

[8] T. Tanaka, M. Narazaki, and T. Kishimoto, "IL-6 in Inflammation, Immunity, and Disease," *Cold Spring Harb Perspect Biol*, vol. 6, no. 10, pp. a016295–a016295, Oct. 2014, doi: 10.1101/cshperspect. a016295.

[9] T. Kishimoto and K. Ishizaka, "Regulation of Antibody Response in Vitro," *The Journal of Immunology*, vol. 116, no. 2, pp. 534–541, Feb. 1976, doi: 10.4049/jimmunol.116.2.534.

[10] J. Scheller, C. Garbers, and S. Rose-John, "Interleukin-6: From basic biology to selective blockade of pro-inflammatory activities," *Semin Immunol*, vol. 26, no. 1, pp. 2–12, Feb. 2014, doi: 10.1016/j.smim.2013.11.002.

[11] C. A. Hunter and S. A. Jones, "IL-6 as a keystone cytokine in health and disease," *Nat Immunol*, vol. 16, no. 5, pp. 448–457, May 2015, doi: 10.1038/ni.3153.

[12] Y. Takeshita *et al.*, "New BBB Model Reveals That IL-6 Blockade Suppressed the BBB Disorder, Preventing Onset of NMOSD," *Neurol Neuroimmunol Neuroinflamm*, vol. 8, no. 6, Nov. 2021, doi: 10.1212/NXI.0000000000001076.

[13] H. A. M. Wols, G. H. Underhill, G. S. Kansas, and P. L. Witte, "The Role of Bone Marrow-Derived Stromal Cells in the Maintenance of Plasma Cell Longevity," *The Journal of Immunology*, vol. 169, no. 8, pp. 4213–4221, Oct. 2002, doi: 10.4049/jimmunol.169.8.4213.

[14] G. Tosato *et al.*, "Monocyte-derived human B-cell growth factor identified as interferon-beta 2 (BSF-2, IL-6)," *Science (1979)*, vol. 239, no. 4839, pp. 502–504, Jan. 1988, doi: 10.1126/science.239.4839.502.

[15] I. Madhloom, R. Othman, and H. Al-bayati, "Molecular detection of coronavirus in camelids and bovines using real-time quantitative polymerase chain reaction in Wasit Province, Iraq" *Open Vet J*, no. 0, p. 1, 2025, doi: 10.5455/OVJ. 2025.v15.i2.25.

[16] A. Raheem Hashim, H. Ala Farid, Z. Adil, N. Haikaz Hasrat, Z. Adil Yakob, and Y. Ala Sabeeh, "The occurrence of cytokine storm syndrome among ICU patients with covid-19 in Basrah city, southern of Iraq: Does Tocilizumab affect the outcome?", doi: 10.37896/sr8.7/013.

[17] W. Hafez *et al.*, "Interleukin-6 and the determinants of severe COVID-19: A retrospective cohort study.," *Medicine*, vol. 102, no. 45, p. e36037, Nov. 2023, doi: 10.1097/MD.0000000000036037.

[18] J. X. Yin *et al.*, "Increased interleukin-6 is associated with long COVID-19: a systematic review and meta-analysis," *Infect Dis Poverty*, vol. 12, no. 1, p. 43, Apr. 2023, doi: 10.1186/s40249-023-01086-z.

[19] E. A. Said *et al.*, "Defining IL-6 levels in healthy individuals: A meta-analysis," *J Med Virol*, vol. 93, no. 6, pp. 3915–3924, Jun. 2021, doi: 10.1002/jmv.26654.

[20] S. Hara *et al.*, "Comparison of the levels of neopterin, CRP, and IL-6 in patients infected with and without SARS-CoV-2," *Heliyon*, vol. 8, no. 5, p. e09371, May 2022, doi: 10.1016/j.heliyon. 2022.e09371.

[21] S. Mollazadeh *et al.*, "Investigation of IL-6 serum level in COVID-19 patients with positive COVID-19 IgG/IgM antibody titers to check inflammation and disease progression," *Cytokine*, vol. 177, p. 156564, May 2024, doi: 10.1016/j.cyto.2024.156564.

[22] R. S. Rasheed and S. Salim, "Interleukin 6 Levels and their Correlation with Various Hematological and Biochemical Parameters in Covid-19 Patients," *AL-Kindy College Medical Journal*, vol. 19, no. 1, pp. 75–80, Apr. 2023, doi: 10.47723/kcmj. v19i1.893.

[23] M. Pal, M. A. Febbraio, and M. Whitham, "From cytokine to myokine: the emerging role of interleukin-6 in metabolic regulation," *Immunol Cell Biol*, vol. 92, no. 4, pp. 331–339, Apr. 2014, doi: 10.1038/icb.2014.16.

[24] H. T. Thwiny, T. A. Al Hamed, and A. R. Nazzal, "Seroepidemiological study of Middle East Respiratory Syndrome (MERS) virus infection in Iraqi dromedary camels," *Vet Arh*, vol. 88, no. 2, pp. 191–200, Mar. 2018, doi: 10.24099/vet.arhiv.161224.

[25] M. El-Boshy, H. Abbas, S. El-Khodery, and S. Osman, "Cytokine response and clinicopathological findings in Brucella infected camels (Camelus dromedarius)," *Vet Med (Praha)*, vol. 54, no. 1, pp. 25–32, Jan. 2009, doi: 10.17221/3044-VETMED.

[26] X. Yu, Y. Wu, J. Zhang, Jirimutu, A. Zulipikaer, and J. Chen, "Pre-evaluation of humoral immune response of Bactrian camels by the quantification of Th2 cytokines using real-time PCR," *The Journal of Biomedical Research*, vol. 34, no. 5, p. 387, 2020, doi: 10.7555/JBR.34.20190035.

[27] J. Hussen and H. J. Schuberth, "Recent Advances in Camel Immunology," *Front Immunol*, vol. 11, Jan. 2021, doi: 10.3389/fimmu.2020.614150.