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Gender Differences in Cytokine Response to Post-COVID 19 Vaccination: Insights from Iraqi Subjects

Roua J. AbdulKhaliq[1], Mohammed YI Al-Hamadani[1] and Wael M. El-Sayed[2]*

[1]*Department of Pathological Analysis, College of Applied Science, University of Fallujah, Iraq*
[2]*Department of Zoology, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt*

ABSTRACT

Since the emergence of SARS-CoV-2 in late 2019, COVID-19 has posed unprecedented global challenges, prompting urgent vaccine development to mitigate its health, economic, and social impacts. This cross-sectional study was conducted in Baghdad, Iraq from September 2021 to February 2021 to evaluate cytokine responses in 80 participants (47 males, 33 females) following complete COVID-19 vaccination. Venous blood samples assessed for INF-γ and TNF-α levels showed significantly elevated INF-γ levels in vaccinated individuals compared to non-vaccinated, regardless of COVID-19 status, underlining robust vaccine-induced immunity. Notably, vaccinated males had significantly higher INF-γ levels than females, driven by non-infected cases. Gender differences in TNF-α levels were also observed among non-vaccinated individuals and non-infected where females had higher TNF-α levels than males. These gender differentiations warrant further investigation into the underlying mechanism. The strong immune response by the elevated levels of INF-γ observed in vaccinated individuals could serve as a marker to help deploy vaccines for COVID-19 or other variants. Further studies are required to explore the mechanisms underlying the gender differences reported in this study.

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Key words
Cytokines, Interferon-gamma, SARS-CoV-2, TNF-α, Vaccines

INTRODUCTION

In late 2019, the world witnessed the emergence of a new and highly virulent pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or coronavirus disease of 2019 (COVID-19). On March 11, 2020, the World Health Organization (WHO) officially declared COVID-19 a global pandemic, recognizing its widespread impact and severity. By January 2020, the toll of this pandemic had become starkly evident:s globally, there were 28.32 million confirmed cases and 616,465 fatalities (Smith and Jones, 2020). Global collaborative efforts significantly accelerated the development of COVID-19 vaccines. By January 9, 2020, the landscape of vaccine development was remarkable, with 63 candidate vaccines undergoing clinical trials and another 173 in the preclinical stages of development (WHO, 2021).

Assessing the practical effectiveness of COVID-19 vaccination in real-world settings is crucial for evaluating the risks and benefits associated with vaccination strategies (Patel *et al*., 2020). COVID-19 vaccines aim to enhance immunity against the disease, potentially reducing severe cases, overall incidence rates, and fatalities in urban areas through widespread vaccination (WHO, 2021).

This cross-sectional study aimed to evaluate specific cytokines (INF-γ and TNF-α) in vaccinated individuals and assess seroconversion rates following complete COVID-19 vaccination from September 2021 to February 2021 in 80 participants (47 males and 33 females) selected from a clinical laboratory in Baghdad, Iraq. The ongoing challenges in understanding COVID-19 immunity and assessing vaccine effectiveness underscore the importance of evaluating cytokine profiles, as these can offer critical insights into the immune mechanisms that underlie both protection and susceptibility to the virus.

INF-γ and TNF-α were selected as key biomarkers due to their critical roles in the immune response to infections, particularly in the context of viral infections like COVID-19. INF-γ is known for its role in activating

* Corresponding author: wael_farag@sci.asu.edu.eg, waelelhalawany@hotmail.com
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macrophages and enhancing antigen presentation, making it a key cytokine in the adaptive immune response (Alspach *et al*., 2019). TNF-α, on the other hand, is a potent pro-inflammatory cytokine involved in the regulation of immune cells and the development of immune responses (El-Maraghi *et al*., 2020; Haroun *et al*., 2023; Mahmoud *et al*., 2024). Together, these cytokines provide insight into both innate and adaptive immune processes, which are essential for understanding the body's response to SARS-CoV-2 and evaluating the effectiveness of vaccines.

MATERIALS AND METHODS

Subjects

This cross-sectional and descriptive study was conducted from September 2021 to February 2021 at the Department of Biotechnology, College of Science, Al-Fallujah University, and the International Research and Development Center in Baghdad, Eighty randomly selected patients comprising of 47 males and 33 females, aged 15 to 63 years were involved in the study. The inclusion criteria were: newly confirmed diagnosis of COVID-19, no prior receipt of antiviral drugs, and no chronic inflammatory diseases.

Blood sample collection

Venous blood (5ml) was collected using sterile reusable syringes. Of this, 3 ml was transferred into a plain tube with a gel clot activator and allowed to clot for one hour at room temperature to facilitate clot formation. Following this, the tubes were centrifuged at 1500 xg for 10 min. The resulting serum was carefully aspirated using a Pasteur pipette and transferred into sterilized Eppendorf tubes, which were then stored at -20°C until further analysis.

Assay for interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α)

The ELISA assay for IFN-γ was conducted using Goat Interferon γ purchased from Sunlong Biotech (Zhejiang, China), Cat# SL0068Gt, following the manufacturer's instructions. The ELISA assay for TNF-α utilized Goat Interferon γ purchased from My BioSource (San Diego, USA), cat# MBS2502004, according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± SEM. The data passed the Kolmogorov-Smirnov test for normal distribution, and two tailed unpaired T-test was used for comparisons between groups (Van Emden, 2019). To identify outliers, we utilized the ROUT test in GraphPad Prism software (version 8.2) with a Q value of 1%. Statistical analyses were conducted using GraphPad Prism (version 8.02, 2020) for Windows software. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Adaptive and innate immune responses to respiratory virus pollution can be detrimental to an otherwise healthy respiratory system and exacerbate pulmonary diseases (Newton *et al*., 2016). While COVID-19 shows promising initial indicators during infection compared to respiratory syncytial virus (RSV) (Azekawa *et al*., 2020; Schwartz and Dhaliwal, 2020), its transmissibility and destructiveness far exceed those of influenza and RSV. The singular host immune response in COVID-19 patients likely significantly contributes to the disease's high transmissibility and severity (Grifoni *et al*., 2020).

Statistical analysis indicated no significant difference in the age between vaccinated and non-vaccinated groups across the cases examined. Therefore, the findings reported in this study are not biased by age or COVID-19 infection status (Supplementary Table S1).

SARS-CoV-2 pollution may not consistently trigger interferon production but rather delay or suppress interferon III or II production in lung tissues (Winkler *et al*., 2020). The variability in immune responses observed among COVID-19 patients can lead to significantly diverse clinical outcomes. Vaccines have demonstrated efficacy in preventing severe illness caused by the SARS-CoV-2 Omicron variant, similar to previous strains (Thompson, 2022).

T cell-mediated immunity and immune memory against SARS-CoV-2 are believed to be more robust and longer-lasting than antibody responses post-infection (Dan *et al*., 2021), and they appear to be more resistant to variants of concern even after vaccination (Redd *et al*., 2021; Liu *et al*., 2022). The clinical implications of the T cell response are considerable. Research indicates protective cross-reactivity between SARS-CoV-2 and common human coronaviruses within memory T cells (Kundu *et al*., 2022). The presence of SARS-CoV-2-specific T cells in peripheral blood predicts protection in individuals with low anti-S IgG responses (Wyllie *et al*., 2021).

INF-γ levels

The serum level of significantly ($p< 0.0001$) elevated by 172% in the non-infected vaccinated volunteers compared to the non-infected and non-vaccinated control (Table I). In the infected patients, the level of INF-γ significantly ($p< 0.001$) elevated by 278% in the vaccinated

subjects compared to the non-vaccinated (Table I).

Table I. Comparison of INF-γ levels (pg/ml) and TNF-α in vaccinated and non-vaccinated patients with and without COVID-19 infection.

Status	Non-vaccinated	Vaccinated
INF-γ		
Non-infected	239.6±34.6 (n=34)	653.1±34.1* (n=29)
Infected	180.3±74.3 (n=6)	681.1±63.2* (n=11)
TNF-α		
Non-infected	749.5 ± 55.9 (n=34)	1676.2 ± 146.6* (n=29)
Infected	550.6 ±72.7 (n=6)	1785.3 ± 190.6* (n=11)

Data are expressed as mean ± SEM, n is given in parenthesis. *: Significant difference between vaccinated and non-vaccinated at $p < 0.05$.

IFN-γ plays a significant role in the immune response to COVID-19. IFN-γ is a cytokine that is crucial for the activation of macrophages and plays a central role in the immune response against viral infections, including SARS-CoV-2, the virus that causes COVID-19 (Schroder *et al*., 2004). IFN-γ is involved in initiating an antiviral state in infected cells and in neighboring cells. It helps in controlling viral replication and spread by enhancing the immune response. Studies have shown that levels of IFN-γ can vary among individuals infected with COVID-19 (Hwang *et al*., 1995). Higher levels of IFN-γ have been associated with a stronger immune response and better control of the virus, potentially leading to milder disease outcomes (Channappanavar *et al*., 2017). The effectiveness of IFN-γ in combating COVID-19 may vary with different viral variants, such as the Omicron variant. However, it remains an important part of the immune response against SARS-CoV-2 (Arunachalam *et al*., 2020). Given its role in antiviral immunity, IFN-γ has been considered as a potential therapeutic agent or target in the treatment of COVID-19. Its levels and activity are critical factors in understanding individual immune responses and developing targeted therapies against the virus (Hadjadj *et al*., 2020; Mantlo *et al*., 2020).

Vaccination induces a robust T cell immunity effective against the Omicron variant (Keeton *et al*., 2022; Moss, 2022), and potentially against future variants (Tarke *et al*., 2022). In our study, although most participants developed a SARS-CoV-2-specific INF-γ response after the second dose, patients with low or no response showed significantly lower INF-γ levels. With the Omicron variant becoming predominant, this reduced response could pose challenges. The Omicron variant has shown significant immune evasion from antibodies induced by other variants or vaccines, thereby reducing serum neutralization ability (Schmidt *et al*., 2022). However, T cell epitopes targeted by the virus have remained relatively unchanged. Studies suggest that T cell response to Omicron remains intact, despite diminished B cell/antibody responses to the variant and its sub-lineages. Therefore, T cell responses may play a critical role in combating Omicron. Patients with low or non-responsive INF-γ levels may be more susceptible to symptomatic and severe infection and potentially have higher mortality rates compared to controls and responsive patients when exposed to this viral variant (Iketani *et al*., 2022).

Table II. Assessment of INF-γ and TNF-α levels (pg/ml) by gender in infected and non-infected patients

Gender	Infected	Non- infected
INF-γ		
Female	418.7 ±74.5 (n=6)	397.8±36.1 (n=27)
Male	442.7 ±62.1 (n=11)	494.6±31.6 (n=36)
TNF-α		
Female	1119.5±300.7 (n=6)	1346.1±99.7 (n=27)
Male	1475.0±259.5 (n=11)	1048.6±144.5 (n=36)

Data are expressed as mean ± SEM, n is given in parenthesis

The assessment of INF-γ concentrations, stratified by gender and infection status, revealed no significant changes in the INF-γ between infected female versus non-infected females, the same applied to the males (Table II).

INF-γ levels based on gender and vaccination status showed significant differences between non-vaccinated and vaccinated females and males, emphasizing the impact of vaccination on INF-γ responses. INF-γ level in the vaccinated females was significantly ($p = 0.0003$) higher than that of the non-vaccinated females by 142%. Similarly, INF-γ level in the vaccinated males was significantly ($p < 0.0001$) higher than that of the non-vaccinated males by 317% (Table III).

Figure 1A provides comparative analysis of INF-γ levels between vaccinated and non-vaccinated individuals, stratified by gender and infection status. Vaccinated infected females showed significantly ($p = 0.006$) higher INF-γ levels by 176% compared to non-vaccinated infected peers. Similarly, vaccinated infected males exhibited significantly ($p = 0.0007$) higher INF-γ levels by 443% than non-vaccinated infected males. Vaccination also increased INF-γ levels in non-infected subjects regardless of gender, with vaccinated non-infected females showing significantly ($p = 0.0005$) higher INF-γ levels by 113% compared to non-vaccinated non-infected females. In males, the difference was more pronounced, with vaccinated non-infected males displaying significantly ($p <$

0.0001) higher INF-γ levels by 240% than non-vaccinated non-infected males (Fig. 1A).

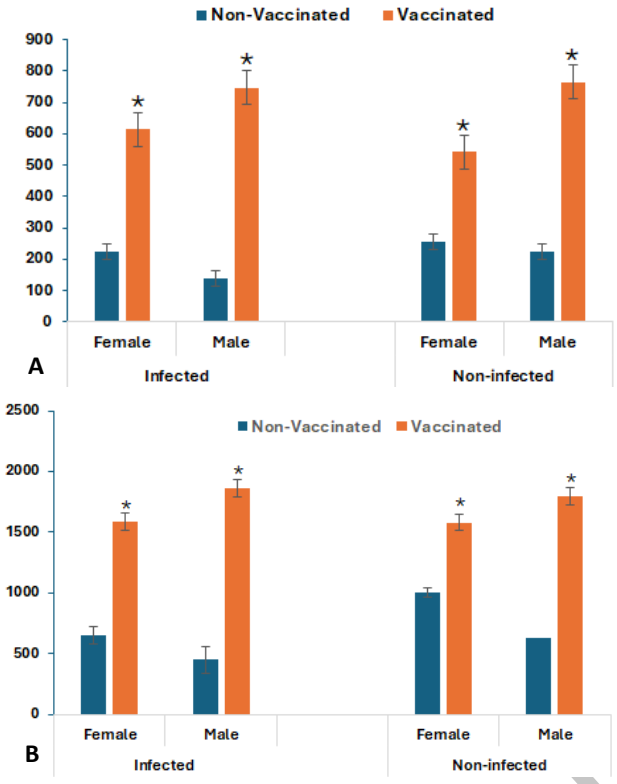

Fig. 1. Comparison of serum INF-γ levels (pg/ml) (A) and TNF-α levels (pg/ml) (B) between vaccinated and non-vaccinated patients, stratified by infection status. Data are expressed as mean ± SEM. *: Significant difference between vaccinated and non-vaccinated at $p < 0.05$.

The vaccinated subjects had higher serum INF-γ levels than the non-vaccinated whether they were infected or non-infected. The gender and infection status had no significant effect on the INF-γ levels. The vaccinated subjects regardless of the gender had significantly higher INF-γ levels than the non-vaccinated subjects. Interestingly, the total vaccinated males had significantly ($p = 0.017$) higher INF-γ levels than the total vaccinated females. The main contributor to this significant elevation is the level of INF-γ in non-infected vaccinated males. This gender differentiation was not evident in the infected group, probably due to the small sample size.

A limitation of our study is that we only measured INF-γ production in patient blood. While T cells are a primary source of INF-γ, other cells such as B cells and antigen-presenting cells can also produce INF-γ (Burke and Young, 2019; Siegel *et al*., 2021; Alexander *et al*., 2021). This method does not encompass the full spectrum of T cell responses, which may be indicated by other markers of a protective immune response following vaccination.

Table III. Assessment of INF-γ (pg/ml) and TNF-α by gender in vaccinated and non-vaccinated patients.

Gender	Non-vaccinated	Vaccinated
INF-γ		
Female	238.5 ±59.3 (n=14)	577.9 ±57.3* (n=19)
Male	181.4 ±56.4 (n=26)	756.3 ±43.6* (n=21)
TNF-α		
Female	928.3±109.9 (n=14)	1582.5±103.2* (n=19)
Male	607.4±116.8 (n=26)	1818.2±207.0* (n=21)

Data are expressed as mean ± SEM, n is given in parenthesis. *: Significant difference between vaccinated and non-vaccinated at $p < 0.05$.

TNF-α levels

In the current study, we have also assessed serum levels of tumor necrosis factor-alpha (TNF-α). TNF-α is a pivotal cytokine involved in the inflammatory response and immune regulation, with significant implications in COVID-19 pathophysiology. TNF-α is released by immune cells in response to viral infections, including SARS-CoV-2, initiating and amplifying the inflammatory cascade. This cytokine is particularly implicated in the cytokine storm observed in severe COVID-19 cases, where elevated TNF-α levels, alongside other cytokines, lead to extensive inflammation and tissue damage, notably in the lungs. Studies have consistently demonstrated that elevated TNF-α levels correlate with more severe COVID-19 symptoms, including acute respiratory distress syndrome (ARDS) and multi-organ dysfunction, thus impacting disease outcomes adversely (Liu *et al*., 2020; Del-Valle *et al*., 2020).

The serum level of TNF-α significantly ($p < 0.0001$) increased by 124% in non-infected vaccinated volunteers compared to non-infected, non-vaccinated controls (Table I). Among infected patients, TNF-α levels significantly ($p = 0.0003$) rose by 224% in vaccinated subjects compared to non-vaccinated ones (Table I). Assessment of TNF-α concentrations, stratified by gender and infection status, showed no significant changes between infected and non-infected females, as well as males (Table II).

However, TNF-α levels based on gender and vaccination status demonstrated significant differences between vaccinated and non-vaccinated females and males, highlighting vaccination's impact on TNF-α responses. TNF-α levels in vaccinated females were significantly ($p = 0.0002$) higher by 71% compared to non-vaccinated females, while in vaccinated males, levels were significantly ($p < 0.0001$) higher by 199% (Table III).

Figure 1B provides a comparative analysis of TNF-α levels between vaccinated and non-vaccinated individuals, stratified by gender and infection status. Vaccinated infected females showed significantly ($p= 0.016$) higher TNF-α levels by 145% compared to non-vaccinated infected peers. Similarly, vaccinated infected males exhibited significantly ($p= 0.006$) higher TNF-α levels by 311% than non-vaccinated infected males. Vaccination also increased TNF-α levels in non-infected subjects regardless of gender, with vaccinated non-infected females showing significantly ($p= 0.002$) higher TNF-α levels by 57% compared to non-vaccinated non-infected females. In males, the difference was more pronounced, with vaccinated non-infected males displaying significantly ($p < 0.0001$) higher TNF-α levels by 186% than non-vaccinated non-infected males (Fig. 1B). The non-vaccinated non-infected females had significantly higher TNF-α levels than the counter males. These gender differentiations in TNF-α and INF-γ warrant further investigation into the underlying mechanism.

Given its role in COVID-19 pathogenesis, TNF-α has emerged as a promising therapeutic target. Therapies aimed at blocking TNF-α activity, such as monoclonal antibodies like infliximab, have been investigated to mitigate the cytokine storm and potentially alleviate disease severity in critically ill COVID-19 patients (Tracey *et al*., 2008). Clinical studies have underscored the utility of TNF-α as a biomarker for disease progression and a predictor of clinical outcomes in COVID-19 patients, shedding light on its complex interplay with other cytokines and immune responses during infection (Chen *et al*., 2020; Zhang *et al*., 2020). TNF-α plays a critical role in driving inflammation and exacerbating disease severity in COVID-19. Ongoing research efforts continue to elucidate its precise mechanisms and therapeutic implications, aiming to develop targeted interventions that can effectively manage severe manifestations of the disease.

The findings of our study are consistent with prior research on COVID-19 vaccine effectiveness in preventing infection among patients receiving anti-TNF therapy, demonstrating no heightened incidence of COVID-19 in these individuals (Feng *et al*., 2021). Extensive research has investigated the correlation between vaccine efficacy and antibody titers across various COVID-19 vaccines, (Krammer, 2021; AbdulKhaliq *et al*., 2023; El-Bakri *et al*., 2023), highlighting the protective function of antibodies against COVID-19. TNF-α and INF-γ, as utilized in our study, have demonstrated consistent results aligned with the WHO International Standard and anti-SARS-CoV-2 antibody (WHO, 2020; Rubio-Acero *et al*., 2021).

CONCLUSION

The study highlights the complex interactions among vaccination status, immune markers (INF-γ and TNF-α), gender, and COVID-19 infection. Vaccinated individuals, regardless of infection status, showed higher INF-γ levels, indicating robust T cell-mediated immunity against variants like Omicron. Lower INF-γ levels in non-responsive individuals may increase susceptibility to severe COVID-19 outcomes, especially with emerging variants. Notably, vaccinated males had significantly higher INF-γ levels than females, driven by non-infected cases. Gender differences in TNF-α levels were observed among non-vaccinated individuals and non-infected where females had higher TNF-α levels than males. These findings underscore the need for further investigation into immune response mechanisms. Overall, vaccination induces strong seroconversion, protecting against COVID-19. Ongoing vigilance and adaptive vaccination strategies, including boosters, are crucial amid evolving variants. The gender differentiations reported in this study warrant further investigation into the underlying mechanism.

DECLARATIONS

Ethical statement and IRB approvals

The experimental procedures and protocols were approved by the institutional ethics committee of the College of Applied Sciences at the University of Fallujah (UF/CAS/12/23/20) and were conducted in accordance with the standards outlined in the "Guide to Ethics of Scientific Research on Human Subjects".

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20240917130242

Statement of conflicts of interest

The authors have declared no conflict of interest.

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Supplementary Material

Gender Differences in Cytokine Response Post-COVID-19 Vaccination: Insights from Iraqi Subjects

Roua J. AbdulKhaliq[1], Mohammed YI Al-Hamadani[1] and Wael M. El-Sayed[2]*

[1]Department of Pathological Analysis, College of Applied Science, University of Fallujah, Iraq
[2]Department of Zoology, Faculty of Science, Ain Shams University, Abbassia 11566, Cairo, Egypt

Supplementary Table S1. Characteristics (gender, age, vaccination, and infectious status) of the participants in the study.

Status	Non-vaccinated (n=40)	Vaccinated (n=40)	Total (80)
Female: Male	14:26	19:21	33:47
Age (Year) range	15-56	17-63	15-63
No. of non-infected with COVID	34	29	63
No. of infected with COVID	6	11	17

* Corresponding author: wael_farag@sci.asu.edu.eg, waelelhalawany@hotmail.com
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