

Monitoring of IFN- γ level in Pfizer/BioNTech vaccinated Iraqi's people

Mayyadah Rafeeq¹, Majid Jabir²

^{1,2}Applies science department, University of technology- Iraq

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (Covid-19) have afflicted tens of millions of people in a worldwide pandemic. The most reliable approach to stop the spread of infectious illnesses is through vaccination. Safe and effective vaccines are needed urgently.

Methods: The study had eighty-one (81) participants ranging from 18 to 66 years old who were recently injected with COVID-19 mRNA Pfizer/BioNTech [BNT162b2] vaccines. They received two vaccine doses of 30 μ g, 0.3 mL injections twenty-one (21) days apart. Before the first vaccination, blood samples were collected. This procedure was repeated on days 7-10 following the first immunization, and on 7-10 days following the second dosage. All samples were tested for IFN- γ using a High Sensitivity Human ELISA Kit corresponding to each marker (Elabscience/United State).

Results: Compared to before vaccination and the first dosage, IFN-g levels were higher after the second dose.

Conclusions: Our study demonstrated that vaccinations caused Th1 biases in all groups.

Keywords: Covid-19, Pfizer/BioNTech, vaccine, IFN- γ .

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has resulted in a public health crisis with serious long-term social and economic impact. Its growth has had catastrophic impacts in many nations. The pathogenic mechanism of the extremely contagious COVID-19 has received a lot of interest. COVID-19 is caused by an infection from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus [1], The main method of transmission for SARS-CoV-2 is airborne respiratory droplets [2]. SARS-CoV-2 is a combination of serious pathogenicity (i.e., able to cause disease) and being highly infectious. This infectivity is made worse by the fact that virus transmission can be done by asymptomatic and pre-symptomatic individuals.

This is unlike SARS-CoV-1 and MERS-CoV, which were transmitted by symptomatic patients and, thus could be contained more efficiently [3,4].

The "cytokine storm" occurrence, which is brought on by the too much production of pro-inflammatory cytokines, is associated with the COVID-19 disease. The root cause of CS is an uncontrolled immune response that result from immune cells, such as lymphocytes and macrophages, to continually activate and expand as well as release vast quantities of cytokines, causing a cytokine storm.

Address for correspondence: Majid Jabir
Applies science department, University of technology- Iraq
Email: 100131@uotechnology.edu.iq

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: pnrjournal@gmail.com

How to cite this article: Mayyadah Rafeeq, Majid Jabir, Monitoring of IFN- γ level in Pfizer/BioNTech vaccinated Iraqi's people, J PHARM NEGATIVE RESULTS 2022;13: 612-616.

Access this article online

Quick Response Code:



Website:
www.pnrjournal.com

DOI:
10.47750/pnr.2022.13.04.081

The clinical results of CS are related to activity of proinflammatory cytokines such IL-1, IL-6, IL-18, IFN- γ and TNF- α [5].

The SARS-CoV-2 virus triggers a strong host defense that causes an excessive inflammatory response. According to a research by Ruan et al., of a 68 fatal instances, 36 patients (53%) died from respiratory failure, five (7%) from circulatory failure due to myocardial injury, 22 (33%) from both, and the last five from undetermined causes, and they hypothesized that fulminant myocarditis or virus-activated "cytokine storm syndrome" would be the causes of COVID-19 death [6]. The immune system will launch a fierce attack on the body in response to the cytokine storm, in acute cases of SARS-CoV-2 infection, similar to what happens with SARS-CoV and MERS-CoV infection, the virus can induce ARDS, multiple organ failure, and ultimately death [7]. Therefore, it is of utmost importance for all of humanity to find effective vaccinations against SARS-CoV-2 infection.

Countries all over the globe are competing with one another to find and implement the best preventative, curative, and prophylactic measures to lower the morbidity and death brought on by this virus [8,9]. The most reliable, cost-efficient, and safe method of pandemic control is vaccination. Vaccines work by triggering the body's natural immunological response. As soon as the Chinese government published the genetic sequence of the new coronavirus in early 2020, manufacturing companies for vaccines started to take action all around the world. Among the top brands are Sinovac, CanSino, AstraZeneca, Moderna from the United States and a vaccine developed jointly by the United States and Germany by Pfizer-BioNTech. A novel method for creating SARS-CoV-2 vaccines is messenger RNA (mRNA) [10]. In this study, we compared the IFN- γ responses to the 1st and 2nd dose of the BNT162b2 mRNA (Pfizer/BioNTech) vaccine in vaccinated participants with diseases (hypertension, diabetes, and individuals with hypertension, diabetes, and heart disease) to healthy participants.

Materials and Methods

Study Design and Participants

The present study included eighty-one (81) participants who were recently vaccinated with mRNA Pfizer/BioNTech [BNT162b2] vaccines. This exploratory analysis used samples from healthy participants from 18 to 66 years old who have already received two vaccine injections 21 days apart at a dose of (30 μ g, 0.3 mL). The participants were divided into four groups, first the healthy subjects, the second with hypertension, the third with diabetes, and finally those with hypertension, diabetes, and heart disease. All participants received their vaccination between October 2021 and March 2022. Blood samples were collected as previously described. Samples collected at baseline (before first vaccination), (Day 7-10, after first vaccination), and

(Days 7-10, after second vaccination), were analysed. Informed consent in oral and written form were obtained from all the participants.

Specimen Collection and Preparation

A. Specimen collection

Participants' serum samples were extracted from blood samples in serum collection tubes by centrifugation for 10 minutes at 1,000–2,000 \times g., and serum fractions were organized, collected, and frozen for later use. Before analysis, frozen materials were thawed at room temperature for 1 hour. Before analysis, thawed samples were vortexed. Analyzing preserved samples from multiple time periods of the same donor was done in parallel studies.

B. ELISA technique

Levels IFN- γ were quantified using High Sensitivity Human ELISA Kit identical for each marker (Elabscience/United State). Briefly, 50 μ L of standard or sample was added to each well and incubated for 90 minutes at 37 $^{\circ}$ C. Then, the liquid was eliminated and 50 μ L of biotinylated detection Ab was added and incubated for one hour at 37 $^{\circ}$ C. Next, the solution is aspirated from the wells and washed three times. Then, 50 μ L of HRP Conjugate was added and incubated for 30 minutes at 37 $^{\circ}$ C. After that, the solution was aspirated from the wells and washed five times. Then, 50 μ L of substrate reagent was added and incubated for 15 minutes at 37 $^{\circ}$ C. Finally, 25 μ L of stop solution was added and the OD value was determined at 450 nm instantly.

Statistical analysis

GraphPad prism7 was used to analyze the data [11, 12]. Results are represented as mean \pm SD [13].

Result

Eighty-one were vaccinated with mRNA Pfizer/BioNTech [BNT162b2] COVID-19 vaccine. The age of entire groups (mRNA Pfizer/BioNTech) ranged between (18-66) years old; 69 were women and 12 were men. vaccinated individuals were included 45 healthy individuals, 15 with hypertension, 12 with diabetes, and 9 with hypertension, diabetes, and heart disease. IFN- γ measured for all participants (before the first vaccination), (Days 7–10, following the first immunization), and (Days 7–10, following the second immunization) using High Sensitivity Human ELISA Kit corresponding for each marker (Elabscience/United State) as shown in Figures 1-4 respectively.

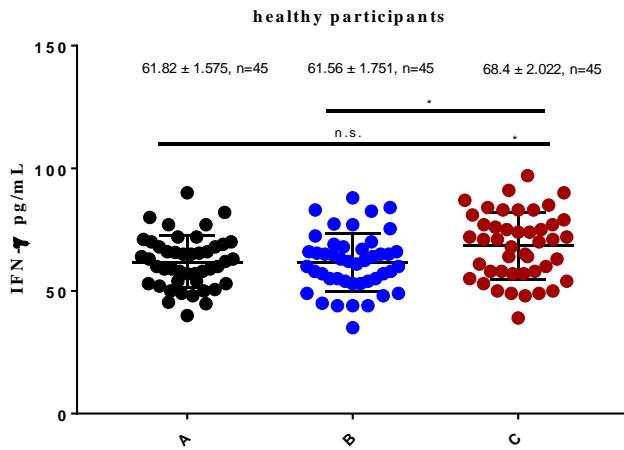


Figure 1: Dynamic changes in IFN- γ in COVID-19 vaccinated healthy participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

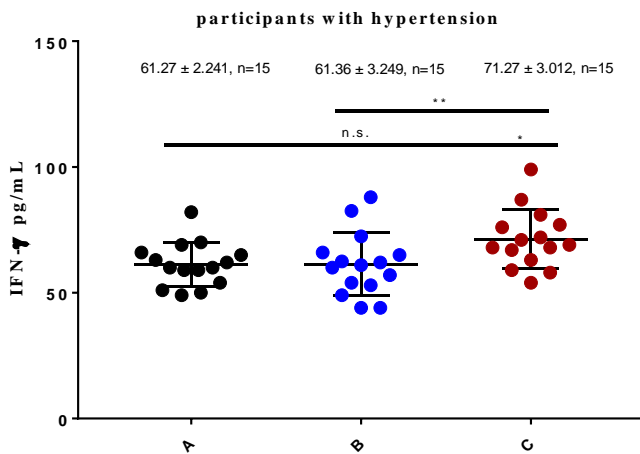


Figure 2: Dynamic changes in IFN- γ in COVID-19 vaccinated hypertension participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

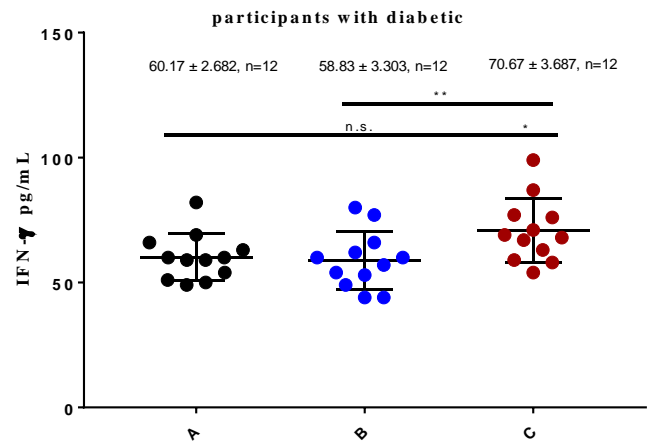


Figure 3: Dynamic changes in IFN- γ in COVID-19 vaccinated diabetic participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

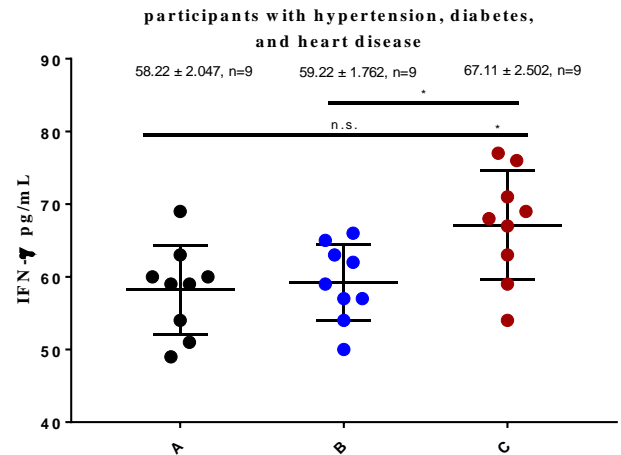


Figure 4: Dynamic changes in IFN- γ in COVID-19 vaccinated hypertension, diabetic, and heart diseases participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

Discussion

The creation of a COVID-19 vaccine is believed to be a necessary and essential part of the worldwide effort to contain this pandemic, and several businesses are working to provide a safe and efficient vaccine [8,9]. Anti-SARS CoV-2 mRNA a breakthrough vaccine called BNT162b2 is being used to immunize countless numbers of individuals worldwide. It is based on a genetically modified RNA that can produce a protein in the treated people that triggers an immune response, providing the recipients of the vaccine with immunity versus SARS CoV-2.

The generation of interferon-gamma (IFN- γ) by T cells is a crucial element of the Th1 response. IFN- γ is also necessary

for the establishment and maintenance of type 1 and antiviral immune responses [13,14]. Natural killer (NK) cells, CD8 T cells, and Th1 effector CD4 T cells all generate substantial amounts of the inflammatory cytokine IFN- γ . To begin the development of activated T cells into the IFN-producing Th1 state, IFN- itself must be present [15]. It has also been shown that IFN-g influences humoral responses by regulating the Ig isotypes that B cells generate and by promoting long-lived antibody-secreting cells [16,17]. Increased levels of proinflammatory cytokines, such as IFN-g, TNF-a, IL-6, and IL-8, have been linked to severe lung damage and unfavorable outcomes from SARS-CoV or MERS-CoV infection, according to earlier research [18-21]. Decreased IFN- expression in CD4+ T cells and lymphopenia (in CD4+ and CD8+ T cells) have also been linked to severe COVID-19 in a number of studies [22,23].

The majority of researches have reported the clear immunological dysfunction in moderate and severe illness, including decreased expression of IFN- γ by CD4 + T, CD8 + T, and NK cells [24]. The same author discovered that IFN- production by CD4 + T cells was often lower in severe cases compared to moderate instances. It has recently been shown that IFN- γ secretion levels by CD4+ and CD8+ T cells from individuals with moderate illness are comparable to those in critically sick patients. In this instance, IFN- γ levels reduced in moderate patients while increasing with time in severely sick patients. The virus may boost the release of these cytokines, though, since there was a significant link between viral load and IFN- γ . [25]. In patients with severe COVID-19 illness, IFN- α , IFN- γ , and TNF- α levels were shown to be high and to be linked with viral load [26]. According to Zhou et al., ISGs have been shown to be highly expressed in COVID-19 patients, to the point where they may be detrimental [27].

IFN- γ has a significant role in cellular immunity, has immunomodulatory actions that improve antigen presentation and processing, stimulate leukocyte trafficking, induce an antiviral status, promote antimicrobial responses, and affect cellular proliferation and apoptotic [28]. In 59 nations, research by Hussein et al. assessed their possible correlation with the BCG, HAV, and flu vaccines as well as their natural infections against COVID-19 infection. They also looked at the associated death rate. It is suggested that the BCG vaccination, together with the high prevalence of viral diseases such hepatitis A and influenza, which cause a greater level of IFN- γ among people in countries with lower COVID-19 mortality, may assist to explain the mortality variations between nations [29].

In contrast to Th2 (IL-13+, GATA3+) or Th17 (IL-17+, ROR γ T+) cells, mRNA COVID-19 vaccines are known to primarily generate Th1 CD4+ T cells expressing IL-2, IFN-gamma, and the transcription factor Tbet [30]. During adaptive immunity, T cells are the main generator of IFN- γ . In a research by Lozano-Ojalvo et al., it was shown that the SARS-CoV-2 spike and peptide pools triggered the release of IFN-gamma in COVID-19 recovered effector

antigen-specific CD4+ effector T cells, but not in naive donors, and COVID-19 recovered patients mount a greater IFN-gamma response compared to naive participants, according to IFN-gamma produced 10 days following the initial BNT162b2 vaccine dosage. While recovered people reduced their IFN-gamma production after day 10 of the second dosage, naive individuals had a rise in IFN-gamma to substantial levels [31].

In a study by Bergamaschi et al. that examined the cytokine reactions to the first and second doses of the BNT162b2 mRNA vaccine (Pfizer/BioNtech) in antigen-naive and previously infected participants, it was discovered that there was a similar pattern of cytokine expression at 24 hours after vaccination between vaccine recipients with pre-existing SARS-CoV-2 immunity who received the first vaccine dose and antigen-naive individuals after the second vaccine dose. For the quick attraction and activation of effector immune cells, it is suggested that anamnestic responses be induced with increased levels of IFN- γ and TNF-a [32]. According to our findings, INF-g levels in all groups rose after the second dosage in comparison to both before and after the first dose of the vaccination.

Conclusions:

The vaccine polarizes the T-cell response towards type 1 immunity by inducing IFN- γ production, which inhibits Th2 cell differentiation and induce antiviral immune responses.

REFERENCES

- [1] Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., and Collaboration, H. L. H. Across Speciality (2020). COVID-19: Consider cytokine storm syndromes and immunosuppression. *The Lancet* (London, England), 395(10229), 1033.
- [2] Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., ... & Feng, Z. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *New England journal of medicine*.
- [3] Hoehl, S., Rabenau, H., Berger, A., Kortenbusch, M., Cinatl, J., Bojkova, D., Behrens, P., Böddinghaus, B., Götsch, U., Naujoks, F., Neumann, P., Schork, J., Tiarks-Jungk, P., Walczok, A., Eickmann, M., Vehreschild, M. J. G. T., Kann, G., Wolf, T., Gottschalk, R., and Ciesek, S. (2020). Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. *New England Journal of Medicine*, 382(13), 1278-1280.
- [4] Li, R., Pei, S., Chen, B., Song, Y., Zhang, T., Yang, W., and Shaman, J. (2020). Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science*, 368(6490), 489-493.
- [5] Shimizu, M. (2019). Clinical features of cytokine storm syndrome. In *Cytokine storm syndrome* (pp. 31-41). Springer, Cham.
- [6] Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive care medicine*, 46(5), 846-848.
- [7] Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., ... & Wang, F. S. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet respiratory medicine*, 8(4), 420-422.
- [8] Amanat, S. A. R. S., and Krammer, F. S. CoV-2 Vaccines: Status Report. *Immunity*, (52).
- [9] Dhama, K., Sharun, K., Tiwari, R., Dadar, M., Malik, Y. S., Singh, K.

- P., & Chaicumpa, W. (2020). COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. *Human vaccines & immunotherapeutics*, 16(6), 1232-1238.
- [10] Bukhari, M. H., Syed, M., and Zain, S. (2021). The Differences between Traditional Vaccines and RNA Vaccines: Safety, Efficacy, Reliability and Future of COVID-19 Vaccines. *Annals of King Edward Medical University*, 27(2).
- [11] Ali, I. H., Jabir, M. S., Al-Shmgani, H. S., Sulaiman, G. M., and Sadoon, A. H. (2018, May). Pathological and immunological study on infection with *Escherichia coli* in ale balb/c mice. In *Journal of Physics: Conference Series* (Vol. 1003, No. 1, p. 012009). IOP Publishing.
- [12] Younus, A., Al-Ahmer, S., and Jabir, M. (2019). Evaluation of some immunological markers in children with bacterial meningitis caused by *Streptococcus pneumoniae*. *Research Journal of Biotechnology*, 14, 131-133.
- [13] Radhi, M. M., Abdullah, H. N., Jabir, M. S., and Al-Mulla, E. A. J. (2017). Electrochemical effect of ascorbic acid on redox current peaks of CoCl₂ in blood medium. *Nano Biomed. Eng*, 9(2), 103-106.
- [14] Schroder, K., Hertzog, P. J., Ravasi, T., and Hume, D. A. (2004). Interferon- γ : an overview of signals, mechanisms and functions. *Journal of leukocyte biology*, 75(2), 163-189.
- [15] Baumgarth, N. (2021). The shaping of a B cell pool maximally responsive to infections. *Annual review of immunology*, 39, 103-129.
- [16] Stone, S. L., Peel, J. N., Scharer, C. D., Riskey, C. A., Chisolm, D. A., Schultz, M. D., Yu, B., Ballesteros-Tato, A., Wojciechowski, W., Mousseau, B., Misra, R. S., Hanidu, A., Jiang, H., Qi, Z., Boss, J. M., Randall, T. D., Brodeur, S. R., Goldrath, A. W., Weinmann, A. S., Rosenberg, A. F., & Lund, F. E. (2019). T-bet transcription factor promotes antibody-secreting cell differentiation by limiting the inflammatory effects of IFN- γ on B cells. *Immunity*, 50(5), 1172-1187.
- [17] CHIEN, J. Y., HSUEH, P. R., CHENG, W. C., YU, C. J., and YANG, P. C. (2006). Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. *Respirology*, 11(6), 715-722.
- [18] Chu, H., Zhou, J., Wong, B. H. Y., Li, C., Cheng, Z. S., Lin, X., Poon, V. K., Sun, T., Lau, C. C., Chan, J. F., To, K. K., Chan, K., Lu, L., and Yuen, K. Y. (2014). Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. *Virology*, 454, 197-205.
- [19] Zhou, J., Chu, H., Li, C., Wong, B. H. Y., Cheng, Z. S., Poon, V. K. M., Sun, T., Lau, C. C. Y., Wong, K. K. Y., Chan, J. Y. W., Chan, J. F. W., To, K. K. W., Chan, K. H., Zheng, B. J., and Yuen, K. Y. (2014). Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. *The Journal of infectious diseases*, 209(9), 1331-1342.
- [20] Kong, S. L., Chui, P., Lim, B., and Salto-Tellez, M. (2009). Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. *Virus research*, 145(2), 260-269.
- [21] Pedersen, S. F., and Ho, Y. C. (2020). SARS-CoV-2: a storm is raging. *The Journal of clinical investigation*, 130(5), 2202-2205.
- [22] Lagunas-Rangel, F. A., and Chávez-Valencia, V. (2020). High IL-6/IFN- γ ratio could be associated with severe disease in COVID-19 patients. *Journal of medical virology*.
- [23] Lucas, C., Wong, P., Klein, J., Castro, T. B., Silva, J., Sundaram, and Iwasaki, A. (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature*, 584(7821), 463-469.
- [24] Chen, G., Wu, D. I., Guo, W., Cao, Y., Huang, D., Wang, H., Wang, T., Zhang, X., Chen, H., Yu, H., Zhang, X., Zhang, M., Wu, S., Song, J., Chen, T., Han, M., Li, S., Luo, X., Zhao, J., and Ning, Q. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation*, 130(5), 2620-2629.
- [25] Zhou, Z., Ren, L., Zhang, L., Zhong, J., Xiao, Y., Jia, Z., Guo, L., Yang, J., Wang, C., Jiang, S., Yang, D., Zhang, G., Li, H., Chen, F., Xu, Y., Chen, M., Gao, Z., Yang, J., Dong, J., Liu, B., Zhang, X., Wang, W., He, K., Jin, Q., Li, M., and Wang, J. (2020). Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell host & microbe*, 27(6), 883-890.
- [26] Li, Q., Li, J., Tian, J., Zhu, B., Zhang, Y., Yang, K., Ling, Y., and Hu, Y. (2012). IL-17 and IFN- γ production in peripheral blood following BCG vaccination and *Mycobacterium tuberculosis* infection in human. *European review for medical and pharmacological sciences*, 16(14), 2029-2036.
- [27] Schepers, K., Dirix, V., Mouchet, F., Verscheure, V., Lecher, S., Lochter, C., and Mascart, F. (2015). Early cellular immune response to a new candidate mycobacterial vaccine antigen in childhood tuberculosis. *Vaccine*, 33(8), 1077-1083.
- [28] Kak, G., Raza, M., and Tiwari, B. K. (2018). Interferon-gamma (IFN- γ): Exploring its implications in infectious diseases. *Biomolecular concepts*, 9(1), 64-79.
- [29] Husseini, A. A., Kamil, A. A., and Aloudal, M. R. (2021). COVID-19 Disease and Interferon-[gamma]: Has it a Protective Impact on Mortality?. *Erciyes Medical Journal*, 43(2), 116-122.
- [30] Sahin, U., Muik, A., Vogler, I., Derhovanessian, E., Kranz, L. M., Vormehr, M., ... & Türeci, Ö. (2021). BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. *Nature*, 595(7868), 572-577.
- [31] Lozano-Ojalvo, D., Camara, C., Lopez-Granados, E., Nozal, P., del Pino-Molina, L., Bravo-Gallego, L. Y., Paz-Artal, E., Pion, M., Correa-Rocha, R., Ortiz, A., Lopez-Hoyos, M., Iribarren, M. E., Portoles, J., Rojo-Portoles, M. P., Ojeda, G., Cervera, I., Gonzalez-Perez, M., Bodega-Mayor, I., Montes-Casado, M., Portoles, P., Perez-Olmeda, M., Oteo, J., Sanchez-Tarjuelo, R., Pothulova, V., Schwarz, M., Brahmachary, M., Tan, A. T., Bert, N. L., Berin, C., Bertoletti, A., Guccione, E., and Ochando, J. (2021). Differential effects of the second SARS-CoV-2 mRNA vaccine dose on T cell immunity in naive and COVID-19 recovered individuals. *Cell reports*, 36(8), 109570.
- [32] Bergamaschi, C., Terpos, E., Angel, M., Bear, J., Rosati, M., Stellars, D., Karaliota, S., Apostolakou, F., Bagratuni, T., Patseas, D., Gumeni, S., Trougakos, I., Dimopoulos, M. A., Felber, B. K., and Pavlakis, G. N. (2021). Systemic IL-15, IFN- γ and IP-10/CXCL10 Signature Associated With Effective Immune Response in BNT162b2 mRNA Vaccine Recipients.