Evaluation Of TLR-4 Level Among Pfizer/Biontech Vaccinated People

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Abstract
Background: Over 221 million illnesses and over 4.6 million fatalities caused by the coronavirus disease 2019 (COVID-19) were confirmed as of September 2021, according to the WHO. Accelerated vaccine development initiatives and widespread immunization campaigns were implemented to address the enormous morbidity and death load. The most reliable approach to stop the spread of infectious illnesses is through vaccination. 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 µg, 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 TLR-4 using a High Sensitivity Human ELISA Kit (Elabscience/United State). Results: TLR-4 levels did not significantly rise in any of the groups. Conclusions: The elevated TLR-4, was not significantly rise in any of the studied groups of our study. Further clinical studies are needs to understand the role of other immunological factors in safety of COVID-19 vaccines.
Key words: Covid-19, Pfizer/BioNTech [BNT162b2] vaccine, TLR-4

1. Introduction

A pneumonia of an unknown origin surfaced in Wuhan, China, around the end of 2019. Acute respiratory failure was the primary reason why most patients arrived at the hospital, while some also experienced serious consequences [1]. It was eventually determined that the new coronavirus SARS-CoV-2(severe acute respiratory syndrome coronavirus 2) was the cause of this illness, (COVID-19, coronavirus disease 2019) [2]. A non-segmented, enveloped, positive-sense RNA virus at approximately 30-kilobase, SARS-CoV-2 is a developing virus with enormous worldwide relevance. Belonging to the family Coronaviridae (group beta - coronavirus in the subfamily Orthocoronavirinae) [3,4], SARS-CoV-2 virions (i.e., entire virus particles) are spherical in shape like other coronaviruses. With a 65–125 nm diameter [5], its most notable feature is the spike projection coming from the virions' surface. These spike projections make the virus resemble a crown, and thus the name coronavirus [6,7]. TLRs are the primary immune system controllers and are a subclass of pathogen-recognition receptors (PRR). The phylogenetically maintained TLR family triggers an innate immune response in response to SARS-CoV-2 infection. The protecting and maladaptive responses to lower respiratory viral infection are thought to depend on TLRs [8]. The nasal cavity and the submucosa of the respiratory tract both include mast cells that express TLR. These function as a sort of microbial defense barrier. Additionally, SARS-CoV-2 can activate mast cells, which results in the early release of inflammatory substances such as protease and histamine. Late activation results in the production of pro-inflammatory IL-1 family members, such as IL-1 and IL-33, as well as IL-6 and TNF-α, which further activate the macrophages and cause airway inflammation [9]. According to Conti et al. [10], activation of the TLRs during COVID-19 infection may cause the release of IL-1β and other pro-inflammatory cytokines. People suffering from severe COVID-19 have greater serum levels of pro-inflammatory cytokines and chemokines, including as IL-2, IL-6, IL-1β, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α, and TNF-α, compared to individuals who are not infected [11–13]. Organ damage and host cell death result from this pro-inflammatory state and the virus's direct infection. DAMPS like HMGB1 and oxPAPC are released by damaged host cells and are able to increase inflammation via TLR binding, through the MyD88-NF-B pathway. A negative feedback loop results when pro-inflammatory conditions are followed by an increase in the production of anti-inflammatory cytokines. For downstream of MyD88, IL-10 acts as a suppressor of the immunological response. According to translational research, IL-10 is raised in COVID-19 and is greater in patients who are severely sick, just like pro-inflammatory cytokines [14,15]. Furthermore, the interaction of TLRs with virus particles results in immunopathological effects that lead to mortality in COVID-19 patients [16]. Another SARS-CoV2 investigations have demonstrated the pathogenic function of TLR4 in COVID19 patients' increased inflammation, which resulted in NETosis and inflammasome activation [17-19]. The most reliable, cost-efficient, and safe method of pandemic control is vaccination. 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

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messenger RNA (mRNA) [20]. Here, we characterized the TLR-4 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) in comparison with healthy participants.

2. 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 µg, 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 x 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 of TLR-4 were quantified using a High Sensitivity Human ELISA Kit identical for each marker (Elabscience/United State). Briefly, 50µL of standard or sample was added to each well and incubated for 90 minutes at 37 ℃. Then, the liquid was eliminated and 50µL of biotinylated detection Ab was added and incubated for one hour at 37 ℃. Next, the solution is aspirated from the wells and washed three times. Then, 50µL of HRP Conjugate was added and incubated for 30 minutes at 37 ℃. After that, the solution was aspirated from the wells and washed five times. Then, 50µL of substrate reagent was added and incubated for 15 minutes at 37 ℃. Finally, 25µL of stop solution was added and the OD value was determined at 450 nm instantly.

3. Statistical Analysis

GraphPad prism7 was used to analyze the data [21,22]. Results are represented as mean± SD [23].

4. Results

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. TLR-4 was 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 Figure 1-4. All results are displayed by GraphPad Prism 7.

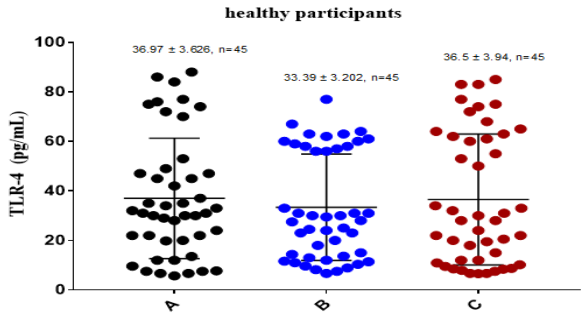

Figure1: Dynamic changes in TLR-4 in COVID-19 vaccinated healthy participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

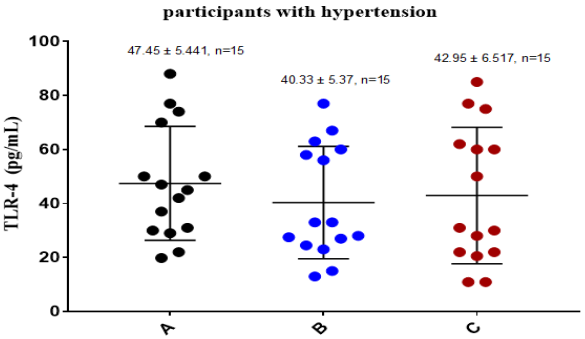

Figure 2: Dynamic changes in TLR-4 in COVID-19 vaccinated hypertension participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

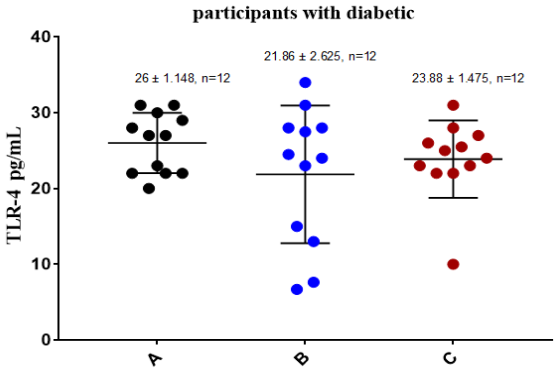

Figure 3: Dynamic changes in TLR-4 in COVID-19 vaccinated diabetic participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

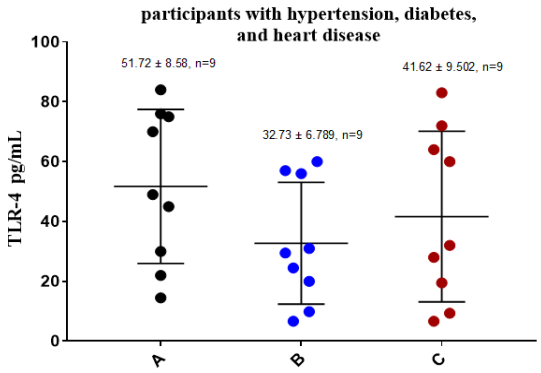

Figure 4: Dynamic changes in TLR-4 in COVID-19 vaccinated hypertension, diabetic, and heart diseases participants. A: before the first vaccine, B: after (7-10 days) of vaccine dose, C: after (7-10 days) of the second vaccine dose.

5. 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 [24]. 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 acute type of COVID-19 is characterized by an aberrant and exaggerated immune host reaction that favors a bad prognosis in a large percentage of patients, particularly those with obesity, diabetes, hypertension, and atherosclerosis. Tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) are two pro-inflammatory cytokines that are overexpressed, which are byproducts of the Toll-Like receptors 4 (TLR4) pathway, describes the ongoing inflammation that occurs in various cardiometabolic disorders [25]. According to a recent study, the full-length S protein of the SARS-CoV-2 virus has antigenic epitopes that can bind to the TLR4/MD-2 complex and trigger an immune response [26]. This relationship was shown in an in-silico investigation, which also suggested the possibility that the COVID-19 inflammatory effects may be influenced by the TLR pathway. The scientists' molecular docking investigation showed strong interaction between the viral S protein and human innate immune receptors TLR1, TLR4, and TLR6, with TLR4 having the greatest reported binding energy [27]. As ACE2 expression is increased by TLR4 activation in cells that would normally express it at relatively modest levels, this study offers a mechanism for the increased virulence of SARS-CoV-2 and shows that the influence of PAMPs and VAMPs on TLR4 is extensive. Two scenarios most likely play a part in these outcomes: (i) Through their spike glycoproteins, the SARS-CoV-2 viruses themselves bind to TLR4 to increase ACE2 expression and so improve infectivity and (ii) TLR4 in tissues would be activated by the cleaved S1 glycoprotein subunits that are released into the interstitial space and blood stream. By furin/TMPRSS2 or cathepsins B/L in the endosomes, the outer S1 subunits are separated from the spike [28], During the process of viral entrance, for instance, after cell interaction with ACE2 [29, 30]. Thus, cleaved spike S1 may potentially activate TLR4 intracellularly (perhaps in endosomes), however if it were exported from the cell via the secretory channel during the viral departure phase, it would perform a more important function [31, 32]. Additionally, there is proof that the S1 domain was shed into the extracellular environment, where TLR4 could be activated, especially considering that COVID-19 patients' blood and urine both included spike S1 [33, 34]. Aboudounya et al work's established that the SARS-CoV-2 spike protein S1 domain activates TLR4, suggesting that TLR4 may function as a receptor or accessory factor for the virus [35]. Most individuals with severe COVID-19 have pre-existing illnesses [36], such as diabetes [37]. TLR4 may be responding to both exogenous (PAMPs) and endogenous (DAMPs) ligands during SARS-CoV-2 infection, however a definitive mechanism of activation has not yet been established. Diabetes patients already have increased amounts of PAMPs [38–40], and DAMPs [41], in their blood, which might go worse with a diagnosis of COVID-19. Föhse et al investigation's revealed that following BNT162b2 vaccination, innate immune cells responded less strongly to TLR4 and TLR7/8 ligands while responding more strongly to cytokines produced by fungus [42]. A more balanced inflammatory response to SARS-CoV-2 infection may be indicated by the induction of tolerance towards stimulation with TLR7/8 (R848) or TLR4 (LPS) ligands following BNT162b2 vaccination. One would wonder if such an impact could help to reduce the likelihood of excessive inflammation in COVID-19, one of the leading causes of mortality [43]. In our study, TLR-4 levels did not significantly rise in any of the research groups.

6. Conclusions

In the current study, 81 participants ranging from 18 to 66 years old who were recently vaccinated by mRNA Pfizer/BioNTech [BNT162b2] vaccines. They received two vaccine doses of 30 µg, 0.3 mL injections twenty-one (21) days apart. Before the first vaccination, and after first and second vaccine dose blood samples were collected. TLR-4 was elevated using ELISA test. The results showed that the TLR-4 levels did not significantly rise in any of the groups.

References

[1] Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., ... & Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet, 395(10224), 565-574.
[2] Jiang, S., Shi, Z., Shu, Y., Song, J., Gao, G. F., Tan, W., & Guo, D. (2020). A distinct name is needed for

the new coronavirus. The Lancet, 395(10228), 949.
[3] Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., Meng, J., Zhu, Z., Zhang, Z., Wang, J., Sheng, J., Quan, L., Xia, Z., Tan, W., Cheng, G., and Jiang, T. (2020). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell host & microbe, 27(3), 325-328.
[4] Wang, N., Shang, J., Jiang, S., and Du, L. (2020). Subunit vaccines against emerging pathogenic human coronaviruses. Frontiers in microbiology, 11, 298.
[5] Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell, 181(2), 281-292.
[6] COVID, M. A. S. (2020). infection: Emergence, transmission, and characteristics of human coronaviruses/Muhammad Adnan Shereen, Suliman Khan, Abeer Kazmi, Nadia Bashir, Rabeea Siddique. Journal of Advanced Research, 24(3), 91-98.
[7] Sheahan, T. P., Sims, A. C., Leist, S. R., Schäfer, A., Won, J., Brown, A. J., Montgomery, S. A., Hogg, A., Babusis, D., Clarke, M.O., Spahn, J. E., Bauer, L., Sellers, S., Porter, D., Feng, J. Y., Cihlar, T., Jordan, R., Denison, M. R., and Baric, R. S. (2020). Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nature communications, 11(1), 1-14.
[8] Akira, S., Takeda, K., & Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. Nature immunology, 2(8), 675-680.
[9] Kritas, S. K., Ronconi, G., Caraffa, A. L., Gallenga, C. E., Ross, R., & Conti, P. (2020). Mast cells contribute to coronavirus-induced inflammation: new anti-inflammatory strategy. J Biol Regul Homeost Agents, 34(1), 9-14.
[10] Conti, P., Ronconi, G., Caraffa, A. L., Gallenga, C. E., Ross, R., Frydas, I., & Kritas, S. K. (2020). Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. J Biol Regul Homeost Agents, 34(2), 327-331.
[11] Orlov, M., Wander, P. L., Morrell, E. D., Mikacenic, C., & Wurfel, M. M. (2020). A case for targeting Th17 cells and IL-17A in SARS-CoV-2 infections. The Journal of Immunology, 205(4), 892-898.
[12] Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet, 395(10223), 497-506.
[13] Tan, M., Liu, Y., Zhou, R., Deng, X., Li, F., Liang, K., & Shi, Y. (2020). Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Immunology, 160(3), 261-268.
[14] Han, H., Ma, Q., Li, C., Liu, R., Zhao, L., Wang, W., ... & Xia, Y. (2020). Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerging microbes & infections, 9(1), 1123-1130.
[15] Zhao, Y., Qin, L., Zhang, P., Li, K., Liang, L., Sun, J., ... & Zhang, Y. (2020). Longitudinal COVID-19 profiling associates IL-1RA and IL-10 with disease severity and RANTES with mild disease. JCI insight, 5(13).
[16] Patra, R., Das, N. C., & Mukherjee, S. (2021). Targeting human TLRs to combat COVID-19: A solution? Journal of medical virology.
[17] Cicco, S., Cicco, G., Racanelli, V., & Vacca, A. (2020). Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): two potential targets for COVID-19 treatment. Mediators of inflammation, 2020.
[18] Khadke, S., Ahmed, N., Ahmed, N., Ratts, R., Raju, S., Gallogly, M., ... & Sohail, M. R. (2020). Harnessing the immune system to overcome cytokine storm and reduce viral load in COVID-19: a review of the phases of illness and therapeutic agents. Virology Journal, 17(1), 1-18.
[19] Sohn, K. M., Lee, S. G., Kim, H. J., Cheon, S., Jeong, H., Lee, J., ... & Jo, E. K. (2020). COVID-19 patients upregulate toll-like receptor 4-mediated inflammatory signaling that mimics bacterial sepsis. Journal of Korean medical science, 35(38).
[20] 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).
[21] 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.
[22] 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.
[23] 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.
[24] 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.
[25] Brandao, S. C. S., Ramos, J. D. O. X., Dompieri, L. T., Godoi, E. T. A. M., Figueiredo, J. L., Sarinho, E. S. C., Chelvanambi, S., and Aikawa, M. (2021). Is Toll-like receptor 4 involved in the severity of COVID-19 pathology in patients with cardiometabolic comorbidities? Cytokine & Growth Factor Reviews, 58, 102-110.
[26] Bhattacharya, M., Sharma, A. R., Mallick, B., Sharma, G., Lee, S. S., and Chakraborty, C. (2020). Immunoinformatics approach to understand

molecular interaction between multi-epitopic regions of SARS-CoV-2 spike-protein with TLR4/MD-2 complex. Infection, Genetics and Evolution, 85, 104587.
[27] Choudhury, A., and Mukherjee, S. (2020). In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. Journal of medical virology, 92(10), 2105-2113.
[28] Evans, J. P., and Liu, S. L. (2021). Role of host factors in SARS-CoV-2 entry. Journal of Biological Chemistry, 297(1).
[29] Al-Horani, R. A., Kar, S., and Aliter, K. F. (2020). Potential anti-COVID-19 therapeutics that block the early stage of the viral life cycle: structures, mechanisms, and clinical trials. International Journal of Molecular Sciences, 21(15), 5224.
[30] Xia, S., Lan, Q., Su, S., Wang, X., Xu, W., Liu, Z., Zhu, Y., Wang, Q., Lu, L., and Jiang, S. (2020). The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin. Signal transduction and targeted therapy, 5(1), 1-3.
[31] Duan, L., Zheng, Q., Zhang, H., Niu, Y., Lou, Y., and Wang, H. (2020). The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: implications for the design of spike-based vaccine immunogens. Frontiers in immunology, 11, 576622.
[32] Belouzard, S., Millet, J. K., Licitra, B. N., and Whittaker, G. R. (2012). Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses, 4(6), 1011-1033.
[33] George, S., Pal, A. C., Gagnon, J., Timalsina, S., Singh, P., Vydyam, P., Munshi, M., Chiu, J. E., Renard, I., Harden, C. A., Ott, I. M., Watkins, A. E., Vogels, C. B. F., Lu, P., Tokuyama, M., Venkataraman, A., Casanovas-Massana, A., Wyllie, A. L., Rao, V., Campbell, M., Farhadian, S. F., Grubaugh, N. D., Cruz, C. S. D., Ko, A. I., Perez, A. Z. B., Akaho, E. H., Moledina,, D. G., Testani, J., John, A. R., Ledizet, M., Mamoun, C. B., and Yale IMPACT Team. (2021). Evidence for SARS-CoV-2 spike protein in the urine of COVID-19 patients. Kidney360, 2(6), 924.
[34] Ogata, A. F., Maley, A. M., Wu, C., Gilboa, T., Norman, M., Lazarovits, R., Mao, C., Newton, G., Chang, M., Nguyen, K., Kamkaew, M., Zhu, Q., Gibson, T. E., Ryan, E. T., Charles, R. C., Marasco, W. A., and Walt, D. R. (2020). Ultra-sensitive serial profiling of SARS-CoV-2 antigens and antibodies in plasma to understand disease progression in COVID-19 patients with severe disease. Clinical chemistry, 66(12), 1562-1572.
[35] Aboudounya, M. M., Holt, M. R., and Heads, R. J. (2021). SARS-CoV-2 Spike S1 glycoprotein is a TLR4 agonist, upregulates ACE2 expression and induces pro-inflammatory M1 macrophage polarisation. BioRxiv.
[36] Yang, J., Zheng, Y. A., Gou, X., Pu, K., Chen, Z., Guo, Q., Ji, R., Wang, H., Wang, Y., and Zhou, Y. (2020). Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. International journal of infectious diseases, 94, 91-95.
[37] Muniyappa, R., and Gubbi, S. (2020). COVID-19 pandemic, coronaviruses, and diabetes mellitus. American Journal of Physiology-Endocrinology and Metabolism, 318(5), E736-E741.
[38] Jayashree, B., Bibin, Y. S., Prabhu, D., Shanthirani, C. S., Gokulakrishnan, K., Lakshmi, B. S., Mohan, V., and Balasubramanyam, M. (2014). Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. Molecular and cellular biochemistry, 388(1), 203-210.
[39] Gomes, J. M. G., de Assis Costa, J., and Alfenas, R. D. C. G. (2017). Metabolic endotoxemia and diabetes mellitus: A systematic review. Metabolism, 68, 133-144.
[40] Trøseid, M., Nestvold, T. K., Rudi, K., Thoresen, H., Nielsen, E. W., and Lappegård, K. T. (2013). Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery. Diabetes care, 36(11), 3627-3632.
[41] Shin, J. J., Lee, E. K., Park, T. J., and Kim, W. (2015). Damage-associated molecular patterns and their pathological relevance in diabetes mellitus. Ageing Research Reviews, 24, 66-76.
[42] Föhse, F. K., Geckin, B., Overheul, G. J., van de Maat, J., Kilic, G., Bulut, O., Dijkstra, H., Lemmers, H., Sarlea, A., Reijnders, M., Hoogerwerf, J., Simonetti, T., van Crevel, R., Li, Y., GeurtsvanKessel, C., Domínguez-Andrés, J., and Netea, M. G. (2021). The BNT162b2 mRNA vaccine against SARS-CoV-2 reprograms both adaptive and innate immune responses.
[43] Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., and Wen, C. (2020). Cytokine storm in COVID-19: the current evidence and treatment strategies. Frontiers in immunology, 11, 1708.