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Does Prior Immunization with Measles, Mumps, and Rubella Vaccines Affect the Antibody Response to COVID-19 Antigens?

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ABSTRACT

Background: Incidence and severity of SARS-CoV2 infection are significantly lower in children and teenagers proposing that certain vaccines, routinely administered to neonates and children may provide cross-protection against this emerging infection.

Objective: To assess the cross-protection induced by prior measles, mumps and rubella (MMR) vaccinations against COVID-19.

Methods: The antibody responses to MMR and tetanus vaccines were determined in 53 patients affected with SARS-CoV2 infection and 52 age-matched healthy subjects. Serum levels of antibodies specific for NP and RBD of SARS-CoV2 were also determined in both groups of subjects with ELISA.

Results: Our results revealed significant differences in anti-NP ($P<0.0001$) and anti-RBD ($P<0.0001$) IgG levels between patients and healthy controls. While the levels of rubella- and mumps-specific IgG were not different in the two groups of subjects, measles-specific IgG was significantly higher in patients ($P<0.01$). The serum titer of anti-tetanus antibody, however, was significantly lower in patients compared to healthy individuals ($P<0.01$).

Conclusion: Our findings suggest that measles vaccination trigger those B cells cross-reactive with SARS-CoV2 antigens leading to production of increased levels of measles-specific IgG.

Keywords: Antibody response, Cross-protection, MMR, SARS-CoV2, Tetanus

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INTRODUCTION

Based on statements from the World Health Organization (WHO) and reports from different countries, COVID-19 is a pandemic disease with a greater incidence in the elderly population. So far, more than 25 million people have been infected and almost 850 thousand died from this disease worldwide (1). The severity of COVID-19

increases with age and children have milder clinical symptoms with very low mortality rates. In a study in 2135 reverse transcription polymerase chain reaction (RT-PCR) positive children in an outbreak region in China, only one child died while most cases had no or mild clinical manifestations (2, 3).

There are some probable hypotheses to explain the differences in immune response, severity, and duration of the disease in children compared to adults and elderly people. Among them, one may consider differences in the serum sex hormone levels (4) and differences in the neuro-immune-endocrine interface; for instance, the anterior pituitary hormone responses to environmental and inflammatory stresses via cytokines like interleukin 6 (IL-6) (4-6). Another hypothesis is the influence of prior vaccinations in children who receive a large number of vaccines as part of the Expanded Program of Immunization (EPI) integrated into the national vaccination scheme proposed for all infants and children by the WHO. Many viral and bacterial vaccines are routinely administered to all children after birth, such as BCG, pertussis, tetanus, diphtheria, hepatitis B, measles, mumps and rubella (7). Waning of the immune response occurs for most of these vaccines after several years, depending on the vaccine formulations and physicochemical characteristics of the immunogens (8, 9). Base on the durability of the immune response to these vaccines, they may cross-protect the immunized subjects against other viral or bacterial infections.

Vaccination with the triple measles, mumps, and rubella (MMR) vaccines started in the early 1970s following pandemics of viral infections like measles that caused an estimated 2.6 million deaths each year (7, 10, 11). All these infections are caused by single-stranded ribonucleic acid (ssRNA) viruses similar to the causative agent of COVID-19 infection. We hypothesized that the lower incidence, severity, and mortality of COVID-19 in children might be associated with cross-reactivity of the MMR antibodies produced in children after vaccination with the SARS-CoV 2 antigens. However, these MMR antibodies are likely to gradually decrease with age, particularly in elderly persons.

The present study was designed to examine this hypothesis by analyzing MMR specific antibody titers as well as the serum titers of specific antibodies for the nucleocapsid protein (NP) and receptor-binding domain (RBD) of SARS-CoV2 in the affected patients and healthy subjects. The antibody response to the tetanus vaccine was also investigated in patients and healthy subjects as a control.

MATERIALS AND METHODS

Patients and Healthy Controls

One hundred and five individuals, including 53 COVID-19 patients (38 males, 15 females; mean age, standard deviation: 59.97, 12.30) and 52 healthy subjects (48 male, 4 female; mean age, standard deviation: 52.86, 4.79) were enrolled in this study. The healthy control serum samples were selected from archived sera that were collected before August 2019 with a documented history of MMR and tetanus vaccination. Patients' sera were collected from subjects with RT-PCR positive results who were admitted to the Masih Daneshvari Hospital affiliated to Shahid Beheshti University of Medical Sciences and later fully recovered from the disease. Fever, respiratory distress, and cough were the most frequent symptoms observed in the patients. Blood samples were collected 2-5 weeks after approval of their RT-PCR results for routine paraclinical analysis.

Enzyme-linked Immunosorbent Assay (ELISA)

Measles, mumps, and rubella-specific IgG antibodies were measured using commercial ELISA kits (measles and mumps, Euroimmun, Lubeck, Germany; rubella, Pishtazteb, Karaj, Iran). Tetanus-specific IgG antibodies were measured using an indirect ELISA, as previously described (12).

COVID-19 IgG antibodies were measured using an indirect ELISA. Briefly, the nucleocapsid and RBD spike proteins (Sino Biological, China) were coated at 1 µg/ml in PBS on the wells of a microplate (MaxiSorp, Nunc, Denmark). After blocking the microplate with 3% skim milk-PBS, 1/200 diluted sera were added to the plate. Subsequently, HRP-conjugated mouse monoclonal anti-human IgG antibody (Sina Biotech Co., Tehran, Iran) was added to the microplate. The optical densities (OD) of the samples were measured at 450 nm after addition of tetramethylbenzidine (TMB) (Pishtazteb) substrate solution followed by 0.5 M sulfuric acid stop solution.

Statistical Analysis

Graph Pad Prism version 8 (Graph Pad Software, San Diego, California) and Statistical Package for Social Sciences (SPSS v.22) were used for statistical analyses. The Kolmogorov-Smirnov test was done for normal distribution. Differences in serum IgG levels between patients and healthy individuals were analyzed with the Mann-Whitney U test and the correlation of antibody titers within each group was determined by Pearson correlation test. P values of less than 0.05 were considered statistically significant.

RESULTS

Determination of Serum Titers of the Viral Nucleocapsid and RBD Specific Antibodies

NP and RBD specific IgG antibody levels were shown to be significantly higher in patients compared to the healthy control group (P<0.0001 and P<0.0001, respectively) (Figure 1). None of the control subjects displayed elevated levels of these antibodies.

Determination of Serum Levels of MMR and Tetanus Antibodies

Anti-mumps and anti-rubella IgG antibody levels were found to be similar in patients and healthy subjects. Serum levels of measles-specific IgG, however, were significantly higher in patients (P<0.01), whereas tetanus antibody titer was significantly higher in healthy subjects compared to the patients' group (P<0.01) (Figure 2).

A highly significant correlation was found between RBD and NP antibodies in patients (r=0.576, P<0.0001). Such correlations could not be established for other antibodies in patients or healthy subjects, except for the measles-specific antibody which was found to be significantly correlated with the NP antibody titer in the patients (r=0.293, P<0.01) (Figure 3).

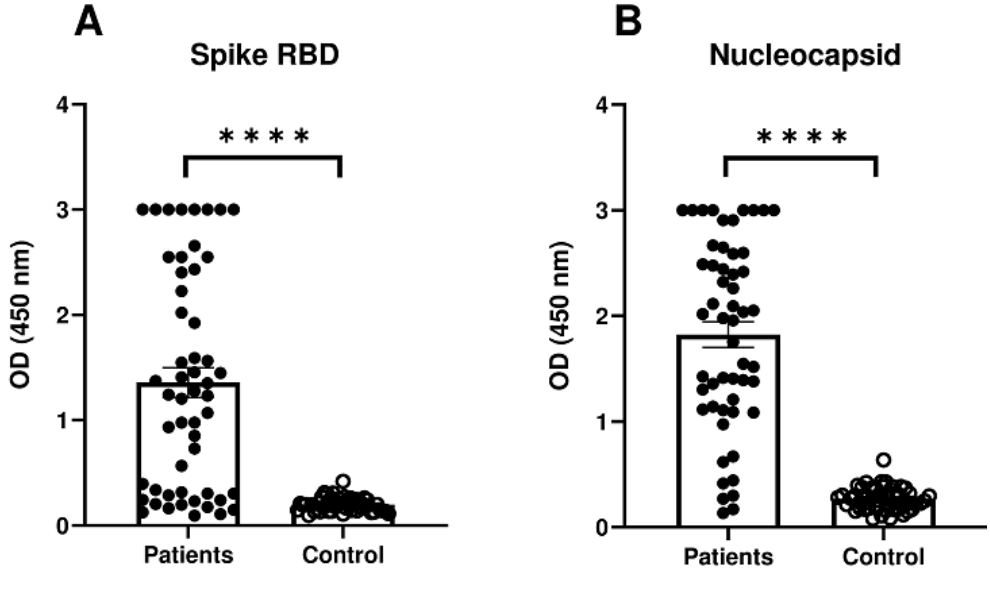

Figure 1. Anti-spike RBD and anti-nucleocapsid IgG antibody levels in serum of COVID-19 patients and healthy controls; (****P<0.0001).

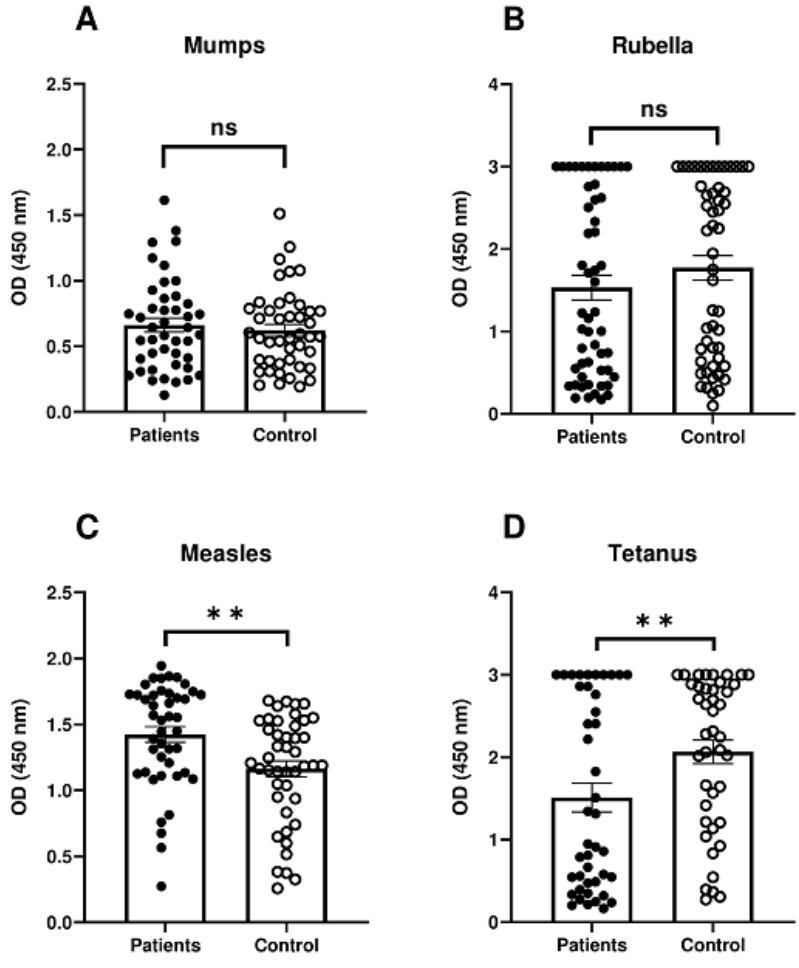

Figure 2. Serum IgG levels against (A) mumps, (B) rubella, (C) measles, and (D) tetanus in COVID-19 patients and healthy controls; (**P<0.01).

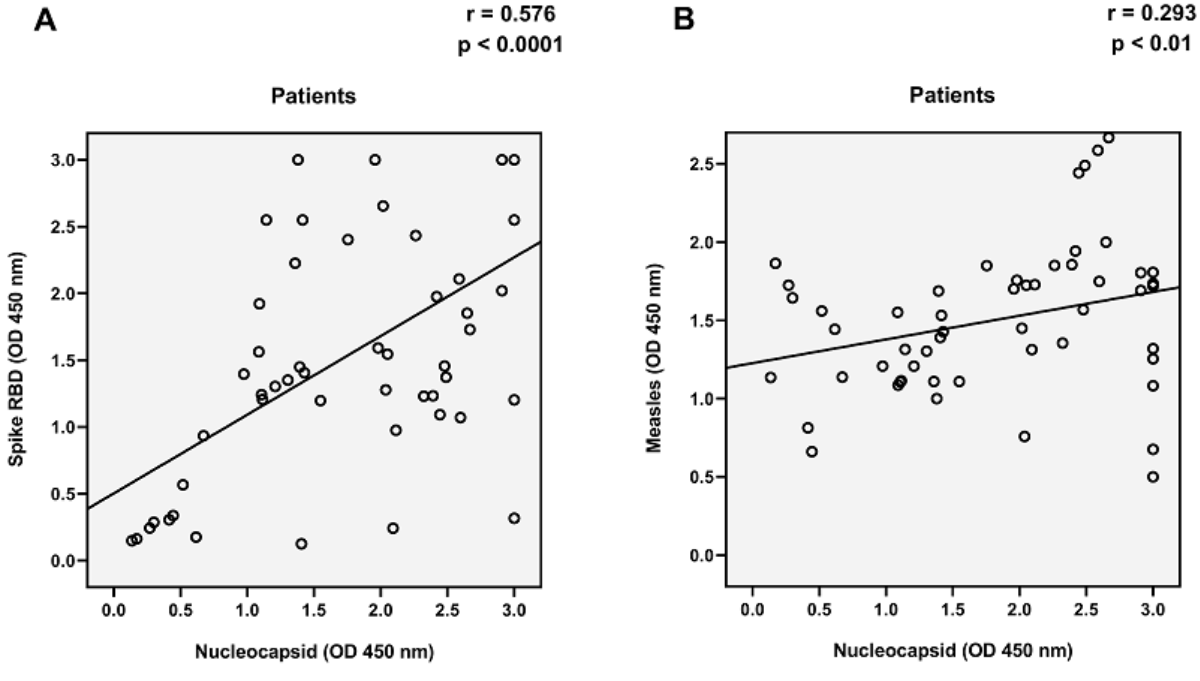

Figure 3. Statistical correlation between serum levels of (A) anti-spike RBD with anti-nucleocapsid antibody and (B) anti-measles with anti-nucleocapsid antibody in COVID-19 patients.

DISCUSSION

Difference in mortality and symptom severity between children and elderly subjects in COVID-19 patients is an unresolved and mysterious issue. One of the essential factors for variations in immune responses at population and also at individual levels is the major histocompatibility complex (MHC) diversity (13, 14). This complex, however, is not expected to act differentially in children and adults.

Historically, such a difference have also been reported for other viral respiratory epidemics and pandemics such as seasonal influenza, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (15). One reason for this difference could be associated with the more recent vaccinations of the children by a variety of vaccines, such as Bacillus Calmette–Guérin (BCG), diphtheria, pertussis, and tetanus (DPT), hepatitis B, polio, rotavirus and MMR. Some of these vaccines might be able to induce cross-protection against other pathogens. BCG, for example, has been shown to confer non-specific protection against pathogens unrelated to Mycobacterium tuberculosis, such as Candida albicans, Staphylococcus aureus and a variety of respiratory viruses (16-20), through a process known as trained immunity. This type of immunity refers to an enhanced non-specific immune response to an unrelated infection mediated by innate immune cells, such as monocytes, macrophages and natural killer (NK) cells (21, 22). Epidemiological data suggest that countries that implement BCG vaccine in their neonatal vaccination scheme, experience less contagion and mortality rates due to COVID-19 infection (23, 24).

Mass neonatal vaccination with MMR vaccines globally might also results in innate immune responses leading to induction of interferons (IFN)s and NK cells, thereby offering non-specific immunity against SARS-CoV2 in children. MMR vaccines consist of attenuated enveloped ssRNA viruses that have glycoprotein spikes, similar to SARS-CoV2. There is 32%, 31% and 33% homology between the spike amino acid sequences of measles, mumps and rubella, respectively, with that of the SARS-CoV2 (25). Thus, the chance of cross-protection provided by the combined effects of IFNs and NK cells together with cross-reactive antibodies induced by MMR is higher than vaccines that are not expected to induce cross-reactive antibodies, such as BCG.

We intended to assess whether COVID-19 infection may induce an anamnestic antibody response to MMR viruses through activation of MMR-specific memory B cells cross-reacting with SARS-CoV2; or by induction of primary B cells specific for SARS-CoV2 proteins, particularly the spike protein, with cross-reactivity with the MMR antigens. If this is the case, then we should expect a rise in MMR-reactive antibodies in serum of COVID-19 patients. To test this hypothesis, we measured the IgG level against MMR viruses in the serum of 53 recovered COVID-19 patients and compared it with that of the age-matched normal individuals.

Nucleocapsid and RBD specific antibodies were initially measured in the serum of both groups. The results indicate significantly higher levels of these COVID-19 specific antibodies in patients. None of the healthy subjects demonstrated elevated levels of these antibodies (Figure 1), suggesting that they have not been recently contaminated with SARS-CoV2 or other members of the Coronavirus family. Indeed, the serum samples of healthy subjects were collected several months before the outbreak of COVID-19 in Iran.

We then measured the antibody titer against MMR viruses in the serum of both groups. Our data demonstrated significantly higher levels of antibody against measles, but not rubella or mumps viruses (Figure 2). We also measured the antibody response to tetanus toxin as a control. Vaccination against tetanus is also included in the national vaccination scheme and all infants are given this vaccine early after birth (26). Surprisingly, higher

antibody levels were observed in the serum of healthy subjects compared to the patients group (Figure 2). Interpretation of this finding is challenging, but it could be due to general suppression of the immune response during the course of COVID-19 infection in affected patients (27, 28).

Our findings with regards to elevated titers of measles antibody in serum of COVID-19 patients is supported by epidemiological data, which show the correlation between routine MMR vaccination and lower COVID-19 death rates (29). it was reported that milder symptoms observed in the 955 sailors on the USS Roosevelt navy who tested positive for COVID-19 (only one hospitalization) may have been due to the fact that all US navy recruits were compulsorily vaccinated with MMR (30). Taken together, such findings may support initiation of clinical trials using measles or the triple MMR vaccines in high-risk groups such as the health care workers to fight against COVID-19 infection.

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Conflicts of Interest: None declared.

REFERENCES

1. WHO Coronavirus Disease (COVID-19) Dashboard; https://covid19.who.int.
2. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 among children in China. Pediatrics. 2020;145(6):1-12.
3. Okyay RA, Sahin AR, Aguinada RA, Tasdogan AM. Why are children less affected by COVID-19? Could there be an overlooked bacterial co-Infection? EJMO. 2020;4(1):104-5.
4. Chesnokova V, Melmed S. Minireview: Neuro-immuno-endocrine modulation of the hypothalamic-pituitary-adrenal (HPA) axis by gp130 signaling molecules. Endocrinology. 2002;143(5):1571-4.
5. Chesnokova V, Auernhammer CJ, Melmed S. Murine leukemia inhibitory factor gene disruption attenuates the hypothalamo-pituitary-adrenal axis stress response. Endocrinology. 1998;139(5):2209-16.
6. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev. 1999;79(1):1-71.
7. Hamborsky J, Kroger A, Wolfe C. Epidemiology and prevention of vaccine-preventable diseases. 13th ed. Washington D.C.: Public Health Foundation; 2015.
8. Mossong J, Putz L, Schneider F. Seroprevalence of measles, mumps and rubella antibodies in Luxembourg: results from a national cross-sectional study. Epidemiology & Infection. 2004;132(1):11-8.
9. Patic A, Strbac M, Petrovic V, Milosevic V, Ristic M, Hrnjakovic Cvjetkovic I, et al. Seroepidemiological study of rubella in Vojvodina, Serbia: 24 years after the introduction of the MMR vaccine in the national immunization programme. PLoS One. 2020;15(1):1-14.
10. Mirchamsy H, Shafyi A, Bassali Y, Bahrami S, Nazari F. A comparative study of two live measles vaccines in Iran. J Hyg (Lond). 1970;68(1):101-10.
11. Zahraei SM, Gouya MM, Mokhtari Azad T, Soltanshahi R, Sabouri A, Naouri B, et al. Successful control and impending elimination of measles in the Islamic Republic of Iran. J Infect Dis. 2011;204(suppl_1):305-11.
12. Manghi MA, Pasetti MF, Brero ML, Deluchi S, di Paola G, Mathet V, et al. Development of an ELISA for measuring the activity of tetanus toxoid in vaccines and comparison with the toxin neutralization test in mice. J Immunol Methods. 1994;168(1):17-24.
13. Radwan J, Babik W, Kaufman J, Lenz TL, Winternitz J. Advances in the evolutionary understanding of MHC polymorphism. Trends Genet. 2020;36(4):298-311.
14. Borsetti A, Ferrantelli F, Maggiorella MT, Sernicola L, Bellino S, Gallinaro A, et al. Effect of MHC haplotype on immune response upon experimental SHIV SF162P4cy infection of mauritian cynomolgus macaques. PLoS One. 2014;9(4):1-8.
15. Liu W, Zhang Q, Chen J, Xiang R, Song H, Shu S, et al. Detection of COVID-19 in children in early January 2020 in Wuhan, China. N Engl J Med. 2020;382(14):1370-1.
16. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific

protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A. 2012;109(43):17537-42.
17. Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun. 2014;6(2):152-8.
18. Bueno SM, González PA, Cautivo KM, Mora JE, Leiva ED, Tobar HE, et al. Protective T cell immunity against respiratory syncytial virus is efficiently induced by recombinant BCG. Proc Natl Acad Sci U S A. 2008;105(52):20822-7.
19. Cautivo KM, Bueno SM, Cortes CM, Wozniak A, Riedel CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guerin promotes virus clearance and protects from infection. J Immunol. 2010;185(12):7633-45.
20. Covian C, Retamal-Diaz A, Bueno SM, Kalergis AM. Could BCG vaccination induce protective trained immunity for SARS-CoV-2? Front Immunol. 2020;11:1-7.
21. Netea MG, Quintin J, Van Der Meer JW. Trained immunity: a memory for innate host defense. Cell Host Microbe. 2011;9(5):355-61.
22. Sanchez-Ramon S, Conejero L, Netea MG, Sancho D, Palomares o, Subiza JL. Trained immunity-based vaccines: a new paradigm for the development of broad-spectrum anti-infectious formulations. Front Immunol. 2018;9:1-11.
23. Miller A, Reandelar MJ, Fasciglione K, Roumenova V, Li Y, Otazu GH. Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study. MedRxiv. 2020. doi: 10.1101/2020.03.24.20042937.
24. Klinger D, Blass I, Rappoport N, Linial M. Significantly improved COVID-19 outcomes in countries with higher BCG vaccination coverage: A multivariable analysis. Vaccines (Basel). 2020;8(3):378-91.
25. Sidiq KR, Sabir DK, Ali SM, Kodzius R. Does early childhood vaccination protect against COVID-19? Front Mol Biosci. 2020;7:1-6.
26. Moradi-Lakeh M, Esteghamati A. National immunization program in Iran: whys and why nots. Hum Vaccin Immunother. 2013;9(1):112-4.
27. Liu R, Wang Y, Li J, Han H, Xia Z, Liu F, et al. Decreased T cell populations contribute to the increased severity of COVID-19. Clin Chim Acta. 2020;508:110-4.
28. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterize COVID-19 infections. JCI insight. 2020. doi: 10.1172/jci.insight.140329.
29. Anbarasu A, Ramaiah S, Livingstone P. Vaccine repurposing approach for preventing COVID-19: can MMR vaccines reduce morbidity and mortality? Hum Vaccin Immunother. 2020. doi: 10.1080/21645515.2020.1773141.
30. Fidel PL, Noverr MC. Could an unrelated live attenuated vaccine serve as a preventive measure to dampen septic inflammation associated with COVID-19 infection? mBio. 2020;11(3):1-4.