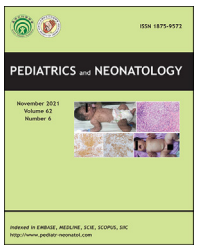
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Pediatrics and Neonatology 62 (2021) 661–663

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.pediatr-neonatol.com



Short Communication

Neutralization antibody titers against SARS-CoV-2 in an infant born to a mother with COVID-19

Yi-Ching Chen [a,1], Yu-An Kung [b,1], Rajendra-Prasad Janapatla [c], Mei-Hua Hsu [c], Reyin Lien [d], Jeng-Chang Chen [e], Shin-Ru Shih [b,f,*], Cheng-Hsun Chiu [a,c,**]

[a] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[b] *Research Center for Emerging Viral Infections, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[c] *Molecular Infectious Disease Research Center, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[d] *Division of Neonatology, Department of Pediatrics, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[e] *Division of Pediatric Surgery, Department of Surgery, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*
[f] *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan*

Received Jun 8, 2021; received in revised form Jul 6, 2021; accepted Jul 9, 2021
Available online 10 September 2021

1. Introduction

Infants/neonates are usually less susceptible to SARS-CoV-2 infection.[1,2] Like other viral infections such as varicella, the outcome of intrauterine or neonatal infection is strongly correlated with mothers. During the pregnancy, fetuses born to pregnant women with SARS-CoV-2 infection are prone to intrauterine growth restriction, prematurity, or even miscarriage because of maternal placenta insufficiency caused directly by the infection or inflammation.[3] In contrast, neonates may be protected by effective placental antibody transfer if the expectant mother acquired SARS-CoV-2 infection in the late trimester.[4,5] Here, we describe the serial detection of antibodies and associated neutralizing activity in a mother–neonate pair, in which the mother contracted mild SARS-CoV-2 infection in the third trimester.

* Corresponding author. Research Center for Emerging Viral Infections, College of Medicine, Chang Gung University, Taoyuan, Taiwan.
** Corresponding author. Division of Pediatric Infectious Diseases, Department of Pediatrics, Chang Gung Children's Hospital, 5 Fu-Hsin Street, Kweishan, Taoyuan, 333, Taiwan.
E-mail addresses: srshih@mail.cgu.edu.tw (S.-R. Shih), chchiu@adm.cgmh.org.tw (C.-H. Chiu).
[1] These authors contributed equally to this research.

https://doi.org/10.1016/j.pedneo.2021.07.007

2. Methods

A healthy newborn with a gestation age (GA) of 38 weeks and birth bodyweight of 2630 gm was born to a mother who had coronavirus disease 2019 (COVID-19) in the early third trimester (GA = 30 weeks and four days). The mother and newborn were confirmed negative by nasopharyngeal RT-PCR before and after the delivery. Serum samples from the mother before delivery (day 0) and cord blood (child, day 0) were collected. We also obtained sera from the mother (day 180) and child (days 7, 30, 60, 120, 180) after birth. We used an enzyme-linked immunosorbent assay (ELISA) to evaluate antibodies against the SARS-CoV-2 spike glycoprotein S1, receptor-binding domain (RBD), and nucleocapsid (N) protein (Sino) for these samples and incubated them overnight at 4 °C. Each well was blocked with StartingBlock™ T20 blocking buffer (Thermo Fischer Scientific, Waltham, MA, USA). Samples were added to the wells and incubated for 1 h at 37 °C. Horseradish peroxidase-tagged anti-human antibodies (IgG, IgM, and IgA, Abcam, Cambridge, UK) were added to the wells for 1 h at 37 °C. The chromogenic reagent was 3,3′,5,5′-tetramethylbenzidine (R&D Systems, Minneapolis, MN, USA). In our ELISA method, the cut-off value for the antibody against S1, RBD, and N protein was 0.15, 0.2, and 0.3 (optical density at 450 nm), respectively. A virus neutralization assay with an inhibitory concentration of >99% ($IC_{>99\%}$) was performed to measure the functional antibody titers of these samples at a biosafety level 3 by the protocol described previously.[6,7] Briefly, Vero E6 cells were seeded in a 96-well plate and incubated at 37 °C for 24 h. All sera were heat-inactivated at 56 °C for 30 min, and then serially diluted twofold with Dulbecco's modified Eagle's medium (Gibco) and 2% fetal bovine serum (Gibco). The 100 50% tissue culture infectious doses (100 TCID50) of SARS-CoV-2 were incubated with a serial dilution of serum at 37 °C for 1 h before infection of Vero E6 cells. The infected cells were incubated at 37 °C for five days, then fixed with 10% formaldehyde and stained with crystal

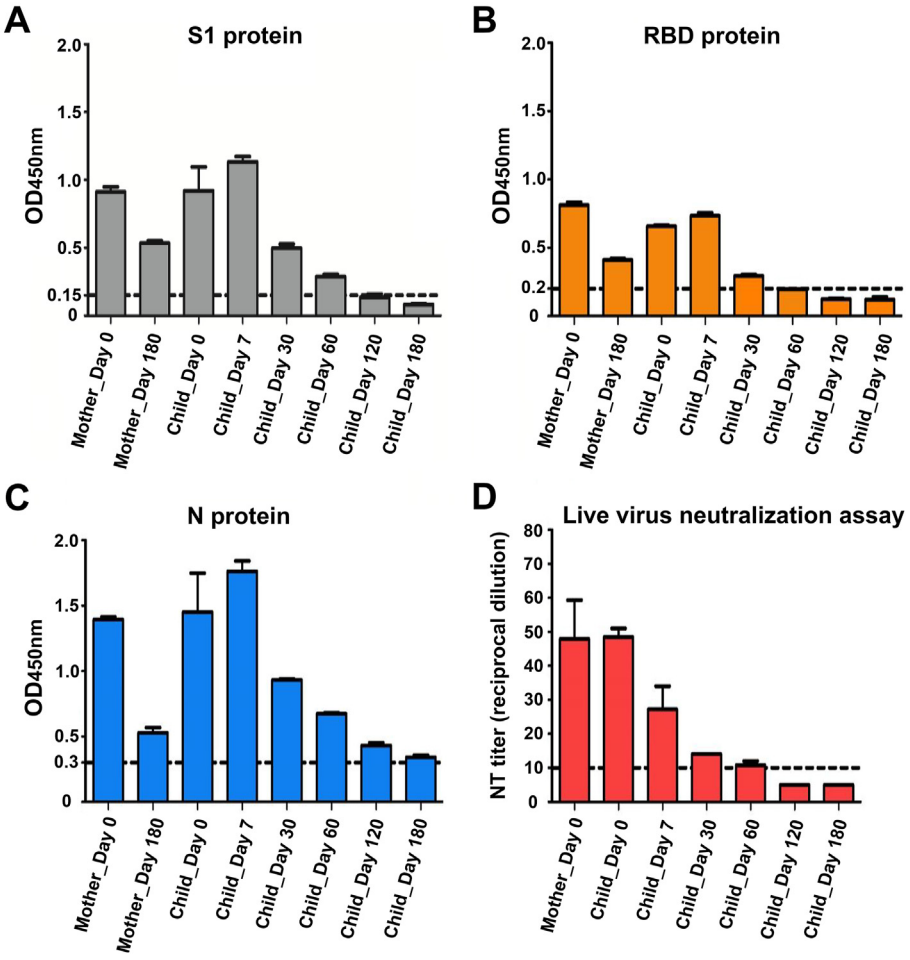


Figure 1 Antibody Responses Against SARS-CoV-2 in a Mother—Neonate Pair. An indirect ELISA based on coating with different viral antigens, including glycoprotein S1 (A), RBD (B), and N protein (C), was used to detect antibodies in serum. A live virus neutralization test was conducted in a biosafety level 3 laboratory to determine the neutralizing antibody titers in the mother and infant (D). Serum samples were from the mother before delivery (day 0), cord blood (child, day 0), and from the mother (day 180) and child (days 7, 30, 60, 120, and 180) after birth.

violet. The neutralizing titer determination is based on the presence or absence of the cytopathic effect, and then the logarithm of the 50% endpoint is calculated using the Reed—Muench method. Each serum sample was tested in four replicates.

3. Results

Using an indirect ELISA assay, serum antibody titers of the newborn against S1, RBD, and N protein lasted nearly two months after birth. However, antibodies against S1 and RBD decayed to an undetectable level four months after birth. Regarding the mother, antibodies against SARS-CoV-2 remained detectable until six months postpartum. The results demonstrated that the antibodies declined over time in both the mother and infant (Fig. 1A—C).

A live virus neutralization assay was conducted in a biosafety level 3 laboratory to evaluate whether the serum contained neutralizing antibodies (NAbs) to prevent SARS-CoV-2 infection. The serum of the mother right after delivery showed a NAb titer of 1:48 against SARS-CoV-2 (Fig. 1D). However, we could not detect the NAb titer from the mother's serum at six months postpartum because serum alone caused cell death at the dilution of 1:20. Therefore, we started at the dilution of 1:40. Although the serum control did not cause cell death at this dilution, the mother's serum at six months postpartum showed no neutralizing activity. Serum from the infant at birth showed a higher NAb titer than the mother. Like the ELISA result, the NAb titers declined in the infant seven days after birth; however, the NAb titers lasted for two months (Fig. 1D). These results indicate that serum of the infant born to a SARS-CoV-2-infected mother contained NAbs against SARS-CoV-2, but a decline in the neutralizing antibody response was subsequently observed.

4. Discussion

This study demonstrated effective placental antibody transfer in a mother—neonate pair. The placental antibody transfer ratio was around one with the ELISA method. However, the NAb titer determined by the neutralization assay using the wild-type virus was higher in the neonate at birth than the mother. We further confirmed that NAbs could last almost two months in the neonate, suggesting the potential of passive protective immunity for two months, even though the mother had only mild disease. This result is a promising observation for maternal immunization to protect neonates against COVID-19. Vaccine-induced NAb titer is usually stronger than natural infection.[8] This study provides evidence that maternal immunization with the SARS-CoV-2 vaccine may confer protection in neonates for two months or longer. This hypothesis requires more studies for validation in the current vaccine era.

Funding statement

This work was supported by Ministry of Science and Technology (grant MOST 109-2327-B-182-002), and Chang Gung Memorial Hospital (grant CORPG3K0311), Taiwan, the Research Center for Emerging Viral Infections from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan and the MOST, Taiwan (grant MOST 110-2634-F-182-001), and the National Institutes of Health USA grant U01 AI151698 for the United World Antiviral Research Network (UWARN).

Ethical information

The study was approved by Institutional Review Board, Chang Gung Hospital, Taiwan (202000837A3).

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

We thank the study participants who donated their time and samples. We thank Kuan-Ting Liu, Sheng-Yu Huang, and Peng-Nien Huang in Research Center for Emerging Viral Infections, Chang Gung University College of Medicine, Taoyuan, Taiwan for their assistance in performing the experiments and Yu-Bin Pan at Biostatistical Unit, Clinical Trial Center, Chang Gung Memorial Hospital, Taoyuan, Taiwan for the help in statistical analysis.

References

1. Cao Q, Chen YC, Chen CL, Chiu CH. SARS-CoV-2 infection in children: transmission dynamics and clinical characteristics. *J Formos Med Assoc* 2020;**119**:670—3.
2. Hong H, Wang Y, Chung HT, Chen CJ. Clinical characteristics of novel coronavirus disease 2019 (COVID-19) in newborns, infants and children. *Pediatr Neonatol* 2020;**61**:131—2.
3. Dumitriu D, Emeruwa UN, Hanft E, Liao GV, Ludwig E, Walzer L, et al. Outcomes of neonates born to mothers with severe acute respiratory syndrome coronavirus 2 infection at a large medical center in New York City. *JAMA Pediatr* 2021;**175**:157—67.
4. Flannery DD, Gouma S, Dhudasia MB, Mukhopadhyay S, Pfeifer MR, Woodford EC, et al. Assessment of maternal and neonatal cord blood SARS-CoV-2 antibodies and placental transfer ratios. *JAMA Pediatr* 2021;**175**:594—600.
5. Edlow AG, Li JZ, Collier AY, Atyeo C, James KE, Boatin AA, et al. Assessment of maternal and neonatal SARS-CoV-2 viral load, transplacental antibody transfer, and placental pathology in pregnancies during the COVID-19 pandemic. *JAMA Netw Open* 2020;**3**:e2030455.
6. Gong YN, Tsao KC, Hsiao MJ, Huang CG, Huang PN, Huang PW, et al. SARS-CoV-2 genomic surveillance in Taiwan revealed novel ORF8-deletion mutant and clade possibly associated with infections in Middle East. *Emerg Microbes Infect* 2020;**9**:1457—66.
7. Kuo TY, Lin MY, Coffman RL, Campbell JD, Traquina P, Lin YJ, et al. Development of CpG-adjuvanted stable prefusion SARS-CoV-2 spike antigen as a subunit vaccine against COVID-19. *Sci Rep* 2020;**10**:20085.
8. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020;**396**:467—78.