


ORIGINAL RESEARCH

Serological description of neonatal umbilical cord blood from pregnant women confirmed with positive COVID-19 by RT-PCR at Rumah Sakit Umum Pusat H. Adam Malik, Medan, Indonesia

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Article Info	ABSTRACT
<p>Received Sep 3, 2022 Revised Oct 25, 2022 Accepted Nov 1, 2022 Published Dec 1, 2022</p> <p>Corresponding author: Benjamin Sihite benjaminsihite718@gmail.com</p> <p>Keywords: COVID-19 RT-PCR Vertical transmission Neonatal umbilical serology IgG IgM</p> <p>This is an open access article under the CC BY-NC-SA license (https://creativecommons.org/licenses/by-nc-sa/4.0/)</p> 	<p>Objective: To evaluate the serological description of the neonatal umbilical cord in COVID-19 mothers confirmed by RT-PCR at Rumah Sakit Umum Pusat (RSUP) H. Adam Malik Medan in January-June 2021.</p> <p>Materials and Methods: This study was an observational study with a case series approach where the cases were mothers infected with SARS CoV-2 confirmed by RT-PCR. The study was conducted at Universitas Sumatera Utara (USU) Hospital Laboratory and RSUP H. Adam Malik for 6 months, from January 2021 to June 2021. The data collected were analyzed using descriptive statistics. If the data were normally distributed, they were presented as mean \pm SD; otherwise, they were presented as median (min-max) for each variable. The Statistical Package for Social Sciences version 22.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis.</p> <p>Results: Neonatal umbilical cord serology results (IgM and IgG) were predominantly non-reactive, where IgM was non-reactive in 43 neonates (97.7%) and IgG was non-reactive in 37 neonates (84.1%). In mothers without COVID-19 symptoms, neonate umbilical cord serology results were dominated by non-reactive IgM (88.6%) and IgG (79.5%). In mothers who recovered from COVID-19, neonate umbilical cord serology results were also dominated by non-reactive IgM (95.5%) and IgG (81.8%).</p> <p>Conclusion: The neonatal umbilical cord serology results from the mother confirmed with positive COVID-19 were nonreactive IgG and IgM in the majority of 35 (79%) samples, reactive IgG in 7 (15.9%), and reactive IgM in 1 (2%) sample.</p>

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INTRODUCTION

Current COVID-19 pandemic raises concern about its effect on pregnancy. Reports of neonatal COVID-19 infection immediately after delivery indicate either transplacental migration or horizontal transmission by

direct surface contact during delivery¹ or during breastfeeding with virus migration.¹⁻²

The placenta is a physical and immunological defense against fetal infection. Maternal natural killer (NK) cells, decidual macrophages, and T cells inhabit the placenta decidua. Immune cells in the decidua are

essential for placenta remodeling and implantation. Their absence is linked to miscarriage and other negative pregnancy outcomes.³ When the maternal-fetal interface barrier fails, pathogens cleave the maternal innate immune system and placental trophoblast defenses of the fetus by mechanisms that are not fully elucidated.⁴

Several studies have found infected newborns by testing viruses in samples of placenta, amniotic fluid, umbilical cord blood, and neonate throat swabs.⁵ Other publications indicate the possibility of vertical transmission due to the presence of IgM in certain neonates from mothers infected with SARS-CoV-2.⁶ Several studies have shown three cases of neonates with positive anti-SARS-CoV-2 IgM and IgG serology at birth from SARS-CoV-2-infected mothers. While maternal IgG antibodies cross the placenta, IgM comes from the fetus, thus suggesting SARS-CoV-2 virus exposure in the uterus. The sensitivity and specificity of IgM detection would be 88.2%/96.2% and 70.2%/99%, which is much higher than observed for other viral infections.⁷

Transfer of maternal IgG to the placenta is an important mechanism for fetal protection when fetal humoral immune response is insufficient. Given that IgM is not normally transferred from mother to fetus due to its larger macromolecular structure under normal conditions, some studies have speculated that neonates may have been infected with SARS-CoV-2 in utero from mothers with COVID-19.⁸

In North Sumatra, Indonesia, the Central General Hospital (*Rumah Sakit Umum Pusat/RSUP*) H. Adam Malik Medan is one of the referral hospitals for COVID-19 cases from other hospitals in North Sumatra. According to provisional data, at RSUP H. Adam Malik Medan, North Sumatra, from July-November 2020, it received around 34 pregnant patients with suspected or confirmed COVID-19, with confirmed cases ranging from 7–15 patients per month. The authors were interested in evaluating serological description of neonates' umbilical cord blood from mothers with confirmed COVID-19 by RT-PCR examination for evaluation of mother/infant immune reaction and to clarify potential COVID-19 vertical transmission from mother to child.

MATERIALS AND METHODS

This study was an observational study with a case series approach where the case comprised of mothers infected with SARS CoV-2 confirmed by RT-PCR. The study was conducted at RS Universitas Sumatera Utara (USU) Laboratory and RSUP H. Adam Malik for 6 months from January to June 2021. The samples were pregnant

women who came to RSUP H. Adam Malik and RS USU who met inclusion criteria, namely women in 3rd trimester pregnancy and positive COVID-19 confirmed by RT-PCR, while defective blood or serum preparations were excluded from the study. Sampling was done by non-probability sampling, ie. by the consecutive sampling technique.

Tools needed in this research were PPE (Personal Protection Equipment), frozen tube (red cap tube), 3 cc syringe, centrifuge, timer, aliquot, aliquot storage box, refrigerator freezer, YHLO brand rapid test, buffer, stopwatch (timer), and 10-20 mcl micro pipette. The research material was derived from umbilical cord blood/serum of mothers who were confirmed positive with RT-PCR.

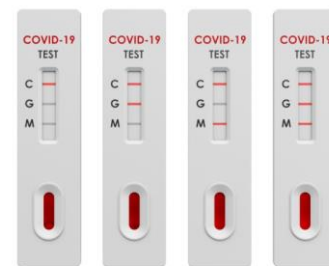


Figure 1. YHLO Antibody

Notes:

1. Negative result: if only C line appears, and G and M lines do not exist.
2. Positive results: if there is a C line and an M or G line, it indicates presence of antibodies to SARS CoV-2 virus.
3. Invalid: if there is no C line, the result is invalid even though there is a G or M line.

Statistical analysis

The data were analyzed descriptively to determine the frequency distribution of the research subjects based on the characteristics of the research sample. The data are displayed in a frequency distribution table with percentage values for each variable. Prior to analysis, a normality test was carried out first with the Kolmogorov-Smirnov test. If the data were normally distributed, they were presented as mean \pm SD; otherwise, they were presented as Median (Min-Max) for each variable. The Statistical Package for Social Sciences version 22.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

The characteristics of the research subjects are presented in Table 1.

Table 1. Characteristics of research subjects

Characteristics	n = 44
1. Maternal characteristics	
Age, years old (Mean±SD)	28.91±5.810
Parity, n(%)	
Primiparous	14 (31.8)
Secundiparous	11 (25.0)
Multiparous	19 (43.2)
Swab PCR, n(%)	
Positive	44 (100.0)
Symptomatic	5 (11.4)
Asymptomatic	39 (88.6)
Negative	0 (0.0)
Hemoglobin, gr/dl (Mean±SD)	11.33 ± 1.86
Hematocrit, % (Mean ±SD)	33.02 ± 5.16
Leukocytes, sel/mm ³ (Mean ±SD)	11.725.45 ± 4612.52
Platelets, sel/mm ³ (Mean ±SD)	262.318.18 ± 92.950.79
Neutrofil/Lymfosit Ratio (NLR)	6.09 ± 1.36
Chest X-ray image, n(%)	
Bronchopneumonia	26 (59.1)
Right pleural effusion	1 (2.3)
Cor and pulmo normal	17 (38.6)
Treatment duration, days	7.63 ± 2.68
Maternal outcome, n(%)	
Cured	42 (95.5)
Death	2 (4.5)
2. Neonates characteristics	
IgM immunoserology	
Reactive	1 (2.3)
Non-Reactive	43 (97.7)
IgG immunoserology	
Reactive	7 (15.9)
Non-Reactive	37 (84.1)
IgM & IgG immunoserology	
Reactive	1 (2.3)
Non-Reactive	43 (97.7)
Swab PCR, n(%)	
Positive	0 (0.0)
Negative	44 (100.0)

Based on mothers' characteristics, the mean age of research subjects was 28.91 years. The majority were multigravida, as many as 19 subjects (43.2%). All study subjects were pregnant women with positive COVID-19 confirmed by RT-PCR swab in as many as 44 patients (100%). Five patients (11.4%) were symptomatic, and 39 patients (88.6%) were asymptomatic. Based on subject outcome, as many as two subjects (4.5%) died, and 42 other subjects (95.5%) were declared cured.

Neonates characteristics based on immunoserological examination of IgM and IgG antibodies found one subject (2.3%) was IgM reactive, seven subjects (15.9%) were IgG reactive, and one subject (2.3%) was reactive to both IgM and IgG. However, on RT-PCR swab examination of neonates, all were negative for COVID-19.

Immunoserology results of neonates from pregnant women with positive COVID-19 confirmed by RT-PCR

Based on clinical manifestations of COVID-19 positive pregnant women as confirmed by RT-PCR, neonates IgM antibodies were found in one baby (2.3%). Of COVID-19 positive pregnant women as confirmed by RT-PCR with symptoms, neonates IgG antibodies were found in three babies (6.8%). Based on the condition of COVID-19 positive pregnant women as confirmed by RT-PCR who were died, neonates IgM antibodies were found in one baby (2.3%) and neonates IgG antibodies were also found in one baby (2.3%).

Table 2. IgM and IgG immunoserology result of neonates from pregnant women with positive COVID-19 confirmed by RT-PCR based on clinical manifestations.

		Neonates immunoserology IgM (N=44)		Neonates immunoserology IgG (N=44)		Total
		Reactive (%)	Non Reactive (%)	Reactive (%)	Non Reactive (%)	
Clinical Manifestations	Symptomatic	1 (2.3)	4 (9.1)	3 (6.8)	2 (4.5)	5 (11.4)
	Asymptomatic	0 (0.0)	39 (88.6)	4 (9.1)	35 (79.5)	39 (88.6)
Maternal Outcome	Cured	0 (0.0)	42 (95.5)	6 (13.6)	36 (81.8)	42 (95.5)
	Death	1 (2.3)	1 (2.3)	1 (2.3)	1 (2.3)	2 (4.5)
Total		1 (2.3)	43 (97.7)	7 (15.9)	37 (84.1)	44 (100)

Table 3. Immunoserological test result of neonates from pregnant women with positive COVID-19 confirmed by RT-PCR against neonates PCR swab examination results.

Immunoserological test result		Neonates PCR Swabs (N=44)		Total
		Positive (%)	Negative (%)	
IgM	Positive	0 (0.0)	1 (2.3)	1 (2.3)
	Negative	0 (0.0)	43 (97.7)	43 (97.7)
IgG	Positive	0 (0.0)	7 (15.9)	7 (15.9)
	Negative	0 (0.0)	37 (84.1)	37 (84.1)
IgG & IgM	Positive	0 (0.0)	1 (2.3)	1 (2.3)
	Negative	0 (0.0)	43 (97.7)	43 (97.7)
Total		0 (0.0)	44 (100.0)	44 (100.0)

From neonates of pregnant women positive for COVID-19 as confirmed by RT-PCR who underwent immunoserological examination, one baby (2.3%) had reactive IgM, seven (15.9%) had reactive IgG, and another baby (2.3%) had reactive IgM and IgG, but all tested negative for COVID-19 on RT-PCR swab examination.

Of pregnant women with positive COVID-19 confirmed by RT-PCR in this study, neonates' IgM antibodies were found in one baby (2.3%). Of pregnant women with positive COVID-19 confirmed by RT-PCR with symptoms, neonates IgG antibodies were found in three babies (6.8%). Based on the condition of women with positive COVID-19 confirmed by RT-PCR who died, neonates IgM antibodies were found in one baby (2.3%). From pregnant women with positive COVID-19 confirmed by RT-PCR who died, neonates with IgG antibodies were found in one baby (2.3%).

Zeng et al. examined six infants born to six women with confirmed COVID-19 and discovered that they all had higher IgG and IgM antibodies (10 AU/mL) than normal levels, with IgG levels of 125.5 AU/mL and IgM levels of 39.6 AU/mL. IgG is passively transferred across the placenta from mother to fetus, beginning at the end of the second trimester and reaching high levels at birth. However, the detected IgM is usually not transferred from mother to fetus due to its larger macromolecular structure.⁹ ACE2 expression is seen in many tissues directly associated with developing pregnancy, whereas a recent single-cell RNA sequencing analysis showed ACE2 expression in maternal-fetal stromal, perivascular, placental, and decidua cells. Kotlyar et al. hypothesized the possibility of in utero transmission because IgM cannot cross the placenta.¹⁰

A recent journal reviews also stated that IgG or IgM was detected in 90% of infants with negative PCR swabs from mothers with confirmed COVID-19 (10/11; 95% CI: 73.9%–107.9%). For IgG and IgM, the mean antibody levels detected were 75.49 AU/ml (range, 7.25-140.32 AU/ml) and 3.79 AU/ml (range, 0.16-45.83 AU/ml), respectively ($p = 0.0041$). A total of 19 studies were reported in China and the other countries. China reported 4.2% of infants contracted COVID-19 vertically from their mothers, while outside China it was 10.5%. The method of delivery by cesarean section was found in 10% of positive babies, while vaginal delivery was 10.3%. In addition, asymptomatic infants from infected mothers have a strong immune response to COVID-19 in the second trimester of pregnancy because of IgG formation potential.¹¹

Based on the condition of women with positive COVID-19 confirmed by RT-PCR who died in this study,

neonate IgM antibodies were found in one baby (2.3%) and neonate IgG antibodies were also found in one baby (2.3%). A study of 9 third-trimester pregnant women with COVID-19 discovered that none of the patients had severe COVID-19 or died, and that there were no severe outcomes in neonates or fetal infections that could have been caused by intrauterine vertical transmission.¹² A review of 26 journals on neonate COVID-19 infection stated that demographically, 44 neonates from 35 confirmed COVID-19 mothers, only 1 neonate with confirmed COVID-19 was found and the rest had higher IgM and IgG serology levels than normal, and overall, the mothers were able to recover completely.¹³

In this study, from neonates of pregnant women with positive COVID-19 confirmed by RT-PCR who underwent immunoserological examination, one (2.3%) had reactive IgM, seven (15.9%) had reactive IgG, and one (2.3%) had reactive IgM and IgG, but all tested negative for COVID-19 on RT-PCR swab examination. Zhu et al. studied neonates of 9 pregnant women with confirmed COVID-19 with various mild-moderate symptoms. Of these neonates, 6 had a Pediatric Critical Illness Score (PCIS) of less than 90.

Clinically, symptoms encountered in neonates were shortness of breath, fever, thrombocytopenia, abnormal liver function, tachycardia, vomiting, and pneumothorax. All of the neonates had pharyngeal swabs and the results were negative for COVID-19, but the possibility of false-negative results could not be ruled out.¹⁴

This is in line with a case report by Dong et al. in which IgM, IgG, and RT-PCR swab examinations were carried out on neonates from a mother with confirmed COVID-19. The results found that the neonates had high serological levels, but swab results were negative.¹⁵ In contrast to Liu et al.'s study, who examined 51 neonates, neither IgG, IgM, nor positive RT-PCR swab results were found.¹⁶ Yang et al. also found that none of the 23 infants had a positive nasopharyngeal swab; one preterm infant was found to have a positive result for IgM anti-SARS-CoV-2 within 2 hours of birth.¹⁷

This study showed that there was potential for antibodies inherited from pregnant women to provide protection for neonates against SARS-CoV-2 infection. Another evidence suggests that vertical transmission in the uterus is responsible for COVID-19 in neonates that makes neonatal infection through the umbilical cord unlikely.¹⁸⁻²⁰ Further studies are needed to determine whether COVID-19 antibodies protect neonates from COVID-19 infection; the concentration of IgG that can provide prevention; and whether the transplacental kinetics of vaccine-derived antibodies are similar to

those obtained naturally. In addition, the results of this study were expected to be useful in determining the management of neonates from mothers infected with COVID-19.

There were limitations in this study because it was an observational one, therefore it could not draw some conclusions in terms of testing and two-group comparison. Therefore, further research is needed to provide an in-depth review of COVID-19 outcomes among mothers and neonates in Type A hospital scale.

CONCLUSION

The neonatal umbilical cord serology results from the mothers confirmed with positive COVID-19 revealed that the majority (79%) of the samples, comprising 35 mothers, had nonreactive IgG and IgM; seven (15.9%) had reactive IgG, and one (2%) had reactive IgM. All the babies' PCR swab results were negative. Therefore, research with different methods is needed to assess potential vertical transmission of COVID-19.

DISCLOSURES

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Conflict of interest

All authors have no conflict of interest.

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This research has received no external funding.

Author Contribution

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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