Vertical transmission and humoral immune response following maternal infection with SARS-CoV-2 - A prospective multicenter cohort study

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Abstract

Objective: To explore maternal humoral immune responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the rate of vertical transmission. Design: A prospective cohort study. Setting: Two university-affiliated medical centers in Israel. Population: Women positive for SARS-CoV-2 reverse-transcription-polymerase-chain-reaction (RT-PCR) test, during pregnancy were enrolled just prior to delivery. Methods: Levels of anti-SARS-CoV-2 spike-IgM, spike-IgG and nucleocapsid-IgG were tested in maternal and cord blood at delivery, and neonatal nasopharyngeal swabs were subjected to PCR testing. Main outcomes measures: The primary endpoint was the rate of vertical transmission, defined as either positive neonatal IgM, positive neonatal IgG with sero-negative mother or positive neonatal PCR. Results: Among 72 women, 36 (50%), 39 (54%) and 30 (42%) were positive for anti-spike-IgM, anti-spike-IgG and anti-nucleocapsid-IgG, respectively ($p<0.0001$ for IgG antibodies-comparison). At least 8/72 (11%) neonates were infected in utero; one had a positive PCR result and seven had positive IgG antibodies while their mothers were seronegative for the same IgG. IgM was not detected in cord blood. Anti-nucleocapsid-IgG and anti-spike-IgG were detected in 83% and 85% of neonates of seropositive mothers, respectively (Pearson coefficient correlation 0.8, $p<0.001$). The highest rate of positive maternal serology tests was 8-12 weeks post-infection (89% anti-spike IgG, 78% anti-spike-IgM and 67% anti-nucleocapsid-IgG). Thereafter, the rate of positive serology tests declined gradually; at 20 weeks post-infection, only anti-spike-IgG was detected in 33-50%. Conclusions: The rate of vertical transmission was at least 11%. Vaccination should be considered 3 months post-infection in pregnant women due to a decline in antibody levels.

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Abstract

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Design: A prospective cohort study.

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Results: Among 72 women, 36 (50%), 39 (54%) and 30 (42%) were positive for anti-spike-IgM, anti-spike-IgG and anti-nucleocapsid-IgG, respectively ($p<0.0001$ for IgG antibodies-comparison).

At least 8/72 (11%) neonates were infected in utero; one had a positive PCR result and seven had positive IgG antibodies while their mothers were seronegative for the same IgG. IgM was not detected in cord blood. Anti-nucleocapsid-IgG and anti-spike-IgG were detected in 83% and 85% of neonates of seropositive mothers, respectively (Pearson coefficient correlation 0.8, $p<0.001$). The highest rate of positive maternal serology tests was 8-12 weeks post-infection (89% anti-spike IgG, 78% anti-spike-IgM and 67% anti-nucleocapsid-IgG). Thereafter, the rate of positive serology tests declined gradually; at 20 weeks post-infection, only anti-spike-IgG was detected in 33-50%.

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Funding

This study was performed in collaboration with the Israeli Ministry of Health.

Tweetable abstract

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Key words: Antibodies**;** COVID-19; pregnancy; neonates; SARS-CoV-2.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus that usually causes mild respiratory illness (Corona virus disease; COVID-19) in humans. Severe illness can develop, especially in high-risk populations, such as elderly, immunosuppressed patients, and patients with comorbidities.

The SARS-CoV-2 virion has four structural proteins known as the spike, envelope, membrane, and nucleocapsid proteins; the nucleocapsid protein holds the RNA genome, and the spike, envelope and membrane proteins create the viral envelope[1].

Diagnosis of SARS-CoV-2 infection is based on reverse-transcription polymerase chain reaction (RT-PCR) performed on nasopharyngeal samples[2]. Serologic tests may also be used for COVID-19 diagnosis. Anti-spike IgG and IgM and anti-nucleocapsid IgG detection kits are commercially available. Time from positive SARS-CoV-2 nasopharyngeal swab correlated with SARS-CoV-2 IgG antibody levels and antibody titers gradually decline within 6-9 months, until stabilization[3].

The effect of pregnancy on humoral response to SARS-CoV-2 infection as well as the rate of vertical transmission are not fully understood. At the beginning of the current COVID-19 pandemic, evidence pointed to a lack of vertical transmission, as determined by amniocentesis, umbilical cord blood, placenta, neonatal secretion and breast milk sampling[4-9]. However, recent data, mostly from case reports and case series, demonstrated the presence of SARS-CoV-2 in the placenta[10-12], positive RT-PCR of nasopharyngeal swabs of newborns and evidence of seropositivity in neonates[13-17].

Evidence for vertical transmission is suggested in either positive neonates for SARS-CoV-2 RT-PCR, the presence of IgM-type antibodies in the newborn since these antibodies do not cross the placenta, and positive neonatal IgG serology with seronegative mother.

The present study explored maternal humoral immune responses to SARS-CoV-2 infection and the rate of vertical transmission.

Patients and Methods

Design

This prospective multicenter cohort study was conducted between July 3rd 2020 and January 24th 2021 at Emek and Baruch-Padeh Medical Centers, two university-affiliated medical centers in north Israel. The study protocol was approved by the Local Institutional Review Boards (60-20-EMC and 90-20-POR). Informed consent was obtained from all individuals who participated in the study.

The study cohort consisted of pregnant women[?]18 years old who had a positive nasopharyngeal swab for SARS-CoV-2, as determined by RT-PCR, during pregnancy.

Women were enrolled at admission to the delivery ward, before delivery, by one of the team investigators. After enrollment, SARS-CoV-2 anti-nucleocapsid-IgG, anti-spike-IgG and anti-spike-IgM levels in maternal and cord blood were measured near delivery. Nasopharyngeal samples were collected from the neonates in the Department of Neonatology and were subjected to SARS-CoV-2 PCR testing. Participants were excluded from the study if both cord blood serology tests and neonatal RT-PCR could not be obtained due to technical reasons.

Determination of SARS-CoV-2 antibody levels

Serum was separated from clot and blood cells by centrifugation (1000 xg , 10 min) using gel separator tubes. Samples were either directly tested for SARS-CoV-2 anti-nucleocapsid-IgG antibodies by the Architect i2000 analyzer on the day of sample collection or were separated into a secondary tube and frozen at -20^{0}C until the test was performed. After performing the test, samples were frozen at -20^{0}C. For determination of SARS-CoV-2 anti-spike (S1/S2) IgG and IgM antibody titers, samples were thawed and mixed by vortex, and then subjected to ready-to-use assays on automated analyzers, as detailed in Table 1.

Study endpoints

The primary endpoint was the rate of vertical transmission, defined as either positive neonatal IgM serology, positive neonatal IgG serology with a seronegative mother or positive neonatal SARS-COV-2 PCR. Humoral immune response was also evaluated, including the rate of positive mothers for each tested antibody and antibody levels by time between infection and delivery.

Correlation between antibody levels and clinical manifestation of COVID-19 was also evaluated as well as demographic and pregnancy characteristics and data regarding fetal malformations.

Statistical analysis

Sample size was calculated using the binomial proportion test. The rate of vertical transmission was estimated to be 7% when defined by RT-PCR[9]. Assuming that using serology tests increases the rate to 10% versus 0% in non-infected population, 71 women were required (80% power, 5% one-sided alpha).

Categorical variables were analyzed using the chi-squared test or Fisher's exact test. The correlation between maternal and neonatal IgG antibody levels was assessed by the Pearson coefficient. The locally scatter plot smoothing (LOESS) non-parametric regression model was utilized to compare the mean drop in antibody levels over time from COVID-19 diagnosis and delivery. Antibodies levels were normalized by dividing each value by the largest value. Anti-spike antibodies were also multiplied by 3 in order to fit the scale. Antibodies levels below the threshold for positive results according to the kit instructions were set to be zero.

Statistical analyses were carried out with SAS version 9.4 (SAS Institute, Cary, NC, USA). Significance was set at a p value of <0.05. Data were analyzed by the authors E.Y and Z.N.

Results

Among the 79 women who participated in the study, 72 had available serology test results. Seven women did not have serology information due to technical reasons. Thirty six neonates did not undergo nasopharyngeal swabbing for SARS-CoV-2 PCR due to parental refusal. One woman did not have enough serum to determine anti-spike IgM levels.

Patient characteristics are presented in Table 2. SARS-CoV-2 antibody profiles of women who had COVID-19 during pregnancy and of their neonates, are presented in Table 2. Among the 72 women, 36 (50%), 39 (54%) and 30 (42%) women tested positive for anti-spike-IgM, anti-spike-IgG and anti-nucleocapsid-IgG, respectively ($p<0.0001$ for the comparison of IgG antibodies; Table 3). At least 8/72 (11%) neonates were infected with SARS-CoV-2 in utero; one neonate had a positive PCR test and 7/48 neonates carried anti-SARS-CoV-2 IgG while the mothers were sero-negative for the same IgG. The rest of the neonates were either seronegative or had the same IgG as their mothers, and therefore whether the IgG transferred from the mothers or was self-produced by the fetus could not be determined. Of the seronegative women for whom vertical transmission was identified, 8/48 (17%) neonates were infected with SARS-CoV-2 in utero. No fetal malformations were detected.

Anti-nucleocapsid-IgG and anti-spike-IgG were detected in 83% and 85% of neonates of mothers seropositive for those antibodies, respectively. Maternal and neonatal IgG antibody levels were positively correlated (Pearson coefficient 0.8, $p<0.001$). With regards to the interval between infection and delivery, the highest rate of maternal positive serology tests was when the interval was between 8-12 weeks (89% anti-spike IgG, 78% anti-spike IgM and 67% anti-nucleocapsid IgG). Thereafter, the rate of positive serology tests declined gradually. After 20 weeks, only anti-spike IgG was detected in 33-50% (Figure 1A).

Maternal anti-spike-IgG responses were the longest compared with anti-nucleocapsid-IgG and anti-spike-IgM responses (Figure 1,B).

The rate of women with positive IgG serology was higher if COVID-19 was symptomatic compared to asymptomatic (anti-nucleocapsid-IgG 29 (50%) versus 1 (7%), $p=0.004$; anti-spike-IgG 35 (60%) versus 4 (29%), respectively, $p=0.03$).

Discussion

Main Findings

The present study explored the rate of vertical transmission and humoral immune responses following maternal SARS-CoV-2 infection during pregnancy. Evidence of a vertical transmission rate of at least 11% was observed. In addition, the highest rate of positive serology tests was among women who delivered 8-12 weeks post-infection, after which, the rate of positive serology tests declined gradually. Women delivering 20 or more weeks after infection, only carried anti-spike IgG antibodies.

Strengths and limitations

The strengths of the study lay in its prospective design, the large sample size with statistical justification, and evaluation of several antibodies against SARS-CoV-2. The limitations of the study were the lack of serial maternal serology sampling, insufficient sample size for assessing neonatal complications related to COVID-19 and parental refusal to allow neonatal SARS-CoV-2 nasopharyngeal swabbing. It should be noted that only five neonates were born within 2 weeks of maternal SARS-CoV-2 infection, when serology test sensitivity and specificity might be lower.

Clinical interpretation

Data regarding vertical transmission of SARS-CoV-2 is scarce. Most data are based on case reports and small case series. A recent review analyzed 38 studies that assessed COVID-19 and pregnancy. The rate of vertical transmission of SARS-CoV-2 differed by sample source and test type; rates were 2.9%, 7.7%, 2.9%, and 3.7% for neonatal nasopharyngeal swab testing (N=936), placental sampling (N=26), cord blood IgM serology (N=34) and neonatal IgM serology (N=82), respectively. Amniotic fluid (N=51) and neonatal urine (N=17) analyses showed no evidence of vertical transmission[18]. The highest vertical transmission rates (9.7% of N=31) were observed when testing neonatal fecal/rectal samples[19,20]. In our study the rate of vertical transmission measured by neonatal nasopharyngeal swab testing only, was 3%, while serology analysis added at least an additional 10% of vertical transmission. These findings are similar to those reported in the literature and emphasize the importance of analyzing serology for the assessment of fetal infection. It is particularly important in mothers who had COVID-19 disease whose baby showed a negative neonatal nasopharyngeal swab test. Since positive neonatal serology may suggest fetal infection, these neonates should undergo close surveillance for possible long-term implications.

SARS-CoV-2 binds to host cells through the angiotensin-converting enzyme-2 (ACE-2) receptor, after which, serine proteases TMPRSS2 contribute to priming the spike protein, to enable it to fuse with the host cell membrane and replicate[1, 21]. Vertical transmission is possible as the ACE-2 receptor is expressed on various cells, such as cells of the ovary, uterus, vagina, placenta[22] and venous and arterial endothelium, as well as of the smooth muscle of the umbilical cord[14].

Research interpretation

Approximately 50% of the mothers in our cohort had SARS-CoV-2 antibodies. In a study that examined 392 COVID-19 convalescent subjects, 366 (93.4%) were positive for SARS-CoV-2 IgG antibodies[3]. Time from positive SARS-CoV-2 nasopharyngeal swab correlated with SARS-CoV-2 IgG antibody levels (Pearson r –0.281, $p < 0.001$), with a 50% decline in antibody levels within 6 months; however, levels were still above the cut-off for positive serology result. Thereafter, antibody levels stabilized and remained similar up to 9 months post-infection. In 15% of participants with tests at two time points (N=59), SARS-CoV-2 anti-spike antibodies decreased below the positive cut-off[3]. According to our results, during pregnancy, anti-SARS-CoV-2 antibody titers declined more rapidly, with the rate of women with positive anti-spike IgG declining from 89% at 2-3 months post-infection to 38% by 5 months post-infection. These data suggest that while immune responses to SARS-CoV-2 infection during pregnancy are similar to those measured in non-pregnant women[3], antibody titer decline more rapidly during pregnancy. One explanation is due to the transition to a Th2 anti-inflammatory environment during pregnancy that may attenuate the immune response[23-25]. Future

studies should explore this hypothesis. This observation suggests that SARS-CoV-2 vaccination should be considered 3 months post-infection in pregnant women due to a decline in antibody levels.

Conclusions

Taken together, the rate of vertical transmission was at least 11% when assessed both with RT-PCR of nasopharyngeal samples and serology tests. The highest rate of maternal seropositivity was 8-12 weeks post-infection. Anti-spike IgG levels remained high for the longest period of time and therefore should be used in serology testing, to avoid false negative results.

The authors report no conflict of interest.

Authorship Contributions

M.M, E.Y, O.R, S.S, J.H, T.S, A.A, Y.P and Z.N participated in the study design and data collection. E.Y and Z.N analyzed the data and wrote the manuscript. M.M, O.R, S.S, J.H, T.S, A.A, and Y.P critically reviewed the manuscript.

Details of ethics approval

Not applicable.

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Table 1. Anti-SARS-CoV-2 Antibody Detection Assays.

Table 2. Demographic characteristics and pregnancy course of women with COVID-19 during pregnancy.

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Legends of Figure

Figure 1. Maternal humoral immune response following infection with SARS-CoV-2 according to time from infection to delivery

(A) The rate of pregnant women with positive serum anti-spike IgG (IgG-S), anti-spike IgM (IgM-S) and anti-nucleocapsid IgG (IgG-N) antibodies over time (weeks) from infection.

(B) LOESS smooth curve (smoothing parameter 0.4) of mean antibody levels according to time between COVID-19 disease and delivery (weeks). Antibodies levels were normalized by dividing each value with the highest value measured. Anti-spike antibodies were also multiplied by 3 in order to fit the scale. Antibody levels below the seropositivity threshold are shown as zero. Note that the graph represents the rate of antibody level decline and not their actual values.

Reference List

(1) Kumar M, Al Khodor S. Pathophysiology and treatment strategies for COVID-19. J Transl Med 2020; 18(1):353.

(2) Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020; 9(1):386-389.

(3) Achiron A, Gurevich M, Falb R, Dreyer-Alster S, Sonis P, Mandel M. SARS-CoV-2 antibody dynamics and B-cell memory response over time in COVID-19 convalescent subjects. Clin Microbiol Infect 2021.

(4) Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet 2020; 395(10226):809-815.

(5) Breslin N, Baptiste C, Gyamfi-Bannerman C, Miller R, Martinez R, Bernstein K et al. Coronavirus disease 2019 infection among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. Am J Obstet Gynecol MFM 2020; 2(2):100118.

(6) Chen Y, Peng H, Wang L, Zhao Y, Zeng L, Gao H et al. Infants Born to Mothers With a New Coronavirus (COVID-19). Front Pediatr 2020; 8:104.

(7) Li N, Han L, Peng M, Lv Y, Ouyang Y, Liu K et al. Maternal and Neonatal Outcomes of Pregnant Women With Coronavirus Disease 2019 (COVID-19) Pneumonia: A Case-Control Study. Clin Infect Dis 2020; 71(16):2035-2041.

(8) Wang L, Shi Y, Xiao T, Fu J, Feng X, Mu D et al. Chinese expert consensus on the perinatal and neonatal management for the prevention and control of the 2019 novel coronavirus infection (First edition). Ann Transl Med 2020; 8(3):47.

(9) Fan C, Lei D, Fang C, Li C, Wang M, Liu Y et al. Perinatal Transmission of 2019 Coronavirus Disease-Associated Severe Acute Respiratory Syndrome Coronavirus 2: Should We Worry? Clin Infect Dis 2021; 72(5):862-864.

(10) Baud D, Greub G, Favre G, Gengler C, Jaton K, Dubruc E et al. Second-Trimester Miscarriage in a Pregnant Woman With SARS-CoV-2 Infection. JAMA 2020; 323(21):2198-2200.

(11) Penfield CA, Brubaker SG, Limaye MA, Lighter J, Ratner AJ, Thomas KM et al. Detection of severe acute respiratory syndrome coronavirus 2 in placental and fetal membrane samples. Am J Obstet Gynecol MFM 2020; 2(3):100133.

(12) Algarroba GN, Rekawek P, Vahanian SA, Khullar P, Palaia T, Peltier MR et al. Visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy. Am J Obstet Gynecol 2020; 223(2):275-278.

(13) Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, La Rosa M. Severe COVID-19 during Pregnancy and Possible Vertical Transmission. Am J Perinatol 2020; 37(8):861-865.

(14) Dong L, Tian J, He S, Zhu C, Wang J, Liu C et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. JAMA 2020; 323(18):1846-1848.

(15) Zamaniyan M, Ebadi A, Aghajanpoor S, Rahmani Z, Haghshenas M, Azizi S. Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infection. Prenat Diagn 2020; 40(13):1759-1761.

(16) Ferrazzi E, Frigerio L, Savasi V, Vergani P, Prefumo F, Barresi S et al. Vaginal delivery in SARS-CoV-2-infected pregnant women in Northern Italy: a retrospective analysis. BJOG 2020; 127(9):1116-1121.

(17) Zeng H, Xu C, Fan J, Tang Y, Deng Q, Zhang W et al. Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. JAMA 2020; 323(18):1848-1849.

(18) Saadaoui M, Kumar M, Al Khodor S. COVID-19 Infection during Pregnancy: Risk of Vertical Transmission, Fetal, and Neonatal Outcomes. J Pers Med 2021; 11(6).

(19) Kotlyar AM, Grechukhina O, Chen A, Popkhadze S, Grimshaw A, Tal O et al. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. Am J Obstet Gynecol 2021; 224(1):35-53.

(20) Novazzi F, Cassaniti I, Piralla A, Di Sabatino A, Bruno R, Baldanti F. SARS-CoV-2 positivity in rectal swabs: implication for possible transmission. J Glob Antimicrob Resist 2020; 22:754-755.

(21) Valdes G, Neves LA, Anton L, Corthorn J, Chacon C, Germain AM et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta 2006; 27(2-3):200-207.

(22) Jing Y, Run-Qian L, Hao-Ran W, Hao-Ran C, Ya-Bin L, Yang G et al. Potential influence of COVID-19/ACE2 on the female reproductive system. Mol Hum Reprod 2020; 26(6):367-373.

(23) Dashraath P, Wong JLJ, Lim MXK, Lim LM, Li S, Biswas A et al. Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. Am J Obstet Gynecol 2020; 222(6):521-531.

(24) Aghaeepour N, Ganio EA, Mcilwain D, Tsai AS, Tingle M, Van Gassen S et al. An immune clock of human pregnancy. Sci Immunol 2017; 2(15).

(25) Enninga EA, Nevala WK, Creedon DJ, Markovic SN, Holtan SG. Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period. Am J Reprod Immunol 2015; 73(3):251-262.

Table 1. Anti-SARS-CoV-2 Antibody Detection Assays

Kit no.	Tradename	Measured analyte	Assay Manufacturer	Method	Analyzer	Cut-off	Clinical performance (>15 days after positive PCR result)
1	SARS-CoV-2 IgG	SARS-CoV-2 nucleocapsid IgG antibodies	Abbott Diagnostics, Sligo, Ireland	Chemiluminescence Microparticle Immunoassay	Architect i2000R	1.4 index (Sample/Cut-off)	Sensitivity 100.0% Specificity 99.6%
2	Liaison SARS-CoV-2 S1/S2 IgG	SARS-CoV-2 IgG anti S1 and Anti S2 antibodies	DiaSorin S.p.A., Saluggia, Italy	Chemiluminescence immunoassay	Liaison XL	12.0 AU/mL	Sensitivity 97.4% Specificity 98.5%
3	Liaison SARS-CoV-2 IgM	SARS-CoV-2 IgM antibodies	DiaSorin S.p.A., Saluggia, Italy	Chemiluminescence immunoassay	Liaison XL	1.1 index value	Sensitivity 94.0% Specificity 99.3%

Table 2: Demographic characteristics and pregnancy course of women with COVID-19 during pregnancy

Age	30.2 (4.6)
BMI (kg/M^2)	26.2 (5.1)
Number of children	1.7 (1.5)
Place of residency	
City>20,000	36 (50%)

Age	30.2 (4.6)
Town<=20,000	30 (42%)
Village	6 (8%)
Ethnicity	
Jew	27 (38%)
Arab	45 (63%)
Gestational week at COVID-19 disease	29.27 (8.94)
Trimester at COVID 19 disease	
1	5 (7%)
2	21 (29%)
3	46 (64%)
Illness duration (days)	8 (12)
Symptoms	
Asymptomatic	14 (19%)
Fever	12 (17%)
Cough	23 (32%)
Dyspnea	20 (28%)
Rhinorrhea	9 (13%)
Loss of smell sensation	39 (54%)
Fatigue	28 (39%)
Myalgia	23 (32%)
Vomiting	3 (4%)
Diarrhea	6 (8%)
Headache	17 (24%)
Interval between COVID-19 infection and delivery (weeks)	9.6 (8.9)
Delivery week	38.9 (1.8)
Preterm delivery	6 (8%)
Gestational hypertension/preeclampsia	4 (6%)
GDM	7 (10%)
Neonate gender	
Male	43 (60%)
Female	29 (40%)
Cesarean delivery	16 (22%)
Birth weight	3281 (457)
SGA neonate	2 (3%)
APGAR at 1 minute	3 (4%)
APGAR at 5 minute	1 (1%)
Cord pH	7.3 (0.1)

Values are presented as Mean (standard deviation) or number (percent)

Missing values: BMI-2, coed pH - 8

Abbreviations: BMI; body mass index; COVID, Corona virus disease; GDM, gestational diabetes mellitus; SGA, small for gestational age

Table 3: SARS-COV-2 antibody profile of women with COVID-19 disease during pregnancy and of their neonates

Maternal SARS-CoV-2 nucleocapsid IgG	
Negative	42 (58%)

Maternal SARS-CoV-2 nucleocapsid IgG	
Positive	30 (42%)
Maternal SARS-CoV-2 spike IgG	
Negative	33 (46%)
Positive	39 (54%)
Maternal SARS-CoV-2 nucleocapsid and spike IgG	
Negative	30 (42%)
Both positive	27 (38%)
One positive	15 (21%)
Maternal SARS-CoV-2 spike IgM	
Negative	35 (49%)
Positive	36 (51%)
Neonatal SARS-CoV-2 nucleocapsid IgG	
Negative	42 (58%)
Positive	30 (42%)
Neonatal SARS-CoV-2 spike IgG	
Negative	35 (49%)
Positive	37 (51%)
Neonatal SARS-CoV-2 nucleocapsid and spike IgG	
Negative	32 (44%)
Both positive	27 (38%)
One positive	13 (18%)
Neonatal SARS-CoV-2 spike IgM	
Negative	72 (100%)
Positive	0 (0%)
Neonatal PCR for SARS-CoV-2	
Negative	35 (97%)
Positive	1 (3%)

Values are presented as number (percent)

Missing values: Neonatal PCR for SARS-CoV-2 n=36, Maternal SARS-CoV-2 anti-spike IgM n=1