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ORIGINAL ARTICLE

The metabolic and immunological characteristics of pregnant women with COVID-19 and their neonates

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Abstract

Our aim was to investigate whether SARS-CoV-2 infection raised high risks of late pregnancy complications, and posed health problems in fetuses and neonates. We analyzed the data of COVID-19 pregnant women with COVID-19 during late pregnancy and their neonates. Eleven out of 16 (69%) pregnant women with COVID-19 had ++ or +++ of ketone body in urine. The blood uric acid of pregnant patients was 334 μmol/L (IQR, 269–452). D-dimer and FDP in pregnant patients were 3.32 mg/L (IQR, 2.18–4.21) and 9.6 mg/L (IQR, 5.9–12.4). Results of blood samples collected at birth showed that 16 neonates had leukocytes (15.7×10^9/L (IQR, 13.7–17.2)), neutrophils (11.1×10^9/L (IQR, 9.2–13.2)), CK (401 U/L (IQR, 382–647)), and LDH (445 U/L (IQR, 417–559)). Twenty-four hours after birth, a neonate from COVID-19 woman had fever and positive of SARS-CoV-2 gene. Another woman had strongly positive for SARS-CoV-2 gene (+++) for 4 weeks, and delivered one neonate who had SARS-CoV-2 IgM (46 AU/mL) and IgG (140 AU/mL) on day 1 after birth. In the third trimester, COVID-19 infection in pregnant patients raised high risks of ketonuria, hypercoagulable state, and hyperfibrinolysis, which may lead to severe complications. COVID-19 increased the inflammatory responses of placenta, and fetuses and neonates had potential organ dysregulation and coagulation disorders. There was a potential intrauterine transmission while pregnant women had high titer of SARS-CoV-2, but it is necessary to detect SARS-CoV-2 in the blood cord, placenta, and amniotic fluid to further confirm intrauterine infection of fetuses.

Keywords COVID-19 · SARS-CoV-2 · Pregnancy metabolic complications · Intrauterine transmission · Immune responses · Neonates

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Abbreviations

COVID-19	Coronavirus disease-19
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
STP	Serum total protein
ALB	Albumin
TCO2	Total carbon dioxide
UA	Uric acid
GLU	Glucose
Na +	Sodium
AG	Anion gap
OSM	Osmotic pressure
TAG	Triacylglycerol
LDH	Lactate dehydrogenase
FDP	Fibrinogen degradation products
WBC	White blood cell
GGT	Gamma-glutamyl transpeptidase

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Glb	Globulin
A/G	Albumin-globulin ratio
TBIL	Total bilirubin
Mg^{2+}	Magnesium
P	Phosphorus
Ca × P	Calcium-phosphorus product
HDL-C	High-density leptin cholesterol
CK	Creatine kinase
PT	Prothrombin time
PS	Prothrombin time activity
APTT	Activated partial thromboplastin time
FIB	Fibrinogen
AT-III	Antithrombin III
PO2	Partial pressure of oxygen
SO2	Oxygen saturation
SB	Standard bicarbonate
TCO2	Total carbon dioxide
BE	Base Excess
HL	2-Hydroxypropanoic acid (lactic acid)
RBC	Red blood cell

Introduction

In December 2019, a series of pneumonia cases were initially observed in Wuhan City, Hubei province, China, then a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the pathogen for the epidemic pneumonia [1]. On February 12, the World Health Organization (WHO) officially named the infectious disease as coronavirus disease 2019 (COVID-19) [2]. On March 12, 2020, the COVID-19 viral disease has swept into at least 117 countries and killed more than 4000 people, and WHO officially announced a pandemic of COVID-19 viral disease [3]. As of March 22, 2020, many studies reported the clinical and immunological characteristics of the general population with COVID-19, but the studies on pregnant women with COVID-19 and their fetuses were relatively rare [4, 5]. More investigations are needed to explore the effects of the SARS-CoV-2 on pregnant women and their fetuses and neonates.

On one side, due to the changes of pregnancy hormones and metabolic disturbance, some pregnant women during the third trimester experience several metabolic complications. Gestational diabetes mellitus (GDM) is a common metabolic complication during pregnancy, which presents with insulin resistance and beta-cell dysfunction [6]. Besides that, the changes of pregnancy hormones can prevent the cells from using up the glucose, and the body starts using up the fat reserves to attain the required energy, which leads to the production of ketones and pregnancy complications like ketonuria [7]. In the first and early second trimesters, healthy pregnancy remains immune quiescence and inhibition to allow the immunologically distinct fetoplacental unit to develop and grow, and in the third trimester, immune quiescence and inhibition are reversed in association with immune activation and functional immune responses [8, 9]. So how did the metabolic characteristics and the immune responses during the third trimester affect the outcomes of COVID-19 infection in pregnant women?

On the other side, some viruses, including rubella virus, EB virus, and cytomegalovirus (CMV), can spread through intrauterine transmission and pose high risks of health problems in fetuses and neonates [10–12]. When the virus crosses the placenta to the fetus, we can detect the present of viruses in the amniotic fluid, cord blood, and placenta. Additionally, antivirus IgM antibody might present in the serum of fetus while the fetus infects the virus in the uterus [13]. Previous studies described that no evidence of intrauterine transmission was observed in SARS or MERS, but abortion and preterm birth have been reported in cases of infection with SARS and MERS during pregnancy [14, 15]. So far, whether SARS-CoV-2 can spread through intrauterine transmission and how COVID-19 infection affect the fetuses during pregnancy remain largely unknown.

Sixteen pregnant women with COVID-19, 35 to 39 weeks of gestation, were admitted to Renmin Hospital of Wuhan University and delivered 16 neonates by cesarean section. In this study, we investigated 16 pregnant patients and their neonates to explore the risks of metabolic complications and outcomes of COVID-19 pregnant women. In the meanwhile, we examined whether SARS-CoV-2 can spread to fetuses through intrauterine transmission, and explored whether COVID-19 infection in pregnant women posed health problems of fetuses and neonates.

Material and methods

Study oversight

In this study, the data from 16 pregnant women with SARS-CoV-2 infection were collected and analyzed, including clinical characteristics, CT scan images, blood and urine tests, and other primary data. And all these tests were applied to their 16 neonates delivered by cesarean section. All the data recorded from COVID-19 pregnant women and their neonates have been reviewed by a group of professional doctors. The Ethics Committee of Renmin Hospital of Wuhan University had approved the study design.

Distinguishing SARS-CoV-2 infection from the infections of 13 other respiratory tract pathogens

Diagnosis of all the cases was based on the WHO interim guidance. Nasopharyngeal swab specimens were harvested from pregnant women with suspected SARS-CoV-2 infection,

and were detected for ORF1ab and N genes of SARS-CoV-2 by RT-PCR. In order to eliminate the infection of other respiratory tract pathogens, nasopharyngeal swab samples were tested for the genes of respiratory tract pathogens, including influenza A virus types of H1N1 and H3N2, influenza A virus (2009), influenza B virus, parainfluenza virus, respiratory syncytial virus, metapneumovirus, common coronavirus, rhinovirus, adenovirus, bocavirus, mycoplasma pneumoniae, and chlamydia pneumoniae.

Assessing the potential metabolic complications of pregnant women with COVID-19

Blood samples and urine samples were collected from 16 pregnant women with COVID-19. Blood tests were performed for coagulation profile (FDP, D-dimer, and FIB, etc.) and serum biochemical analysis (AST, ALP, CK, LDH, UA, and procalcitonin, etc). The urine samples were tested for bilirubin, glucose, and ketone body, etc. These metabolic indicators were assessed for metabolic complications in pregnant women with COVID-19.

Assessing the immunological characteristics in COVID-19 pregnant women during the acute stage and recovery stage

During the acute stage and recovery stage of COVID-19, blood samples from 16 pregnant women were collected and examined complete blood cell (CBC) and different lymphocyte subsets, as well as humoral immune function (IgM, IgG, IgA, IgE, C3, and C4). C reactive protein (CRP), IL-6, IL-10 and procalcitonin (PCT) were measured for the assessment of inflammatory responses. The titers of SARS-CoV-2 IgM and IgG in pregnant women with COVID-19 were tested by enzyme-linked immunosorbent assay (Vazyme Biotech, Nanjing, China). The titers of IgM and IgG in healthy person are < 10 AU/mL.

Analyzing the impact on fetuses while pregnant women infected with SARS-CoV-2 during late pregnancy

For assessing the potential impact on fetuses of pregnant women with COVID-19, the neonatal blood samples were collected at birth, and were examined for complete blood cell (CBC) and coagulation profile, as well as serum biochemical analysis. Nasopharyngeal swab samples of neonates were harvested at the time of giving birth, and were detected the N gene and ORF1ab genes of SARS-CoV-2 by RT-PCR. For the suspected intrauterine infection of SARS-CoV-2, the titers of SARS-CoV-2 IgM and IgG were measured by enzyme-linked immunosorbent assay, and CT scan images were taken for further assessment.

Statistical analysis

Continuous variables were described as medians and interquartile ranges (IQR), and categorical variables were described as percentages. These variables were compared using the unpaired *t* test with Welch's correction. For comparative analyses, a p value of < 0.05 was considered statistically significant, while < 0.01 was considered highly statistically significant. All statistical analyses were performed using GraphPad Prism 8.0.2 and SPSS 25.0.

Results

The clinical characteristics of 16 pregnant women with COVID-19 in the third trimester

Pregnant women suspected of SARS-CoV-2 infection were admitted to hospitals after January 30, 2020. Sixteen pregnant women, gestation ages of 35–39 weeks, were confirmed with SARS-CoV-2 infection by RT-PCT results, in conjunction with CT scan images. The symptoms of these pregnant women included fever, cough, diarrhea, and minor shortness of breath (Table 1). In 13 out of 16 (81.2%) pregnant patients, the images of chest CT scan presented multiple patchy, ground glass opacity in the left and/or right lungs, which illustrated the typical image features of COVID-19. Three out of 16 (18.8%) showed untypical images, including multiple patchy consolidations with high density shadows in the lobes of lungs. Four out of 16 (25%) patients had small amount of pleural effusion and partial pleural thickening (Fig. 1).

The potential metabolic syndromes in pregnant women with SARS-CoV-2 infection

In order to explore the metabolic changes in pregnant women during the period of SARS-CoV-2 infection, we assessed various metabolic indicators of blood and urine samples. In 16 pregnant women with COVID-19 in the third trimester, 3 patients had gestational diabetes milletus. The blood uric acid of patients was 334 μmol/L (IQR, 269–452), which are much higher than those of healthy pregnant women (288 μmol/L (IQR, 232–308)). In the meanwhile, the osmotic pressure was lower than that of healthy pregnant women. In urine samples, 11 out of 16 (69%) pregnant women with COVID-19 had ++ or +++ of ketone body, which indicated the disorders of glucose and lipid metabolism (Table 1). These pregnant patients may have a potentially high risk of metabolic complication, ketonuria. The levels of D-dimer and FDP in pregnant women with COVID-19 were 3.32 mg/L (IQR, 2.18–4.21) and 9.6 mg/L (IQR, 5.9–12.4), which were much higher than those of healthy pregnant women, 1.70 mg/L (IQR, 1.07–2.60) and 4.9 mg/L (IQR, 3.3–7.2), respectively (Table 1).

Table 1 The metabolic and immunological characteristics of healthy maternities and 16 maternities with COVID-19 in the third trimester

The clinical characteristics of 16 maternities with COVID-19			
Age (years)	24–34		
Weeks of gestation	35–39		
with fever	4(*n*)	25%	
with cough	8(*n*)	50%	
with diarrhea	2(*n*)	12.5%	
with minor shortness of breath	2(*n*)	12.5%	
Exposure to transmission source	6(*n*)	37.5%	
Typical image of COVID-19 (CT scan)	13(*n*)	81.2%	
Delivery by cesarean section	16(*n*)	100%	
Gestational diabetes mellitus	3(*n*)	18.8%	
Ketone body in urine (++ or ++)	11(*n*)	69%	
EBV VCA-IgM(+)	1(*n*)	6.3%	
Treatment with AebidoI/Oseltamivir	14(*n*)	87.5%	
Outcome (recovery)	16(*n*)	100%	
Metabolic parameters	Healthy maternity	Maternity with COVID-19	*P*1 value
AST (U/L)	18(15–20)	21 (16–35)	0.046
ALP(U/L)	144(117–184)	140(123–171)	0.463
STP(g/L)	58(57–63)	57 (56–62)	0.492
ALB(g/L)	37 (35–39)	37 (34–38)	0.341
TC02 (mmol/L)	22 (21–23)	20 (19–21)	0.001
UA (μmol/L)	288 (232–308)	334 (269–452)	0.045
GLU (mmol/L)	4.9 (4.3–5.3)	3.9 (3.5–4.9)	0.401
Na + (mmol/L)	138 (137.5–139)	140 (137.8–145.3)	0.017
Calcium-Calibration (mmol/L)	2.4 (2.2–2.5)	2.5 (2.4–2.6)	0.023
AG(mmol/L)	13 (12–15)	17 (16–19)	0.001
OSM (mmol/L)	293 (292–294)	276 (271–286)	< 0.001
TAG (mmol/L)	3.6 (3.0–5.2)	3.3 (2.7–5.5)	0.777
LDH (U/L)	196 (169–210)	202 (185–258)	0.079
D-dimer (mg/L)	1.70 (1.07–2.60)	3.32 (2.18–4.21)	0.038
FDP(mg/L)	4.9 (3.3–7.2)	9.6 (5.9–12.4)	0.046
Maternity with COVlD-19	Acute stage	Recovery stage	*P*2 value
WBC(10^9/L)	8.7 (6.9–10.5)	7.2 (5.9–9.1)	0.086
Lymphocyte (%)	13.2 (10.1–18.1)	21.3 (15.6–26.0)	0.004
Monocyte (%)	5.8 (4.6–7.3)	6.6 (4.7–7.9)	0.671
Neutrophil (%)	80.4 (73.2–83.8	68.1 (61.6–78.0)	0.004
Eosinophil (%)	0.15 (0–1.12)	2.05 (1.15–3.25)	0.005
Basophil (%)	0.20 (0.10–0.28)	0.50 (0.20–0.80)	< 0.001
Lymphocyte) (10^9/ L)	1.1 (0.9–1.5)	1.6 (1.2–1.9)	0.045
Monocyte (10^9/ L)	0.53 (0.37–0.67)	0.48 (0.37–0.55)	0.179
Neutrophil(10^9/ L)	6.8 (5.2–8.7)	5.1(3.7–6.5)	0.038
Eosinophil(10^9/ L)	0.01 (0–0.10)	0.14 (0.09–0.23)	0.007
Basophil(10^9/ L)	0.02 (0.01–0.03)	0.04 (0.02–0.05)	0.009

1. SI conversion factors: To convert AST values to μkat/L, multiply by 0.0167; ALP values to μkat/L, multiply by 0.0167; LDH values to μkat/L, multiply by 0.0167

2. Data available for healthy pregnant women and 16 pregnant women with COVID-19 in the third trimester

3. Data are presented as number (percentage) and median (IQR, interquartile range)

4. *P*1 values indicate differences between healthy maternity and maternity with COVID-19

5. *P*2 values indicate differences between acute infection and recovery of COVID-19 in maternity

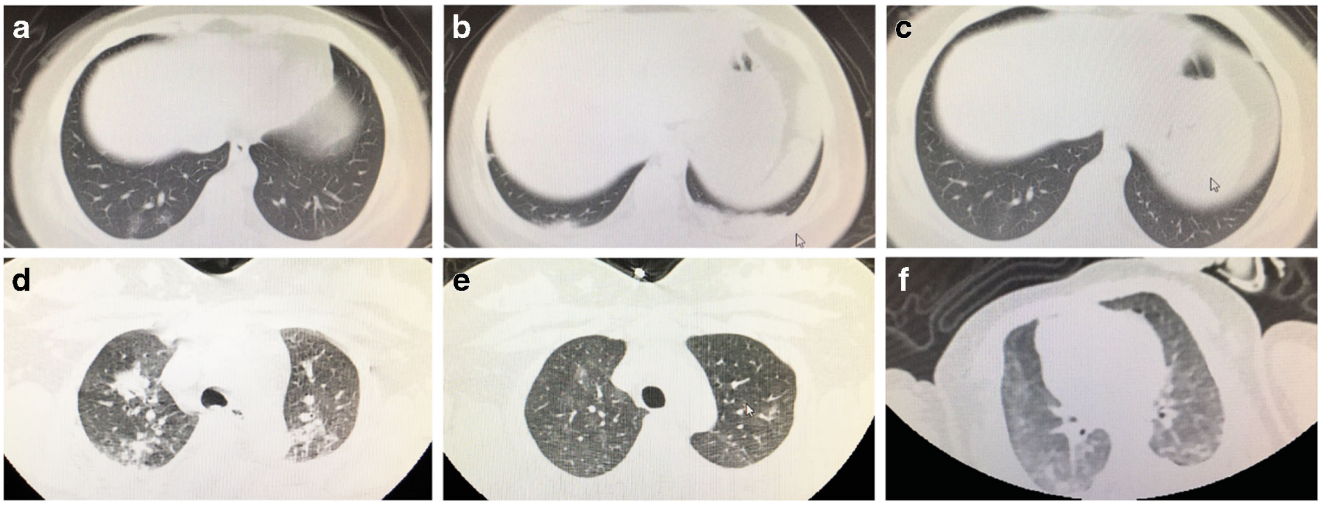

Fig. 1 Chest CT scan features of two pregnant patients with COVID-19 and one neonate with COVID-19 24 h after birth. (a) Pregnant patient 1 during the acute stage: Multiple patchy, ground glass opacity in both lungs, especially in the lower lobes; (**b**) Pregnant patient 1 during the acute stage: A small amount of pleural effusion and partial pleural thickening on both sides; (**c**) Pregnant patient 1 during the recovery stage: Obvious absorption of ground glass opacity; (**d**) Pregnant patient 2 during the acute stage: Multiple patchy shadows with increased density in both lungs; (**e**) Pregnant patient 2 during the recovery stage: Obvious improvement of lung lesions; (**f**) A neonate diagnosed with COVID-19 24 h after birth from pregnant patient: Increased lung markings, spotted and patchy shadows with high-density in the outer area of both lungs, mainly in the back of the upper lobes and the dorsal segment of the lower lobes

These results demonstrated that pregnant women with COVID-19 experienced the state of hypercoagulability and hyperfibrinolysis.

The immunological characteristics and the outcomes of COVID-19 infection in pregnant women in the third trimester

In these pregnant women with COVID-19, lymphocytes were 1.1×10^9/L (IQR, 0.9–1.5) during the acute stage. The analysis of lymphocytes subsets reflected that these patients had $CD4^+$ T cells (428/μL (IQR, 288–487)) and $CD8^+$ T cells (430/μL (IQR, 405–456)), NK cells (133/μL (IQR, 56–185)), and B cells (112/μL (IQR, 85–141)), which remained in the normal range. Besides that, the examinations of IgM, IgG, IgA, IgE, C3, and C4 showed that these pregnant patients held normal humoral immune responses (Table 2).

Further investigation found that these pregnant patients had a high level of monocytes during the acute stage and recovery stage of COVID-19, which might have the benefit to eliminate SARS-CoV-2. During the period of recovery, these pregnant patients had much more lymphocytes (1.6×10^9/L (IQR, 1.2–1.9)) than those in the early stage of COVID-19. These patients also had high titers of SARS-CoV-2 antibodies, 102.9 AU/mL (IQR, 63.6–117.6) of IgM and 125.1 AU/mL (IQR, 88.2–151.7) of IgG, respectively. On day 7 after the onset of COVID-19, one pregnant woman already had 35.1 AU/mL of SARS-CoV IgM (Table 2). The lymphocyte count and the titer of IgG antibodies could be considered as the immunological indicators to monitor the recovery of COVID-19. During the period of recovery, images of chest CT scan reflected that obvious improvement of lung lesions, and absorption of pleural effusion (Fig. 1).

The vital impact on fetuses and neonates while pregnant women infected with SARS-CoV-2 in the third trimester

Sixteen neonates were delivered by cesarean section at the gestational age of 37–39 weeks. Sixteen neonates had 1-min Apgar scores of 7 to 9 and 5-min Apgar scores of 8 to 10. The median birth weight of these neonates was 3.13 kg (IQR, 2.85–3.54). To elucidate whether SARS-CoV-2 infection of pregnant women affected fetuses, we collected the blood samples of neonates at birth from the mothers with COVID-19. The comparative analysis indicated that the leukocytes of these neonates were 15.7×10^9/L (IQR, 13.7–17.2), exceptionally higher than those of neonates from healthy pregnant women (12.3×10^9/L (IQR, 9.0–13.2)) ($p < 0.01$). Similar to the high level of neutrophils (11.1×10^9/L (IQR, 9.2–13.2)), the high ratio of neutrophil and lymphocytes, 4.3 (IQR, 3.2–5.6), presented in these neonates. In the meanwhile, PCT was high than 4.95 in 4 out of 16 (25%) neonates (Table 3). The findings indicated that the inflammatory responses and pathological changes might present in placenta before neonates born from mother with COVID-19.

Further analyses of metabolic parameters showed that these 16 neonates from mother with COVID-19 had low level of PO2 (74 mmHg (IQR, 50–80)) and SO2 (93% (IQR, 82–95)). Extremely high levels of CK 401 U/L (IQR, 382–647) and LDH 445 U/L (IQR, 417–559), presented the potential cardiac dysregulation. These 16 neonates had a high level of GGT

Table 2 The immune functions of 16 maternities with COVID-19 in the third trimester

Lymphocyte subsets	Reference range	Median (IQR) maternity with COVID-19
$CD3^+$ (%)	56–86	78 (75–81)
$CD3^+$ (/μL)	723–2737	790 (719–972)
$CD4^+$ (%)	33–58	37 (29–40)
$CD4^+$ (/μL)	404–1612	428 (288–487)
$CD8^+$ (%)	13–39	37 (32–46)
$CD8^+$ (/μL)	220–1129	430 (405–456)
CD4/CD8	0.9–2.0	1.0 (0.8–1.1)
$CD19^+$ (%)	5–22	9.7 (7.6–12.1)
CD19 (/μL)	80–616	112 (85–141)
$CD16^+56^+$(%)	5–26	9.9 (7.9–15.4)
$CD16^+56^+$(/μL)	84–724	133 (56–185)
Humorial immunity	Reference range	Maternity with COVID-19
lgG (g/L)	7–16	9.5 (7.6–10.3)
lgM (g/L)	0.4–2.3	1.0 (0.6–1.5)
lgA (g/L)	0.7–4.0	1.8 (1.4–2.2)
lgE (IU/mL)	< 100	52 (36–60)
Complement 3 (g/L)	0.9–1.8	1.2 (1.1–1.4)
Complement 4 (g/L)	0.1–0.4	0.2 (0.2–0.4)
SARS-CoV-2 antibody*	Healthy maternity	Maternity with COVID-19
lgM (AU/mL)*	1.6 (0.7–6.7)	102.9 (63.6–117.6)
lgG (AU/mL)*	1.9 (0.4–2.4)	125.1 (88.2–151.7)

*P value < 0.001

1. Data available for 16 pregnant women with COVID-19 in their third trimester
2. Data are presented as median (IQR, interquartile range)

(115 U/L (IQR, 97–231)) and TBIL (50 μmol/L (IQR, 42–62)), and high level of UA (384 μmol/L (IQR, 325–471)) and low OSM (275 mosm/L (IQR, 270–279). Besides that, these 16 neonates also had low level of FIB (1.4 g/L (IQR, 1.2–1.7)), AT-III (48% (IQR, 43–52)), and PS (67% (IQR, 60–70)), and high level of D-dimer (1.26 mg/L (IQR, 0.85–2.78) and FDP (4.5 mg/L (IQR, 2.8–9.2)) (Table 3).

Investigation of the potential intrauterine transmission during pregnancy with SARS-CoV-2 infection

In this study, we thoroughly assessed the potential intrauterine transmission among these 16 neonates. One pregnant woman with COVID-19 delivered a neonate by cesarean section and the neonate had fever 24 h after birth. This neonate tested positive for SARS-CoV-2 genes in the nasopharyngeal swab, and CT scan images also reflected the features of virus infection (Fig.1), which indicated that this neonate might have intrauterine infection with SARS-CoV-2.

Another pregnant woman, 35 weeks of gestation, had typical CT scan images of virus infection and was admitted to the hospital on day 7 after the onset of COVID-19. After the treatment with Arbidol and Oseltamivir for 3 weeks, the N gene and ORF1ab genes of SARS-CoV-2 still showed strongly positive (+++) in the nasopharyngeal swab, and high titers of IgM (108 AU/mL) and IgG (98 AU/mL) presented in the blood sample of this pregnant patient. At 38 weeks of gestation, a neonate was delivered from this pregnant woman with COVID-19 by cesarean section. On day 1 after birth, this neonate had the titers of SARS-CoV-2 IgM (46 AU/mL), and SARS-CoV-2 IgG (140 AU/mL). And on day 15 after birth, this neonate had the titers of SARS-CoV-2 IgM (12 AU/mL), and SARS-CoV-2 IgG (70 AU/mL).

Discussion

With significant changes in endocrine hormones and receptors, as well as the level of progesterone, pregnant women in the third trimester are prone to experience some complications, such as GDM, ketonuria, heart disease, and coagulation disorders [6, 7]. This study found that 11 out of 16 (68.8%) pregnant women with COVID-19 had ++ or +++ of ketone body in urine. The blood glucose levels were monitored for these patients, and the patients were administrated with iv

Table 3 The characteristics of 16 neonates from pregnant women with COVID-19

The clinical characteristics of 16 neonates			
Gestationa1 age (weeks)	37–39		
Weight (kg)	3.13 (median)	2.85–3.54 (IQR)	
1 min Apgar score	8 (median)	7–9 (IQR)	
5 min Apgar score	9 (median)	8–10 (IQR)	
SARS-CoV-2 genes(+)	1 (*n*)	24 h after birth	
SARS-CoV-2 lgM/lgG	1 (*n*)	46 AU/mL, 140 AU/mL on day 1 after birth	
PCT	0.63 (median)	0.16–4.95 (IQR)	
Outcome (recovery)	16 (*n*)	100%	
Metabolic parameters	Reference range	Neonates from COVID-19 maternity	
AST (U/L)	13–35	38 (35–68)	
GGT (U/L)	7–45	115 (97–231)	
STP (g/L)	65–85	56 (51–58)	
ALB (g/L)	40–55	38 (36–41)	
Glb (g/L)	20–40	17 (16–18)	
A/G	1.2–2.4	2.2 (2.1–2.6)	
TBIL(μmol/L)	0–23	50 (42–62)	
TC02 (mmol/L)	22–33	19 (17–21)	
UA (μmol/L)	155–357	384 (325–471)	
GLU (mmol/L)	3.9–6.1	3.4 (3.1–4.5)	
Mg2+ (mmol/L)	0.75–1.02	0.72 (0.67–0.79)	
P (mmol/L)	0.85–1.51	1.97 (1.69–2.20)	
Ca xP (mmol2/L2)	< 4.52	4.61 (4.03–5.83)	
OSM (mosm/L)	280–310	275 (270–279)	
HDL-C (mmol/L)	≥ 1	0.77 (0.66–0.92)	
CK (U/L)	40–200	401 (382–647)	
LDH (U/L)	120–250	445 (417–559)	
PT (s)	9–13	14 (13–14)	
PS (%)	75–135	67 (60–70)	
APTT (s)	25–31.3	54 (45–67)	
FIB (g/L)	2–4	1.4 (1.2–1.7)	
D-dimer (mg/L)	0–0.55	1.26 (0.85–2.78)	
FOP (mg/L)	0–5	4.5 (2.8–9.2)	
AT-III (%)	80–120	48 (43–52)	
P02(mm Hg)	80–100	74 (50–80)	
S02 (%)	95–98	93 (82–95)	
Supercarbonate (mmol/L)	23–31	21 (18–22)	
SB (mmol/L)	23–31	21 (20–22)	
TC02 (mmol/L)	24–32	22 (19–23)	
BE (mmol/L)	–3–3	–4.2 (− 5.8–3.1)	
Blood cells	Neonate from healthy maternity	Neonate from COVID-19 maternity	*P* value
WBC (10^9/L)	12.3 (9.0–13.2)	15.7(13.7–17.2)	0.001
Lymphocyte (%)	26.8 (19.5–37.2)	16.9 (13.7–20.9)	0.014
Monocyte (%)	10.4 (8.8–13.2)	8.5 (7.5–10.1)	0.091
Neutrophil (%)	62.6 (48.5–66.2)	71.6 (64.6–74.6)	0.005
Eosinophil (%)	1.55 (0.50–2.53)	1.70 (0.68–3.53)	0.388
Basophil (%)	0.70 (0.50–1.13)	0.70 (0.50–0.83)	0.534
Lymphocyte (10^9/L)	3.0 (2.2–3.8)	2.5 (2.2–3.0)	0.338
Monocyte (10^9/L)	1.19 (0.90–1.74)	1.34 (1.18–1.61)	0.495

Table 3 (continued)

Neutrophil (10^9/L)	6.2 (4.9–8.9)	11.1 (9.2–13.2)	< 0.001
Neutrophil/lymphocytes	2.3 (1.3–3.2)	4.3 (3.2–5.6)	0.003
Eosinophil (10^9/L)	0.16 (0.06–0.30)	0.26 (0.10–0.55)	0.039
Basophil (10^9/L)	0.09 (0.06–0.12)	0.10 (0.09–0.13)	0.256
RBC (10^{12}/L)	4.8 (4.4–5.3)	4.8 (4.3–5.3)	0.607
Platelet (10^9/L)	255 (243–353)	299 (285–340)	0.259

1. SI conversion factors: To convert GGT values to μkat/L, multiply by 0.0167; CK values to μkat/L, multiply by 0.0167

2. Data available for 16 neonates from COVID-19 pregnant women, and the neonatal blood samples were collected within 24 h after birth

3. Data are presented as median (IQR, interquartile range)

4. *P* values indicate differences between neonates from healthy pregnant women and neonates from COVID-19 pregnant women

injection of glucose while they had low of blood glucose and urine ketone body ++ or +++. The blood uric acid of patients was 334 μmol/L (IQR, 269–452), which were much higher than those of healthy pregnant women (288 μmol/L (IQR, 232–308)). In the meanwhile, the osmotic pressure was lower than that of healthy pregnant women. These results showed that in the third trimester, the COVID-19 infection aggravated the metabolic disturbance. We need to pay attention on the high risk of metabolic complication, ketonuria in patients with COVID-19 during late pregnancy.

Late pregnancy experiences the changes of coagulation profile, including the increase of clotting factors, the decrease of natural anticoagulants and the reduced activity of the fibrinolytic enzyme. These changes are associated with the state of hypercoagulability, and pregnant women are prone to have the potential risk of thrombosis and DIC [16, 17]. The levels of D-dimer and FDP in pregnant women with COVID-19 were 3.32 mg/L (IQR, 2.18–4.21) and 9.6 mg/L (IQR, 5.9–12.4), which were much higher than those of healthy pregnant women. COVID-19 infection increased the high risk of hypercoagulability and hyperfibrinolysis, even potential DIC during late pregnancy. The COVID-19 patients with high risk of hypercoagulability and hyperfibrinolysis were administrated with low-molecular-weight heparin during the perinatal period.

B cells and NK cells, as well as $CD4^+$ T cells and $CD8^+$ T cells reduced slightly, and these pregnant patients held healthy humoral immune, which were similar to the immunological characteristics of non-pregnant persons with mild COVID-19 infection [18, 19]. Further investigation found that these pregnant women had a high level of monocytes during the acute stage and recovery stage, which might have the benefit to eliminate SARS-CoV-2. During the recovery period, these pregnant patients had much more lymphocytes (1.6×10^9/L (IQR, 1.2–1.9)) than those of acute stage of COVID-19, and also had high titers of SARS-CoV-2 antibodies, 102.9 AU/mL (IQR, 63.6–117.6) of IgM and 125.1 AU/mL (IQR, 88.2–151.7) of IgG, respectively. The functional immune responses presented in the third trimester could contribute to the recovery of COVID-19 pregnant women.

Previous studies suggest that the virus infections during the pregnancy are associated with the high rate of complications in fetuses, such as the spontaneous abortion, the premature birth, and the intrauterine growth restriction [20, 21]. To further elucidate whether SARS-CoV-2 infection of pregnant women affected fetuses, we collected the blood samples of neonates at birth from mothers with COVID-19. The comparative analysis indicated that these neonates had exceptionally high level of leukocytes and neutrophils (15.7×10^9/L (IQR, 13.7–17.2)) and (11.1×10^9/L (IQR, 9.2–13.2)), respectively. The ratio of neutrophil and lymphocytes was 4.3 (IQR, 3.2–5.6) in these neonates and PCT of 25% neonates was higher than 4.64 mg/L. These findings suggest that inflammatory responses might occur in placenta before neonate born from mother with COVID-19. Previous study reported that the placentas had pathological changes and abnormal weight when the mothers infected SARS-CoV during late pregnancy [22].

Further analysis indicated that these 16 neonates had low level of PO2 (74 mmHg (IQR, 50–80)) and SO2 (93% (IQR, 82–95)), and high levels of CK 401 U/L (IQR, 382–647) and LDH 445 U/L (IQR, 417–559), as well as the changes of coagulation profile. These results of the blood samples collected on sooner after of neonate birth, indicated that organ dysregulations might happen in the fetuses and neonates during pregnancy with COVID-19.

One of the most critical questions is whether the virus can transmit to fetuses while the pregnant women infected with SARS-CoV-2 [14, 15]. In our study, a pregnant woman with COVID-19 delivered a neonate. 24 h after birth, this neonate had fever. The neonate had positive result of SARS-CoV-2 genes, and CT scan images also showed the features of virus infection. Yu et al. reported that the neonate was confirmed SARS-CoV-2 infection 36 h after birth [23]. A study tested positive of E, RdRP, and N genes of SARS-CoV-2 in a placenta of pregnant women with COVID-19 [24]. These findings suggest that fetuses might have potential intrauterine infection while mother infected with SARS-CoV-2. Another pregnant woman with 35 weeks of gestation was admitted to the hospital on day 7 after the onset of COVID-19, and strongly positive for SARS-CoV-2 (+++) for 4 weeks. A neonate

was delivered from this pregnant patient at 38 weeks of gestation by cesarean section. This neonate had 46 AU/mL of SARS-CoV-2 IgM and 140 AU/mL of IgG on day 1 after birth. Generally, as the largest of immunoglobulin, IgM antibody cannot cross the placenta due to its size [13]. Zeng et al. reported 2 neonates had high titer of SARS-CoV-2 IgM at the time of birth [25]. SARS-CoV-2 IgM in the neonates might have been produced by the fetuses if the high titer of virus crossed the placenta to fetuses. COVID-19 infection induced inflammatory responses and might cause the pathological changes of placentas, and the increased SARS-CoV-2 IgM in fetus might be transferred from mother through placenta under pathological condition.

In our study, chest CT scan images of 16 pregnant patients with COVID-19 showed multiple patchy, ground glass opacity, or multiple patchy shadows with increased density in the lungs. Four of 16 (25%) pregnant patients with COVID-19 had small amount of pleural effusion and partial pleural thickening on both sides. Chest CT represents the good standard to assess COVID-19 pneumonia. Buonsenso et al. reported the results using lung ultrasound for diagnosis and monitoring of COVID-19 pneumonia in pregnant women and children. These studies showed that lung ultrasound examination can be a valid alternative to CT scan and X-ray, particularly for reducing unnecessary radiation in pregnant women and children [26, 27].

Conclusions

In this study, we presented the metabolic and immunological changes of 16 pregnant women with COVID-19 and their neonates. During the third trimester and peripartum, COVID-19 infection raised the high risk of metabolic complication, including ketonuria, hypercoagulability and hyperfibrinolysis in pregnant women. In order to avoid severe complications during the third trimester and peripartum, it is very important for patients with COVID-19 to monitor the levels of blood glucose and urine ketone body, and to administrate with injection of glucose and heparin, etc. The functional immune responses in the third trimester contributed to the recovery of COVID-19 in pregnant patients. On the other side, fetuses and neonates might suffer from the inflammatory responses and metabolic dysregulation while their mother infected with SARS-CoV-2 during late pregnancy. Our investigation indicated that there might be a potential intrauterine infection of fetuses while pregnant women had high titer of SARS-CoV-2, but it is necessary to detect SARS-CoV-2 in the blood cord, placenta, and amniotic fluid to further confirm the intrauterine infection of fetuses.

Author contributions J Zhou and BH developed the concept and designed this study. J Zhou, BH, MW, YW, and J Zhao contributed the acquisition, analysis, and interpretation of data. J Zhou, BH, YW, and LG contributed drafting of the manuscript and critical revision of the manuscript for important intellectual content. JW, YW, J Zhao, and CY conducted statistical analysis. CY, JW, HZ, YT, HT, LG, and DL contributed the administrative, technical, and material support. All authors reviewed and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was established according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Renmin Hospital of Wuhan University on February 10, 2020.

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