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ORIGINAL ARTICLE

SARS-CoV-2 Infection Increases High Risk Rate of Down Syndrome Screening Test by Reducing Alpha-Fetoprotein

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SUMMARY

Background: **The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a significant global health issue in recent years. Numerous studies indicate that COVID-19 during pregnancy is associated with an increased likelihood of pregnancy complications. Additionally, pregnancy itself is known to elevate the risk of severe SARS-CoV-2 infection. To explore the potential impact of SARS-CoV-2 infection on the probability of Down syndrome in fetuses, we conducted serological testing of Down syndrome markers in pregnant women who had contracted the virus.**
Methods: **Serological experiments were conducted utilizing a particle chemiluminescence test. The cohort of pregnant women was categorized into three groups: a control group with no infection, a group infected with SARS-CoV-2 Omicron within the first six weeks of gestation, and a group infected beyond the sixth week of gestation.**
Results: **In the group of individuals infected within 6 gestational weeks, the infection resulted in a decrease in alpha-fetoprotein (AFP) levels and a higher positive rate of Down syndrome screening tests ($p < 0.05$). However, in this study, SARS-CoV-2 infection did not lead to an increase in the occurrence of Down syndrome in the fetus. The positive rate of women infected beyond 6 gestational weeks was slightly higher than the non-infected group (6.2% vs. 5.7%), but these differences were not statistically significant ($p > 0.05$). Within the group infected beyond 6 gestational weeks, there was, compared to the control group, a decrease in free beta human chorionic gonadotropin (β-hCG) levels ($p < 0.05$).**
Conclusions: **This study presents a novel investigation into the impact of SARS-CoV-2 infection on AFP and β-hCG levels. It has been observed that pregnant women who contract SARS-CoV-2 may exhibit an increased likelihood of positive results in serum tests conducted for Down syndrome screening. However, it is important to note that the occurrence of Down syndrome in the developing fetus does not appear to be elevated. To validate these findings, additional research involving larger and diverse cohorts is necessary.**
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INTRODUCTION

In previous years, the global COVID-19 pandemic was caused by the SARS-CoV-2 virus. This infection has been found to have various effects on immunological, respiratory, coagulation, endothelial cell, placental, and vertical transmission mechanisms in pregnant women [1]. Limited evidence exists regarding the potential consequences of COVID-19 during the early stages of pregnancy, specifically within the first 12 weeks of gestation. Previous viral infections, such as Influenza A (H1N1), have been associated with significant maternal mortality rates of 8% and preterm birth rates of 30% [2]. The SARS coronavirus has been associated with a maternal mortality rate of 25.8% [3]. Numerous studies conducted thus far provide reassurance regarding the risk of COVID-19 during pregnancy, indicating no significant difference compared to the general population [4]. However, the impact of SARS-CoV-2 infection on the development of Down syndrome remains inconclusive. Down syndrome, a prevalent chromosomal abnormality, typically arises from fetuses carrying three copies of chromosome 21, known as Trisomy 21 [5]. The etiology of this disease exhibits a high degree of complexity. Individuals diagnosed with Down syndrome frequently present with cognitive impairment, stunted growth, instability of the atlantoaxial joint, decreased neuronal density, congenital heart anomalies, muscular hypotonia, and cerebellar underdevelopment [6]. Moreover, individuals with Down syndrome are at an increased risk of developing various medical conditions, such as autoimmune disorders, recurrent infections, obstructive sleep apnea, auditory and visual impairments, hypothyroidism, epilepsy, hematological disorders, anxiety disorders, and early-onset Alzheimer's disease [5]. Prenatal screening and prenatal diagnosis are crucial strategies for mitigating the incidence of this particular ailment. The identification of Down syndrome risk in fetuses is accomplished through early maternal screening, which involves assessing the concentration of specific substances in the pregnant woman's serum and conducting an ultrasound examination to evaluate the thickness of the fetus's neck zona pellucida [7,8]. The indicators are frequently linked to the presence of Down syndrome in the fetus. The primary objective of Down syndrome screening is to offer an initial evaluation of the fetus's well-being and to offer recommendations for subsequent steps, if deemed necessary. The present study aims to examine the impact of SARS-CoV-2 Omicron on the prevalence of Down syndrome by analyzing the results of Down syndrome screening tests.

MATERIALS AND METHODS

Subjects

A retrospective analysis was done of the clinical records of pregnant women who had undergone Down syndrome screening tests at the Prenatal Diagnosis Center, Maternity and Child Healthcare Hospital of Anyang, Anyang City, Henan Province, China, from October 1, 2022, to May 30, 2023. Pregnant women who were not infected by SARS-CoV-2 Omicron were divided into a control group (n = 1,108), 28.7 ± 4.2 (19 - 35) years old, 16.6 ± 1.0 (15 - 20) gestational weeks, pregnant women who were infected by SARS-CoV-2 Omicron within 6 gestational weeks were divided into test group 1 (n = 1,101), 27.9 ± 4.3 (18 - 35) years old, 16.5 ± 0.9 (15 - 20) gestational weeks, and pregnant women who were infected by SARS-CoV-2 Omicron beyond 6 gestational weeks were divided into test group 2 (n = 2,185), 28.2 ± 4.3 (18 - 35) years old, 16.5 ± 0.9 (15 - 20) gestational weeks. This study has been conducted in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. Approval from the Ethics Committee was obtained for this retrospective study (ID: 2023022 5002).

SARS-CoV-2 Omicron infection test

The *in vitro* diagnostic kit was purchased from Sansure Biotech, Inc., Changsha, China. The pregnant women had a confirmed SARS-CoV-2 diagnosis by a positive result on real-time polymerase chain reaction (qRT-PCR) testing of nasopharyngeal and/or oropharyngeal samples. The samples were sequenced by Anyang City CDC and identified as Omicron variant (BA.1 branch).

Serum test

Down syndrome tests were performed on UniCel Dx-I800 Access Immunoassay System (Bechman Coulter, Inc. Brea, CA92821, USA) by measuring the concentration of β-hCG, unconjugated Estriol (uE_3), AFP, and Inhibin A (Inh A) in the pregnant woman's serum. Lumi-Phos 530 substrate buffer, β-hCG, unconjugated Estriol (uE_3), AFP, and Inhibin A test kit were purchased from Bechman Coulter, Inc. The usable detection limit of β-hCG is 0.6 mIU/mL. The usable detection limit of uE_3 is 0.017 ng/mL. The usable detection limit of AFP is 0.5 ng/mL. The usable detection limit of Inhibin A is 1.0 pg/mL.

Amniotic fluid cell karyotype analysis

Amniotic fluid was cultured using Amniocyte Culture Medium (catalogue number: #20220701, Guangzhou Baiyunshan Baidi Biotechnology Co., Ltd., Guangzhou, China). G-banded karyotyping tests were performed on cultured amniotic fluid according to the standard protocols from Professor Kong's laboratory [9]. The karyotype was determined at a resolution of 320 - 500 band level.

Copy number variation sequencing (CNV-seq) test

CNV-seq was used to detect microdeletion and microduplication in chromatin of fetus. Genomic DNA was extracted from uncultured amniotic fluid, and the CNV-seq was according to Kong et al. [10]. Briefly, after li-

brary construction, samples were sequenced on the NextSeq 550AR platform (ANOROAD Gene Technology (Beijing) Co., LTD, Beijing, China). Bioinformation analyses were done using AnnoCNV system (ANOROAD Gene Technology (Beijing) Co., LTD, Beijing, China). CNVs were classified as one of 5 different types according to the then current American College of Medical Genetics and Genomics (ACMG) guidelines. All abnormal patients were followed by a genetic counselling session before test reports were given.

Statistics

All analyses were performed using SPSS 16.0. A p-value less than 0.05 was considered significant.

RESULTS

Baseline results

Between October 1, 2022, and May 30, 2023, a total of 4,440 pregnant women consented to Down syndrome screening. Among them, 46 pregnant women were excluded from the study due to various reasons, including 20 cases of declined follow-up visits, 12 cases of unexplained miscarriages, 11 cases of long-term medication, and 3 cases involving ethical considerations. Furthermore, 1,108 pregnant women remained uninfected by SARS-CoV-2 Omicron during their pregnancy, while 1,101 pregnant women contracted the virus within the first six weeks of gestation. 2,185 pregnant women were infected by SARS-CoV-2 Omicron during 7 - 12 gestational weeks. For recruitment flowchart, see Figure 1, while the baseline characteristics of the pregnant women in each group are shown in Table 1.

SARS-CoV-2 infection caused higher positive rate of Down syndrome screening tests

The results of Down syndrome screening are categorized as low risk, borderline risk, and high risk. In this study, the incidence of high risk was found to be higher in the group with infection within 6 gestational weeks compared to both, the group with no infection and the group with infection beyond 6 gestational weeks ($p < 0.05$), as shown in Table 2. The high-risk rate in the group with infection beyond 6 gestational weeks was slightly higher than the group with no infection (6.2% vs. 5.7%), yet the difference was statistically nonsignificant ($p > 0.05$). There was no significant difference in the rate of borderline risk among the three groups ($p > 0.05$).

SARS-CoV-2 infection caused AFP decrease in pregnant women

The study utilized measurements of AFP, β-hCG, uE3, and Inh A to evaluate the likelihood of a fetus having Down syndrome [11]. The concentrations of these indicators were measured, and a comprehensive assessment report was generated by incorporating factors such as age, gestational age, weight, and others. Any alterations in the levels of these indicators may result in anomalies in the detection report. The findings of this investigation revealed a decrease in AFP within the group of pregnancies at 6 gestational weeks, indicating a potential association between reduced AFP levels and an increased risk of high-risk outcomes, as previously observed. At the same time, β-hCG was found to decrease in infection beyond 6 gestational weeks group ($p < 0.05$) (Table 3). But the decrease β-hCG did not influence the risk level assessed by Down syndrome screening. As previous research reported, slight fluctuations of the indicators did not affect the assessment of risk levels [12]. As Table 3 shows, uE_3 and Inh A were not significantly different between groups ($p > 0.05$).

SARS-CoV-2 infection did not cause an increase in Down syndrome

The karyotype of the fetal chromosomes of pregnant women at high risk was analyzed using cytogenetics and molecular genetics methods. The data indicated a higher incidence of high risk in the Down syndrome screening test. However, there was no significant association between fetuses of pregnant women infected within 6 gestational weeks and Down syndrome ($p > 0.05$). Additionally, there were no significant differences observed in the detection of fetal chromatin microdeletion and microduplication, as shown in Table 4.

DISCUSSION

Initially, a correlation between maternal age and Down syndrome was established, followed by the discovery of biochemical markers in pregnant women carrying fetuses with Down syndrome [13]. Presently, it is customary to employ biochemical markers and algorithms to assess the risk of Trisomy 21. A screening protocol involving four biochemical markers, namely alpha-fetoprotein, free β-hCG, unconjugated estriol, and Inhibin A, has been extensively utilized during the second trimester [11]. This screening strategy has been extensively employed in numerous countries and regions. Pregnant women who yield positive screening results are required to undergo additional examinations, such as the detection of peripheral blood free DNA. During the process of serum biomarker testing, various factors have the potential to impact the concentrations of the discussed biochemical markers. The presence of abnormalities in any of the four indicators can lead to a positive screening outcome. Conversely, the accuracy of serum screening tests is not as elevated as that of peripheral blood free DNA tests [14]. The serum screening method offers the benefit of being cost-effective and easily applicable, thus it emphasizes the significance of quantifying the influence of positive or negative screening outcomes on patient experience and anxiety. It is crucial for pregnant women with negative screening results to remain vigilant about additional tests associated with Down screening to avoid being misguided by false negative test out-

Table 1. Baseline characteristics of the pregnant women in each group.

Category	No infection group (n = 1,108)	Infection group 1 (n = 1,101)	Infection group 2 (n = 2,185)
Age *			
≤ 25 years	258 (23.3%)	335 (30.4%)	579 (26.5%)
26 - 30 years	381 (34.4%)	371 (33.7%)	721 (33.0%)
31 - 35 years	469 (42.3%)	395 (35.9%)	885 (40.5%)
Gestational age, week			
15 - 15 + 6	99 (8.9%)	100 (9.1%)	193 (8.8%)
16 - 16 + 6	507 (45.8%)	563 (51.1%)	1,047 (47.9%)
17 - 17 + 6	316 (28.5%)	306 (27.8%)	650 (29.7%)
18 - 18 + 6	121 (10.9%)	105 (9.5%)	218 (10.0%)
19 - 19 + 6	49 (4.4%)	21 (1.9%)	62 (2.8%)
20 - 20 + 6	16 (1.4%)	6 (0.5%)	15 (0.7%)
Weight, kg	62.3 ± 10.7	62.6 ± 10.8	61.7 ± 10.8
Cigarette	0	0	0
Alcohol	0	0	0
Assisted reproduction *	119 (10.7%)	242 (22.0%)	434 (19.9%)

Data are n (%) or mean ± SD.
*** There were significant differences between groups ($p < 0.05$).**

Table 2. Distribution of Down syndrome screening results in different groups of pregnant women.

Category	Low risk (n, %)	Borderline risk (n, %)	High risk (n, %)	χ^2 (vs. control group)	p-value (vs. control group)
Control group	927 (83.6%)	117 (10.5%)	64 (5.7%)	-	-
Test group 1	873 (79.2%)	120 (10.8%)	108 (9.8%)	12.89	0.002 *
Test group 2	1,852 (84.6%)	197 (9.2%)	136 (6.2%)	2.15	0.34

*** There were significant differences between groups ($p < 0.05$).**

comes.

In this study, it was observed that the serum AFP level exhibited a decrease in individuals with SARS-CoV-2 infection within the 6 gestational weeks group, as compared to the group without infection. Additionally, in the cohort of women infected beyond 6 gestational weeks, a decrease in β-hCG levels was observed when compared to the control group. The noteworthy reduction in AFP levels resulted in an elevated positive rate of serum screening outcomes. This study represents the first instance of demonstrating a modification in AFP levels among pregnant women during the COVID-19 era. It is important to note that AFP synthesis plays a crucial role in embryonic hematopoiesis within the yolk sac. The production of alpha-fetoprotein (AFP) is initiated by the liver during the onset of fetal hematopoiesis. Over time, the liver assumes the primary role in AFP synthesis, while the developing gastrointestinal tract may produce limited amounts of AFP [15]. AFP is classified within the albumin family, which encompasses vitamin D-binding protein, alpha-albumin, and serum albumin (SA) [16]. In humans, AFP can be detected in fetal serum as early as 29 days post-conception, and it traverses the placenta from the fetus to the mother. During the first trimester of pregnancy, there is a noticeable increase. In a typical pregnancy, there is a direct relationship between the age of the pregnancy and the levels of maternal AFP [16]. Existing literature indicates that pregnant women who tested positive for SARS-CoV-2 had considerably lower vitamin D levels compared to the control group at the moment of childbirth. Since all serum proteins, including those that bind to vitamin D, are produced in the yolk sac during the early stages of embryo development, it is possible that CO-

Table 3. Changes in Down syndrome screening test indicators after SARS-CoV-2 infection.

Category		No infection group (n = 1,108)	Infection group 1 (n = 1,101)	Infection group 2 (n = 2,185)
AFP	Mean ± SD (ng/mL)	47.44 ± 19.00	42.97 ± 18.32	45.99 ± 18.52
	t (vs. control group)	-	5.64	2.12
	F (vs. control group)	-	8.14	1.34
	p (vs. control group)	-	0.004 *	0.25
β-hCG	Mean ± SD (mIU/mL)	(4.46 ± 2.80) x 10^4	(4.83 ± 2.94) x 10^4	(4.39 ± 2.69) x 10^4
	t (vs. control group)	-	-3.00	0.79
	F (vs. control group)	-	0.195	7.97
	p (vs. control group)	-	0.66	0.005 *
uE_3	Mean ± SD (ng/mL)	1.38 ± 0.50	1.29 ± 0.51	1.38 ± 0.52
	t (vs. control group)	-	3.90	-0.46
	F (vs. control group)	-	0.025	3.93
	p (vs. control group)	-	0.88	0.05
Inh A	Mean ± SD (pg/mL)	216.68 ± 104.04	207.13 ± 109.64	208.23 ± 100.49
	t (vs. control group)	-	2.10	2.25
	F (vs. control group)	-	2.46	0.028
	p (vs. control group)	-	0.12	0.868

*** There were significant differences between groups ($p < 0.05$).**

Table 4. Fetus chromosome karyotype of high-risk pregnant women.

Category	No infection group (n = 64, 5.7%)	Infection group 1 (n = 108, 9.8%)	Infection group 2 (n = 136, 6.2%)
seq [hg19] 46, XN (n, %)	61, 5.5%	104, 9.4%	128, 5.9%
Aneuploidy (n, %)	Trisomy 21 (1, 0.09%)	47, XXY (1, 0.09%)	Trisomy 21 (2, 0.09%)
Microdeletion (n, %)	1, 0.09%	2, 0.18%	3, 0.14%
Microduplication (n, %)	1, 0.09%	1, 0.09%	3, 0.14%

VID-19 disrupts the yolk sac's functions. This observation prompts further investigation into the impact of SARS-CoV-2 infection on yolk sac development, and additional research will aid in elucidating the mechanism behind the decline of alpha-fetoprotein (AFP) in pregnant women with COVID-19 during early pregnancy.

In the group of women infected beyond 6 gestational weeks was a slight decrease in β-hCG levels. However, this decrease in β-hCG did not result in significant changes in the serum test of the Down syndrome screening evaluation system. This suggests that the influence of COVID-19 on β-hCG secretion in late pregnancy may not be significant. However, the presence of COVID-19 during the later stages of pregnancy has been identified as a risk factor for various adverse out-

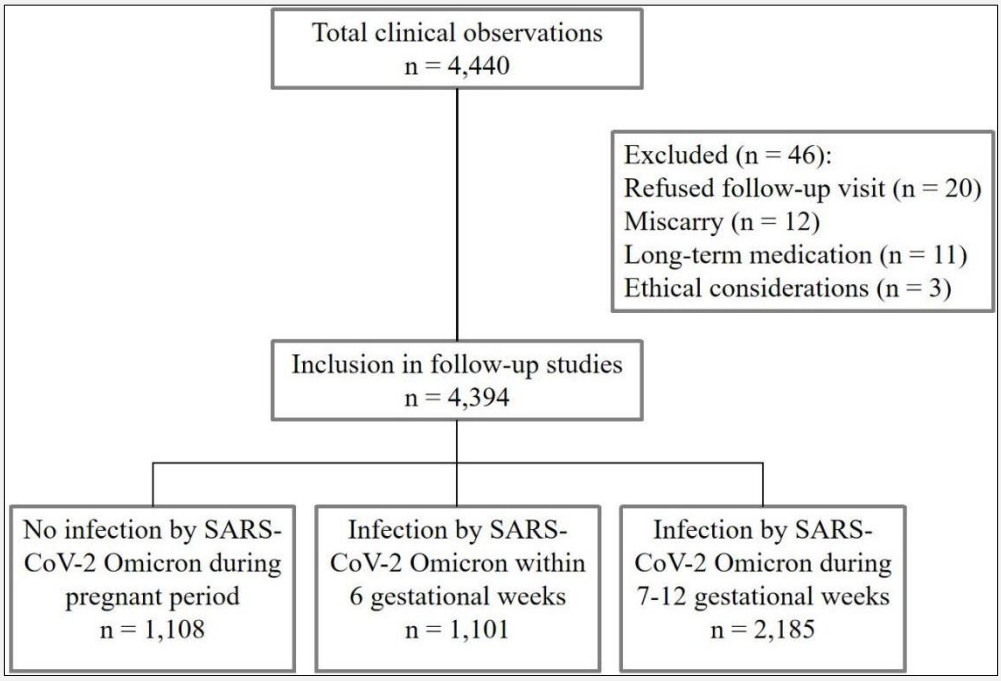
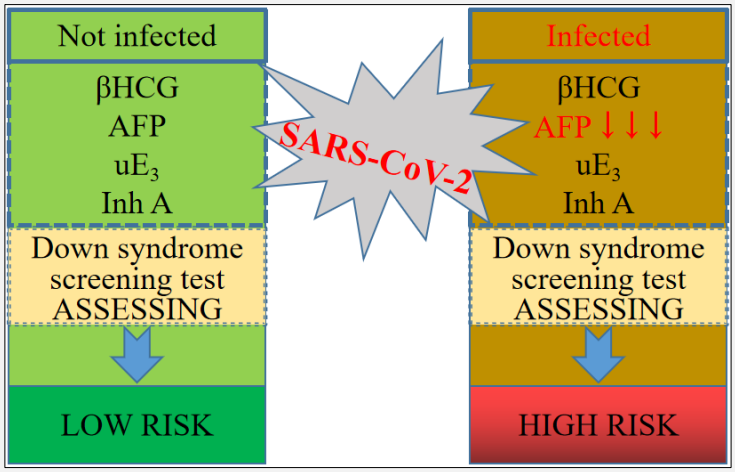


Figure 1. Recruitment flowchart of the current study.

SARS-CoV-2 infection increases high risk rate of Down syndrome screening test by reducing AFP.



1. SARS-CoV-2 infection caused higher positive rate of Down syndrome screening tests.
2. SARS-CoV-2 infection caused AFP decrease in pregnant women.
3. SARS-CoV-2 infection did not cause increase of Down syndrome.

comes, such as preterm birth, pre-eclampsia, stillbirth, intrauterine growth restriction, and developmental defects in newborns [13]. Despite an elevated rate of high-risk results in Down syndrome screening tests, there was no significant association between infection during the first six weeks of gestation and the occurrence of Down syndrome in the fetuses of infected pregnant women. Additionally, there were no significant differences observed in the detection of chromosomal microdeletions and microduplications in the fetal chromatin. In summary, this study has discovered a relatively unique occurrence wherein pregnant women infected with SARS-CoV-2 during the early stages of pregnancy exhibit a notable decrease in serum AFP levels. This finding suggests a potential interference of SARS-CoV-2 with the synthesis of AFP in the yolk sac, thereby offering valuable insights for future investigations.

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Declaration of Interest:
The authors declare that they do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted and that generative AI and AI-assisted technologies were not applied in this study.

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