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Original article

Seroprevalence of antibodies against to SARS-CoV 2 in umbilical cord blood in two hospital centers in Córdoba and Sucre, Colombia



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ABSTRACT

Introduction: Pregnant women continue to be vulnerable to COVID-19, and their immunosuppressed state could put them at greater risk of developing more severe forms of the disease. In Colombia and Latin America, there are few studies on the immune response of the newborn against SARS-CoV-2.

Aim: To determine the prevalence of SARS-CoV-2 infection in umbilical cord blood in two hospital centers in Córdoba and Sucre.

Methods: Between March and June 2021, a prospective descriptive cross-sectional study was carried out. Two hospitals from the departments of Córdoba and Sucre, located in the Northwest Caribbean area of Colombia, participated. Three hundred sixty umbilical cord blood samples were taken at the two hospitals. A commercial ELISA was performed to detect total IgG, IgM, and IgA antibodies against the N protein of SARS-CoV-2. The ethics committee approved the study of the participating institutions.

Results: Of 3,291 women who gave birth in the hospital centers included in the study, 360 (11%) participated. Complete clinical data were obtained for 223 women. The mean age of the women was 24 years (range, 15–42). 29.4% (106/360) of the umbilical cord samples had total antibodies against SARS-CoV-2. Pregnant women did not have blood samples taken. 58% of the women were asymptomatic. There was no association between umbilical cord samples, clinical, epidemiological characteristics, and serological response to antibodies to SARS-CoV-2 ($p > 0.05$).

Conclusions: The prevalence of umbilical cord blood samples was 29.4% for total SARS-CoV-2 antibodies. The study provides essential aspects for the epidemiological approach to neonates infected with SARS-CoV-2.

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1. Introduction

At the end of December 2019, the SARS-CoV-2 virus spread rapidly in the population of Wuhan, China. Since then, there has been a

dramatic increase in the number of new cases and deaths from the COVID-19 disease. Currently, the pandemic is a serious global public health problem, and the appearance of different variants of the virus leads to its persistence. As in other viral infectious diseases, pregnant women continue to be vulnerable to COVID-19. Their immunosuppressed state could expose them to a higher risk of developing more severe forms of the disease [1].

More than 59 million people in the United States and 300 million worldwide have been diagnosed with COVID-19 disease, including more than 155,500 pregnant women in the United States [2]. With approximately 140 million live births annually worldwide, the

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prevalence of SARS-CoV2 in pregnant women is high, with 15% in urban centers. The number of children exposed to maternal COVID-19 infection worldwide could reach up to 20 million per year, particularly in low vaccination coverage for pregnant populations [2].

Maternal-fetal-neonatal SARS-CoV-2 infection can occur in utero after maternal viremia and subsequent placental infection. In intrapartum, it happens through cervical or vaginal secretions or after birth through breast milk. Virus detection by PCR in umbilical cord blood or neonatal blood collected within the first 12 h of birth or amniotic fluid collected before membrane rupture demonstrates congenital infection [3].

Although vertical transmission by SARS-CoV-2 is rare, given the potential for profound maternal immune activation, it is likely to impact the developing fetal brain [4]. Spontaneous abortions, premature births, and neonatal infections have also been described [5,6]. SARS-CoV-2 has been associated with deaths in neonates, stillbirths, chorioamnionitis, small gestational age, and hypoxic-ischemic encephalopathy [4,7]. SARS-CoV and Middle East respiratory syndrome coronavirus has also been reported in pregnant women. These coronaviruses, phylogenetically related to SARS-CoV-2, increased hospitalization in the intensive care unit and case fatality. However, the prenatal transmission of SARS could not be confirmed at that time [6].

During the COVID-19 pandemic, pregnant women were shown to have interrupted their regular prenatal care, with many being admitted to emergency obstetric services requiring immediate intervention. This increased the number of cases diagnosed with small for gestational age and hypoxic-ischemic encephalopathy in newborns [7].

In Colombia, 6,070,616 confirmed cases of COVID-19 and 139,037 deaths (2.5%) had been reported. Being one of the countries with one of the highest mortality rates in Latin America [8]. In pregnant women, 20,902 cases have been reported, with 232 (1.1%) deaths. Regarding newborns, 1,892 have been confirmed, and 9 (0.4%) have died in the country. In the department of Córdoba, the confirmed cases of COVID-19 are 118,827, with 3,953 (3.3%) deaths during the entire pandemic. In the department of Córdoba, 1,215 cases of SARS-CoV-2 and 19 (1.6%) deaths have been reported in pregnant women, and 49 cases in newborns have been reported [8]. In the department of Sucre, 418 cases of COVID-19 have been reported, 3 (0.7%) deceased pregnant women, and 32 cases of COVID-19 in neonates [8].

The serological and seroprevalence studies are helpful to identify asymptomatic infections and contribute to clinical diagnosis. They are essential in the current epidemiological context where molecular tests are only performed on high-risk populations in Colombia, which shows a growing curve of infections and limited resources. The detection of IgM and IgG immunoglobulins against SARS-CoV-2 has been reported in neonates of mothers confirmed by COVID-19 [9]. This serological evidence generates interest in the role of antibodies against SARS-CoV-2 in maternal and child health. Studies carried out on umbilical cord samples indicate that maternal infection has a minimal impact on the immunological profile of the newborn. Studies of stem cells extracted from the umbilical cord have proven safe and effective in treating severe COVID-19 [10].

In Colombia and Latin America, there are few studies on the epidemiological burden of SARS-CoV-2 in pregnant women and the subsequent impact of the immune response on the newborn. The objective of this study was to determine the prevalence of SARS-CoV-2 infection in umbilical cord blood in two hospital centers in Córdoba and Sucre.

2. Materials and methods

2.1. Type of study, population, geographical location, and umbilical cord blood samples

A cross-sectional prospective descriptive study was carried out between March and June 2021 during the third epidemic wave in

Colombia. Two hospitals from the departments of Córdoba and Sucre, located in the Caribbean area in the Northwest of Colombia, participated. These two centers serve a population of 3500 births per year. Three hundred sixty umbilical cord blood samples were taken at the two hospitals. The umbilical cord blood was obtained in the delivery room, the blood was refrigerated and transferred in less than two hours to the laboratory of the Tropical Biological Research Institute of the University of Córdoba. The Apgar test (Appearance, Pulse, Grimace, Activity, and Respiration) was also performed. Five variables are used to check a baby's health in the test.

2.2. Serological test

An ELISA Ingezim® COVID 19 DR (Ref Eurofins, Ingenasa, Madrid) was performed. The test detects total IgG, IgM, and IgA antibodies against the N protein of SARS-CoV-2. According to the manufacturer, the test has a sensitivity of 92.5% against PCR compared to other commercial serological tests. It is 98.3%. Sensitivity is 85% between days 7 and 16 post symptoms and 100% from day 17. Specificity is 99.2% (95% CI: 97.3%–99.9%). Previous studies in the Caribbean region of Colombia showed a sensitivity of 90.1% and a specificity of 74% for this test [11].

2.3. Statistical analysis

The clinical, epidemiological, and demographic data of the women included in the study were taken from the medical records. All participating women completed a questionnaire about symptoms of COVID-19 disease and primary epidemiological data. Data on the neonates were obtained from the electronic health records of the participating clinical centers. An analysis of descriptive statistics, Chi-Square test, was carried out, the significance of the p-value was set at < 0.05 was included in the Infostat® program (version 6, 2019, USA).

2.4. Ethical aspects

The ethics committee approved this study of the participating institutions and the IIBT. The study considered the Declaration of Helsinki that establishes ethical principles for medical research in human beings, including protecting the participants' dignity, autonomy, privacy, and confidentiality [12]. Resolution 008430 of 1993 of the Ministry of Health of Colombia, which regulates studies in health sciences [13], was also taken into account.

3. Results

A total of 3,291 women gave birth in the hospital centers included in the study; 360 (11%) participated. Complete clinical data of 223 women were obtained. Table 1 shows the clinical and epidemiological characteristics of pregnant women and neonates. The mean age was 24 years (range, 15–42). The mean week of gestation was 38 weeks (range, 34–41). Sixty-nine percent (122/175) had a cesarean delivery. The mean birth weight of the babies was 3.186 g (range, 2.340–4.470). The mean APGAR scores at one minute and five minutes after birth were 8.8 (range, 5–9) and 9.8 (8–10), respectively. At the date of the study in Colombia, pregnant women were not prioritized for vaccination against COVID-19.

The 29.4% (106/360) of the umbilical cord samples had total IgG/IgM/IgA antibodies against SARS-CoV-2. An additional test to detect individual IgM antibodies was not performed. Pregnant women did not have blood samples taken. Three neonates were preterm between 34 and 35 weeks of gestation. The month of SARS-CoV-2 infection in mothers is unknown. So far, the children have not been followed up to establish their health status. The study did not consider early or late cord clamping.

Table 1
Clinical and epidemiological characteristics of pregnant women and neonates.

	Mean +SD	Coefficient of variation	Min/Max
Maternal age (years) (n = 218)	24.42 + 6.15	25.18	(15–42)
Gestational age (week) (n = 220)	38.54 + 1.07	2.76	(34–41)
birth weight (g) (n = 222)	3186.36 + 392.4	12.32	(2340–4470)
Size (cm) (n = 215)	50.3 + 1.96	3.9	(45–57)
APGAR score 1 min/10 (n = 218)	8.86 + 0.47	5.3	(5–9)
APGAR score 5 min/10 (n = 207)	9.86 + 0.38	3.88	(8–10)

SD: standard deviation; n = number of subjects with complete clinical data. APGAR (Appearance, Pulse, Grimace, Activity, and Respiration).

The main symptoms related to COVID-19 reported by the 223 participants with a complete clinical history were: 28% cough (n = 63), 23% flu-like symptoms (n = 52), 6.72% fever (n = 15/), 4.9% sore throat (n = 11), 4.9% ageusia (n = 11), 4.5% diarrhea (n = 10), 3.6% anosmia (n = 8), 2.2% digestive disorders (n = 5), and 1.34% respiratory distress (n = 3) (Fig. 1). No association was found between umbilical cord samples, clinical and epidemiological characteristics, and serological response for total antibodies to the SARS-CoV-2 (Fig. 1) (p > 0.05). APGAR values were normal in the neonates.

The comorbidities identified were arterial hypertension (n = 6), syphilis (n = 4), diabetes (n = 1), no patients with HIV were found. Preeclampsia occurred in six pregnant women, being the only obstetric complication reported in the study. The medications used by the participants during pregnancy were antibiotics (n = 85), ivermectin (n = 3), corticosteroids (n = 2), and anticonvulsants (n = 1).

4. Discussion

The prevalence of the present study was 29.4% for total SARS-CoV-2 antibodies in umbilical cord blood samples. A similar study done in Denmark, with 17 cord blood samples, found antibodies against SARS-CoV-2 in 94% [14]. The high seroprevalence could be because the number of Danish samples was low compared to our work that included 360 umbilical cord samples. The time of seroconversion of the neonate is unknown. However, the Danish study demonstrated that the neonate is not protected against infection in the acute phase of maternal SARS-CoV-2 infection. In addition, the Danish study managed to detect antibodies in the mothers, which demonstrates the transmission of some gamma globulins via the placenta [14]. A limitation of the present work is that blood samples

were not taken from the 360 mothers to compare it with the umbilical cord serology.

Many countries were included in a meta-analysis [3], including China, the USA, Italy, Iran, Switzerland, Spain, Turkey, Australia, India, Germany, France, Canada, Honduras, Brazil, and Peru. Three hundred twenty-nine pregnant women with positive RT-PCR and 331 neonates were analyzed. The study demonstrated congenital, intrapartum, and postnatal maternal-fetal-neonatal infections by SARS-CoV-2. The findings were based on the molecular detection of SARS-CoV-2 in amniotic fluid samples, umbilical cord blood, placentas, cervical secretions, and breast milk [3]. The present study has another limitation since RT-PCR tests were not performed. However, antibodies against the N protein confirm natural infection and maternal exposure to SARS-CoV-2. Antibodies found in 29.4% of the umbilical cord demonstrate the highly efficient transplacental transfer of IgG from the mother to the umbilical cord blood [15].

SARS-CoV-2 RNA and IgM antibodies have been found in neonates and this evidences vertical transmission and intrauterine infection by SARS-CoV-2 [16,17]. Studies have found neonates that were seropositive for IgM. However, these findings were not sufficient to confirm the vertical transmission of SARS-CoV-2 since no RT-PCR test was performed [18]. It is unknown whether neonates with positive RT-PCR results have high levels of IgM or IgG, whether the antibodies are protective, and how long antibodies last against infection [19]. Further work is needed to understand SARS-CoV-2 immunity in neonates. Such findings could have implications for possible vaccination guidelines in that population.

Studies suggest that the clinical characteristics and prognosis of pregnant women with COVID-19 could be the same as the general population [20]. Nevertheless, pregnant women appear to be at increased risk of admission to an intensive care unit [21]. In the

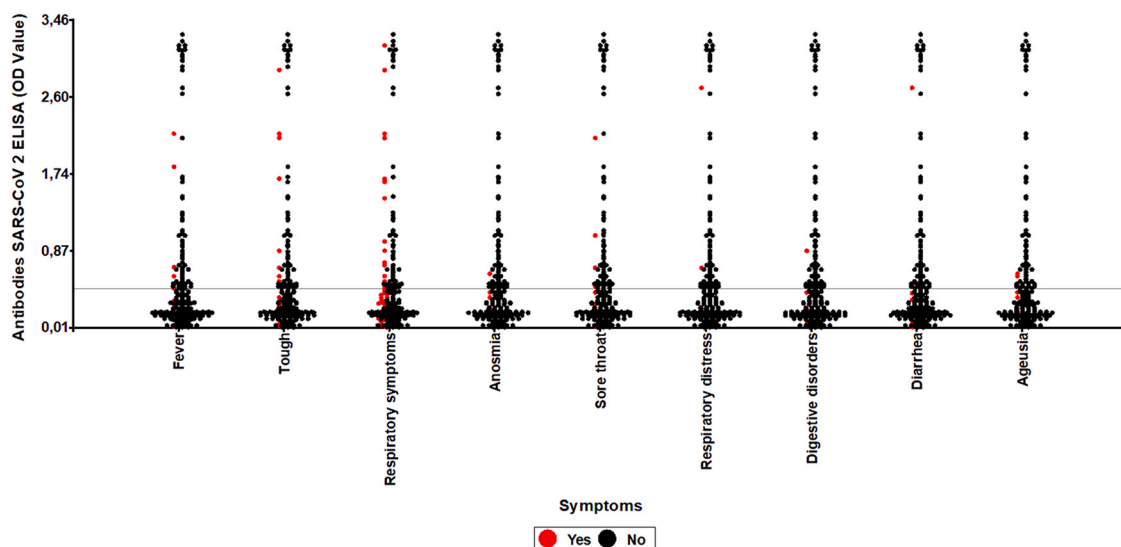


Fig. 1. Relationship between clinical manifestations and the serological test for total antibodies against SARS-CoV-2.

present study, pregnant women did not present obstetric complications, and 58% did not report symptoms related to COVID-19.

In Perú, 2419 pregnant women found a prevalence of 7.0% anti-SARS-CoV-2 [22], much lower than our work, which was 29.4%, possibly due to the lower number of patients included in our study. In the Peru study, IgM was found in 10%, IgM/IgG in 78.8%, and IgG in 11.2%. Our work did not carry out a differentiation of gamma globulins. In the Peruvian study, 89.4% of pregnant women were asymptomatic, contrasting with our study, which was 58%. In the study from Peru, obstetric complications such as premature rupture of membranes (11.8%) and preeclampsia (6.5%) were observed. We have only six pregnant women with preeclampsia, and it was the only obstetric complication reported in the study.

Although the full epidemic peak in Colombia was during the study, of the 360 neonates in this study, all were born healthy with non-respiratory disease compatible with SARS-CoV-2. This is likely because the receptors ACE2 and TMPRSS2, used by SARS-CoV-2, are not expressed in the placenta. Thus, it might be rare for SARS-CoV-2 to cause infection via this route [23,24].

However, our study did not perform a biannual follow-up of neonates during the COVID pandemic. Follow-up is essential because a study of more than 200 pregnant women exposed to SARS-CoV-2 suggested that prenatal infection is associated with an increased risk of neurodevelopmental-related problems [2].

On the other hand, several studies [7] compared the demographic characteristics, morbidity, and mortality of COVID-19 cases in neonates. They found no difference between the groups regarding gestational week and birth weight ($p > 0.05$). Results similar to those of the present study (Table 1). Our study found no association between the clinical and epidemiological characteristics for the SARS-CoV-2 virus ($p > 0.05$) APGAR values were expected in the neonates. In Colombia in 2021, according to the National Administrative Department of Statistics (DANE) population projections, women of childbearing age were 13,472,182 between the ages of 15 and 49. In 2020, the number of births was 629,402. The national birth rate is 12.5 children per 1000 inhabitants, and in the departments of Córdoba and Sucre, it was 13.5 and 14.8 respectively for the year 2020 [25]. Essential data demonstrate that women in Colombia are at risk. Continuing with studies in the current pandemic allows a better understanding of the risks exposed to pregnant women and newborns.

The present study did not analyze the use of drugs used for COVID-19. However, some participants received treatment with antibiotics ($n = 85$), ivermectin ($n = 3$), corticosteroids ($n = 2$), and anticonvulsants ($n = 1$). None of them have shown evidence for the treatment of COVID-19, but in Colombia, the sale of drugs in pharmacies is not strictly controlled by the Ministry of Health.

In conclusion, the present work demonstrated that the high seroprevalence in the umbilical cord indicates that COVID-19 hit the population of Colombian pregnant women hard. The study provides essential aspects for the epidemiological approach to neonates infected with SARS-CoV-2.

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Author contributions

JB, SM, VT, JM, and AM conceived the study, analyzed the data, and wrote and revised the article. DE, GR, GA, and DS assisted with data analysis, interpreted the results, and revised the article. All authors reviewed and approved the final version of the article.

Competing interests

All authors declare no conflict of interest.

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