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A CASE-CONTROL STUDY ON THE MODE OF DELIVERY IN PREGNANT WOMEN WITH AND WITHOUT COVID-19 AND INFLAMMATORY MARKERS CRP AND FERRITIN

ESTUDO CASO-CONTROLE SOBRE O MODO DE PARTO EM GESTANTES COM E SEM COVID-19 E OS MARCADORES INFLAMATÓRIOS PCR E FERRITINA

ESTUDIO DE CASOS Y CONTROLES SOBRE LA VÍA DE PARTO EN GESTANTES CON Y SIN COVID-19 Y LOS MARCADORES INFLAMATORIOS PCR Y FERRITINA

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ABSTRACT

Objective: This study aimed to evaluate the association between COVID-19 infection, inflammatory biomarkers, and the mode of delivery in pregnant women, identifying potential markers associated with obstetric outcomes. Methods: A paired case-control study was conducted with 152 pregnant women (76 cases with confirmed COVID-19 infection and 76 age-matched controls). Sociodemographic, clinical, and laboratory data (serum ferritin and C-reactive protein - CRP) were collected from institutional records. Statistical analysis included Chi-square, G, Z, and Mann-Whitney tests. Odds Ratios (OR) were calculated to assess the likelihood of clinical outcomes and the impact of comorbidities. Results: A significant association was observed between COVID-19 infection and the mode of delivery, with cesarean sections being significantly more frequent in the infected group (OR = 3.0; p = 0.0008). Pregnant women with pre-existing comorbidities had a 63-fold higher likelihood of COVID-19 infection (p < 0.001). While CRP levels showed no significant difference (p = 0.2060), serum ferritin levels were significantly higher in the COVID-19 group (p = 0.0131). Subgroup analysis demonstrated that hyperferritinemia remained a significant finding among cesarean deliveries in the COVID-19 group (p = 0.0185 and p = 0.0406). Conclusions: COVID-19 infection in pregnancy is strongly associated with an increased probability of cesarean delivery. Serum ferritin, rather than CRP, serves as a robust inflammatory biomarker for identifying infected patients and may assist in the clinical stratification of gestational outcomes.

KEYWORDS: *COVID-19. Pregnancy. Biomarkers.*

RESUMO

Objetivo: Este estudo teve como objetivo avaliar a associação entre a infecção por COVID-19, biomarcadores inflamatórios e o modo de parto em gestantes, identificando possíveis biomarcadores para desfechos obstétricos. Métodos: Foi realizado um estudo caso-controle pareado com 152 gestantes (76 casos com infecção confirmada por COVID-19 e 76 controles pareados por idade). Dados sociodemográficos, clínicos e laboratoriais (ferritina sérica e proteína C-reativa - PCR) foram coletados a partir de registros institucionais.

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A análise estatística incluiu os testes Qui-quadrado, G, Z e Mann-Whitney. Odds Ratios (OR) foram calculados para avaliar a probabilidade de desfechos clínicos e o impacto das comorbidades. Resultados: Foi observada associação significativa entre a infecção por COVID-19 e o modo de parto, sendo as cesarianas significativamente mais frequentes no grupo infectado (OR = 3,0; p = 0,0008). Gestantes com comorbidades pré-existentes apresentaram uma probabilidade 63 vezes maior de infecção por COVID-19 (p < 0,001). Enquanto os níveis de PCR não mostraram diferença significativa (p = 0,2060), os níveis de ferritina sérica foram significativamente mais elevados no grupo COVID-19 (p = 0,0131). A análise de subgrupos demonstrou que a hiperferritinemia se manteve como um achado significativo entre os casos de cesariana no grupo COVID-19 (p = 0,0185 e p = 0,0406). Conclusões: A infecção COVID-19 na gestação está fortemente associada a uma maior probabilidade de parto cesáreo. A ferritina sérica, ao contrário da PCR, mostra-se um biomarcador inflamatório robusto para identificação de pacientes infectadas e pode auxiliar no rastreio clínico dos desfechos gestacionais.

PALAVRAS-CHAVE: COVID-19. Gravidez. Biomarcadores.

RESUMEN

Objetivo: Evaluar la asociación entre la infección por COVID-19, los biomarcadores inflamatorios y la vía de parto en gestantes, identificando marcadores predictores de resultados obstétricos. Métodos: Estudio de casos y controles emparejado con 152 gestantes (76 casos con infección confirmada por COVID-19 y 76 controles emparejados por edad). Se recopilaron datos sociodemográficos, clínicos y de laboratorio (ferritina sérica y proteína C reactiva - PCR) de historias clínicas. El análisis incluyó las pruebas de chi-cuadrado, G, Z y de Mann-Whitney. Se calcularon odds ratios (OR) para evaluar la probabilidad de resultados clínicos y el impacto de comorbilidades.Resultados: Se observó una asociación significativa entre la infección por COVID-19 y la vía de parto; las cesáreas fueron más frecuentes en el grupo infectado (OR = 3,0; p = 0,0008). Gestantes con comorbilidades preexistentes presentaron una probabilidad 63 veces mayor de infección por COVID-19 (p < 0,001). Los niveles de PCR no mostraron diferencias significativas (p = 0,2060), pero la ferritina sérica fue significativamente más alta en el grupo con COVID-19 (p = 0,0131). El análisis de subgrupos demostró que la hiperferritinemia se mantuvo como hallazgo significativo en casos de cesárea dentro del grupo infectado (p = 0,0185 y p = 0,0406).Conclusiones: La infección por COVID-19 en el embarazo está fuertemente asociada con una mayor probabilidad de parto por cesárea. La ferritina sérica, a diferencia de la PCR, se presenta como un biomarcador inflamatorio robusto para identificar a las pacientes infectadas y puede ayudar en la estratificación clínica de los resultados gestacionales.

***PALABRAS CLAVE**: COVID-19. Embarazo. Biomarcadores.*

INTRODUCTION

SARS-CoV-2 is the etiological agent of coronavirus disease 2019 (COVID-19), a systemic inflammatory condition characterized by high infectivity and a predilection for the respiratory tract. Pregnant women constitute a high-risk population particularly vulnerable to respiratory pathogens due to physiological adaptations in their immune and cardiopulmonary systems. In this cohort, COVID-19 is associated with increased risks of severe pneumonia, acute respiratory distress syndrome (ARDS), intensive care admission, and maternal mortality [1-4].

The complex immunological milieu of pregnancy, marked by the modulation of cytokine cascades, altered regulatory cell activity, and fluctuations in human chorionic gonadotropin (hCG), estrogen, and progesterone, exacerbates maternal susceptibility to severe COVID-19 infection. Clinically, infected pregnant women show higher rates of preterm birth and cesarean delivery, highlighting the virus's substantial impact on obstetric outcomes [5-8].

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Early risk stratification through reliable laboratory biomarkers is essential for timely clinical intervention. While C-reactive protein (CRP) is widely used to monitor disease progression in obstetric patients, emerging evidence suggests that serum ferritin (SF) may offer superior prognostic value. In severe COVID-19, hyperferritinemia is a hallmark of the hyperinflammatory state and correlates significantly with poor clinical outcomes and mortality [9-12]. However, the specific role of SF in predicting the mode of delivery remains insufficiently explored.

Identifying appropriate biomarkers is crucial for assessing disease severity and guiding the management of the maternal-fetal dyad. Given the potential for adverse outcomes, understanding the relationship between inflammatory markers and obstetric interventions is vital for optimizing maternal and neonatal care.

The objective of this study was to analyze the relationship between COVID-19 infection, inflammatory biomarkers (CRP and SF), and the mode of delivery to establish reliable predictors of obstetric and gestational outcomes.

METHODS

This is an observational case-control study with 152 pregnant women, where the case group consisted of 76 pregnant women with positive diagnoses for COVID-19 and the control group consisted of 76 women with negative diagnoses for this infection.

Population and sample

This paired case-control observational study involved pregnant women aged 18 years or older, categorized into COVID-19 infected (cases) and non-infected (controls) groups. Participants were recruited from the inpatient and outpatient clinics of the University Hospital of the Federal University of Maranhão (HUUFMA) between January 2021 to June 2022. Inclusion criteria for cases required a laboratory-confirmed diagnosis of COVID-19 via RT-PCR. Controls were matched 1:1 to the case group. Exclusion criteria included multiple gestations and co-infections with other known viral pathogens.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) / Research Ethics Committee of the University Hospital of the Federal University of Maranhão (CEP/HUUFMA) under protocol number [CAAE: 32226420.9.0000.5086]. The study was conducted in strict accordance with the Declaration of Helsinki. All participants were fully informed regarding the research objectives and procedures, and written informed consent was obtained from all individuals prior to their inclusion in the study.

Inclusion and exclusion criteria

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This study included all pregnant women (aged over 18 years) who sought HUUFMA services for the treatment or diagnosis of COVID-19 (pregnant women with or without COVID-19). Pregnant women with previously diagnosed immunosuppressive diseases and those who refused to sign the written consent were excluded. Participants with incomplete or missing data in their hospital medical records were also excluded. Pregnant women were divided into two groups: the test group (case) and the control group, according to the result of the COVID-19 diagnosis.

Data Collection

The participants' sociodemographic, computed tomography (CT), and health data (e.g., anemia, comorbidities, C-reactive protein, ferritin) were obtained from the Medical Archive Service (SAME) and/or University Hospital Management Application (AGHU) of HUUFMA and/or the registration system of the Clinical Analysis and Histocompatibility Laboratory Unit (ULACH). Laboratory data, such as real-time reverse transcription polymerase chain reaction (RT-PCR) test results for COVID-19, were collected from ULACH.

Diagnosis for COVID-19

The criteria for the positive diagnosis of the COVID-19 virus were: positivity in the real-time reverse transcription polymerase chain reaction (RT-PCR) test. In cases where patients did not have detectable viral loads by RT-PCR, the COVID-19 Clinical Management Protocol was followed, as outlined by the Ministry of Health. Patients who presented imaging findings on chest computed tomography (CT-thorax) such as bilateral infiltrates and/or ground-glass opacities, accompanied by clinical manifestations such as fever (≥37.8°C), cough, fatigue, dyspnea, malaise, myalgia, upper respiratory symptoms (e.g., shortness of breath), and rare gastrointestinal symptoms, were considered positive.

Laboratory Analysis

The determination of CRP and SF was performed using serum samples collected in a vacutainer-type tube without anticoagulants, by a trained team that followed all biosafety protocols in accordance with hospital guidelines. The blood tests were performed using the Cobas 6000 auto-analyzer system. Serum ferritin assays were conducted using an electro-chemiluminescence immunoassay (ECLIA) with the Elecsys Ferritin kit (Cobas modules e601- Roche®). C-reactive protein assays were performed using an immunoturbidimetric assay, using the Integra CRP HS (ultrasensitive), a method that provides a more accurate measurement, as it can detect minute values of CRP in the blood (Cobas modules e501- Roche®). All tests were carried out using commercial kits following the manufacturer's instructions.

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Data Analysis

All information was entered into an Excel spreadsheet for further data analysis and variable cross-referencing, using BioStat 5.0 and Epi Info software. A descriptive analysis of the sociodemographic and clinical characteristics of both groups was performed: age, skin color, marital status of pregnant women, and gestational age at delivery. The results were expressed in frequency and percentage. For categorical variables, the Chi-square and Odds Ratio tests were used, while numerical variables were analyzed using Normality and Mann-Whitney tests, as they had a non-normal distribution. The analysis was performed on two groups: the test group (pregnant women with a positive COVID-19 diagnosis) and the control group (pregnant women with a negative test for the virus). A significance level of 5% was used for all statistical tests.

RESULTS AND DISCUSSION

The study comprised 152 pregnant women, including 76 patients in the COVID-19 group and 76 age-matched controls. The mean and median ages were 27.32 and 26 years, respectively. Sociodemographic characteristics and gestational age at delivery ($p < 0.001$) are summarized in Table 1. A significant association was observed between COVID-19 infection and the mode of delivery; specifically, pregnant women with COVID-19 were three times more likely to undergo a cesarean section compared to the control group (OR = 3.0; $p = 0.0008$). Furthermore, the presence of comorbidities was strongly associated with COVID-19 status, with a 63-fold higher likelihood of infection in patients with pre-existing conditions ($p < 0.001$; Table 2).

Table 1. Sociodemographic data, gestational age during COVID-19, and neonatal outcomes of pregnant women attended at a university hospital in São Luís, Maranhão, Brazil

Variable	**Total**	**Case group**	**Control group**	***p*-value**
	n=152	(with COVID-19) n=76	(without COVID-19) n=76	
Age*	27.32 (6.40)	28.43(6.46)	26.21(6.19)	0.0323
Skin color**				
White	30 (39.48%)	17 (22.37%)	13 (17.11%)	0.1862
Not white	113 (48.68%)	57 (75.00%)	56 (73.68%)	
No information	9 (11.84%)	2 (2.63%)	7 (9.21%)	
Marital status**				
No partner	121 (79.60%)	59 (77.63%)	62 (81.58%)	0.8068
With partner	30 (19.74%)	16 (21.05%)	14 (18.42%)	
No information	1 (0.66%)	1 (1.32%)	0 (0.00%)	

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Gestational age** (in weeks)				
1-13	3 (1.97%)	3 (3.95%)	0 (0.00%)	<0.001
14-26	11 (7.24%)	11 (14.47%)	0 (0.00%)	
from 27	137 (90.13%)	62 (81.58%)	75 (96.68%)	
No information	1 (0.66%)	0 (0.00%)	1 (1.32%)	
Results neonatal**				
Abortion	1 (0.66%)	1 (1.32%)	0 (0.00%)	
Pre-term	5 (3.29%)	5 (6.58%)	0 (0.00%)	<0.001
Term	57 (37.50%)	45 (59.21%)	12 (15.79%)	
No information	1 (0.66%)	0 (0.00%)	1 (1.32%)	

*Mean and standard deviation (t-test). **Frequency and percentage (G Test).

Table 2. Association between COVID-19 and mode of delivery and comorbidities in pregnant women attended at university hospital in São Luís, Maranhão, Brazil

Variable	Case Group (with COVID-19)	Control group (without COVID-19)	Odds Ratio	95% IC	*P*
Mode of delivery					
Cesarean delivery	52 (68.42%)	36 (47.37%)	3.1296	1.6439-5.9581	0.0008
Vaginal	24 (31.58%)	40 (52.63%)			
Comorbidities					
Yes	55 (72.37%)	3 (3.95%)	63.7302	18.0892-224.5276	<0.0001
No	21 (27.63%)	73 (96.05%)			

* Chi-square, α=0.05.

The most prevalent comorbidities among the case group were anemia (7.89%), urinary tract infections (7.89%), cardiovascular diseases (5.26%), and systemic arterial hypertension (2.63%). Regarding inflammatory biomarkers, no significant differences were observed in C-reactive protein levels between the two groups (p = 0.2060). Conversely, serum ferritin levels were significantly higher in pregnant women with COVID-19 (p = 0.0131, Z-test). The distribution of these biomarkers is illustrated in the BoxPlot diagrams (Figures 1A and 1B; Table 3).

Table 3. Summary statistics of C-reactive protein and ferritin levels among pregnant women with COVID-19 at a university hospital in São Luís, Maranhão, Brazil

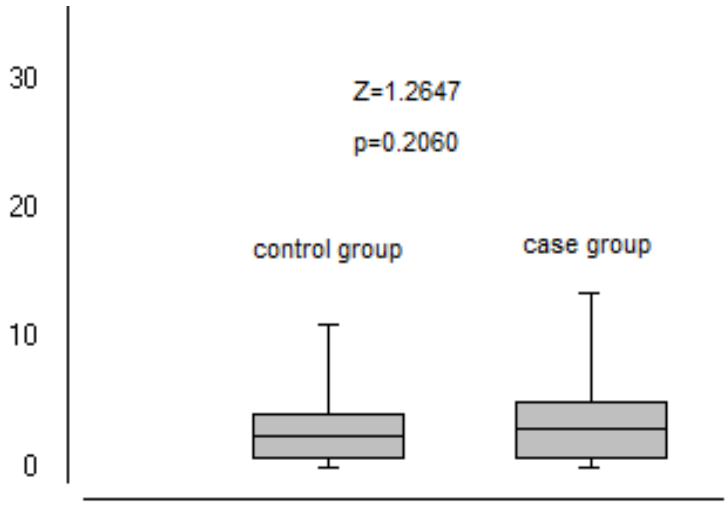
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		Case group (with COVID-19)	Control group (without COVID-19)	Test Z*
CRP				
	Average	4.0271	3.0076	Z=1.2647
	Median	1.2800	1.0250	p=0.2060
	Standard deviation	5.5454	4.3163	
	Amplitude	27.4300	22.1800	
	Variance	30.7513	18.6304	
Ferritin				
	Average	66.8076	45.7204	Z=2.4822
	Median	45.0900	33.3800	p=0.0131
	Standard deviation	65.9762	33.6703	
	Amplitude	277.0800	138.0800	
	Variance	4352.8542	1133.6903	

CRP: C-reactive protein. * α=0.05

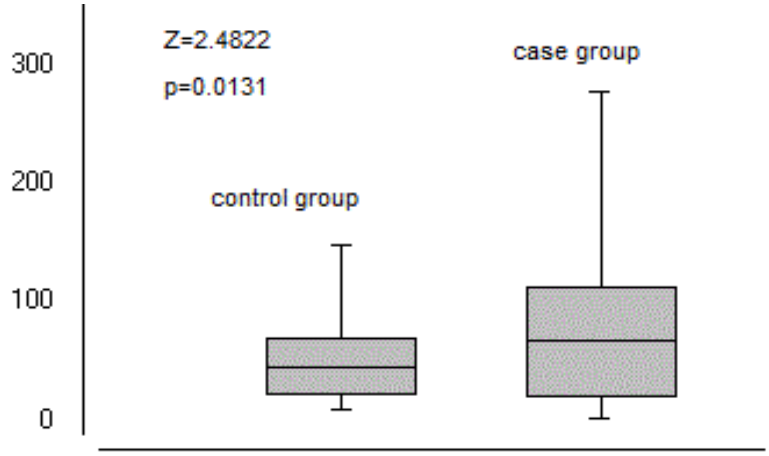
Figure 1A. BoxPlot graph with the distribution of data on the inflammatory biomarker C-reactive protein in pregnant women with (case group) and without COVID-19 (control group), attended at a University Hospital in São Luís/Maranhão, Brazil.



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Figure 1B. BoxPlot graph with the distribution of data from the inflammatory biomarker ferritin in pregnant women with (case group) and without COVID-19 (control group), attended at a University Hospital in São Luís/Maranhão, Brazil



Subgroup analysis based on the mode of delivery revealed that ferritin levels remained significantly elevated in the COVID-19 group regardless of the delivery method. Statistically significant differences were maintained for both vaginal delivery (p = 0.0185) and cesarean section (p = 0.0406), as detailed in Table 4.

Table 4. Ferritin levels in pregnant women with and without COVID-19, according to mode of delivery, attended at a University Hospital in São Luís/Maranhão, Brazil

Measures of Ferritin Levels	with COVID-19		without COVID-19	
	Vaginal	Cesarean delivery	Vaginal	Cesarean delivery
	g1	g2	g3	g4
Median	45.2100	54.4050	27.5500	46.5350
Average	57.2904	120.4504	44.3028	47.2956
Standard deviation	67.3026	189.3814	35.8441	31.5096
Mann-Whitney test, α =0.05				
g1 x g2 – p=0.1374		g1 x g4 – p=0.8445		**g2 x g4 – p= 0.0406**
g1 x g3 – p=0.6978		**g2 x g3 – p=0.0185**		g3 x g4 – p= 0.7016

A pivotal finding of this study was the robust association between COVID-19 and the mode of delivery. Patients infected with COVID-19 were three times more likely to undergo a cesarean section compared to non-infected pregnant women. This aligns with global observations of increased surgical

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interventions during the pandemic [1], although some smaller cohorts have reported statistically non-significant trends in preterm births and cesarean rates [2]. While acute COVID-19 should not inherently mandate a cesarean delivery, unless maternal or fetal decompensation occurs, the high rates observed may reflect clinical caution regarding potential vertical transmission or the instability of the maternal respiratory status [3,13-15].

Interestingly, while CRP is a well-established predictor of ICU admission and disease severity [16], our data did not show a statistically significant difference in CRP levels between the groups. In contrast, our findings suggest that serum ferritin was associated with COVID-19 status and delivery outcomes, showing higher sensitivity in this cohort. Ferritin regulation is intricately linked to hepcidin, a liver-synthesized hormone stimulated by pro-inflammatory cytokines [17,18]. Our results demonstrated significantly elevated ferritin levels in COVID-19 patients across both delivery modes, suggesting it may be more strongly associated than CRP with adverse obstetric outcomes in this population.

The pathogenic role of ferritin in COVID-19 warrants particular attention. The COVID-19 spike protein can mimic hepcidin, leading to hyperferritinemia and an influx of free iron. This process triggers oxidative stress, ferroptosis, and hypoxia, all of which can severely compromise fetal development [17,18]. Furthermore, extreme hyperferritinemia is a hallmark of cytokine storm syndrome and hemophagocytic lymphohistiocytosis (HLH), conditions associated with poor prognosis and multiorgan failure [10,11,19]. Beyond being a byproduct of inflammation, ferritin may actively drive the inflammatory process by binding to T-cell immunoglobulin and mucin domain 2 (TIM-2), further amplifying the expression of pro-inflammatory mediators [12,19].

The significantly higher ferritin levels observed in our cesarean group suggest that hyperferritinemia may serve as a surrogate biomarker for the systemic instability that prompts surgical intervention. Despite limitations such as a relatively modest sample size and the analysis of only two biomarkers due to financial constraints, this study's strengths include rigorous RT-PCR-based diagnosis and standardized clinical management. Given that the pandemic has been correlated with increased cesarean and prematurity rates in Brazil [20], our findings reinforce the importance of monitoring serum ferritin as a dual diagnostic and prognostic tool.

FINAL CONSIDERATIONS

In conclusion, this study establishes a clinical association between maternal COVID-19 infection, a prevalence of pre-existing comorbidities, and a threefold increase in the likelihood of cesarean delivery. While C-reactive protein demonstrated limited sensitivity and failed to differentiate between patient cohorts, serum ferritin emerged as a robust, highly sensitive biomarker for COVID-19 status and adverse obstetric outcomes, maintaining statistical significance across all delivery modalities. COVID-19 infection in pregnancy is strongly associated with an increased probability of cesarean

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delivery. Serum ferritin, rather than CRP, serves as an inflammatory biomarker for identifying infected patients. Therefore, monitoring serum ferritin levels provides objective clinical data for risk assessment, helping to guide maternal-fetal medical interventions and prevent gestational complications.

Conflict of interest: The authors declare no conflict of interest.

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