

Research Article

Estimation of Immunoglobulins in Iraqi SARS-CoV-2-Infected Mothers and Their Infants

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ABSTRACT

Objectives: The study aimed to estimate the levels of specific immunoglobulins (IgG, IgM, and IgA) in mothers and their infants affected by COVID-19 and to examine the impact of vaccination on antibody levels.

Method: It was a case-control study conducted among Iraqi pregnant women who gave birth at Saint Raphael Hospital in Baghdad. A total of 148 Iraqi pregnant women were included, with 122 females previously infected with SARS-CoV-2 (group 1) and 26 healthy controls (group 2). Blood samples were collected from mothers before birth and from newborns after birth. The diagnosis of COVID-19 in patients was based on clinical characteristics and PCR results. IgG, IgM, and IgA were detected using the ELISA technique. Real-time PCR was used to detect SARS-CoV-2 RNA in nasopharyngeal swabs.

Results: IgG was detected in both mothers and their infants, while IgA was detected in mothers only. IgM was not detected in either mothers or infants. The study also examined the impact of vaccination on antibody levels.

Conclusions: The study concludes that IgG and IgA were identified only in mothers, with IgM being undetectable in both mothers and their infants. The most robust immune responses were observed in mothers who had received the AstraZeneca vaccine.

Keywords: IgG, IgM, IgA, Sars-Cov-2 Infection, Pregnancy, Infants

Introduction

The new SARS-CoV-2, which emerged in Wuhan, China in December 2019, is a beta coronavirus that belongs to the same family as the earlier severe acute respiratory syndrome (SARS) virus.¹ and the Middle East Respiratory Syndrome (MERS) virus.² Because SARS-CoV-2 is a new virus, there is a lack of data on the impact of the infection in pregnant women, including differences from other infections in adults, the risk of vertical transmission to the foetus, and the impact on foetal health, if any. Most pregnant women with COVID-19 disease have mild or moderate flu-like symptoms.³ Understanding the neonatal immune response to SARS-CoV-2 and the transmission of maternally derived passive immunity to newborns is critical to inform current infection prevention and vaccination strategies against COVID-19. This knowledge is essential to protecting pregnant women and children. A crucial aspect of immunity against infectious pathogens in young children depends on the sufficient production of maternal antibodies, their passage through the placenta to the foetus, and the persistence of passive immunity in

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the newborn. Recent publications have shown evidence of the transmission of maternal antibodies against SARS-CoV-2 across the placenta.⁴⁻⁶

Protection against early neonatal infection depends on the extent of passage of the infectious agent across the placenta and the transfer of maternal antibodies to the foetus. Transplacental transfer of maternal IgG may protect the newborn from infection.⁷

Our aim was to examine the transmission of maternal antibodies to infants born to women infected with SARS-CoV-2 during pregnancy. In this evaluation, SARS-CoV-2 antibodies were measured in maternal serum, infant blood samples, and breast milk during postpartum hospitalisation.

Method

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This case-control study was conducted at Saint Raphael Hospital (Al-Rahibat) in Baghdad, Iraq, from October 20, 2022, to March 1, 2023. A total of 148 pregnant Iraqi women, aged 18 to 37 years, were included, regardless of their SARS-CoV-2 status or symptoms. Upon admission, all women underwent SARS-CoV-2 screening with nasopharyngeal (NP) swab. A venous blood sample was taken from the mother shortly before delivery. The diagnosis was based on clinical characteristics and PCR results. Approval for the study was obtained from the Ethics Committee of the Faculty of Biology (University of Baghdad) [CSEC/1022/0126 on October 17, 2022], and consent was obtained from patients.

Inclusion Criteria

Mothers with a negative SARS-CoV-2 PCR test result (previously infected) were assigned to the patient group, while those without an infection formed the control group.

Exclusion Criteria

History of cancer

History of autoimmune disease

Real-Time PCR

SARS-CoV-2 RNA was detected using a qualitative real-time PCR test kit (Biocomma® Nucleic Acid Purification Kit, CMC Medical Devices & Drugs S.L.C./Horacio, Malaga, Spain). RNA isolated from a nasopharyngeal swab was detected using the Real Line SARS-CoV-2 kit. Internal controls were included in all PCR tests. PCR analyses were conducted within 24 hours of sample collection.

Serum Collection

Venous blood samples were collected from mothers before birth and from newborns after birth. Serum was isolated by centrifugation and stored at -20°C until analysis. IgG, IgM, and IgA were detected using ELISA.

IgG, IgM, and IgA Detection by ELISA

ELISA tests were performed using kits from Siemens (Germany), following the manufacturer's instructions. The tests detect specific antibodies targeting the nucleocapsid and spike proteins of SARS-CoV-2.

SERION ELISA Agile Test Principle

Microplate test strips coated with antigens specific to the pathogen are used. Diluted samples are incubated in the coated wells, and specific antibodies present in positive samples bind to antigens. Secondary antibodies labelled with alkaline phosphatase detect these bound antibodies. The enzymatic reaction produces a coloured product, which is measured photometrically at 405 nm to determine antibody concentration.

Results and Discussion

One hundred and forty-eight women were included, with 122 previously infected with SARS-CoV-2 and 26 uninfected controls, non-smokers, without prior abortions, chronic diseases, or vaccine doses. Most women underwent caesarean sections at Saint Raphael Hospital. Table 1 shows detailed characteristics of women and infants.

Amid the widespread transmission of SARS-CoV-2 globally, infections among pregnant women are becoming more common. Despite this, there are limited reports on infections in pregnant women and their newborns, for instance, among 34 pregnant women diagnosed with SARS-CoV-2 in Wuhan hospitals, 30 were in the third trimester and four were in the second trimester. Foetal health was closely monitored in eight cases, revealing complications such as vaginal bleeding and premature rupture of membranes (PROM) in some instances, alongside other conditions like gestational hypertension and preeclampsia.⁸

In our study, all participants had a history of exposure to COVID-19, with the majority showing negative findings on chest CT scans. Notably, caesarean delivery was prevalent, accounting for 92.6% of cases, while vaginal delivery occurred in only nine instances. Fortunately, no maternal deaths were recorded.

Our findings indicate a notable elevation in IgG levels among mothers previously infected with SARS-CoV-2 compared to the control group (Table 2 and Figure 1). Conversely, IgM results were consistently negative across both patient and control groups.

Protection against early neonatal infection relies on the transfer of maternal antibodies across the placenta.⁹ During the second trimester, IgG antibodies are typically passed from mother to foetus, with umbilical cord levels often higher than maternal levels, especially post-vaccination.^{10,11} However, in cases of infection, this transfer may be less

efficient. The transfer ratio varies based on factors like placental health, time of infection to delivery, and maternal antibody levels.¹²

Detection of IgM occurs within 3–6 days post-SARS-CoV-2 infection, while IgG detection follows after eight days.^{13,14} IgM indicates recent exposure, while IgG suggests prior infection, aiding in clinical diagnosis, especially when RT-PCR results are negative.^{15,16}

Both natural infection and vaccination trigger the production of SARS-CoV-2-specific antibodies, notably IgA, IgM, and IgG against viral proteins.^{17,18} IgG levels may remain elevated for over a year, with titers correlating with disease severity.¹⁹ Notably, symptomatic patients tend to have higher and longer-lasting antibody responses.²⁰

Infants born to mothers with COVID-19 often carry anti-COVID-19 antibodies, primarily IgG, suggesting placental transfer. Studies report varying antibody prevalence among newborns, irrespective of maternal symptoms, indicating a consistent transfer of antibodies across the placenta.^{21,22}

Table 3 exhibits IgA levels in previously infected mothers and their infants. The results display that 87.7% were positive in serum and 86.1% in milk, while it was not detected in the serum of infants (Figure 2).

Infants typically have low IgA levels in serum samples taken immediately after birth, necessitating follow-up to assess antibody titers. Passive immunity, acquired from maternal antibodies via the placenta and breast milk, offers protection for 3–9 months until the infant's active immune response develops.²³

Breast milk, a complex mixture of nutrients and antibodies, primarily contains IgA, crucial for mucosal defence.^{24,25} However, IgG and other antibodies are also present, con-

tributing to immune protection. The exact amounts and quality of transferred antibodies remain uncertain but breastfeeding has been linked to protection against various infections, potentially including SARS-CoV-2.²⁶

A significant anti-SARS-CoV-2 response was observed in both serum and breast milk of infected mothers, characterised by IgA predominance in milk and IgG dominance in serum.^{27,28}

Table 4 outlines data from 64 vaccinated women and 58 non-vaccinated mothers, with the highest IgG concentration observed in non-vaccinated mothers (29.8 g/L). Differences in serum IgA and IgA levels in milk were non-significant across vaccinated and non-vaccinated groups.

Table 5 reveals that there is a significant (P > 0.05) rise in IgG concentration in babies delivered from non-vaccinated moms (36.5 g/dL) previously infected with the SARS-CoV-2 virus as compared to the vaccinated group (27.2 g/dL). No significant differences were recorded in the levels of IgG, IgA, or weight of the babies delivered from mothers suffering from chronic diseases and intact moms infected with the SARS-CoV-2 virus. The AstraZeneca vaccine induced an immune response in vaccinated mothers, as evidenced by increased serum IgG levels in previously infected patients who received the AstraZeneca vaccine in Iraq.²⁹ Additionally, studies have demonstrated efficient transmission of SARS-CoV-2 IgG antibodies across the placenta in mothers vaccinated with the Pfizer mRNA vaccine during pregnancy, suggesting potential immunity transfer to newborns. This suggests that maternal vaccination not only protects mothers from COVID-19 but also confers immunity to newborns.³⁰ However, further research is needed to assess the effectiveness of humoral response in this context.

Variables		Mother	Duchahilitu		
		Patients group n (%)	Control group n (%)	Probability	
Age mean ± SE (Years)		28.99 ± 0.49	28.50 ± 0.49	P > 0.05	
	1st time	48 (39.3)	0(0.0)		
	2nd time	32 (26.2)	0(0.0)	Uncountable	
Pregnancy	3rd time	23 (18.9)	0(0.0)		
	4th time	12 (9.8)	0 (0.0)		
	5th time	6 (4.9)	0 (0.0)		
	> 5 times	1 (0.8)	0 (0.0)		
Previous abortions	Yes	37 (30.3)	0 (0.0)	D : 0.001	
	No	85 (69.7)	26(100)	Ρ<0.001	
Smoking status	Yes	9 (7.4)	0 (0.0)	P < 0.001	
	No	113 (92.6)	26 (100)		

Table I.Characteristics of Sars-Cov-2 Mothers and Their Infants at Birth

N = 122

	None	85 (69.7)	26(100)	
Previous abortions	1 time	22 (18.0)	0 (0.0)	
	2 times	8 (6.6)	0 (0.0)	P > 0.05
	3 times	2 (1.6)	0 (0.0)	
	4 times	2 (1.6)	0 (0.0)	
	> 4 times	3 (2.5)	0 (0.0)	
	Nil	114 (93.4)	26 (100.0)	
	Diabetes	2 (1.6)	0 (0.0)	
Chronic diseases	Asthma	5 (4.1)	0 (0.0)	P < 0.001
	Epilepsy	1 (0.8)	0 (0.0)	
COVID-19	Yes	64 (52.5)	0 (0.0)	D : 0.001
vaccination status	No	58 (47.5)	26 (100)	P < 0.001
	Nil	58 (47.5)	0 (0.0)	
COVID-19	Pfizer	51 (41.8)	26 (100)	P < 0.001
vaccination status	AstraZeneca	5 (4.1)	0 (0.0	
	Sinopharm	8 (6.6)	0 (0.0)	
	Artificial	9 (7.4)	19 (73.1)	
Type of feeding	Natural	105 (86.1)	7 (26.9)	P < 0.001
	Mixed	8 (6.6)	0 (0.0)	
-	Natural	9 (7.4)	6 (23.1)	5
Birth type	Caesarean	113(92.6)	20 (76.9)	P < 0.001
Variables		Infants' group		
		Patients group n (%)	Control group n (%)	Probability
Weight (kg)		3.25 ± 0.03	3.26 ± 0.03	P > 0.05
Infants' sex	Males	67 (54.9%)	19 (73.1%)	
	Females	55 (45.1%)	7 (26.9%)	P < 0.001
	Intact	121 (99.2)	26 (100)	D
Infants' status	Premature	1 (0.8)	0(0.0)	P < 0.05

Table 2.Means of IgM and IgG Levels of Mothers Infected with Sars-Cov-2 Virus and Healthy Control

Mothers/Infants	COV IgG (mean ±	Drobobility	
	Patient group	Control group	Probability
Mothers	24.46 ± 2.39	0.0 ± 0.0	P < 0.001
Infants	31.66 ± 2.22	-	
(-		
Mothers	0.11 ± 0.02	0.0 ± 0.0	P < 0.001
Infants	-	-	-



Figure I.IgG Levels in IgG, (a) Mothers and (b)Their Infants

Та	Table 3.IgA Levels in Previously Infected Mothers and Their Infants				
	Mothors' group	Infonto			

IgA	Mother	Infants' Group	
	Serum n (%)	Milk n (%)	(Serum) n (%)
Positive	107 (87.7)	105 (86.1)	0 (0.0)
Negative	15 (12.3)	17 (13.9)	122 (100.0)



Figure 2.Levels of IgA i+++n (a).Serum of Mothers, (b).Mothers' Milk, and (c).Their Infants

Table 4.Means of Serum IgM, IgG, IgA, and IgA Levels in the Milk of Mothers Infected Previously with SARS-CoV-2 Virus and Vaccinated Previously with Pfizer, AstraZeneca, Sinopharm, and Non-Vaccinated Mothers

Group	No. of samples	lgM (BAU/mL)	lgG (BAU/mL)	lgA (IU/mL)	lgA milk (IU/mL)
Vaccinated with Pfizer	51	0.138 ± 0.2	21.6 ±10	0.41 ± 0.1	0.51 ± 0.2
Vaccinated with AstraZeneca	3	0.76 ± 0.3	24 ± 11	0.56 ± 0.2	0.63 ±0.2
Vaccinated with Sinopharm	10	0.06 ± 0.1	7.8 ± 8	0.50 ± 0.1	0.66 ± 0.1
Non-vaccinated	58	0.07 ± 0.1	*29.8±12	0.46 ± 0.1	0.58 ± 0.2

* Significant at $P \le 0.05$

Table 5.Means of IgG, IgA of Babies from Vaccinated and Non- Vaccinated Mothers

Group	No. of samples	IgA baby serum (IU/mL)	lgG baby (BAU/mL)
Babies from vaccinated mothers	64	0.06 ± 0.3	27.2 ± 40
Babies from non-vaccinated mothers	58	0.07 ± 0.04	*36.5 ± 50

* Significant at P \leq 0.05

Conclusion

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IgG was detected in both mothers and their infants, IgA was detected in mothers only, while IgM was undetectable in both mothers and their infants. Additionally, the most robust immune responses were observed in mothers who had received AstraZeneca vaccines, and these responses were passed on to their infants, resulting in elevated levels of IgG.

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Conflict of Interest: None

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