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RESEARCH ARTICLE

ASSESSMENT OF MATERNAL AND NEONATAL IgG ANTIBODIES TITRES AGAINST SARS-CoV-2 – A CROSS SECTIONAL COHORT STUDY FROM A TERTIARY CARE HOSPITAL

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ABSTRACT

The impact of the COVID-19 pandemic on maternal and neonatal health has been insidious, yet comparatively less studied in present research. Reports have shown that IgG antibodies against SARS-CoV-2 can be transmitted vertically, but the efficacy and rate remain insufficiently evaluated. Some studies approximated 50% SARS-CoV-2 IgG antibody transfer from mother to child, whereas others showed higher titres in mothers infected with COVID-19 rather than vaccinated. The present study aimed to assess maternal and neonatal SARS-CoV-2 antibody titres and find correlations between them. A total of 92 mother/child dyads (total n=182) were recruited from Vani Vilas Hospital, a specialised women's and children hospital in Bangalore. The maternal blood and neonatal blood samples were drawn after informed consent from the mother was taken. Qualitative detection of IgG antibodies against S1 protein was done by standard ELISA method using COVID KAWACH IgG MICROLISA, J. Mitra & Co. Pvt. Ltd. Basic demographic details and SARS-CoV-2 vaccine history were obtained from the pregnant women. The data was analysed using SPSS software version 23.0, and appropriate parametric and non-parametric tests such as Chi-square test, paired and unpaired t-test were used wherever applicable. A p-value less than 0.05 was considered significant. Of the 92 women in the present study, the ages were 19 to 33 years with a mean of 25 ± 3 years. 71 out of 92 women had received the Covishield vaccine, and the rest with Covaxin. Their mean antibody titre was 1.7124 ± 0.9429. Almost all the mothers (91 out of 92) had positive antibody titres post vaccination. Positive IgG antibody titres against SARS-CoV-2 were found in neonatal serum, confirming the transplacental transfer of IgG antibodies to the neonates. 78 (84.8%) neonates had positive IgG titres. There was a strong positive correlation (ρ=0.887) between maternal and neonatal titres (p-value <0.0001). There was no significant correlation between blood group, type of vaccine and the antibody titres. The results of the present study implicate the transplacental transfer of IgG antibodies against SARS-CoV-2. This supports the current recommendation for pregnant women to receive the SARS-CoV-2 vaccination. Thus, boosting maternal immunity during pregnancy translates into measurable seropositivity in the child, which may result in a milder course of clinical neonatal disease. This data aids in developing an optimal vaccine schedule for both pregnant mother and child.

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INTRODUCTION

Since March 2020, the world has grappled with the COVID-19 pandemic, experiencing multiple waves. Extensive efforts within the scientific community have focused on understanding the virus's genetics, pathogenicity, and how the host immune system responds to combat SARS-CoV-2 infection (1). The first confirmed case of COVID-19 in India was reported on 30 January 2020(2). By 26 January 2022, India had documented 40,085,116 confirmed cases and 491,154 deaths, making it the country with the second-highest number of COVID-19 cases globally, following the United States (3). Research into the SARS-CoV-2 infection and immunity among pregnant women and newborns is an aspect of SARS-CoV-2 research that remains less explored. Typically, neonates safeguard themselves against infection through either their innate immune response or maternal antibodies passed to them through the placenta (4).

Understanding the quantity of SARS-CoV-2 neutralizing antibodies transferred from mother to baby is crucial for developing vaccination strategies for pregnant women (5,6). Recently, the Indian Council of Medical Research approved vaccination for pregnant women. However, data on the transfer of SARS-CoV-2 specific antibodies from mothers to newborns remains inconclusive (7-11). Immunization of mothers during pregnancy facilitates the transfer of immunity to the newborn, which has proven effective in reducing mortality and morbidity from many infectious diseases (7). A recent study in India confirmed that approximately 50% of SARS-CoV-2 specific IgG antibodies are effectively transferred from mothers to neonates, and about 75% of the IgG positives provide neonatal protection by neutralizing the virus (13). The transfer of maternal IgG to the foetus is influenced by various factors, including maternal IgG levels and gestation period. Detection of SARS-CoV-2 S protein-specific IgG with negative IgM in neonates confirms the passive transmission of IgG from the exposed mother (7). Neonates born to mothers infected during the last trimester tend to develop seroconversion and high IgG levels during delivery. The severity of maternal infection correlates with the IgG titre value, which peaks both in maternal serum and umbilical cord blood; however, severity does not affect the transfer ratio of IgG (14). Interestingly, despite similar maternal serum IgG levels as in the umbilical cord blood, the neutralizing antibodies are reduced in the umbilical cord blood. Thus, comprehending this process is vital for a better understanding of SARS-CoV-2 antibodies produced in pregnant women compared to non-pregnant individuals. A crucial aspect of understanding this phenomenon is to ascertain the antibodies specific to SARS-CoV-2 detected in neonates, whether acquired through vaccination or infection, and their impact on preventing infection. Moreover, developing an optimal vaccine schedule is essential to prevent both pregnant mothers and infants after delivery from SARS-CoV-2 infection (7). This research aims to investigate the antibody titre against SARS-CoV-2 in pregnant women and neonates at a tertiary healthcare hospital to inform and tailor our healthcare system accordingly.

METHODOLOGY

This was a cross-sectional study, encompassing pregnant women and their neonates. The study was carried out at the State-level VRDL, Department of Microbiology, within the Bangalore Medical College and Research Institute, a tertiary care hospital. This institute is affiliated with Vani Vilas Maternity and Children's Hospital, serving as the source for recruiting study participants. Ethical approval from the institutional ethics committee and informed consent were obtained from both the study subjects and the parents of neonates before sample collection.

The study included 92 mother and neonate dyads.

Inclusion Criteria: Postnatal mothers and their neonates who have been either vaccinated against COVID-19 or have contracted COVID-19. Postnatal mothers with neonates aged less than 28 days.

Exclusion Criteria: Postnatal mothers who have not been infected with COVID-19 and have received only one dose of vaccination.

RESULTS

The research was conducted over 2 months. Blood samples were collected from mothers and neonates during routine testing at the time of delivery. These samples were used to detect SARS-CoV-2 neutralizing antibodies. Serum was isolated through centrifugation, and IgG antibodies against the S1 fragment of SARS-CoV-2 were qualitatively detected using the standard ELISA method, following the guidelines outlined in the ELISA kit literature. The ELISA procedure in this study utilized the COVID KAWACH IgG MICROLISA by J. MITRA & Co. The strip-holder was loaded with COVID-19 antigen-coated strips. Coated strips were washed three times with a wash buffer, followed by inversion and tapping on dry absorbent paper. 100µl of diluted Negative Control (NC) was added to wells A-1 & B-1, and 100µl of diluted Positive Control (PC) was added to wells C-1 & D-1. Diluted samples (100µl) were then added into the wells, commencing from E-1. A cover seal was applied, and the plate was placed in a container with a lid dampened to create a humidity chamber, followed by an incubation period at 37°C for 60 minutes. After the incubation, the plate was removed, washed with Wash Solution, and then dried. Subsequently, 100µl of Enzyme Conjugate Solution was added to each well, and the plate was once again sealed and incubated at 37°C for another 60 minutes within the humidity chamber. Following this, aspiration and washing were carried out as detailed earlier. Following this, 100µl of working substrate solution was introduced into each well, and an incubation period at room temperature (20-30°C) for 10 minutes in the dark ensued. Stop Solution (100µl) was added, and the absorbance was measured at 450 nm employing the Robonik ELISA Reader.

The cut-off value was calculated as the mean absorbance of Negative Controls + 0.2. The interpretation of the results was based on the OD value, cut-off value, and P/N ratio, classified as "Positive" or "Negative". The obtained data was recorded and analysed utilizing MS Excel and SPSS software version 23.0. Appropriate parametric and non-parametric tests were employed, considering p-value < 0.05 as significant. The research spanned from August 15th to October 15th, encompassing a total of 92 mothers and neonates who met the specified inclusion criteria. Blood samples were collected as part of routine laboratory examinations for the study. Positive IgG antibody levels against SARS-CoV-2 were detected in the neonatal serum, confirming the passage of IgG antibodies from the mother to the neonate through the placenta. Most of the participants were within the age range of 19 to 33 years, with an average age of 25.05 and a standard deviation of 3.090.

The maternal blood group distribution is shown in **Table 1**, and the type of vaccine received is shown in **Table 2**.

Table 1: **Distribution of maternal blood groups**

Blood Group	Frequency	Percentage (%)
A-	2	2.2
A+	23	25.0
AB+	5	5.4
B-	1	1.1
B+	36	39.1
O-	3	3.3
O+	22	23.9
Total	92	100.0

Table 2. Distribution of type of vaccine received by mothers

Type of Vaccine Taken	Frequency	Percent
Covishield	71	77.2
Covaxin	21	22.8
Total	92	100.0

Figure 1: Distribution of maternal blood groups among study participants (n = 92). The most common blood group was B+ (39.1%), followed by A+ (25%) and O+ (23.9%).

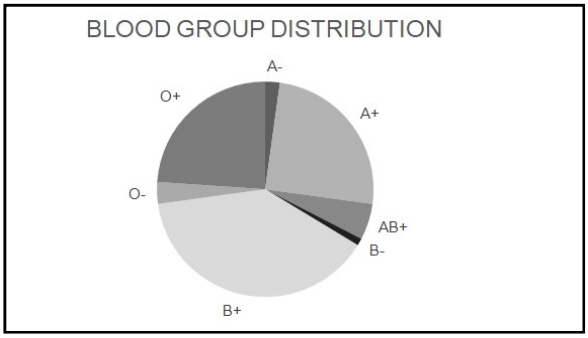
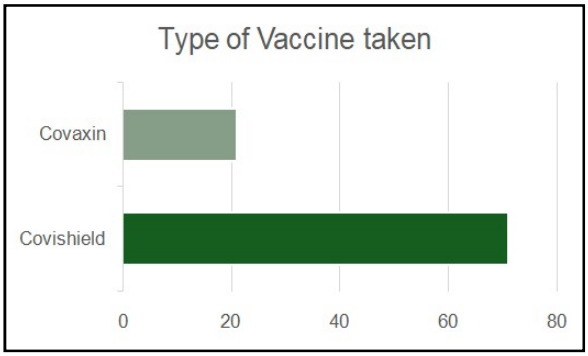


Figure 2: Distribution of COVID-19 vaccine types received by mothers (n = 92). The majority received Covishield (77.2%), while 22.8% received Covaxin.



ANTIBODY TITERS

Maternal Titre: The mean antibody titres of the mothers were found to be 1.7124 with a standard deviation of 0.9429.

According to the methods followed the cut-off value for IgG antibody titre was found to be 0.265. Only 1 (1.1%) was found to be below the cut off value (i.e negative titre) and 91 (98.9%) above the cut-off value (i.e, positive titre) (Table 3).

Table 3. Maternal antibody titres and positivity status

	N	Mean	Std. Deviation
Maternal Titre (Cut-off =0.265)	92	1.71238	0.942926

Maternal Titre Result	Frequency	Percent (%)
Negative	1	1.1
Positive	91	98.9
Total	92	100.0

Neonatal Titre: The mean antibody titres of the neonates were found to be 1.11115 with a standard deviation of 0.7506.

According to the methods followed the cut off value for IgG antibody titre was found to be 0.265. Out of the 92, 14 (15.2%) were found to be negative and 78 (84.8%) were positive for SARS-CoV-2 antibodies (Table 4).

Table 4. Neonatal antibody titres and positivity status

	N	Mean	Std. Deviation
Neonatal Titre (Cut-off =0.265)	92	1.11115	.750606

Maternal Titre Result	Frequency	Percent (%)
Negative	14	15.2
Positive	78	84.8
Total	92	100.0

Correlation between Maternal and Neonatal Antibody Titre: When comparing the antibody titres of mothers and their neonates, we found a significant correlation between maternal and neonatal titres with a p value of <0.0001 (Table 5).

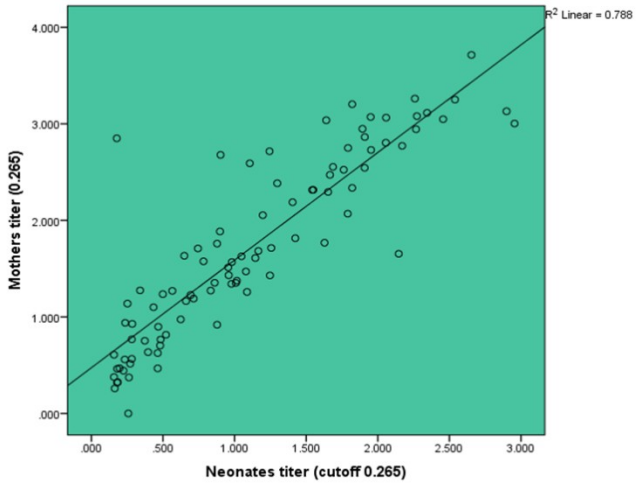
Table 5. Correlation between maternal and neonatal antibody titres

		Maternal titre (Cut-off =0.265)	Neonatal titre (Cut-off =0.265)
Maternal titre (Cut off=0.265)	Pearson Correlation	1	.887**
	Sig. (2-tailed)		< 0.0001
	N	92	92
Neonatal titre (cutoff 0.265)	Pearson Correlation	.887**	1
	Sig. (2-tailed)	0	
	N	92	92

** Correlation is significant at the 0.01 level (2-tailed).

Since the correlation coefficient is 0.887 which is very strong and positive (Figure 3).

Figure 3: Correlation between maternal and neonatal antibody titres. A strong positive correlation was observed (r = 0.887, p < 0.0001).



Correlation Between Type of Vaccine Taken and Antibody Titres

No significant relation was found when comparing different types of vaccine taken with maternal (p=0.971) (Table 6) and neonatal (p=0.758) antibody titre (Table 7).

Table 6. Correlation between type of vaccine and Mother's antibody titres

		Vaccine Type	Mothers' titre (0.265)
Vaccine Type	Pearson Correlation	1	-0.004
	p-value		0.971
	N	92	92
Maternal titre (Cutoff = 0.265)	Pearson Correlation	-0.004	1
	p-value	0.971	
	N	92	92

Since the correlation co-efficient is - 0.004 which is very weak and negative.

Since the p-value is not < 0.05 the correlation is not significant.

Table 7: Correlation between type of vaccine and Neonate's antibody titres

		Vaccine Type	Neonates' titre (Cutoff 0.265)
Vaccine Type	Pearson Correlation	1	0.032
	p-value		0.758
	N	92	92
Neonates' titre (cutoff 0.265)	Pearson Correlation	0.032	1
	p-value	0.758	
	N	92	92

Since the correlation coefficient is 0.032 which is very weak and positive. Since the p-value is not < 0.05 the correlation is not significant.

Correlation Between Maternal Antibody Titres and Maternal Blood Group: No significant relation was found when comparing the maternal blood groups with maternal antibody titres (p = 0.299) (Table 8).

Table 8. Correlation between maternal blood group and maternal antibody titres

		Blood Group	Maternal Antibody titre (0.265)
Maternal Blood Group	Pearson Correlation	1	-0.11
	p-value		0.299
	N	91	91
Mothers' titre (0.265)	Pearson Correlation	-0.11	1
	p-value	0.299	
	N	91	92

The correlation coefficient is -0.11, which is very weak and negative.

Since the p-value is not < 0.05 the correlation is not significant.

DISCUSSION

Vaccinated women displayed a reactive antibody titre for SARS-CoV-2, and their neonates also exhibited reactive antibody titres due to the transfer of IgG antibodies across the placenta. Notably, women who received vaccinations displayed higher antibody titres, suggesting that boosting immunity during pregnancy can lead to discernible serological advantages and potentially result in milder neonatal disease courses (15). The findings provide confirmation of IgG antibody transfer against SARS-CoV-2 through the placenta, affirming the recommendation for administering SARS-CoV-2 vaccination to pregnant women, especially considering that the women were vaccinated months prior to conception. While antibody titres may diminish over time in both mothers and neonates, they remain a crucial protective mechanism for neonates, especially when their innate protective mechanisms are not yet fully developed. It is plausible that over time, other protective mechanisms like cell-mediated immunity may evolve alongside antibody-mediated immunity.

Kashani-Ligumsky et al.(8) conducted a cohort study comparing neonatal immunity following maternal exposure to SARS-CoV-2 during pregnancy. They examined cord blood from three groups: women infected during pregnancy, those vaccinated during pregnancy, and a control group. The findings revealed higher antibody titers against SARS-CoV-2 in neonates born to vaccinated mothers compared to those born to infected mothers, suggesting potentially more prolonged protection. This study highlights the potential advantages of maternal vaccination during pregnancy in conferring stronger immunity against SARS-CoV-2 to newborns.

Paul and Chad(9) presented a case report where a healthy, full-term female infant was born to a COVID-19 naive mother who had received a single dose of mRNA vaccine for SARS-CoV-2 three weeks before delivery. At birth, IgG antibodies specific to SARS-CoV-2 were detected in the cord blood, marking the first known case of an infant exhibiting detectable SARS-CoV-2 IgG antibodies after maternal vaccination. Gill et al.(2) reported a case of a 34 year old multigravid healthcare worker receiving the mRNA vaccine during the third trimester. Both the patient and neonate showed SARS-CoV-2 specific antibodies at a titer of 1:25,600, indicating transplacental antibody passage after vaccination. This aligns with the current study's finding of neonatal antibody transfer after maternal vaccination. This finding is corroborated by Dustin D et al. who conducted a cohort study at Pennsylvania Hospital involving 1714 women who delivered between April 9 and August 8, 2020. Of 1471 mother-newborn pairs assessed, 72 of 83 seropositive pregnant women showed SARS-CoV-2 IgG transfer across the placenta, with antibody concentrations directly linked between maternal and cord blood.

Similarly, Mithal et al showed transplacental IgG transfer to infants from women in their third trimester, with increasing transfer ratios observed with vaccination latency. Also, Rottenstreich et al. investigated maternal and neonatal SARS-CoV-2 antibody levels after antenatal mRNA vaccination. They found efficient transplacental transfer post vaccination, suggesting potential maternal and neonatal protection against SARS-CoV-2.

Nir et al. studied vaccinated parturient women, demonstrating efficient SARS-CoV-2 immunoglobulin G transfer across the placenta to neonates. They compared this transfer to COVID-19 recovered patients and observed a positive correlation between maternal and cord blood antibody concentrations. This also aligns with the present study.

Conversely, some findings have conflicted with this study. Joseph et al. conducted a prospective cohort study on pregnant patients with SARS-CoV-2 infection during pregnancy. Despite robust maternal immune responses, they found lower than expected efficiency in transplacental antibody transfer, notably reduced neutralization between maternal and cord blood.

The discussion provided emphasizes the findings related to IgG antibody transfer against SARS-CoV-2 from vaccinated pregnant women to their neonates via the placenta. It underscores the importance of SARS-CoV-2 vaccination in pregnant women, particularly noting that the vaccinated women exhibited positive antibody titres, leading to neonatal antibody transfer and potentially milder disease courses in newborns. The study suggests that higher antibody titres in vaccinated pregnant women may confer notable serological advantages and bolster neonatal protection, especially considering their relatively immature innate immune systems.
However, limitations noted in the present discussion, such as sample size, absence of cord blood samples, lack of milk antibody measurements, and the safety of vaccines during pregnancy, were not explicitly addressed or reported uniformly in these papers. Additionally, while the current study does not evaluate the response to SARS-CoV-2 infection, other studies mentioned maternal infection and its implications for neonatal immunity.

This highlights the nuanced aspects and varied focuses across studies regarding maternal vaccination, infection, antibody transfer, and their implications for neonatal immunity against SARS-CoV-2.

CONCLUSION

In summary, this study demonstrated positive IgG antibody titres against SARS-CoV-2 in neonatal serum, confirming the transplacental transfer of IgG antibodies from vaccinated mothers. A significant correlation was observed between maternal and neonatal antibody titres.

Therefore, it can be inferred that maternal vaccination against SARS-CoV-2 leads to passive immunity in neonates through the transplacental transfer of IgG antibodies, contributing to the maintenance of herd immunity. Further research is warranted to determine the optimal timing for vaccination and assess the vaccine's safety during pregnancy.

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