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Research Article

Maternal-Fetal Outcome and Characterisation of SARS-CoV-2 RNA in Amniotic Fluid and Breast Milk of Obstetric Patients with SARS-CoV-2 Infection during the Pandemic in Mizoram, India: Single-Center Retrospective Data Analysis

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ABSTRACT

Introduction: The impact of COVID-19 and its mutations has impacted the health system while pregnant women were among the vulnerable population. Studies are limited especially in the northeast states of India. The study aims to report the maternal- fetal outcome as well as evidence of transmission of COVID-19 through other biological samples. ***Method:*** This is a single-center, retrospective data analysis of obstetric patients who were infected with SARS-CoV-2 and their newborns during the early onset of the pandemic in Mizoram. The study also investigates the presence of SAR-CoV-2 RNA in amniotic fluid and breast milk from the patients. The study data such as the demographic and clinical characteristics were obtained through questionnaires and from the medical records department. ***Results:*** A total of 145 obstetric patients with SARS-COV-2 infection were admitted to a tertiary care hospital in Mizoram. About 53.8% of patients were 39 weeks gestation during the time of admission where 60.1% were asymptomatic and diagnosed immediately. A total of 142 infants were successfully delivered and the mean birthweight was 2.9 2.9± 0.54 SD. Among the tested infants 15.2 % were positive of COVID-19 infection. Infant mortality was 2.1 %. Detection of SARS-Cov-2 RNA from breastmilk and amniotic fluid were observed. The analysis shows that there is no significance between the neonates and the two samples. Nevertheless, analysis was performed between the two samples and found to be significant with P-value = 0.003 ***Conclusion:*** The findings of this research provide add valuable insights into the limited knowledge about the role of maternal health in the context of the epidemic of coronavirus virus virus-infected pregnant women in Mizoram, India and also the association of positive COVID-19 breast milk and amniotic fluid from infected mothers to their infants. However, due to the lack of resources and the retrospective nature of our study, more investigation is required.

INTRODUCTION

The emergence of the novel coronavirus, SARS-CoV-2, in late 2019, marked a watershed moment in global healthcare, presenting unparalleled challenges to healthcare systems across the world [1, 2]. As the virus spread rapidly and unpredictably, it posed a unique set of concerns for various segments of the population, and among these, pregnant women emerged as a particularly vulnerable and noteworthy group [3, 4].Pregnant women have always been a special demographic within the field of healthcare, but the advent of the COVID-19 pandemic cast them into an even brighter spotlight [5, 6]. The dynamic interplay between maternal health and the well-being of the developing fetus was thrust into the forefront of scientific research, inviting a closer examination of how COVID-19 impacted this delicate equilibrium [7, 8]. This ongoing inquiry has revealed critical insights into the challenges faced by pregnant women during the pandemic and the far-reaching consequences it has on both mother and child [9,11].Scientific research played a pivotal role in understanding the un-

-ique challenges faced by pregnant women during the pandemic [12-14].Studies focused on how COVID-19 affected pregnancy outcomes, fetal development, and the long-term health of both the mother and child[15-19].These investigations unveiled complex and multifaceted relationships that extended beyond the direct impact of the virus [20-23].

One of the key findings was that pregnant women with COVID-19 were at a higher risk of experiencing severe illness, such as acute respiratory distress syndrome, than their non-pregnant counterparts[24, 25]. The physiological changes that occur during pregnancy, such as increased heart rate and oxygen consumption, can exacerbate respiratory distress. Furthermore, pregnant women who developed severe COVID-19 symptoms often required more aggressive treatments, including hospitali-

-zation and, in some cases, intensive care unit (ICU) admission. In addition to the immediate health risks, researchers delved into the impact of COVID-19 on pregnancy outcomes. Studies found an increased risk of preterm birth among pregnant women with COVID-19. Preterm birth, defined as birth before 37 weeks of gestation, can lead to various health issues for the newborn, including respiratory distress syndrome, developmental delays, and other complications. The reasons behind this increased risk were complex and multifaceted, involving both the direct impact of the virus and the physiological changes in the mother's body[26].This study delves into the unique context of Mizoram, a north-eastern state in India known for its rich cultural diversity has its own set of socioeconomic and healthcare dynamics, aims to comprehensively investigate the clinical course of pregnant women infected with COVID-19 within the region during the

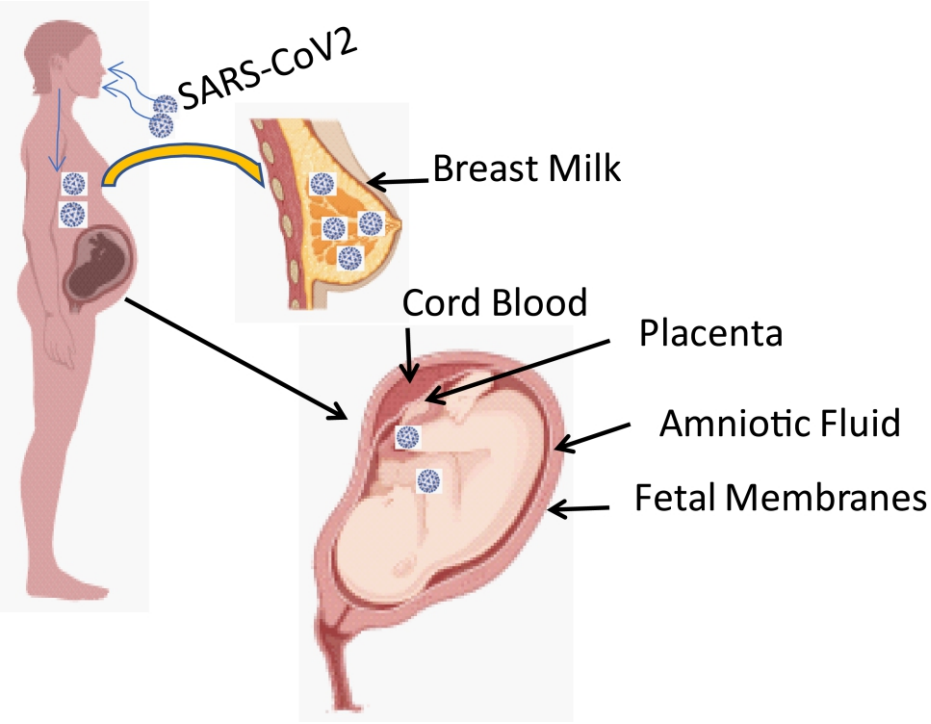

Figure 1: The Transfer of SARS-CoV-2 from Mother to Fetus through Vertical Transmission.

early days of the pandemic. The state's remote and hilly terrain, coupled with variations in healthcare infrastructure, necessitates a dedicated study that caters to the region's specific needs [27]. Furthermore, it explores the presence and characteristics of SARS-CoV-2 in amniotic fluid and breast milk among infected pregnant women. These two bodily fluids play pivotal roles in maternal-fetal health, and understanding the presence and characterization of the virus within them is of paramount importance**(Figure 1).** The potential for vertical transmission and the safety of breastfeeding have been subjects of intense interest and debate in the global medical community. By conducting in-depth investigations within Mizoram context, we aim to add valuable insights into the ongoing aspects and foster a more nuanced understanding of the virus's impact on the mother and child [32].

MATERIALS & METHODS

Study setting

The study is a single-center, retrospective study of obstetric patients who were infected with SARS-CoV-2 and their newborns during the epicenter of the outbreak. The study took place in a tertiary care hospital in Mizoram located in the northeastern region of India during March 2021- September 2022. Throughout this investigation, the clinical course of pregnant women diagnosed with COVID-19 in Mizoram, taking into account factors such as maternal age, comorbidities, gestational age, and access to healthcare will be explored. By analyzing the demographic, clinical, and obstetric characteristics of these individuals, we aim to provide a comprehensive assessment of the risks and outcomes associated with COVID-19 during pregnancy. Demographic and clinical characteristics were obtained through questionnaires and from the medical records department. Informed consent was also obtained from

the patients and the present study was also approved by the Institutional Ethics Committee (No. F20016/1/18-ZMC/IEC/83). The study also investigates the presence of SAR-CoV-2 RNA in amniotic fluid and breast milk from the subjects.

Collection of samples

Nasopharyngeal and oropharyngeal swabs were obtained from subjects at the time of admission. Throat swab was also obtained from the neonates. Other samples such as amniotic fluid and breast milk were also collected in sterile conditions from the subjects. All specimens were obtained in viral transport media (VTM) which were immediately transported for further investigation.

Isolation of RNA, real-time RT-PCR

All the samples collected in VTM were processed in a biosafety cabinet claclinical samples using QIAamp Viral RNA Kits for viral RNA isolation (Qiagen India Pvt. Ltd) using manufacturer's instructions for use. VIRALDTECT-II Multiplex Real-Time PCR kit for COVID-19 (Gene2me Pvt. Ltd) a quantitative detection of SARS-CoV-2 according to manual instructions.

Statistical analysis

Descriptive statistics (absolute number and percentage) were used to show the characteristics of the study population. The Chi-square test was performed to analyse the association of the patient's characteristics with the clinical presentation and livelihood. Risk factors associated with clinical presentation, comorbidities, lifestyle, and respiratory infections were explored by fitting the multivariate logistic regression model to the data. All estimates were examined by using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, N.Y., USA), and the significance level was set at $\alpha < 0.05$.

RESULT

The study included 145 obstetric patients confirmed with SARS-COV-2 infection who were admitted to a tertiary care hospital in Mizoram. The record showed that the highest admission was in July 2021 (n=26). The mean age of the patients was 27.95 ± 5.516 and the age group (30-34 years) was the most frequently observed. Out of the total number of patients, 60.1% were asymptomatic and diagnosed immediately following the guidelines. Detailed results of the clinical characteristics of the patients and their outcomes are given in **Table1.**

Table 1: Baseline characteristics of obstetric patients in our study

Baseline characteristics		Frequency / Percentage
Age (Mean ±SD)		27.95± 5.516
Age group		
	<20	13/9
	20-24	27/18.6
	25-29	39/26.9
	30-34	51/35.2
	35-39	15/10.3
District		
	Aizawl	114/78.6
	Champhai	3/2.1
	Khawzawl	1/0.7
	Kolasib	7/4.8
	Lunglei	5/3.4
	Mamit	7/4.8
	Serchhip	8/5.5

Education		
	High School	24/16.6
	Higher Secondary School	51/35.2
	Graduation	66/45.5
	Post Graduation	4/2.8
Occupation		
	Employed	24/16.6
	Unemployed	107/73.8
	Self employed	14/9.7
Status of Symptomatic patients		
	Asymptomatic	88/60.6
	Mild	49/33.7
	Severe	8/5.5
Symptoms		
	Fever	52/35.9
	Anosmia	35/24.1
	Rash	4/2.8
	Headache	22/15.2
	Fatigue	18/12.4
	Cough	28/19.3
	Sore throat	40/27.6
	Shortness of breath	21/14.5
History to tobacco consumption		21/14.5
Gestation week at admission		
	37 weeks	13/9
	39 weeks	78/53.8
	40 weeks	54/37.2
Delivery Method		
	Vaginal Delivery	43/29.7
	Emergency C section	102/70.3
Vaccination History		
	Not vaccinated	104/71.7
	Vaccinated	41/28.3
Pregnancy outcome		
	Term	87/60.0

	Preterm	54/37.2
	Abortion/miscarriage/ new born death/ still birth	4/2.7
Presences of COVID-19 in the family during pregnancy		76/52.4
Breastfeeding exposure during pregnancy		114/78.6

In the present study, a total of 142 infants were successfully delivered and discharged, where 51.7% and 48.3% were male and female respectively. The mean birthweight was 2.9 2.9± 0.54 SD. Evaluation of the infant's APGAR score showed 91% obtaining a good score as described in **table2.** Unfortunately, 2.1 % succumbed to their illness while COVID-19 investigation was also done by RT PCR resulting in 15.2 % positively confirmed SARS-CoV2 infection.

Table 2: Infant baseline characteristics and outcome included in the study.

Baseline characteristics (n=142)		**Frequency / Percentage**
Gender		
	Male	75/51.7
	Female	70/48.3
Birth weight		2.9± 0.54
Apgar score		
	Good score (7-10)	132/91
	Medium score (4-6)	6/4.1
	Poor score (0-3)	7/4.8
Status of Symptomatic patients		
	Mild	21/14.5
	Severe	4/2.8
Total No. of Infant Covid-19 positive by RT-PCR (n=101)		22/15.2
Infant mortality		3/2.1

Other biological samples such as breastmilk (n=107) and amniotic fluid (n=46) were obtained from the patients at the time of hospital stay. The results have shown that 6.2% and 6.9% detected the presence of SARS-CoV-2 RNA by real-time RT-PCR respectively. Ct value were found to be low ranging between 26-30. Although these findings did not have direct association to the neonates, analysis of the two samples were found to be significant with p-value = 0.003 as shown in the **table3 & 4.**

Table 3: Association between the infants and the two biological sample.

	Amniotic fluid RT-PCR (n=35)		**Breastmilk RT-PCR (n=69)**	
Infant Covid -19 RT-PCR	Positive	Negative	Positive	Negative
Positive	1	8	1	12
Negative	7	19	8	48
Total	8	27	9	60
p-value	0.648		1.000	

Table 4: Analysis between breastmilk and amniotic fluid from COVID-19 positive obstetric patients.

	Breastmilk RT-PCR		
Amniotic fluid RT-PCR	Positive	Negative	Total
Positive	5	2	7
Negative	2	21	23
Total	7	23	30
p-value	0.003		

DISCUSSION

The impact of COVID-19 on pregnant women and their neonates has been a subject of growing concern and extensive research. In this retrospective study, a total of 145 pregnant women with COVID-19 infection in their third trimester were examined along with characteristics of COVID-19 in amniotic fluid and breastmilk[33]. In our study, the average maternal age is much younger than in other countries as the majority of the population is much younger which is in accordance to [34,35]. Majority of the patients were from the capital district Aizawl with a graduation education degree where 16.6% are employed. About 53.8% of patients were 39 weeks gestation during the time of admission and responded to taking the COVID-19 test only because of the close calling of their due date[36-39]. About 60.1% were reported asymptomatic which is in line with the study done by Smith et al. Therefore, it is impossible to know how long they have been infected [40, 41]. Among the symptomatic patients' the most common presenting symptoms were fever, sore throat, and anosmia which is coherent with other studies[42-44]. The present study reports 5.5% of severely ill patients requiring supplementary oxygen and 70.3% had undergone emergency cesarean section out of which 5 cases were not related to COVID-19 illness complications. Out of the five cases, three cases were due to pregnancy rupture of the membrane while the other two cases were due to fetal distress and cephalopelvic disproportion. The outcome of the infants resulted in three cases of intrauterine fetal demise and a case of miscarriage. A study by Ferazzi et al reported that vaginal delivery is associated with a low risk of intrapartum SARS-Cov-2 transmission while other studies report that due to the risk of fecalcontamination, rectal swab test should be considered before vaginal delivery. The findings of this research are in parallel with other studies from different countries. Research studies, such as the one conducted by Sutton et al have examined the potential for vertical transmission of SARS-CoV-2 from mother to neonate [45, 46]. Sutton et al. found limited evidence of vertical transmission, with minimal cases of neon-

-ates testing positive for the virus. This suggests that while the virus can cross the placenta, the risk of vertical transmission appears to be relatively low [47-49].

Several studies have investigated neonatal outcomes in COVID-19-positive pregnant women. The study by Allotey et al explored the risk factors associated with adverse neonatal outcomes, finding that maternal COVID-19 increased the likelihood of preterm birth and low birth weight[50]. In the present study, 37.2% were recorded as preterm birth with an average normal birth (**Table 1).** A total of 101 neonates 15.2% weretested positive and about 16.6% were admitted to the NICU due to acute neonatal respiratory distress. Although, breastfeeding was a subject of debate among the COVID-19-positive mothers. In the current study, 78% were continuously breastfeeding during the pandemic. Research conducted a study examining breast milk samples from COVID-19-positive mothers and found no evidence of viral RNA in the breast milk. Studies such as the research conducted by Penfield et al. have explored the presence of SARS-CoV-2 in amniotic fluid from COVID-19-positive pregnant women. In our study, 10/107 breastmilk and 9/46 amniotic fluid were positively confirmed of the presence of SARS-Cov-2 RNA using RT-PCR. The analysis shows that there is no significant linkage between the neonates and the two samples(**Table 4).** This suggests that breastfeeding and the presence of SARS-CoV-2 in breast milk and amniotic fluid from COVID-19-positive pregnant women may not pose a significant risk of viral transmission from mother to neonate through this route. Evidently, analysis shows that there is an association between breastmilk and amniotic fluid as both of them share a bioactive component that can stimulate cell growth and play a vital role in infant development (Table 4).

Beyond the presence of viral RNA in breast milk, research has also explored the potential benefits of breastfeeding for neonates born to COVID-19-positive mothers. A study highlighted the presence of SARS-CoV-2-specific antibodies in breast milk, which may provide passive immunity to neonates such as the source of anti-SARS-CoV-2 IgA and IgG and neutralizes SARS-CoV-2 activity, potentially protecting them from infection or mitigating its severity.

These findings collectively contribute to the understanding of maternal-infant transmission of SARS-CoV-2. They suggest that the

risk of vertical transmission through amniotic fluid or breastfeeding is relatively low, especially when adequate infection control measures are in place. However, clinical recommendations continue to evolve, emphasizing the importance of appropriate precautions, maternal hygiene, and vigilant monitoring of both mothers and neonates.

CONCLUSION

This study represents a critical step toward ensuring the well-being of pregnant women and their new-borns during the COVID-19 pandemic, particularly in regions with unique healthcare challenges like Mizoram. The findings of this research provide valuable insights into the association of positive COVID-19 breast milk and amniotic fluid from infected mothers to their infants. While the risk of transmission via these routes appears limited, the presence of protective antibodies in breast milk is an encouraging finding. This knowledge is vital for healthcare professionals and policymakers in designing effective preventive and management strategies.

LIMITATION

Since this is a retrospective study our ability to obtain additional data was limited. Additionally, the examination of other samples to detect antibodies indicative of potential vertical transmission was not within the scope of our research.

CONFLICT OF INTEREST

The authors have indicated they have no potential conflicts of interest to disclose.

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