ORIGINAL RESEARCH

Microbiological profile of neonatalsepsis among babies born to COVID-19 positive mothers- A cross-sectional study

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ABSTRACT

Introduction: The SARS-CoV-2 pandemic has had significant impact worldwide, particularly in middle and low income countries. While this impact was well recognised in certain age groups, its effect on neonatal population is largely unknown. Although COVID-19 was described as mild disease in newborns, various concerns associated with the effect of infection on newborns are still there. **Aim and Objectives:** The aim of this study was to study the microbiological profile and COVID status of neonates having sepsis born to COVID-19 positive mothers in a tertiary care hospital. **Material and Methods:** Around 1000 clinically suspected cases of neonatal sepsis cases were reported among neonates born to COVID-19 positive mothers admitted to neonatal intensive care unit at a COVID dedicated tertiary care hospital during the period of July2021 to December2021. Around 2 ml of blood was collected and inoculated aseptically from the neonates into a brain heart infusion broth containing blood culture bottle. Identification of the organism was done conventionally on the basis of microscopy, colony characteristics and biochemical properties. Antimicrobial susceptibility testing was performed on all bacterial isolates as per CLSI guidelines. **Results:** Out of 100 neonates examined, bacteria was isolated from 80 neonates (80%), while rest showed yeast (*Candida* spp.) growth. The most common etiology for neonatal sepsis was Staphylococcus aureus species (20%), followed by Pseudomonas species (17%),Acinetobacter and CONS Coagulase-negative Staphylococcus (CONS (4%) each, followed by e.coli and Klebsiella (3%) each, Candida species were (20%),(Table 2) Of the 20 Candida isolates, non Candida albicans species were more common (66 %) than C. albicans (33%). Of these, Candida tropicalis (33%), Candida glabrata (16.5%), and Candida parapsilosis (16.5%) were the predominant speciesGram positive organisms showed most susceptibility to Doxycycline and Clindamycin while gram negative organisms to aminoglycosides and co-trimoxazole. All the 04 *Candida* isolates were non- albicans *Candida*. **Conclusion:** The current study suggested that there was no significance difference observed in the clinical presentation and outcome of newborns with sepsis and COVID-19 positive status versus non COVID-19 babies. But co-infection of COVID-19virus and other microbes was observed here.

INTRODUCTION

The novel corona virus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a highly contagious RNA virus that was first detected in Wuhan City [1]. It has resulted in 675 million infections and 6.7 million deaths by2022, globally [2]. India has witnessed 44 million infections and .53 million deaths during this period [1,2] The SARS- CoV-2 pandemic has had significant impact worldwide, particularly in middle and low income countries. While thisimpact was well recognised in certain age groups, its effecton neonatal population is largely unknown.[3]

Although COVID-19 was described as mild disease in newborns, various concerns associated with the effect of infection on newborns are still there Vertical maternal-fetal transmission is a major concern with SARS- CoV-2 infection .There is limited information on the existence of SARS-CoV-2 in the female genital tract, and it may be important for evaluating both vertical and sexual transmission.

In a recent Indian study, samples from a COVID infected mother's placenta, cord blood, amniotic fluid, and vaginal secretions testedfor SARS CoV-2 were found to be negative [4].

There is, however, no consensus on the mode of delivery and optimal time of delivery in COVID-19 infected women and whether cesarean delivery is the preferred mode to prevent vertical transmission [5,6,7]

It is important to investigate whether and how, SARS-CoV-2 reaches the fetus, to optimize pregnancy management, prevent neonatal infection, improve pregnancy outcomes, and eventually understand SARSCoV-2 path physiology in the fetus and neonate
In addition, viral respiratory infections are known to predispose patients to bacterial infections, which mayworsen the outcome as the disease progresses [8].
As true with other viral pneumonia, bacterial and fungal infections are common complications seen in prolonged hospitalized COVID-19 adult patients. Recent studies documented 3.1–3.5% of bacterial co-infections upon admission in COVID-19 patients, while secondary bacterial infections following hospitalization occurred in 15% of patients [9].

AIM AND OBJECTIVES

The aim of this study was to study the microbiological profile and COVID status ofneonates having sepsis born to COVID-19 positive mothers in a tertiary care hospital.

MATERIAL AND METHODS

Around 100 clinically suspected cases of neonatal sepsis cases were reported among neonates born to COVID-19 positive mothers admitted to neonatal intensive care unitat a COVID dedicated tertiary care hospital during the period of July 2021 to December 2021.
Around 2 ml of blood was collected and inoculated aseptically from the neonates into a brain heart infusion broth containing blood culture bottle. Bottles were incubated at 37°C as per the conventional techniques. The bottles were incubated for 5 days before reporting a negative. A Gram stain and a subculture on blood agar and MacConkey agar were performed at 48 h and 72 h (if sterileat 48 h) from each blood culture bottle.
Identification of the organism was done conventionally on the basisof microscopy, colony characteristics and biochemical properties according to standard protocol[10]. Antimicrobial susceptibility testing was performed on all bacterial isolates as per CLSI guidelines.
All the isolates of yeast were subjected to Gram staining and werefurther identified by the germ tube test, growth on Hichrome media, sugar fermentation, and sugar assimilation tests for yeast identification [11,12]. Antifungal susceptibility test (AFST) for yeastwas not performed.
The SARS-CoV-2 RT-PCR (real-time polymerase chain reaction) test on nasopharyngeal or oropharyngeal swab samples collected from neonates at birth was used to assess evidence of vertical transmission. The SARS-CoV-2 RT-PCR test was performed in the ICMR approved COVID laboratory, Department of Microbiology, ofour tertiary care center.

RESULTS

The study, conducted during a 6 month period from July to December 2021,observed a male predominance(3:1) in neonates with sepsis.[Table 1]
Of the 100 samples from neonates with septicemia born to COVID-19 positive mothers, 48 (48%) babies were COVID positive. The risk factors forsepsis among neonates born to COVID-19 positive mothers are shown in[Table 1]
The majority of newborns (86%) were <7 days of birth. [Table 1]
Bacteremia was observed in 80patients (80%), whereas cultures from 20 patients (20%) grew yeast, though the difference was not statistically significant. (Unpaired t-test, Degree of Freedom: 1, p>0.372).(Table 2)
Among the positive blood cultures, Gram-positive and Gram negative bacteria contributed to 73% (73 isolates) and 7% (07isolates), respectively, while 20% (20 isolates) of the isolates were Candida species (Table 2).
The most common etiology for neonatal sepsis was Staphylococcus aureus species (20%), followed by Pseudomonas species (17%), Acinetobacter and CONS Coagulase-negative Staphylococcus (CONS (4%) each, followed by e.coli and Klebsiella(3%) each, Candida species were (20%),(Table 2)
Of the 20 Candida isolates, non Candida albicans species were more common (66 %) than C. albicans (33%). Of these, Candida tropicalis (33%), Candida glabrata (16.5%), and Candida parapsilosis (16.5%) were the predominant species (p=0.02, Mann WhitneyU Test; Nonparametric test, Degree of Freedom=1
In the present study, Grampositive microorganisms accounted for greater sepsis among the COVID- 19 positive cases (45.8%) compared to COVID-19 negative cases (34.6%);though the difference was not statistically significant.
Gram positive organisms showed most susceptibility to Doxycycline and Clindamycin while gram negative organisms to aminoglycosides and co-trimoxazole.(Table 3 andTable 4)
None of the risk factors were found to be significant in COVID-19 positive and negative neonates with sepsis, (Mann Whitney U Test; Degree of freedom=1, p>0.05, 95% confidenceinterval) (Table 5).
The overall outcome of the babies born witha COVID 19 positive or negative status was satisfactoryand all but 3 (3%) survived.

Table 1: The demographic profile and risk factors for sepsisamong neonates born to COVID-19 positive mothers

Demographic parameters and risk factors among newborns	n=100 (%)
Gender	7

Male	5
Females	2 5
Age of Newborn babies	8
<7 days of Birth	6
>7 days of Birth	1 4
Clinical parameters	5
COVID status of newborns	0
Small for gestational age	4 3
Respiratory distress syndrome	3 4

Table 2: Microbiological etiology among cases with COVID-19

Microorganisms	**COVID-19 positive cases (n=48)**		**COVID-19 negative cases (n=52)**	
Gram-positive cocci (73)				
Staphylococcus aureus	22 (30)	23/48 (46%)	9(15)	**18/52 (35%)**
Coagulase negative Staphylococcus (CONS)	13 (17)		1 (1)	
Streptococcus pneumoniae	0		4(8)	
Enterococcus spp.	3 (4)		0	
Unpaired t-test, P>0.283, statistically insignificant				
Gram Negative Bacilli (07)				
Escherichia coli	2 (8)	21/48 (42%)	4 (8)	**28/52 (54%)**
Klebsiella pneumoniae	3 (12)		12(23)	
Pseudomonas aeruginosa	1 (4)		4 (8)	
Acinetobacter species	4 (17)		8 (15)	
Unpaired t test, P>0.583, statistically insignificant				
Yeast (20)				
Candida species	7 (14)	7/48 (14%)	13(25)	**13/52 (25%)**
Unpaired t-test, P>0.228, statistically insignificant				
*p<0.05 is statistically significant				

Table 3: Antibiotics susceptibility pattern among Gram-positive cocci blood culture isolates in neonatal septicemia cases(n=100)

Sensitivity to antibiotic	**No. of isolate(n=100)**
Teicoplanin	96
Chloramphenicol	12
Cotrimoxazole	42
Gentamicin	86
Clindamycin	100
Tetracycline	40
Azithromycin	50

Table 4: Antibiotics susceptibility pattern among Gram-negative bacilli blood culture isolates from babies with neonatal septicemia (n=100)

Sensitivity to antibiotic	**No. of isolate(n=100)**
Mer0penem	60
Cefotaxime	96
Ceftazidime	40
Tobramycin	24
Ceftriaxone	96
Tigecycline	90
Cotrimoxazole Gentamicin Ciprofloxacin Piperacillin+Tazobactam	50 50

	50 100

Table 5: Neonatal risk factors among COVID positive and negative cases in neonatal sepsis(n=100)

Neonates risk factors	COVID-19 positive newborns n=48(%)	COVID-19 negative newborns n=52(%)	Mann Whitney UTest (Non parametric test)Degree of freedom=1 95% in Ctoenrfnidaelnce
Small for gestational age	24 (50)	20 (38.4)	**p=0.31**
Respiratory distress syndrome	16(33)	20 (38.4)	**p=1.00**
Preterm baby	14 (29)	14 (27)	**p=1.00**
Meconium stainedliquor	10 (21)	10 (19)	**p=1.00**
Low birth weight baby	12 (25)	8(15)	**p=0.37**
Neonatal jaundice	6 (12)	6 (11)	**p=0.31**

DISCUSSION

Neonatal septicemia is a clinical syndrome of bacterimia withsystemic infection in 1st month oflife.

It is estimated that 20% of all neonates develop sepsis, and NS is responsible for 30–50% of total neonatal deaths in developing countries [13,14].

It is the main cause of infant mortality and morbidity in the NICU, and it often leads to various complications, which not only increase the hospital burden but also have a serious negative impact on children's physical and mental growth [15,16].

In the present study, 100 blood culture isolates obtained from neonates born to COVID-19 positive mothers, were taken. Males were slightly more affected than females (3:1). However, no effectof gender has been reported on blood culture positivity rate. Our findings correlate with the study done by Sharma *et al*. from Delhi(M: F=2.5:1) [17].

Of the 50 samples from patients with NS born to COVID-19 positive mothers, 48 babies (48%) tested COVID -19 positive. Most neonatal SARS-CoV-2 infections are acquired after birth by Horizontal virus transmission from the mother, healthcare workers, or other family members [18]. Limited reports document the potential mechanisms for vertical transmission as placental and umbilical cord during intrauterine life, cervicovaginal secretions during delivery, or through breastfeeding in the postpartum period [19,20].

A single positive RT-PCR test in a respiratory sample from a newborn can have several conclusions. It may indicate active viral replication or the presence of viral fragments acquired during passage through the birth canal or from external environmental contact soon after birth, or may even be a result of surface contamination that does not necessarily resultin neonatal infection [21].

he presence of IgG antibodies in the newborn atbirth may simply reflect the transfer of maternal antibodies and does not confirm the diagnosis of intrauterine infection. Consequently, a positive serological test in a newborn at birth always requires confirmation with a molecular test. Therefore, future studies should include SARS- CoV-2 RNA on vaginal samples and include direct testing of intrauterine tissue samples such as amniotic fluid, cord blood, placenta, viral load, and other variables to further determine the risk of COVID-19 vertical transmission to the neonate.

In our study, among the premature and low-birth-weight neonates, gram-positive sepsis due to *Staphylococcus aureus* (30%), was more common than Pseudomonasaeruginosa and *Acinetobacter baumannii,similar to other studies.* [18,19].

Although BSI due to Candida species in the neonate is less frequent, it is often associated with higher morbidity and mortality rates. Among newborns with a birth weight of <1000 g, candidemia has been reported in 4–8% of cases, with 30%mortality [20]. The overall incidence of candidemia in the present study was 20%, though certain studies have reported a higher incidence of candidiemia of up to 20.29% [7,21,22].

The increased use of invasive devices, broad-spectrum antibiotics in neonates have been the major risk factors leading to candidemia in NICU [23,24].

The predominance of non *C. albicans* (66%) over *C. albicans* (33%) was conspicuous in our study, with *C. tropicalis* as the most predominant (33%), followed by *C. glabrata*, and *C. parapsilosis*. A similar study by Zarei et *al.* observedin Iran that non *C. albicans* (63.3%), with *C. tropicalis* in 16%, *C. glabrata* in 10% and *C. krusei* in 6.6% as the commonisolates [25]

Gram positive organisms showed most susceptibility to Doxycyclineand Clindamycin while gram negative organisms to aminoglycosidesand co-trimoxazole ,similar to study by Asrat and Amanuel et al[26].

The current body of evidence from high-burden COVID-19 areas globally suggests that co- infections are common, particularly in severe cases. In a study conducted in Wuhan, of the total 41 patients, co-infections were reported in 31% of the ICU patients andin 10% of the patients admitted to the wards. Preterm birth was found to be the most common adverse pregnancy outcome in patients with COVID-19 [27,28].

In our study, the rate of preterm delivery was 28% of the total 100 newborns born with sepsis. This is certainly significant and clinicallyrelevant when compared to the incidence of preterm delivery in

theUnited States, estimated at 9.9% [29].

In addition, ICMR reports a high prevalence of antimicrobial resistance (AMR) in Indian hospitals inthe pre-COVID times.

The practice of initiating empiric or prophylacticantibiotics often allows the selection of MDR pathogens.

On the contrary, clinicians' choice of targeted antibiotic therapy over empiric prophylaxis would be a promising step to prevent the emergence of secondary infections due to MDR pathogens and result in favorable patient outcomes among COVID-19 patients [30].

CONCLUSION

The current study suggested that there was no significance difference observed in the clinical presentation and outcome of newborns with sepsis and COVID-19 positive status versus non COVID- 19 babies. But co-infection of COVID-19 virus and other microbes was observed here.

Infected infants can shed the SARS-CoV-2 virus through the respiratory tract as well as in stool. Therefore, caregivers should take caution with good hand hygiene.

Prompt recognition of illness in this population is essential to limit further transmission in the community.

Standard and additional infection control measures andguidelines should therefore be mandatory in hospitals handling COVID-19 positive mothers and newborns [31].

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