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ESTIMATION OF VARIATION OF BIOCHEMICAL PARAMETERS OF COVID-NEGATIVE NEONATES BORN OF COVID INFECTED MOTHERS

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ABSTRACT The World Health Organization (WHO) declared the outbreak of COVID-19 as a Public Health Emergency of International Concern on January 30, 2020. Due to the sudden incidence and high pathogenicity, there is little knowledge about the risk of infection by the virus to pregnant women and parturient. The issues pertaining to transplacental transfer and also there is lack of clarity on potential complications of SARS-CoV-2 coagulopathy.The report about the influence of COVID-19 on pregnant women and their babies is very limited . Moreover, it is not clear the effects covid infected mothers (anytime in the 2nd or 3rd trimester of the gestational period) will have on the physiological status of the infants who themselves have not been infected by Covid-19. This study has been designed to assess any difference or deviation in the biochemical parameters of a COVID negative infant of a COVID infected mother during any time of her pregnancy from the values usually observed from the study of biochemical parameters of a neonate born of non-COVID infected mother based on history taking and checking of reports for RTPCR and Rapid Antigens Test for COVID 19. At the initial level the study population was distributed into two groups of 50 mothers each, 1st group consisted of mothers infected by COVID 19 and 2nd group consisted of mothers not infected by COVID 19 during pregnancy. The neonatal biochemical parameters were evaluated based on the data gathered from cord blood sample analyses. The primary objective for conducting this study will be to estimate the incidence of any altered amount of D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, PT/INR in non-covid infants born of covid mothers versus non-covid infants born of non-covid mothers. This study found that the neonates who were COVID negative despite having COVID infected mothers did not have any stark variation in the values of biochemical parameters as compared to the control neonates of this study group. However, since COVID is a relatively new disease with little knowledge about its consequences the maternal COVID status may have an impact on neonatal health in the long run. So, as far as possible the regular periodic health check up and screening of the aforementioned biochemical parameters of these neonates should be followed consistently.

KEYWORDS :

INTRODUCTION

The World Health Organization (WHO) declared the outbreak of COVID-19 as a Public Health Emergency of International Concern on January 30, 2020 [1]. Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2 primarily causing damage to lower respiratory tract but post Covid patients have been known to show long term complications of heart, breathing problems, chronic kidney failure and stroke. So far following the trend of infection people of all ages are susceptible to SARS-CoV-2. The SARS-CoV-2 has also been associated with hypercoagulability, development of ischemic changes, D-dimer elevation and disseminated intravascular coagulation in some [2].

Due to the sudden incidence and high pathogenicity, there is little knowledge about the risk of infection by the virus to pregnant women and parturient [1]. The issues pertaining to transplacental transfer and also there is lack of clarity on potential complications of SARS-CoV-2 coagulopathy[3]. The report about the influence of COVID-19 on pregnant women and their babies is very limited [1]. Moreover, it is not clear the effects covid infected mothers (anytime in the 2nd or 3rd trimester of the gestational period) will have on the physiological status of the infants who themselves have not been infected by Covid-19 [1]. Any derangement in the blood parameters of the neonates may result in the abnormal functioning of any vital organs and cause deterioration in terms of long-term health. So, it is important to relate how the covid infected mother may affect the biochemical parameters like (D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, Prothrombin/INR) of the child in comparison to non-covid infected mothers [4], [5]. The necessity for this study is because it has been observed that the above-mentioned parameters has shown derangement most of the time in the mothers who were infected by covid from various observational studies [6] so whether it will have similar effects on the neonates who are not infected by COVID 19. This study was accomplished by collecting a sample of 5 ml cord blood of the neonate from the maternal side of the umbilicus just after parturition when the umbilical cord is clamped and running the laboratory tests on the above-mentioned parameters after taking appropriate consent of the mother and guardian of the neonate.

This study has been designed to assess any difference or deviation in the biochemical parameters of a COVID negative infant of a COVID infected mother during any time of her pregnancy from the values usually observed from the study of biochemical parameters of a neonate born of non-COVID infected mother based on history taking and checking of reports for RTPCR and Rapid Antigens Test for COVID 19. At the initial level the study population was distributed into two groups of 50 mothers each, 1st group consisted of mothers infected by COVID 19 and 2nd group consisted of mothers not infected by COVID 19 during pregnancy.

The neonatal biochemical parameters were evaluated based on the data gathered from cord blood sample analyses. Phlebotomy was not chosen as it is an invasive procedure and neonates are often burdened with laboratory phlebotomies for other illnesses. The parameters used for comparison from cord blood are D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, Prothrombin/INR. The data from this study will help to throw some light on the association between maternal COVID and the effects it may have on future health status of the neonates. This will help on getting a precautionary screening for these children infuture.

Literature Review

Due to the sudden incidence of SARS-CoV-2 not much information is clear regarding the consequences of the disease. Moreover, especially not about itslong-term impact on maternal and consequently on long term neonatal health status. Several studies are ongoing about the possible effects of COVID 19 on neonates due to the mother being affected during pregnancy and also chances of vertical transmission and change in blood picture of neonate.

Literature data about neonatal health born of symptomatic and asymptomatic COVID mothers is primarily obtained from China based on study from small sized population which may not be generalized [2]. According to studies vertical transmission propensity is low currently accompanied by low rates of testing-based vertical or perinatal transmission along with no evidence clinically present about neonatal SARS-CoV-2 infection [2]. Since this study involves studying of biochemical parameters using cord blood it has been found that there is a significant drop in the level of RBD (Receptor Binding Domain) specific binding and neutralizing antibodies in cord blood

compared to the maternal blood [7].

One such case study in Wuhan, China recorded the biochemical parameters of neonates who tested COVID negative but their mothers were COVID positive compared with a study done in Jaipur, Rajasthan where a population of 120 mothers were taken with 5 neonates testing positive and 115 having negative RT-PCR result [8,9]. The CRP level, D-dimer, Procalcitonin, PT/INR, Alanine Aminotransferase and Aspartate Amino transferase was within normal range for a neonate unlike when a neonate was found to be COVID positive [8,9]. However, Bilirubin and Alkaline Phosphatase was increased [10, 11] that can be explained physiologically due to immaturity of liver in neonates so decreased conjugation and excretion of bilirubin and transplacental transfer of placental isoenzyme and increased bone isoenzyme of ALP in neonates. The results of this study will further help in validating the findings about the clinicolabaratory outcomes in neonates born of COVID infected mothers and will accordingly help in taking future precautionary measures.

AIMS AND OBJECTIVE

The primary objective for conducting this study will be to estimate the incidence of any altered amount of D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, PT/INR in non-covid infants born of covid mothers versus non-covid infants born of non-covid mothers.

MATERIALS AND METHODS

Study Type and Site

This will be a cross-sectional, descriptive, hospital-based study conducted in a tertiary healthcare center including laboratory investigations.

Study Population

The study population will include all neonates born within a span of two months in a tertiary healthcare center to covid infected and non-covid mothers. Only term deliveries will be included.

Pregnant mothers having comorbidity such as diabetes, hypertension, cancer, chronic debilitating diseases, tuberculosis, interstitial lung disease, congenital cardiac, nephrotic and hepatic disease, etc. and on drugs like insulin, blood thinners, bronchodilators, hypertensives, diuretics, etc. will be excluded.

Study Duration

The study was conducted over a period of 2 months, from 3rd August, 2022 to 30th September, 2022.

Sample Size

The sample size will include 50 non-covid neonates born to covid positive mothers and 50 non-covid neonates born to covid negative mothers.

Selection Criteria

Only apparently healthy neonates will be selected for the study. Neonates with any major illness affecting normal biochemical parameters of D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, Prothrombin/INR like thromboembolism, congenital cardiac, kidney and liver diseases like Crigler Najjar, Gilbert Syndrome, etc. will be excluded. Preterm deliveries will be excluded due to immature liver functions.

Data Collection

Proper history of mother will be taken as to at what time of the pregnancy she contracted the covid infection and whether she was asymptomatic or mildly symptomatic (severely symptomatic is excluded due to unavailability of cord blood to be taken during the caesarean section). Proper history of covid negative mothers to be taken to ensure she did not contract the covid infection in the past 2 years.

Sample Collection

Immediately after delivery, the umbilical cord will be clamped. 5ml of venous cord blood will be collected using sterile syringe from the maternal side of the umbilical cord and divided into two parts. A part of sample transferred to Coagulation Study vial containing trisodium citrate for Prothrombin/INR, D-Dimer test and rest to Serum Separator vial containing silica and serum separating gel for evaluating Procalcitonin, ALT, AST, Direct bilirubin, Total Bilirubin and CRP.

Laboratory Tests

D-Dimer: Test specimen reacts with D-dimer latex reagent(R2) and activation buffer(R1) based on agglutination reaction in a turbidimetric assay. If present D-Dimer results in formation of an insoluble complex with increased turbidity, measured at 630nm wavelength. D-Dimer concentration is proportional to increase in turbidity. [12]

Prothrombin/INR: Plasma is extracted and transferred to a measuring test tube. Next excess of calcium is added to reverse anticoagulating effect of citrate followed by factor III to activate the clotting cascade. INR: Ratio of patient's PT to control raised to power of ISI value (depends on analytical system). [13]

Following tests are done in fully automated COBAS analyzer which is a closed system using COBAS reagents.

Procalcitonin: 1st incubation: Sample(antigen), biotinylated monoclonal and ruthenium complex labeled monoclonal PCT-specific antibodies react to form sandwich complex. [14]

2nd incubation: After adding Streptavidin-coated microparticles, interaction of biotin and streptavidin bind complex to solid phase.

Microparticles get magnetically captured onto electrode surface after aspirating reaction mixture into measuring cell. Chemiluminescent emission induced by voltage application to electrode is measured by photomultiplier.

CRP: On combining with This buffer, specimen is incubated. Second reagent (latex particles coated with mouse anti-human CRP antibodies) is then added. Latex particles aggregate to form immune complexes in presence of circulating CRP. CRP concentration is proportional to the light scattering capacity of complex with the resulting light absorbance being read against a stored CRP standard curve. The concentration of CRP is measured at a primary wavelength of 546 nm (secondary wavelength 800 nm). The process used is two-reagent turbidimetric system.[15]

Alanine Aminotransferase: Reaction between L-alanine and 2-oxoglutarate catalyzed by ALT in sample. The pyruvate formed is reduced by NADH catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+ . The catalytic activity of ALT is directly proportional to NADH oxidation rate. So, there is decrease in absorbance which is measured at maximum wavelength of 340nm. [16]

Aspartate Aminotransferase: The transfer of an amino group from L-aspartate to 2-oxoglutarate is catalyzed by AST in sample. The oxaloacetate formed is reduced by malate dehydrogenase (MDH), to form NAD+ from NADH. The catalytic activity of AST is directly proportional to NADH oxidation rate. So, there is decrease in absorbance which is measured at maximum wavelength of 340nm. [17]

Alkaline Phosphatase: For assessing tissue non-specific Alkaline Phosphatase diethanolamine-L-phenylalanine (DEA-Phe) methodis used. The sum of liver and bone Alkaline phosphatase activities are assayed as determined by an inhibiting IAP activity method with Phe [18].

Direct Bilirubin: Acidified sodium nitrite produces nitrous acid reacts with sulfanilic acid (in acidic solution) to form diazotized sulfanilic acid which reacts with bilirubin to form isomers of azobilirubin. In this assay, only direct (conjugated) bilirubin is converted to azobilirubin isomers. the direct bilirubin concentration is proportional to the intensity of the red color of azobilirubin, measured photometrically at 546nm. [19]

Total Bilirubin: Total bilirubin is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium, in the presence of a suitable solubilizing agent. Total bilirubin concentration is proportional to the red colour intensity of azo dye, determined photometrically at 546nm. [20]

Statistical Analysis

- All data will be first put in Excel sheet.
- Accordingly, normality of distribution will be determined by Kolmogorov-Smirnov Goodness of fit test. [21]
- Then the comparison of the two sets of data will be done by

Student-T Test or Mann-Whitney U-Test. [22]
- The tests will be done by SPSS recent edition software. [23]

Confidentiality
All data collected and test results obtained will be anonymous.

Consent
Only those who have read and signed the Informed Consent Form will be taken into account. All procedures will be duly explained before taking consent.

OBSERVATION AND RESULTS

A total of 100 COVID negative neonates were taken out of which 50 neonates were born of COVID infectedmothers and 50 of non-COVID infected mothers. The Average values, Standard Deviation t test result, p value of mother's biochemical parametersalong of two groups are shown in Table 1.

The values of Average and Standard Deviation of D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, Prothrombin/INR from cord blood of COVID negative neonates born of COVID infected mothers and non-COVID infected mothers are represented in Table 2 along with their independent T-Test values (with p value) which shows the comparison between the 2 groups.

Table 1: Mean±SD of maternal biochemical parameters of COVIDand non COVID infected mothers with t-test between 2 groups:

Parameter	Mean for Covid Mother	Standard Deviation for Covid Mother	Mean for Non Covid Mother	Standard Deviation for Non-Covid Mother	T Test Value	P Value
PT/INR	13.55/0.95	13.55±0.58/0.95±0.04	13.46/0.95	13.46±0.61/0.95±0.04	-0.763/-0.775	0.767/0.781
D-Dimer (µg/ml)	2	2±0.46	0.13	0.13±.07	-28.137	<0.001
CRP (mg/l)	25.67	25.67±15.55	1.44	1.44±0.63	-11.012	<0.001
Procalcitonin (ng/ml)	0.36	0.36±0.23	0.01	0.01±0.01	-10.595	<0.001
ALT (IU/l)	20.26	20.26±6.39	26.02	26.02±5.84	4.703	0.316
AST (IU/l)	24.42	24.42±8.57	31.4	31.4±8.31	4.134	0.525
ALP (IU/l)	489.78	489.78±106.01	429.44	429.44±124.93	-2.604	0.327
Direct Bilirubin (mg/dl)	0.16	0.16±0.05	0.15	0.15±0.06	-0.509	1.471
Total Bilirubin (mg/dl)	0.92	0.92±0.09	0.9	0.9±0.11	-1.441	1.895

Comments: The parameters of CRP, D-Dimer and Procalcitonin are significantly higher in the COVID mothers as compared to the non-COVID ones (p<0.001)

Table 2: Mean±SD of COVID negative neonatal biochemical parameters born of COVID and non-COVID infected mothers with t-test between 2 groups:

Parameter	Mean for Case Neonate	Standard Deviation for Case Neonate	Mean for Control Neonate	Standard Deviation for Control Neonate	T Test Value	p Value
PT/INR	14.39/1.01	14.39±1.37/1.01±0.1	14.58/1.03	14.58±1.4/1.03±0.1	0.687/0.688	0.718/0.738
D-Dimer (µg/ml)	0.25	0.25±0.09	0.24	0.24±0.09	-0.607	0.88
CRP (mg/l)	1.94	1.94±0.64	2.29	2.29±0.78	2.442	0.146
Procalcitonin (ng/ml)	0.02	0.02±0.02	0.01	0.01±0.01	-0.823	0.343
ALT (IU/l)	21.7	21.7±9.03	21.94	21.94±9.87	0.127	0.802
AST (IU/l)	35.54	35.54±19.36	38.22	38.22±26.35	0.58	0.247
ALP (IU/l)	184.06	184.06±118.79	149.38	149.38±45.27	-1.929	<0.001
Direct Bilirubin (mg/dl)	0.16	0.16±0.05	0.28	0.28±0.1	7.433	<0.001
Total Bilirubin (mg/dl)	1.55	1.55±0.51	1.4	1.4±0.6	1.292	0.72

Comments: The parameters of ALP and Direct Bilirubin are observed to be significant in neonates (p value <0.001)

A comparative analysis is being done between the neonate's biochemical parameters born of COVID infected and non-infected mothersby representing the means of parameters on bar diagrams

On comparison of the PT/INR values between 2 groups of neonates, it is found not to be significantly different (p value 0.718/0.738)

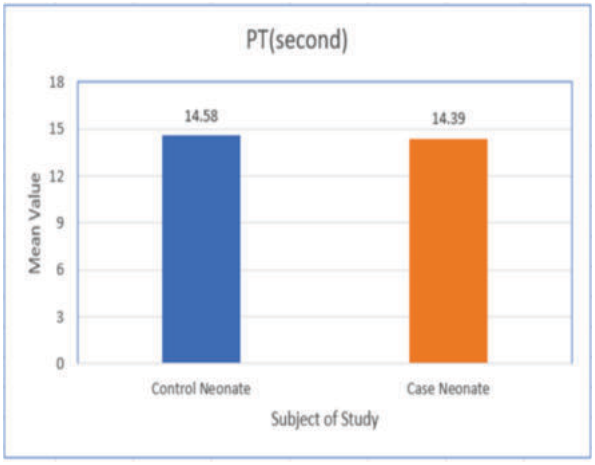

Fig 1- Bar Diagram showing comparison between PT values of neonates born of non-COVID infected and COVID infected mothers

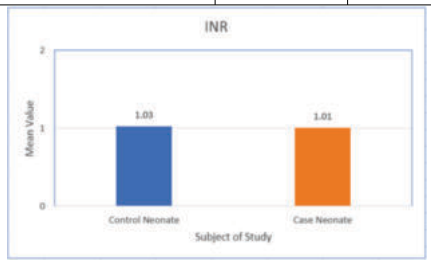

Fig 2- Bar Diagram showing comparison between INR values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the D-dimer values between 2 groups of neonates, it is found not to be significantly different (p value 0.88)

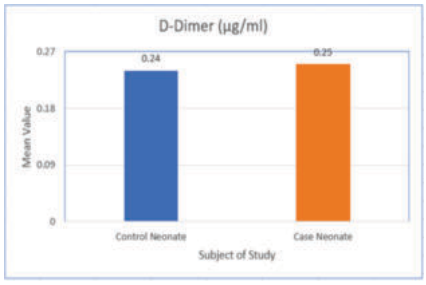

Fig 3- Bar Diagram showing comparison between D-dimer values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the CRP values between 2 groups of neonates, it is found not to be significantly different (p value 0.146)

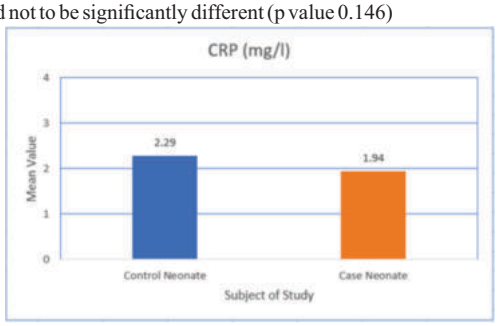

Fig 4- Bar Diagram showing comparison between CRP values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the Procalcitonin values between 2 groups of neonates, it is found not to be significantly different (p value 0.343)

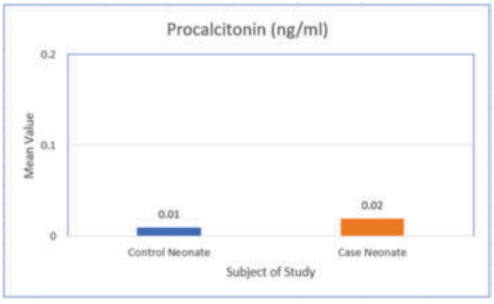

Fig 5- Bar Diagram showing comparison between Procalcitonin values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the ALT values between 2 groups of neonates, it is found not to be significantly different (p value 0.802)

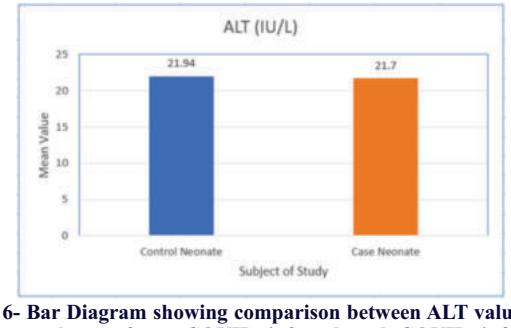

Fig 6- Bar Diagram showing comparison between ALT values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the AST values between 2 groups of neonates, it is found not to be significantly different (p value 0.247)

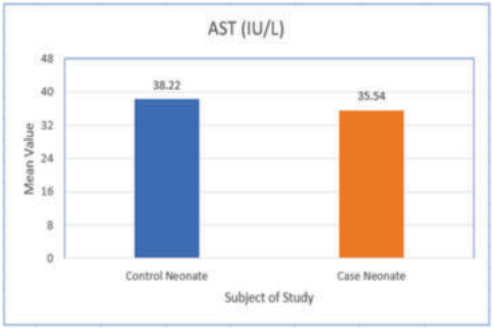

Fig 7- Bar Diagram showing comparison between AST values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the ALP values between 2 groups of neonates, it is found to be significantly different (p value <0.001)

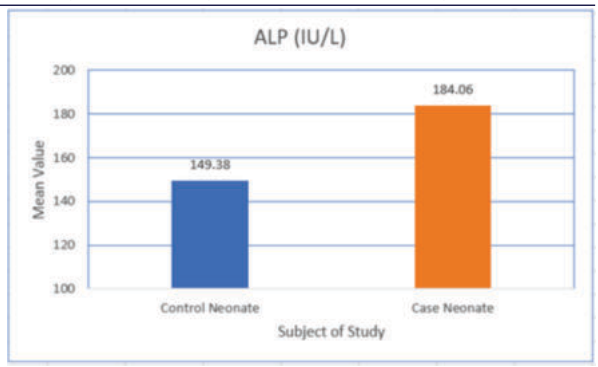

Fig 8- Bar Diagram showing comparison between ALP values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the Direct Bilirubin values between 2 groups of neonates, it is found to be significantly different (p value <0.001)

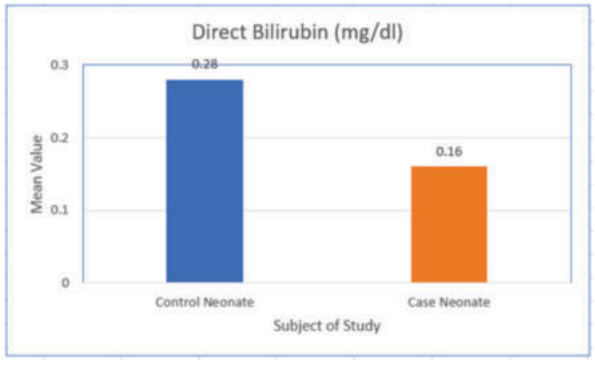

Fig 9- Bar Diagram showing comparison between Direct Bilirubin values of neonates born of non-COVID infected and COVID infected mothers

On comparison of the Total Bilirubin values between 2 groups of neonates, it is found not to be significantly different (p value 0.72)

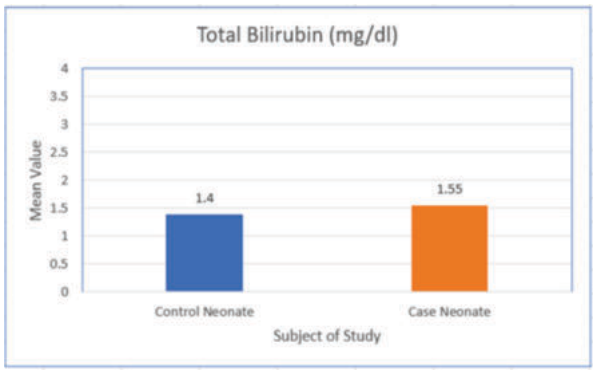

Fig 10- Bar Diagram showing comparison between Direct Bilirubin values of neonates born of non-COVID infected and COVID infected mothers

DISCUSSION

As already mentioned, that COVID 19 being a relatively new disease long term consequences of the infection are still clouded. Therefore, it is very much pertinent to assess the biochemical parameters which includes the pro-inflammatory mediators and liver enzymes of the neonates of COVID mothers and compare the same with those of neonates with normal mother. This assessment will caution us about any problems that may arise in the immunological status and also chronic diseases of the neonates in the future.

This study found that the neonates who were COVID negative despite having COVID infected mothers did not have any stark variation in the values of biochemical parameters as compared to the control neonates of this study group. This study can be compared to a similar case study found to be done in Wuhan, China and Jaipur, Rajasthan in India where the biochemical parameters showed the same variation of parameters

of neonates born of COVID mothers unlike those who got infected after birth and had variations in D-dimer and CRP specifically and also changes in Procalcitonin [8,9] just like in between COVID infected and non-infected mothers in this study.

The significantly different variations with p value <0.001 of Direct Bilirubin and ALP in neonates has a physiological basis. The ALP having various isoenzymes in the bone and placenta besides the ones found in liver results in very high levels [10]. On the other hand, Bilirubin is significant as the neonatal livers just after birth are yet to mature and are not competently able to handle the excessive hemolytic load of erythrocytes and accordingly metabolize the hemoglobin [11].
Due to the time constraint and difficulty in compliance of the subjects in this study, the neonates could not be followed up for further assessment of the biochemical parameters and the physiological health status of the neonates. However, it will be wise to screen these neonates, if possible, to check any clinical manifestations that might otherwise go unnoticed.

CONCLUSION

This study can be concluded on the basis that there are no significant changes in the biochemical parameters (D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, PT/INR) of neonates due to having mothers getting infected by COVID during their pregnancy. However, it will always be advisable to keep a tab on the health status of these if possible and periodic testing at least till the infantile stage. As not much information is known related to the health status of neonates as a consequence of COVID 19 this study can provide a base for further clinical investigation and establishment of facts for other studies.

Summary

Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2 primarily cause damage to lower respiratory tract. Due to the sudden incidence and high pathogenicity, there is little knowledge about the risk of infection by the virus to pregnant women and parturient. The aim was of this study was to find if the biochemical parameters which includes D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, PT/INR of non-COVID infected neonates born of COVID mothers had increased or significant variation as compared to non-COVID neonates born of non-COVID infected mothers. The study included 100 neonates divided into 2 groups of 50 each as non-COVID neonates born of COVID infected mothers during their gestational period and non-COVID neonates born of non-COVID infected mothers. Cord blood was collected after delivery and estimation of D-dimer, Procalcitonin, CRP, ALT, AST, ALP, Direct bilirubin, Indirect Bilirubin, PT/INR were done for both the case and control group of neonates. No significant variation has been observed in the biochemical parameters between the case and control group of subjects. However, since COVID is a relatively new disease with little knowledge about its consequences the maternal COVID status may have an impact on neonatal health in the long run. So, as far as possible the regular periodic health check up and screening of the aforementioned biochemical parameters of these neonates should be followed consistently.

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