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Maternal COVID-19 Exposure Risk during Pregnancy is Associated with Altered Placental Histopathology: An Exploratory Study from Kolkata, India

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Abstract: Background: The impact of epidemiological exposure risk to COVID-19 remains unexplored, especially in pregnant women. This study, therefore, aims to investigate how exposure risk to COVID-19 during pregnancy influences the outcome and placental histopathology. Methods: A cohort of 60 pregnant women (30 control and 30 exposed) from Calcutta Medical Hospital and Calcutta National Medical College Hospital was studied. An exposure risk scoring system was assigned based on common COVID exposure routes such as contact frequency, clinical risk factors and symptom history. Clinical parameters were recorded and correlated with histopathological findings of haematoxylin-eosin-stained placental sections. Statistical analyses included Mann-Whitney U test, Chi-square test, Odds ratio estimation and Logistic regression. Findings: Higher pregnancy complications were observed in women with higher COVID-exposure risk women (odds ratio=4.5, $p<0.05$). Placental abnormalities were markedly elevated in the exposed-risk group with increased fibrin deposition (73.4% vs 13.15%, $p<0.01$), crowding of villi (9.4% vs 0%, $p<0.01$) and calcification (8.9% vs 2.1%, $p<0.05$). Normal histology was dramatically reduced among patients with higher exposure risk (7.83% vs 67%, $p<0.01$). Further, correlation analysis revealed that fibrin deposition, crowding of villi and syncytial knots were positively associated. While a higher exposure risk scores correlated with alterations in placental morphology, it did not serve as an independent predictor of overall clinical outcomes within this cohort. Conclusion: Even without active infection at delivery, maternal exposure risk to COVID-19 during pregnancy could be statistically associated with specific histopathological changes in the placenta, suggesting a potential subclinical impact. The innovative exposure risk scoring model developed here provides an effective framework for quantifying infection risk and its biological implications.

Keywords: COVID exposure, placental abnormalities, histopathological changes, vertical transmission.

INTRODUCTION

The COVID-19 pandemic caused by SARS-CoV-2 was a destabilizing event in the history of human life (1). The high transmissibility of the virus has always been a matter of concern. Droplets and aerosol are the most common modes of horizontal transmission of COVID. However, vertical transmission of SARS-CoV-2 is an area that requires thorough research, especially the broader impact of environmental exposure and maternal systematic stress on

placental architecture even without documented foetal infection. There are reports that suggest vertical transmission in SARS-CoV-2 is debatable. A case report from Texas indicated possible transplacental transmission of SARS-CoV-2 where a premature infant born to a COVID infected female showed signs of respiratory distress and low levels of oxygen in her blood. The infant had tested COVID-19 positive 24 hours after birth (2). Yet another study conducted in 2023 on a cohort of patients diagnosed with SARS-CoV-2 during their pregnancy revealed chronic villitis as a distinct histopathologic manifestation seen in their placenta vs control (3). However, few reports discard potential transmission of SARS-CoV-2 from mother to foetus through placenta. One such study mentioned one neonate born to the COVID infected mother having mild respiratory distress. However, a series of SARS-CoV-2 nucleic acid tests performed were negative in samples from both the mother and neonate (4). Another report suggested that COVID-19 infection during childbirth did not affect the general condition of the newborn (5).

Given the nebulous nature of the effect of a COVID infection during childbirth, it was equally important to evaluate the impact of direct and indirect exposure of SARS-CoV-2 infection on the histology of the placenta. Therefore, our study acts as an exploratory, hypothesis-generating investigation to evaluate the implementation of an epidemiological risk-scoring framework to assess the wider impact of COVID exposure risk on women. In this study an exposed group was classified based on a scoring mechanism where women who either had symptoms or had a higher chance of being exposed to the infection during pregnancy as per their daily lifestyle. This structured exposure risk model was utilized as a proxy for exposure probability. This pilot study correlates standardized risk scores with semi-quantitative histopathological evaluations of term placentae and identifies preliminary association between exposure risk and maternal delivery complications as well as placental histopathological alterations.

METHODS

Sample Design

As underreporting of COVID- 19 cases exists in India, we allocated exposure risk scores to participants. Given that the prevalence of COVID-19 and its spread and deaths due to the disease vary according to the strain of SARS CoV-2 and the time, the study was conducted following a balanced experimental design. Survey was conducted randomly among a cohort of pregnant women, till we obtained 30 participants with high exposure score (6). The clinical samples from these participants were evaluated against the clinical samples of 30 participants with low exposure risk score, bringing the total sample size of the study to n=60. This is an exploratory and hypothesis-generating study. This exploratory study provides effect size estimates that may inform power calculations for future confirmatory investigations.

Clinical Data Collection

Patient survey as well as the placenta sample collection began after obtaining the human ethical clearance from Calcutta Medical College Hospital, Kolkata and Calcutta National Medical College Hospital, Kolkata (vide ethical certificate: MC/KOL/IEC/NON_SPON/1290/03/2022 dated 16/03/2022 and EC-CNMC/2024/403 dated

04/05/2024). As COVID testing was not routine at the time of sample collection, we were unable to obtain a confirmation of infection. To solve the problem, we developed a scoring method as described below. COVID exposure history of the pregnant women was collected by questionnaire method. Data/ samples were collected upon signed informed consent. The data was collected between April 2022-June, 2024. The following features were recorded during the survey: patients' age, duration of pregnancy, vaccination status of the pregnant women, mode of delivery (vaginal vs. caesarean section), and maternal hospital course (complicated vs. uncomplicated). Further, patients' occupations, family members present and their occupations were also noted via the survey. Whether they were COVID infected or exposed during their pregnancy from infected family members, or their family members were exposed to COVID was also recorded. If the pregnant woman had any COVID-like symptoms such as cough, cold and fever during their pregnancy. Different foetal abnormalities and comorbidities of the patients were also recorded. The patient details are tabulated in Table I.

Table I: Details of participants.

Parameters		**Control**	**Exposed**
Sample size		30	30
Vaccines dose	Single dose	7	9
	Double dose	16	16
	None	7	5
Mother's age		17-34(p>0.05)	18-33(p>0.05)
Duration of pregnancy		30-40 weeks	30-42 weeks

At the time of data collection, COVID-19 testing was not performed among asymptomatic women during delivery. The ethical approval protocol did not include serological testing, as participants were clinically COVID-negative at the time of delivery. This also indicates that if a serological test were done retrospectively, it would not reliably determine the timing of infection during pregnancy, particularly in a largely vaccinated population, where antibody presence may reflect vaccination rather than natural infection. Therefore, rather than confirming retrospective infection, the study was designed to examine associations between a structured epidemiological exposure risk and placental morphology in a pandemic context.

Development of Scoring Mechanism

The exposure risk scoring system was developed based on epidemiological plausibility rather than arbitrary assignment and does not correspond to confirmed infection or direct viral burden. The exposure of every participant in the study was scored based on the following three factors. These factors were chosen as they were the most common forms of exposure to the disease (7–9)

Contact-based Avenues of exposure: This was determined based on occupation of the participants and their family members living under the same roof. If a participant or their family member had to work out of home, they were scored higher. More the social interaction, higher was the exposure score assigned.

Criteria: Contact based scores were assigned on a scale of 0-3, concurrent with increasing frequency, duration and diversity of extra-household interactions. Literature reveals that the risk of COVID-19 infection to be higher in essential workers like individuals in healthcare and having customer-facing jobs (10,11). A score of 0 was assigned for no outdoor exposure; a score of 1 was assigned for incidental interaction like interacting with neighbours or going to the marketplace; individuals involved in stand-alone non-essential services with limited one-on-one exposure and government / public officials/health workers who had to continue delivering essential services every day with prolonged and high-throughput interaction with others, were scored 3, similarly, individuals working in the marketplace who had limited social isolation were also scored 3.

Clinical risk factors: They were further scored based on the presence of documented risk factors associated with COVID 19 such as diabetes, cardiovascular diseases, high blood pressure, kidney disease, anaemia, respiratory diseases and hypothyroidism (12). The presence or absence of these risk factors has been assigned a low weightage of 1 or 0 respectively to prevent the burden of comorbidities to overwhelm the exposure.

Symptom history during pregnancy: If the patients had symptoms such as cough, cold and fever during their pregnancy, they were allotted a high score of 5, absence of such symptoms was concurrent with score allotment of 0. Symptom history during pregnancy was given the highest weightage given that they are indicators of COVID-19 infection. Presence of COVID-19 symptoms during a previous pregnancy was also considered and scored 3 for their presence, 0 for absence.

The aggregate of these three components was the final exposure score of all participants. Considering the fact that COVID-19 pandemic is global, (13), all individuals can be categorized as high risk, moderate risk or low risk, no one as “no risk”. Hence, all participants were categorized as either- high exposure to COVID-19 (referred to as “exposed” participants hereon) and low exposure to COVID-19 (referred to as “control” participants hereon) based on 50th percentile value of the scores (Table II).

Table II: Percentiles for the exposure score of the participants

Percentiles	**5th**	**10th**	**25th**	**50th**	**75th**	**90th**	**95th**
Exposure score	3.0500	5.0000	7.0000	10.0000	15.7500	21.0000	24.9000
Number of cases (n)	3	4	10	15	15	8	5

Haematoxylin-eosin Staining of Placenta

Placenta tissues were collected right after delivery. Due to limitations of biosafety protocols, placenta was collected from patients who were COVID negative at the time of delivery. The placental tissue was obtained after vertical incision, 2 centimetres away from the umbilical cord to get the syncytiotrophoblast of chorionic villi region as it was our area of interest because it forms the junction of transmission for various pathogens. The following steps were done in accordance with standard protocol (14).The tissues were fixed in 10% formalin solution. Tissue blocks were made using paraffin wax and 4-micron tissue sections were cut using Leica microtome. The tissue sections were fixed on glass slides and deparaffinized using xylene, followed by gradual rehydration steps of 100%, 90%, 70% and 50% ethanol. Post haematoxylin, gradual dehydration was done using different

concentrations- 50%, 70%, 90% of alcohol, followed by eosin staining and 100% ethanol wash. Each alcohol step was conducted for 5 minutes. The slides were soaked in xylene for 10 minutes, followed by mounting of the slides by DPX solution. Images were taken under 10X magnification using Zeiss (Lab.A1-AXIO model) microscope. The images were taken using ISCapture v3.6 software at 10X objective.

Semi-quantification of Histopathology

The histopathological scoring of placental tissue abnormalities was conducted through a structured, semi-quantitative approach to ensure reproducibility and minimize subjective bias. A total of 10 independent microscopic fields were analysed per sample at uniform magnification and orientation to ensure consistency across all observations. Both COVID-exposed risk and control placental samples were examined following the Amsterdam Placental Workshop Group 2014 Classification [12] . Each field was evaluated for distinct histopathology. To standardize the data, for each histopathology, total number of abnormalities observed was divided by total fields, i.e., 600 (30 control/exposed x 2 slides x 10 fields) and then a percentage was calculated to represent the frequency of that placental abnormality. To establish inter-observer reliability, the scoring was independently confirmed by a second observer. These percentage scores were then used to compare the incidence of each histopathological abnormality between COVID-exposed and control groups and correlate with the clinical data obtained through hospital survey via questionnaire. This scoring system allowed for semi-quantitative comparison across samples.

Statistical Analysis

Statistical analyses were done using MS-Excel, IBM SPSS STATISTICS20 and R4.4.2 software. Mann-Whitney U test was done to find significant difference between two groups- with high and low exposure scores. Odds ratio, Pearson χ^2 test and contingency was done to find association between different variables. Multivariate logistic regression was done to predict placental abnormalities based on exposure score and controlled for maternal age and comorbidities. A correlogram was done to further visually represent the significance between two groups. Images were processed using Adobe Photoshop 7.0.

RESULTS

Clinical Features of Participants Reveal More Complications upon COVID Exposure Risk

Survey of pregnancy complications further revealed that pregnant women who were at risk of exposure to COVID during their pregnancy had more complications during delivery. The complications were noted from clinical reports from physicians and included conditions such as preeclampsia, preterm birth, improper contraction, plummeting blood pressure, and extreme labour pain with cough and cold. Complication during delivery was observed to be significantly higher in the study participants with risk of COVID exposure compared to the control group (Mann-Whitney U standardized test statistic= 2.175, $p=0.03$, $p<0.05$). Higher exposure-risk score was associated with a 4.5-fold increase in delivery complications (OR= 4.5, 95% CI: 1.094-18.503). Although the intervals are wide, indicating small sample size and limited precision, the Chi-square test and contingency coefficient was observed to be

significant (Chi-square= 4.812 (p=0.028, p<0.05), contingency coefficient= 0.272 (p=0.028, p<0.05)). However, the exact magnitude of significance requires validation in a larger cohort.

Pathological Features of the Placenta Reveal more Abnormalities upon Exposure Risk to COVID-19

Various histopathologies were observed along with normal cytology of the placenta. Pathological features such as syncytial knots, increased fibrin deposition, calcification, crowding of villi, chorangiosis and extravasation of RBCs were observed after heamatoxylin-eosin staining. Representative images of each type are shown in Figure 1.

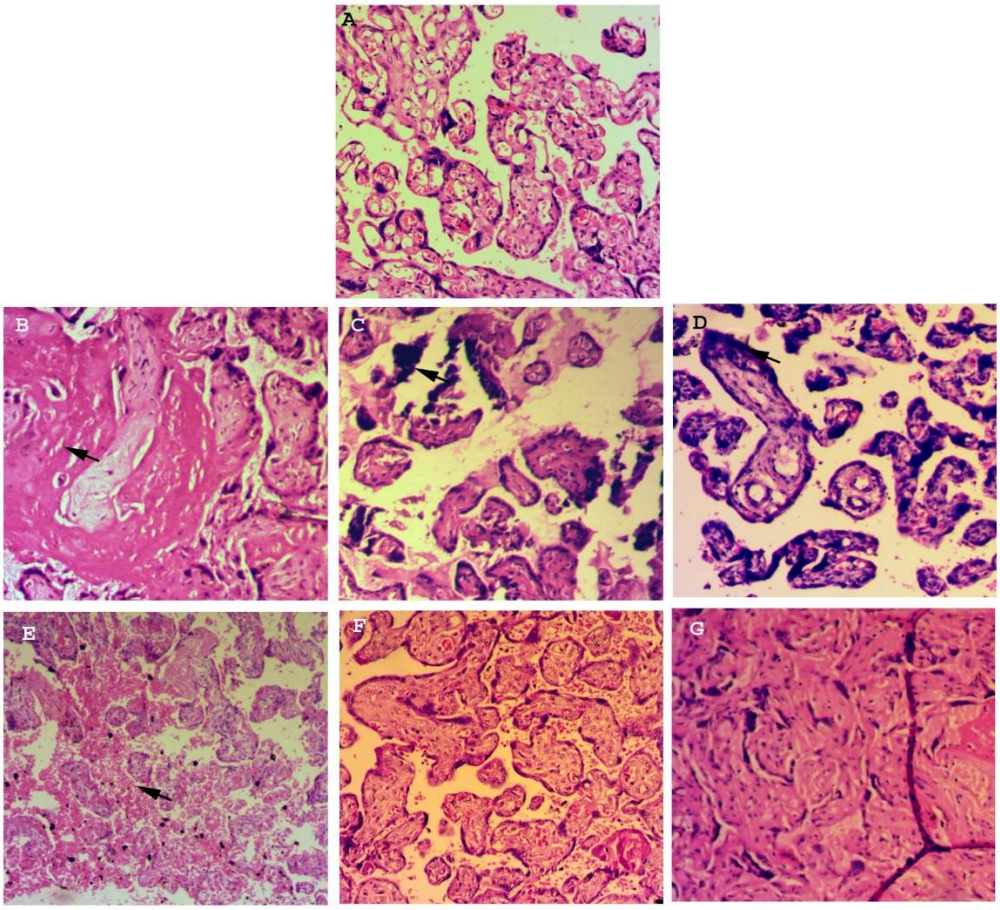

Figure 1: Representative images of histopathological changes in Normal (A) vs COVID exposed placentae (B-G). Histological abnormalities observed are shown with black arrows. (B): fibrin deposition, (C): calcification, (D): syncytial knots, (E): extravasation of RBCs, (F): chorangiosis, (G): crowding of villi.

A higher occurrence of the histopathological abnormalities was observed in the placenta from women at risk of COVID exposure than the control (Table III). Both crowding of villi and calcification was positively associated with exposure-risk score validated by odds ratio and χ^2 (Table IV). Whereas, the chances of having normal cytology decreased in pregnant women with higher exposure-risk scores.

Table III: Quantification of histopathological abnormalities observed in COVID exposed vs control placentas.

Histopathological abnormalities	Exposed patients N=30	Control patients N=30	Mann-Whitney U Standardized Test Statistic	Asymptotic sig. (2-sided test)	Independent samples t-test for observer 1 vs observer 2
Normal histology	7.83% (n=5)	67% (n=27)	-5.320	p=0.000; p<0.01	0.95 (p>0.05)
Calcification	8.9% (n=6)	2.1%(n=2)	2.340	p=0.019; p<0.05	0.98 (p>0.05)
Crowding of villi	9.4%(n=7)	0	3.003	p=0.003; p<0.01	0.98 (p>0.05)
Chorangiosis	5.8%(n=2)	0	1.426	p=0.154; p>0.05	0.95 (p>0.05)
Extravasation of RBCs	1.24%(n=1)	1.74%(n=2)	-0.587	p=0.557; p>0.05	0.95 (p>0.05)
Fibrin deposition	73.4%(n=27)	13.15%(n=9)	4.943	p=0.000; p<0.01	0.96 (p>0.05)
Syncytial knots	23.25%(n=14)	22%(n=15)	0.394	p=0.694; p>0.05	0.91 (p>0.05)

Table IV: Statistical analysis of histopathological changes.

	Odds ratio	Lower limit	Upper limit	Pearson chi-square (significance)	Contingency (significance)
Normal	0.028	0.006	0.123	29.697(p=0.000; p<0.01)	0.575(p=0.000; p<0.01)
Fibrin deposition	18	4.378	74.012	20.376(p=0.000; p<0.01)	0.503(p=0.000; p<0.01)
Calcification	8.826	1.012	76.960	5.192(p=0.023; p<0.05)	0.282(p=0.023; p<0.05)
Crowding of villi	1.364	1.099	1.697	9.231(p=0.002; p<0.01)	0.365(p=0.002; p<0.01)

NOTE: The wide confidence intervals denote limited precision

To assess whether complications during delivery and all the histopathologies observed could be associated with each other, correlation statistics was performed (Figure 2). As per the correlogram, crowding of villi was associated with fibrin deposition and syncytial knots and crowding of villi, syncytial knots and calcification were negatively correlated with normal cytology. Furthermore, binary logistic regression with Hosmer Lemeshow goodness-of-fit was carried out and it was observed that fibrin deposition could be predicted by exposure-risk score. The regression model has been tabulated below (Table V [a-d]). Multivariate logistic regression was done to assess the independent predictors of placental histopathology. The model portrayed overall significance and explained a modest proportion of variance. Within the model, exposure-risk score was observed to be a significant predictor, but age and comorbidity, with wide confidence intervals indicating limited precision, were not.

Table V: Fibrin deposition predicted by exposure score.

Table V(a): Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	8.615	3	.035
	Block	8.615	3	.035
	Model	8.615	3	.035

Table V(b): Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	71.265[a]	.134	.182

[a] Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

Table V(c): Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	11.000	8	.202

Table V(d): Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Exposure Score	.178	.074	5.755	1	.016	1.194	1.033	1.381
	Age	-.029	.070	.167	1	.682	.972	.847	1.115
	Comorbidities	-.057	.614	.009	1	.926	.945	.284	3.147
	Constant	-.604	1.941	.097	1	.756	.547		

a. Variable(s) entered on step 1: Exposure Score, Age, Comorbidities.

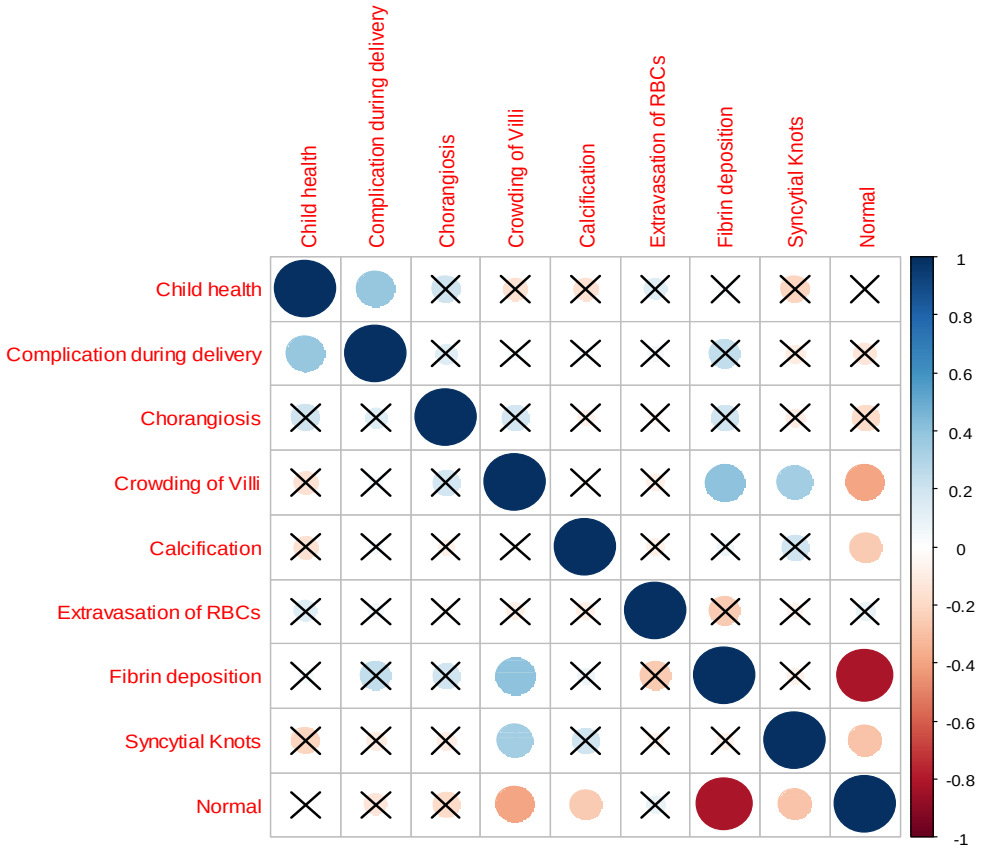

Figure 2: Correlelogram showing correlations between various observed parameters. Blue indicates a significant positive correlation and red indicates a significant negative correlation. The intensity of the correlation is indicated by the size of the dots and colour intensity. The cross indicates a correlation that was not statistically significant.

DISCUSSION

Our exploratory study investigated whether a higher epidemiological exposure risk to SARS-CoV-2 during pregnancy is associated with changes in placental histopathology, even without an active infection at delivery, based on a structured, questionnaire-based exposure-risk score. Increased placental abnormalities specifically fibrin deposition, calcification, crowding of villi were exhibited by the women classified as having higher exposure risk scores together with higher occurrence of pregnancy complications. A statistically significant association between exposure risk scores and particular histopathological alterations, notably a 4.5-fold increase in delivery complications (OR=4.5, 95% CI: 1.094-18.503) was revealed by our analysis.

Substantial literature detailed different effects of COVID infection on pregnant women most commonly maternal vascular malperfusion and syncytial knot formation. But sparse data exists on indirect maternal exposure risk. Our observations of placental abnormalities are mostly consistent with these reports despite the fact our cohort was confirmed by exposure risk and not active infection. Both crowding of villi and calcification had positive association with COVID exposure risk. The higher odds ratio value further suggested chances of fibrin deposition increase due to cumulative exposure risk. The

chances of having normal cytology decreased in women with lower COVID exposure risk. These findings align with previous reports describing placental lesions in COVID infected women, such as maternal and foetal vascular malperfusion, chronic histiocytic intervillositis [13,14]. The study further assessed if these villous abnormalities could be correlated with complications during delivery. Although this study cannot establish that these complications directly resulted from COVID infection, our findings suggest women with higher exposure risk to SARS-CoV-2 may be associated with increased obstetric risk. Further, the correlogram revealed significant correlations between fibrin deposition, villous crowding and syncytial knots, suggesting these lesions may be a part of a common pathological cascade triggered by maternal inflammation, endothelial injury or immune dysregulation secondary to COVID exposure. However, without confirmatory molecular analyses, these tissue alterations could be viewed general markers of placental stress rather than lesions unique to SARS-CoV-2.

The current study offers a notable hypothesis on the impact of COVID-19 pandemic on maternal physiology by investigating the histopathological changes in placenta from women with higher epidemiological exposure risk as well as complications during delivery. It may be noted that our work introduces a quantitative exposure scoring model that integrates contact history, comorbidities and symptomatic data to objectively classify risk levels among pregnant women. This innovative approach enables a more refined understanding of how indirect SARS-CoV-2 exposure risk may influence pregnancy outcomes and might act as an important epidemiological tool for identifying vulnerable pregnant populations during infectious disease outbreaks.

Our study is limited by the fact that we could not confirm an active infection during pregnancy, as testing was not routine at the time of sample collection and hence we could not identify viral antigens. Therefore, the underlying biological and molecular mechanisms driving our findings remain exploratory and suggestive. Furthermore, there was always under-reporting amongst the population we studied. Secondly, the study was conducted in Indian tertiary-care hospitals because they serve a large and socio-demographically diverse obstetric population, allowing evaluation of exposure patterns in a real-world setting during the pandemic period. Given differences in healthcare access, population density, and public health response across countries, findings are most directly applicable to similar low- and middle-income healthcare contexts. Also, cohort studies with larger sample size should be conducted to validate the exposure scoring system proposed in this study. Finally, we were also unable to investigate any strain-specific differences in pregnancy outcomes.

The findings of this study highlight the need for clinicians to consider exposure history—not just infection status—when assessing pregnancy risk. Additionally, these findings reinforce the importance of preventive strategies such as maternal vaccination, infection control measures, and timely clinical intervention.

To conclude from this hypothesis-generating pilot study, we can say that SARS-CoV-2 exposure risk, even in the absence of an active infection, is associated with obstetric complications and key histopathological alterations in the placenta. Also, the development of an exposure risk scoring system further highlights its possible use in determining at-risk pregnancies. However, the findings require validation in larger cohorts including laboratory-based confirmation of infection, molecular analyses, precise vaccination timelines and neonatal follow-up data to definitely isolate the impacts of viral exposure from wider environmental and maternal confounding factors.

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Author Contributions:

- Sulagna Mandal: Methodology, Validation, Formal analysis, Investigation, data curation, Writing-original, review and editing,
- Ruchira Mukherjee: Formal analysis, Writing- review and Editing
- Aditi Bhattacharyya: Resources, Investigation
- Sukumar Mitra: Resources
- Pranab Kumar Biswas: Resources
- Aparna Mukhopadhyay: Conceptualisation, Writing - Review & Editing, Supervision, Project administration

Ethical Approval: This work involves human samples. Ethical clearance was obtained from Calcutta Medical College Hospital, Kolkata and Calcutta National Medical College Hospital, Kolkata vide ethical certificate: MC/KOL/IEC/NON_SPON/1290/03/2022 dated 16/03/2022 and EC-CNMC/2024/403 dated 04/05/2024. Signed informed consents were obtained from all patients participating in the study. No animals were used.

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Declaration on use of AI: AI was used for paraphrasing and English corrections. For such purpose Gemini was used.

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