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Unlocking the mystery of the role of Vitamin D in iron deficiency anemia in antenatal women: a case control study in a tertiary care hospital in New Delhi

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Abstract

Background Vitamin D deficiency and anemia are clinical conditions that coexist during pregnancy. A high prevalence of Vitamin D deficiency ranging from 50 to 94% is seen throughout the country. The aim of the study was to discover the association between Vitamin D status and iron deficiency anemia during pregnancy. Improving the vitamin D status of pregnant women is crucial to prevent iron deficiency anemia and can improve maternal and fetal outcomes.

Methods A case–control study including 94 primigravida women of age within the age group 18 to 30 years, divided into two groups: a Case Group of 48 patients with already diagnosed iron deficiency anemia (mild to moderate) and a Control Group of 46 antenatal women with normal hemoglobin levels. Data on sociodemographic, clinical characteristics, and the levels of 25(OH) Vitamin D was estimated in both the groups. The association of 25(OH)D levels and anemia was then determined using suitable statistical analysis.

Results Among pregnant women affected with anemia, 75% of women had serum Vitamin D concentrations < 20 ng/ml compared to 52.2% of women in the controls. Maternal serum vitamin D level was significantly lower in pregnant women affected with anemia (19.61 ± 13.12) as compared to control (29.43 ± 24.05); ($p = 0.024$). A positive correlation was found between hemoglobin and vitamin D levels in pregnant women (Pearson's $r = 0.200$, $p = 0.05$).

Conclusions These findings provide evidence suggesting that Vitamin D deficiency or insufficiency during pregnancy may be a risk factor for anemia and correction of Vitamin D levels can improve hemoglobin levels. Educational efforts should be made to include safe vitamin D intake in antenatal care.

Keywords Pregnant women, Vitamin D deficiency, Anemia, Obstetrics, Maternal, Developing countries, Hemoglobin

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Background

Vitamin D deficiency is recognized as a public health concern characterized by low levels of serum 25 hydroxy Vitamin D [25(OH)D] below 20 ng/ml. A high prevalence of Vitamin D deficiency ranging from 50 to 94% is seen throughout the country [1]. Major factors that are significantly associated with its deficiency include dietary habits and inadequate sunlight exposure [2]. Increasing pollution due to escalating urbanization, and lifestyle with poor outdoor activities are likely to worsen the condition [3]. In addition, its requirement further increases during pregnancy as it is crucial for maternal health, foetal skeletal growth, and optimal maternal and foetal outcomes.

Anemia is also a common problem during pregnancy, especially in developing countries. In India, as per the National Family Health Survey (NFHS-4) data, the prevalence of anemia during pregnancy is around 50% [4]. Maternal anemia has been linked with an increased risk of preterm delivery, low birth weight, intrauterine growth retardation, and adverse perinatal outcomes [5].

Vitamin D deficiency and anemia are clinical conditions that coexist during pregnancy. Studies suggest an association between Vitamin D deficiency and upregulation of mRNA expression of hepcidin, a molecule resulting in decreased level of ferroportin responsible for iron absorption, which indicates adequate Vitamin D serum levels can provide additional protection against iron deficiency in pregnant women [6]. Vitamin D receptors have already been demonstrated in bone marrow and its concentration is several 100- fold higher in bone marrow as compared to plasma [7]. Vitamin D also has an important role in erythropoiesis and it is also demonstrated that Vitamin D supplementation may improve anemia management [8].

However, the association between vitamin D deficiency and iron deficiency anemia among antenatal women remains underexplored. This study aims to bring out the potential relationship between vitamin D deficiency and iron deficiency anemia in pregnant women. Additionally, this study will provide data related to the magnitude of Vitamin D Deficiency in iron-deficient pregnant women. This study emphasized the need to improve vitamin D status in pregnant anemic women through Vitamin D supplementation along with iron supplements.

To the best of our knowledge, clinical evidence is still scarce in this field to comprehend their correlation.

Aims and objectives

- **Primary Objective:** To assess the association of Vitamin D levels between iron-deficient anemic and nonanemic pregnant women.
- **Secondary Objectives:**
    - To assess the association of various socio-demographic factors among the study subjects.
    - To assess the association of selected maternal characteristics with iron deficiency anemia among the study subjects.
    - To assess the association of anthropometric and biochemical parameters with iron deficiency anemia among the study subjects.

Methods

- **Design and Study Population:** A Hospital based Case Control Study was conducted among pregnant women between 28th August 2022 to 15th October 2022 in the Maternity Ward/Antenatal Clinic of the Department of Obstetrics and Gynaecology at the Dr. Baba Saheb Ambedkar Medical College and Hospital, Delhi.

  The sample size was calculated by taking the proportion of Vitamin D Deficiency in non-anemic and anemic pregnant women as 40% and 75% respectively, at 95% level of confidence and 80% power with the same number of cases and controls [9]. The minimum sample size calculated by the Fleiss formula was 31 subjects for cases and controls each. We enrolled 107 pregnant women who came for antenatal check-up or at the time of delivery and were willing to participate in the study. They were thoroughly screened by history and examination. Thirteen subjects were excluded from the study due to incomplete medical records or their samples were hemolyzed. The remaining 94 pregnant women were divided into two groups: a total of 48 antenatal women with already diagnosed iron deficiency anemia (mild to moderate) were included in the Case group and 46 antenatal women with normal hemoglobin levels were included in the Control group.
- **Eligibility Criteria:** The included subjects in both groups were primigravida women within the age group of 18 to 30 years, with confirmed singleton uncomplicated pregnancy at or after 28 weeks of gestation defined through their last menstrual period or through their first-trimester ultrasound results were included. The inclusion criteria also included the ability to provide the consent, no history of blood transfusion during current pregnancy. Exclusion criteria included pregnant women with any active

comorbid disease (e.g.: thyroid diseases, chronic hypertension, seizure disorder, renal diseases, diabetes mellitus, chronic liver diseases, etc.) and anemia other than iron deficiency anemia. Patients already taking Vitamin D supplementation during pregnancy were also excluded. Patients with thalassemia (as per Mentzer Index) or history of thalassemia, sickle cell anemia, hemolytic anemia, peptic ulcer disease, gastritis, malabsorption syndrome, chronic blood loss, presence of chronic hematological diseases were also not recruited.

- **Information and Sample Collection:** Detailed history and examination were performed with regard to current pregnancy. A pre-validated and semi-structured data collection form was used to collect information on socio-economic, demographic details, personal details, pregnancy history which comprised past/ongoing medical and treatment history, iron, folic acid and Vitamin D supplement intake, regular milk consumption and information regarding present pregnancy. Antenatal investigations were noted which included blood investigations and Mentzer Index, where Mentzer Index was calculated to detect any pregnant women of β thalassemia trait. Those pregnant women who provided informed written consent, 5 ml peripheral venous blood was collected under strict aseptic conditions by a licensed laboratory technician and 2 ml of the blood was sent for. The serum was separated in a cold centrifuge within 30 min and was kept in frozen aliquots at -40 °C on the same day until analyzed. 25(OH) Vitamin D_2 and D_3 were measured with the help of commercially available kit using chemiluminescent immunoassay technology (CLIA) (interassay coefficient of variation, 12%). Rest of the blood was used to measure other standard blood parameters using impedance method measurement (Sysmex XP-100, Japan). The serum Vitamin D test done was an addition to the standard antenatal investigation performed in the third trimester with no extra needle prick. The 25(OH) Vitamin D levels were estimated in both groups. The association of 25(OH)D levels and anemia was then determined using suitable statistical analysis.
- **Criteria for Analysis:** Anemia in pregnant women was defined as a hemoglobin level of equal and more than 11 g/dl as normal, 10–10.9 g/dl as mild anemia, 7–9.9 g/dl as moderate anemia and 4–6.9 g/dl as severe anemia as per the guidelines of Indian Council of Medical Research (ICMR) [10].

  Vitamin D deficiency was defined as serum 25(OH)D levels less than or equal to 20 ng/ml and Vitamin D insufficiency is considered as more than 20 ng/ml but less than 30 ng/ml, while more than or equal to 30 ng/ml is considered as sufficient or normal [11].
- **Statistical Analysis:** Entries were performed and data cleaning was done from the case study form into our customized Excel Sheet (Microsoft Corp., USA). Differences in maternal characteristics and serum Vitamin D concentrations between both groups were calculated by χ2 test (for categorical variables), Mann-Whitney test (for Vitamin D concentrations), and Odds Ratio (for strength of association). All analyses were conducted using the trial version of the statistical package for social sciences (version 27.0; SPSS Inc., Chicago, IL) software, and $p \leq 0.05$ is statistically significant.

Results

We successfully analyzed serum 25(OH)D concentrations from all 94 samples (48 pregnant women affected with anemia and 46 controls not having anemia). The selected characteristics and sociodemographic details of the anemic women (Case) and non-anemic women (Controls) are summarized in Table 1. The mean age was 23.2 ± 3.3 years (median 22 years) for anemic pregnant women and 24.1 ± 3 years (median 24 years) for the controls. Almost all the cases were housewives (97.9%), but 8.7% of controls were employed. According to the Modified Kuppuswamy scale, nearly three-quarters (70.8%) of the cases fall under the upper lower class, while almost half (47.3%) are above the lower middle class [12]. While 56.5% of the controls have finished their intermediate class, 70.9% of the cases have not finished up to that point in their schooling. Supplementation with Iron and Folic Acid (IFA) was lacking in 43.8% of cases but present in 78.3% of controls. In contrast to the 73.9% of controls who regularly consume milk, regular milk consumption was found in only 45.8% of cases. Majority of women in both groups (89.6% in the case group and 93.5% in the control group) have more than 4 antenatal care visits to monitor the progress of their pregnancy. Nearly more than half of the studied pregnant women in both groups (58.3% in the case group and 56.5% in the control group) are non-vegetarian by diet. Among pregnant women affected with anemia, 75% of women had serum Vitamin D concentrations ≤ 20 ng/ml compared to 52.2% of women in the controls.

Independent Sample Mann Whitney U Test has been applied to check whether the distribution of BMI, Hemoglobin, RBC count, TLC, Platelet Count, MCV, MCH, MCHC, Hematocrit, and serum Vitamin D levels is same across cases and controls. We found that there is

Table 1 Distribution of study subjects according to socio-demographic and maternal characteristics

Maternal Characteristics	Variable	Cases (*n* = 48) [Frequency (%)]	Controls (*n* = 46) [Frequency (%)]
Age	18–21	17(35.4%)	10(21.7%)
	22–25	20(41.7%)	20(43.5%)
	≥ 26	11(22.9%)	16(34.8%)
Occupation	Housewife	47(97.9%)	42(91.3%)
	Employed	1(2.1%)	4(8.7%)
Education	Till Primary School	20(41.7%)	10(21.7%)
	High School	14(29.2%)	10(21.7%)
	Intermediate	8(16.7%)	11(23.9%)
	Graduate	6(12.5%)	9(19.6%)
	Post Graduate	0	6(13.0%)
Religion	Hindu	41(85.4%)	40(87%)
	Others	7(14.6%)	6(13%)
Socio economic status	Lower	10(20.8%)	5(10.9%)
	Upper Lower	24(50.0%)	19(41.3%)
	Lower Middle	13(27.1%)	17(37.0%)
	Upper Middle	1(2.1%)	4(8.7%)
	Upper	0	1(2.2%)
Iron / Folic Acid Supplementation	Present	27(56.2%)	36(78.3%)
	Absent	21(43.8%)	10(21.7%)
ANC Visits	< 4 visits	5(10.4%)	3(6.5%)
	≥ 4 visits	43(89.6%)	43(93.5%)
Dietary Habits	Non-Vegetarian	28(58.3%)	26(56.5%)
	Vegetarian	20(41.7%)	20(43.5%)
Milk Consumption	Present	22(45.8%)	34(73.9%)
	Absent	26(54.2%)	12(26.1%)
Serum Vitamin D Level	Deficient	36(75.0%)	24(52.1%)
	Insufficient	3(6.3%)	9(19.6%)
	Sufficient	9(18.7%)	13(28.3%)

significantly lower levels of Hemoglobin ($p = 0.000$), RBC count ($p = 0.000$), MCV ($p = 0.015$), MCH ($p = 0.000$), MCHC ($p = 0.000$), and Hematocrit ($p = 0.000$) in case group as compared to the control group. The study did not find any statistical significant difference in the distribution of Body Mass Index (BMI) ($p = 0.762$), Total Leucocyte Count (TLC) ($p = 0.264$) and platelet count ($p = 0.213$) among case and control group. Maternal serum vitamin D level was significantly lower in pregnant women affected with anemia (19.61 ± 13.12) as compared to control (29.43 ± 24.05); ($p = 0.024$) [Table 2].

Table 3 summarizes the association of maternal characteristics and lifestyle habits among study subjects which includes iron and folic acid supplementation, Dietary habits, and milk consumption. The study did not find any statistically significant association between religion as well as dietary habits among study subjects. Subjects who are not taking Iron/Folic Acid supplementation are at 2.8 times more risk for being anemic (Odds ratio: 2.8; 95% C.I. 1.13–6.91). Subjects who are not consuming milk are at 3.35 times more risk for being anemic (Odds ratio: 3.35; 95% C.I. 1.40–7.98).

A weak positive correlation was found between hemoglobin and vitamin D levels among study subjects (Pearson's $r = 0.200$, $p = 0.05$) (Fig. 1).

Discussion

Vitamin D deficiency has been linked to a variety of health consequences during pregnancy and is a global public health concern. In this hospital-based case–control study conducted on pregnant women, our main aim was to find the association between Vitamin D status and iron deficiency anemia during pregnancy. We found maternal serum Vitamin D concentrations were significantly less in women affected with anemia as compared to normal pregnant women (mean: 19.61 ng/ml in the case group vs. 29.43 ng/ml in the control group). Our results are in line with recent studies suggesting a strong association of low

Table 2 Distribution of BMI, blood and serum parameters among anemic and non-anemic pregnant women

Measurements	Cases($N=48$)		Controls ($N=46$)		*p*- value[a]
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
BMI(kg/m^2)	23.94 ± 3.88	23.24 (4.73)	23.99 ± 3.17	23.33 (3.17)	0.762
Haemoglobin (g/dL)	9.35 ± 1.03	9.50 (1.03)	12.13 ± 0.83	12.05 (0.83)	0.000
RBC count (x 10^6/μL)	3.62 ± 0.59	3.62 (0.59)	4.06 ± 0.50	4.05 (0.50)	0.000
TLC (Total Leucocyte Count) (x 10^3/μL)	11.32 ± 2.99	10.45 (2.99)	11.69 ± 2.63	11.25 (2.63)	0.264
Platelet Count (x 10^3/μL)	234.48 ± 103.72	222.50 (103.72)	202.46 ± 72.30	202.50 (72.31)	0.213
MCV (fL)	86.20 ± 11.78	85.30 (85.31)	90.76 ± 8.72	89.65 (8.72)	0.015
MCH (pg)	26.33 ± 4.08	26.25 (4.08)	30.22 ± 3.50	29.90 (3.50)	0.000
MCHC (g/dL)	30.43 ± 2.95	31.05 (2.95)	33.27 ± 1.63	33.10 (1.63)	0.000
Hematocrit (%)	30.64 ± 3.66	31.25 (3.66)	36.50 ± 2.49	36.45 (2.49)	0.000
Serum Vitamin D Levels (ng/ml)	19.61 ± 13.12	15.18 (13.13)	29.43 ± 24.05	19.59 (24.05)	0.024

BMI Body Mass Index, *RBC* Red Blood Cell, *MCV* Mean Corpuscular Volume, *MCH* Mean Corpuscular Hemoglobin

[a] *p*-value < 0.05 (Mann–Whitney test)

Table 3 Association of maternal characteristics and lifestyle habits among anemic and non-anemic pregnant women

Maternal Characteristics	Variable	Cases ($n=48$) [Frequency (%)]	Controls ($n=46$) [Frequency (%)]	Odds Ratio (95% CI)
Religion	Hindu	41(85.4%)	40(87.0%)	1
	Others	7(14.6%)	6(13.0%)	1.138 (0.352–3.683)
Iron / Folic Acid Supplementation	Present	27(56.3%)	36(78.3%)	1
	Absent	21(43.7%)	10(21.7%)	2.800 (1.135–6.910)
Dietary Habits	Non-Vegetarian	28(58.3%)	26(56.5%)	1
	Vegetarian	20(41.7%)	20(43.5%)	0.929 (0.410–2.104)
Milk Consumption	Present	22(45.8%)	34(73.9%)	1
	Absent	26(54.2%)	12(26.1%)	3.348 (1.404–7.986)
Serum Vitamin D Level	Deficient	36(75.0%)	24(52.2%)	1
	Insufficient	3(6.3%)	9(19.6%)	0.222 (0.055–0.906)
	Sufficient	9(18.7%)	13(28.2%)	0.462 (0.171–1.248)

Vitamin D levels with anemia in pregnant women. Several studies reported low maternal vitamin D levels may be a risk factor for gestational anemia [13–17].

There are various possible explanations for the link between vitamin D deficiency and maternal anemia. Calcitriol, the active form of vitamin D, may upregulate erythropoietin receptor expression on erythroid progenitor cells and may have a proliferative impact on erythroid burst generating units, which is synergistic with erythropoietin [18, 19]. Calcitriol has an essential part in the immune function control by reducing the activity of pro-inflammatory cytokines which could be critical to its involvement in anemia prevention [20]. Studies suggest an association between Vitamin D deficiency and upregulation of mRNA expression of hepcidin, a molecule resulting in decreased level of ferroportin responsible for iron absorption, which indicates adequate Vitamin D serum levels can provide additional protection against iron deficiency in pregnant women [6]. Vitamin D receptors have already been found in bone marrow, where its concentration is hundreds folds higher than in plasma [7].

One of the most important findings of our study was the unexpectedly high prevalence of deficient Vitamin D levels among both groups (75% in case and 52.2% in control group). Hypovitaminosis D among North Indian pregnant women has also been widely reported [21–24]. The rationale might be owing to insufficient sun exposure, dark skin complexion and a lack of dietary calcium consumption, as Vitamin D synthesis initiates in the skin after sun exposure [25, 26]. High amounts of atmospheric pollution in Indian metropolitan cities could also be an important factor [27].

We also accounted for several parameters as a risk factor for maternal anemia where we reported the education status and socioeconomic status of the mother can

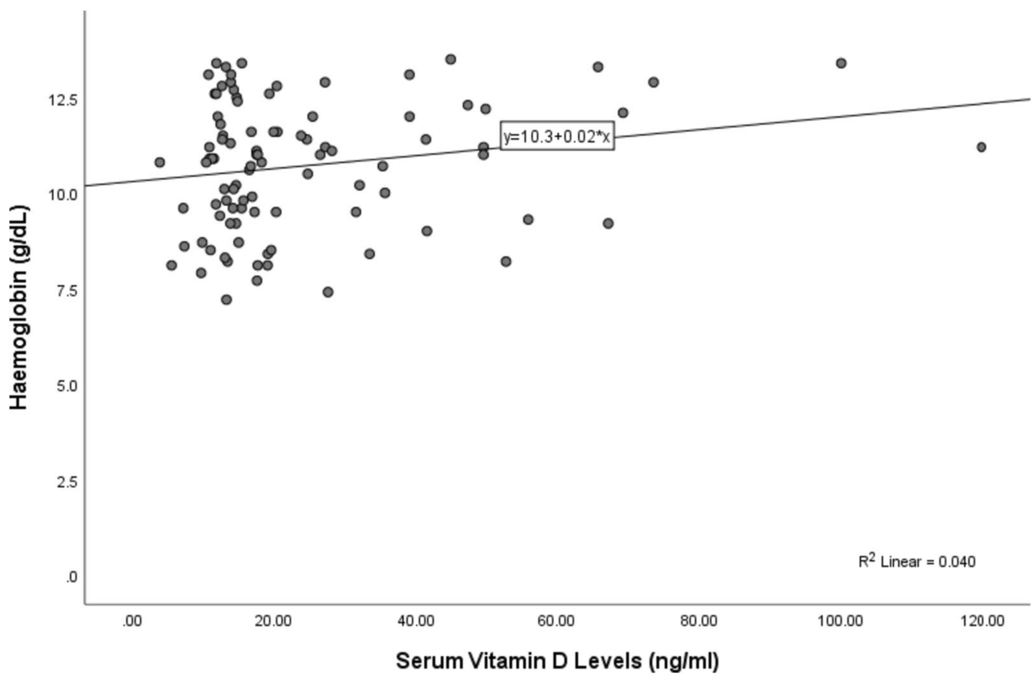

Fig. 1 Graph showing the correlation between hemoglobin and serum vitamin D levels for pregnant women

also be a risk factor for anemia. This may be attributed to the fact that educated women belonging to middle socioeconomic status tend to prioritize self-care, actively engage in antenatal care, maintain a nutritious diet, supplement with iron and calcium, and take measures to prevent potential parasitic infections. The educational component makes the subject group more aware and responsive to new concepts and contents of public health campaigns, making them more likely to adopt good healthy living and treatment-seeking behaviour. On the other hand, uneducated and economically disadvantaged mothers are expected to lack proper nutrition [28]. This explains education and socioeconomic status are linked with maternal anemia.

Our results show a positive correlation of the effect of iron and folic acid (IFA) supplementation on maternal anemia which is consistent with various other studies [29, 30]. Although India has incorporated iron and folic acid supplementation schemes for pregnant women since 1970, these programmes have been hampered by a variety of problems and supply and coverage restrictions [31]. These schemes were relaunched in 2018 as Anemia Mukt Bharat (AMB) Abhiyan to address basic difficulties such as programme finance, execution, and last mile delivery but still the prevalence of anemia was very high during pregnancy [32].

We discovered a link between regular milk consumption and anemia. In contrast to our findings, coffee and milk have been recognized as possible iron absorption inhibitors, but only when high amounts of these items are ingested in the same meal as the iron [33]. This also shows that pregnant women are better aware of the need to take calcium and iron supplements not in the same meal. Still pregnant women should be concerned about their milk consumption as milk is an excellent source of protein and minerals like calcium, and it is linked to an increased birth weight [34].

Pregnant women identified with Vitamin D deficiency were proactively contacted, and they were provided with appropriate vitamin D supplements as per our institutional protocol. Through this study, we were able to conclude that future research should focus on more unified approaches to vitamin D testing and preventative strategies that can be integrated into already existing antenatal care settings to prevent Iron Deficiency Anemia.

A limitation of our study was the relatively small sample size and we lacked information on the iron status of the pregnant women. As a result, we were unable to explore the potential relationship between vitamin D and these markers. Further research that takes into account the above potential markers and includes a larger population size with long-term impacts is necessary and would have a significant impact on public healthcare policy.

Conclusion

The purpose of this study was to compare vitamin D levels in iron-deficient anemia and non-anemic pregnant women. These findings provide evidence suggesting that

Vitamin D deficiency or insufficiency during pregnancy may be a risk factor for anemia and correction of Vitamin D levels can improve hemoglobin levels. This study emphasized the need of improving vitamin D levels in pregnant anemic women by supplementing with vitamin D and iron. Further investigations at a larger scale on national level and multicentric trials need to be conducted to evaluate the direct relation between Vitamin D deficiency and iron deficiency anemia. Currently, Vitamin D supplementation is not recommended as a part of antenatal care programs in India. In view of the high prevalence of low Vitamin D levels in pregnancy, educational efforts need to be made to include regular safe Vitamin D and other micronutrients intake as a part of public health strategy to improve the health of both mothers and their offspring.

Abbreviations

25(OH)D	25-Hydroxyvitamin D
NFHS	National Family Health Survey
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
HIV	Human Immunodeficiency Virus
CLIA	Chemiluminescent Immunoassay Technology
SPSS	Statistical Package for Social Sciences
ANC	Antenatal Care
BMI	Body Mass Index
IQR	Interquartile Range
RBC	Red Blood Corpuscle
TLC	Total Leucocyte Count
AMB	Anemia Mukt Bharat
ICMR	Indian Council of Medical Research
STS	Short Term Studentship

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Authors' contribution

T.H., R.K. and D.D. were responsible for conception of the study. T.H., R.K., D.D. and P.A. were advised to design. T.H. and R.K. collected the data. T.H., R.K., D.D. and R.P.J. analysed the data and interpreted the findings. T.H., P.R. and R.K. drafted and revised the manuscript. All authors approved the final version.

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Availability of data and materials

The datasets used in the present study are available with the ICMR STS-2022 registry (ICMR STS ID: 2022–02037) and with the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval has been obtained from the Institutional Research Review Committee and Institutional Ethical Committee of Dr. Baba Saheb Ambedkar Medical College and Hospital (IRRC-5/2022), Delhi prior to the start of the study, and the methods were carried out in accordance with the approved guidelines. Informed written consent has been taken from all subjects before including them in the study. Prior to recruitment, pregnant women were given information about the study using the Participation Information Sheet. Confidentiality of all the data is ensured by keeping the responses and the collected data under a secure setting.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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