Association of high cortisol levels in pregnancy and altered fetal growth. Results from the MAASTHI, a prospective cohort study, Bengaluru

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Summary

Background The role of maternal stress levels on mothers' mental health and fetal growth has been previously studied. However, the evidence linking cortisol exposure during pregnancy to growth outcomes in infants is sparsely available from lower and middle-income countries. We aim to investigate the association of serum cortisol levels in pregnancy with infant birth outcomes and postpartum depressive symptoms in a public health facility in India.

Methods The current study is a part of the maternal antecedents of adiposity and studying the transgenerational role of hyperglycemia and insulin (MAASTHI) prospective cohort. We assessed the relationship between maternal exposure to serum cortisol and adverse neonatal outcomes and postpartum depressive symptoms. Serum cortisol levels in stored blood samples were measured in 230 pregnant women as a biomarker for stress during pregnancy. Pregnant women between 18 and 45 years of age were recruited for the study, presenting at ≥14 weeks of gestation and providing voluntary written informed consent. The Edinburgh Postnatal Depression Scale assessed postpartum depressive symptoms, and detailed infant anthropometric measurements were carried out at birth.

Findings We found that higher levels (>17.66 μg/L) are significantly associated with low birth weight (OR = 2.28; 95% CI 1.21–4.32) and lower weight for length (OR = 2.16; 95% CI 1.07–4.35). The odds of developing postpartum depressive symptoms in pregnant women with higher mean cortisol cut-off levels is 2.3-fold [OR: 2.33, 95% CI (1.17, 4.64)] compared than women with lower cortisol levels. No significant association was found between serum cortisol and infants' birth weight for gestational age, head circumference, the sum of skinfold thickness, and crown-rump length.

Interpretation Our results support the hypothesis that higher maternal cortisol levels may adversely impact birth weight, weight for length in newborns, and postpartum depressive symptoms in mothers.

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Keywords: Pregnancy; Serum cortisol; Fetal growth; Stress; Biomarker; Anthropometry

Introduction

Pregnancy induces significant changes in the levels and function of the endocrine systems; these changes affect the mother's mental health[1] and fetal development.[2] Women may experience stress, anxiety, depression, social isolation, and pathological conditions. Pregnancy-specific stress may be in any form, including worries about prenatal screening results, concern about the

Abbreviations: MAASTHI, Maternal antecedents of adiposity and studying the transgenerational role of hyperglycemia and insulin; EPDS, Edinburgh Postnatal Depression Scale; HPA axis, Hypothalamic-pituitary-adrenal axis; 11β-HSD, 11beta hydroxysteroid dehydrogenase 2; LMICs, Lower and middle-income countries; NFHS, National Family Health Survey; ISO, International Organization for Standardization; NABL, National Accreditation Board for testing and calibration Laboratories; LBW, Low birth weight; WLZ, Weight for length Z-score; SGA, Small for gestational age; HC, Head circumference; CRL, Crown-rump length; SPSS, Statistical Package for the Social Science; BMI, Body mass index; GDM, Gestational diabetes mellitus; DNA, Deoxyribose nucleic acid; IQ, Intelligence quotient

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Research in context

Evidence before this study

Studies examining depression and cortisol levels during pregnancy and their effects on birth outcomes and maternal mental health have yielded mixed results, with limited sample sizes for serum cortisol assessments. Evidence is lacking from LMICs linking cortisol levels during pregnancy to infants' physical growth outcomes. Standardized questionnaires have limitations in assessing depression during pregnancy, as perspectives can change. Combining questionnaires and biomarker assessments could help identify individuals at risk.

Added value of this study

We show that higher cortisol levels (>17.66 μg/L) during pregnancy are significantly associated with lower birth weight and weight for length in infants. Pregnant women with higher cortisol levels also have a 2.3-fold increased risk of developing postpartum depressive symptoms. These findings add to the limited evidence from LMICs on the relationship between cortisol levels during pregnancy and birth outcomes, particularly for postpartum depressive symptoms.

Implications of all the available evidence

While cortisol levels demonstrate the physiological effects of stress, further research is needed to determine the psychopathology related to the HPA axis. This study highlights the need to build the evidence base for neonatal or maternal interventions to mitigate adverse maternal and infant outcomes. These findings raise awareness about mental health issues during pregnancy and the need for improved prenatal mental health services in public and private healthcare settings. This study underscores the urgency of early interventions to mitigate adverse maternal and infant outcomes.

health and development of the baby, and ambiguity about the life changes associated with motherhood.[3] Stress during pregnancy is harmful to the health of the mother and the fetus. Recent evidence indicates that it may have long-term consequences[4] related to growth retardation, organ maturation, attachment difficulties, infant motor and cognitive development, and decreased intelligence quotient (IQ).[4,5]

Fetal development ensures the optimal health of the child in the future. Mental health status during pregnancy significantly impacts the onset and persistence of depression. Previous studies have shown that elevated psychosocial stress in pregnancy increases the risk of poor birth outcomes[6] and postpartum depressive symptoms.[7] Poor maternal mental health due to depression in pregnancy may lead to lowered fetal growth.[8] Infants born to women suffering from postpartum depressive symptoms are more likely to develop mental health problems later in life.[9]

Several putative mechanisms have been hypothesized to explain elevated cortisol levels during pregnancy on maternal mental health and child development. Cortisol is a hormone released in response to stress.[10] First, elevated cortisol induces vasoconstriction of the uterine artery, reduces the uterine blood flow, and results in poor growth and adverse birth outcomes.[11,12] Second, the cortisol levels are higher in pregnant women than in fetal circulation and are regulated by the hypothalamic-pituitary-adrenal (HPA) axis. When maternal cortisol crosses the maternal–fetal barrier, the placental enzyme 11beta hydroxysteroid dehydrogenase 2 (11β-HSD2) inactivates the cortisol to inert cortisone and minimizes fetal exposure.[13] Hence, the usual hypercortisolemia exposure during pregnancy is not deleterious to the fetus. However, excessive stress-induced cortisol levels to reduce the activity of 11β-HSD2 increases the transplacental passage of active maternal cortisol, leading to premature fetal exposure and a detrimental impact on the fetal tissues that respond to the cortisol.[11,14] Cortisol level during pregnancy is also linked to mental health difficulties in mothers.[7] The sudden decline in the cortisol level due to the disappearance of the placenta after delivery results in the HPA axis dysregulation, reflecting signs and symptoms of postpartum depressive symptoms such as fatigue, hypersomnia, lethargy, and hyperphagia.[15,16]

Studies during pregnancy investigating the effects of the HPA on birth outcomes and maternal mental health status have shown mixed results. Among the few studies that have assessed the relationship between serum cortisol and birth outcomes, most were assessed in a small sample size (between 6 and 98).[17,18] Results of studies exploring a relationship between cortisol and postpartum depressive symptoms were inconclusive and have shown both hypercortisolemia and hypocortisolemia to be associated with postpartum depressive symptoms.[7] The majority of the existing literature on stress was obtained using a questionnaire. Standardized questionnaires, when used alone, have limitations in assessing depression as they only reflect perspectives that change during pregnancy.[19] Hence, they need to be combined with biomarker assessments to identify those at risk of stress. The evidence from lower- and middle-income countries (LMICs) linking cortisol levels during pregnancy to infants' physical growth outcomes is lacking.

We aimed to understand the association of elevated serum cortisol during pregnancy on birth outcomes and postpartum depressive symptoms. We hypothesized that fetal exposure to higher pregnancy cortisol levels is associated with a higher risk of altered growth parameters in infants and a higher risk of developing

postpartum depressive symptoms in mothers assessed after delivery.

Methods

Design

The study participants were nested in the 'Maternal antecedents of adiposity and studying the transgenerational role of hyperglycemia and insulin' (MAASTHI) cohort. The objective of the cohort study was to assess the association between the nutritional and psychosocial environment in pregnancy with adverse neonatal outcomes, including adiposity. The MAASTHI cohort consisted of 4862 pregnant women with a mean age of 24.25 years (SD ± 4.06). A baseline questionnaire assessment was conducted among eligible participants who were >14 weeks of gestation, aged between 18 and 45 years and resided in the study location for five years. The overall study design, methods, and outcomes have been previously published elsewhere.[20] For the present study, a serum cortisol assessment was conducted among a sub-sample of pregnant women who had completed 24 weeks of gestation. The required sample size for the current study is 222 calculated using open-Epi software with 17.5% of low birth weight from the National Family Health Survey estimate for the year 2015–16,[21] at a 95% confidence interval and 5% precision level; a final sample of 230 was recruited.

Data collection

Demographic and obstetric characteristics

At enrollment, trained research staff obtained socio-demographic details, smoking status and alcohol habits, obstetric history, and detailed anthropometric measurements from the participants. Obstetric information on gravida, parity, history of abortion and stillbirth, morbidities during pregnancy, gestational diabetes, and hypertension was obtained from each participant. Additionally, participants' blood hemoglobin and gestational diabetes status were assessed, and blood samples were stored for advanced analysis. We used 230 samples from the biorepository for cortisol estimation from the stored samples. Samples from the biorepository were shipped to a nationally accredited laboratory, certified by the International Organization for Standardization (ISO) and the National Accreditation Board for testing and calibration Laboratories (NABL).

Exposure assessment

The research team informed the pregnant women who completed 24 weeks of gestation before the day of blood collection and advised them to come early in the morning, between 8 am and 9 am. A trained phlebotomist ensured that each pregnant woman rested for at least 30 min prior to blood collection and performed a venipuncture using a vacutainer after locating a healthy vein in the antecubital fossae. The blood was obtained in a single prick on pregnant women who were instructed to rest in bed, and a 6-ml blood sample (single sample) was collected for serum preparation. The vacutainer was wrapped in aluminum foil to prevent sunlight exposure and kept for 45 min for coagulation. It was subsequently centrifuged for 10 min at 1500 rpm/min. The clear supernatant serum was transferred into freeze-resistant black-colored aliquots. The aliquots are placed in cryo boxes which are later stored in a deep freezer (–80 °C) through the step-down procedure. The aliquots containing serum samples were stored at –80 °C at the biorepository maintained at the study site until shipment to a central laboratory for cortisol assays. The subsample of 230 study respondents stored serum samples were selected randomly for serum cortisol estimation. Before processing the samples for laboratory analysis, the samples were kept at –80 °C for 1–2 years. The analytical work was conducted by NABL accredited external laboratory using an electrochemiluminescence immune assay method, a fully automated analyzer determined serum cortisol concentration, reported in μg/L. The lower and upper limits of measurements were 2.16 μg/L and 32.86 μg/L, respectively.

Primary outcome assessment

Primary outcomes.

Birth outcomes:

- Low birth weight (Yes/No); low birth weight was defined as a birth weight less than 2.5 kg.
- Lower weight for length (Yes/No); lower weight for length was defined as WLZ scores below the 10th percentile for gestational weight based on gender.

Infant anthropometric measurements. The trained research staff recorded anthropometric measurements within 72 h after delivery. The child's weight was recorded using SECA 354, and length and the crown-rump length were measured with SECA 417 Infantometer. Chasmors body circumference tape was used to measure circumferences such as chest, waist, hip, and mid-upper arm circumference, and skinfold thickness was measured using Holtain Calliper (Holtain, UK). The research staff was periodically tested and certified for anthropometry assessment from St. John's Research Institute, Bangalore. We measured weight in kilograms, and readings were taken to the nearest 0.5 g, length in centimeters, circumferences in centimeters, and skinfold thickness in millimeters. We obtained three readings for each measurement.

Secondary outcome assessment

1. Infant outcomes. Small for gestational age (SGA) was defined as birth weight below the 10th percentile for gestational age and gender (Yes/No). Lower head circumference (HC) was defined as HC Z-scores below the 10th percentile for gestational age and gender (Yes/

No); Lower skinfold thickness was defined as the sum of skinfold thickness below the 10th percentile (Yes/No) and Crown-rump length (CRL) was defined as CRL below the 10th percentile (Yes/No).

2. Maternal postpartum depressive symptoms. Depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS). EPDS has been extensively used to screen depression during pregnancy and postpartum periods within 72 h of delivery. It uses a 10-item scale, with each item having a score from 0 to 3 and a maximum score of 30. For this study, we considered a score of 0–12 to indicate 'no depressive symptoms and a score of 13 or higher to indicate clinically relevant depressive symptoms.

Covariates

Based on a priori, we included the following covariates: gestational age at testing, obstetric morbidity, age, education, socioeconomic status, gravida, parity, gestational diabetes, and hypertension, body mass index, history of previous abortion, social support, depressive symptoms during pregnancy, gestational age at delivery, type of delivery and gender of the child. Information on these covariates was obtained from participants during enrollment and follow-up assessment. Among those selected covariates were considered confounders. A questionnaire used to assess social support includes emotional, instrumental, informational, and appraisal components.[22] This questionnaire comprised a 12-item scale, with each item having a score from 0 to 3 and a maximum score of 36. A cut-off score of 24 was considered to classify the scores into "good" social support and "low" social support.

Statistical analysis

The null hypothesis tested in our statistical models is that altered growth in infants or postpartum depressive symptoms are not associated with elevated cortisol levels during pregnancy. The data checked for assumptions of regression that include: normality (outcome variables), multicollinearity, outliers, and linear relationship between independent and dependent variables. Serum cortisol is considered an independent variable, whereas infant anthropometric markers and postpartum depressive symptom scores were dependent variables. Independent variables and listed confounders were tested for the assumptions except for normality and proceeded with regression analysis. The distribution of mother and newborn characteristics across the participants was examined using a univariate regression analysis with serum cortisol recorded in terms of mean cut-off. The regression analysis of maternal exposure to serum cortisol on both birth outcomes and maternal postpartum depressive symptoms was tested. Linear regression analysis was performed to examine the independent association between serum cortisol and infant outcomes by initially adjusting for gestational age, infant gender, maternal age, parity, BMI, and gestational age at delivery. To determine the sensitivity of serum cortisol exposure to different threshold levels, the exposure variable was categorized based on percentiles and mean cut-off (mean cortisol = 17.66 μg/L), and the association was estimated using logistic regression analysis. A similar set of analyses was carried out for maternal cortisol levels to evaluate its association with maternal postpartum depressive symptoms, including continuous and categorical serum cortisol variables adjusting for potential confounders. We presented the correlation coefficients, the odds ratio (wherever applicable), the 95% confidence interval, and the p-value. All analyses were performed using the Statistical Package for the Social Science (SPSS) version 23 statistical software.

Ethics approval and consent to participate

The study was reviewed and approved by the institutional ethical review board (IEC) at the Bangalore campus of Indian Institute of Public Health. Only participants willing to participate voluntarily and those who have provided written informed consent are enrolled.

Role of funding source

The funding agency had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Results

The baseline maternal characteristics of the participants are displayed in Table 1. Most of the participants were above 30 years of age (65.7%) and practiced Islam religion (52.6%), followed by participants who identified themselves as Hindus (43.5%). A relative majority had a high school education (42%). Nearly 65% of the participants belonged to an upper-class family; 37.5% reported smoking habits among their spouses. Nearly 18% of them were found to have depressive symptoms during the current pregnancy. The mean gestational age at cortisol assessment was 27.6 weeks (±2.28).

We found a statistically significant difference for the following variables; maternal age, gravida, infant's gender, and infant's weight for length z-score below the 10th percentile in women with higher cortisol levels compared with normal cortisol levels (Table 1).

The results of the linear regression analysis between cortisol exposure and birth outcomes (Table 2) showed no significant association with birth weight ($\beta = 0.015$; p-value > 0.05) or weight for length ($\beta = 0.009$, p-value > 0.05). When cortisol was categorized into low (<25th percentile), moderate (25th–75th percentile), or high (>75th percentile) levels, there was no significant

Variables	Categories	Cortisol levels		p-value
		Low (≤17.66 µg/L) (n = 116)	High (>17.66 µg/L) (n = 114)	
Maternal age (years)	Continuous (mean & SD)	24.93 (±4.47)	23.51 (±3.8)	**0.012**
Maternal age (years)	18–25 (Ref)	11 (4.8)	07 (3)	
	26–30	36 (15.7)	25 (10.8)	0.221
	>30	69 (30)	82 (35.7)	0.08
Gravida	1 (Ref)	36 (15.7)	49 (21.3)	
	2	50 (21.7)	46 (20)	**0.037**
	≥3	30 (13)	19 (8.3)	0.296
Parity	Nullipara (Ref)	41 (17.8)	55 (23.9)	
	Primi	62 (27)	52 (22.6)	0.075
	Multi	13 (5.7)	07 (3)	0.38
BMI	Normal (18.5–22.9) (Ref)	36 (15.7)	41 (17.8)	
	Underweight (<18.5)	05 (2.2)	09 (3.9)	0.223
	Overweight (23–24.9)	19 (8.3)	20 (8.7)	0.162
	Obese (≥25)	56 (24.3)	44 (19.1)	0.44
spousal smoking cigarette	No (Ref)	70 (31.3)	70 (31.3)	0.863
	Yes	41 (18.3)	43 (19.2)	
Spousal alcohol habit	No (Ref)	91 (40.6)	100 (44.6)	0.172
	Yes	20 (8.9)	13 (5.8)	
History of abortion	No (Ref)	90 (39.1)	95 (41.3)	0.273
	Yes	26 (11.3)	19 (8.3)	
GDM	No (Ref)	90 (39.1)	96 (41.7)	0.203
	Yes	26 (11.3)	18 (7.8)	
HYP	No	106 (46.1)	108 (47)	0.322
	Yes	10 (4.3)	6 (2.6)	
Respondent's sum of skin fold	<90th percentile (Ref)	107 (46.5)	100 (43.5)	0.257
	≥90th percentile	9 (3.9)	14 (6.1)	
Depressive symptoms	No (0–12) (Ref)	97 (42.2)	91 (39.6)	0.457
	Yes (≥13)	19 (8.3)	23 (10)	
Social support	≥24 (Ref)	55 (23.9)	53 (23.1)	0.889
	<24	61 (26.5)	61 (26.5)	
GA at delivery	<37 weeks	07 (3)	08 (3.5)	0.763
	≥37 weeks (Ref)	109 (47.4)	106 (46.1)	
Infant characteristics				
Infant gender	Female (Ref)	47 (20.4)	66 (28.7)	
	Male	69 (30)	48 (20.9)	**0.008**
Low birth weight (<2.5 kg)	No (Ref)	76 (33)	88 (38.3)	
	Yes	40 (17.4)	26 (11.3)	**0.05**
SGA	No (Ref)	63 (27.5)	63 (27.5)	
	Yes	53 (23.1)	50 (21.8)	0.826
WLZ (<10th percentile)	No (Ref)	86 (37.6)	96 (41.9)	
	Yes	30 (13.1)	17 (7.4)	**0.043**
HC (<10th percentile)	No (Ref)	102 (44.5)	103 (45)	
	Yes	14 (6.1)	10 (4.4)	0.426
SSF (<10th percentile)	No (Ref)	103 (44.8)	104 (45.2)	
	Yes	13 (5.7)	10 (4.3)	0.539

The bold values are the p-values obtained for the listed variables. Maternal factors such as maternal age, gravida and infant characteristics such as gender, weight for length are the factors significantly differ between the cortisol groups. GA-gestational age, BMI-Body mass index, GDM-pregnancy gestational diabetes mellitus, HYP-pregnancy hypertension, SGA-Small for gestational age, WLZ-weight for length z-score, HC-head circumference, SSF-sum of skinfold thickness.

Table 1: **Maternal and infant characteristics and their association with maternal cortisol levels (n = 230).**

Exposure variable	Birth weight/low birth weight (β/OR (95% CI))		WLZ score/lower weight for length (β/OR (95% CI))	
	Univariate	Adjusted	Univariate	Adjusted
Cortisol (continuous)	0.01 (−0.02, 0.04)	0.01 (−0.01, 0.01)	0.01 (−0.03, 0.04)	0.01 (−0.05, 0.03)
Cortisol (percentiles) Low (<25th percentile) = ref				
Moderate	1.13 (0.42, 3.04)	1.02 (0.37, 2.85)	NA	NA
High	1.32 (0.36, 4.81)	1.48 (0.38, 5.73)	NA	NA
Cortisol (mean cut-off) Low (≤17.66 µg/L) = Ref				
High (>17.66 µg/L)	1.78[a] (0.99, 3.18)	2.28[a] (1.21, 4.32)	1.97[a] (1.01, 3.82)	2.16[a] (1.07,4.35)

β-Coefficient, OR-odds ratio. The adjusted covariates are Gestational age, gender, maternal age, parity, BMI, and gestational age at delivery. [a]Denotes p-value <0.05.

Table 2: **Regression results on the association between maternal cortisol level and birth weight, weight for length at birth (n = 230).**

association with low birth weight or lower weight for length. However, cortisol levels were categorized using a mean cut-off of 17.66 µg/L and after adjusting for confounders, showed a significant association with low birth weight (OR = 2.28; 95% CI 1.21–4.32) and lower weight for length (OR = 2.16; 95% CI 1.07–4.35). Pregnant women with cortisol levels above 17.66 µg/L had twice the odds of delivering a baby with low birth weight and lower weight for length compared to those with cortisol levels below the mean cut-off.

The results of the linear regression analysis between serum cortisol and other infant birth outcomes (Table 3) showed no significant association with birth weight for gestational age score (–0.010, p-value > 0.05), sum of skin fold thickness (–0.01, p-value > 0.05), head circumference (0.006, p-value > 0.05), or crown-rump length (0.031, p-value > 0.05). The categorization of cortisol levels into three groups (low, moderate, and high) or two groups (mean cut-off) also showed no significant association with other infant outcomes, including small for gestational age, lower skinfold thickness, lower head circumference, and lower crown-rump length.

After adjusting for sociodemographic, obstetric, and infant variables, we observed that cortisol levels during pregnancy with a higher mean cut-off of >17.66 µg/L were substantially associated with postpartum depressive symptoms (Table 4). The adjusted model results show a 2.3-fold increased risk of developing postpartum depressive symptoms in pregnant women with higher mean cortisol cut-off levels than women with lower (than mean cut-off) cortisol levels [AOR: 2.33, 95% CI (1.17, 4.64)]. The findings suggest an increased risk of postpartum depressive symptoms among pregnant women with higher serum cortisol after adjusting for a set of covariates.

Discussion

We evaluated the association between maternal cortisol levels in pregnancy with birth outcomes and postpartum depressive symptoms status. Even though our results for the continuous serum cortisol variable were non-significant for all the listed primary and secondary outcome variables, we found that primary outcomes such as low birth weight, lower weight for length, found to be significantly related to higher serum cortisol levels categorized as per the mean cut-off levels and found a significant association between high mean cortisol and maternal postpartum depressive symptoms, suggesting hypercortisolemia leads to postpartum depressive symptoms.

The findings of our study on the effect of cortisol on primary outcomes are consistent with previous findings. The systematic review and meta-analysis findings revealed a negative correlation between maternal salivary cortisol and birth weight.[23] The results from the rural Malawi trial showed a strong association between pregnancy salivary cortisol levels and birth weight.[24] Several other studies have reported similar results.[25,26] Our results differ from the studies conducted in developed countries like Netherlands and California, where maternal cortisol levels in pregnancy had no significant associations with infant birth weight.[27,28]

This association could be a proxy for hormonal impact as inadequate consumption of food, essential fatty acids, or vitamins like folic acid and vitamin B12 could play a potential role in this link. Higher cortisol level during pregnancy is also associated with lower newborn weight for length. This finding is consistent with the results of multiple studies, including the findings from Germany.[18] A study conducted among a cohort of Singaporean pregnant women found an association between depression and low birth weight for length.[29] Our findings differ from the rural Malawi trial, which found no association between cortisol level during pregnancy and size at birth.[24]

There is no clear evidence in the literature from India and other LMICs on stress biomarkers and their impact on birth outcomes. However, studies that used questionnaires to assess mental health status during pregnancy found a substantial impact on small for gestational age.[30] Moreover, there is insufficient

Exposure variable	Birth weight for gestational age/SGA (β/OR (95% CI))		Sum of skinfold thickness/lower skinfold thickness (β/OR (95% CI))		HC score/lower HC (β/OR (95% CI))		CRL/lower CRL (β/OR (95% CI))	
	Univariate	Adjusted	Univariate	Adjusted	Univariate	Adjusted	Univariate	Adjusted
Cortisol (continuous)	−0.01 (−0.02, 0.00)	−0.01 (−0.02, 0.00)	−0.01 (−0.09, 0.06)	−0.01 (−0.09, 0.07)	0.12 (−0.02, 0.05)	0.01 (−0.03, 0.04)	0.006 (−0.08, 0.09)	0.031 (−0.06,0.12)
Cortisol (percentiles)								
Low (<25th percentile) = ref								
Moderate	1.57 (0.63,3.89)	1.48 (0.58,3.75)	1.27 (0.27, 5.83)	1.1 (0.22,5.38)	4.02 (0.91,17.79)	3.65 (0.79,16.87)	2.226 (0.28,17.55)	1.748 (0.20,14.68)
High	1.87 (0.56, 6.21)	1.95 (0.56,6.75)	0.5 (0.04, 5.94)	0.46 (0.03,6.17)	3.93 (0.70,22.15)	3.51 (0.58,21.02)	3.474 (0.33,36.24)	3.861 (0.32,45.43)
Cortisol (mean cut-off)								
Low (≤17.66 µg/L) = Ref								
High (>17.66 µg/L)	1.06 (0.63, 1.78)	1.09 (0.63, 1.89)	1.31 (0.55, 3.12)	1.36 (0.54, 3.54)	1.21 (0.67, 2.19)	1.26 (0.66, 2.40)	2.098 (0.81, 5.40)	2.298 (0.81, 6.46)

β-Coefficient, OR-odds ratio. The adjusted covariates are Gestational age, gender, maternal age, parity, BMI, and gestational age at delivery.

Table 3: **Regression results on the association between cortisol level and small for gestational age (SGA), sum of skinfold thickness, head circumference and crown rump length (n = 230).**

evidence on the effect of higher cortisol exposure during pregnancy on an infant's head circumference, skinfold thickness and crown-rump length. This suggests the need for further research to explore more on this relationship as well as clinical expert advice on the probable associations. The probable reason for the non-significant association with other anthropometric measurements may be that most of the study participants were in their second and third trimesters since we assessed blood cortisol levels in all women who completed 24 weeks of gestation. Researchers have found that elevated stress hormone levels during early pregnancy have more significant effects on offspring than elevated stress hormone levels later in pregnancy.[31]

Our study's strengths include using a prospective study design to assess early morning cortisol during pregnancy. There is reduced variability in the actual cortisol concentration during this time of the day. We standardized the timing of sample collection during the early morning and restricted the participants who arrived late. The limitations of this study include a one-time assessment of cortisol at ≥24 weeks compared to previous studies that have assessed cortisol concentration during different time points of gestation, i.e., in the first, second and third trimesters. The EPDS scale is the only screening tool for identifying women with depressive symptoms. The clinical psychologist or more qualified professionals must make the clinical diagnosis. The postpartum depressive symptoms assessed early after delivery may not be an accurate estimate of postpartum depressive symptoms as the hormonal-emotional adjustments happen during the first seven days after delivery. The study findings cannot be generalized beyond the source population. We recommend further research to be explored in a representative population with frequent cortisol measurements.

This study's findings illustrate higher threshold of maternal serum cortisol assessed during pregnancy is linked to infant growth outcomes (low birth weight and lower weight for length), reflecting an alteration in in-utero programming. Our findings revealed that a threshold serum cortisol level of >17.66 µg/L is linked with low birth weight, lower weight for length z-scores of the infants at birth, and maternal postpartum depressive symptoms. While cortisol levels demonstrate the physiological effects, further research is needed to establish whether the HPA axis, closely linked with psychopathology, has altered in function. Hence, a better understanding of tropic hormones from the hypothalamus and pituitary glands, along with cortisol levels, is warranted. Further studies in this direction can guide neonatal or maternal interventions to mitigate adverse infant and maternal outcomes. These findings could be used to promote awareness of mental health issues and their adverse consequences during pregnancy. They could potentially

Exposure variable	Postpartum depressive symptoms (n = 230)	
	Unadjusted (β estimate/Odds ratio &CI)	Adjusted (β estimate/Odds ratio &CI)
Cortisol (continuous)	−0.18 (−0.53, 0.16)	−0.23 (−0.59, 0.12)
Cortisol (trichotomous) (Cortisol <25th percentile = Ref)		
Medium (25–75th percentile)	0.79 (0.31, 2.01)	0.68 (0.22, 2.09)
High (>75th percentile)	1.14 (0.32, 4.08)	1.36 (0.30, 6.20)
Cortisol (mean cut off) Low (≤17.66 µg/L) = Ref		
High (>17.66 µg/L)	1.53 (0.88, 2.63)	2.33[a] (1.17, 4.64)

The model is adjusted for age, socioeconomic status, maternal education, gravida, H/o abortion, BMI, gestational diabetes, hypertension, depressive symptoms during pregnancy, social support, gestational age at delivery, type of delivery, and the gender of the child. [a]Denotes p-value is <0.05.

Table 4: **Regression analysis for elevated cortisol during pregnancy and postpartum depression (n = 230).**

promote efforts to improve prenatal mental health services in public and private healthcare settings. The findings will also aid in developing evidence-based solutions, such as stress management programs, to reduce maternal stress and enhance birth outcomes. Social support and other stress-reduction educational activities should be explored in places where access to mental health services is limited.

The results of our study support the hypothesis that maternal cortisol level assessed during pregnancy affects fetal birth outcomes and increases the risk of developing postpartum depressive symptoms. These results have significant implications for understanding early developmental psychopathology, which is well-known but poorly understood. More research is required to study different outcomes about possible mechanisms, maternal physiological parameters, and biochemical changes and assess a possible association between stress biomarkers, birth outcomes, and postpartum depressive symptoms. Given the challenges of measuring the HPA axis during pregnancy, we focused on a blood biomarker as a proxy rather than the placenta as a source of potential biomarkers. Future studies should focus on reliable markers such as total, free and salivary cortisol and epigenetic markers such as DNA methylation to assess HPA activity during pregnancy to clinically confirm the association. Risk stratification and further assessments should be done based on the contextual needs of the pregnant women. Our goal is to present the findings to the healthcare community with the hope that mental health status in pregnant women is prioritized, especially in LMICs, where it is neglected.

Contributors

Prafulla Shriyan was involved in the conceptualization, Investigation, analysis, Writing – original draft. Paulomi Shridhar was involved in writing- review & editing. Onno C.P. van Schayck did critical review, editing of the manuscript. Giridhara R. Babu supervised the study, design the methodology and involved in writing, review and editing. All authors have read and approved the manuscripts.

Data sharing statement

The data set used in the current study contains participants identifiable information and thus accessible upon request from corresponding author.

Declaration of interests

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