

NEUROENDOCRINE AND INFLAMMATORY BIOMARKER IN IMMEDIATE POST-PARTUM STATE WITH STILL BIRTH AND ITS COMPARISON WITH LIVE BIRTH AS PREGNANCY OUTCOME

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ABSTRACT

Objectives: Stillbirth remains a significant contributor to global and perinatal mortality, with a rate of 13.9/1,000 total births worldwide and 11.79/1,000 in India. The contributing factors include hypertensive disorders, diabetes, infections, and congenital anomalies. The immediate postpartum period provides an opportunity to evaluate the maternal physiological and biochemical changes that may be linked to stillbirth. Neuroendocrine and inflammatory biomarkers, such as dehydroepiandrosterone sulfate (DHEA-S) and homocysteine (HCY), are essential in understanding the stress and inflammation associated with adverse pregnancy outcomes, including stillbirth. This study aimed to estimate and compare the levels of DHEA-S and HCY in women with stillbirth and live births to explore their potential association with adverse pregnancy outcomes.

Methods: A case-control study was conducted at a tertiary care center involving 60 women aged 20–35 years. The participants were divided into two groups: 30 women with stillbirth and 30 controls with live births. Detailed demographic, obstetric, and clinical data were collected and venous blood samples were analyzed for DHEA-S and HCY using the enzyme-linked immunosorbent assay and immunoturbidimetric methods, respectively. Data analysis was performed using the Statistical Packages for the Social Sciences v23.0, with statistical significance set at $p < 0.05$.

Results: The mean gestational age was significantly lower in the stillbirth group (34.8 ± 4.8 weeks) compared to the controls (38.7 ± 1.0 weeks, $p = 0.01$). The stillbirth cases exhibited significantly higher levels of DHEA-S (2.74 ± 0.48 $\mu\text{g/mL}$) and HCY (12.25 ± 5.27 $\mu\text{mol/L}$) compared to the controls (0.98 ± 0.45 $\mu\text{g/mL}$, $p = 0.01$; 8.64 ± 2.28 $\mu\text{mol/L}$, $p = 0.01$). Abnormal levels of DHEA-S were observed in all stillbirth cases, while only 50% of controls had abnormal levels ($p = 0.01$). Obstetric complications in the stillbirth group included anemia (13.3%), hypothyroidism (6.7%), intrauterine growth restriction (3.3%), oligohydramnios (3.3%), preeclampsia (3.3%), type 2 diabetes with polyhydramnios (3.3%), and breech presentation (3.3%). Significant differences in physical characteristics such as height and body mass index were noted between the groups.

Conclusion: Elevated levels of DHEA-S and HCY in the stillbirth group suggest distinct pathophysiological responses in the postpartum period, which may indicate the adverse pregnancy outcomes. These biomarkers could potentially serve as useful tools for identifying and managing pregnancies at risk for stillbirth and related complications. Further studies are required to validate their role in predicting and improving the pregnancy outcomes.

Keywords: Stillbirth, Dehydroepiandrosterone sulfate, Homocysteine, Biomarkers, Neuroendocrine, Inflammatory markers.

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INTRODUCTION

The immediate postpartum period is a pivotal phase, characterized by significant physiological and emotional shifts, especially following adverse pregnancy outcomes such as stillbirth [1,2]. Neuroendocrine and inflammatory biomarkers are essential for deciphering maternal responses to childbirth, shedding light on the effects of stress and inflammation. Analyzing these biomarkers provides valuable insights into the unique biological processes that differentiate stillbirth from live birth [3-5]. Stillbirths account for a significant proportion of perinatal deaths worldwide. Recent data report a global stillbirth rate of 13.9/1,000 total births, while in India, the health management information system 2021–2022 recorded a stillbirth rate of 11.79/1,000 births [6,7]. This rate serves as an indicator of the quality of antenatal care in a given region [8]. The leading maternal causes of stillbirth include hypertensive disorders, severe anemia, uncontrolled diabetes, and TORCH infections such as toxoplasma, rubella, cytomegalovirus, and herpes simplex virus, as well as chorioamnionitis [9-11]. Placental factors such as abruptio placenta, umbilical cord complications, and vascular malperfusion in both the mother and fetus also contribute significantly. The fetal causes include birth defects, being small for gestational age, and congenital anomalies [12]. Stress hormones such as dehydroepiandrosterone sulfate (DHEA-S) are the critical regulators of

pregnancy and childbirth. They influence maternal and fetal physiology by modulating the hypothalamic-pituitary-adrenal (HPA) axis, a key pathway that governs the body's stress response. Activation of the HPA axis under prenatal stress triggers the release of these hormones, which help adapt to stress and maintain homeostasis. In addition, cortisol and DHEA-S serve as the valuable biomarkers for evaluating HPA axis functionality and predicting stress-related pregnancy outcomes [13]. Maternal plasma homocysteine (HCY) concentration is proposed to be associated with certain pregnancy complications. Alterations in maternal HCY levels cause placental vasculopathy which leads to the various placenta-mediated complications [14]. The present study aimed to estimate and compare the levels of neuroendocrine marker DHEA-S and inflammatory marker HCY in women presenting with stillbirth and those presenting with live birth.

METHODS

Study area and study subjects recruitment

An observational and analytical case-control study was conducted in the Department of Biochemistry in association with the Department of Obstetrics and Gynecology, ESIC Medical College and Hospital, Faridabad. The study was conducted from July 01st, 2023 to November 30th, 2024 after obtaining institutional ethical committee clearance.

The study involved 60 women aged 20–35 years who were in labor or admitted for safe confinement after 20 weeks of gestation. Participants were categorized into two groups: 30 women with singleton pregnancies experiencing stillbirth (cases) and 30 women with live births without obstetric complications (controls).

Table 1: Comparison of age and other demographic details between the groups

Variables	Case		Controls		p-value
	Mean±SD		Mean±SD		
Age	26.10±3.94		25.93±3.49		0.86
Gestational age	34.80±4.80		38.70±1.00		0.01*
Weight in kg	64.73±5.10		64.97±2.99		0.83
Height in cm	163.53±3.01		158.13±2.40		0.02*
BMI in kg/m ²	24.30±2.40		26.01±0.97		0.01*
Pulse rate per min	75.00±6.00		74.00±3.00		0.72
Systolic blood pressure	119.50±11.64		115.93±7.15		0.159
Diastolic blood pressure	79.23±7.13		78.00±5.17		0.466
Respiratory rate per min	15.00±2.00		15.00±1.00		0.609

There was no significant difference in the mean age of participants between the two groups. BMI: Body mass index

Table 2: Comparison of the parity between the groups

Parity	Case		Controls		Chi-square (p-value)
	Count	N %	Count	N %	
Multi para	16	53.3	17	56.7	2.07 (0.355)
Grand multipara	2	6.7	0	0.0	
Primi	12	40.0	13	43.3	

There is no significant difference in the distribution of parity between the groups

Table 3: Comparison of the obstetric details between the groups

Obstetric complications	Case		Controls		Chi-square (p-value)
	Count	n%	Count	n%	
Anemia	4	13.3	0	0.0	-
GDM	0	0.0	0	0.0	
GHTN	0	0.0	0	0.0	
Hypothyroidism	2	6.7	0	0.0	
IUGR	1	3.3	0	0.0	
Low AFI (oligohydramnios)	1	3.3	0	0.0	
Nil	19	63.3	30	100	
Preeclampsia	1	3.3	0	0.0	
Breech presentation	1	3.3	0	0.0	
Type 2 DM, polyhydramnios	1	3.3	0	0.0	
Other details					
Socioeconomic status					
Lower middle	19	63.3	16	53.3	0.61 (0.43)
Upper middle	11	36.7	14	46.7	
Relevant past history					
Abortion	7	23.3	3	10.0	6.38 (0.173)
DM	0	0.0	1	3.3	
Neonatal death	1	3.3	0	0.0	
Nil	20	66.7	26	86.7	
Stillbirth	2	6.7	0	0.0	
Diet history					
Mixed diet	11	36.7	15	50.0	1.08 (0.29)
Vegetarian	19	63.3	15	50.0	
Family history					
Father DM	2	6.7	1	3.3	4.35 (0.36)
Father HTN	1	3.3	0	0.0	
Maternal hypothyroid	1	3.3	0	0.0	
Mother DM	0	0.0	2	6.7	
Nil	26	86.7	27	90.0	

Type 2 DM: Diabetes mellitus, IUGR: Intrauterine growth restriction

Exclusion criteria

Multifetal pregnancy, conceived through assisted reproductive treatment, age above 35 years and age below 20 years, on immunosuppressive treatment, autoimmune diseases.

Sample collection and processing

Informed consent was taken from all the subjects of the study population after which, detailed information regarding different characteristics of study participants such as age, parity, body mass index (BMI), socioeconomic condition, and relevant obstetric history and clinical examination was collected from the patient record file. 6 mL of venous blood was collected from antecubital vein following all aseptic measures (4 mL in yellow capped vacutainers and 2 mL in grey capped vacutainer) as soon as possible within 24 h of delivery. Following that, the clotted and whole blood was separated by centrifuging sample at 3500 rpm for 15 min. The serum samples for carrying out special investigations were stored in deep freezer at -20°C and analyzed later.

The parameters such as serum DHEA-S and HCY levels were analyzed. Enzyme-linked immunosorbent assay (ELISA) and immunoturbidimetric methods, respectively, were utilized for precise quantification.

HCY levels were measured using the semi autoanalyzer Randox Daytona (Randox Laboratories) using the HCY 2 reagent system, which employs a dual-chambered pack with ready-to-use reagents. Oxidized HCY is reduced to its active form by Tris (2-Carboxyethyl) phosphine hydrochloride. The reduced HCY reacts with serine in the presence of cystathionine β-synthase to form L-cystathionine, which is further broken down by cystathionine β-lyase to yield HCY, pyruvate, and ammonia. Pyruvate is then reduced to lactate by lactate dehydrogenase using nicotinamide adenine dinucleotide (NADH) as a coenzyme. The HCY concentration is directly proportional to the conversion of NADH to NAD⁺, measured spectrophotometrically at 340 nm. Calibration with

a standard curve enables the accurate quantification of HCY levels in samples.

For DHEA-S measurement, ELISA method (DHEA-S ELISA KIT (Calbiotech kit) was used, a competitive binding principle was employed using the CBI DHEA-S kit. In this method, DHEA-S in the sample competes with horseradish peroxidase-conjugated DHEA-S for binding to anti-DHEA-S antibodies immobilized on wells coated with Goat-anti-Rabbit-immunoglobulin G. After incubation and washing, tetramethylbenzidine reagent was added to produce a color change, which was stopped, and the absorbance was measured at 450 nm. A standard curve was used to determine DHEA-S concentrations in the test samples.

Statistical analysis

Statistical analysis was conducted using the Statistical Packages for the Social Sciences v23.0 on Windows 10. Data collected in the pro forma were entered into Excel and summarized as mean, standard deviation, frequency, and percentage. Results were presented through tables, figures, bar diagrams, and pie charts. The unpaired t-test was used to compare the mean differences in continuous data, while the Chi-square test analyzed categorical data. p<0.05 was considered statistically significant.

RESULTS

The present study included a total of 60 patients with 30 as cases and 30 as controls.

However, the mean gestational age was significantly lower in the cases compared to the controls (p<0.05). The physical characteristics, including height and BMI, showed significant differences between the groups, with the mean height being significantly greater in the cases and the BMI being significantly lower in the cases compared to the controls.

Table 4: Comparison of the gender of baby between the groups

Sex of baby	Cases		Controls		Chi-square (p value)
	Count	N %	Count	N %	
Female	11	36.7	14	46.7	0.62 (0.43)
Male	19	63.3	16	53.3	

Table 5: Comparison of the biomarkers between the groups

Biomarkers	Case		Controls		p-value
	Mean	SD	Mean	SD	
DHEAS	2.74	0.48	0.98	0.45	0.01*
HCY	12.25	5.27	8.64	2.28	0.01*

There was a significantly higher mean level of DHEAS and homocysteine in the cases compared to the controls (p<0.05). HCY: Homocysteine, DHEA-S: Dehydroepiandrosterone sulfate

Table 6: Comparison of presence of abnormal level of biomarkers between the groups

Biomarkers	Case		Controls		Chi-square (p-value)
	Count	Column N %	Count	Column N %	
DHEAS					
Normal	30	100.0	15	50.0	20.0 (0.01)*
Abnormal	0	0.0	15	50.0	
HCY					
Normal	28	93.3	30	100.0	2.06 (0.15)
Abnormal	2	6.7	0	0.0	

There was a significantly higher incidence of abnormal levels of DHEAS and HCY in the cases compared to the controls (p<0.05). HCY: Homocysteine, DHEA-S: Dehydroepiandrosterone sulfate

There were no significant differences in the demographic characteristics of the participants between the two groups, including socioeconomic status, pertinent medical history, dietary habits, and familial health history. The cases exhibited a range of obstetric complications, including anemia (13.3%), hypothyroidism (6.7%), intrauterine growth restriction (IUGR) (3.3%), oligohydramnios, preeclampsia (3.3%), type 2 diabetes mellitus with polyhydramnios (3.3%), and breech presentation (3.3%).

The distribution of neonatal gender between the groups was comparable.

DISCUSSION

The postpartum period is a crucial time for evaluating biochemical and physiological changes that may signal underlying health issues impacting pregnancy outcomes. Neuroendocrine and inflammatory biomarkers have emerged as the essential tools for understanding adverse events, such as stillbirth. Biomarkers such as DHEA-S offer valuable neuroendocrine insights, while inflammatory markers such as HCY provide a deeper understanding of the role of inflammation in pregnancy complications [3,15]. This study investigated neuroendocrine and inflammatory biomarkers in the immediate postpartum period, comparing outcomes between stillbirths (cases) and live births (controls) in a cohort of 60 patients, with 30 participants in each group.

Similar to the present study, Sundararajan *et al.* documented a comparable mean age of pregnant mothers, with a mean age of 24.43±0.14 years. In addition, the patients were comparable in terms of gestational age, obstetric scores, comorbid conditions, and vital parameters [13]. In the current study, age was comparable between the groups, with no significant differences observed. However, the cases exhibited a significantly lower mean gestational age compared to the controls. Physical characteristics varied, with cases showing a significantly higher mean height but a lower BMI compared to the controls, while mean weight remained consistent across both groups. Vital parameters, including pulse rate, blood pressure, and respiratory rate, showed no significant differences between the groups. Similarly, in the study by Suman *et al.*, comparable demographic details, vital parameters, and obstetric characteristics were documented between the groups. In the present study, it was observed that stillbirth occurred in patients who had obstetric complications, including anemia (13.3% of cases), hypothyroidism (6.7% of cases), IUGR (3.3% of cases), oligohydramnios (3.3% of cases), preeclampsia (3.3% of cases), type-2 diabetes mellitus with polyhydramnios (3.3% of cases), and breech presentation (3.3% of cases). In contrast, 63.3% of stillbirths occurred in patients without any obstetric complications. This further emphasizes the need for biomarkers to identify women at increased risk for stillbirth.

In a study conducted by McClure *et al.* to assess the causes of stillbirth in India, it was concluded that hypertensive diseases, such as preeclampsia, were the leading maternal cause of stillbirth, followed

by severe anemia. The primary fetal causes of stillbirth were identified as fetal malperfusion leading to IUGR and placental abnormalities [16].

In the present study, the mean serum DHEA-S level in the cases was $2.74 \pm 0.48 \mu\text{mol/L}$, whereas the controls showed a mean value of $0.98 \pm 0.45 \mu\text{mol/L}$. This difference was found to be statistically significant. Serum levels of DHEA-S can be used to assess the HPA axis, as these hormones undergo significant changes during pregnancy. Any alterations or imbalances in the levels of these hormones can lead to prenatal stress and adverse pregnancy outcomes, such as stillbirth. In accordance with the present study, Sundararajan *et al.* documented the higher levels of DHEA-S among the cases of stillbirth and abortion compared to normal deliveries. Another prospective study conducted in pregnant women found that circulatory DHEA-S levels were higher in women with adverse birth outcomes than in those with normal birth outcomes. Receiver operating characteristic analysis showed that circulatory DHEA-S levels and the cortisol/DHEA-S ratio had acceptable accuracy for diagnosing stillbirth [13].

Our current study demonstrated a statistically significant higher mean HCY level ($12.25 \pm 5.27 \mu\text{mol/L}$) in the cases compared to the controls ($8.64 \pm 2.28 \mu\text{mol/L}$). Similarly, a study by Suman *et al.* also reported significantly higher mean HCY levels among the cases compared to the controls [17]. These biochemical abnormalities in cases indicate higher levels of metabolic and inflammatory stress compared to the controls.

Limitations

While this study provides valuable insights into the role of neuroendocrine and inflammatory biomarkers in pregnancy outcomes, several limitations need to be acknowledged:

Sample size

The sample size of 60 participants, with only 30 cases and 30 controls, is relatively small. Larger studies with more diverse populations would help to validate these findings and provide more generalizable results.

Single-center design

The study was conducted at a single tertiary care center, which may limit the generalizability of the results to broader populations, particularly in different geographic regions or healthcare settings. Multi-center studies would provide a more comprehensive understanding.

Case Control nature

The study design was observational and case control study, meaning that it can identify associations but not causality. Longitudinal studies are needed to explore the causal relationships and changes in biomarker levels over time, especially in response to interventions or clinical management.

Absence of genetic factors

The study did not assess the genetic factors that could contribute to stillbirth or pregnancy complications. Future research could benefit from including genetic screening to better understand the genetic predispositions to adverse pregnancy outcomes.

Potential confounding factors

While the study accounted for several important variables such as age, BMI, and comorbid conditions, other potential confounders (e.g., lifestyle factors, medication use, or environmental exposures) were not comprehensively controlled. These factors could influence the biomarkers being studied and their relationship with pregnancy outcomes.

Biomarker variability

Biomarkers such as DHEA-S and HCY can be influenced by various factors including time of sample collection, stress, diet, and medication. Variability in these external factors may have affected the measurements, and more controlled conditions could provide more reliable results.

Lack of longitudinal follow-up

This study assessed the biomarkers only during the immediate postpartum period. However, biomarker levels could vary throughout pregnancy and postpartum and longitudinal follow-up of participants would provide a more comprehensive view of how these markers change over time and correlate with pregnancy outcomes.

Exclusion of other pregnancy complications

The study focused on stillbirth and did not include other types of pregnancy complications, such as preterm birth or miscarriage that might also show altered levels of these biomarkers. Including a wider range of complications would help broaden the scope of the findings.

Implications

Despite these limitations, the findings of this study highlight the potential value of DHEA-S and HCY as biomarkers for assessing pregnancy outcomes. Elevated levels of these biomarkers could help identify high-risk pregnancies and allow for earlier interventions, potentially improving maternal and fetal health outcomes. The results suggest that monitoring neuroendocrine and inflammatory markers during pregnancy could become an essential part of prenatal care, particularly for women at risk of stillbirth. Future research with larger, multi-center, and longitudinal studies is needed to further validate these findings and explore the utility of these biomarkers in the clinical practice.

CONCLUSION

The study underscores the significant differences in physical characteristics, neuroendocrine markers (DHEA-S), and inflammatory markers (HCY) between stillbirth and live birth outcomes, suggesting distinct postpartum pathophysiological responses associated with stillbirth. Elevated levels of both DHEA-S and HCY were notably higher in the stillbirth cases compared to the controls. These findings highlight the potential role of these biomarkers as the valuable indicators for understanding and managing adverse pregnancy outcomes. The identification of an effective predictive biomarker could enable the early detection of high-risk pregnancies, facilitating enhanced surveillance, comprehensive antenatal care, and timely deliveries, ultimately improving the pregnancy outcomes.

AUTHOR'S CONTRIBUTION

Kavya Sasank and Harnam Kaur were responsible for data analysis, draft preparation, plagiarism removal, and proofreading of the manuscript. S.B. Sharma and J.C. Sharma contributed to data collection, compilation, and statistical analysis, as well as conducting the literature review for the study. Each author played a crucial role in ensuring the quality and integrity of the research and manuscript.

CONFLICT OF INTEREST

None.

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