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ORIGINAL RESEARCH

Erythrocyte Folate and Homocysteine in Hypertensive Disorders of Pregnancy: Associations with Pregnancy Outcomes

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Background: Hypertensive disorders complicating pregnancy (HDCP) are a major cause of maternal and fetal morbidity. This study aims to examine the relationship between red blood cell folate (RBCF) and homocysteine (Hcy) levels and HDCP severity, as well as to assess their predictive value for pregnancy outcomes.

Methods: This retrospective study included 326 HDCP patients and 153 controls. The diagnostic criteria for HDCP were based on the American College of Obstetricians and Gynecologists. RBCF and Hcy levels were measured at diagnosis using automated immunoassays. ROC curve analysis was performed to assess the predictive value of RBCF and Hcy for poor pregnancy outcomes.

Results: A total of 326 women with HDCP and 153 normotensive controls were included. Blood pressure was significantly higher in the HDCP group (both $p < 0.001$). HDCP patients exhibited markedly lower RBC folate (286.08 ± 59.04 vs 334.83 ± 51.95 ng/mL, $p < 0.001$) and higher Hcy levels (8.41 ± 1.39 vs 7.58 ± 1.40 μmol/L, $p < 0.001$) than controls. Across HDCP severity, RBCF declined progressively from gestational hypertension to mild and severe preeclampsia, while Hcy increased correspondingly. Among HDCP patients, adverse pregnancy outcomes occurred in 44.5%, including prematurity (34.4%), low birth weight (19.3%), and fetal distress (12.9%). Compared with women with good outcomes, those with poor outcomes had significantly lower RBCF (255.61 ± 51.56 vs 310.45 ± 53.13 ng/mL, $p < 0.001$) and higher Hcy (9.04 ± 1.36 vs 7.90 ± 1.25 μmol/L, $p < 0.001$). ROC analysis showed that combined RBCF–Hcy testing provided the highest predictive performance for adverse outcomes (AUC = 0.85, 95% CI 0.81–0.89; sensitivity 85.52%; specificity 75.14%), outperforming either biomarker alone.

Conclusion: RBCF and Hcy are crucial biomarkers for assessing the severity of HDCP and predicting adverse pregnancy outcomes.

Keywords: hypertensive disorders complicating pregnancy, red blood cell folate, homocysteine, pregnancy outcomes, predictive biomarkers

Introduction

Hypertensive disorders complicating pregnancy (HDCP), including gestational hypertension and preeclampsia, affect approximately 10% of pregnancies and remain a leading cause of maternal and perinatal morbidity worldwide.[1,2] This condition is characterized by the clinical features of edema, hypertension, and proteinuria, with severe cases potentially leading to headaches, visual disturbances, and even seizures or coma.[3] HDCP is a leading cause of maternal and perinatal mortality, often leading to multi-organ dysfunction such as pulmonary edema, placental abruption, liver and renal failure, disseminated intravascular coagulation, and stroke.[4] Despite extensive research, the underlying mechanisms of HDCP are not fully understood, and current clinical management primarily relies on monitoring blood pressure and determining the optimal timing of delivery. These limitations underscore the need for early biomarkers that can identify women at elevated risk of disease progression and adverse pregnancy outcomes.[5]

A growing body of evidence suggests that disruptions in one-carbon metabolism, particularly involving folate and homocysteine (Hcy), may contribute to endothelial dysfunction, oxidative stress, and abnormal placental vascular remodeling, all of which play pivotal roles in the pathogenesis of HDCP.[6–8] Folate deficiency reduces methylation

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capacity and promotes Hcy accumulation, while elevated Hcy impairs nitric oxide bioavailability and endothelial function.[9,10] These mechanistic insights support the hypothesis that folate–Hcy imbalance may precede clinical deterioration and could therefore serve as a metabolic signature of early disease risk.

Evidence suggests that MTHFR gene polymorphisms, such as rs3753584, independently influence RBC folate concentrations and are strongly associated with Hcy metabolism.[11] Studies have demonstrated a negative correlation between folate and Hcy levels in HDCP patients. Folate supplementation effectively reduces Hcy levels.[4] Furthermore, elevated Hcy levels are an independent risk factor for HDCP and are associated with greater disease severity.[3,12] It is important to follow the recommended dosage of folate supplementation, as excessive intake may increase the risk of hypertensive disorders and insulin resistance during pregnancy.[13,14]

Although previous studies have reported associations between folate status, Hcy levels, and HDCP, the evidence supporting their predictive value remains limited.[15] Most existing studies examined these biomarkers separately, used small or heterogeneous cohorts,[16] or lacked stratification by disease severity[17] making it difficult to determine whether folate- and Hcy-related metabolic changes can be translated into reliable clinical predictors. In addition, few studies have investigated how genetic factors, such as MTHFR polymorphisms, interact with metabolic biomarkers to influence HDCP risk, leaving important mechanistic questions unresolved. Moreover, prior work has rarely evaluated the combined predictive utility of RBCF and Hcy or systematically examined biomarker gradients across HDCP severity categories,[18,19] representing a meaningful gap in the current literature.

In China, women of childbearing age are advised to begin daily supplementation with 400 μg of folic acid three months before conception and continue throughout pregnancy.[20] Studies have shown that appropriate supplementation ensures most pregnant women reach RBC folate levels sufficient for preventing neural tube defects.[21,22] However, the predictive value of folate metabolic markers (RBC folate and Hcy) for HDCP severity and pregnancy outcomes still requires further high-quality evidence.[23] Understanding these associations is particularly important in settings with standardized folate intake, where metabolic variability is more likely to reflect underlying physiological differences rather than dietary disparity.

This study was conducted in a Chinese population, where nationwide folic-acid supplementation policies provide a consistent baseline for folate intake.[24] In addition, the prevalence of MTHFR C677T polymorphisms is particularly high among Chinese women, leading to greater susceptibility to elevated Hcy levels.[25,26] These genetic and policy-related characteristics make this cohort uniquely suitable for investigating how folate–Hcy metabolism relates to HDCP severity and adverse pregnancy outcomes. Taken together, this context offers an opportunity to clarify unresolved questions regarding the metabolic, genetic, and clinical determinants of HDCP risk.

This retrospective study analyzed the relationship between serum RBCF and Hcy levels and the severity of HDCP at diagnosis in pregnant women at our hospital. It also evaluated the ability of RBCF and Hcy to predict pregnancy outcomes in HDCP patients.

Materials and Methods

Study Design

This retrospective study was conducted between June 2022 and December 2024. Data collection proceeded in predefined phases. From June 2022 to October 2023, patient screening, enrollment, clinical registration, and baseline blood sampling, including red blood cell folate and homocysteine measurements, were completed. Longitudinal follow-up was performed through December 2024 to monitor pregnancy progression, document clinical manifestations, classify HDCP severity, and ascertain pregnancy outcomes. Data cleaning, verification, and statistical analyses were carried out from January to June 2025. All clinical and laboratory data were extracted from the hospital's electronic medical record system, ensuring standardized documentation. Cases with incomplete clinical records or missing biomarker data (RBCF or Hcy) were excluded from the analysis; therefore, a complete-case approach was used. This study was approved by the Ethics Committee of Zibo Central Hospital. Written informed consent was obtained from all participants prior to enrollment.

Diagnostic Criteria for HDCP

The study was approved by Zibo Central Hospital, and informed written consent was obtained from the participants. The diagnostic criteria for HDCP were based on the 2013 guidelines of the American College of Obstetricians and Gynecologists (ACOG).[27] Preeclampsia was diagnosed in patients after 20 weeks of gestation with a history of normal blood pressure, based on the following criteria: (1) Hypertension, defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg, measured at least twice with a minimum of 4 hours apart; (2) Proteinuria, defined as 24-hour urinary protein ≥300 mg or a urine protein-to-creatinine ratio ≥0.3. In the absence of proteinuria, the diagnosis could still be confirmed with one or more of the following features: thrombocytopenia (platelets < 100,000/μL), liver dysfunction (serum transaminases > 2 times the normal limit or persistent right upper quadrant pain), renal impairment (serum creatinine >1.1 mg/dL or a twofold increase without pre-existing kidney disease), pulmonary edema, or neurological/visual disturbances.

Severe preeclampsia was diagnosed when any of the following criteria were present in patients with established preeclampsia: (1) Severe hypertension, defined as SBP ≥160 mmHg or DBP ≥110 mmHg, confirmed twice 4 hours apart with the patient resting and no prior antihypertensive therapy; (2) Thrombocytopenia (platelets <100,000/μL); (3) Liver dysfunction (serum transaminases >2 times the normal limit or persistent right upper quadrant pain); (4) Progressive renal dysfunction (serum creatinine >1.1 mg/dL or doubled without underlying kidney disease); (5) Pulmonary edema; (6) New-onset neurological symptoms, including seizures or visual disturbances.

Inclusion Criteria

Pregnant women diagnosed with HDCP were enrolled if they met all of the following conditions: (1) age greater than 18 years; (2) a confirmed singleton pregnancy; (3) diagnosis of HDCP based on established clinical criteria; (4) completion of clinical registration and blood sampling at the Obstetrics Department of our institution; and (5) documented intake of 400 μg/day of oral folic acid during both the first and third trimesters. All participants provided written informed consent before enrollment.

Pregnant women without HDCP were enrolled as the control group if they met the following conditions: (1) age greater than 18 years; (2) a confirmed singleton pregnancy; (3) absence of any clinical diagnosis of HDCP; (4) completion of clinical registration and blood sampling at the Obstetrics Department of our institution; and (5) documented intake of 400 μg/day of oral folic acid during both the first and third trimesters. Written informed consent was obtained from all participants.

Exclusion Criteria

Women were excluded from both groups if they met any of the following criteria: (1) failure to take folic acid (≥ 400 μg/d) during both the first and third trimesters; (2) a history of hypertension before pregnancy; (3) multiple gestation; (4) gestational diabetes; (5) cerebrovascular disease; (6) severe hepatic or renal insufficiency with acute or chronic damage; (7) active infection; (8) or autoimmune diseases, such as rheumatoid arthritis or other systemic inflammatory conditions.

Confounding Control Strategy

We implemented systematic measures to minimize potential confounding by maternal age, pre-pregnancy BMI, and folic acid intake. Confounding was first reduced through strict enrollment criteria and stratified baseline matching. Maternal age was categorized into <28, 28–35, and >35 years, and pre-pregnancy BMI into <18.5, 18.5–24, 24–28, and ≥28 kg/m^2. Case distribution across these strata was balanced between the HDCP group (n = 326) and the control group (n = 153). Additional sources of confounding were minimized by excluding women with pre-existing hypertension, multiple gestation, gestational diabetes, severe hepatic or renal dysfunction, or autoimmune disease, as these conditions may influence vascular or metabolic status and thus affect RBCF and Hcy levels. Although socioeconomic status, detailed dietary folate intake, and parity may also influence folate–Hcy metabolism, these variables were not available for all participants and therefore were not included in the matching process. Their potential influence cannot be fully excluded and is acknowledged as residual confounding.

RBC Folate Measurement

The timing of biomarker sampling was tightly standardized to the clinical diagnosis of HDCP and gestational age to ensure that measured RBCF and Hcy levels accurately reflected the metabolic state at disease onset. For women in the HDCP group, blood samples were obtained within 48 hours of confirmed diagnosis, with all diagnoses made at ≥20 gestational weeks. Gestational age at sampling was matched between the HDCP and control groups, minimizing potential bias related to physiological changes across gestation. RBC folate levels were measured using an Abbott fully automated immunoassay system (model 12000SR) with the Abbott Red Blood Cell Folate Assay Kit.

Homocysteine Measurement

Hcy levels were measured using a fully automated biochemical analyzer (Hitachi LABOSPECT 008AS).

Follow-Up of HDCP Patients

HDCP patients were followed up and classified into Good Outcomes and Poor Outcomes groups based on pregnancy outcomes. Pregnancy outcomes were classified using internationally recognized perinatal criteria. Favorable outcomes included: term delivery (≥37 weeks), absence of HDCP-related complications (placental abruption, pulmonary edema, postpartum hemorrhage), normalization of blood pressure within 72 hours postpartum, neonatal birth weight ≥2500 g, Apgar score ≥8, and no fetal distress or congenital anomalies.

Adverse outcomes included all complications, such as prematurity, low birth weight, fetal distress, neonatal asphyxia, placental abruption, postpartum hemorrhage, neonatal death.

Study Sample Size

The sample size was estimated using G*Power 3.1 based on the primary endpoint examining the association between RBCF levels and the occurrence and adverse outcomes of HDCP. An effect size of Cohen's $d = 0.53$, derived from prior literature and preliminary data from our team, was used for the calculation. With a two-tailed α of 0.05 and a statistical power $(1–\beta)$ of 0.80, the minimum required sample size was determined to be ≥270 participants in the HDCP group and ≥135 participants in the control group to detect significant differences in RBCF and Hcy levels. The final study population exceeded these thresholds, with 326 HDCP patients and 153 controls, ensuring adequate statistical power for the analyses.

Statistical Analysis

The data are presented as mean ± standard deviation (SD) or n (%). Normality of continuous variables was assessed using the Shapiro–Wilk test. For normally distributed variables with homogeneous variances, unpaired t-tests were used; Welch's correction was applied when variances were unequal. For non-normally distributed data, the Mann–Whitney *U*-test was selected. For comparisons across more than two groups, the Brown–Forsythe ANOVA was used due to unequal variances, followed by Games–Howell post-hoc tests. Categorical variables were analyzed using Fisher's exact test. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Analysis of Demographic and Clinical Characteristics

A total of 326 women with HDCP and 153 normotensive controls were included (Table 1). No significant differences were observed in maternal age, gestational age at enrollment, pre-pregnancy BMI, or parity between the two groups. However, significant differences in blood pressure were observed, with the HDCP group demonstrating higher SBP (150.23 ± 13.54 vs 113.87 ± 8.56 mmHg, $p < 0.001$) and DBP (101.87 ± 10.32 vs 78.23 ± 6.47 mmHg, $p < 0.001$) compared to the control group. Among the 326 HDCP patients, 161 were diagnosed with gestational hypertension, 98 with mild preeclampsia, and 67 with severe preeclampsia.

Table 1 Demographic and Clinical Characteristics of Pregnant Women with Hypertensive Disorders Complicating Pregnancy (HDCP) or Normal Pregnancy (Control)

Characteristics	Control (n = 153)	HDCP (n = 326)	p value
Maternal age (years)			
< 28	43 (28.1%)	92 (28.2%)	0.733
28-35	91 (59.5%)	185 (56.7%)	
> 35	19 (12.4%)	49 (15.1%)	
BMI before pregnancy (kg/m^2)			
< 18.5	12 (7.8%)	35 (10.7%)	0.403
18.5-24	81 (52.9%)	184 (56.5%)	
24-28	46 (30.1%)	76 (23.3%)	
≥ 28	14 (9.2%)	31 (9.5%)	
Gestational weeks	32.08 ± 2.86	32.34 ± 2.73	0.481
SBP (mmHg)	113.87 ± 8.56	150.23 ± 13.54	< 0.001
DBP (mmHg)	78.23 ± 6.47	101.87 ± 10.32	< 0.001
Parity, %			
Nulliparous	99 (64.7%)	220 (67.5%)	0.604
Multiparous	54 (35.3%)	106 (32.5%)	
Clinical classification			
GH	–	161 (49.4%)	–
mPE		98 (30.1%)	
sPE		67 (20.5%)	

Notes: The data are presented as mean ± SD or n (%). The comparisons of data between the two groups were done by Mann–Whitney test, Unpaired *t* test with Welch's correction or Fisher's exact test.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GH, Gestational hypertension; mPE, mild preeclampsia; sPE, severe preeclampsia.

Comparisons of RBCF and Serum Hcy at Diagnosis

The RBCF and serum Hcy levels at diagnosis were compared between 326 pregnant women with HDCP and 153 women with normal pregnancies. RBCF levels were significantly lower in the HDCP group compared to controls (Figure 1A, Control: 334.83 ± 51.95 ng/mL, HDCP: 286.08 ± 59.04 ng/mL, $p < 0.001$, F* (DFn, Dfd) = 1.292 (325, 152)), while Hcy levels were significantly higher in the HDCP group (Figure 1B, Control: 7.58 ± 1.40 μmol/L, HDCP: 8.41 ± 1.39 μmol/L, $p < 0.001$, F* (DFn, Dfd) = 1.016 (325, 152)). Additionally, within the HDCP group, RBCF levels decreased progressively with disease severity, with lower levels observed in gestational hypertension (GH, 303.16 ± 57.19 ng/mL), mild preeclampsia (mPE, 281.92 ± 53.92 ng/mL), and severe preeclampsia (sPE, 251.08 ± 54.61 ng/mL) (Figure 1C, $p = 0.009$ between GH and mPE, $p = 0.001$ between mPE and sPE, F* (DFn, DFd) = 21.61 (2.000, 253.4)). In contrast, Hcy levels increased with the severity of the disease (Figure 1D, $p = 0.018$ between GH, 8.03 ± 1.34 μmol/L and mPE, 8.52 ± 1.39 μmol/L, $p = 0.009$ between mPE and sPE, 9.16 ± 1.31 μmol/L, F* (DFn, DFd) = 17.01 (2.000, 251.4)).

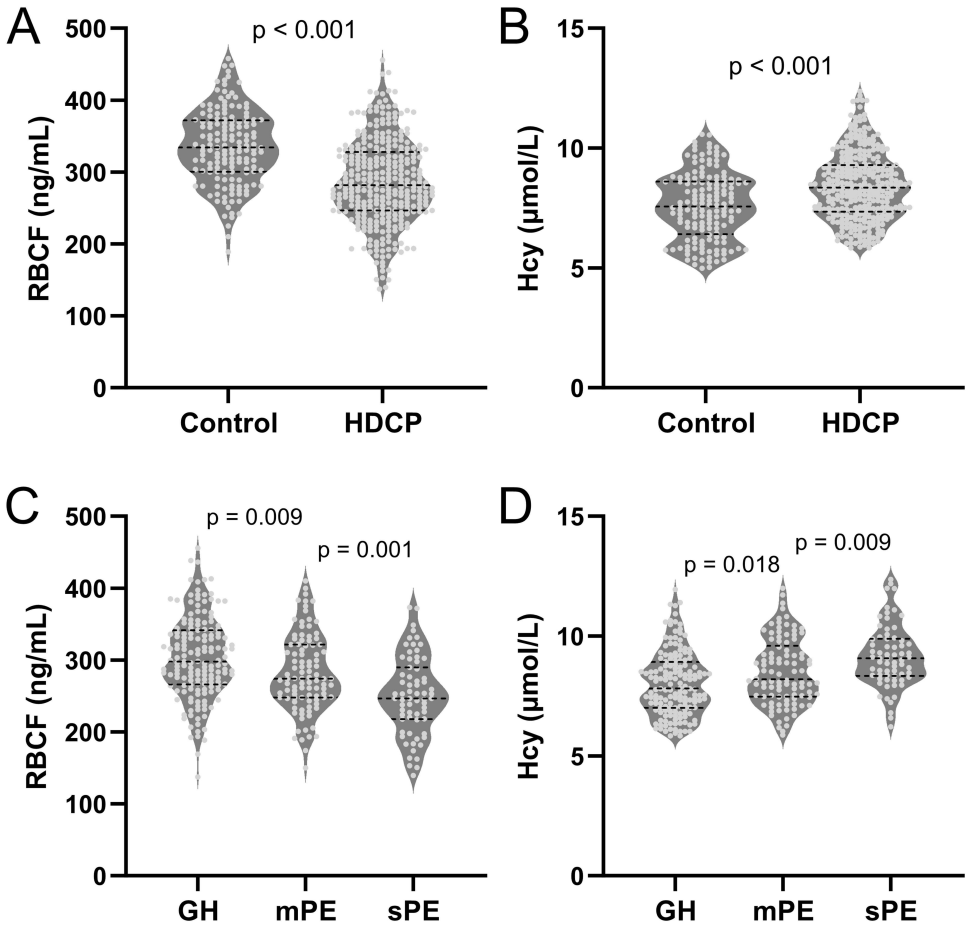

Figure 1 Comparisons of RBCF (**A**) and serum homocysteine (Hcy, **B**) at diagnosis between pregnant women with HDCP (n = 326) and normal pregnancy (Control n = 153). The data are presented as a Volin plot. P-values were calculated from an Unpaired *t*-test with Welch's correction. Comparisons of RBCF (**C**) and Hcy (**D**) at diagnosis among gestational hypertension (GH, n = 161), mild preeclampsia (mPE, n = 98), and severe preeclampsia (sPE, n = 67). The data are presented as a Volin plot. P-values were calculated from the Brown-Forsythe ANOVA test followed by a Games-Howell's multiple comparisons test.

Adverse Pregnancy Outcomes in Women with HDCP

Subsequently, to evaluate the impact of HDCP on pregnancy outcomes, the adverse outcomes in 326 women with HDCP were summarized in Table 2. A total of 145 cases (44.5%) of adverse outcomes were observed (Table 2). Prematurity

Table 2 Pregnant Outcomes of Pregnant Women with Hypertensive Disorders Complicating Pregnancy (HDCP)

	HDCP (n = 326)
Prematurity	112 (34.4%)
Low birth weight	63 (19.3%)
Neonatal asphyxia	28 (8.6%)
Fetal distress	42 (12.9%)
Neonatal mortality	3 (0.9%)
Placental abruption	17 (5.2%)

(*Continued*)

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Table 2 (Continued).

	HDCP (n = 326)
Premature rupture of membranes	21 (6.4%)
Post-partum hemorrhage	34 (10.4%)
Total poor outcomes	145 (44.5%)

Note: The data are presented as n (percentage).

occurred in 34.4% (n = 112), low birth weight in 19.3% (n = 63), and neonatal asphyxia in 8.6% (n = 28) (Table 2). Fetal distress was observed in 12.9% (n = 42), neonatal mortality in 0.9% (n = 3), and placental abruption in 5.2% (n = 17) (Table 2). Premature rupture of membranes was reported in 6.4% (n = 21), and postpartum hemorrhage in 10.4% (n = 34) (Table 2).

Clinical Characteristics and Pregnancy Outcomes in Women with HDCP

To investigate the factors influencing pregnancy outcomes in HDCP, we compared the demographic and clinical characteristics between 181 women with favorable outcomes and 145 women with poor outcomes. Significant differences were identified in SBP, DBP, and the severity of HDCP. The poor outcome group had significantly higher SBP (154.36 ± 14.18 mmHg vs 147.12 ± 12.08 mmHg, $p < 0.001$) and DBP (105.61 ± 9.92 mmHg vs 98.86 ± 0.65 mmHg, $p < 0.001$) (Table 3). A greater proportion of sPE was observed in the poor outcome group (32.4% vs 11.1%, $p < 0.001$), while GH was more prevalent in the favorable

Table 3 Demographic and Clinical Characteristics Between Good Pregnant Outcomes and Poor Pregnant Outcomes in Pregnant Women with Hypertensive Disorders Complicating Pregnancy (HDCP)

Characteristics	Good (n = 181)	Poor (n = 145)	p value
Maternal age (years)			
< 28	55 (30.4%)	37 (25.5%)	0.138
28-35	105 (58.0%)	80 (55.2%)	
> 35	21 (11.6%)	28 (19.3%)	
BMI before pregnancy (kg/m^2)			
< 18.5	19 (10.5%)	16 (11.0%)	0.173
18.5-24	111 (61.3%)	73 (50.3%)	
24-28	38 (21.0%)	38 (26.2%)	
≥ 28	13 (7.2%)	18 (12.4%)	
SBP (mmHg)	147.12 ± 12.08	154.36 ± 14.18	< 0.001
DBP (mmHg)	98.86 ± 0.65	105.61 ± 9.92	< 0.001
Parity, %			
Nulliparous	123 (67.9%)	97 (66.9%)	0.905
Multiparous	58 (32.1%)	48 (33.1%)	

(*Continued*)

Table 3 (Continued).

Characteristics	Good (n = 181)	Poor (n = 145)	p value
Clinical classification			
GH	94 (51.9%)	67 (46.2%)	< 0.001
mPE	67 (37.0%)	31 (21.4%)	
sPE	20 (11.1%)	47 (32.4%)	

Notes: The data are presented as mean ± SD or n (%). The comparisons of data between the two groups were done by Mann–Whitney test, Unpaired *t* test with Welch's correction or Fisher's exact test.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GH, Gestational hypertension; mPE, mild preeclampsia; sPE, severe preeclampsia.

outcome group (51.9% vs 46.2%, p < 0.001) (Table 3). No significant differences were found between the groups in terms of maternal age, pre-pregnancy BMI, parity, or mPE.

Association of RBCF and Serum Hcy Levels with Pregnancy Outcomes in HDCP

To investigate the relationship between RBCF and serum Hcy levels and pregnancy outcomes, we compared these biomarkers at diagnosis between 181 women with good pregnancy outcomes and 145 with poor pregnancy outcomes in HDCP. The results revealed that women with poor pregnancy outcomes had significantly lower RBCF levels (Good: 310.45 ± 53.13 ng/mL, Poor: 255.61 ± 51.56 ng/mL) and significantly higher Hcy levels (Good: 7.90 ± 1.25 μmol/L, Poor: 9.04 ± 1.36 μmol/L) at diagnosis compared to those with good outcomes (p < 0.001, Figure 2A and B).

Predictive Value of RBCF and Hcy for Pregnancy Outcomes in HDCP

To assess the predictive value of RBCF, Hcy, and their combined testing for poor pregnancy outcomes in women with HDCP, ROC curve analysis was conducted, as shown in Figure 3. Both RBCF and Hcy demonstrated significant predictive value for adverse outcomes. The area under the curve (AUC) for RBCF was 0.77 (95% CI: 0.72 to 0.82, p < 0.001), with a sensitivity of 80% and specificity of 63.54%. For Hcy, the AUC was 0.74 (95% CI: 0.68 to 0.79, p < 0.001), with a sensitivity of 69.66% and specificity of 70.72% (Table 4). When RBCF and Hcy were combined, the AUC increased to 0.85 (95% CI: 0.81 to 0.89, p < 0.001), with a sensitivity of 85.52% and specificity of 75.14% (Table 4).

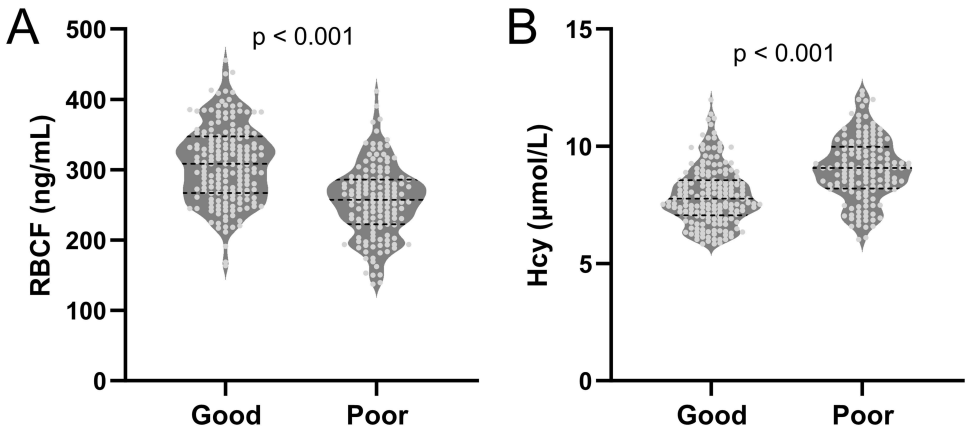

Figure 2 Comparisons of RBCF (**A**) and serum homocysteine (Hcy, **B**) at diagnosis between good pregnant outcomes (n = 181) and poor pregnant outcomes (n = 145) in pregnant women with HDCP. The data are presented as a Volin plot. P-values were calculated from an Unpaired *t*-test with Welch's correction.

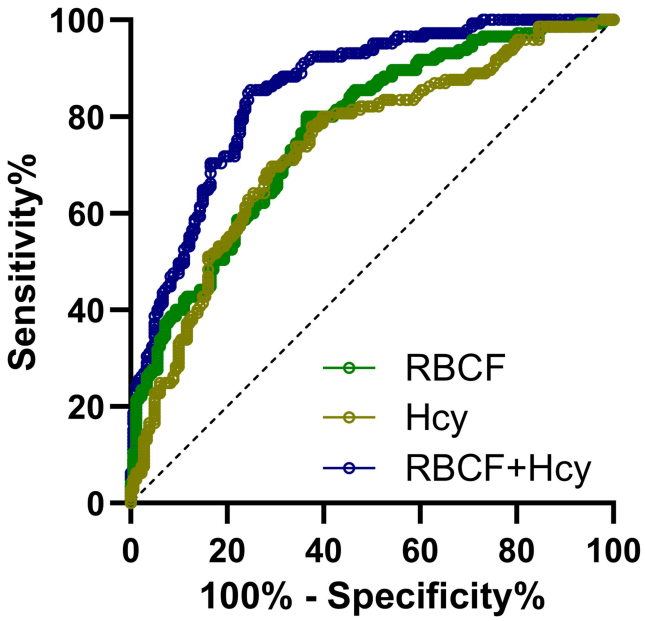

Figure 3 ROC analysis of the predictive values of RBCF, homocysteine (Hcy), and their combined test for poor outcomes in pregnant women with HDCP.

Discussion

HDCP remains a significant cause of maternal and fetal morbidity and mortality, with increasing blood pressure being a hallmark of the condition. In this study, we aimed to explore the relationship between RBCF and Hcy levels and HDCP severity, as well as their potential for predicting adverse pregnancy outcomes. Our results revealed that RBCF levels were significantly reduced, whereas Hcy levels were elevated in women with HDCP compared with normotensive controls, with a consistent gradient across disease severity. These findings suggest that RBCF and Hcy may serve as clinically informative biomarkers for early identification and risk stratification in HDCP.

Hypertension in HDCP is known to induce systemic vascular dysfunction and placental hypoperfusion, ultimately increasing maternal and neonatal complications.[3,4] Previous studies have reported similar metabolic abnormalities in folate and Hcy pathways among patients with preeclampsia.[28–31] Folate deficiency, by impairing one-carbon metabolism and methylation, may compromise placental development, while elevated Hcy promotes oxidative stress and endothelial injury.[32–35] Our results extend these mechanistic observations by demonstrating that the metabolic gradient in RBCF–Hcy levels parallels clinical progression from gestational hypertension to mild and severe preeclampsia, supporting their potential use as biomarkers reflecting disease trajectory.

We observed that HDCP was associated with substantial adverse pregnancy outcomes, including prematurity, fetal growth restriction, and neonatal asphyxia. Although these results were expected, our study adds quantitative evidence linking low RBCF and high Hcy at diagnosis with an increased likelihood of poor pregnancy outcomes, suggesting that

Table 4 ROC Analysis of the Predictive Values of Red Blood Cell Folate (RBCF), Homocysteine (Hcy) at Diagnosis and Their Combined Test for Poor Outcomes in Pregnant Women with Hypertensive Disorders Complicating Pregnancy (HDCP)

	AUC (95% CI)	p value	Cut Off	Sensitivity	Specificity	Youden Index
RBCF	0.77 (0.72 to 0.82)	< 0.001	290.8	80.00%	63.54%	0.44
Hcy	0.74 (0.68 to 0.79)	< 0.001	8.43	69.66%	70.72%	0.40
RBCF+Hcy	0.85 (0.81 to 0.89)	< 0.001	–	85.52%	75.14%	0.61

Note: Logit (RBCF+Hcy) = −0.02 × RBCF + 0.674 × Hcy.
Abbreviation: CI, confidence interval.

metabolic dysregulation may precede clinical deterioration. These associations are consistent with prior findings on placental vascular impairment in folate deficiency and Hcy excess.[4,30]

The combined assessment of RBCF and Hcy demonstrated superior predictive accuracy for adverse outcomes compared with either biomarker alone. This supports the hypothesis that folate deficiency and hyperhomocysteinemia act through partially independent yet complementary pathways, and their joint evaluation provides a more comprehensive picture of metabolic and vascular risk in HDCP. This pattern aligns with multicenter studies advocating combined biomarker testing to improve prediction of HDCP severity and complications.[4,36] Furthermore, RBCF—which reflects long-term folate status—outperformed serum folate in predictive value, consistent with WHO recommendations for using RBCF as a more stable indicator of folate sufficiency.

Importantly, our findings underscore the relevance of investigating a Chinese population, where nationwide periconceptional folic acid supplementation and the high prevalence of the MTHFR C677T polymorphism create a distinct metabolic context that may influence folate–Hcy dynamics. Within this setting, our study offers new evidence by demonstrating a clear severity-dependent gradient in RBCF and Hcy across HDCP subtypes, showing that the combined assessment of these biomarkers provides superior predictive performance compared with individual markers, and generating data from a cohort with uniformly regulated folic acid intake, thereby minimizing nutritional variability and strengthening the validity of the observed associations.

Folate and homocysteine levels are influenced by multiple external and biological factors. Variations in dietary folate intake can affect both RBC folate reserves and circulating Hcy concentrations.[37,38] Genetic factors, particularly the MTHFR C677T polymorphism that is common in East Asian populations, may further impair folate metabolism and increase Hcy levels, thereby altering susceptibility to HDCP.[39] Differences in folic acid supplementation adherence add additional variability to folate–Hcy status.[40] Although our strict inclusion/exclusion criteria and baseline stratified matching minimized several confounders, residual confounding due to unmeasured dietary patterns, genetic heterogeneity, or adherence issues cannot be completely excluded. Future studies incorporating detailed nutritional assessment, objective monitoring of supplement adherence, and genotyping will help clarify these influences.

RBCF and Hcy testing also have potential clinical utility. Both assays are inexpensive, widely available, and require no specialized equipment, making them practical for large-scale risk assessment in routine prenatal care. In contrast to angiogenic markers such as sFlt-1/PlGF, which require specialized platforms, folate–Hcy testing may be especially valuable in low-resource settings.[41] Because these biomarkers reflect modifiable metabolic pathways, they could guide early dietary or metabolic interventions, offering an advantage over non-modifiable clinical risk factors. Although direct evidence for cost-effectiveness in HDCP screening remains limited, related interventions in other populations suggest favorable cost profiles.[42]

This study has several limitations. The retrospective design limits causal inference and introduces potential selection bias. The single-center setting may restrict generalizability, and unmeasured factors such as detailed dietary intake, genetic variation, and long-term folate adherence were not captured. Additionally, RBCF and Hcy were measured only once at diagnosis; future longitudinal studies should examine dynamic changes across gestation. Larger, multiethnic, prospective cohorts are needed to validate the predictive utility of these biomarkers and determine optimal screening thresholds.

Conclusion

In conclusion, this study shows that lower RBC folate and higher homocysteine levels are associated with greater HDCP severity and an increased risk of adverse pregnancy outcomes. The combined assessment of RBCF and Hcy demonstrated better predictive performance than either marker alone, suggesting that these biomarkers may serve as useful tools for early risk stratification in HDCP. However, these findings should be interpreted in light of the study's retrospective, single-center design and the possibility of unmeasured confounding factors. Future multicenter, prospective studies with more comprehensive nutritional and genetic assessments are needed to validate these associations and further clarify the clinical utility of combined RBCF–Hcy testing in HDCP management.

Data Sharing Statement

Data are available on reasonable request to the corresponding author.

Ethical Approval

The study was approved by Zibo Central Hospital, and informed written consent was obtained from the participants. The study was performed in strict accordance with the Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects.

Informed Consent

All patients signed the informed consent.

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Disclosure

The authors report no conflicts of interest in this work.

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