


# Maternal and Postnatal Umbilical Cord Blood Alpha-1-Acid Glycoprotein and Protein-Bound Hexose Levels as Inflammatory Biomarkers in Preeclampsia: A Case-Control Study

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Received: 23 August 2025

Revised: 17 October 2025

Accepted: 11 November 2025

## What's Known

- Preeclampsia is a multisystem disorder linked to maternal and postnatal morbidity and mortality. It is associated with systemic inflammation, endothelial dysfunction, and oxidative stress.
- In preeclampsia, exaggerated inflammation damages endothelial cells. Alpha-1-acid glycoprotein and protein-bound hexoses increase, reflecting immune dysregulation and altered glycosylation during pregnancy and inflammatory disease states.

## What's New

- This study investigated  $\alpha$ 1-AGP and PBH levels in maternal and postnatal umbilical cord blood of normotensive and preeclamptic women.
- Unlike previous studies assessing these two levels separately, our findings reveal their association in both compartments, offering new insights into the combined inflammatory role, potentially shedding light on novel diagnostic or predictive biomarkers.

## Abstract

**Background:** Preeclampsia (PE) is a multisystem disorder of unknown cause, leading to maternal and fetal morbidity and mortality. This study aimed to measure the inflammatory biomarkers alpha-1-acid glycoprotein ( $\alpha$ 1-AGP) and protein-bound hexose (PBH) in the maternal circulation of women with PE and normotensive pregnant women, as well as in postnatal umbilical cord blood, to compare levels between groups and assess their association with PE.

**Methods:** A hospital-based case-control study was conducted from June 2024 to July 2025 at the Maternity Teaching Hospital in Erbil, Kurdistan Region, Iraq, including 104 preeclamptic and 99 normotensive pregnant women. Maternal and postnatal umbilical cord  $\alpha$ 1-AGP levels were measured using a BiotecSunlong ELISA kit (cut-off: 71.3 and 50.64 ng/mL, respectively), while PBH was measured using the orcinol colorimetric method (cut-off: 111 and 67.36 mg/dL, respectively).

**Results:** Maternal  $\alpha$ 1-AGP and PBH levels were significantly higher in preeclamptic women ( $P < 0.001$ ). In postnatal umbilical cord blood,  $\alpha$ 1-AGP was elevated in PE, while PBH showed no significant difference. Maternal and postnatal  $\alpha$ 1-AGP demonstrated a moderate positive correlation ( $\rho = 0.522$ ,  $P < 0.001$ ), whereas PBH showed a weak correlation ( $\rho = 0.242$ ,  $P = 0.016$ ). Receiver Operating Characteristic (ROC) analysis indicated excellent discrimination between groups, with an Area Under the Curve (AUC) of 1.00 for maternal  $\alpha$ 1-AGP and 0.964 for maternal PBH.

**Conclusion:** Maternal  $\alpha$ 1-AGP and PBH are significantly elevated in preeclampsia, with  $\alpha$ 1-AGP also higher in postnatal umbilical cord blood. The high AUC supports their relevance as biomarkers of maternal and postnatal inflammation in PE.

Please cite this article as: Hussein J, Ahmed S, Alalaf S. Maternal and Postnatal Umbilical Cord Blood Alpha-1-Acid Glycoprotein and Protein-Bound Hexose Levels as Inflammatory Biomarkers in Preeclampsia: A Case-Control Study. Iran J Med Sci. doi: 10.30476/ijms.2025.108410.4331.

**Keywords** • Acute-phase proteins • Glycoprotein • Orosomucoid • Pregnancy complications

## Introduction

Preeclampsia (PE), a disorder involving multiple organ systems, occurs in roughly 2–8% of pregnancies, typically emerging after the midpoint of gestation. It is recognized as a serious global

health threat, related to severe maternal and fetal complications.<sup>1</sup> Despite a comprehensive understanding of the clinical presentation and diagnostic criteria, the precise underlying pathogenesis of this condition remains largely undefined. Therefore, there is currently limited progress in developing effective screening and preventive strategies for this condition.<sup>2, 3</sup> PE frequently originates from a poorly implanted placenta experiencing hypoxia, which subsequently triggers an immune response. This cascade releases inflammatory mediators that severely damage the vascular endothelium. The resultant widespread endothelial dysfunction manifests clinically as hypertension, proteinuria, and oedema. At sites of vascular injury, immune-related cells become activated and accumulate, with increased systemic levels of cytokines and adhesion molecules. Specifically, immune-related cytokines, including interleukin-10 (IL-10) and interleukin-6 (IL-6), stimulate hepatic production of high-sensitivity C-reactive protein (hs-CRP), contributing to the systemic immune response and maternal endothelial activation characteristic of PE.<sup>4</sup> Moreover, these cytokines promote hepatic synthesis of other acute-phase reactants, such as alpha-1-acid glycoprotein ( $\alpha$ 1-AGP).<sup>5</sup> Previous studies have reported that PE is related to altered placental glycosylation patterns, including increased carbonylation of key receptors, such as the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R).<sup>6-8</sup>

While numerous prior investigations have evaluated  $\alpha$ 1-AGP levels in the maternal serum of women with PE, a notable research void exists concerning the direct investigation or comparison of this marker's levels in umbilical cord blood within the context of PE.<sup>9-11</sup> This observational deficit similarly extends to protein-bound hexose (PBH), the second biomarker of interest. To our current knowledge, no studies have previously quantified  $\alpha$ 1-AGP or PBH levels in the serum of postnatal umbilical cord affected by PE.<sup>12</sup> Furthermore, existing studies that do measure these biomarkers often focus solely on maternal serum or incorporate them into broader multi-marker panels, without specifically isolating the individual contributions of  $\alpha$ 1-AGP or PBH in the postnatal umbilical cord circulation. This lack of focused investigation highlights a significant gap in understanding the precise role of these proteins and their potential involvement in the pathophysiology of PE. In contrast, previous research has successfully measured both  $\alpha$ 1-AGP and PBH levels in

normotensive pregnant women, with postnatal umbilical cord analyses consistently utilizing an umbilical cord blood sample.<sup>12-14</sup>

This study aims to evaluate and compare  $\alpha$ 1-AGP and PBH levels in maternal blood and postnatal umbilical cord blood samples collected from both preeclamptic women and normotensive pregnant women.

## Patients and Methods

### Study Design and Setting

This hospital-based case-control study was conducted at the high-risk ward, labor ward, and operating theatre of Maternity Teaching Hospital in Erbil City, Kurdistan Region, Iraq. Participant recruitment and data collection were carried out from June 2024 to July 2025. Maternity Teaching Hospital in Erbil serves as the primary public maternity hospital and a major tertiary care and referral center for obstetrics and gynecology in the Erbil Governorate. This facility operates continuously, providing comprehensive maternal and neonatal care, including emergency services, vaginal and Caesarean section deliveries, and specialized interventions, handling approximately 13000 deliveries annually.

### Study Population and Sampling

**Participants and Recruitment:** A total of 203 pregnant women presenting for delivery were enrolled in the study using a non-probability Convenience Sampling technique. Participants were recruited based on their availability and eligibility at the time of admission. They were subsequently categorized into two distinct groups based on the presence or absence of PE.

**Inclusion Criteria:** Participants in the study had to be pregnant women aged 18 years or older who delivered at  $\geq 26$  weeks of gestation, irrespective of delivery mode (vaginal or Caesarean section) or parity. Furthermore, all included women needed to have their pre-pregnancy body weight recorded, possess documentation of a first-trimester ultrasound, and provide voluntary informed consent before enrollment.

**Exclusion Criteria:** Women were excluded from the study if they presented with any pre-existing chronic conditions known to affect inflammatory markers, including diabetes mellitus, chronic hypertension, kidney disease, autoimmune diseases, or active infections. Additionally, women not scheduled for delivery (e.g., emergency or unscheduled cases where timely consent or assessment was not possible) were excluded.

### *Case and Control Definitions*

**Preeclamptic Women (Cases):** This group consisted of 104 pregnant women diagnosed with PE according to the American College of Obstetricians and Gynecologists (ACOG) guidelines<sup>2</sup> as new-onset hypertension after 20 weeks of gestation, with a systolic blood pressure (SBP)  $\geq 140$  mm Hg or  $\geq 160$  mm Hg, or a diastolic blood pressure (DBP)  $\geq 90$  mm Hg or  $\geq 110$  mm Hg, measured on two separate occasions at least 4 hours apart, accompanied by proteinuria—either  $\geq 300$  mg in a 24-hour urine collection or  $\geq 1+$  on a urine dipstick. In cases without proteinuria, the diagnosis of PE required evidence of maternal organ dysfunction, including thrombocytopenia, renal impairment (serum creatinine  $>1.1$  mg/dL or a doubling from baseline), increased liver aminotransferase enzymes ( $\geq 2$ ×the reference range), pulmonary oedema, and new-onset cerebral or visual disturbances.<sup>2</sup>

**Normotensive Pregnant Women (Controls):** This group, designated as normotensive pregnant women, included 99 participants who did not meet the diagnostic criteria for PE and maintained normal blood pressure throughout their entire pregnancy.

### *Sample Size Estimation*

The required sample size was calculated using A Priori Power Analysis via G\*Power software, aligning with the primary study objective of comparing two independent means (biomarker levels in preeclamptic versus normotensive women). Based on a pooled standard deviation ( $\alpha$ ) of 23.47 mg/dL derived from preliminary unpublished pilot data from 20 participant (10 pregnant women with PE and 10 pregnant normotensive women), and setting the significance level ( $\alpha$ ) at 0.05 (two-sided) and the desired statistical power ( $1-\beta$ ) at 80%, the calculation indicated that a sample of 100 participants per group was required to reliably detect a mean difference of 8.96 mg/dL or greater in Maternal Protein-Bound Hexose (PBH) levels. The final enrolled cohort of 203 participants (104 preeclamptic and 99 normotensive women) comfortably exceeded this minimum requirement, thereby providing sufficient statistical power to detect the hypothesized effect size.

### *Data Collection and Sample Processing*

**Data Collection:** Data collection commenced upon participant recruitment through direct interviews using a custom-designed checklist. This checklist was developed specifically for this study to consistently capture relevant maternal

and fetal clinical and laboratory information. Although it is not a standardized or previously validated questionnaire, the checklist was reviewed by the research team to ensure clarity and completeness of the collected data. This encompassed basic characteristics and clinical information, including maternal age and parity, which was defined by pregnancies reaching 24 weeks' gestation and more. Parity was categorized as primiparous (Para 1), multiparous (Para 2-4), and grand multiparous (Para  $\geq 5$ ). Gestational age at delivery was determined from the first-trimester ultrasound performed between 11 and 14 weeks. Blood pressure measurements (systolic and diastolic) were taken to diagnose PE. All participants, from both normotensive pregnant women and preeclamptic women groups, were followed until delivery, and their mode of delivery (vaginal or cesarean section) was recorded. Postnatal umbilical cord blood samples were subsequently collected for biomarker analysis.

**Sample Processing:** Prior to blood collection, participants were identified, and the procedure was briefly explained to ensure their comfort and cooperation. Blood was drawn from a peripheral vein (antecubital fossa) using standard venipuncture technique into Vacutainer serum tubes. Approximately 5 mL of maternal venous blood was collected from each participant upon admission to the labor ward, within 24 hours of diagnosis (for both cases and controls), and before the delivery of the postnatal umbilical cord.

Five mL of venous blood was collected using a sterile technique and transferred into plain Vacutainer serum tubes. The samples were allowed to clot at room temperature (25 °C), after which the serum was separated by centrifugation using a ROTOFIX 32 A Centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 2000–3000 rpm for 10–15 min. Only clear, non-hemolyzed serum was then aliquoted into labeled Eppendorf tubes and stored at  $-20$  °C until biochemical analysis.

### *Chemicals and Reagents*

Analytical-grade chemicals and reagents were obtained from the following sources: mannose, galactose, ethanol, and sodium hydroxide (NaOH) were supplied by Fluka (U.K.); concentrated sulfuric acid ( $H_2SO_4$ ) was purchased from Hopkin & Williams Ltd. (U.K.); orcinol was obtained from Biochem Chemopharma (product code: 518190010, Cosne-sur-Loire, France). All chemicals were of analytical grade and used without further purification.

*Biomarker Analysis***Measuring  $\alpha$ 1-AGP Levels and Establishing a Cut-off for Preeclamptic Women and Postnatal Umbilical Cord:**

$\alpha$ 1AGP concentrations were measured using the BiotecSunlong ELISA kit (Catalogue Number: SL1845Hu, BiotecSunlong, China) following the manufacturer's guidelines. Absorbance was read using a BioTek ELx800 Microplate Reader (BioTek Instruments, ELx800, Winooski, VT, USA), with all samples measured in duplicate. Due to differences in measurement methods and units used to determine the  $\alpha$ 1-AGP biomarker in preeclamptic women and postnatal umbilical cord blood in previous studies, we established a cut-off value of 71.3 ng/mL based on the mean serum  $\alpha$ 1-AGP level in normotensive pregnant women. Levels exceeding this threshold were observed in preeclamptic women, suggesting a potential increase in this biomarker in this group. Similarly, the mean  $\alpha$ 1-AGP concentration of 50.64 ng/mL, measured in postnatal umbilical cord blood from normotensive pregnant women, was used as a surrogate normal value, with any  $\alpha$ 1-AGP level above this considered elevated.

**Measuring Protein-Bound Hexose and Establishing a Cut-off for Preeclamptic Women and Postnatal Umbilical Cord:**

PBH levels were determined using the orcinol colorimetric method. First, protein-carbohydrate conjugates were precipitated from samples using 99% ethanol at 25 °C. These samples were then hydrolyzed to release oligosaccharides. Next, hexose was converted to furfural by heating in an alkaline solution for 45 min. The resulting chromogen, formed with orcinol, was measured spectrophotometrically (EMCLAB, Germany) at 520 nm, as previously described.<sup>15</sup> Given that some studies have determined PBH levels in maternal and postnatal umbilical-cord blood, particularly in the context of PE. As demonstrated by Horowitz,<sup>12</sup> the PBH biomarker was measured in normal maternal and postnatal umbilical-cord blood according to the method described by Winzler.<sup>16</sup> In contrast, our study measured PBH using the method described by,<sup>15</sup> and therefore, the reference range for PBH was determined based on our own control group. From these samples, a cut-off value of 111 mg/dL was established, with levels above this threshold observed in preeclamptic women. Similarly, the mean PBH concentration of 67.36 mg/dL in postnatal umbilical-cord blood from normotensive pregnant women was used as a surrogate normal value, and values exceeding this level were considered elevated in preeclamptic cases.

*Ethical Approval*

This study was approved by the Ethics Committee, College of Medicine, Hawler Medical University (Ref. No. 6/2, May 27, 2024). Written informed consent was obtained from all participants before enrolment. Confidentiality and anonymity were maintained throughout the study. The study was conducted in accordance with the Declaration of Helsinki.

*Statistical Analysis*

Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, New York, USA). The normality of the data was tested with the Shapiro-Wilk test; accordingly, non-parametric tests were used when appropriate. The chi-square test of association was employed to compare proportions. Fisher's exact test was used when the expected count in more than 20% of the table cells was less than 5. The Mann-Whitney test was applied to compare the mean ranks of two groups. The Spearman ( $\rho$ ) correlation coefficient was calculated to assess the strength of the correlation between two numerical variables. ROC (Receiver-Operator-Characteristic) curve analysis was performed to determine a biomarker cutoff value, above which the test can be considered positive. Youden's index (sensitivity+specificity-1) was calculated. The highest value of this index indicates the optimal balance of sensitivity and specificity. The McNemar test was used to show whether there is a significant difference between the results of the biomarkers and PE. A P value of less than 0.05 was considered statistically significant.

**Results**

The demographic characteristics of the study participants are summarized in table 1. More than one-third of women in the PE group were aged  $\geq$ 35 years (35.6%), compared with (21.2%) in the normotensive group ( $P=0.073$ ). All normotensive pregnant women delivered at  $\geq$ 37 weeks of gestation, whereas only (51.9%) of the preeclamptic group reached term gestation ( $P<0.001$ ). Primiparity was observed in (40.4%) of the normotensive group and (31.7%) of the preeclamptic group ( $P=0.350$ ). All women in both groups conceived spontaneously. Overall, the groups were comparable in parity and demonstrated no statistically significant difference in age, but differed significantly in gestational age at delivery.

Preeclamptic women exhibited higher mean biomarker levels than normotensive pregnant women. For Postnatal umbilical cord biomarkers,

**Table 1: Basic characteristics and obstetric history of Normotensive pregnant women and Preeclamptic women groups**

		Normotensive pregnant women	Preeclamptic women	Total	P value
		n (%)	n (%)	n (%)	
Age (years)	<20	8 (8.1)	8 (7.7)	16 (7.9)	0.073*
	20-34	70 (70.7)	59 (56.7)	129 (63.5)	
	≥35	21 (21.2)	37 (35.6)	58 (28.6)	
Gestational age	<37	0 (0.0)	50 (48.1)	50 (24.6)	<0.001*
	≥37	99 (100.0)	54 (51.9)	153 (75.4)	
Mean±SD		39.01±0.91	36.23±3.23		<0.001†
Parity	Primiparous	40 (40.4)	33 (31.7)	73 (36.0)	0.350*
	Multiparous	34 (34.3)	45 (43.3)	79 (38.9)	
	Grand multiparous	25 (25.3)	26 (25.0)	51 (25.1)	
Total		99 (100.0)	104 (100.0)	203 (100.0)	

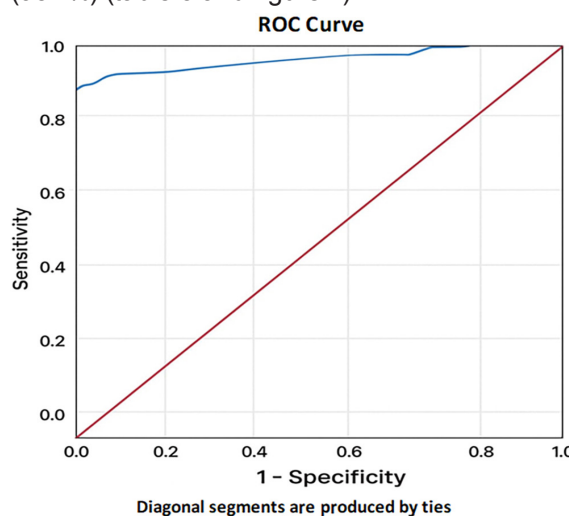
\*Calculated by Chi square test; †Calculated by Mann-Whitney test; P<0.05 is considered significant.

the mean α1-AGP was significantly elevated in the preeclamptic pregnant women group compared to the normotensive pregnant women group (P<0.001). However, no significant difference was observed in the mean postnatal umbilical cord PBH between these two groups (P=0.807; table 2).

The findings indicated that α1-AGP levels were significantly higher in preeclamptic women than in normotensive pregnant women, with an area under the curve (AUC) of 100% (P<0.001) at a cut-off of 71.3 ng/mL. Accuracy indicators, including sensitivity, specificity, positive predictive value, negative predictive value, and total agreement, were all 100% (table 3).

Mothers' PBH levels were significantly elevated in preeclamptic women compared to normotensive pregnant women, with an AUC of 96.4% at a cut-off value of 111 mg/dL. At this cut-off, the accuracy indicators of mothers' PBH for identifying elevated levels were as follows: sensitivity (90.4%), specificity (100%),

PV+ (100%), PV- (90.8%), and total agreement (95.1%) (table 3 and figure 1).



**Figure 1:** ROC curve analysis of mothers' protein-bound hexose (PBH) levels in preeclamptic women versus normotensive pregnant women. ROC curve analysis of mothers' protein-bound hexose (PBH) levels in preeclamptic women.

**Table 2: Biomarker characteristics of study participants**

Biomarkers		Normotensive pregnant women		Preeclamptic women		P value*
		Mean±SD	Median (95% CI)	Mean±SD	Median (95% CI)	
Mothers	α <sub>1</sub> -AGP (ng/mL)	47.6±13.2	50.0 (45.0-50.3)	102.7±32.4	89.8 (95.0-107.8)	<0.001
	PBH (mg/dL)	98.5±5.2	100.0 (97.6-99.6)	136.9±23.4	131.0 (132.3-141.9)	<0.001
PUC	α <sub>1</sub> -AGP (ng/mL)	50.6±13.9	54.2 (47.9-53.4)	97.8±28.3	90.0 (92.2-103.5)	<0.001
	PBH (mg/dL)	67.3±16.7	60.0 (64.0-70.7)	67.5±16.3	63.3 (64.2-70.8)	0.807

\*Calculated by the Mann-Whitney test. α<sub>1</sub>-AGP (α<sub>1</sub>-Acid glycoprotein); PBH: Protein-bound hexose; PUC: Postnatal Umbilical Cord; P<0.05 is considered significant.

**Table 3: Accuracy of maternal α1-acid glycoprotein and protein-bound hexose in differentiating preeclampsia from normotensive pregnancy based on ROC analysis**

Test variable	AUC	95% CI	P value	Sen. (%)	Sp. (%)	PPV (%)	NPV (%)	Accuracy (%)	P value*
Maternal α <sub>1</sub> -AGP (ng/mL)†	1.000	1.000–1.000	<0.001	100	100	100	100	100	1.000
Maternal PBH (mg/dL)††	0.964	0.937–0.990	<0.001	90.4	100	100	90.8	95.1	0.002

\*Calculated by McNemar test (comparing the test variable results with preeclampsia). †A positive test indicates values ≥111 mg/dL, and a negative test indicates values <111 mg/dL. ††A test is considered positive when it is ≥71.3 ng/mL, and considered negative when it is <71.3 ng/mL. AUC: Area Under the Curve; CI: Confidence interval; Sen: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; α<sub>1</sub>-AGP: α<sub>1</sub>-Acid glycoprotein; PBH: Protein-bound hexose; P<0.05 is considered significant.

**Table 4:** Correlations between the mothers' and postnatal umbilical cord biomarkers

Mothers' biomarkers	PUC biomarkers	$\rho$	Sample	P value
Mothers' $\alpha$ <sub>1</sub> -AGP (ng/mL)	PUC $\alpha$ 1- AGP (ng/mL)	0.522	Preeclamptic women	<0.001
Mothers' PBH (mg/dL)	PUC PBH (mg/dL)	0.242	Preeclamptic women	0.016
Mothers' $\alpha$ <sub>1</sub> -AGP (ng/mL)	PUC $\alpha$ 1- AGP (ng/mL)	0.173	Normotensive pregnant women	0.087
Mothers' PBH (mg/dL)	PUC PBH (mg/dL)	0.296	Normotensive pregnant women	0.003

$\alpha$ <sub>1</sub>-AGP:  $\alpha$ <sub>1</sub>-Acid glycoprotein; PBH: Protein-bound hexose; PUC: Postnatal umbilical cord; P<0.05 is considered significant.

When only the preeclamptic women's data were analyzed, the correlation between mothers'  $\alpha$ 1-AGP and postnatal umbilical cord  $\alpha$ 1-AGP was positive, of medium strength, and significant ( $\rho=0.522$ ,  $P<0.001$ ), while the correlation between mothers' and postnatal umbilical cord PBH was weak significant correlation ( $\rho=0.242$ ;  $P=0.016$ ). When the normotensive pregnant women were considered, there was a weak, significant positive correlation between mothers' and postnatal umbilical cord PBH ( $\rho=0.296$ ;  $P=0.003$ ), but the correlation between mothers' and postnatal umbilical cord I  $\alpha$ <sub>1</sub>-AGP was not significant ( $P=0.087$ ) (table 4).

## Discussion

In our study, all women in the normotensive pregnant women group had a gestational age of  $\geq 37$  weeks, whereas only 51.9% of women in the preeclamptic women group reached this gestational age ( $P<0.001$ ). This significant difference reflects the clinical reality that PE often necessitates early delivery to avoid maternal and fetal complications. Therefore, this disparity does not indicate a gestational age mismatch between the two groups but rather highlights the disease's effect on gestational duration.<sup>17</sup>

The results indicated that women diagnosed with PE exhibited significantly higher  $\alpha$ 1-AGP levels, with a calculated cut-off value of 71.3 ng/mL, compared to the normotensive pregnant women group. These results demonstrate that  $\alpha$ 1-AGP serves as a biomarker of inflammation.  $\alpha$ 1-AGP is a circulating protein produced by the liver and other tissues in response to inflammatory stimuli. It is classified as an acute-phase protein and functions as an immunomodulatory molecule, regulating the immune system's response.<sup>18, 19</sup>

Elevated levels of  $\alpha$ 1-AGP in the non-pregnant female reproductive system often signal an underlying inflammatory state. These increased levels can be related to various conditions, including chronic inflammatory disorders such as polycystic ovary syndrome (PCOS) and systemic lupus erythematosus.<sup>20</sup> They may also indicate more acute or serious systemic illnesses, such as infections, sepsis, and various types of cancer. Furthermore,

elevated  $\alpha$ 1-AGP levels can serve as a marker of autoimmune diseases and endothelial dysfunction, reflecting a compromised state of the vascular endothelium.<sup>21</sup>

However, normal pregnancy does not significantly alter  $\alpha$ 1-AGP levels in the plasma, according to research. Based on the study by Chu and colleagues,<sup>22</sup>  $\alpha$ 1-AGP levels in pregnant women with normal pregnancies remain stable and comparable to those of healthy, non-pregnant women. This evidence suggests that pregnancy itself does not alter baseline  $\alpha$ 1-AGP levels. However, the study also found that in a small group of pregnant women experiencing acute inflammation,  $\alpha$ 1-AGP levels were significantly elevated. Therefore, an elevated  $\alpha$ 1-AGP level in a pregnant patient is a strong indicator of an underlying inflammatory condition, rather than a byproduct of the pregnancy itself.<sup>21</sup> The levels of  $\alpha$ 1-AGP can potentially increase with complications during pregnancy, such as PE or gestational diabetes.<sup>23</sup>

The pathophysiology of PE, a high-risk pregnancy complication, has been an actual challenge for the maternal-fetal line.<sup>24</sup> The etiology of the condition can be attributed to an excessive maternal systemic inflammatory response during pregnancy, which is initiated by the activation of both innate and adaptive immune systems.<sup>25</sup>

Postnatal umbilical cord  $\alpha$ 1-AGP levels were significantly elevated in newborns of preeclamptic women compared with those of the normotensive pregnant group ( $P<0.001$ ); the mean postnatal umbilical cord  $\alpha$ 1-AGP level in the normotensive group was  $50.6 \pm 13.9$  ng/mL. This finding is consistent with previous research characterizing PE as a state of heightened inflammation affecting both the mother and the postnatal umbilical cord, as reported by Catarino and colleagues, who also observed similar inflammatory elevations in preeclamptic pregnancies, directly supporting this concept, demonstrating that various acute-phase reactants, which are indicative of a systemic inflammatory response, are significantly elevated in the umbilical cord blood of infants born to preeclamptic mothers.  $\alpha$ 1-AGP, being an established acute-phase protein, aligns with their conclusion that the fetus is not merely

a passive recipient but actively participates in an inflammatory response to the maternal condition.<sup>26</sup>

The present study shows significantly elevated levels of PBH in the preeclamptic women group compared to the normotensive pregnant women group, using a cut-off value of 111 mg/dL. In a healthy individual, the body keeps the concentration of serum glycoproteins, including PBH, within a very specific and narrow range.<sup>27</sup> Elevated levels of PBH are often indicative of an underlying inflammatory or pathological process. While this information is not specific to any gender, it is particularly relevant in several conditions affecting women's health. For instance, abnormal cell growth in ovarian cancer can alter protein glycosylation, leading to higher PBH levels.<sup>28</sup> Similarly, the systemic inflammation linked to conditions such as severe depression and anxiety can lead to an increase in glycoprotein production, which results in elevated PBH concentrations. Therefore, PBH can serve as a broad indicator of these pathological changes, offering a valuable, albeit non-specific, insight into various disease states.<sup>29, 30</sup>

Our ROC analysis showed AUC for  $\alpha$ 1-AGP and PBH, demonstrating their significant elevation in preeclamptic women compared to normotensive pregnancies. High sensitivity and specificity further reflect the significant increase in these biomarker levels among preeclamptic women. While no previous studies have investigated the use of these biomarkers specifically for PE, the precise functions of  $\alpha$ 1-AGP, particularly in relation to pregnancy, are not yet fully understood. Despite this,  $\alpha$ 1-AGP constitutes an important component of normal plasma, accounting for approximately 1–3% of total plasma proteins, and its concentration can increase up to tenfold during inflammatory states.<sup>13</sup> In addition to maternal inflammatory markers, placental factors such as soluble fms-like tyrosine kinase-1 (sFlt1) have recently been proposed as potential circulating endothelial-damaging factors in PE. According to Staff and colleagues' trophoblast-derived sFlt1 has little or no transfer into the fetal circulation, supporting the view that elevated maternal sFlt1 levels in PE are predominantly of placental origin.<sup>31</sup> While sFlt1 transfer across the placental barrier appears negligible, overexpression of trophoblast glycoprotein (TPBG) has been shown to impair trophoblast migration and invasion toward the uterine spiral arteries, leading to inadequate vascular remodeling and placental ischemia.<sup>32</sup> The current study found a weakly significant correlation ( $\rho=0.242$ ,  $P=0.016$ ) between the

PBH levels of mothers and those of their postnatal umbilical cord, with a mean value of  $(67.36\pm 16.70)$  (mg/dL). This suggests that the postnatal umbilical cord blood does not simply receive these substances from the mother. Instead, the fetus likely has its own regulatory mechanisms, as its PBH levels are significantly lower than the mother's, a finding that holds true even in cases of preterm birth and PE.<sup>33, 34</sup> The clinical significance of these elevated PBH levels is substantial. The data indicate that PBH levels are markedly increased in preeclamptic women, reflecting disease-related changes. Further research is warranted to better understand the clinical implications and the extent of these elevations.

The strength of the study lies in the simultaneous analysis of both maternal and postnatal umbilical cord blood samples, allowing investigation of inflammatory responses in the mother–postnatal umbilical cord pair and providing insight into the potential impact of maternal PE. The use of derived reference (cut-off) values for  $\alpha$ 1-AGP and PBH enhances the relevance and applicability of the findings to the studied population.

The study's primary weakness is its single-center design, which limits the generalizability of the findings. While we found a strong link between the biomarkers and PE, these results may not apply to the wider population. A further limitation is the lack of directly comparable existing research, as most prior studies on  $\alpha$ 1-AGP used different methods and focused only on maternal serum samples.<sup>9-11</sup> Finally, these biomarkers were measured only once during pregnancy, meaning the findings cannot be used to predict PE. Future prospective multi-center studies are needed to assess these biomarkers' potential as an early predictive tool. To fully establish their role, these markers should be estimated early in pregnancy, with frequent measurements taken throughout gestation, well before a PE diagnosis is made in the ordinary way. This serial assessment would confirm if women who eventually develop PE show an increased level of these biomarkers before clinical onset.

## Conclusion

Maternal  $\alpha$ 1-AGP and PBH levels are significantly elevated in preeclamptic women, with  $\alpha$ 1-AGP also higher in postnatal umbilical cord blood. Maternal and postnatal umbilical cord  $\alpha$ 1-AGP showed a moderate positive correlation, whereas PBH correlations were weak. ROC analysis showed high AUC values for maternal  $\alpha$ 1-AGP and PBH, indicating their relevance in maternal

and postnatal inflammation associated with PE.

### Authors' Contribution

J.H, S.A, and Sh.A: participated in conception and design; acquisition, analysis, and interpretation of data for the work, drafting the manuscript, and reviewing it critically for important intellectual content. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

### Declaration of AI

The authors utilized QuillBot (Academic Mode, latest available version), an AI language tool, to assist with rephrasing and grammar correction, enhancing the manuscript's clarity and readability. The authors have reviewed, edited, and approved the final text, and take full responsibility for its content.

**Conflict of Interest:** None declared.

### References

- Chappell LC, Brocklehurst P, Green ME, Hunter R, Hardy P, Juszczak E, et al. Planned early delivery or expectant management for late preterm pre-eclampsia (PHOENIX): a randomised controlled trial. *Lancet*. 2019;394:1181-90. doi: 10.1016/s0140-6736(19)31963-4. PubMed PMID: 31472930; PubMed Central PMCID: PMC6892281.
- Shields LE, Goffman D, Caughey AB ACOG practice bulletin: Clinical management guidelines for obstetrician-gynecologists. *Obstetrics and gynecology*. 2020;135:e237-60.
- Jung E, Romero R, Yeo L, Gomez-Lopez N, Chaemsaitong P, Jaovisidha A, et al. The etiology of preeclampsia. *Am J Obstet Gynecol*. 2022;226:S844-s66. doi: 10.1016/j.ajog.2021.11.1356. PubMed PMID: 35177222; PubMed Central PMCID: PMC8988238.
- Sowmya S, Ramaiah A, Sunitha T, Nallari P, Jyothy A, Venkateshwari A. Evaluation of Interleukin-10 (G-1082A) Promoter Polymorphism in Preeclampsia. *J Reprod Infertil*. 2013;14:62-6. PubMed PMID: 23926566; PubMed Central PMCID: PMC3719316.
- Roy S, Kumar V, Kumar V, Behera BK. Acute Phase Proteins and their Potential Role as an Indicator for Fish Health and in Diagnosis of Fish Diseases. *Protein Pept Lett*. 2017;24:78-89. doi: 10.2174/0929866524666161121142221. PubMed PMID: 27903234.
- Aziz SB. High sensitivity C-reactive protein in preeclamptic (PE) women. *Tikrit Med J*. 2015;20:1-5.
- Cresswell JA, Alexander M, Chong MYC, Link HM, Pejchinovska M, Gazeley U, et al. Global and regional causes of maternal deaths 2009-20: a WHO systematic analysis. *Lancet Glob Health*. 2025;13:e626-e34. doi: 10.1016/s2214-109x(24)00560-6. PubMed PMID: 40064189; PubMed Central PMCID: PMC11946934.
- Robajac D, Masnikosa R, Miković Ž, Mandić V, Nedić O. Oxidation of placental insulin and insulin-like growth factor receptors in mothers with diabetes mellitus or preeclampsia complicated with intrauterine growth restriction. *Free Radic Res*. 2015;49:984-9. doi: 10.3109/10715762.2015.1020798. PubMed PMID: 25812587.
- Kronborg CS, Allen J, Vittinghus E, Knudsen UB. Pre-symptomatic increase in urine-orosomucoid excretion in pre-eclamptic women. *Acta Obstet Gynecol Scand*. 2007;86:930-7. doi: 10.1080/00016340701371322. PubMed PMID: 17653877.
- Bottorff MB, Pieper JA, Boucher BA, Hoon TJ, Ramanathan J, Sibai BM. Lidocaine protein binding in preeclampsia. *Eur J Clin Pharmacol*. 1987;31:719-22. doi: 10.1007/bf00541302. PubMed PMID: 3556378.
- Kristensen K, Wide-Swensson D, Lindstrom V, Schmidt C, Grubb A, Strevens H. Serum amyloid a protein and C-reactive protein in normal pregnancy and preeclampsia. *Gynecol Obstet Invest*. 2009;67:275-80. doi: 10.1159/000214081. PubMed PMID: 19390201.
- Horowitz RN. Glycoprotein and other serum proteins of maternal serum. *Am J Obstet Gynecol*. 1969;105:216-9. doi: 10.1016/0002-9378(69)90061-1. PubMed PMID: 5810451.
- Liu XI, Green DJ, van den Anker JN, Rakhmanina NY, Ahmadzia HK, Momper JD, et al. Mechanistic Modeling of Placental Drug Transfer in Humans: How Do Differences in Maternal/Fetal Fraction of Unbound Drug and Placental Influx/Efflux Transfer Rates Affect Fetal Pharmacokinetics? *Front Pediatr*. 2021;9:723006. doi: 10.3389/fped.2021.723006. PubMed PMID: 34733804; PubMed Central PMCID: PMC8559552.
- Laurell CB. Orosomucoid and alpha 1-antitrypsin in maternal and fetal sera at parturition. *Scand J Clin Lab Invest*. 1968;21:136-8.

- doi: 10.3109/00365516809084275. PubMed PMID: 5706636.
- 15 Rimington C. Seromuroid and the bound carbohydrate of the serum proteins. *Biochem J.* 1940;34:931-40. doi: 10.1042/bj0340931. PubMed PMID: 16747238; PubMed Central PMCID: PMC1265364.
  - 16 Winzler RJ. Glycoproteins. In: Putnam FW, editor. *The Plasma Proteins*. Vol 1. New York: Academic Press; 1960. p. 309-47. doi: 10.1016/B978-1-4832-2810-5.50016-5.
  - 17 Beardmore-Gray A, Seed PT, Fleminger J, Zwertbroek E, Bernardes T, Mol BW, et al. Planned delivery or expectant management in preeclampsia: an individual participant data meta-analysis. *Am J Obstet Gynecol.* 2022;227:218-30. doi: 10.1016/j.ajog.2022.04.034. PubMed PMID: 35487323.
  - 18 Ceciliani F, Lecchi C. The Immune Functions of  $\alpha(1)$  Acid Glycoprotein. *Curr Protein Pept Sci.* 2019;20:505-24. doi: 10.2174/1389203720666190405101138. PubMed PMID: 30950347.
  - 19 Khamchun S, Thakaeng C, Na Lampang R. Serum alpha-1-antitrypsin level in the severity prognosis of systemic lupus erythematosus patients: Systematic exploration of novel biomarker. *Biomedicine (Taipei).* 2022;12:19-30. doi: 10.37796/2211-8039.1297. PubMed PMID: 35836976; PubMed Central PMCID: PMC9236717.
  - 20 Durmuş M, Uzunlar Ö, Çelik H, Çandar T. Does alpha-1-acid glycoprotein determine for infertility in polycystic ovary syndrome? *Eur J Obstet Gynecol Reprod Biol.* 2022;274:155-9. doi: 10.1016/j.ejogrb.2022.05.023. PubMed PMID: 35653904.
  - 21 Tarkun I, Arslan BC, Cantürk Z, Türemen E, Sahin T, Duman C. Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. *J Clin Endocrinol Metab.* 2004;89:5592-6. doi: 10.1210/jc.2004-0751. PubMed PMID: 15531516.
  - 22 Chu CY, Singla VP, Wang HP, Sweet B, Lai LT. Plasma alpha 1-acid glycoprotein levels in pregnancy. *Clin Chim Acta.* 1981;112:235-40. doi: 10.1016/0009-8981(81)90382-x. PubMed PMID: 7016376.
  - 23 di Masi A, Trezza V, Leboffe L, Ascenzi P. Human plasma lipocalins and serum albumin: Plasma alternative carriers? *J Control Release.* 2016;228:191-205. doi: 10.1016/j.jconrel.2016.02.049. PubMed PMID: 26951925.
  - 24 Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens.* 2014;4:97-104. doi: 10.1016/j.preghy.2014.02.001. PubMed PMID: 26104417.
  - 25 Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in pre-eclampsia. *Mol Aspects Med.* 2007;28:192-209. doi: 10.1016/j.mam.2007.02.006. PubMed PMID: 17433431.
  - 26 Catarino C, Santos-Silva A, Belo L, Rocha-Pereira P, Rocha S, Patrício B, et al. Inflammatory disturbances in preeclampsia: relationship between maternal and umbilical cord blood. *J Pregnancy.* 2012;2012:684384. doi: 10.1155/2012/684384. PubMed PMID: 22685662; PubMed Central PMCID: PMC3366239.
  - 27 Bork K, Horstkorte R, Weidemann W. Increasing the sialylation of therapeutic glycoproteins: the potential of the sialic acid biosynthetic pathway. *J Pharm Sci.* 2009;98:3499-508. doi: 10.1002/jps.21684. PubMed PMID: 19199295.
  - 28 Thakkar V, Patel P, Prajapati N, Kaur R, Nandave M. Serum Levels of Glycoproteins are Elevated in Patients with Ovarian Cancer. *Indian J Clin Biochem.* 2014;29:345-50. doi: 10.1007/s12291-013-0380-6. PubMed PMID: 24966484; PubMed Central PMCID: PMC4062676.
  - 29 Dheebalakshmi N, Poonguzhali B. Serum glycoproteins in patients with severe depression compared with normal subjects. *Med-Pulse Int J Biochem.* 2018;7:56-9. doi: 10.26611/1002731.
  - 30 Dheebalakshmi N, Poonguzhali B. Serum glycoproteins in patients with severe depression compared with normal subjects. *Med-Pulse Int J Biochem.* 2018;7:56-9. doi: 10.26611/1002731.
  - 31 Staff AC, Braekke K, Harsem NK, Lyberg T, Holthe MR. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol.* 2005;122:33-9. doi: 10.1016/j.ejogrb.2004.11.015. PubMed PMID: 15935542.
  - 32 Sun L, Shi M, Wang J, Han X, Wei J, Huang Z, et al. Overexpressed Trophoblast Glycoprotein Contributes to Preeclampsia Development by Inducing Abnormal Trophoblast Migration and Invasion Toward the Uterine Spiral Artery. *Hypertension.* 2024;81:1524-36. doi: 10.1161/hypertension.124.22923. PubMed PMID: 38716674.
  - 33 DiPrisco B, Kumar A, Kalra B, Savjani GV,

- Michael Z, Farr O, et al. Glycosylated Fibronectin and Inhibin Are Lower and Anti-Müllerian Hormone Is Higher in Umbilical Cord Blood When Mothers Have Preeclampsia. *Endocr Pract.* 2020;26:318-27. doi: 10.4158/ep-2019-0448. PubMed PMID: 31859547.
- 34 Tea I, Le Gall G, Küster A, Guignard N, Alexandre-Gouabau MC, Darmaun D, et al. <sup>1</sup>H-NMR-based metabolic profiling of maternal and umbilical cord blood indicates altered materno-foetal nutrient exchange in preterm infants. *PLoS One.* 2012;7:e29947. doi: 10.1371/journal.pone.0029947. PubMed PMID: 22291897; PubMed Central PMCID: PMC3264558.