ORIGINAL ARTICLE

Assessment of the Diagnostic Value of the Terminal Complement Complex, Factor D and Factor H in Preeclampsia

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ABSTRACT

Background: *To assess the diagnostic role of serum complement factor H (CFH), complement factor D (CFD), and terminal complement complex (C5b-9) and urine C5b-9 in women diagnosed with preeclampsia (PE) and severe PE compared to normal pregnancy.* ***Methodology****: A cross-sectional study involved 89 women divided into three groups: Group I, thirty women with PE, and Group II, thirty women with severe PE. Group III consisted of twenty-nine women with normal pregnancies as a control group. Estimation of serum levels of CFH, CFD, C5b-9, and urine C5b-9 levels in different study groups was done by ELISA test. A correlation analysis was carried out between levels of complement factors, clinical data, and laboratory tests.* ***Results:*** *Serum CFD, CFH, C5b-9, and urine C5b-9 levels were significantly elevated in patients with PE and severe PE compared to a normal pregnancy ($p < 0.001$). In addition, severe PE patients showed a significant increase compared to the PE group ($p < 0.001$). A significant positive correlation was found between serum CFD, CFH, C5b-9, and urine C5b-9 with both systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patient groups. The accuracy of serum CFH, C5b-9, and urine C5b-9 in the diagnosis of severe PE was found to be 100%, while serum CFD had an overall accuracy of 98.9%.* ***Conclusion:*** *Serum CFH, CFD, C5b-9, and urine C5b-9 can serve as diagnostic markers for severe PE, aiding in early detection of high-risk patients, disease monitoring, and appropriate treatment.*

INTRODUCTION

Preeclampsia (PE), is a hypertensive pregnancy disorder, that has a substantial impact on the health and well-being of both mothers and newborns worldwide, with a prevalence ranging from 2% to 8% among all pregnancies[1]. PE is a multi-system disorder that begins at the maternal-fetal interface[2]. Classically, PE has been associated with high blood pressure, endothelial injury, and impaired kidney function accompanied by proteinuria[3].

When PE is complicated by severe hypertension, proteinuria, thrombocytopenia, pulmonary edema, signs of impaired central nervous system function, liver cell damage, oliguria, it is termed PE with severe features[4].

The exact role of the immune system in the development of preeclampsia (PE) is not well comprehended and necessitates further investigations[5]. It is acknowledged that complement activation, which is essential for maintaining a healthy pregnancy, can contribute to the development of PE if it becomes excessive or improperly regulated [6].

The complement cascade, which plays a crucial role in immune response, can be initiated through three pathways: the classical, lectin, and alternative pathways[7]. At the convergence point of these pathways, C5 is proteolytically cleaved by C3b, resulting in the generation of C5a and C5b. Subsequently, C5b initiates the sequential assembly of C5b-6, C5b-7, C5b-8, and C5b-9, known as the terminal complement complex[8]. Limited data suggest that patients with severe preeclampsia (PE) may exhibit elevated levels of the terminal complement complex; however, further research is needed to fully understand this association [9].

C3 is a key component of the complement cascade and is essential for the activation of both the classical and alternative pathways of the complement system. It plays a crucial role in placental development, highlighting its significance in pregnancy [10,11]. Complement factor H (CFH) is a glycoprotein that serves as the primary regulator of C3 complement activation. When CFH is deficient, it can result in excessive activation of C3, disrupting the normal regulation of the complement system[12,13].

Complement factor D (CFD), commonly referred to as adipsin or C3 convertase activator, is a serine protease primarily synthesized by adipose tissue and is necessary for activating the complement alternative pathway[14]. The primary role of CFD is to split factor B, a component of the alternative pathway, into a non-

catalytic unit called Ba and a catalytic unit called Bb. The Bb joins with complement C3b to form the C3-converting enzyme[15].

Each of CFD and CFH regulates the C3 protein, which is involved in the development of severe PE[16]. Therefore, we proposed that changes in CFD and CFH serum levels would be valuable diagnostic information for severe PE. Therefore, the aim of this study was to assess serum CFD, CFH, C5b-9, and urine C5b-9 levels among PE and severe PE patients, as well as in women with uncomplicated pregnancies, to investigate their diagnostic role and correlate their values with clinical examination and laboratory data.

METHODOLOGY

Ethical statement:

The "Institutional Ethical Review Board" at Faculty of Medicine, Assiut University, Egypt, reviewed and approved the study protocol (IRB no. 17101573). A written consent was supplied by all the participants prior to their inclusion in the study.

Study design and participants:

This study was a cross-sectional study that was carried out at the Woman's Health Hospital of Assiut University from January to June 2022. During the study period, each pregnant woman >20 weeks who visited the hospital's labor unit was clinically assessed; Individuals diagnosed with PE or severe PE were requested to participate in the study. Exclusion criteria were multiple pregnancies, fetal death, chronic renal affection, untreated bacterial or viral infection, women complain of preexisting or gestational diabetes mellitus, autoimmune disease, and immunosuppressive agents.

The participants in this study were identified as having preeclampsia (PE) or severe preeclampsia based on the 2019 guidelines of the American College of Obstetricians and Gynecologists (ACOG) [17].The participants were categorized into three groups: Group I: thirty women with PE, and Group II: thirty women with severe PE. Group III was the control group, consisting of twenty-nine women with uncomplicated pregnancies who were matched in terms of age and gestational age.

Study tools & intervention:

At enrollment, all participants who were eligible were asked about maternal age, gestational age, number of children, previous abortions, and previous cesarean sections. In addition, clinical examination was done by BP measurement. CBC, liver functions, and urine protein assessment were investigated. To ensure fetal viability, an ultrasound examination was done.

At admission, under strict aseptic precautions, blood samples were collected from patients in the labor unit. Blood was drawn from a vein and placed in serum-separating tubes at a volume of around 5 ml. It was centrifuged at 2000–3000 rpm for 20 minutes after being allowed to coagulate at room temperature for 10–20 minutes. The serum was separated into two Eppendorf tubes and stored at −20°C for subsequent utilization.

Using an indwelling catheter or a random clean-catch specimen, urine was collected into a sterile container for the dipstick test then immediately transferred. To remove any impurities, urine was centrifuged, and the supernatant was stored in Eppendorf tubes at -20°C for ELISA.

The enzyme linked immunosorbent assay (ELISA) kits used the double-antibody sandwich technique to detect human serum CFD, CFH, C5b-9, and urine C5b-9 (ELK Biotechnology, Co., Ltd., Wuhan; Cat. Nos. ELK1128, ELK1720, and ELK3025, respectively). The immunoassays were performed following recommendations supplied by the manufacturer.

The microtiter plate included in these kits was pre-coated with antibodies that are specific to CFD, CFH, or C5b-9. Standards or samples were put in the correct wells of a microtiter plate along with a biotin-conjugated antibody that specifically targets CFD, CFH, or C5b-9. After following the manufacturer steps finally, a solution of sulfuric acid was introduced to stop the enzyme-substrate reaction, and a change in color was measured at a range of 450nm ± 10nm spectrophotometrically. The concentrations of complement factors CFD, CFH, or C5b-9 in the samples were then assessed by comparing the optical density to the standard curve.

Statistical analysis:

Data were analyzed using SPSS (Statistical Package for the Social Sciences, version 20, IBM, and Armonk, New York). The Shapiro-Wilk test was done to evaluate whether the data were in line with the normal distribution. Means ± standard deviations (SDs) were calculated for normally distributed quantitative data, and for comparison between groups, an ANOVA was followed by a post-hoc test. The medians (minimum-maximum) were calculated for abnormally distributed quantitative data, and for group comparison, the Kruskal-Wallis and Mann-Whitney U tests were utilized.

Correlation analysis of variables was assessed by Pearson correlation test. The diagnostic accuracy of serum CFD, CFH, C5b-9, and urine C5b-9 in the diagnosis of severe PE was assessed by the receiver operator characteristics (ROC) curve. The confidence level was maintained at 95%, and therefore, a p value was considered statistically significant if it was < 0.05.

RESULTS

Participant characteristics:

Different study groups had no significant variations in clinical data ($p > 0.05$), except for blood pressure. The two patient's groups had significantly higher SBP

and DBP compared to the control group. Also, SBP and DBP were considerably elevated in the severe PE in comparison to those with PE group. Women with severe PE exhibited significantly lower total protein levels compared to those with PE. Also, the lymphocytic count was higher in the women with severe PE compared to the other two groups, as shown in **table 1**.

Table 1: Baseline data for the study groups

	Preeclampsia (PE) group (n=30)	**Severe PE group (n= 30)**	**Control group (n= 29)**	***P*1**	***P*2**	***P*3**
Age (years)*	29.13 ± 6.92	30.70 ± 6.63	29.55 ± 5.28	0.34	0.80	0.48
Gestational age* (wk)	33.43 ± 4.56	33.57 ± 4.21	34.76 ± 1.90	0.89	0.12	0.09
Number of children**	2 (0-7)	2 (0-6)	2 (0-6)	0.46	0.38	0.88
Previous abortion**	1 (0-6)	1 (0-3)	1 (0-4)	0.06	0.38	0.30
Previous cesarean section**	1 (0-6)	1 (0-5)	1 (0-4)	0.74	0.52	0.75
Systolic blood pressure (mmHg)*	135.33 ± 8.60	166.67 ± 17.48	122.41 ± 11.23	**< 0.001#**	**< 0.001#**	**< 0.001#**
Diastolic blood pressure (mmHg)*	86.67 ± 7.11	99.67 ± 8.50	77.59 ± 8.72	**< 0.001#**	**< 0.001#**	**< 0.001#**
Total protein in blood (mg/dl)*	61.91 ± 9.06	58.56 ± 5.90	62.13 ± 7.76	**0.03#**	0.77	0.06
Lymphocytes (10^3/ul)*	1.97 ± 0.68	2.48 ± 0.45	1.79 ± 0.23	**0.04#**	0.48	**< 0.001#**

*Data have been expressed as mean ±SD, **median (range). # Statistical significant difference. ***P*1** compares preeclampsia (PE) vs. severe PE groups; ***P*2** compares preeclampsia (PE) vs. control groups; and ***P*3** compares severe PE vs. control groups.

Levels of serum CFD, CFH, C5b-9, and urine C5b-9:

Regarding serum levels of CFD, patients with severe PE had significantly higher levels compared to both PE and control groups (44.75 ± 2.81 vs. 34.32 ± 7.28; p1 < 0.001 and 15.93 ± 2.36; p3 < 0.001), respectively. There was also a considerably higher difference in CFD between the PE group and the control group (34.32 ± 7.28 vs. 15.93 ± 2.36; p2< 0.001).

Patients with severe PE had significantly higher serum CFH levels compared to those in PE group and control group (59.02 ± 11.87 vs. 33.93 ± 2.33; p1 < 0.001 and 27.26 ± 2.41; p3 < 0.001), respectively. Also, serum CFH levels were significantly higher in the PE group than control group (33.93 ± 2.33 vs. 27.26 ± 2.41; p2 = 0.02).

A significantly higher level of serum C5b-9 was found in severe PE patients in comparison to PE and control groups (22.59 ± 2.22 vs. 11.41 ± 1.93; p1 < 0.001 and 7.88 ± 1.11; p3 < 0.001), respectively. The PE group also had significantly elevated serum C5b-9 levels compared to the control group (11.41 ± 1.93 vs. 7.88 ± 1.11; p2= 0.01).

Additionally, the urinary C5b-9 level in the severe PE group was also significantly increased when compared to the PE group and control group (100.97 ± 2.79 vs. 88.67 ± 5.04; $p < 0.001$ and 31.87 ± 4.87; $p < 0.001$), respectively. Also, the PE group was significantly higher level of urinary C5b-9 than the control group (88.67 ± 5.04 vs. 31.87 ± 4.87; $p < 0.001$), the results are indicated in **figure 1** and **table 2**.

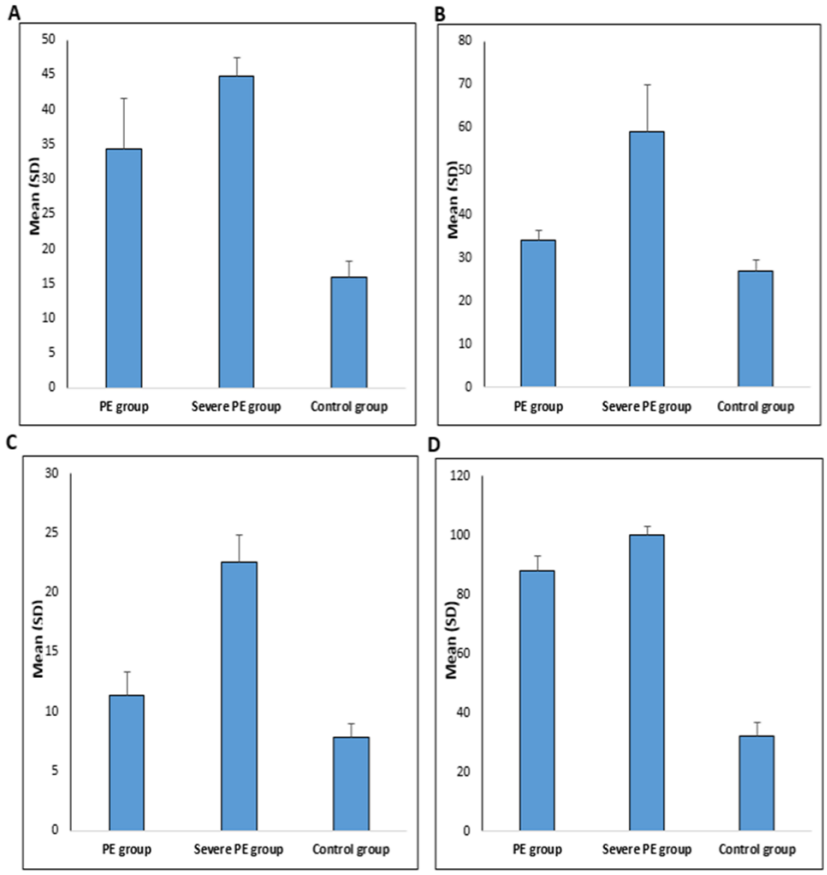

Fig.1: A: Level of factor D among the studied groups. B: Level of factor H among the studied groups. C: Level of serum C5b-9 among the studied groups. D: Level of urine C5b-9 among the studied groups.

Table 2: Factors D, H, and C5b-9 among the study groups

	Preeclampsia (PE) group (n=30)	**Severe PE group (n= 30)**	**Control group (n= 29)**	***P*1**	***P*2**	***P*3**
Factor D (ng/mL)	34.32 ± 7.28	44.75 ± 2.81	15.93 ± 2.36	**< 0.001***	**< 0.001***	**< 0.001***
Factor H (ng/mL)	33.93 ± 2.33	59.02 ± 11.87	27.26 ± 2.41	**< 0.001***	**0.02***	**< 0.001***
Serum C5b-9 (ng/mL)	11.41 ± 1.93	22.59 ± 2.22	7.88 ± 1.11	**< 0.001***	**0.01***	**< 0.001***
Urine C5b-9 (ng/mL)	88.67 ± 5.04	100.97 ± 2.79	31.87 ± 4.87	**< 0.001***	**< 0.001***	**< 0.001***

Data have been expressed as mean ±SD. The *P* value was statistically significant if it was < 0.05. ***P*1** compares preeclampsia (PE) vs. severe PE groups; ***P*2** compares preeclampsia (PE) vs. control groups; and ***P*3** compares severe PE vs. control groups.

Results of correlation and ROC curve analysis:

There was a significant positive moderate correlation between CFD and CFH with SBP ($r = 0.510$, $p < 0.001$, $r = 0.426$, $p = 0.001$, respectively). Also, CFD and CFH were significantly positively correlated with DBP ($r = 0.454$, $p < 0.001$, $r = 0.402$, $p = 0.001$, respectively) in all patients.

While a significantly positive strong correlation was found between urine C5b-9 and serum C5b-9 levels with SBP in all patients ($r = 0.640$ and $r = 0.614$, respectively; both $p < 0.001$), a significant positive moderate correlation between urine C5b-9 and serum C5b-9 levels with DBP was detected ($r = 0.464$ and $r = 0.492$, respectively; both $p < 0.001$), as illustrated in **table 3**.

Table 3: Correlation between studied factors and blood pressure in all patients

	SBP (mmHg)		DBP (mmHg)	
	r	P	r	p
Factor D	**0.510***	**< 0.001**	**0.454***	**< 0.001**
Factor H	**0.426***	**0.001**	**0.402***	**0.001**
Urine C5b-9	**0.640***	**< 0.001**	**0.464***	**< 0.001**
Serum C5b-9	**0.614***	**< 0.001**	**0.492***	**< 0.001**

Data are expressed as *r* (strength of correlation), *P* (significance of correlation and was significant if < 0.05). **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure

The present study used the ROC curve to assess the diagnostic accuracy of serum CFD, CFH, C5b-9, and urine C5b-9 in severe PE. It was found that each of serum CFH, C5b-9, and urine C5b-9 had 100% accuracy.

The sensitivity and specificity were both 100% at a cutoff point of serum CFH levels > 38.5 ng/ml, and the area under the curve (AUC) was 1. Also, at a cutoff point > 15.7 ng/ml of serum C5b-9 levels, the sensitivity and specificity were 100%, with an AUC of 1. Similarly, at a cutoff point of urine C5b-9 levels > 94.5 ng/ml, the sensitivity and specificity were both 100% and the AUC was 1. CFD showed reliable diagnostic significance with an AUC of 0.99 (98.9% accuracy). CFD had a sensitivity of 100% and a specificity of 98.3% as a marker for severe PE at a cutoff point of > 40.1, as indicated in **table 4**.

Table 4: Accuracy of factor D, factor H, urine C5b-9, and serum C5b-9 in the diagnosis of severe PE:

	Factor D	Factor H	Urine C5b-9	Serum C5b-9
Sensitivity	100%	100%	100%	100%
Specificity	98.3%	100%	100%	100%
PPV	96.8%	100%	100%	100%
NPV	100%	100%	100%	100%
Accuracy	98.9%	100%	100%	100%
Cutoff point (ng/mL)	**> 40.1***	**> 38.5***	**> 94.5***	**> 15.7***
Area under curve	0.99	1	1	1
P value	< 0.001	< 0.001	< 0.001	< 0.001

P value was statistically significant if it was < 0.05. **PPV**: positive predictive value; **NPV**: negative predictive value

DISCUSSION

Baseline data showed a significantly higher blood pressure among patients' groups. In addition, the severe PE group had considerably lower serum total protein levels than the PE group. It is known that elevated blood pressure and proteinuria are diagnostic criteria for PE. Additionally, the lower serum total protein level in severe PE was due to more proteinuria than PE[18].

In the present study, women with severe PE were found to have a higher lymphocytic count compared to both the PE and the control groups. In agree with our study, Wang et al.[19]stated that women with PE and severe PE had considerably greater levels of leukocytes and lymphocytes than those in the control group.

Increased lymphocyte and neutrophil numbers and surface marker activation indicate a moderate systemic immune response that is significant for sustaining a healthy pregnancy[20]. However, excessive amounts of inflammatory cytokines released by placental stromal and trophoblast cells stimulate neutrophil and lymphocyte cells, which amplify the response in PE[21]. Conversely, Liu et al.[22] found that preeclamptic patients' placental mesenchymal stem cells secrete soluble factors that suppress lymphocyte proliferation and alter immune balance.

The present study concluded that levels of CFD in serum increased significantly in women diagnosed with PE and severe PE compared to the control group. Also, it was significantly increased in severe PE than PE. Our findings are consistent with Liu et al. [23], they stated that PE patients exhibited a significantly higher CFD level than healthy pregnant women. It has been established that end-stage renal failure is linked with a 10-fold increase in plasma levels of CFD, showing a close association between CFD metabolism and renal functions[24,25]. This explains our findings that higher

levels of CFD are linked to the severity of PE, which could be due to renal affection in severe cases.

The alternative complement pathway was found to be activated in preeclamptic patients[26]. A prior investigation illustrated that CFH functions as a regulatory protein, preventing alternative pathway overactivation by competing with factor B[27]. Our findings showed an increase in CFH serum levels in severe PE and PE groups compared to the control group. A previous research demonstrated that preeclamptic women have been observed to have higher serum CFH levels throughout the first trimester[28]. This was consistent with our findings and could be explained by the increase in CFH levels serving as a compensatory mechanism to regulate the harmful effects of complement system activation in PE[29].

Another study revealed that women with PE early or late onset showed considerably decreased CFH serum levels than those with a healthy pregnancy[30]. These different results may be because measuring serum soluble complement factor levels isn't always a good way to assess if the complement system is activated or not, as the levels of these factors change during production and during consumption[31].

In current study, serum and urine C5b-9 levels differed significantly between the groups. Patients with severe PE had the highest value, followed by patients with PE and normotensive women. Burwick et al.[32] found that urine levels of C5b-9 in women with severe PE were significantly greater than those of PE women without severe features or those without hypertension. Also, levels of plasma C5b-9 were significantly elevated in all pregnant women with any hypertensive disorder than in women without hypertension. This was in line with our study.

Furthermore, previous studies showed that higher concentrations of upstream complement proteins, such as C3a and Bb, were related to increased risk of PE in women[33,34]. The continuous activation of pathways located upstream, possibly initiated during early pregnancy, results in the subsequent activation of the terminal complement pathway, which is accountable for the complement system's damaging inflammatory and lytic actions[35].

Normally, C5b-9 in urine is not detected as it has large molecular size. But when complement proteins are activated, they can directly induce kidney damage, which causes C5b-9 to be released into the urine due to impaired glomerular or tubular function[36]. As severe PE patients have higher urine C5b-9 levels than PE patients, some degree of renal affection in severe PE may be found.

Valencia et al.[37] conducted a recent study involving 298 women with different pregnancy hypertensive disorders. The study revealed an increase in urine C5b-9 concentrations and was observed only in PE participants who had acute kidney injury.

Regarding the correlations of different study factors with blood pressure, it is known that increased blood pressure is the main diagnostic criteria for PE. A previous study showed that PE can be diagnosed with either high SBP or high DBP[38]. Our present study found, for the first time, that levels of serum CFD, CFH, C5b-9, and urine C5b-9 correlated positively with both SBP and DBP in all patients. Thus, provide further evidence that the complement system is directly correlated with the pathogenesis and severity of PE.

Similarly, Burwick et al. [36] found that there was a correlation between a decrease in C5b-9 levels in both plasma and urine and clinical improvement, resolution of symptoms, and longer pregnancy in patients with severe PE.

We also evaluated the diagnostic significance of levels of serum CFD, CFH, C5b-9, and urine C5b-9 in severe PE. Among these factors, CFD showed the lowest Area Under the Curve (AUC). The best AUC results came from the other three factors. Therefore, these complement factors can serve as diagnostic markers for severe PE.

The limitations of the current study were its small sample size and being conducted in a single center. Also, we didn't perform follow-up on those patients for a long time to link those biomarkers with both maternal and fetal outcomes.

CONCLUSIONS

In women with PE, higher levels of serum CFD, CFH, C5b-9, and urine C5b-9 were detected. Each of CFH and C5b-9 had 100% accuracy for the diagnosis of severe PE, while CFD had 98.9% overall accuracy. Therefore, there is possibility to be used as diagnostic markers for severe PE. Thus, using these biomarkers for the diagnosis and subclassification of PE can help identify high-risk patients, monitor disease development, and supply appropriate treatments.

Conflict of Interest: The authors declare that they have no **conflict of** interests

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