Maternal serum SHARP1 and hypoxia inducible factor-1α as biomarkers for preeclampsia

Original Article

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ABSTRACT

Objectives: Preeclampsia remains a major cause of maternal mortality and morbidity worldwide, with an increased risk of cardiovascular disease later in life. Many previous studies have examined several biomarkers. We aimed to assess the role of Split and hairy-related protein-1 (SHARP1) together with hypoxia-inducible factor-1α (HIF-1α) as biomarkers for the detection of preeclampsia.

Materials and Methods: This case-control study included 150 pregnant women, 75 healthy women and 75 with preeclampsia, who were recruited for delivery during the third trimester of pregnancy and subjected to full clinical and laboratory testing. This testing included compete blood analysis, and plasma SHARP1 and HIF-1α measurement using ELISA. The main outcome was the association of SHARP1 and HIF-1α with preeclampsia.

Results: A significant decrease in SHARP1 ($P < 0.0001$) and a significant increase in HIF-1α ($P < 0.0001$) were observed in patients versus the controls. The SHARP1 and HIF-1α levels were independent indicators of preeclampsia after adjusting for maternal age, body mass index, and parity (the odds ratio for SHARP1 was 0.04, 95% confidence interval 0.321–0.791; $P < 0.0001$, and the OR for HIF-1α was 30.222, 95% CI 6.219 – 146.877; $P < 0.0001$).

Conclusion: SHARP1 and HIF-1α may have be used as biomarkers for the proper recognition of preeclampsia. The synergistic actions of SHARP1 and HIF-1α might play a key role in the pathogenesis of preeclampsia. Larger studies are likely to help verify the data and justify the wider application of these markers.

Key Words: Hypoxia inducible factor-1α; preeclampsia; SHARP1.

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INTRODUCTION

Gestational weight Preeclampsia is a multifactorial pathology defined by the association of elevated blood pressure and significant proteinuria after 20 weeks of gestation[1]. This condition complicates three-to-eight percent of all pregnancies[2], and it is one of the primary causes of maternal and neonatal morbidity and mortality worldwide[3]. Although the exact etiology of preeclampsia is still elusive, the role of the placenta in preeclampsia pathogenesis has been well described[4]. Preeclampsia is supposed to occur by means of insufficient trophoblastic invasion during pregnancy, which leads to inconsistent remodeling of the myometrial spiral arteries, resulting in placental hypoperfusion and an ischemic microenvironment[5], which in turn induces the upregulation of hypoxia-inducible factor 1 alpha (HIF-1α)[6].

During early gestation, HIF-1α is highly expressed in the hypoxic environment of the placenta. HIF-1α decreases at approximately nine weeks of gestation when placental oxygen levels increase[7]. Persistently elevated HIF-1α levels have been recognized to be associated with preeclampsia. Nevertheless, the molecular basis of the chronic elevation in HIF-1α in preeclampsia and its underlying role in the pathogenesis of preeclampsia are poorly understood[8].

Currently, there is growing evidence that oxygen-independent mechanisms, such as cytokines, hormones, or growth factors, can induce HIF-1α[6,9]. Split and hairy-related protein-1 (SHARP1) is a member of the transcriptional repressor subfamily of basic helix-loop-helix transcription factors[10]. SHARP1 is expressed in many embryonic and adult tissues associated with the placenta[11]. SHARP1 is a tumor suppressor in thyroid, breast and endometrial cancers. Therefore, SHARP1 downregulation may contribute to the proliferation, invasion and migration of malignant cells through mechanisms that include HIF-1α overexpression[12-14]. SHARP1 has a role in HIF-1α degradation, which led to the hypothesis that SHARP1 acts

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as a global inhibitor of HIF-1α activity, and a reduction in SHARP1 might induce high levels of HIF-1α[13].

To our knowledge, no previous studies have evaluated the correlation between SHARP1 and HIF-1α in patients with preeclampsia. Hence, we aimed to examine the hypothesis that preeclampsia was associated with low maternal serum levels of SHARP1 and high maternal serum levels of HIF-1α.

PATIENTS AND METHODS

We conducted a case-control study between November 2018 and February 2019. We enrolled 150 pregnant women who were admitted to the delivery unit of Bugshan Hospital in Jeddah.

The participants were divided into two even groups: preeclampsia patients (n=75) were in the study group, and healthy-normotensive pregnant women (n=75), who were randomly selected from among patients hospitalized for labor during the latent phase in the same hospital in the same period, were in the control group. The women in the control group had no evidence of preeclampsia and were matched for maternal age, body mass index (BMI), and gestational age at delivery. The present study excluded patients with complications such as fetal congenital malformation, multiple gestations, chorioamnionitis, a maternal history of diabetes mellitus, cardiovascular, renal, hepatic diseases, or systemic lupus erythematous, or proven autoimmune disease or patients on medications, such as antiplatelet, corticosteroids or nonsteroidal anti-inflammatory drugs.

Preeclampsia was diagnosed based on the recently revised criteria of the American College of Obstetrics and Gynecology in 2013[1]: (i) blood pressure ≥ 140 mmHg systolic or 90 mmHg diastolic on two occasions at least 4 hours apart after 20 weeks of gestation with a previously normal blood pressure or blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic and (ii) proteinuria (protein/creatinine ratio ≥ 0.3 or dipstick reading of 1+) or (iii) in the absence of proteinuria, new-onset hypertension with the new onset of any of the following: thrombocytopenia (platelet count ≤ 100,000/microliter), renal insufficiency (serum creatinine concentrations ≥ 1.1 mg/dl, elevated blood concentrations of liver transaminases to twice normal concentration, pulmonary edema, cerebral or visual symptoms.

We obtained 3 mL venous blood samples into ethylene-diamine-tetra-acetic acid plasma sample tubes for complete blood count with differentials, including platelets and mean platelet volume. Additionally, we collected 3 mL blood samples into nonheparinized tubes for the assessment of serum SHARP1 and HIF-1α. After centrifugation at 3,000 g for 5 minutes, the serum samples were aliquoted and stored at -80°C until further analysis. All samples were processed within 2 hours after venipuncture. For the preeclampsia group, blood samples were obtained when preeclampsia was diagnosed.

The serum SHARP1 and HIF-1α levels were determined using a commercially available enzyme-linked immunosorbent assay kit developed for SHARP1 and HIF-1α (Cloud-Clone Corp, Katy, TX, USA, Product No.: SEL257Hu for SHARP1 and SEA798Hu for HIF-1α) according to the manufacturer's instructions and given as ng/mL. The detection range of both kits was 0.156-10 ng/mL, and the sensitivity of the assay was 0.056 ng/mL for SHARP1 and 0.059 ng/mL for HIF-1α. The intra-assay and interassay coefficients of variation were less than 10% and 12%, respectively, for both kits.

STATISTICAL ANALYSIS

Data analysis was performed using the SPSS 17.0 software package (SPSS, Chicago, IL). A *P value <0.05* was considered to be significant. The Shapiro-Wilk test was used to analyze the distribution of the data. Variables are given as the mean ± standard error of the mean, median (interquartile range), or number (percent). Independent Student's t-test was used to compare continuous variables. Moreover, nonparametric variables were compared by the Mann-Whitney test. Categorical variables were compared using the Chi square or Fisher exact test, as appropriate. Receiver operating characteristic curve analysis was performed to examine the required levels of SHARP1 and HIF-1α to discriminate between cases and controls. The area under this curve was estimated, and the optimum cutoff value of SHARP1 and HIF-1α was defined according to the highest Youden index. The diagnostic validity of the optimum cutoff value was evaluated and expressed in terms of sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios.

Sample size calculation was performed using G*Power version 3.1.9.2 (Heinrich Heine Universität; Dusseldorf; Germany) using reference values of SHARP1 previously reported[15]. Based on a two-tailed alpha error of 0.05, a power of 0.8, and an effect size of 0.462, a total of 150 subjects (75 cases and 75 controls) were calculated as the minimally required sample size to achieve statistical power.

RESULTS

In total, the data collected from the 150 women in the two study groups (preeclampsia and control) were analyzed and compared to examine the maternal levels of SHARP1. Demographic characteristics are shown in Table 1. There was no significant difference in maternal age, BMI, parity, or gestational age between the two groups. Participants with preeclampsia had a significantly higher systolic and

diastolic blood pressure at the time of admission than control patients ($p < 0.0001$). Figure 1 shows the SHARP1 and HIF-1α serum levels. The mean serum SHARP1 was significantly lower, while the mean serum HIF-1α was significantly higher among women with preeclampsia than among control individuals ($P < 0.0001$).

The data regarding the capability of SHARP1 and HIF-1α levels to distinguish between the cases of preeclampsia and control participants are shown in Table 2 and Figure 2. The area under the receiver operating characteristic curve indicated excellent discriminative value. The optimum cutoff for the use of SHARP1 as a marker of preeclampsia was set at a level less than 3.85 ng/mL. Meanwhile, the optimum cutoff for the use of HIF-1α was set at a level greater than 2.73 ng/mL. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of these cutoff levels are outlined in Table 2.

Figure 3 shows the correlation between the serum levels of SHARP1 and HIF-1α. There was a significant inverse correlation between the serum levels of SHARP1 and HIF-1α (r = - 0.640, *P value* < *0.0001*).

The multivariable logistic regression model for risk factors for preeclampsia is shown in Table 3. After adjusting for maternal age, BMI, and nulliparity, the SHARP1 and HIF-1α levels were found to be independent indicators of preeclampsia. The model had good overall fit, as evidenced by the −2 log likelihood test ($P < 0.0001$), the Hosmer and Lemeshow test ($P = 0.542$), and the correct classification rate (71.7%).

Table 1: Comparison of women with or without preeclampsia[a]

Variable	Case group (n=75)	Control group (n=75)	*P value*[b]
Age	27.19 ± 0.68	26.69 ± 0.70	0.616
Pre-pregnancy body mass index[c]	29.21 ± 0.64	27.98 ± 0.63	0.176
Parity	0 (0-2)	1 (0-2)	0.272
Nulliparous	41 (54.7%)	32 (42.7%)	0.254
Smoking	2 (2.7%)	3 (4%)	1.0
GA at delivery, days	243.40 ± 2.64	248.2 ± 1.35	0.108
Delivery at GA < 37weeks	52 (69.3%)	47 (62.7%)	0.389
Socioeconomic status Low High	 48 (64%) 27 (36%)	 44 (58.7%) 31 (41.3%)	0.5024
Residence Urban Rural	 53 (73.3%) 22 (26.7%)	 56 (74.7%) 19 (25.3%)	0.5826
Systolic blood pressure, mmHg	154.67 ± 1.37	113.34 ± 1.28	< 0.0001
Diastolic blood pressure, mmHg	100.20 ± 0.89	69.27 ± 0.85	< 0.0001
Hemoglobin, g/dL	10.3 ± 0.15	10.62 ± 0.18	0.21
Platelets, x 10^9/L	245.83 ± 7.35	254.19 ± 7.81	0.384
Mean platelet volume, fL	11.11 ± 0.14	10.92 ± 0.13	0.311
SHARP1, ng/mL	2.97 ± 0.12	5.31 ± 0.18	< 0.0001
HIF-1α ng/mL	3.26 ± 0.06	2.44 ± 0.03	< 0.0001

Abbreviation: GA, gestational age; SHARP1, Split and hairy related protein-1; HIF-1α, Hypoxia inducible factor-1α.
a Values are given as are mean±SD, median (interquartile range), or number (percentage), unless otherwise indicated.
b Student t test, Mann-Whitney U test, or Chi-squared test was used as appropriate.
c Calculated as weight in kilograms divided by the square of height in meters.

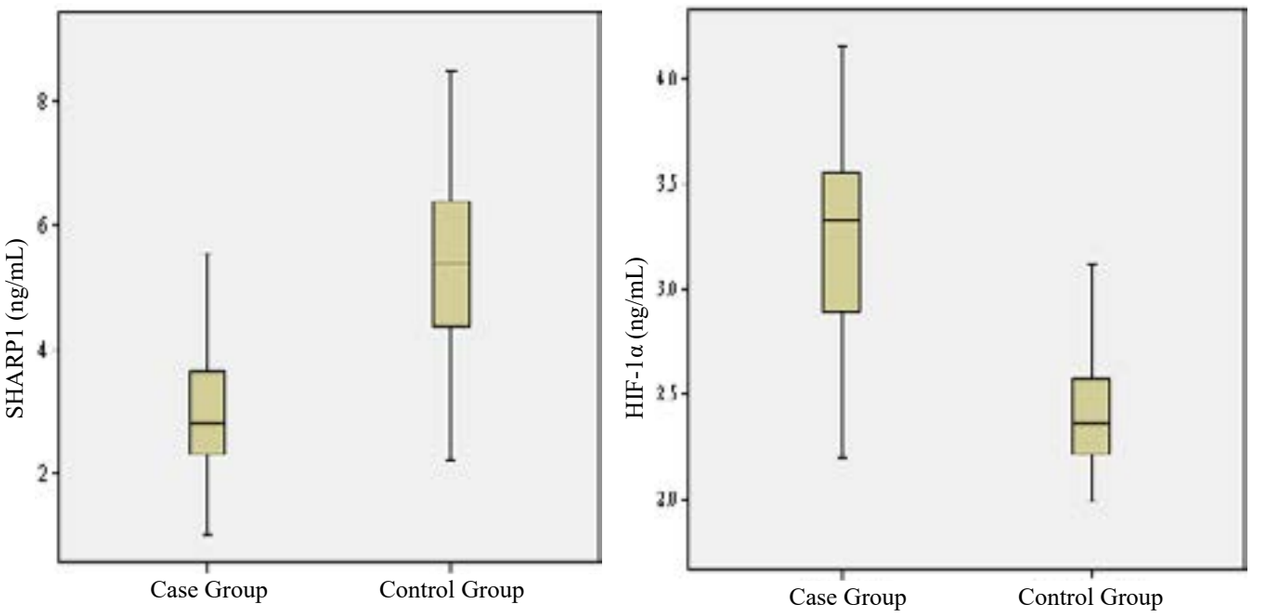

Fig. 1: Split and hairy related protein-1 (SHARP1) and Hypoxia inducible factor-1α (HIF-1α) levels in case group compared with control group.

Table 2: Use of serum SHARP1 and HIF-1α levels to discriminate between women with or without preeclampsia.

Measure	SHARP1		HIF-1α	
	Estimate	95% CI	Estimate	95% CI
Area under the receiver operating characteristic curve	0.879	0.825 - 0.933	0.920	0.878 - 0.961
Youden index, J	0.654	NA	0.667	NA
Optimum cutoff level, ng/mL	3.85	NA	2.73	NA
Sensitivity, 95% CI	86.67	76.84 - 93.42	86.7	76.84 - 93.42
Specificity, 95% CI	78.67	67.68 - 87.29	80	69.17 - 88.35
Positive Predictive Value, 95% CI	80.25	72.28 – 86.36	81.25	73.21 - 87.30
Negative Predictive Value, 95% CI	85.51	76.60 – 91.40	85.71	76.92 - 91.53
Positive Likelihood Ratio, 95% CI	4.06	2.61 – 6.33	4.33	2.73 - 6.87
Negative Likelihood Ratio, 95% CI	0.17	0.09 - 0.31	0.17	0.09 - 0.30

Abbreviation: SHARP1, Split and hairy related protein-1; HIF-1α, Hypoxia inducible factor-1α; CI, confidence interval; NA, not applicable.

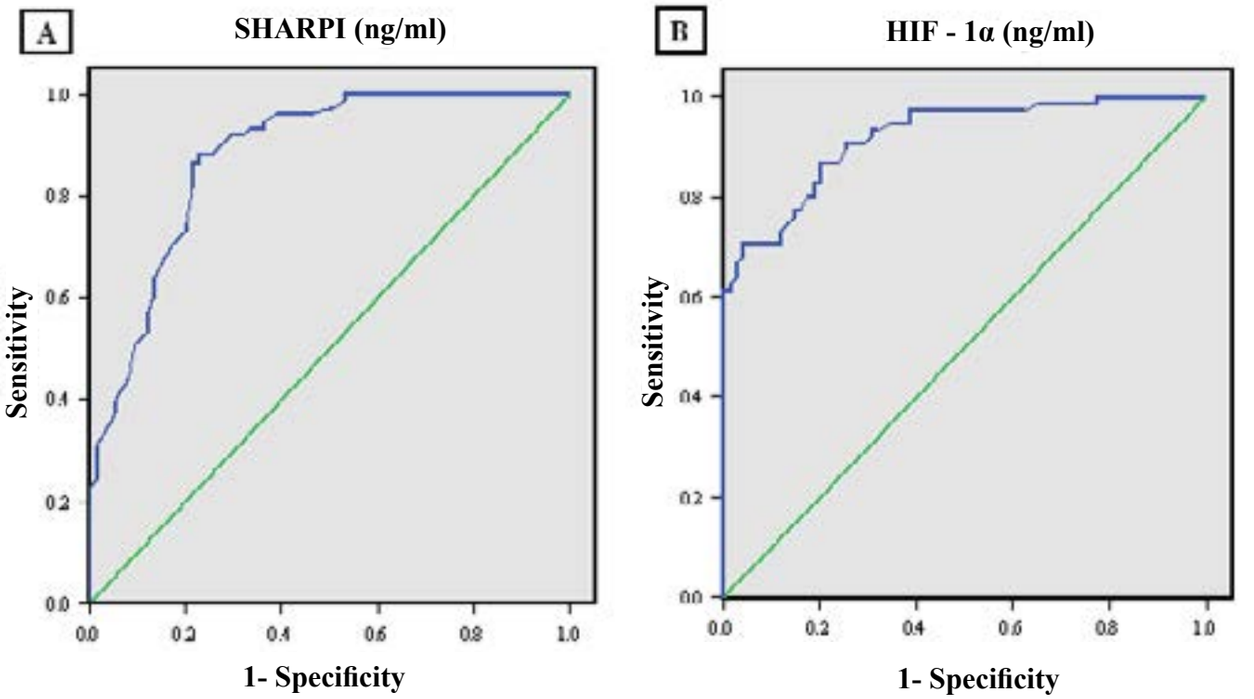

Fig. 2: Receiver-operating characteristic (ROC) curve derived from Split and hairy related protein-1 (SHARP1) and Hypoxia inducible factor-1α (HIF-1α).

Table 3: Multivariable logistic regression model to identify risk factors for preeclampsia

Variable	Regression coefficient (standard error)	Wald	Odds ratio (95% confidence interval)	P value
SHARP1, ng/mL	-0.686 ± 0.230	8.860	0.504 (0.321 – 0.791)	0.003
HIF1a, ng/mL	3.409 ± 0.807	17.855	30.222 (6.219 – 146.877)	< 0.0001
Age, years	0.018 ± 0.045	0.158	1.018 (0.932 – 1.112)	0.691
Body mass index, kg/m^2	9.47 ± 0.13	0.109	2.504 (0.819 – 7.656)	0.741
Parity	9.47 ± 0.13	2.589	1.018 (0.932 – 1.112)	0.108
Constant of the regression equation	-7.214 ± 3.335	4.860	0.001	0.031

Abbreviation: SHARP1, Split and hairy related protein-1; HIF-1α, Hypoxia inducible factor-1α.

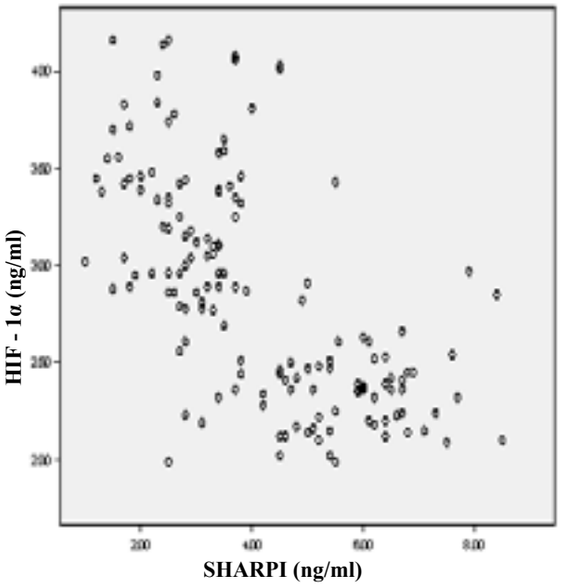

Fig. 3: Correlation between serum Split and hairy related protein-1 (SHARP1) and Hypoxia inducible factor-1α (HIF-1α) levels.

DISCUSSION

The present study compared maternal serum HIF-1α and SHARP1 levels between preeclampsia patients and healthy control patients. Our results showed that maternal serum SHARP1 levels were lower and serum HIF-1α levels were higher in the preeclampsia group than in the healthy group. In addition, there was a significant negative correlation between serum SHARP1 and HIF-1α levels, suggesting a mechanistic link in their roles in the progression of preeclampsia.

Preeclampsia is a multisystem disorder whose etiology is poorly understood; therefore, effective treatment and diagnostic strategies for preeclampsia are not available, and this disorder remains a leading cause of maternal and fetal mortality[16]. Evidence indicates that preeclampsia originates from the placenta and resolves with delivery and the removal of the placenta[17].

Normally, early stages of placental development affect trophoblast invasion of the uterine spiral arteries, transforming them into enlarged capacitance vessels[18]. Increasing evidence advocates that HIF-1α is the key regulator of the invasiveness of trophoblasts[19]. In early pregnancy, the placenta has a hypoxic environment that enhances HIF-1α expression, which in turn inhibits trophoblast invasion and guards trophoblast DNA from damage by reducing oxidative stress[20]. However, by the seventh week of gestation, villous trophoblast proliferation starts pushing cells away from the basal membrane of the chorionic villi toward areas with higher oxygen levels. Gradually, trophoblasts increase their invasiveness potential. Ultimately, at 14 weeks of gestation, invasive trophoblasts remodel the spiral arteries into enlarged capacitance vessels[21]. Conversely, in preeclampsia, there is a persistent hypoxic milieu that creates a vicious cycle with high levels of HIF-1α constantly repressing trophoblast invasiveness that leads to improper spiral artery remodeling and poor placentation[20].

The role of HIF-1α in preeclampsia pathogenesis has been studied by immunohistochemical and serum analyses both in vitro and in vivo[8,22-24]. Akhilesh *et al*. showed that the HIF-1α levels of mothers with preeclampsia were higher than those of normal controls[25]. Likewise, Verma and colleagues demonstrated increased placental expression of HIF-1α in preeclampsia compared to normotensive pregnancies[26]. In contrast, Demircan-Sezer *et al*. conducted a study on 25 preeclampsia patients and 21 normal pregnant women as a control group. They found that maternal blood HIF-1α levels were significantly lower in the preeclampsia group than in the control group[27]. This contradictory finding might be due to the limited number of enrolled cases in their study.

SHARP1 is a basic helix-loop-helix transcription factor that is involved in different cellular processes, including proliferation, apoptosis, differentiation, and regulation of circadian rhythm[28,29]. A scant number of studies in endometrial, breast, and thyroid cancers detected a simultaneous inverse regulatory effect of SHARP1 on HIF-1α[12-14]. In our study, we found a significant decrease in maternal serum SHARP1 in preeclampsia patients compared with controls. Moreover, we demonstrated a negative correlation between the expression patterns of SHARP1 and HIF-1α. Previous molecular studies have speculated that SHARP1 binds to the HIF-1α subunit and directly directs HIF-1 to the proteasome for degradation under normal or low oxygen conditions with subsequent downregulation of HIF-1 responsive genes[30].

Indeed, few studies have evaluated the role of SHARP1 in preeclampsia. Ersoy and colleagues found that maternal serum SHARP1 levels in the second and third trimesters were lower in women with preeclampsia than in controls[15]. Moreover, Prakansamut & Phupong demonstrated that serum SHARP1 levels in the first trimester were effective for predicting preeclampsia. The authors showed significantly lower first trimester serum SHARP1 levels in preeclampsia women than in nonpreeclampsia women[31]. Similar to these findings, our study reveals a significant decrease in serum SHARP1 levels in preeclampsia compared to the control group.

The novelty of this study is the simultaneous use of a combination of SHARP1 and HIF-1α as markers for the detection of preeclampsia. In the current study, for the first time, we suggested that serum SHARP1 and HIF-1α may have the potential to be used as biomarkers for the detection

of preeclampsia. The cooperative action between HIF-1α and SHARP1 might play a key role in the pathogenesis of preeclampsia. Limitations of the study include that it was a single medical center and enrolled a relatively small number of patients.

CONCOLUSION

In conclusion, we demonstrated that SHARP1 and HIF-1α are candidate biomarkers for the detection of preeclampsia. In addition, both had potential in the pathogenesis of preeclampsia. We recommend larger, multicenter studies to establish a foundation for wider use of our results.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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