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Maternal serum perlecan and ischemia modified albumin levels as biomarkers of preeclampsia severity

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Abstract

Preeclampsia (PE) is a pregnancy disorder characterized by hypertension and end-organ damage. Reliable biochemical markers for diagnosis and prediction of PE severity can improve maternal health, and several of these markers have been suggested till now. The goal of our study was to evaluate maternal serum levels of Perlecan and Ischemia modified albumin (IMA) in PE patients, and to investigate their relationship with the severity. This study included 45 pregnant women, who were divided into three groups: mild PE (n=15), severe PE (n=10), and normal pregnant females (n=20) as a control group. Maternal serum levels of Perlecan and IMA were determined by the enzyme linked immunosorbent assay (ELISA). Preeclamptic women with severe features have significantly higher serum Perlecan and IMA levels than women with mild PE and control (P<0.001 for both). Serum levels of Perlecan and IMA were significantly increased in patients with mild PE as compared with control (P<0.001 for both). Serum Perlecan levels were positively correlated with systolic blood pressure (SBP), diastolic blood pressure (DBP), ALT, AST, creatinine, urea, uric acid, and proteinuria, but negatively correlated with platelet count and fetal birth weight. Serum IMA level was positively correlated with SBP, DBP, but negatively correlated with Albumin, and fetal birth weight. The receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of Perlecan and IMA in the prediction of PE severity. Serum Perlecan had greater sensitivity and lower specificity for severe PE than for mild PE. Serum IMA had greater sensitivity and lower specificity for severe PE than for mild PE. In conclusion, maternal serum Perlecan and IMA levels were biomarkers for monitoring PE and the increase in serum Perlecan levels was in accordance with the severity of PE. Also, Perlecan was superior to IMA as a predictor for PE severity.

Keywords: Preeclampsia, Perlecan, Ischemia modified albumin

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Introduction

Preeclampsia (PE) together with the other hypertensive disease of pregnancy is a major contributor to maternal mortality worldwide, PE complicating up to 2 to 8% of all pregnancies. Despite that maternal mortality in high-income countries is much lower than in developing countries, 16% of maternal deaths can be related to hypertensive disorders.[1]

PE is a dangerous pregnancy disorder, characterized by extensive vascular endothelial damage and vasospasm that occurs after the 20th week of pregnancy and continues until 4–6 weeks after birth. PE is characterized by gestational hypertension combined with one or more of the following conditions detected after ≥20 weeks of gestation: proteinuria, acute kidney injury, liver dysfunction, placental dysfunction, neurologic and hematologic features.[2] Unfortunately, the only treatment for PE is still delivery, and PE causes iatrogenic preterm birth in about 15% of pregnancies.[3]

PE may be life-threatening for both mother and child and is still a major cause of maternal-fetal morbidity and mortality. After preeclamptic pregnancies, the mother may develop premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life.[1] The children born to preeclamptic mothers are relatively small at birth. In addition, the children have an increased risk of developing coronary heart disease, stroke, and metabolic syndrome in adult life.[4]

Although the pathophysiology underlying the disease has been partially elucidated, the definitive mechanisms of PE remain unknown.[5] Many causes, such as an abnormal trophoblastic invasion of uterine blood vessels, placental ischemia, diffuse vasospasm, coagulation anomalies, oxidative stress, vascular endothelial damage, abnormal nitric oxide and lipid metabolism, genetic and nutritional factors, immunological intolerance between maternal and fetoplacental tissue, are attributed for etiology of PE.[6]

The presence of severely elevated blood pressure or other systemic features is defined as PE with severe features and can complicate up to 1% of all pregnancies. The accompanying severe features are associated with an increased risk of morbidity and mortality.[7]

Biochemical markers not only allow early detection of patients at risk of PE but also can help in classifying patients into different categories according to their severity for timely intervention. Many different biochemical markers have been investigated based upon the pathophysiology of the disease but their accuracy in predicting PE severity has been inconsistent.[8]

Perlecan is a proteoglycan found in the extracellular matrix that maintains endothelial cell function. It is abundant in the basement membrane and helps to maintain the endothelial barrier function and vascular homeostasis.[9] Perlecan can be detected at the exterior surface of trophectoderm during implantation and may play a regulatory role in the early phases of human trophoblast invasion which is a key pathway in the development of PE.[10] Autophagy plays an important role in trophoblast functions for normal placental development and involves in the pathophysiology of PE. Perlecan can enhance trophoblast invasion processes by binding to heparan sulfate interacting protein.[11]

One of the crucial roles of Perlecan is to promote angiogenesis. Furthermore, Perlecan appears to have an inhibitory effect on autophagy which may contribute to the pathophysiology of PE.[12,13] Placental hypoxia is a major feature in PE pathogenesis, and it has been found that Perlecan expression was increased in hypoxic conditions.[6]

Albumin is an important component of the antioxidant system. During oxidative stress, its structure is constantly subjected to modifications, resulting in a reduction in its ability to bind metals[14]. Subsequently, it is transformed into a new modified molecule known as ischemia modified albumin (IMA). The generation of reactive oxygen species (ROS) and free radicals temporarily changes the N-terminal region of albumin, resulting in an increase in IMA concentration.[15]

Pregnancy is always associated with oxidative stress and ROS generation. IMA

produced by ROS is found to be a sensitive and early biochemical marker of ischemic heart disease and can be utilized as an important marker to distinguish between ischemic and non-ischemic diseases.[16]

Oxidative stress could play a role in the development of PE. Defective endovascular trophoblast invasion and inadequate remodeling of uterine spiral arteries have been regarded as the causal factor. This defective invasion creates a hypoxic intrauterine environment, which results in the generation of oxidative free radicals.[16,17] Accumulation of biomarkers of oxidative stress accompanied by depletion of antioxidant reserves is considered a hallmark of PE.[18] IMA is an oxidatively modified form of albumin protein that is formed under oxidative stress. Because PE is associated with placental hypoxia and oxidative stress is implicated in its pathogenesis, maternal serum IMA could be a potential biomarker of PE.[19]

Because PE remains one of the most important obstetric complications, there have been ongoing studies on the biochemical markers for the early diagnosis of this disease and prediction of its severity. In the light of these previous data, the goal of this study was to evaluate maternal serum levels of Perlecan and IMA as biomarkers for monitoring PE patients and to investigate their correlation with the severity.

Subjects and Methods

Study design

This case-control study was conducted on 45 pregnant women, who were recruited from the outpatient clinic and the Department of Obstetrics and Gynecology of Al- Zahraa University Hospital, Faculty of Medicine for Girls, Al- Azhar University. The study was carried out during the period from July 2021 to March 2022. All preeclamptic patients enrolled in this study were diagnosed according to the standard criteria in the guidelines published by the American College of Obstetricians and Gynecologists (ACOG) in 2013.[20] Pregnant females with the following criteria were included in our study: A single-tone pregnancy, primigravida or multiparas, their age ranged from 20 to 40 years, with gestational age from 28 to 40 weeks; calculated according to the date of last menstrual cycle and confirmed by ultrasound.

Pregnant women with a bad obstetric history, twin pregnancy, previous medical diseases like diabetes mellitus, essential hypertension, peripheral vascular diseases, ischemic heart disease were excluded from the study. Also, thyroid, blood, renal, and hepatic disorders, and chronic autoimmune disease, hypercholesterolemia, smoking, and any associated disorders like urinary tract infection were excluded from this study. The participants of this study were divided into three groups: a mild PE group (n=15), a severe PE group (n=10) according to the standard criteria in the guidelines published by ACOG in 2013[20]. In addition, 20 apparently healthy pregnant females, matched for age, the gestational weeks, and demographic characteristics of the patient group were included as a control group.

The protocol of the study was reviewed and approved by the local ethical committee, Faculty of Medicine for Girls, Al- Azhar University, (approval number 912 dated 30/6/2021). Before enrollment in this study, informed written consent was obtained from all subjects after explaining the purpose of the study. The demographic and obstetric data were recorded on admission and the data was kept confidential. All subjects had the right to withdraw from the study without affecting their management. Full history taking, complete general and obstetrical evaluations of all pregnant women were performed, and fetal development was evaluated according to ultrasonographic measurements. Body mass index (BMI) was calculated through height and weight measurements (kg/m^2). Their systolic and diastolic blood pressure (SBP and DBP) values were recorded. Pregnancy outcomes were obtained by reviewing medical records of study subjects.

Blood sampling and measurements

Under a complete aseptic condition, 5 ml of whole venous blood was obtained by anti-cubital vein puncture of each study subject,

using a sterile disposable syringe. The collected blood sample was divided into three aliquots: the first one was evacuated into an EDTA tube as an anticoagulant for complete blood count (CBC) determination using a fully automated cell counter (Sysmex XP300, Kobe, Japan). The second aliquot was evacuated into a gel tube, allowed to clot and the serum obtained by centrifugation at 1776 xg for 10 minutes. Obtained sera were used for assessment of random blood sugar, albumin, uric acid, Na, K, liver, and kidney function tests using a fully automated auto-analyzer (COBAS INTEGRA 400 plus, Roche Diagnostics GmbH, Mannheim, Germany). The third aliquot was used to separate sera, kept frozen at -20 °C for further measurement of maternal serum Perlecan and IMA. Perlecan and IMA were measured using commercially available ELISA kits (Cat. No E1460Hu, Lot No 202111007 and Cat. No: E1172Hu, Lot No 209211009, respectively, both supplied by Bioassay Technology Laboratory and Shanghai Crystal Day Biotech CO., LTD, China) according to the manufacturer's instructions. An ELISA system which included a plate shaker-incubator (Thermo-Shaker from EU for Grant Instruments Ltd, Cambs, England) an ELISA washer (ELx50 Biokit, Italy) and a plate reader (AS 1851 from DAS, Italy), was used according to the manufacturer's instructions.

Random midstream urine was collected for protein estimation. The samples were collected and transported according to the standard guidelines[21]. Semi-quantitative dipstick method (Lot No 210323, Combo Stik urine reagent strip ™ series DFI CO., Ltd. Korea) was used to estimate the urine proteins. Two readings of 1+ for samples on two different occasions were considered diagnostic of PE. Serum IMA/albumin ratios (IMAR) were calculated. Pregnant women were followed till delivery for maternal and fetal outcomes. Fetal birth weight was obtained on a digital weight scale.

Ultrasonography

All cases underwent transabdominal ultrasound (US) examination using (LOGIQ V5 US equipped with a 3.5 Hz transabdominal probe, GE Healthcare MEDICAL SYSTEMS, CO., LTD, China) at admission for assessment of fetal viability and number, fetal biometry [Biparietal diameter (BPD)-Femur length (FL)-Abdominal circumference (AC)], placenta (site and maturity), and Liquor (amount described as an amniotic fluid index (AFI) and turbidity).

Statistical analysis

Data were collected, revised, coded, and entered in a computer using the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations, and ranges when their distribution was found parametric. Also, qualitative variables were presented as numbers and percentages. Paired-wise comparison (post hock test) between groups was done by using a one-way analysis of variance (ANOVA) test. Correlation between two quantitative parameters in the same group was done by using Spearman correlation coefficients. The best cut-off point with sensitivity, specificity, positive and negative predictive value, and area under the curve (AUC) for the prediction of severe PE patients was done by using the receiver operating characteristic (ROC) curve. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *P*-value was considered significant at the level of < 0.05.

Results

Statistically significant differences were found between the studied groups in terms of blood pressure; SBP (*P*< 0.001), DBP (*P*< 0.001), platelet (Plt) count (*P*< 0.001), ALT (*P*< 0.001), AST (*P*< 0.001), urea (*P*< 0.001), creatinine (*P*< 0.001), uric acid (*P*= 0.022), S albumin (*P*< 0.001), Na (*P*< 0.001), K (*P*< 0.001), proteinuria (*P*< 0.001) IMRA (*P*< 0.001), There were no statistically significant differences between the groups in terms of age, gravidity, parity, recurrent abortion, BMI, hemoglobin (Hb), number of red blood cells (RBCs), and white blood cells (WBCs) (Tables 1, 3).

Gestational age was significantly lower in severe PE than in other groups. The fetal birth weight was significantly lower in the severe PE group when compared with mild PE and control groups (*P*< 0.001) (Table 1, 2).

Table 1. Comparison between studied groups regarding pregnancy data.

Pregnant data		Control	Mild PE	Severe PE	*P* value	*P*1	*P*2	*P*3
Age (y) (Mean ±SD)		27.55±5.3	27.13±4.8	28.30±5.3	NS	NS	NS	NS
Gravidity (N, %)	PG	3 (15%)	8 (53.3%)	6 (60%)	NS	--	---	--
	G2	4 (20%)	2 (13.3%)	3 (30%)				
	G3	6 (30%)	4 (26.7%)	1 (10%)				
	G4	3 (15%)	0	0				
	G5	2 (105)	1 (6.7%)	0				
	G6	2 (10%)	0	0				
Recurrent Abortion (N, %)	No	19 (95%)	12 (80%)	7 (70%)	NS	---	---	---
	1	0	0	1 (10%)				
	2	0	2 (6.7%)	0				
	3	0	1 (6.7%)	0				
	4	1 (5%)	0	0				
GA (weeks) (Mean ±SD)		36.45±1.50	36.47±2.77	32.25±3.45	NS	0.001	0.009	NS
BMI (kg/m^2) (Mean ±SD)		26.13±2.01	28.13±4.21	29.15±2.35	NS	NS	NS	NS
Free medical history		15 (100%)	20 (100%)	10 (100%)	NS	--	--	--
Past history (N, %)	Free	8 (40%)	11 (73.3%)	9 (90%)	0.0160	--	--	--
	Positive	12 (60%)	4 (26.7%)	1 (10%)				
SBP (mmHg) (Mean ±SD)		107.50±7.16	146.67±4.8	165±5.27	< 0.001	0.033	0.0312	0.0220
DBP (mmHg) (Mean ±SD)		68±5.23	92.67±4.58	111 ±3.16	< 0.001	0.041	0.002	0.0045

*P*1: Normal vs severe PE, *P*2: Mild PE vs severe PE, *P*3: Normal vs mild PE, *$P \geq 0.05$ is not significant (NS). GA: Gestational age, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 2. Comparison between groups regarding fetal data, pregnancy data, and pregnancy outcome.

Fetal data	Control	Mild PE	Severe PE	*P*. value	*P*1	*P*2	*P*3
BPD (weeks) (Mean ±SD)	36.15 ±1.46	35.47±2.85	32.30 ±3.83	0.002	0.001	0.003	NS
FL (weeks) (Mean ±SD)	35.90 ±1.61	35.21 ±2.48	31.50 ±3.62	<0.001	<0.001	<0.001	NS
AC (weeks) (Mean ±SD)	36.05 ±1.70	34.46 ±2.85	31.30 ±3.77	<0.001	<0.001	<0.001	NS
HC (weeks) (Mean ±SD)	33.15 ±1.59	33.40 ±2.52	31.20 ±3.85	0.087	NS	NS	NS
AF Index (ml) (Mean ±SD)	13.83 ±1.07	12.40 ±2.08	11.10 ±1.52	<0.001	<0.001	<0.001	< 0.001
Fetal weight (gm) (Mean ±SD)	3020 ±347.32	2820 ±298.08	2005 ±332.02	<0.001	<0.001	<0.001	NS

Table 2. (Continued).

Fetal data		Control	Mild PE	Severe PE	*P*. value	*P*1	*P*2	*P*3
Apgar score (1 min) (Mean ±SD)		1 ±0.00	1 ±0.00	1.30 ±0.67	<0.001	<0.001	<0.001	NS
Apgar score (5 min) (Mean ±SD)		8.45 ±0.82	7.80 ±1.08	5.10 ±0.87	<0.001	<0.001	< 0.001*	< 0.001*
Placenta (N, %)	Fundal	0	3 (20%)	5 (50%)	0.0138	--	--	--
	Fundal ant	10 (50%)	6 (40%)	4 (40%)		--	--	--
	Fundal post	10 (50%)	6 (40%)	1 (10%)		--	--	--
Gender (N, %)	Male	12 (60%)	8 (33.3%)	4 (40%)	NS	--	--	--
	Female	8 (40%)	7 (46.7%)	6 (60%)		--	--	--
Mode of delivery (N, %)	CS	18 (90%)	13 (86.7%)	10 (100%)	NS	--	--	--
	SVD	2 (10%)	2 (13.3%)	0		--	--	--
NICU (N, %)		0	1 (6.7%)	8 (80%)	<0.0001	--	--	--
Fetal outcome (N, %)	Normal	20 (100%)	14 (93.3%)	2 (20%)	<0.0001	--	--	--
	Preterm	0	1 (6.7%)	8 (80%)		--	--	--
ICU admission (N, %)	Yes	0	0	10 (100%)	<0.0001	--	--	--
	No	20 (100%)	15 (100%)	0		--	--	--

*P*1: Normal vs severe PE, *P*2: Mild PE vs severe PE, *P*3: Normal vs mild PE, *$P \geq 0.05$ is not significant (NS). BPD: Bi-parietal diameter, FL: Femur length, HC: Head circumference, AF Index: Amniotic fluid index. CS: cesarean section, VD: vaginal delivery, NICU: Neonatal intensive care unit.

Serum sodium levels were decreased significantly in the preeclamptic cases (mild and severe) as compared with the control group (*P*<0.001). There was a significant decrease in serum potassium levels (*P*<0.001) between the groups. IMAR was significantly increased in the severe PE group when compared with mild PE and control groups (*P*< 0.001) (Table 3).

Table 3. Comparison between studied groups regarding laboratory data.

Laboratory data (Mean ±SD)	Control	Mild PE	Severe PE	*P* value	*P*1	*P*2	*P*3
Hb (gm/dl)	10.80 ±0.96	11.07±1.06	11.23 ±1.20	NS	NS	NS	NS
PLT ($x10^3/mm^3$)	298.10±54.01	171.5 ±17.3	113.1 ±12.5	< 0.001	< 0.001	< 0.001	< 0.001
RBCS ($x10^6/mm^3$)	4.15 ±0.40	4.41 ±0.99	4.13 ± 0.46	NS	NS	NS	NS
WBCS ($x10^3/mm^3$)	8.73 ±1.44	9.33 ±1.57	8.11 ±2.05	NS	NS	NS	NS
RBS (mg/dl)	96.65±14.07	97 ±8.67	103.9 ±9.02	NS	NS	NS	NS
Uric Acid (mg/dl)	3.46±0.60	5.39 ±0.54	6.64±0.91	< 0.001	0.012	0.015	0.021
ALT (U/L)	13.15 ±3.21	27.40 ±2.16	36.30 ±3.05	< 0.001	< 0.001	< 0.001	< 0.001
AST (U/L)	11.05 ±1.79	28.60 ±2.84	37.20 ±1.75	< 0.001	< 0.001	< 0.001	< 0.001
Urea (mg/dl)	16.45 ±4.21	30 ±3.85	40.5 ±5.7	< 0.001	< 0.001	< 0.001	< 0.001
Creatinine (mg/dl)	0.69 ±0.15	0.96 ±0.14	1.4 ±0.09	< 0.001	< 0.001	< 0.001	< 0.001

Table 3. (Continued).

Laboratory data (Mean ±SD)	Control	Mild PE	Severe PE	*P value	P1	P2	P3
S. Albumin (gm/dl)	4.04 ±0.29	3.47 ±0.36	2.65 ±0.40	< 0.001	< 0.001	< 0.001	< 0.001
Na (m Eq/L)	144.9 ±1.4	139.7 ±1.6	136.65 ±1.34	< 0.001	< 0.001	< 0.001	< 0.001
K (m Eq/L)	4.30 ±0.38	3.27 ±0.20	2.77 ±0.27	< 0.001	< 0.001	< 0.001	< 0.001
Urine Albumin (+) (proteinuria)	0.00 ±0.00	1.26±0.45	2.70 ±0.48	< 0.001	< 0.001	< 0.001	< 0.001
IMAR	8.36 ±1.63	12.75±3.25	24.65 ±4.18	< 0.001	< 0.001	< 0.001	< 0.001

*P*1: Normal vs severe PE, *P*2: Mild PE vs severe PE, *P*3: Normal vs mild PE, *$P \leq 0.05$ is significant. Hb: Hemoglobin, PLT: platelet, RBCs: Red blood cells, WBCs: White blood cells, RBS: Random blood sugar, ALT: alanine transaminase, AST: aspartate transferase, Na: Sodium, K: Potassium, IMAR: Ischemia modified albumin ratio.

Serum Perlecan and IMA levels were significantly elevated in the severe PE group when compared with mild PE and control groups (*P*< 0.001 for both) (Table 4) (Figures 1, 2).

Table 4. Comparison between groups regarding studied biomarkers.

Biomarkers	Control	Mild PE	Severe PE	*P value	P1	P2	P3
Perlecan (ng/ml) (Mean ±SD)	215.6±32.1	324.8±29.8	454.5±44.8	< 0.001	< 0.001	< 0.001	< 0.001
IMA (ng/ml) (Mean ±SD)	32.1 ±5.2	47.7 ±5.3	62 ±4.9	< 0.001	< 0.001	< 0.001	< 0.001

*P*1: Normal vs severe PE, *P*2: Mild PE vs severe PE, *P*3: Normal vs mild PE, *$P \leq 0.05$ is considered significant. IMA: Ischemia modified albumin.

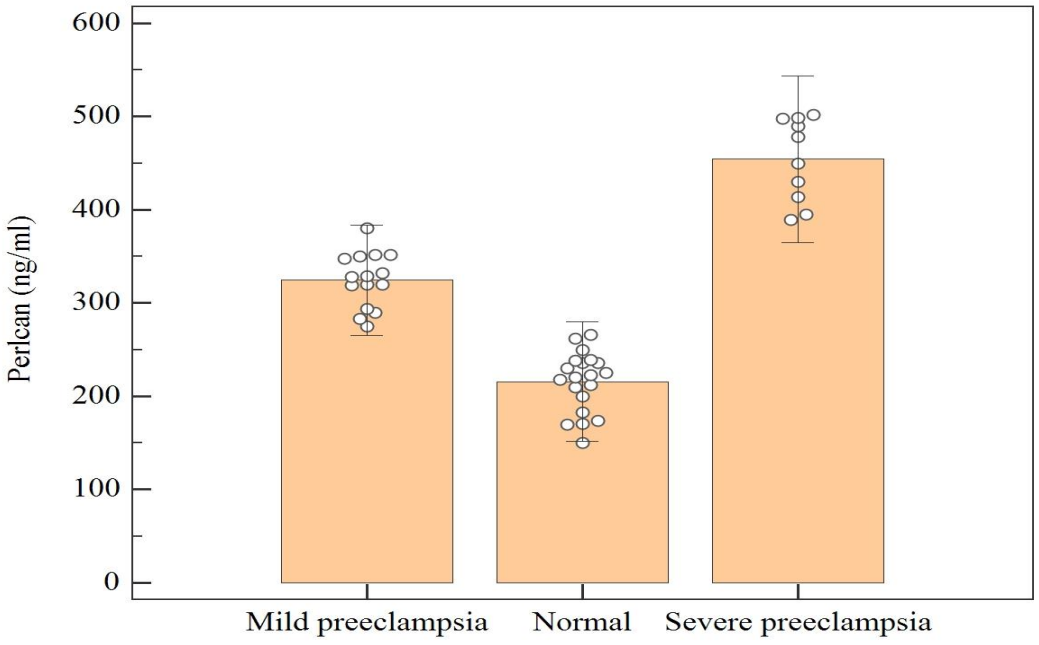

Figure 1. Box-plot graph of Perlecan level in mild PE, normal control, and severe PE groups.

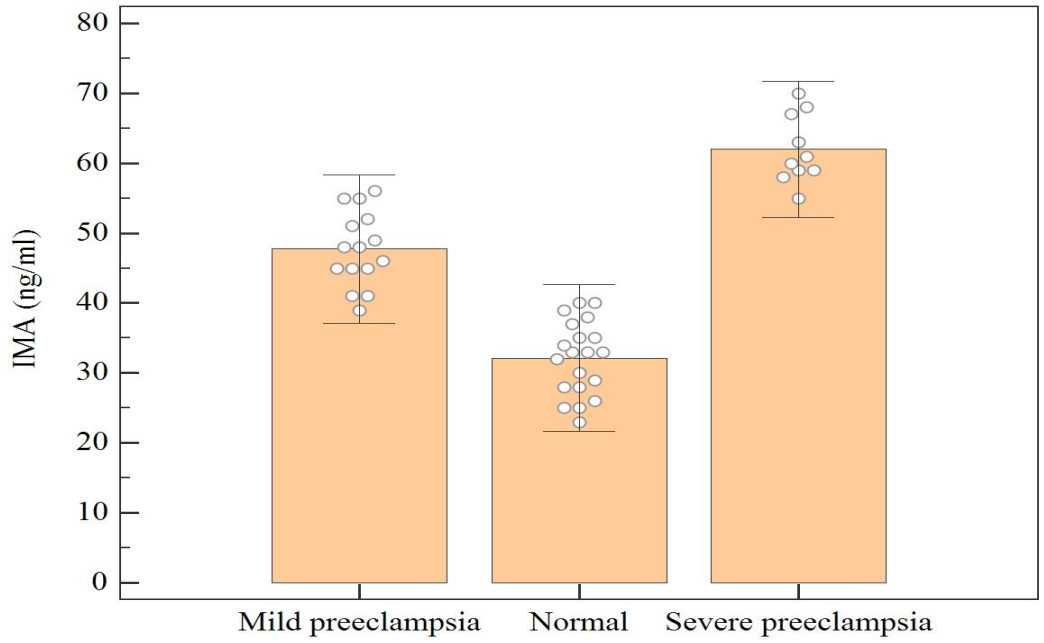

Figure 2. Box-plot graph of IMA level in mild PE, normal control, and severe PE groups.

In severe PE group; serum Perlecan level was positively correlated with SBP (r= 0.878, *P*= 0.0008), DBP (r= 0.924, *P*= 0.0001), ALT (r= 0.917, *P*=0.0002), AST (r=0.948, *P*< 0.0001), urea (r=0.898, *P*< 0.0001), creatinine (r= 0.904, *P*= 0.0003), uric acid (r=0.721 *P*=0.0221) and urinary albumin (proteinuria) (r= 0.850, *P*= 0.001), however, was negatively correlated with PLT (r= -0.936, *P*= 0.0001) and maternal age (r= -0.929, *P*= 0.0001) (Tables 5, 7). Also, similar correlation results were found between serum Perlecan level and the same previous parameters in mild PE group expect for blood pressure, as shown in (Tables 5, 7).

In severe PE group, serum IMA level was positively correlated with SBP (r= 0.778, *P*= 0.008), DBP (r= 0.776, *P*= 0.04), but negatively correlated with Alb (r= -0.931, *P*= 0.0001) and fetal birth weight (r= -0.930, *P*= 0.0001) (Tables 5,6,7). Also, similar correlation results were found between serum IMA level and the same previous parameters in mild PE group as shown in (Table 5, 6, 7).

Table 5. Pearson correlation between biomarkers and pregnancy data.

Pregnant data	Mild preeclampsia				Severe preeclampsia			
	Perlecan		IMA		Perlecan		IMA	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
Age (y)	-0.9043	<0.0001	0.205	NS	-0.929	0.0001	-0.054	NS
GA (weeks)	-0.479	NS	0.212	NS	0.180	NS	-0.259	NS
BMI (kg/m^2)	-0.105	NS	0.113	NS	0.250	NS	-0.478	NS
SBP (mmHg)	-0.396	NS	0.761	0.0010	0.878	0.0008	0.778	0.008
DBP (mmHg)	0.263	0.344NS1	0.793	0.0004	0.924	0.0001	0.776	0.0421

*$P \geq 0.05$ is not significant (NS). GA: Gestational age, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 6. Pearson correlation between biomarkers and fetal data.

Fetal data	Mild preeclampsia				Severe preeclampsia			
	Perlecan		IMA		Perlecan		IMA	
	r	**P* value	r	**P* value	r	**P* value	r	**P* value
BPD (weeks)	-0.495	NS	0.084	NS	0.162	NS	-0.125	NS
FL (weeks))	-0.444	NS	-0.042	NS	0.082	NS	-0.170	NS
AC (weeks)	-0.312	NS	0.094	NS	0.067	NS	-0.163	NS
HC (weeks)	-0.322	NS	0.056	NS	0.109	NS	-0.130	NS
AF Index (ml)	0.329	NS	-0.163	NS	0.587	NS	-0.179	NS
Fetal weight (gm)	-0.146	NS	-0.955	<0.0001	0.005	NS	-0.930	0.0001
Apgar score (1min)	000	---	0.000	--	0.414	NS	-0.169	NS
Apgar score (5min)	-0.033	NS	-0.394	NS	0.251	NS	-0.130	NS

BPD: Bi-parietal diameter, FL: Femur length, HC: Head circumference, AF Index: Amniotic fluid index.
**P* ≥ 0.05 is not significant (NS).

Table 7. Pearson correlation between biomarkers and laboratory data.

Laboratory data	Mild preeclampsia				Severe preeclampsia			
	Perlecan		IMA		Perlecan		IMA	
	r	*P* value	r	*P* value	R	*P* value	r	*P* value
Hb (gm/dl)	0.053	NS	0.165	NS	-0.235	0.081	0.259	NS
Plt ($x10^3/mm^3$)	-0.951	<0.0001	-0.661	0.0073	-0.936	0.0001	0.192	NS
RBCS ($x10^6/mm^3$)	0.157	NS	-0.366	NS	-0.631	NS	0.286	NS
WBCS ($x10^3/mm^3$)	-0.096	NS	0.043	NS	-0.473	NS	-0.134	NS
RBS (mg/dl)	0.247	NS	0.433	NS	0.240	NS	-0.010	NS
Uric Acid (mg/dl)	0.964	<0.0001	0.234	NS	0.721	0.0221	0.088	NS
ALT (U/L)	0.944	<0.0001	0.363	NS	0.917	0.0002	0.246	NS
AST (U/L)	0.969	<0.0001	0.336	NS	0.948	<0.0001	-0.221	NS
Urea (mg/dl)	0.9574	<0.0001	0.264	NS	0.898	<0.0001	0.481	NS
Creatinine (mg/dl)	0.930	<0.0001	0.397	NS	0.904	0.0003	0.307	NS
S. Albumin (gm/dl)	0.453	NS	-0.927	<0.0001	0.309	NS	-0.931	0.0001
Na (mEq/L)	0.388	NS	-0.251	NS	-0.088	NS	-0.442	NS
K (mEq/L)	0.239	NS	-0.371	NS	0.618	NS	-0.401	NS
Urine Albumin (+) (proteinuria)	0.711	0.002	0.382	NS	0.850	0.001	0.412	NS
IMAR	0.092	NS	0.487	NS	-0.235	NS	-0.102	NS

**P* ≥ 0.05 is not significant (NS).Hb: Hemoglobin, Plt: platelet, RBCs: Red blood cells, WBCs: White blood cells, RBS: Random blood sugar, ALT: , AST: , Na: Sodium, K: Potassium, IMAR: Ischemia modified albumin ratio.

The ROC curve was analyzed for Perlecan in the severe and mild PE groups and the sensitivity and specificity were calculated based on the best cut-off. At Perlecan cut-off point of > 380 ng/ml women with severe PE were identified with a sensitivity of 93.3% and specificity of 90%. These showed higher sensitivity and lower specificity than mild PE, where at a cutoff of ≤ 380 ng/ml, the sensitivity and specificity were 90% and 93.3%, respectively. ROC was analyzed for IMA, in the severe and mild PE groups and the sensitivity and specificity were calculated based on the best cut-off. At IMA cut-off point of > 56 ng/ml women with severe PE were identified with a sensitivity of 92.9% and specificity of 72.7%. These showed higher sensitivity and lower specificity than mild PE, where at a cutoff of ≤ 56 ng/ml, the sensitivity and specificity were 72.7% and 92.9%, respectively (Tables 8, 9, and Figures 3,4,5,6).

Table 8. Prognostic value of biomarkers for severe preeclampsia by the receive operating characteristic (ROC) curve analysis.

Severe preeclamsia	Perlecan	IMA
Area under curve (AUC)	0.847	0.831
Cut off level	>380	>56
**P* value	0.0008	0.0004
95% confidence interval	0.647 to 0.958	0.629 to 0.950
Sensitivity %	93.3	92.9
Specificity %	90	72.7
Positive predictive value (PPV %)	93.3	81.2
Negative predictive value (NPV %)	90	88.9

**P* ≤ 0.05 is considered significant.

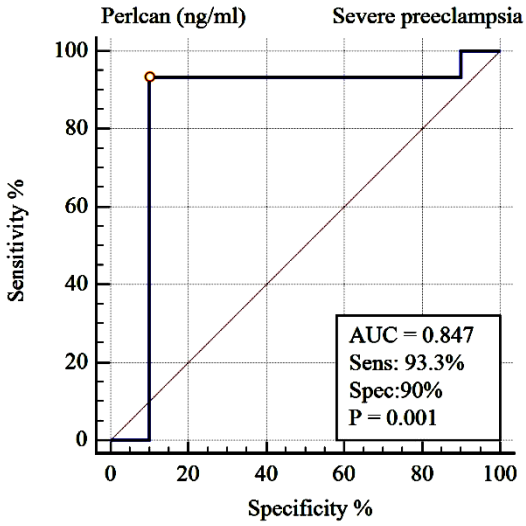

Figure 3. Receiver operating characteristic (ROC) curve showing diagnostic performance of Perlecan biomarker for severe PE. Area under curve (AUC) equal 0.847, with sensitivity 93.3% and specificity 90%.

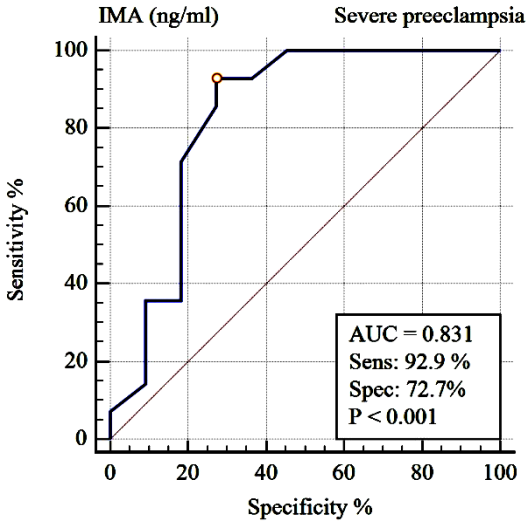

Figure 4. Receiver operating characteristic (ROC) curve showing diagnostic performance of IMA biomarker for severe PE. Area under curve (AUC) equal 0.831, with sensitivity 92.9% and specificity 72.7%.

Table 9. Prognostic value of biomarkers for mild preeclampsia by receiver operating characteristic (ROC) curve analysis.

Mild preeclamsia	Perlecan	IMA
Area under curve (AUC)	0.847	0.831
Cut off level	≤380	≤56
P value	0.0008	0.0004
95% confidence interval	0.647 to 0.958	0.629 to 0.950
Sensitivity %	90	72.7
Specificity %	93.3	92.9
Positive predictive value (PPV %)	90	88.9
Negative predictive value (NPV %)	93.3	81.2

*$P \leq 0.05$ is considered significant.

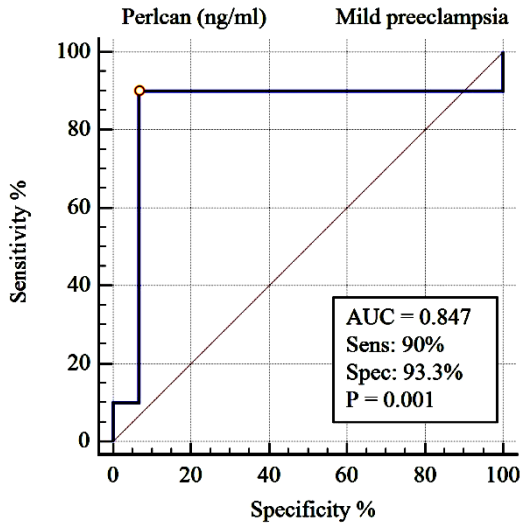

Figure 5. Receiver operating characteristic (ROC) curve showing diagnostic performance of Perlecan biomarker for mild PE. Area under curve (AUC) equal 0.847, with sensitivity 90% and specificity 93.3%.

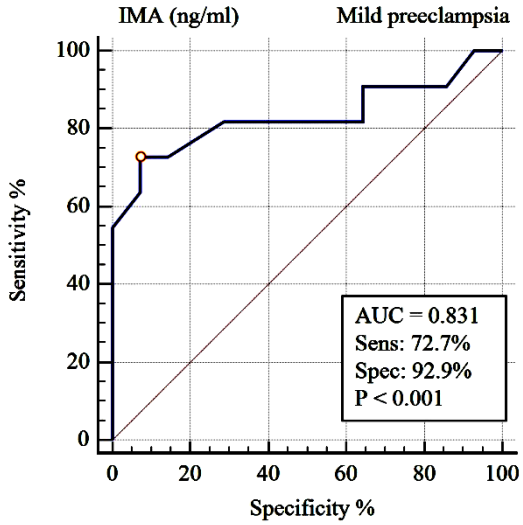

Figure 6. Receiver operating characteristic (ROC) curve showing diagnostic performance of IMA biomarker for mild PE. Area under curve (AUC) equal 0.831, with a sensitivity 72.7% and specificity 92.9%.

Discussion

Preeclampsia is a pregnancy-specific hypertensive disorder characterized by hypertension and proteinuria as well as multisystem involvement. It is one of the most serious clinical conditions that can occur during pregnancy and can seriously affect the life of the mother and the baby.[22,23] Although the pathophysiology of PE is not fully understood, Perlecan contributes to angiogenesis and autophagy inhibition, as well as IMA inducing failure of the trophoblastic invasion of spiral arteries. These two serum biomarkers may play roles in PE pathogenesis, so they may be potential diagnostic biomarkers of PE.[24,25]

In our study, we observed a significantly elevated serum level of Perlecan in severe PE as compared with mild PE and control groups ($P < 0.001$), this was in accordance with a study carried out by Akbas et al., 2020[11] in which

serum Perlecan level was found to be higher in patients with severe PE. Their study is important as it was a preliminary study that suggested that Perlecan, through its effects on placental development and regulation of autophagy, may play a role in the pathogenesis of PE. They explained the increase in its level in PE as circulating concentrations of Perlecan may reflect a combination of both maternal endothelial and placental sources. Infarcted areas of the placenta and endothelial injury may contribute to an increase in its serum level in the severe PE group.[11]

Comparable to our findings, Erkayıran et al., 2021 [24] found that serum Perlecan levels were higher in severe PE cases than in mild PE and control groups, they demonstrated that the reduced nitric oxide bioavailability and the degree of endothelial cell dysfunction might be related to the difference in the serum Perlecan levels between PE with severe features and mild PE groups. The high level in both severe and mild PE cases, as observed in our study, support the notion that Perlecan may play a role in the pathogenesis of PE. The high level of Perlecan in PE may indicate that Perlecan is secreted as a defense molecule against abnormal processes in the body. It is a protective molecule whose level increases as a response to the abnormal autophagic reaction that contributes to the pathogenesis of PE.[26]

Contrary to our study findings, a study by Chui et al., 2012[27] found that protein expression of Perlecan and its mRNA were significantly reduced in PE cases, they explained these findings by saying that Perlecan has an angiogenesis-inducing effect and a decrease in its serum level contributes to the pathogenesis of PE by reducing trophoblast invasion. The small sample size of our groups could explain these unexpected and divergent results

Our study demonstrated that serum Perlecan levels was positively correlated with SBP, DBP, ALT, AST, urea, creatinine, uric acid, and proteinuria but negatively correlated with Plt and maternal age in the severe PE group. Such findings agreed with those of a study conducted by Erkayıran et al., 2021[24] who showed that increased Perlecan levels in severe PE group were in proportion to systolic, diastolic blood pressures, liver, and kidney function tests. They also demonstrated that there was a relationship between serum Perlecan level and PE severity. They suggested that as the severity of PE increases, the severity of autophagy, which may be responsible for the pathogenesis process, also increases, and the level of Perlecan increases as a defense mechanism to prevent it.

We also found a negative correlation between serum Perlecan levels and maternal age, which was consistent with the findings of a study by Akbas et al., 2020.[11] On the other hand, they found no correlation between maternal serum Perlecan levels and other parameters of PE severity in mild and severe cases. This could be explained by differences in the severity of PE and the small sample size of our study groups.

The exact pathogenesis of PE has not been clearly elucidated.[24] However, the oxidative damage that occurs in the placenta causes inflammation, apoptosis, and the release of anti-angiogenic agents and cytokines into the maternal circulation. These placenta-originating substances which enter the maternal circulation cause endothelial dysfunction, which is the key component of PE pathophysiology.

Consequently, the investigation of serum IMA generated by hypoxia/ischemia-driven oxidative stress can be useful in this matter.[3,25] In our study, we observed significantly elevated serum levels of IMA in severe PE as compared with mild PE and control groups ($P< 0.001$). Our results agreed with those of the study done by Karasin and Cift, 2020,[25] which showed that inflammation and endothelial cell activity in PE may alter the albumin molecule in the plasma of preeclamptic patients and may cause an increase in its levels. Also, oxidative stress and a hypoxic environment predominant PE may cause ROS increase, which may cause several changes in IMA levels.

Also, findings of two studies by Bahinipati et al., 2014[28] and D'Souza et al., 2014[29] supported our results as they found, increased serum levels of IMA in PE patients. They demonstrated that maternal serum IMA levels increased in PE, reflecting the oxidative stress associated with placental development. In addition, a study by

Papageoghiour et al., 2008[30] found an early elevation of serum IMA, at 11-12 weeks of gestation in women who developed PE later. They stated that poor placental perfusion causes hypoxia and oxidative stress which leads to PE. These changes occur during the development of the placenta, and hence serum IMA could be an early marker of PE.

However, a study done by Gursoy et al., 2017[31] showed no significant difference in maternal and cord blood IMA levels between the PE and normal pregnant (NP) controls. In addition, a study by Van Rijn et al., 2008[32] found that serum IMA levels were higher in NP controls as compared to the non-pregnant controls, but the IMA levels in PE were comparable to those of NP controls. The discrepancy between these studies could possibly be explained by the smaller number of patients and varying degrees of PE severity.

In our study, we found that serum IMA levels were positively correlated with SBP and DBP, but negatively correlated with albumin, and fetal birth weight which was consistent with data reported by Ustun et al., 2011,[33] who observed a significant positive correlation between serum IMA levels and blood pressure. A study by D'Souza et al., 2014[29] also, found a significant negative correlation between serum IMA levels and albumin and fetal birth weight. They suggested that the negative correlation between IMA and albumin may indicate that IMA is a product of the free radical-mediated oxidative damage to albumin which is associated with PE. Another study by Van Rijn et al., 2008[32] agreed with our results as they showed a negative correlation between serum IMA and fetal birth weight in PE. This could be explained by the notion that impaired placental oxidative stress is commonly related to fetal growth retardation in PE. The oxidative stress may be responsible for the severity of PE and the associated decreased fetal birth weight[34]. Also, the cord blood of neonates of complicated pregnancies was reported to have significantly higher IMA levels.[35]

In our study, no correlation was found between serum IMA levels and parameters of PE severity (except for blood pressure). This observation agreed with that of a study by Karaşin and Cift, 2020[25] who showed no correlation between serum IMA levels and PE severity. One of the reasons for this observation is that PE is a multifactorial disorder. Another possible reason for such observation could be the limited number of our study patients.

We observed significantly elevated serum levels of uric acid, urea, and creatinine in severe PE as compared with mild PE and control groups ($P< 0.001$ for all). Preeclampsia is known to be associated with renal dysfunction due to glomerular endothelial injury causing a reduction in glomerular filtration rate (GFR). Various studies have demonstrated elevated serum levels of renal markers, such as urea, creatinine, and serum uric acid in PE.[36] In agreement with our findings two studies by Padma et al., 2013[37] and Taefi et al., 2008,[38] showed that serum uric acid and creatinine levels were elevated in severe PE, due to decreased urinary clearance secondary to reduced GFR and increased reabsorption. Serum uric acid is not only a marker of the severity of the disease but can also contribute to the pathology of the disease. Uric acid is a product of purine catabolism, and according to Bainbridge & Roberts, 2008[39], hyperuricemia in PE is a multifactorial condition, elevated levels of uric acid are not only attributed to decreased renal clearance but also to increased oxidative stress resulting from placental ischemia and xanthine oxidase activity.

We found that the mean fetal birth weight was significantly lower in severe PE when compared with mild PE and the control group ($P< 0.001$). This agreed with that of the study carried out by Padma et al., 2013,[37] who demonstrated a significant reduction in the fetal birth weight in PE when compared with gestational hypertension and NP. In addition, a study by Vyakaranam et al., 2015[36] found a significant negative correlation between serum uric acid level and fetal birth weight in PE. Also, a study by Hawkins et al. 2012[40] showed that increased uric acid levels more than 5.9 mg/dL were associated with adverse fetal outcomes such as small for gestational age and preterm birth. Because placental hypoxia contributes to high serum uric acid levels in PE, it acts as a better indicator for hypoxia of fetal outcomes

than maternal distress.[37] Two studies by Hawkins et al., 2012[40] and Livingston et al., 2014[42] concluded that uric acid is clinically more beneficial in predicting fetal outcomes than maternal.

In our study, serum sodium and potassium levels were significantly lower in severe PE cases as compared with mild PE and control groups ($P< 0.001$ for both). These findings agreed with those of a study by Roy and Bachu, 2019,[8] they explained such observations as there is an alteration in sodium transport across the cell membrane leading to sodium accumulation extra vascularly with decreased plasma sodium level in PE patients. On the other hand, a study by Tabassum & Al-Jameil, 2015[43] found higher serum sodium and potassium levels in severe PE as compared with mild PE and control groups. Such discrepancy in the study findings may be explained by different sample sizes.

Pregnancy is associated with haemodilution leading to decrease in plasma albumin level.[18] Hence, we normalized IMA to albumin by calculating IMA/Albumin ratio. We noted significantly higher IMA levels and the associated IMAR in the serum of severe PE as compared to mild PE and control groups ($P<0.001$ for both). These observations are in accordance with those of studies done by D'Souza et al., 2014[29] and Roy & Sahana, 2019[18] they found a significantly higher IMA to albumin ratio in severe PE cases. They indicated that this may be due to the increased production of free radicals from the hypoxic placental environment in PE, as IMA is well known to be formed in such conditions. They suggested that measurement of this oxidative biomarker may be beneficial in monitoring pregnancies with respect to the development of PE.

Also, a previous study by Roy & Bachu, 2018[8] found a higher IMRA ratio in severe PE as compared with mild PE and control groups. They demonstrated that PE is associated with placental hypoxia which causes ischemic reperfusion injury resulting in the generation of free radicals which in turn causes alteration of NH2 terminus of human serum albumin resulting in decreased binding of albumin to cobalt and higher IMA level compared to NP control. Also, a study by Aydin et al., 2020 [15] found the IMA/albumin ratio was higher in the early-onset and late-onset PE patients, compared to the control group.

The ROC curve analysis was performed to evaluate the diagnostic performance of Perlecan and IMA in the prediction of PE severity. A study by Vyakaranam et al., 2015 [44] reported comparable data. They observed that serum IMA with a cutoff of 38.33 ng/ml had greater sensitivity (88.9%) and specificity (73.7%) for PE cases than for preeclampsia induced hypertension with a cutoff of 22.9 ng/ml, sensitivity (27.8%), and specificity (89.5%).

In conclusion, maternal serum Perlecan could be used as a novel, simple, and inexpensive indicator of PE severity. Continuous monitoring of Perlecan level could give an indication about the progression of pregnancy and any increase or deviation in its levels may indicate complications of pregnancy. Perlecan outperformed IMA in predicting the severity of PE. An abnormal increase in IMA levels could signal pregnancy complications, including oxidative damage and the development of PE. Measuring these two serum biomarkers may allow earlier intervention to the complications and the reduction of maternal morbidity and mortality.

Author Contributions

ERM, SE; performed the laboratory work. EEE, MMH, and MGA; made the statistical analysis. FAA; examined the patients. FAA, ERM; collected samples. All authors participated in writing and reviewing the paper.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the local ethical committee, Faculty of Medicine for Girls, Al- Azhar University, (approval number 912 dated 30/6/2021).

Informed consent

Informed written consent was obtained from all subjects after explaining the purpose of the study.

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