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ORIGINAL ARTICLE

Evaluation of Maternal Serum Amyloid A as a Biomarker for Preeclampsia

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ABSTRACT

Background*: In current years, there has been a significant improvement in the management of preeclampsia, which has led to a decrease in mortality and morbidity. The challenge lies in its early diagnosis, when there are no apparent clinical signs, serum amyloid-A might be expected to play a role in the early detection and evaluation of preeclampsia. Objective: To investigate and evaluate the maternal serum levels of Amyloid-A as a biomarker for the prediction of preeclampsia.*

Patients and method*: A case-control study carried at Department of Obstetrics and Gynecology/Al-Yarmouk Teaching Hospital. Methods: This study was conducted during the period from January 2021 to October 2021. A convenient sample of 90 pregnant women was enrolled and divided into two groups; Cases group (consisted of 45 pregnant women who presented with preeclampsia) and control group (consisted of 45 normotensive healthy pregnant women matched with the study group for age, body mass index and gestational age). The level of serum amyloid-A, blood urea, serum creatinine, aspartate aminotransferase, alanine aminotransferase, platelets count were measured.*

Results*: The level of Amyloid-A was significantly higher in the preeclampsia group than in the control group (P-value<0.001). According to the cut-off point of 12.25 (ng/dl), 51% of the participants had positive amyloid-A. There was a significant association between the results of Amyloid-A and the development of preeclampsia, the sensitivity and specificity were 95.6, 93.3, respectively (P-value<0.001). The level of Amyloid-A significantly correlated with the level of aspartate aminotransferase and alanine aminotransferase (P-value<0.001 for both).*

Conclusion*: There is significant association between the level of maternal serum amyloid-A and the development of preeclampsia in pregnancy.*

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1. INTRODUCTION

Preeclampsia (PE) is a multi-system disorder characterized by the new onset of hypertension and proteinuria and/or end-organ dysfunction after 20weeks in a patient who was previously noted to be normotensive(1, 2). Clinically, PE is defined as hypertension of at least 140/90 mmHg on two separate occasions ≥4 hours apart accompanied by significant proteinuria of at least 0.3 g in a 24-hour collection of urine, >30 mg/mmol protein/creatinine ratio, or two 'clean-catch – midstream' or catheter specimens of urine collected ≥4 h apart with ≥1+ on reagent strip arising firstly after the 20th week of gestation in a previously normotensive woman and resolving completely by the 6th postpartum week(3, 4). Because of the physiological changes during pregnancy, proteinuria below 300 mg/day is considered normal for pregnant women(5). Protein excretion in the urine increases in normal pregnancy from approximately 5 mg/dL in the first and second trimesters to 15 mg/dL in the third trimester. These low levels are not detected by dipstick. The concentration of urinary protein is influenced by contamination with vaginal secretions, blood, bacteria, or amniotic fluid. It also varies with urine specific gravity and pH, exercise, and posture. Proteinuria usually appears after hypertension in the course of the disease process, but in some women, it may appear before hypertension(6). The gold standard method to quantify proteinuria is a 24-hour urine protein measurement although several methods are introduced to be used universally (5). Hypertensive disorders of pregnancy are a common problem worldwide and one of the three factors of maternal mortality in reproductive age(7). They affect about 5% to 10% of all pregnancies(8), and account for about 18% of maternal deaths worldwide(9). According to the World Health Organization (WHO), the estimated incidence of hypertensive disorders of pregnancy is seven times higher in developing countries as compared to developed countries and the risk of maternal deaths in low- income countries is 300 times that of high-income(10). Hypertensive disorders of pregnancy are classified into four categories include chronic hypertension, gestational hypertension, preeclampsia-eclampsia, and chronic hypertension with superimposed PE(11).

• Chronic hypertension predating pregnancy or diagnosed before 20 weeks of pregnancy or present 12 weeks after delivery(12).

• Gestational hypertension is hypertension firstly arising after 20 weeks gestation in the absence of proteinuria and without biochemical or hematological abnormalities. It is usually not accompanied by fetal growth restriction. Outcomes in pregnancies complicated by gestational hypertension are usually good, but about a quarter of women with gestational hypertension (particularly those who present at <34 weeks) will progress to PE and have poorer outcomes(13).

• Preeclampsia is hypertension developing after 20 weeks gestation with proteinuria and edema, while eclampsia is a severe complication of PE. It's a rare but serious condition where high blood pressure results in seizures during pregnancy(14, 15).

• Chronic hypertension with superimposed PE is associated with increased maternal and perinatal morbidity compared with PE alone. Distinguishing superimposed PE from chronic hypertension can be challenging because, in chronic hypertension, the traditional criteria for the diagnosis of PE, hypertension, and significant proteinuria can often predate the pregnancy. Furthermore, the prevalence of superimposed PE is unlikely to be uniformly distributed across this high-risk group but is related to the severity of preexisting endothelial dysfunction(16).

Serum Amyloid-A:

Homeostasis is essential for most biological systems. Both primordial and adaptable mechanisms exist for reestablishing this important state following perturbations. Among the former, the so-called “acute-phase response” (APR) is prominent. The APR comprises a stereotyped set of physiologic changes that occur as a consequence of inflammation, infection, trauma and other events. The APR involves many physiologic responses including fever, hormonal changes, metabolic alterations. Changes in serum protein levels during the APR are particularly remarkable. Among these, altered serum levels of C-reactive protein (CRP) and serum amyloid-A are the most notable. Proteins and genes of the serum amyloid-A family are particularly prominent in the APR (17). The serum amyloid-A gene family is located on the short arm of chromosome 11, it contains four genes, namely serum amyloid-A 1, serum amyloid-A 2, serum amyloid-A 3 and serum amyloid-A 4. All the genes consist of 4 exons and 3 introns, and their initial transcripts have an 18 amino acids signal sequence that is removed in the serum proteins. Within the serum amyloid-A gene cluster, only the serum

amyloid-A 1 and serum amyloid-A 2 genes encode acute phase serum proteins with approximately 95% sequence identity, which are coordinately induced in response to inflammation (18,19), serum amyloid-A 3 contains an early stop codon suggesting it is a non-translated pseudogene, while serum amyloid-A 4 encodes for a constitutively expressed isoform, meaning it is not induced in the acute phase response (19,20).

2. METHODOLOGY

Study design and setting:

A case-control study was conducted in Iraq, Baghdad, Al-Yarmouk Teaching Hospital, Department of Gynecology and Obstetrics during the period from January 2021 to October 2021.

Ethical considerations:

All ethical issues were approved by the authors. The study has been proposed and subsequently approved by the Scientific Council of Gynecology and Obstetrics of the Iraqi Board of Medical Specializations. Women were informed about the nature of the study and verbal consent was attained from them. All participants were assured of anonymity and confidentiality of information. Data collection and patients enrollment were in accordance with the Declaration of Helsinki of World Medical Association , 2013 for the ethical principles of researches involving human.

Sampling method:

A convenient sample of 90 pregnant women was enrolled in the current study and consisted of two groups:

Case group: Consisted of 45 pregnant women who presented with PE.

Control group: Consisted of 45 normotensive healthy pregnant women matched with the case group for age, body mass index (BMI), and gestational age.

The following criteria were used in the diagnosis of PE;

1. Hypertension: SBP ≥ 140 mmHg or DBP ≥ 90 mmHg on 2 occasions (at least 4 hrs. apart) in a previously normotensive patient or SBP ≥ 160 mmHg or DBP ≥ 110 mmHg on one occasion
2. Proteinuria: Urine dipstick protein ≥1+

Statistical analysis:

The entry and analysis of data were done by Microsoft Excel 2016 and software package of social science (SPSS) version 22. The descriptive analysis focused on frequencies and percentages. Continuous variables were presented as mean (± standard deviation (SD)) and were compared between the two study groups using the Mann–Whitney U test, Pearson's correlation coefficient test was used to calculate the correlation between the continuous variables. A P-value of ≤ 0.05 was considered statistically significant.

3. RESULTS

A total of 90 pregnant women were enrolled in the current study, the mean age was 28.61 (±6.16) years. There was no significant difference between the study groups regarding age and BMI. The SBP and DBP were significantly higher in the case group than the control group (P-value<0.001), as shown in (**Table 1**). There was a significant difference were obtained between the study groups regarding liver function tests (AST and ALT) as P-value <0.05. Regarding the renal function test, there was no significant difference between the study groups in the blood urea and serum creatinine level. In addition, no significant difference was obtained between the study groups regarding the platelet count (**Table 2**). The level of Amyloid-A was significantly higher in the case group than the control group (P-value<0.001), as shown in (**Table 3**). The receiver operating characteristic (ROC) curve was used to estimate the cut- off point best sensitivity and specificity, a cut-off point of 12.25 ng/dl was selected (**Figure 1**). According to the cut-off point of 12.25 (ng/dl), 51% of the participants had positive serum amyloid-A, as shown in (**Figure 2**). There was a significant association between the results of serum amyloid-A and the development of PE according to the cut-off point of 12.25 ng/dl (P-value<0.001). The sensitivity, specificity, positive predictive value, and negative predictive values were 95.6, 93.3, 93.5, and 95.5 respectively, as shown in (**Table 4**). The level of serum amyloid-A significantly correlated with the level of AST and ALT. In contrast, the was no significant correlation between serum amyloid-A and blood urea or serum creatinine. In addition, no significant correlation between serum amyloid-A and platelet count, as shown in (**Table 5 and Figure 3**).

Table 1. Comparison of age ,body mass index, systolic and diastolic blood pressure between the studied groups

Variable	Control group (N=45)		Cases group (N=45)		P. value*
	Mean	SD	Mean	SD	
Age (years)	29.20	6.35	28.02	5.97	0.346
BMI (kg/m^2)	25.24	3.33	26.37	4.25	0.112
Systolic blood pressure (mmHg)	117.17	5.50	157.95	9.20	<0.001
Diastolic blood pressure (mmHg)	73.82	6.80	104.86	8.00	<0.001

SD: Standard deviation, Body mass index. Mann-Whitney U Test used for comparison

Table 2. Comparison of laboratory findings of the studied groups

Variable	Control group (N=45)		Cases group (N=45)		P. value*
	Mean	SD	Mean	SD	
Blood urea (mg/dL)	27.57	4.27	28.22	4.30	0.498
Serum creatinine (mg/dL)	0.74	0.19	0.81	0.15	0.074
Aspartate aminotransferase (U/L)	19.94	2.27	21.25	2.40	0.005
Alanine aminotransferase (U/L)	20.44	3.81	22.44	3.53	0.018
Platelets (× 10^9/L)	261.5	48.4	270.6	40.40	0.449

*SD: Standard deviation, *Mann-Whitney U Test used for comparison*

Table 3. Comparison of Amyloid-A levels of the studied groups

Statistic	Amyloid-A (ng/dl)	
	Control group (N=45)	Cases group (N=45)
Mean	9.11	21.82
SD	3.00	2.29
SE	0.447	0.341
95% CI of mean	8.23 - 10.11	21.15 - 22.49
P. value* < 0.001		

SD: standard deviation, SE: standard error of mean, CI : Confidence interval
**Mann-Whitney test used in comparison*

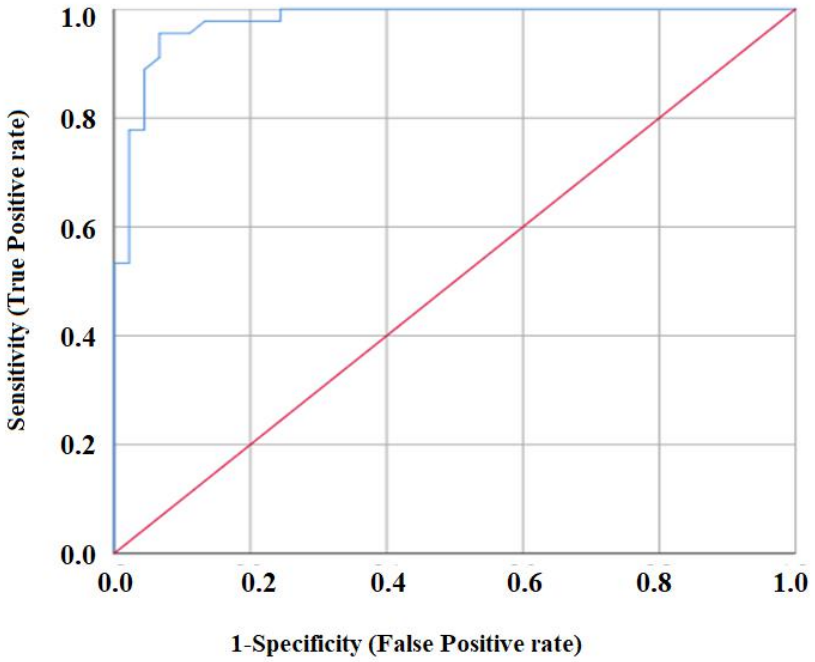

Figure 1. receiver operating Characteristics (ROC) curve analysis for the validity of Amyloid-A level as a biomarker of Preeclampsia (cutoff point= 12.25 ng/d)

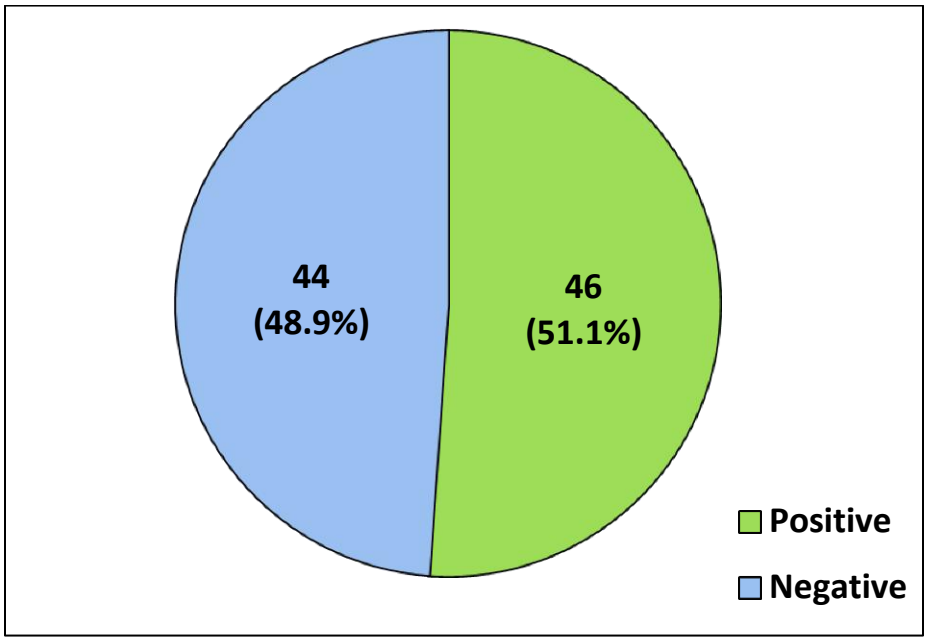

Figure 2. Results of serum amyloid-A according to the cut-off point of 12.25 ng/d.

Table 4. Association between serum amyloid-A level and preeclampsia

Serum amyloid-A	Control group (N=45)		Cases group (N=45)		Total	
	No.	%	No.	%	No.	%
Positive (≥12.25ng/dl)	3	6.5	43	93.5	46	51
Negative (<12.25ng/dl)	42	95.5	2	4.5	44	49
Total	45	50	45	50	90	100
Chi-square test = 67.6, P. value < 0.001						

Validity parameters

Sensitivity	95.60%
Specificity	93.30%
Accuracy	94.4%
Positive Predictive Value	93.5%
Negative Predictive Value	95.5%

Table 5. Results of bivariate Pearson's analysis for the correlation of serum amyloid-A with liver function tests, renal function tests and platelets count

Variable	R	P. value
Aspartate aminotransferase	0.484	<0.001
Alanine aminotransferase	0.492	<0.001
Blood urea	0.176	0.097
Serum creatinine	0.206	0.051
Platelets count	0.169	0.111

R: Pearson's Correlation Coefficient

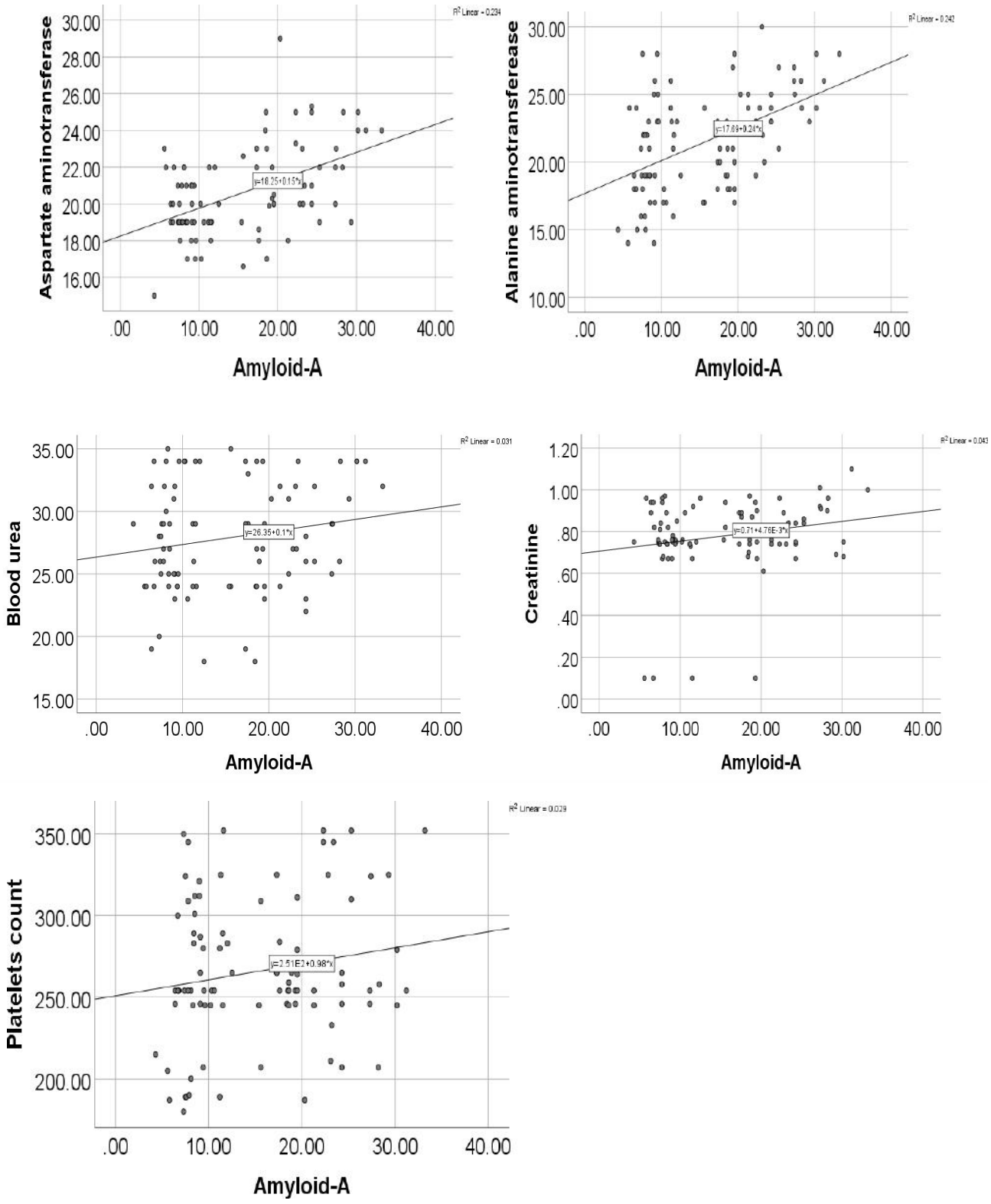

Figure 3. Curve estimation for the correlation of serum Amyloid-A with Aspartate aminotransferase, Alanine aminotransferase, blood urea, serum creatinine, and platelets count

4. DISCUSSION

In recent days, there has been a significant improvement in the management of PE patients, which has led to a decrease in mortality and morbidity caused by PE. However, the challenge lies in its early diagnosis, when there are no apparent clinical signs. Therefore, early intervention could be initiated and maternal mortality and morbidity could be reduced substantially, even in developing countries (21). The first finding of the current study was a significant difference between the study groups regarding the SBP and DBP (P-value <0.001 for both). In comparison, the same findings were obtained by Ekun et al who conducted a cross-sectional study and enrolled 49 preeclamptic women and 50 normotensive healthy pregnant women, the SBP was 172 mmHg and DBP was 112 mmHg among pregnant women with PE compared to SBP of 113 and DBP of 72 among healthy pregnant women(22). Another study was done by Murat et al and included 36 cases with mild preeclampsia, 36 cases with severe preeclampsia and 33 cases of normotensive pregnancy concluded a significant difference in the SBP and DBP between healthy pregnant women, those with mild to moderate, and those with severe PE(23). In the current study, there was no significant difference in the blood urea and serum creatinine between the study groups (P-value was 0.498 and 0.074, respectively). In contrast, a case-control study including 150 pregnant women (75 with preeclampsia and 75 with normal pregnancy) was done in Iraq by Wdad and revealed s significant rise in the mean concentration of serum urea and serum creatinine in preeclamptic pregnant women compared to that of normotensive pregnant women(24). A cross-sectional, hospital based study involving 105 age matched women was done in India by Manjareeka who revealed that the level of serum creatinine was significantly elevated in preeclamptics respectively when compared to normotensives, while there was a statistically insignificant and small increase in serum urea level in preeclamptics compared to normotensive pregnant women(25). In contrast, another study that was done by Ekun et al revealed a significant increase in both blood urea and serum creatinine in PE pregnant women compared to healthy(22). This discrepancy might be related to the severity of PE among the samples of these studies.

There was a significant difference between the study groups regarding the AST and ALT (P-values were 0.005 and 0.018, respectively), and the mean of the AST and ALT was higher in

the case group. The same results were obtained by another case-control study that was done in Iraq by Lamyaa et al and included 130 pregnant women (40 with severe preeclampsia, 40 with non-severe preeclampsia, and 50 with normal pregnancy as a control group)(26). In addition, this agreed with a cross- sectional study among 100 pregnant women after 20 weeks of gestation that was conducted by Munazza et al and concluded that the liver function tests particularly AST and ALT levels were significantly increased among pregnant women with preeclampsia compared to healthy pregnant women(27). In concordance with these results, a study was done in Iran by Kasraeian et al and included 450 women with preeclampsia during the period from 2005 to 2014 revealed a significant increase in the level of AST and ALT in patients with severe PE compared to those with mild PE(28). Another study that was done by Rajoria et al and enrolled 250 women of more than 20 weeks gestation concluded that liver involvement is common in PE and eclampsia and the derangement of parameters of liver function test can be taken as predictors of the disease(29). Regarding the platelet count, no significant difference was obtained between the study groups in the current study (P-value was 0.449). The same results were obtained by another case-control study that was done by Alea et al and included 90 pregnant women (30 with non-severe preeclampsia, 30 with severe preeclampsia, and 30 age-matched normal pregnant women as a control group)(30). Another study was done in Turkey by Kazım Uckan and Hanım Guler Sahin and enrolled 120 women revealed an insignificant decrease in platelet count for pregnant women with PE comparing healthy pregnant(31). In contrast, another study was conducted in the Kingdom of Saudi Arabia by AlSheeha et al and included 120 participants revealed a significant decrease in platelet count in the preeclampsia group (included mild and severe PE) compared to the control group (32). The main finding of the current study was a significant association between the serum amyloid-A level and the development of PE (P_value <0.001). In comparison, another study was conducted in Egypt by Swidan et al and enrolled 75 pregnant women concluded that the serum amyloid-A can be used to discriminate between PE cases and controls(33). This agreed with the results of a case-control study that was also conducted in Egypt by El-Kady et al and included 102 pregnant women who stated that the median serum amyloid-A was statistically significantly higher in women of the PE group in comparison to women of the control group(34). In another study

that was conducted in Turkey by Üstün et al and included 25 normotensive and 25 preeclamptic pregnant women, the serum amyloid-A level in pregnant women with PE was significantly higher than those of normal pregnancy(35). In contrast, another study was done by Kristensen in Denmark which included 295 women with uncomplicated pregnancies, 57 women diagnosed with preeclampsia, and 58 healthy non-pregnant women concluded that serum amyloid-A was not elevated in women with PE compared to women within the control group (36). In the current study, according to the ROC test, the sensitivity and specificity of serum amyloid-A were 95.6% and 93.3% with a cut-off point of 12.25 ng/dl. In comparison to other studies, according to a study that was done in Turkey by Murat et al and included 36 cases with mild preeclampsia, 36 cases with severe preeclampsia and 33 cases of normotensive pregnant, the best cut-off value for amyloid-A was 7.53 mg/L as calculated by ROC curve for serum amyloid-A with a sensitivity of 81.8%, specificity of 69.4%(23). Another study was conducted in Turkey by Üstün revealed that the sensitivity and specificity of serum amyloid-A in identifying patients with preeclampsia were 85% and 75%, respectively with a cut- off value of 9.98 ng/l(35). The mild difference between these studies regarding the mean of amyloid-A and the cut-off points may be related to the individual variation of the participants, the methods of the study, and the accuracy of the investigation.

5. CONCLUSIONS

There is significant association between the level of maternal serum amyloid- A and the development of PE in pregnancy.With a cut-off point of 12.25 ng/dl, the sensitivity and specificity of amyloid- A in detecting PE were 95.6%, 93.3%, respectively.

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