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RESEARCH ARTICLE

Estimation of Obestatin Levels and Liver and Kidney Functions in Pregnant Women with Preeclampsia Compared to Healthy Pregnant Women in Samarra City

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Abstract

Background: Preeclampsia is one of the pregnancy disorders that appear after the 20th week and is characterized by high blood pressure and protein in the urine.
Objective: The study aimed to evaluate the level of obestatin and liver function in women with sepsis and compare it with healthy pregnant women.
Methods: A cross-sectional comparative study was conducted on 90 pregnant women, 45 with normal blood pressure (control group) and 45 with preeclampsia during the second and third trimester of pregnancy.
Results: There were no significant differences in the level of obestatin between women with preeclampsia and healthy pregnant women, as the median value for those with preeclampsia was 92.205 pg/mL (51.832–129.429), compared to 92.205 pg/mL (59.369–128.889) in the healthy group, with $P = 0.773$. Additionally, no significant differences were observed in the levels of total protein and albumin, with respective mean values of 7.730±1.968 g/dL and 2.713±0.888 g/dL in the preeclampsia group, compared to 7.714±1.982 g/dL and 2.865±0.976 g/dL in the healthy group. Similarly, there were no significant differences in creatinine levels, with median values of 1.532±1.174 mg/dL versus 1.451±0.891 mg/dL. An increase in the level of urea in pregnant women with preeclampsia, compared to healthy pregnant women was 13.136±3.392 mg/dL versus 9.435±3.741 mg/dL, with a statistical significance $P < 0.0001^*$. An increase in the level of uric acid in pregnant women with preeclampsia, compared to healthy pregnant women was 5.856±1.552 mg/dL versus 4.097±1.538 mg/dL, with statistical significance $P < 0.0001^*$. The area under the curve (AUC) for obestatin was 0.516 and the sensitivity was 22.22. The liver function AUC was 0.553 and 0.543 and the sensitivity was 55.56 and 42.22. The kidney function AUC was 0.778, 0.797, and 0.526, and the sensitivity was 77.78, 37.78, and 68.89.
Conclusion: There were no significant differences in the level of obestatin between the two groups. No significant differences in the level of liver function, and an increase in the level of urea and uric acid in women with preeclampsia were observed. Obestatin and liver function tests are not diagnostic indicators for preeclampsia; However, urea and uric acid are diagnostic indicators for preeclampsia.

1. Introduction

Preeclampsia is a disorder that affects the placenta, which is the link between the mother and the fetus, and affects 6–8% of pregnant women worldwide. It occurs through high blood pressure in the 20th week of pregnancy with the presence of protein in urine. If there is no protein, it is determined through different manifestations, such as

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low platelets, kidney failure, pulmonary edema, brain and visual problems, or HELLP (**H**emolysis, **E**levated **L**iver enzymes, and **L**ow **P**latelets) syndrome [1]. The main cause of preeclampsia is a decrease in uteroplacental blood flow because the glandular cells do not convert the arteries into blood vessels. As a result, the arteries remain narrow, which limits blood flow to the placenta and thus causes a deficiency in oxygen and nutrients [2]. There are several maternal factors (both genetic and acquired) that cause preeclampsia in pregnancy [3]. It can occur without high blood pressure. For example, about 18% of women with HELLP syndrome have normal diastolic blood pressure, while 5–6% of women with preeclampsia do not have high blood pressure [4]. This study aimed to evaluate the relationship between obestatin levels and some biomarkers of liver and kidney functions in pregnant women with preeclampsia, compared to healthy pregnant women in Samarra city, Iraq. Previous studies related to pregnancy were conducted in Samarra city, Iraq, including Mohammed and Al-Samarrai's study on evaluating the level of hepsin and iron balance in the serum of pregnant women with anemia [5]. The study conducted by Al-Samarrai and Al Samarrai evaluated hepcidin and some biochemical variables in pregnant women [6]. However, these studies did not directly address the issue of preeclampsia, which highlights the importance of this current study. Obesity, albumin, urea, creatinine, and uric acid were chosen because of their role in the physiological changes that accompany this disease. Obestatin is a small protein hormone (peptide) consisting of 23 amino acids [7]. The relationship between obestatin and preeclampsia has not been conclusively established, but several studies indicate that obestatin has an important effect on the endothelium, which directly influences blood pressure regulation. The inner lining of blood vessels (endothelium) plays an important role in regulating blood pressure and maintaining vessel elasticity by secreting substances that promote vasodilation, such as nitric oxide, and prevent inflammation and clot formation. The results of a recent study showed that obestatin has an anti-inflammatory effect on endothelial cells. This effect is evidence of obestatin's ability to support endothelial function [8].

In preeclampsia, blood vessels constrict due to a defect in the lining of blood vessels because of a lack of placental perfusion, which leads to the release of inflammatory and oxidative factors. These factors cause oxidative stress and an inflammatory response that lead to a defect in the secretion of vasodilators, and thus, vasoconstriction and high blood pressure occur [9]. Proteins are multi-polymer biological structures composed of amino acids, and they perform numerous functions, such as structural support, chemical catalysis, and hormonal and enzymatic purposes. Proteins play a vital role in human biochemistry, providing the building blocks to the body [10]. During the first weeks of pregnancy, changes occur in the protein metabolism process to support the growth and development of fetus while maintaining mother's body balance and preparing for breastfeeding. The weight of both mother and fetus increases, and with the fetus's need for food, glucose production increases in mother's body [11,12]. Albumin is one of the most abundant proteins in the human body, with about 40% of it found in the bloodstream, and is a major component of most extracellular fluids [13]. Albumin is used to diagnose many cardiovascular diseases, including preeclampsia, in which a decrease in albumin levels occurs because of deterioration in blood vessel functioning and damage to various tissues and organs, such as the liver and kidneys [14]. Urea is an organic compound and is a secondary metabolic product of protein and nitrogen metabolism [15]. It is highly soluble in water, which helps it to be eliminated through the kidneys [16]. Preeclampsia causes damage to glomerular endothelial cells and glomerular basement membrane, and decreased renal blood flow. These changes lead to a decline in the normal glomerular filtration capacity and a decrease in the glomerular filtration rate, resulting in an increase in the urea level [17]. Uric acid is the end product of purine metabolism and is mainly regulated by the enzyme xanthine oxidase reductase [18]. Uric acid, being a powerful antioxidant in blood plasma, performs several physiological functions [19]. An increase in the level of uric acid occurs in preeclampsia because of weakness in the invasion of trophoblast into the placenta. These products contribute to peripheral vasoconstriction in the glomeruli, and inflammation of glomerular vascular lining leads to a decrease in the glomerular filtration rate and increased reabsorption of uric acid from proximal tubules, thus causing an increase in uric acid [20]. Elevated creatinine levels occur in pregnant women with preeclampsia because of vasospasm of dysfunctional endothelial cells, which leads to shrinkage of intravascular space. The physiological increase in renal blood flow and increased glomerular filtration rate, as in normal pregnancy, does not occur [21]. This study aimed to evaluate serum obestatin, liver and kidney function biomarkers in pregnant women diagnosed with preeclampsia, and to compare these parameters with healthy pregnant women.

2. Materials and Methods

The study was conducted on 90 blood samples of pregnant women, including 45 samples of pregnant women with preeclampsia in the second and third trimesters of pregnancy and 45 samples of healthy pregnant women (as a control group) from August 17, 2023 to November 11, 2023 at Samarra General Hospital and some outpatient clinics. Women with preeclampsia were selected based on a diagnosis of gestational hypertension according to the established medical criteria. Pregnant women with chronic diseases or other health conditions that might affect the results, such as gestational diabetes, toxoplasmosis, hepatitis, severe anemia, and other diseases, were excluded. Preeclampsia patients were aged 25–36 years and were from urban areas only, as women from rural areas were not included in the study. Blood from pregnant women was collected after obtaining their consent and under the supervision and assistance of medical staff. Venus blood was withdrawn from the hand by placing a tourniquet

approximately 8–10 cm above the site. The lower arm was immobilized to taut the skin and stabilize the vein. Blood was withdrawn in the morning to ensure stable vital signs. The blood was withdrawn using plastic syringes and placed in gel tubes for 20 min for clotting; then the blood was separated using a centrifuge for 15 min at a rate of 1,252×*g*. During the process, the blood serum and plasma were separated, free of red and white blood cells and platelets, and serum was used to conduct blood tests. After separation, the serum was divided into Eppendorf tubes (5 mL) and frozen in a freezer at a temperature of -20°C.

Obestatin was determined using the enzyme-linked immunosorbent serological assay (ELISA) based on specific interaction between hormones and antibodies. The antibodies are immobilized on the wells of the microplate. When the sample is added to the wells, any hormone present in the sample binds specifically to the coated antibody. After incubation and washing to remove unbound components, an enzyme- or biotin-labeled secondary antibody is added to form the antibody–antigen–antibody complex. Following additional washes, the appropriate enzyme conjugate and chromogenic substrate are introduced, generating a color reaction proportional to the hormone concentration.

Total protein was estimated using the Biuret reagent method, which involves reacting blood serum containing protein with a Biuret reagent solution to produce a purple complex, whose intensity was measured. Albumin was determined using the Bromocresol Green (BCG) method. Albumin binds to Bromocresol Green reagent (5,5,3,3-tetrabromo-metacresol phthalene sulfate; $C_{21}H_{14}Br_4O_5S$) in an acidic medium (pH 4.3) to form a green albumin–Bromocresol Green complex.

As for urea, ammonium ions react in the presence of sodium salicylate and sodium hypochlorite to form a green compound (indophenol dicarboxylate). The intensity of the color is proportional to the concentration of urea. Enzyme uricase interacts with uric acid to form allantoin and hydrogen peroxide. The peroxidase enzyme is catalyzed by the reaction of 4-aminoantipyrine and dichloro-2,4-phenolsulfonate in the presence of peroxidase to form a quinone dye complex.

Creatinine forms a colored complex with picric acid in an alkaline medium. The rate of formation of this complex is proportional to creatinine. Statistical program SPSS V.25 was used to analyze the study results. Student's *t*-test was used to compare results of pregnant women with preeclampsia and healthy pregnant women at a probability level of 0.05%.

Receiver Operating Characteristic (ROC) analysis is used to evaluate the diagnostic performance of a biomarker by plotting sensitivity against specificity at various cutoff points. The area under curve (AUC) reflects the test's overall accuracy.

3. Results and Discussion

The results of the study showed no significant difference in the level of obestatin between pregnant women with preeclampsia and healthy pregnant women at $P = 0.773$, as the median value in case of pregnant women with preeclampsia was 92.205 pg/mL (51.832–129.429), compared to that of healthy pregnant women, 92.205 pg/mL (59.369–128.889), as shown in Table 1 and Figure 1. The results did not indicate any significant difference between the two groups. The results were not consistent with the results of a study conducted by Wu and colleagues to determine ghrelin–obestatin ratio in maternal serum in cases with preeclampsia, as obestatin level decreases in pregnant women with preeclampsia [22]. On the other hand, obestatin level increased in a study conducted by Ren *et al.* [23], as its level is affected by several disease conditions and factors, such as obesity, type 2 diabetes, and metabolism, because obestatin plays a vital role in regulating energy consumption and burning fat, and any disturbance in the metabolic process affects obestatin levels [24].

There were no significant difference in protein and albumin levels between pregnant women with preeclampsia and healthy pregnant women. The mean±standard deviation (SD) in pregnant women with preeclampsia was 7.730±1.968 g/dL and 2.713±0.888 g/dL, respectively, compared to the mean± SD of healthy pregnant women, that is 7.714±1.982 g/dL and 2.865±0.976 g/dL, respectively, as shown in table 2. Regarding the comparison between total protein, albumin, and phosphorus levels in pregnant women with preeclampsia and women with normal pregnancies, the results of the study were consistent with that of the study conducted by Salari *et al.*, which indicated that there was no relationship between protein levels and preeclampsia [25]. The results of the study conducted by Shahid *et al.* showed that protein levels in case of pregnant women with preeclampsia remain normal [26].

The results of the study conducted by Salari *et al.* showed that there were no significant differences in albumin levels between the two groups of pregnant women [25]. However, Jahan *et al.* found that albumin level could be higher in pregnant women with preeclampsia, compared to normal pregnant women [27]. Hönger's study on albumin metabolism in pregnant women with preeclampsia showed that albumin levels were significantly reduced in preeclampsia group [28]. Preeclampsia affects the kidneys, which could hinder the body's ability to filter albumin normally or lead to albumin deposition through the kidneys [29].

The results of urea and uric acid analysis showed significant differences, as the mean±SD were higher in the group of pregnant women with preeclampsia (13.136±3.392 mg/dL and 5.856±1.552 mg/dL, respectively), compared to the group of healthy pregnant women (9.435±3.741 mg/dL and 4.097±1.538 mg/dL, respectively), as shown in table 2. The elevated levels of urea in pregnant women with preeclampsia were consistent with the results of the study conducted by Saha and Gupta on changes in biochemical parameters in preeclampsia patients [30] and the results of Al-Sultan and Jankeer on liver and kidney function tests in pregnant women with preeclampsia [31].

Urea is the main byproduct of the metabolism of purines, adenine, and guanine, and is an antioxidant formed in the body [32]. Pathological changes, such as

renal ischemia and hypoperfusion, lead to a decrease in glomerular filtration rate and disruption of the functioning of renal tubules, which lead to the accumulation of urea in the blood. In addition, microvascular hemolysis resulting from damage to blood vessels leads to increased urea production, which reflects the severity of renal damage in preeclampsia cases [33]. The results of uric acid analysis were consistent with the findings of Shakarami *et al.* [34] and Hamed *et al.* [35], who discovered a high level of uric acid in pregnant women with preeclampsia, compared to healthy pregnant women. The study done by Le *et al.* also showed that high levels of uric acid in women with preeclampsia could lead to premature birth, restriction of fetal growth inside the womb, and fetal death [36]. Evidence suggests that elevated levels of uric acid in the blood may play a causative role in preeclampsia, disrupting the formation of spiral arteries in the placenta and stimulating the desquamation of trophoblast, leading to failure of normal placental development and oxygen deprivation. Elevated uric acid levels are also associated with damage to blood vessel linings and increased oxidative stress, which exacerbate severity of the condition [37].

Creatinine analysis showed a relative increase in the mean±SD values of creatinine in pregnant women with preeclampsia (1.532±1.174 mg/dL), compared to healthy pregnant women (1.451±0.891 mg/dL), as shown in table 2. However, this increase was not statistically significant as P = 0.713, which indicated no significant differences between the two groups. These results were consistent with the findings of previous studies [38,39], which indicated an increase in the level of creatinine in preeclampsia cases, compared to healthy pregnant women, but this increase was not statistically significant. In contrast, the studies done by Mahmoud *et al.* [38], and Hasan [40] showed a significant increase in the level of creatinine in pregnant women with preeclampsia, which indicates that the results differ depending on nature of the samples, severity of the disease, and stage of infection. The results of ROC analysis (Table 3 and Figures 2–7) showed that obestatin was not a diagnostic indicator for preeclampsia because AUC, sensitivity, and accuracy were low, despite the specificity was high. For protein and albumin, AUC, accuracy, sensitivity, and specificity were low. Therefore, measuring the level of liver function is not a diagnostic indicator for preeclampsia. For urea and uric acid, AUC and sensitivity were high, so measuring their levels is a diagnostic indicator for preeclampsia. However, measuring creatinine levels is not a diagnostic indicator because both AUC and sensitivity are low.

Although some results of the current study were not statistically significant (P > 0.05), this does not necessarily show the absence of a biological or clinical effect. The absence of statistical significance could be due to factors such as limited sample size, large variation within the group, or nature of the disease stage. Therefore, these results must be interpreted while taking into account the surrounding clinical and contextual factors [41].

Table (1): Median (minimum–maximum) of obestatin.

Median (min–max)		P value
Normal pregnancy	Preeclampsia	
92.205 (59.369–128.889)	92.205 (51.832–129.429)	0.773

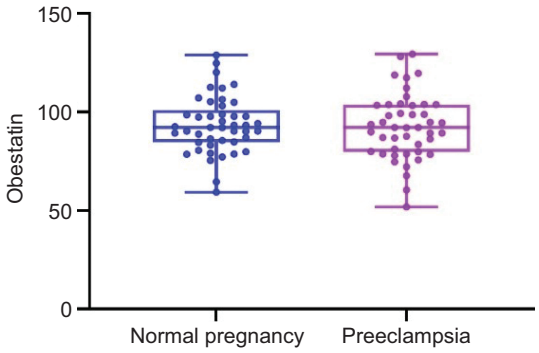

Figure (1): Obestatin levels in a group of pregnant women with preeclampsia and healthy pregnant women.

Table (2): Mean and standard deviation of liver and kidney function levels.

Parameters	Mean ± SD		
	Normal pregnancy	Preeclampsia	*P* value
Protein	7.714±1.982	7.730±1.968	0.970
Albumin	2.865±0.976	2.713±0.888	0.442
Urea	9.435±3.741	13.136±3.392	< 0.0001*
Uric acid	4.097±1.538	5.856±1.552	< 0.0001*
Creatinine	1.451±0.891	1.532±1.174	0.713

Table (3): Area under the curve, accuracy, sensitivity, specificity, *P* value, and cut-off.

Parameters	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy	AUC	*P* value
Obestatin	≤78.706	22.22	86.67	0.08889	0.516	0.7953
Protein	>7.463	55.56	60.00	0.1556	0.543	0.4833
Albumin	≤2.106	42.22	82.22	0.2444	0.553	0.3983
Urea	>10.45	77.78	68.89	0.4667	0.778	<0.001
Uric acid	>4.854	68.89	82.22	0.5111	0.797	<0.001
Creatinine	>1.571	37.78	80.00	0.1778	0.526	0.6697

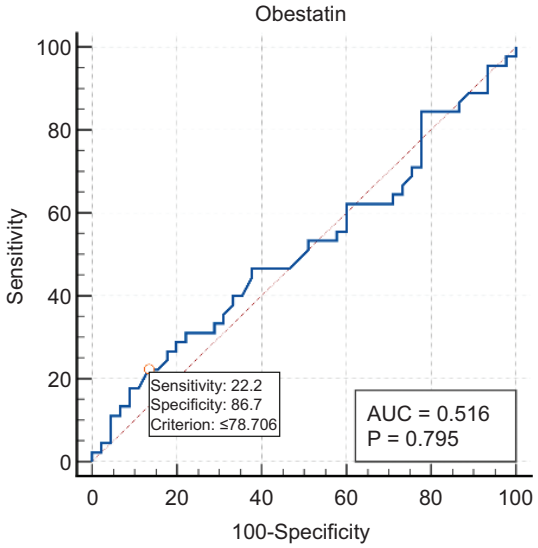

Figure (2): ROC curve for obestatin.

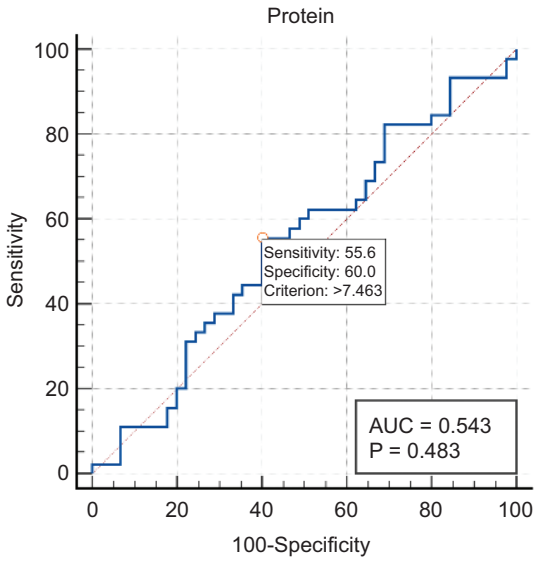

Figure (3): ROC curve for protein.

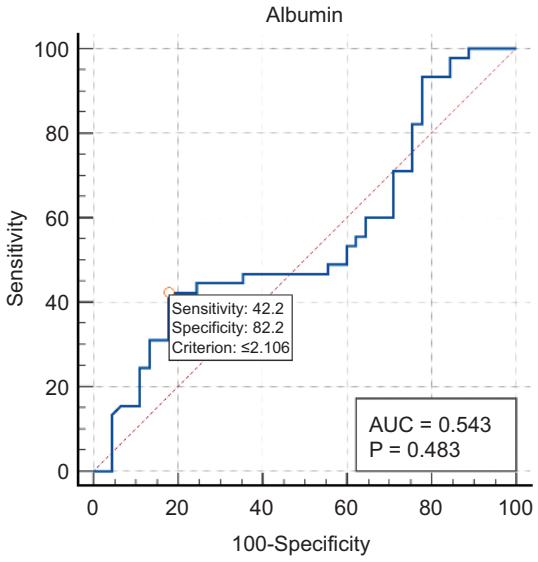

Figure (4): ROC curve for albumen.

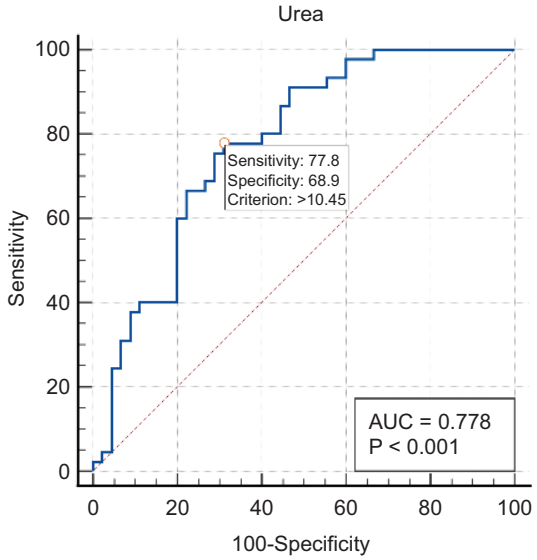

Figure (5): ROC curve for urea.

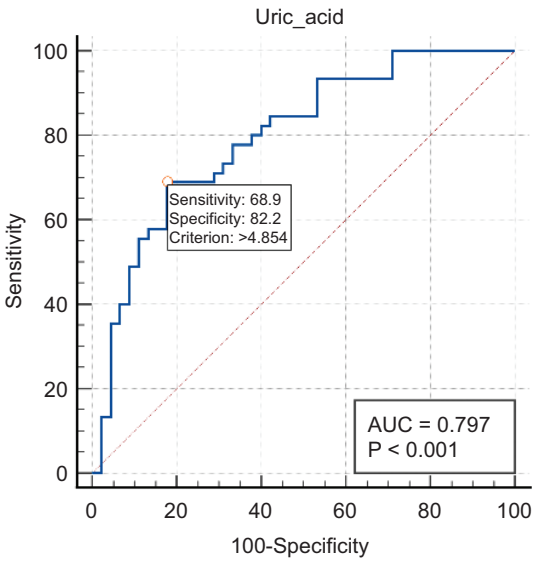

Figure (6): ROC curve for uric acid.

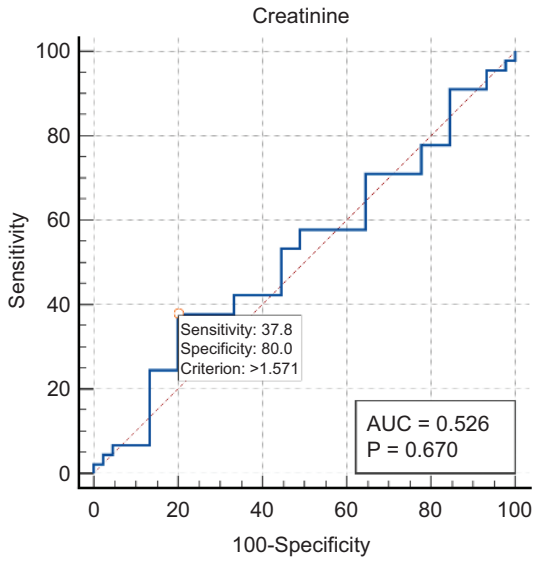

Figure (7): ROC curve for creatinine.

The study results indicate that some biomarkers, such as urea and uric acid, may be used as auxiliary indicators in the diagnosis or monitoring of the severity of preeclampsia, given that their levels are significantly higher in affected women and they have high sensitivity and diagnostic values. Regarding the indicators that did not show sufficient statistical or diagnostic significance, such as obestatin, the results of the current study did not completely rule out its role, but rather open the way for future studies that may examine its relationship to specific stages of the disease or its role in genetic or placental influences. Therefore, these results constitute a preliminary basis for broader and more detailed studies.

Although the study results highlight some biomarkers that may be related to preeclampsia, there are some limitations that must be taken into account when interpreting these results, such as the sample size was limited and the sample was restricted to urban women (from Samarra city) only. In addition, some influencing factors, such as diet or genetic history, were not controlled.

4. Conclusion

The results showed that obestatin couldn't be used as a diagnostic indicator for preeclampsia because. Liver function tests (protein–albumin) are not used as a diagnostic indicator because there are no significant differences in their levels. Urea and uric acid can be used as diagnostic indicators because their levels were higher in women with preeclampsia compared to healthy pregnant women. Creatinine is not used as a diagnostic indicator because there are no significant differences in its levels between the two groups.

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Author Contributions

Both authors contributed to study design and data collection, and approved the final version of the study.

Conflict of interest

The authors declared that there was no conflict of interest. The authors confirmed the ownership of all the figures and tables in the manuscript. Additionally, all figures and images included in the manuscript have the required permission for re-publication attached. The project received approval from the local ethical committee at the University of Samarra.

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