OBSTETRICS

Accuracy of 12-hour versus 24-hour Urine Collection for Diagnosis of Preeclampsia

Sasipimon Sarnnok, M.D.*,
Siraya Kitiyodom, M.D.*

*Department of Obstetrics and Gynecology, Faculty of Medicine, Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima, Thailand

ABSTRACT

Objectives: To evaluate the accuracy of a 12-hour urine collection compared to a 24-hour urine collection for diagnosis of preeclampsia.

Materials and Methods: The diagnostic study was conducted between March and September 2020 at Maharat Nakhon Ratchasima Hospital, Thailand. The participants were pregnant women at ≥ 20 weeks of gestation composed with blood pressure ≥ 140/90 mmHg or clinically suspected preeclampsia. The participants were hospitalized for evaluation of blood and urine protein analysis. The 24-hour urine protein collection was stratified to the two 12-hour urine protein collection samples. The statistical analyses were used to analyze the accuracy of the test and determine the appropriate cut-off value of 12-hour urine protein compared with the gold standard of the 24-hour urine protein value.

Results: The study included 87 participants with 12-hour urine collection, which comprised 174 samples to compare with 24-hour urine collection. The incidence of preeclampsia was 47 patients (54%). The 12-hour urine collection included 174 samples. The appropriate cut-off value was ≥ 143 mg with sensitivity of 89.8%, specificity of 90.8%, and accuracy of 90.2% with near-perfect agreement (Cohen's kappa 0.82). The 12-hour urine collection test offered higher accuracy than urine protein creatinine ratio and urine protein dipstick test.

Conclusion: The 12-hour urine collection provided high accuracy results and was the alternative test to diagnose preeclampsia.

Keywords: preeclampsia, proteinuria, 24-hour urine protein.

ความแม่นยำในการใช้ปริมาณโปรตีนในปัสสาวะ 12 ชั่วโมงเทียบกับปริมาณโปรตีนใน ปัสสาวะ 24 ชั่วโมงในการวินิจฉัยครรภ์เป็นพิษ

ศศิพิมล สารนอก, สิรยา กิติโยดม

บทคัดย่อ

วัตถุประสงค์*: ศึกษาความแม่นยำในการใช้โปรตีนในปัสสาวะ 12 ชั่วโมง วินิจฉัยครรภ์เป็นพิษ*

วัสดุและวิธีการ*: การวิจัยเชิงวินิจฉัยในโรงพยาบาลมหาราชนครราชสีมา ระหว่างมีนาคม-กันยายน พ.ศ.2563 ในสตรีตั้ง ครรภ์ที่อายุครรภ์ตั้งแต่ 20 สัปดาห์ ร่วมกับความดันโลหิตสูง ≥ 140/90 mmHg หรือมีอาการสงสัยภาวะครรภ์เป็นพิษ ผู้เข้า ร่วมงานวิจัยได้ถูกรับไว้ในโรงพยาบาล เพื่อเจาะเลือดและเก็บปริมาณปัสสาวะ 24 ชั่วโมง แบ่งเป็น 2 ช่วง ผู้เข้าร่วมวิจัยหนึ่ง รายจะเก็บปริมาณโปรตีนในปัสสาวะ 12 ชั่วโมง 2 ตัวอย่าง เพื่อนำไปเทียบกับการวินิจฉัยด้วยโปรตีนในปัสสาวะ 24 ชั่วโมง และนำข้อมูลวิเคราะห์เพื่อหาเกณฑ์ปริมาณโปรตีนที่เหมาะสมและมีความแม่นยำในการวินิจฉัยครรภ์เป็นพิษสำหรับการเก็บ ปัสสาวะ 12 ชั่วโมง*

ผลการศึกษา*: จากกลุ่มตัวอย่าง 87 คน มีโปรตีนในปัสสาวะ 12 ชั่วโมงจำนวน 174 ตัวอย่าง พบว่ามีภาวะครรภ์เป็นพิษ 47 คน (ร้อยละ 54) และเกณฑ์วินิจฉัยครรภ์เป็นพิษที่เหมาะสมสำหรับการเก็บโปรตีนในปัสสาวะ 12 ชั่วโมงคือ 143 มิลลิกรัม ความ แม่นยำร้อยละ 90.2, ความจำเพาะร้อยละ 90.8, ความไวร้อยละ 89.8 และ Cohen k 0.802 (Near prefect agreement) โดย แม่นยำมากกว่า urine protein creatinine ratio และ Urine protein dipstick*

สรุป*: ปริมาณโปรตีนในปัสสาวะ 12 ชั่วโมงมีความแม่นยำค่อนข้างสูง สามารถใช้เป็นทางเลือกหนึ่งในการใช้วินิจฉัยครรภ์ เป็นพิษ*

คำสำคัญ*: ครรภ์เป็นพิษ, โปรตีนในปัสสาวะ, ปริมาณโปรตีนในปัสสาวะ 24 ชั่วโมง*

Introduction

Hypertensive disorder in pregnancy is a common disorder and leading cause of maternal and perinatal mortality. In particular, preeclampsia is the most dangerous and complicated condition which is detected approximately 2-8% of all pregnancy and 16% of maternal death worldwide.1 Hypertensive disorder in pregnancy is diagnosed when systolic blood pressure is elevated ≥ 140 mmHg and/or diastolic blood pressure above 90 mmHg and occurs after 20 weeks of gestation[1,2]. Proteinuria remains an important diagnostic criterion for preeclampsia, which differs from other maternal hypertensive disorders[3].

Proteinuria is defined by a few methods, including a 24-hour urine protein collection, or urine protein creatinine ratio (UPCR), or urine protein dipstick test. However, 24-hour urine protein collection is the gold standard to diagnose proteinuria, which is defined as ≥ 300mg/dL without others causes[3,4]. The difficulty of a 24-hr urine collection includes time consuming and required hospital stay. This study aimed to find the method to reduce time of urine protein collection while providing high sensitivity and specificity for the diagnosis of preeclampsia.

According to the prospective observational study conducted by Siva et al[5], a 12-hour urine collection with a 150 mg cut-off values for proteinuria was performed among pregnant women after 20 weeks of gestation with hypertension and suspected preeclampsia. The study illustrated that 42% of samples diagnosed with preeclampsia and the specificity of test reached 91.7% with a sensitivity of 85.9% and no significant difference was found between the time of collection[5].

The aim of this study was to test the hypothesis that a 12-hour urine collection could be used as the gold standard instead of a 24-hour urine collection for diagnosis of preeclampsia.

Materials and Methods

The study was conducted between March and September 2020 at Maharat Nakhon Ratchasima Hospital, Thailand, after approval by the Committee on Human Right Relate to Research Involving Human Subjects. The study included participants who were pregnant women at 20 or more weeks of gestation with high blood pressure, defined by systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg, or clinically suspected preeclampsia conditions including headache, epigastric pain, blurred vision, excessive weight gain, leg edema, or abnormal presence of protein in urine[4,6]. The exclusion criteria limited the factors affecting abnormal urine protein such as previously identified nephropathy, abnormal vaginal discharge, or urinary tract bleeding. The sample size was determined from the following formula to estimate an infinite population proportion. According to Silva et al[5], the equation was derived and substituted with the following values: d=15% of sensitivity at 95% significance level, sensitivity of 12-hour urine collection = 0.85 and prevalence of preeclampsia was 0.42[5]. As a result, the calculated sample size was ≥ 79.

After the informed consents were collected, the participants were admitted to observe their blood pressure levels, routine blood and urine examination (urine protein dipstick, UPCR, and 24-hour urine collection) for diagnosis of preeclampsia. Blood pressure measurement was regularly performed every 4 hours or earlier in those with extremely high blood pressure. The clinically severe feature of preeclampsia was observed.

A 24-hour urine collection period was divided into 2 periods of 12-hour urine collection, using 2 containers for collection. The collection time was divided into the two periods because it aimed to examine whether urine protein values of both periods were correlated for diagnosis of preeclampsia. The time to collection was labeled properly on both containers to avoid confusion during collection and laboratory test.

In this study, the participants must void completely before the collection and urine samples were sent for analysis and UPCR according to the

standard protocol and they were required to void before the collection and to urinate for the final collection to ensure that the samples were collected accurately for 12-hour collection.

Urine samples were sent to laboratory, where the two containers were measured the amount of protein in urine separately. Urine protein values were reported in accordance with the time of collection. The urine samples from both containers were combined to generate a 24-hour urine collection sample and used to analyze 24-hour urine protein values. Finally, the two values of 12-hour urine protein were compared with the 24-hour urine protein levels (2:1 ratio). Moreover, reliability of the findings of 12-hour and 24-hour urine collection were considered by comparing amount of creatinine secretion in terms of lean body mass (in kg). The creatinine secretions in the participants were approximately 15-20 mg/kg[7].

The mean blood pressure values during hospitalization were analyzed. A 12-hour urine protein, 24-hour urine protein, UPCR and urine protein dipstick were collected. The final diagnosis was confirmed after 24-hour urine collection. All participated pregnant women were monitored closely during pregnancy until labor to detect their pregnancy outcomes and observed the incidence of preeclampsia.

The data were analyzed using STATA/IC software version 12.0. The continuous data were expressed to assess normal distribution. The Cohen's kappa test was used to assess the reliability of a 12-hour collection method compared with a 24-hour collection method. To evaluate the validity of the 12-hour test, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and correlation of the two periods of urine collection times were calculated[8].

Results

A total of 92 eligible pregnant women were recruited. Five women were excluded from the study: one participant refused to treatment, two participants diagnosed with chronic hypertension with proteinuria, and two participants diagnosed with nephrotic or nephritis syndrome (Fig. 1).
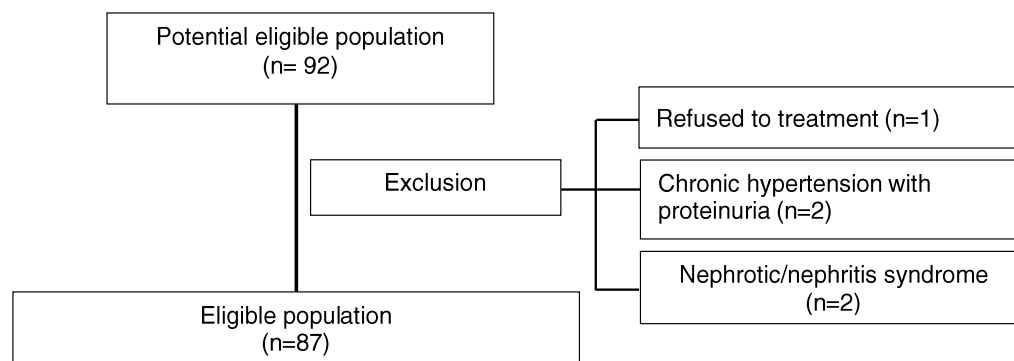
Fig. 1. Flow diagram for the study population.

The remaining 87 women were included in the study. The 12-hour urine collection included 174 samples compared with 24-hour urine collection (ratio 2:1). In which 47 women were diagnosed with preeclampsia (54%). The baseline characteristics of participants were illustrated in Table 1.

As shown in Table 1, participated pregnant women were between the ages of 17-43 years with the mean age of 31 years old and 52.9% of them were obesity (pre-pregnancy body mass index (BMI) ≥ 27 kg/m^2). The prevalence of underlying disease of overt diabetic mellitus and chronic hypertension was found equally at 23% and 8% of patients had both diseases. The rate of women with history of previous pregnancy

with preeclampsia was 8%. After the confirmation of 24-hour urine protein collection for diagnosis preeclampsia, the incidence of preeclampsia and chronic hypertension with superimpose preeclampsia were 47 participants (54%). After pregnancy follow-up, 6 participants (15%) diagnosed with gestational hypertension or chronic hypertensions (without abnormal proteinuria) were categorized as preeclampsia. The study found that the mean gestational age at delivery was 36 weeks, term delivery was 64.4%, and preterm birth was 34.5%. One woman was diagnosed with chronic hypertension with superimposed preeclampsia at 20 weeks of gestation and terminated the pregnancy.

Table 1. Characteristics of the participants (n = 87).

Characteristics	Mean	Median (min-max)
Age, years	30.9 ± 6.11	31 (17-43)
Parity		
Nulliparity	29 (33.3%)	
Multiparity	58 (66.7%)	
Gestational age (GA), weeks	33.7 ± 4.36	35 (20-40)
Singleton pregnancy	85 (97.7%)	
Twin pregnancy	2 (2.3%)	
Body mass index (BMI), kg/m^2		
Pre-pregnancy	27.4 (17.5-49.9)	
During admission	32.7 (20.0-56.4)	
History preeclampsia previous pregnancy	7 (8%)	
Underlying disease		
Overt diabetic mellitus (DM)	20 (23%)	
Chronic hypertension (CHT)	20 (23%)	
Both CHT and DM	8 (9.2%)	
Systemic lupus erythematosus	1 (1.1%)	
Obesity, BMI ≥ 27kg/m^2	46 (52.9%)	
Blood pressure during admission, mmHg		
Mean SBP	144.4 ± 12.97	140 (120-190)
Mean DBP	92.31 ± 9.13	90 (75-120)
Diagnosis		
Gestational Hypertension (GHT)	24 (27.5%)	
Preeclampsia	39 (44.8%)	
Chronic hypertension (CHT)	13 (14.9%)	
CHT with superimpose preeclampsia	8 (9.2%)	
Not pregnancy induced hypertension*	3 (3.6%)	
Preeclampsia after diagnose GHT/CHT	6 (15%)	
GA at delivery, weeks	36.25 ± 3.17	37 (21-40)
Abortion	1 (1.1%)	
Preterm	30 (34.5%)	
Term	56 (64.4%)	

* Not meet eligibility criteria of pregnancy induced hypertension or chronic hypertension

As shown in Table 2, the median represented the findings in this study because the data were not normally distributed. The median 24-hour urine protein was 310 mg and the medians of 12-hour urine protein between the first and the second collection of proteinuria were 151 and 155 mg, respectively.

The receiver operating characteristic (ROC) curve was employed for the analysis of all 174 samples for 12-hour urine protein collection. The purpose was to identify the sensitivity and specificity of 12-hour urine protein method for diagnosis of preeclampsia compared with 24-hour urine protein method. The area under ROC curve was 0.964 (95% confidence interval (CI) 0.942-0.986). (Fig. 2)

Table 2. Urine protein values.

Urine protein values	Median (min-max)
24-hour urine collection (mg)	310 (79.2-12974)
12-hour urine collection (mg)	
The first period of proteinuria	151 (14.5-7906)
The second period of proteinuria	155 (11.6-4191)

Area under ROC curve = 0.964 (95%CI: 0.942 to 0.986)
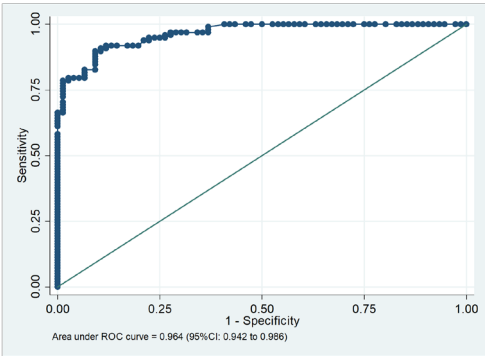
Fig. 2. Sensitivity and specificity of 12-hour urine protein for diagnosis of preeclampsia.

The results appeared that the 12-hour urine protein collection obtained high sensitivity and specificity compared with 24-hour urine protein collection for diagnosis of preeclampsia. The optimal cut-off value of 12-hour urine protein obtained in this study was ≥ 143 mg. This cut-off value presented the highest accuracy of 90.2% and provided a high sensitivity of 89.8%, specificity of 90.8%, PPV of 92.6%, and NPV of 87.3%. Given the cut-off point of ≥ 150 mg as indicated in the study of Silva et al[5] and colleagues, the sensitivity of 85.7% with 90.8% specificity, 92.3% PPV, 83.1% NPV, and 87.9% accuracy were presented in this study.

In addition, Tun et al[9] and Vinayachandran and Darsana[10] suggested the high cut-off value of 12-hour urine protein at ≥ 165 mg, of which it provided high specificity of 100%[9] and 94.7%[10], respectively. The findings from this study were consistent with the previous studies[9,10] (78.6% sensitivity, 97.4% specificity, 97.5% PPV, 77.9% NPV, and 86.8% accuracy). The cut-off value of 12-hour urine protein at ≥ 165 mg illustrated high specificity and PPV, but declined in sensitivity, NPV, and accuracy in comparison to those presented in the cut-off values of 143 and 150 mg.

It was concluded that the cut-off value of 12-hour urine protein of ≥ 143 mg was more appropriate and optimal cut-off to offer higher sensitivity, unchanged

specificity, accuracy with Cohen' kappa value 0.802 (Near perfect agreement) (Table 3).

In addition, this study further assessed the accuracy of the other tests used to verify proteinuria, including UPCR and urine protein dipstick. As illustrated in Table 3, the UPCR test provided 78.8% accuracy, 73% sensitivity, 86% specificity, 87.8% PPV and 70.5% NPV with Cohen' kappa value 0.579 (Moderate agreement). The urine protein dipstick test had the lowest accuracy approximately 67.8% accuracy, 59.2% sensitivity, 78.9% specificity, 78.4% PPV and 60% NPV with Cohen's kappa value 0.368 (Fair agreement). It is illustrated that the 12-hour urine collection test was the best diagnostic test for preeclampsia rather than UPCR and urine protein dipstick test because of high accuracy, specificity, sensitivity, and yielding substantial-near perfect agreement.

Overall, it would appear that the present findings reasonably confirm that the 12-hour urine protein collection provided acceptable accuracy for diagnosis of preeclampsia.

The sensitivity and specificity of 12-hour urine protein by duration were explained by the ROC curve area. The area under the ROC curve of 12-hour urine protein between the first period and the second period of urine collection were 0.964 (95%CI 0.931-0.966) and 0.967 (95%CI 0.938-0.996), respectively and there was no statistically significant difference (p = 0.8726)

Table 3. Reliability and validity of 12-hour urine protein, UPCR, and urine protein dipstick test compared with 24-hour urine protein for diagnosis of preeclampsia.

Cut-off point	Cohen k	p value	Sens (95%CI)	Spec (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
12-hour urine collection							
≥ 143	0.802 (0.713 - 0.891)	< 0.001	89.8 (82 - 95)	90.8 (81.9 - 96.2)	92.6 (85.4 - 97)	87.3 (78 - 93.8)	90.2 (84.8 - 94.2)
≥ 150	0.757 (0.660 - 0.854)	< 0.001	85.7 (77.2 - 92)	90.8 (81.9 - 96.2)	92.3 (84.8 - 96.9)	83.1 (73.3 - 90.5)	87.9 (82.1 - 92.4)
UPCR							
≥ 0.3	0.579 (0.409 - 0.749)	< 0.001	73.5 (58.9 - 85.1)	86.1 (70.5 - 95.3)	87.8 (73.8 - 95.9)	70.5 (54.8 - 83.2)	78.8 (68.6 – 86.9)
Urine protein dipstick							
≥ 1+	0.368 (0.182-0.555)	0.001	59.2 (44.2-73)	78.9 (62.7-90.4)	78.4 (61.8-90.2)	60 (45.2-73.6)	67.8 (56.9-77.4)

Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value, CI: confidence interval, UPCR: urine protein creatinine ratio
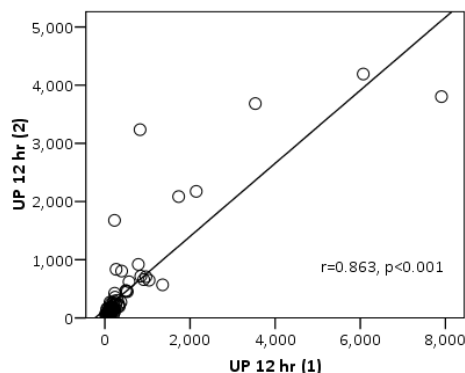
Abbreviation:
UP12hr(1) = The first period of urine collection,
UP12hr(2) = The second period of urine collection,
r = Pearson correlation coefficient

Fig. 3. Correlation of timed urine collection between two periods.

The study found that time to collection of 12-hour urine was correlated with the diagnosis of preeclampsia. The reliability identified by interclass correlation coefficient (ICC) was 0.902 (95%CI 0.850-0.9360) and Pearson correlation was 0.863 (p < 0.001). (Fig. 3)

Discussion

Preeclampsia is the syndrome of a new-onset character of high blood pressure with proteinuria after 20 weeks of gestational age. At present, diagnosis of preeclampsia without proteinuria is acceptable when the presence of extremely high blood pressure measurement at ≥ 160/100 mmHg or clinically severe feature of preeclampsia conditions; including headache, abnormal vision, epigastrium pain, pulmonary edema, or abnormal laboratory consisting of low platelets, hemolysis, elevate liver enzyme, creatinine, or coagulopathy[9].

However, pregnant women with high blood pressure without extremely high blood pressure or absent clinically severe feature form of preeclampsia, the 24-hour urine protein collection is the gold standard test to verify proteinuria[10]. Even if, the UPCR and urine protein dipstick methods provide low accuracy, the tests could be used as a rapid test for diagnosis of preeclampsia and immediate optimal management.

Two-thirds of the participants were multiparity which differ from the previous knowledge, in which nulliparity increased the risk of preeclampsia. The data were analyzed and found that the multiparous participants in this study were significantly associated with high maternal age which increased to 41.4% (p = 0.024) and were not associated with overt diabetes, chronic hypertension, or obesity. Both nulliparous and multiparous participants did not represent the risk of pregnancy hypertension in this study.

The mean blood pressure values during admission included the SBP of 144.4 mmHg (120-190 mmHg) and the DBP of 92.3 mmHg (75-120 mmHg). Most participants who had high blood pressure were included in the study, except for the 3 participants with normal blood pressure consistently. They were included because of the presence of signs and symptoms of preeclampsia; however, their final diagnoses were not eligible for diagnosed pregnancy-induced hypertension and remained in the analysis.

This study stratified the 24-hour urine collection to two periods of collection, using 2 samples of 12-hour urine collection and compared with 24-hour urine protein as a ratio (2:1). The findings revealed that the appropriate cut-off value of 12-hour urine protein was at ≥ 143 mg, yielding the highest accuracy up to 90.2% with reliability indicated by the Cohen's kappa value of 0.802 (Near perfect agreement). Apparently, the findings provided evidence that the accuracy of cut-off value at ≥ 143 mg was higher than the cut-off value at ≥ 150 mg, presented in previous study conducted by Silva et al[5], where the accuracy was 87.9% with the Cohen's kappa value of 0.757 (substantial agreement interpretation).

The study showed that the proper cut-off value at ≥ 143 mg was lower than the cut-off value presented in the previous study. These findings suggested the knowledge to early diagnosis of preeclampsia or consideration of preeclampsia in a case when urine protein value was below 150 mg and the cut-off value ≥ 150 mg of 12-hour urine collection was used.

The 12-hour urine collection test was the best diagnostic test for preeclampsia rather than UPCR and urine protein dipstick test because of high accuracy, specificity, sensitivity, and yielding substantial-near perfect agreement.

Time to collection of 12-hour urine protein was correlated with the diagnosis of preeclampsia. The correlation between the two periods of collection were explained by Pearson correlation coefficient of 0.863 (p <0.001) and ICC = 0.902 (95%CI 0.850-0.936).

The strength of study had several. First, it was the prospective diagnostic study. Second, the findings were examined from urine protein values and other laboratory test results which deliver unbiased results. The limitation of the study was small sample size used in the analysis which could affect reliability.

Conclusions

In conclusion, the 12-hour urine protein collection offered the acceptable accuracy compared with the gold standard of 24-hour urine collection for diagnosis of

preeclampsia. The appropriate cut-off value of 12-hour of urine protein for diagnosis of preeclampsia in this study was ≥ 143 mg with 90.2% accuracy.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. Obstet Gynecol 2019;133:e1-25
2. National Institute for Health and Care Excellence (NICE). Hypertension in pregnancy: diagnosis and management: NICE Guideline [NG133] 2019. Available from: https://www.nice.org.uk/guidance/ng133/resources/hypertension-in-pregnancy-diagnosis-and-management-pdf-66141717671365 [Accessed January 9, 2020].
3. Cunningham FG, Leveno KJ, Bloom SL, Spong C, Dashe JS. Hoffman BL, et al. Williams obstetrics. 25th ed. New York: McGraw-Hill education medical 2018;1344.
4. Lim KH, Steinberg G. Preeclampsia: practical essentials, overview, pathophysiology [Internet]. Emedicine. medscape.com2018 [updated 2018 November 29; cited 2020 Jan 9]. Available from: https://emedicine.medscape.com/article/1476919-overview.
5. Silva RM, Pereira SR, Rego S, Clode N. Accuracy of 12-hour urine collection in the diagnosis of pre-eclampsia. Int J Gynecol Obstet 2018;142:277-82.
6. Leeman L, Dresang ML, Fontaine WP. Hypertensive disorders of pregnancy. Am Fam Physician 2016;93:121-9.
7. Thomas B. Proteinuria workup: approach considerations, renal biopsy [Internet]. Emedicine.medscape.com 2020 [updated 2020 Mar 25; cited 2020 Nov 30]. Available form: https://emedicine.medscape.com/article/238158-overview
8. Watson PF, Petrie A, Method agreement analysis: a review of correct methodology. Theriogenology 2010;73:1167-79.
9. Tun C, Quiñones JN, Kurt A, Smulian JC, Rochon M. Comparison of 12-hour urine protein and protein:creatinine ratio with 24-hour urine protein for the diagnosis of preeclampsia. Am J Obstet Gynecol 2012;207:233.e1-8.
10. Vinayachandran S, Darsana K. Correlation of spot urine albumin and 12-hour urine protein with 24-hour urine protein in pre-eclampsia. J Evid Based Med Health 2017;4:5486-90.