Using 2-hour/6-hour Urine Protein Measurement as Substitute Diagnostic Methods for Evaluation of Pre-Eclampsia

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Citation

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Abstract

Objective: To determine whether the gold standard of 24-hour urine protein value in pre-eclampsia patient can be substituted with 2-hour or 6-hour urine protein values.

Method: We conducted a cross sectional study on women with pre-eclampsia (a positive urinary test strip for protein of at least 1+ and a BP≥140/90 mmHg. Urine samples were collected over 24 hours in subsequent periods: the first 2-hour and the next 6-hour and remaining 16-hour urine , in separate containers . The correlation between both groups was determined by Pearson's correlation.

Result: A total of 36 women were recurited in our study of which 26 had completed urine collection. A total of 16 had mild proteinuria, 2 had severe proteinuria and 8 had no proteinuria. Only 21 of 26 women had the measurements for 6-hour urine collection. There was significant correlation between the 6-hour or 2-hour with 24-hour urine protein.

Conclusion: Total protein values of 6-hour and 2-hour samples, specially 2-hour urine samples, positively correlated with values of 24-hour samples in pre-eclampsia women and could be substituted for assessment of proteinuria instead of 24-hour urine collection in women with pre-eclampsia, as a simpler, faster and cheaper method for diagnosis of pre-eclampsia.

Five to seven per cent of pregnant women are affected with pre-eclampsia, although the incidence of pre-eclampsia is different depending on the geographical location. One of the most common causes of the hospitalization and mortality of pregnant patients in hospitals is eclampsia [1]. According to the national health statistics center, pregnancy-related hypertension is considered the most common serious and dangerous factor for the mother and her fetus [2].

The minimum diagnostic criteria for pre-eclampsia are hypertension (BP 140/90 mmHg) after 20 weeks of gestation and mild proteinuria; more severe forms of pre-eclampsia are characterized by BP 160/110 mmHg and proteinuria >2g/24h or 2+dipstick. Proteinuria is an important sign of pre-eclampsia and Chesly (1985) rightly concluded that the diagnosis of pre-eclampsia is under question in the absence of proteinuria [1].

Repeated Urine Analysis for screening proteinuria is part of standard ante-natal care. These urine analyses are performed on random spot urine specimens using a test strip assay (dipstick) which for pregnant women is more acceptable than 24- hour urine collection. However, if a dipstick is 2+ or more in the absence of bacteria, the next step is usually a 24- hour urine collection for a quantitative measurement of albumin. If a 24-hour urine collection is not possible, a third option, a morning urine sample (semi-quantitative test), is recommended. These recommendations are based on the circadian rhythm in urine albumin excretion [3]. However, for pregnant women, particularly if hospitalized, the circadian variations in albumin excretion are more narrow or even absent, and therefore a shorter urine collection period is possible[4].

When pre-eclampsia is associated with persistent proteinuria, protein excretion is monitored by subsequent 24-

hour urine sample collections. This kind of monitoring can evaluate increases in proteinuria that show the progression of pre-eclampsia and reflect the severeity of nephropathy leading to pre-term labor [3].

The 24- hour urine collection is the gold standard diagnostic method for significant proteinuria in hospitals [5], but it is usually considered difficult and costly and sometimes leads to incomplete collection of urine. In addition, it could delay the diagnosis. This delay in diagnosis of pre-eclampsia and its severity may result in unnecessary hospitalization and an economic burden for patients.

Researchers have suggested faster methods for detection of proteinuria, such using the protein-to-creatinine ratio, and random urinary samples, but these do not reveal the severity of pre-eclampsia as reliably as 24- hour urine collection [6].

It appears that a dipstick is a poor predictive tool for measuring significant proteinuria. Two-thirds of reported cases having mild proteinuria or no proteinuria by dipstick showed significant proteinuria in 24-hour urine collection [7].

We designed this study to evaluate the correlation between 2- and 6-hour proteinuria with 24-hour proteinuria and if possible to suggest 2- and 6-hour-samples as a shorter, acceptable, more affordable substitute for 24-hour samples in the primary evaluation of pre-eclamptic patients.

METHODS

Thirty-six women with pre-eclampsia, admitted to the Obstetrics and Gynecology ward, Qaem Hospital, were included in this cross-sectional study. The inclusion criteria were a positive urinary test for protein of at least 1+ dipstick and BP 140 / 90 after the 20th week of pregnancy. In the case of delivered patients, less than 48 hours should have passed since their delivery. Prior to the urine collection, all women were carefully instructed on the procedure and their permissions had been obtained formally.

A checklist was prepared for each patient asking about her last menstrual period, the number of gestations, gestational sonographic findings, past medical history, family history, symptoms and severe signs on her first visit. Using the results of the routine laboratory tests and other information in the patient's record, other causes of proteinuria such as urinary infection, congestive heart failure, etc., were ruled out to increase the specificity of the study.

Patients who failed to collect their 24- hour urine according to the instructions or who developed a seizure or had any background proteinuric disease were excluded from the study. Finally, 26 patients remained who could complete their urine collection using our instructions. We only included these patients' data.

The patients in this study were on relative bed rest, which means they could get out of bed for at most half an hour per day and their breakfast, lunch and dinner were served to them in their beds. The delivered patients all had urinary catheters so their urinary bag was emptied according to the instructions. The instruction for urine sample collection is as follows:

For collecting of samples, each patient was asked to void before the collection process. For the patients with a urinary catheter, the bags were emptied.

The 2- hour urine sample was placed in a container numbered 1, the following 6 - hour sample in a container labelled No. 2 and the last sample, the 16- hour, was labelled No. 3, plus the patient's name and collection time. The 2-, 6- and 16- hour samples were kept in a refrigerator at a temperature of 40 C until delivered to the Qaem Hospital's laboratory. The urine sample volume of each container was measured separately. The 24- hour urine volume was obtained by adding the 2-hour, 6-hour and 16-hour volumes.

Five ml was taken from each of the 2-, 6- and 24-hour samples for protein measurement. The protein concentration was reported as grams in sample volume for each sample and measured by the turbidometry method.

The gathered data was analyzed with SPSS software. Pearson's correlation was used to obtain the correlation coefficient. Cutoff points for calculating sensitivity and specificity were best determined by ROC and NPV and PPV by crosstab.

RESULT

A total of 36 hospitalized patients were originally included in our study but only 26 of them succeeded in completing the urine collection. The urine collection process of the 10 excluded patients was interrupted by delivery or because their ward changed. Therefore, the study continued with 26 patients. Twenty-one of 26 women had completed the 6-hour urine collection; we missed five 6-hour urine samples due to a mistake by laboratory personnel.

The median age of the studied population was 25 years [Range: 19-38 years]. Of the patients, 92.3 percent were in their third trimester and 7.7% were in the second trimester of their pregnancy. Forty-six percent of the women were nulliparous [n: 12] and 56 percent were multiparous [n: 14]. Sixty-one per cent had a positive family history for hypertension and 19% had a positive past medical history of hypertension. Two patients had a twin pregnancy based on their sonographic reports.

Of the 26 complete urine samples, eight were reported to have no proteinuria, while of the remaining 18 samples, 2 had severe proteinuria and 16 had mild proteinuria. (Since the most severe proteinuric patients lead in pregnancy termination, only 2 severe cases remained in the study). The 6- and 2-hour urine sample results had a meaningful correlation with the results of 24-hour samples, with a correlation coefficient of 0.378 for 6-hour urine protein samples (p:0.00) and 0.77 for 2-hour samples (p :0.00). Figure [1] shows the correlation between 6-hour and 24-hour samples and Figure [2] shows the correlation between 24-hour and 2-hour samples.

The ROC curve for various cutoffs of 6- and 2- hour urine protein samples is shown in Figures [3] and [4]. The value of the area under the ROC curve for 6-hour urine protein is 0.750 and for 2-hour urine protein 0.926.

Figure 1

Figure 1: correlation between 24-hour total urine protein excretion and the 6-hour urine protein excretion(R: 0.378)

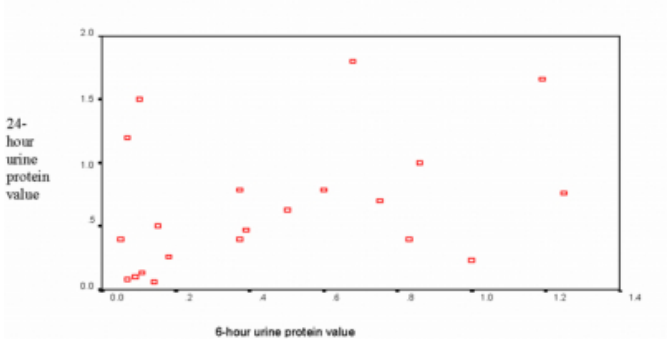


Figure 2

Figure 2: correlation between 24-hour total urine protein excretion and the 2-hour urine protein excretion (R: 0.77).

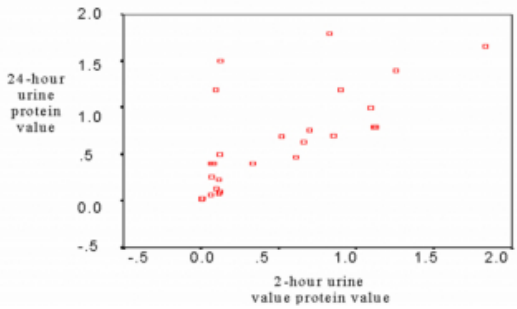


Figure 3

Figure 3: ROC curve for various cutoffs for 6-hour urine protein excretion as a predictor of 24-hour proteinuria . The area under the ROC curve is= 0.750

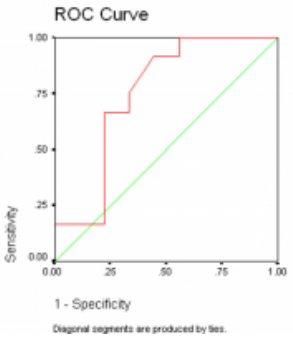
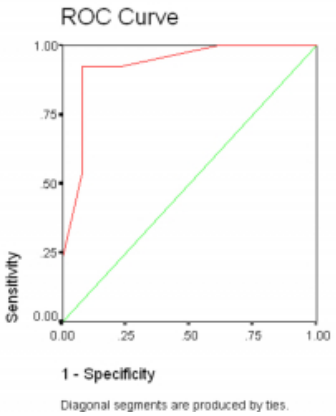


Figure 4

Figure 4: ROC curve for various cutoffs for 2-hour urine protein excretion as a predictor of 24-hour proteinuria . The area under the ROC curve is= 0.926.



The cutoff point of 0.095 g/6-hour yields a sensitivity of 83.3% and specificity of 88.9%, NPV 60% and PPV 64.7% for 6-hour urine collection. Considering the selected cutoff point for 6-hour urine collection, there were 10 false-

negative and 6 false-positive test results. The cutoff point for 2-hour samples was determined to be 0.015 g/2-hour in ROC curve, which has a predictive value for proteinuria with a sensitivity of 77.8% and specificity of 87.5% and NPV and PPV of 63.3% and 93.3%.

The cutoff value of 0.015 g/2-hours for 2-hour urine samples yielded, as well, 8 false-negative and 0 false-positive test results. Most of the false-negative and false-positive test results were respectively within 0.05-0.07 mg and 0.11-0.18 mg for 6-hour samples. For 2-hour urine samples, false-positives were within 0.06-0.07 mg and there was no false-negative.

The mean 24-hour urine protein excretion was 0.722 g/24-hour [SD: 0.129] and the median, 0.55 [Range: 0.04 – 2.5]. The mean urine protein excretion in 6-hour and 2-hour urine collections was 0.161 g/6–hour [SD: 0.034] [Range: 0.008 – 0.500] and 0.053 g/2–hour [SD: 0.009] [Range: 0.001- 0.12].

There was no correlation between 2-hour and 24-hour urine protein results in patients with no proteinuria, which was the same as 6- hour urine protein.

DISCUSSION

In this study, we found meaningful correlations between 6- and 2-hour urinary protein concentrations and traditional 24-hour ones. PPV, NPV, sensitivity and specificity for 6-hour urine protein collections were respectively 64.7%, 60%, 83.3% and 88.95%. The same indices for 2-hour urine protein collections were 93.3%, 63.3%, 77.8% and 87.5%. The analyzed data revealed that 6- and 2-hour urine samples could be used to measure proteinuria in women with pre-eclampsia, but 24- hour urine protein still remains the gold standard test for diagnosis of significant proteinuria.

A shorter time in urine collection can decrease the risk of incomplete urine collection and may have many clinical advantages, such as more rapid diagnosis and, in case of necessity, a shorter delay in glucocoritcoide administration and even termination of pregnancy in these patients.

In severe pre-eclampsia, the increase in protein excretion occurs suddenly and quickly so serial measurements are needed for evaluation of disease progression during patients' hospitalization. In diabetic patients, urinary protein excretion is known to have daily variability; presumably, the same is true in pre-eclampsia [5]. However, there is little likelihood that biologic variability influenced the results as all samples were collected within 24 hours.

Overestimation of proteinuria can lead to unnecessary interventions such as preterm labor. Also, underestimation of urine protein excretion can delay diagnosis of severe nephropathy that ultimately results in kidney damage. Therefore, more attention must be paid to the amount of urine protein as a predictive factor for a patient's condition.

Several studies have been done to recommend the best urine protein measurement. For example, a study shows the protein-to-creatinine ratio in random urine samples in pregnant women to have a correlation with the 24-hour total protein excretion in people with urine protein > 1 g/24- hour [11]. In one other study the protein-to-creatinine ratio in pregnant women with pre-eclampsia was compared with 24-hour urine protein collection and showed that for urine protein > 2g/24h there is poor correlation with the protein-to-creatinine ratio, and therefore the 24-hour collection could not be replaced to identify mild to severe pre-eclampsia in significant proteinuria [8]. Somonthan found significant correlation between 2-hour urine protein and 24-hour urine protein and concluded that 2-hour urine protein collection is more reliable than dipstick [9].

Another study has shown a meaningful correlation between 4-hour and 24-hour urine protein collections in women with hypertensive disorder [10]. Amy et al., comparing the value of 8- and 12- hour urine protein with 24-hour, showed a meaningful correlation in patients with mild and severe proteinuria [5].

Urine protein excretion is variable in a 24-hour cycle that is considered secondary to blood vessel constriction and vasospasm that can cause variation in protein excretion [1]. Protein excretion can be increased in a standing position due to renal vasoconstriction and changing glomerular barrier permeability [2]. A physiological factor known as the circadian rhythm [7] can affect the urine protein collection reliability [4]. In our study, patients were hospitalized and recommended to rest in bed. Patient's 2-hour samples were collected from 8 to 10 in the morning and 6-hour samples from 10 A.M to 4 P.M. Therefore, there was no possibility of evaluating the effect of circadian rhythm on protein excretion value, so another study collecting urine sample at different times of day and night and comparing the value of each sample with 24-hour urine collection should be performed at another time to allow ambulatory follow-up and prevent unnecessary admissions and delays in urine protein collection. In our study we only evaluated the women with pre-eclampsia and ruled out all other diseases

that may cause proteinuria, so we suggest that another study on patients with other pathologic proteinuria disorders, such as diabetes mellitus or other kidney disorders, be conducted to determine if we 24-hour urine collection can be replaced.

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