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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (22): 2584-2588, 2008
ISSN 1028-8880

Urinary Protein Assessment in Preeclampsia: Which Sample is More Suitable?

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Abstract: Mild or severe preeclampsia is responsible for about 70% of hypertensive disorders observed during pregnancy and 24 h urine collection is a gold standard for diagnosis of preeclampsia. This study was performed to determine whether the gold standard of 24 h urine protein value in pre-eclampsia can be substituted with 8 or 12 h urine protein values and to evaluate the effect of ambulation and immobilization on amount of protein excretion. A cross sectional study was conducted on 40 women with pre-eclampsia (BP≥140/90 mmHg), who referred to the Department of Obstetric and Gynecology, Imam Khomeiny hospital in Sari, Iran from April 2005 to September 2005. Positive urinary strip for protein of at least 1+ samples were collected over 24 h in subsequent periods: the first 8 h and the next 4 h and remaining 12 h urine, in separate containers. The correlation between groups was determined by Pearson's correlation. A total of 40 women were recurited in this study of which 36 had completed urine collection. A total of 21 had mild proteinuria, 5 had severe proteinuria and 10 had no proteinuria. There was significant correlation between the 8 or 12 h (day) and 12 h (night) with 24 h urine protein. Total protein values of 8 and 12 h (day) and 12 h (night) samples, positively correlated with values of 24 h samples in pre-eclampsia and could be substituted for assessment of proteinuria instead of 24 h urine collection in women with pre-eclampsia, as a simpler, faster and cheaper method for diagnosis of pre-eclampsia. And ambulation and immobilization in preeclamptic patients has not any effect on protein excretion.

Key words: Hypertension, proteinuria, preeclampsia, diagnosis, 24 h urine

INTRODUCTION

Preeclampsia is a pregnancy-specific multisystem disorder of unknown etiology. The disorder affects approximately 5 to 7% of pregnancies and a significant cause of maternal and fetal morbidity and mortality. Preeclampsia is defined by the new onset of elevated blood pressure and proteinuria after 20 weeks of gestation (Baumwell and Karumach, 2004; Wagner, 2004). The minimum criteria for the diagnosis of preeclampsia is hypertension plus minimal proteinuria, which evident after 20 weeks gestation (Huppertz, 2008). The combination of proteinuria and hypertension during pregnancy markedly increases the risk of perinatal mortality and morbidity (Ferrazzani *et al*., 1990; Chan *et al*., 2005).

Therefore, repeated urine analysis to screen proteinuria are part of standard antenatal care. These urine analysis are performed on random spot urine specimens using a test strip assay. However, if a test strip is positive for protein 2+ or more in the absence of bacteriuria, the next step is usually a 24 h urine collection for quantification of proteinuria. The 24 h urine collection is a time consuming procedure and may result in a delay in diagnosis and treatment or possibly prolonged hospital stay and inaccurate due to incomplete collection. Shortening the period for diagnosis of preeclampsia would be valuable for management, as well as decreasing hospital stay and patient inconvenience. However, for pregnant women, particularly if in hospital, the circadian variation in protein excretion is smaller or absent and it may therefore be possible to use shorter collection period (Kieler *et al*., 2003; Tribl *et al*., 2005). The aim of this study was to evaluate, whether a 24 h urine collection for measuring urinary protein in preeclamptic women could be substituted by a 8 or 12 h collection. In addition we wanted to know if we could collect urine in a short time, which period of time is most suitable, collecting urine in day or night. In other word, we wanted to evaluate the effect of ambulation and immobilization on the amount of proteinuria.

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MATERIALS AND METHODS

Forty women with preeclampsia admitted to the Ward at the Department of Obstetric and Gynecology, Imam Khomeiny hospital Sari, Iran from April 2005 to September 2005 were included in the study. The criteria for inclusion were a positive urinary test for protein of at least 2+ and a planned 24 h urine collection for quantitative protein measurement. Women with upper urinary tract infections, defined as a positive urine culture and fever, were not included. Informed consent was obtained from all women. The patients were not confined to bed rest from 8 am to 8 pm and were allowed to move around in the antenatal ward. The patients were asked to have complete bed rest from 8 pm to 8 am regardless of admitting time. Urine collection started on the first morning after admission to the hospital and all samples were collected within a period of 24 h. Prior to the urine collection, all women were carefully instructed regarding the procedure. At 8 am, the urine was collected to determine RBC, WBC, protein, urine culture and creatinine. In order to increase the accuracy of the test, patients were assisted by a nursing staff for urine collection. The urine was collected in three, separate, clearly marked containers. The first container held the first 8 h of urine, the second container held the next 4 h of urine, while the third container held the remaining 12 h urine sample. Each container was marked with the patient's name, number of the container, collection time and file number (Fig. 1).

The containers were sent to the laboratory of the hospital, where the urine volume of each container was measured with a graduated cylinder and recorded separately. The total 24 h specimen volume was calculated from the summation of all three containers. The urine was stirred to ensure homogeneity and a 6 mL aliquot of urine was taken from the first 8 h collection (sample 1). The remaining urine from the first container was added to the second container and stirred then 6 mL aliquot was obtained as described for the first container (sample NO_2 or day sample). Also 6 mL aliquot of urine was taken from the third container (sample NO_3 or night sample). The remaining urine of the first 12 h (day sample) was added to the second 12 h (night sample), which then represented the entire 24 h urine collection. The urine was stirred and a 6 mL aliquot sample was obtained (sample 4). Then analysis for protein in each of the four aliquot was performed by using modified Fujita method (Sigma Aldrich, 2000, 2001) the absorption that occurs when the Pyrogallol Red-Molybdate reacts with protein to form a bluish purple complex that absorbs at 600 nm proteinuria from the 24 h urine result (sample 4) was categorized as follow: no proteinuria <300 mg, mild proteinuria >300 to <2000 mg and severe proteinuria >2000 mg (Cunningham *et al*., 2005; Tara *et al*., 2008). Data were analyzed using SPSS version 11. Statistical analysis was determined by calculating a correlation coefficient (r) with the p-value less than 0.05 was considered statistically significant. Also prognostic test were analyzed by the use of sensitivity, specificity and positive and negative predictive values.

RESULTS

There were a total of 40 patients with over 20 weeks of gestation and BP = 140/90 mmHg. Two patients were excluded because of positive urine culture and two for delivery prior to collection of urine sample and the data were analyzed statistically.

Patients were classified in three groups based on protein level of 24 h urine: no proteinuria <300 mg (10

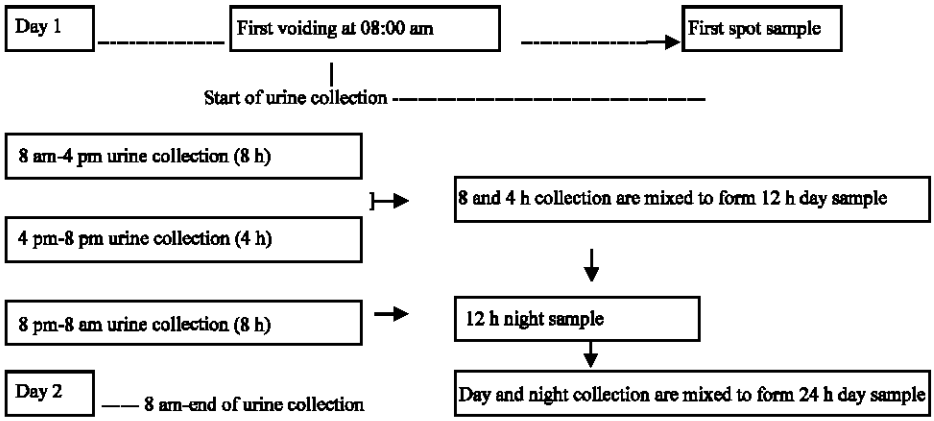

Fig. 1: Flow chart for collection of urinary samples

Table 1: Calculation of Cut of values, Sensitivity, Specificity, PPV, NPV, Pearson correlation coefficient and p-value of different urine samples in mild and sever preeclampsia

Groups	Time period	Cut of values (mg)	Sensitivity (%)	Specificity (%)	PPV	NPV	r	p-value
Mild	8h	105	87	100	100	76	0.98	<0.001
preeclampsia	12 h day	148	94	80	90	88	0.99	<0.001
	12 h night	151	91	100	100	66	0.96	<0.001
Sever	8 h	730	80	100	100	96	0.97	0.000
preeclampsia	12 h day	1100	100	100	100	100	0.99	0.000
	12 h night	>1100	83	100	80	100	0.99	0.000

cases), mild proteinuria ≥300 to <2000 mg (21 cases) and severe proteinuria ≥2000 mg (5 cases). The 8 and 12 h (day) and 12 h (night) of urine protein results correlated with 24 h results for patients with mild and severe proteinuria (p-value = 0.000 and r = 0.97, r = 0.99, r = 0.99) (Table 1).

The cut off value of protein level for 24 h urine as compared to the protein results of 8 and 12 h (day) and 12 h (night) urine was obtained. In this study, the mode was used for determination of cut off value of mild and severe proteinuria. Cut off value for diagnosis of mild preeclampsia was determined to be 105 mg for an 8 h sample, with sensitivity of 87%, specificity and positive predictive value (PPV)of 100% and negative predictive value (NPV) of 76% (r = 0.98, p<0.001). Cut off value for diagnosis of mild preeclamsia was determined to be 148 mg for a 12 h sample (day), with sensitivity of 94%, specificity of 80% and (PPV) of 90% and (NPV) of 88% (r = 0.99, p<0.001). Cut off value for diagnosis of mild preeclamsia was determined to be 151 mg for a12 h sample (night), with sensitivity of 91%, specificity of 100%, (PPV) of 100% and (NPV) of 66% (r = 0.96, p<0.001). Cut off value for diagnosis of severe preeclampsia was determined to be 730 mg for a 8 h sample with sensitivity of 80%, specificity and PPV of 100% and NPV of 96% . Cut off value for diagnosis of severe preeclampsia was determined to be >1100 mg for a 12 h sample (day), with sensitivity, specificity, (PPV) and (NPV) of 100%. Cut off value for diagnosis of severe preeclampsia was determined to be >1100 mg for the 12 h sample (night), with sensitivity of 83%, specificity of 100%, PPVof 80% and NPV of 100%. There was no statistically significant correlation between maternal age and gestational age in patients, with severity of the disease.

DISCUSSION

The measurement of proteinuria has its own problems. The urinary protein excretion in preeclampsia arises due to glomerular endoteliosis. However, it also indicates a generalized increase in capillary permeability in other organ systems of the body. Presence of significant proteinuria acts as a marker for the severity of preeclampsia and patients who are suffering from hypertension and proteinuria are at an increased risk of small for gestational age fetuses and prenatal mortality as well as maternal morbidity (Ferrazzani *et al*., 1990). The most common screening method for detection of proteinuria in preeclampsia is dipstick testing of a random urine sample. The dipstick is inexpensive, easy to use and provides a rapid result, but has been shown to have low sensitivity and specificity for urinary protein excretion over 24 h (Brown and Buddle, 1995; Price *et al*., 2005; Myers *et al*., 2006; Phelan *et al*., 2004; Waugh *et al*., 2003). However, all dipstick testing needs to be confirmed with a 24 h urine collection which is currently accepted as the gold standard for quantification of urinary protein loss. The 24 h urine collection, which has its difficulty, is inconvenient for the patients, costly and may be inaccurate due to incomplete collection. Several investigators explored other means of quantifying proteinuria in a short period of time. The spot urine protein/creatinin ratio is a new method for quantifying protein loss and correlate well with the 24 h urine protein. This technique improves detection of proteinuria and allows for the concentration of the urine in quantifying protein loss. The results are evidently quicker than the 24 h urine collection, which is inconvenient and difficult practically for some of patients (Taherian *et al*., 2006). However, the protein/creatinine ratio of a single urine sample of pregnant woman has been shown to correlate significantly with a 24 h collection of patients with protein values of lesser than 1 g in 24 h. Above this level, the variation between the samples increases (Jaschevatzky *et al*., 1990). Boler *et al*. (1987) studied protein/creatinine ratio in pregnant women with preeclampsia and revealed that the degree of correlation to the 24 h sample was lower in patients with values of greater than 2 g in 24 h.

Therefore, the protein/creatinine ratio is not sensitive enough to determine mild versus severe disease for patients with significant proteinuria and is not suitable to replace the 24 h collection as the standard recommended methodology. Côté *et al*. (2008) on the basis of their systematic review on earlier studies say that we do not advocate use of the spot protein:creatinine ratio or spot albumin:creatinine ratio for monitoring or quantifying proteinuria in pregnancy. The results of this study revealed that the protein values for the 8 and 12 h day and 12 h night are correlated with the entire 24 h sample with high diagnostic value and can predict or diagnose the

preeclampsia in the patients with mild and severe proteinuria. In this study, we calculated cut off values for 8 and 12 (day) and 12 (night) of proteinuria. Total urine protein value of >105 mg in the 8 h sample or 148 mg in the first 12 h sample (day) or >151 mg in the second 12 h sample (night) were predictive of mild preeclampsia, while, 8 h protein values of >730 mg and 12 h values >1100 mg (day and night) were predictive of severe proteinuria. The important point in this study is the low number of patients with severe preeclampsia. Today, due to improvement of prenatal care, there is significant reduction in the incidence of severer preeclampsia and often for the patients who are admitted for severe preeclampsia there is not any sufficient time for the 24 h urinary collection. Several studies have been done for evaluation of proteinuria in a shorter duration of time (2, 4, 6, 8 or 12 h) and all of them revealed that it is possible to determine proteinuria and its severity in a shorter timed urine collections (Adelberg *et al*., 2001; Evans *et al*., 2000; Rinehart *et al*., 1999; Somanathan *et al*., 2003; Wongkitisophon *et al*., 2003) but more studies are needed to generate an exact and reliable cut off values for predicting mild and specially severe preeclampsia. The results of this study in sensitivity and cutoff values of mild preeclampsia were similar to those of Adelberg *et al*. (2001). However, there was a significant difference in cutoff values of 8 and 12 h urine samples for the diagnosis of severe preeclampsia. This difference might be due to this policy in defining severe proteinuria. We categorized present results in severe proteinuria when there was ≤2000 mg protein in 24 h urine (Cunningham *et al*., 2005) versus Adelberg *et al*. (2004), who used 5000 mg as a cut off point.

It is suggested that protein excretion varies. Throughout the day and tends to increase with ambulation and up right position, which produces renal vasoconstriction and alters permeability of the glomerular barrier (Higby *et al*., 1994). These physiologic factors affect protein excretion and produce a circadian rhythm that makes a 24 h collection necessary (Koopman *et al*., 1985; Douma *et al*., 1995). In this study, high correlation between the rate of proteinuria in samples that were collected from 8 am to 8 pm (ambulation period) and from 8 pm to 8 am (immobilization period) revealed that collection of urine can be done at any time (day or night) and daily activity has no effect on proteinuria. This finding is in agreement with McCaw *et al*. (1985), who concluded that proteinuria is not influenced by collection period and bed rest. According to the results of this study, we recommend the 8 h urine collection for the measurement of the peroteinuria in the patient with suspected preeclampsia because of it is faster and more convenience for the patients.

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