JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Title: Comparison of Different Methods to Detect Proteinuria in Normal and Pre-eclamptic Pregnancy

[1]Ummy Salma Munni*, [2]Professor Dr. Khaleda Islam

1. PhD Researcher, Institute of Nutrition and Food Science (INFS), University of Dhaka.
2. Director, Institute of Nutrition and food Science, University of Dhaka

ABSTRACT

Background: Proteinuria is central to the diagnosis of PE and it is an important marker of the severity of progression of the disease. There are several methods of detection of proteinuria, but controversies still exist regarding the most appropriate technique to be used. Moreover, the methods used in our hospitals are still crude in most cases.

Aims: The present study was undertaken to compare the methods used for detection of proteinuria.

Methods: 58 primigravida subjects, aged 17-35 years, with PE were studied along with 100 healthy pregnant women. PE was diagnosed by standard criteria, and proteinuria was measured by heat coagulation (HC), dipstick, sulphosalicylic acid (SSA), protein-creatinine ratio (UPr/Cr) and albumin-creatinine ratio (Alb/Cr). The significance of difference between proportions and medians was measured by Mcnemar chi-square test and Mann-Whitney test respectively.

Results: While all the 100 cases in controls were negative for HC (by definition), there was a significant difference between HC and other tests in the control group. Dipstick detected 2 (χ^2=4.5, $p<0.05$), SSA detected 3 (χ^2=5.33, $p<0.05$), UPr/Cr ratio 5 (χ^2=7.2, $p<0.01$), and Alb/Cr ratio 8(χ^2=10.125, ($p<0.01$) positive cases in the control group. In the case of PE dipstick, SSA and Alb/Cr ratio gave equivalent results. HC was positive in all the 58 cases, but dipstick and SSA detected 41 case (χ^2=15.06, $p<0.001$), and Alb/Cr ratio 42 (χ^2=14.06, $p<0.001$) positive cases. The difference of HC test and SSA with UPr/Cr ratio was statistically significant (χ^2=27.03, $p<0.001$; χ^2=5.5, $p<0.02$). When the microalbuminuria range was considered the Alb/Cr ratio gave additional positive finding in 13 cases of Control and 5 in PE group.

Conclusion:

Dipstick or SSA should be practised which seems to measure albumin accurately as evident from their equivalence with Alb/Cr ratio. But UPr/Cr ratio is a better choice probably due to its capacity to include non-albumin proteins.

INTRODUCTION

Preeclampsia (PE) is the most common hypertensive disorder of pregnancy occurring after 20 weeks of gestation characterized by hypertension, proteinuria and/or edema[1]. The worldwide incidence of the disease is still high in spite of the significant improvement of mother and childcare over the last decades. Its incidence is 5 to 7% among the general population; however, geographic, social, economic and racial differences are responsible for an incidence that is up to three times higher in some populations[2]. Preeclampsia complicates 3-5% first pregnancies and 1% of subsequent pregnancies with around 5-10% cases being severe[3].

Proteinuria is extremely valuable as a prognostic sign in preeclampsia. Frequent monitoring of the amount of protein excreted in the urine must be a part of the evaluation of these patients. A significant increase in proteinuria indicates that the disease has worsened. Accurate measurement of protein in urine is thus, a crucial factor in the management of pregnancy. Only presence or absence of proteinuria changes the management pattern, hospitalization and mode of delivery in different type of hypertensive disorders of pregnancy. Accordingly, protein measurement is a vital part in the diagnosis and management of preeclampsia[4].

In developing world like Bangladesh, we are still using crude methods for detection of proteinuria. It could be argued that the assessment of proteinuria in present routine obstetrical practice is still outdated. The test which we are using may give false negative and false positive results and these false negative results are too dangerous because it places mother and baby at risk. Although it is accepted that proteinuria is assessed most appropriately by the biochemical measurement of total protein excretion over 24-hour period, albumin-creatinine ratio (ACR) and protein-creatinine ratio (UPCR). Most of our public hospitals still use the heat coagulation test; only a few use Sulphosalicylic Acid test (SSA) for detection of proteinuria.

In heat coagulation test urine must be alkaline otherwise protein may not precipitate owing to the formation of alkaline metaprotein and this may hamper the assay. Also there may be considerable individual variation in interpretation. Heat coagulation test is more sensitive than the SSA because it gives positive results with uric acids and some other substances in high concentration.

Sulphosalicylic acid turbidity test is a qualitative screening test for proteinuria. The advantage of this test is its greater sensitivity for proteins. In dipstick urinalysis, proteinuria is detected by the use of reagent strip. It is a semiquantitative measure for the screening of proteinuria[5, 6-8]. This method principally detects negatively charged proteins such as albumin, whereas quantitative method detects all proteins[9]. This test is less sensitive to positively charged proteins such as globulin or part of globulin[10]. It has been found to give high false positive and false negative results, casting doubt on the reliability of dipstick urinalysis for detecting proteinuria in clinical practice.

The false positivity of dipstick for alkaline urine can be eliminated by the addition of SSA[11]. It is a qualitative screening test for proteinuria. It is more accurate than the dipstick in the setting of alkaline urine. However, the SSA measurement of protein are complicated by differences in observer interpretation of turbidity.

Recent studies have documented inaccuracies of these method giving high false positive[5, 12-13] and negative results[5, 14] when compared with the gold standard of 24 hour urine measurement[11]. The result of urine dipstick and

SSA are crude estimation of urinary protein concentration and depends on the amount of urine produced, they correlate poorly with quantitative urinary protein determination[15]. Urine dipstick and SSA tests are crude methods of quantifying proteinuria and should be followed up with a 24 hour urine collection for protein[8].

Most likely problems are often encountered with 24-hour urine collection such as inaccurate timing and incomplete collection. Moreover, it is often difficulty waiting 24-hour or more than hours to know whether proteinuria is truly present[6]. An alternative to the 24-hour urine specimen is the UPCR, determined in a random urine specimen[15-16]. Recent evidence indicates that the UPCR ratio is more accurate and convenient than the 24-hour urine protein measurement[17].

Although PE is a major problem in our country no uniform guideline for detection or management is practiced in our country. Since it has a vast social emotional and economical impact, the early diagnosis of PE is of utmost importance. It may lead to better therapeutic and nutritional management of the patients and thus avoid major catastrophes. To identify the problem in our country the current practice for diagnosing PE need to be explored. Urine from pregnant mother in our population has unique physical and chemical characteristics due to specific nutritional and social (including sanitary) habits and customs. Thus, the detection methods for proteinuria may not be appropriate in our population. Unfortunately, due to poor nutritional status and poor socioeconomic condition in our country a large number of preeclampsia patients are present[18].

There is currently no proven way to prevent PE but good prenatal care and regular visit to the physician will allow for early diagnosis before the condition becomes severe. As preeclampsia is a life-threatening systemic syndrome contributing significantly to serious complications for both fetus and mother[19]. It should be diagnosed accurately. In our population there is no data comparing heat coagulation, dipstick analysis and SSA method with UPCR, which detects the accurate level of proteinuria for the diagnosis of preeclampsia. Also, it is necessary to compare with ACR. Because protein detected in pregnancy and PE may be non albumin in nature. It may vary depending on racial and dietary habits. This study will provide an opportunity to assess the accurate method of detecting the presence of proteinuria. The study has been designed with the above perspective in mind.

SUBJECTS AND METHODS

This case control study was conducted at Dhaka, BIRDEM hospital. One hundred and fifty-eight pregnant women with gestational age of ≥ 20 weeks, who were referred to the hospital for prenatal care, were enrolled in the study. The study examined 58 pregnant women having pre-eclampsia and 100 pregnant women were without preeclampsia. All the women were aged between 17-35 years and primigravidas.

The study was explained to each individual subject and informed consents were taken. Detailed sociodemographic data, family history and medical history were taken on predesigned case record forms.

Urine Protein levels were measured by HC test using a acetic acid method, Dipstick test of urine protein (albumin) by Uriscan strip (YD Diagnostics, Thailand)[20] Sulphosalicylic test by sulfosalicylic acid method.[21] The

concentration of urinary Microalbumin by Immunoturbidimetric method, Urine Creatinine (Alkaline-picrate method), Urine total protein (Pyrogallol red method), The urine protein-creatinine ratio was obtained by dividing the urine protein concentration (mg/l) by the urine creatinine concentration (mol/l) [8].

True proteinuria (≥300mg/day) was detected UPCR ratio of >30 mg protein/mol creatinine[6]. Sensitivity, specificity, positive and negative predictive values were determined for different UPCR ratio values for proteinuria >30 mg protein/mmol creatinine as positive and <30 mg protein/mmol creatinine as negative[6].

The urine albumin-creatinine ratio was obtained by dividing the urine albumin concentration (mg/l) by the urine creatinine concentration (mg/l)[8]. Sensitivity, specificity, positive and negative predictive values were determined for different albumin-creatinine ratio values for proteinuria >25 mg albumin/mmol creatinine as positive and <25 mg protein/mmol creatinine as negative[22].

Statistical Analysis were performed by using statistical package (SPSS, Inc,Chicago,IL, USA). Significant of difference between proportions was measured by Macnemar Chi-Square test and the significance of median (range) was performed by Mann-Whitney test. Receivers operating characteristic (ROC) curve analysis were performed to examine the relative performance of the different methods. Sensitivity, specificity and predictive values of the UPCR and ACR were measured for cut-off point >30 and >25 mg/mmol separately.

RESULTS

One hundred and fifty-eight pregnant women with mean age 28 yrs (range 18-35) were enrolled in this study. Clinical characteristics of the women recruited showed that the median systolic blood pressure was 100 (70-130) mmHg in PE group at the time of recruited.

At the time of study testing, in control group comparison between HC and DP measurement of urinary protein, the result showed that no protein was detected by HC and 2 patients were found as proteinuric in dipstick test. Specificity and NPV werefound 100% and 98%, Again comparison between HC and SSA, 3 patients have shown positive proteinuria and specificity and NPV showed 100% and 97%, when UPCR was compared to HC, 5 patients have found proteinuria. Specificity and NPV was 95% and 95% and 100%. ACR found 8 patients proteinuric and specificity NPV were found 100% and 92%.

On the otherhand, in PE group comparison between HC and measurement of DP urinary protein, the result showed 17 patients were non proteinuric and Sensitivity and PPV were 100% and 70%. SSA method detected same 17 patients, sensitivity and PPV 100% and 70%. UPCR were detected 29 patient's nonproteinuric and sensitivity and PPV were 100% and 50%. ACR detect 16 patients were nonproteinuric and sensitivity 100% and PPV were 72%.

When SSA to detect 41 patients were proteinuric, where as 17 patients were nonproteinuric and 100% showed sensitivity, specificity and NPV. UPCR showed 17 proteinuric and 5 nonproteinuric patients. Sensitivity, specificity, PPV and NPV were 83%, 41%, 58% and 70% respectively. ACR detect to 6 patients were proteinuric.

DISCUSSION

For protein measurement most of our hospitals use the crude method like heat coagulation test; only a few use SSA for detection of proteinuria. How ever the diagnostic accuracy, sensitivity and specificity of heat coagulation test, SSA and dipstick test has been questioned.

The heat coagulation and dipstick methods of urinary protein measurement were compared in the present study. By definition Control group had no proteinuria in the heat coagulation test. It may be expected that dipstick and SSA would give the same results. But unexpectedly dipstick and SSA showed 2 and 3 (out of 100) positivity respectively. This can be explained that strip method and SSA can detect lower levels of protein, that was not detected by heat coagulation method. This observation confirms that strip is more sensitive than heat coagulation test in detection of proteinuria. This finding may also be due to false positive interpretation. Observer error appears to be a significant source of inaccuracy of dipstick analysis. It has been shown that dipstick urinalysis which is usually done as the screening test for proteinuria has been found to give high false positive results.[8] False positive results on conventional dipstick testing occur with alkaline urine, highly concentrated urine gross haematuria, pus, semen or vaginal secretion. The false positivity of dipstick for alkaline urine can be eliminated by the addition of SSA[11]. But the false positivity of this finding to heat coagulation test was excluded by SSA and UPr/Cr ratio.

In comparison between dipstick with SSA method in Control group, SSA is more sensitive than the dipstick result. It may be due to presence of protein other than albumin, such as Bence Jones protein which can not be detected by dipstick method[23].

When UPr/Cr ratio were compared to other method of urinary protein detection, it has been shown that UPr/Cr ratio is more sensitive and specific than other methods. Recent evidences also indicate that the UPr/Cr ratio is more accurate and convenient than the 24 hour urine protein measurement and UPr/Cr ratio determined in a random urine specimen may be an alternative to 24 hour urine specimen.[16-17]

Taking advantages of the availability of a substantial number of PE subjects, the comparison between different methods of protein detection was explored in this work. In PE group, by definition heat coagulation test showed 100% positivity. But both of dipstick and SSA showed 41 (out of 58) positivity and 70% PPV when compared with the heat coagulation test. These 17 (out of 58) negativities of dipstick can be explained that it can only detect albumin and the measured proteins were non albumin or low molecular weight protein. But in SSA this negativity may be due to dilute urine or alkaline urine.

When urinary protein-creatinine ratio was compared with heat coagulation, UPr/Cr ratio showed 29 (out of 58) positivity and 29 (out of 58) negativity. So UPr/Cr ratio is more reliable and accurate than heat coagulation test, SSA and dipstick method from the point of sensitivity, specificity and predictive value.

In Control group Alb/Cr ratio was significantly different from heat coagulation. But no significant differences were observed between Alb/Cr ratio, dipstick and SSA method. But urinary UPr/Cr ratio showed 5 (out of 100) positivity in comparison with 8 (out of 100) positivity of Alb/Cr ratio. In comparison between heat coagulation

and Alb/Cr ratio in PE group 42 (out of 58) positivity was found in Alb/Cr ratio. But when it was compared with UPr/Cr ratio 29 (out of 58) negativity were observed. These 13 (out of 58) discrepancies may be due to microalbuminuria which was not detected by UPr/Cr ratio. It may be reason that these subjects did not develop clinical proteinuria. By follow up of these subjects, we can predict whether these subjects will develop preeclampsia in future or not. There is also controversy regarding this issue. Some suggest in whom abnormal proteinuria develops usually have microalbuminuric phase weeks earlier and this test has some predictive value for predicting the subsequent development of PE[24]. But other studies oppose these findings on the ground of a statistically significant increase in urinary albumin excretion was observed with increasing gestational age as a normal phenomenon and they conclude microalbuminuria can not be used as a predictor of PE[25].

The present data confirms that UPr/Cr ratio is the most sensitive and accurate method but we are still using of crude methods for detection of proteinuria. So it is argued that the assessment of proteinuria in present routine obstetrical practice has not taken a forward step. As PE is a major problem in our country and it has a vast social, emotional and economical impact on population. So early diagnosis of proteinuria by accurate method is utmost importance.

CONCLUSION

Heat coagulation test should be avoided to measure proteinuria in case of pregnancy due to considerable chance of false negativity and positivity. At the least dipstick or SSA should be practiced which seem to measure albumin accurately as evident from their equivalent with Alb/Cr ratio. But UPr/Cr ratio is a better choice problem due to its capacity to include non albumin proteins. In addition to UPr/Cr ratio, Alb/Cr ratio may be suggested as a routine test in pregnancy for detecting microalbuminuria which is a predictor of PE in this state.

REFERENCES

1. *William. C, Mabie, MD, Baha M, Sibai MD.* Hypertensive states of pregnancy, in, Alan H. Decherrney and Martin L. Pernoll (editors), Current Obstetric and Gynaecologic Diagnosis and Treatment. 8th ed. Prentice-Hall International; 1994. p. 380.

2. *Lopez- Jaramilleo P, Casas JP, Serrano N, Braz.* J Med Biol Res 2001 Oct; 34 (10): 1227-35.

3. *Robson SC.* Hypertension and Renal disease in pregnancy. In: Keith Edmonds (Editors), Dewhurst's Text book of Obstetrics and Gynaecology for post-graduates. 6th ed. B black Well Science; 1999. p. 166-167,169.

4. *Hayashi M, Ueda Y, Hoshimoto K, Ota Y, Fukasawa I, Sumori K, et al.* Changes in urinary excreation of six biochemical parameters in normotensive pregnancy and pre-eclampsia. Am J Kidney Dis 2002 Feb 39(2): 392-400.

180.

5. *Halligan AWF, Bell SC, Taylor DJ.* Dipstick Proteinuria: caveat emptor, Br Jl Obstet Gynaecol, 1999 Nov 106: 1113-1115.

6. *Saudan PJ, Brown MA, Farrel T, Shaw L.* Improved methods of assessing proteinuria in hypertensive pregnancy. Br J of Obstet Gynaecol 1997 Nov; 104: 1159-1164.

7. *Bell SC, Halligan AWF, Martin A, Ashmore J, Shennen AH, Lambert PC, et al.* The role of observer error in antenatal dipstick proteinuria analysis. Br J of Obstet Gynaecol 1999 Nov; 106: 1177-1180.

8. *Carroll MF, Temte JL.* Proteinuria in Adults: A Diagnostic Approach. American family physician. University of Wisconsin-Madison Medical School, Madison, Wisconsin. 2000 Sep

9. Curtis *C:* Proteinuria. Medical education, Nursing ceu.com; 2001-2003 February-20.

10. Turner White Communication Inc. "Proteinuria: Harbinger of serious clinical consequence Wayne, PA, 2002.

11. Urine Dipstick Protein, College of Veterinary Medicine, Cornell University. Available at http: //web.vet. cornell.edu/public/popmed/clin path/cpPage /Chemistry/uprotsx.htm.

12. *Kuo VS, Koumantakis G, Gallery EDM.* Proteinuria and its assessment in normal and hypertensive pregnancy, Am J Obstet Gynecol 1992; 17: 723-728.

13. *Brown MA, Buddle ML.* Inadequacy of dipstick proteinuria in hypertensive pregnancy, Aust NZ J Obstat Gynecol 1995; 35: 4: 366-369.

14. *Meyer NL, Mercer BM, Friedman SA, Sibai BM.* Urinary dipstick protein: a poor predictor of absent or sever proteinuria. Am J Obstet Gynecol 1994; 170: 137-141.

15. *Larson TS.* Evaluation of proteinuria. Mayo Clin Proc 1994; 69:1154-8.

16.*Ginsberg JM, Chang BS, Matarese RA, Garella S.* Use of single voided urine samples to estimate quantitative proteinuria. N Engl J Med 1983; 309: 1543-6.

17.*Schwab SJ, Christensen RL, Dougherty K, Klahr S.* Quantitation of proteinuria by the use of protein-to-creatinine ratios in single urine samples. Arch Intern Med 1987, 147: 943-4.

18. Mattar F, Sibai BM. Prevention of preeclampsia. Semin perinatal, 1999, 23(1): 58-64.

19. Report of the working group on research on hypertension during pregnancy. National Heart, Lung, and Blood Institute [online]. 2001 April 27; Available from: http: //www.nhlbi.nih.gov/resources/ hyperten _preg/resources .html

20. Uriscan strip (YD Diagonostics, Thailand). Available from: http://yddia gnostics.com/products/products.asp

21. Blood Safety, Clinical Technology. New Delhi India. http://w3.whosea. org/ micro/21.htm.

22. *Donnelly R.* Should microalbuminuria be a therapeutic goal in hypertensive patients with type 2 diabetes? J Hyperton, 2002; 6 (Sonderheft 3): 36-38.

23. *Laffeyette RA, Perrone RD, Levy AS.* Laboratory evaluation of renal function. In: Schrier RW, Gottlschalk CW. editors. Diseases of the kidney. Boston, Mass; Little Brown, 1996; p. 339.

24. *Bar J, Hod M, Friedman S, Gelerenter I, Kaplan B, Boner G et al.* Microalbuminuria as an early predictor of hypertensive complications in pregnant women at high risk. Am J Kidney Dis 1996 Aug; 28 (2): 220-5.

25. *Kinstantin HKF, Hesseldahi H, Pederson SM.* Microalbuminuria as a predictor of preeclampsia. Acta Obstet Gynecol Scand 1992 Jul; 71 (5): 343-6.