Original Article

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Biochemistry Section

Detection of Proteinuria in Pregnancy: Comparison of Qualitative Tests for Proteins and Dipsticks with Urinary Protein Creatinine Index

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ABSTRACT

Background and Objectives: Excretion of urinary protein increases to 300 mg/d (from up to 150 mg/d) in normal pregnancy. Values above this may be due to disorders that can endanger the patient or her pregnancy. Quantitative analysis of 24-hour urine is considered the gold standard for ascertaining daily protein excretion. Routine laboratory tests performed on spot urine samples indicate protein concentration in the particular sample, and can lead to diagnostic error if urine output is less or more than 1L/d. The Protein Creatinine Index (PCI) shows good correlation with 24-hour protein estimation. However, PCI varies with sex and race. We have correlated the results of qualitative estimation procedures and the dipstick values with protein creatinine index.

Material and Methods: We measured protein and creatinine in spot urine samples obtained from 57 pregnant and 80 non–pregnant healthy women of 18–36 years, and calculated PCI. We also tested the samples qualitatively for proteins by routine tests and dipsticks.

Results: Normal range of PCI in non–pregnant women, determined by a non–parametric method was 30–150. PCI was increased significantly in pregnancy (maximum increase in the third trimester). Amongst the qualitative tests, heat coagulation test gave the lowest percentage of false positives and a slightly higher percentage of false negatives compared to Heller's nitric acid and sulphosalicylic acid tests, and dipsticks.

Interpretations and Conclusions: We conclude that heat coagulation test be used for initial screening, with PCI being performed on all samples testing positive to rule out false positives.

Key words: PCI, Pregnancy, Non-Pregnant Women, Dipsticks

INTRODUCTION

Antenatal tests commonly performed include measurement of weight and blood pressure, estimation of haemoglobin, and qualitative tests for proteins in urine. While the upper limit of the urinary protein excretion is 150 mg/d in normal non–pregnant women [1], it increased up to about 300 mg/d in normal pregnancy, due to increase in blood volume and, therefore, the glomerular filtration rate. Protein excretion exceeding 500 mg/d is central to the diagnosis of preeclampsia in a hypertensive pregnancy and is statistically associated with negative outcomes [2].

Routine examination of random spot urine samples is usually performed by semi-quantitative tests like heat coagulation test, Heller's nitric acid test, and urinary dipsticks. Though easy to perform, these tests indicate the approximate protein concentration at the time of sampling and fail to give an idea about the total daily excretion of protein. Thus, if the urine output in one day is much less or more than 1 litre, misinterpretation of result is likely to occur. Daily excretion of protein can be ascertained only by quantitative analysis of protein in a 24–hour urine sample. This is often cumbersome, inconvenient, involves missing work/school, has poor compliance in ambulatory patients [3], and often delays diagnosis and treatment [4].

The estimation of Protein Creatinine Ratio (PCR) or Protein Creatinine Index (PCI) from spot urine samples obtained from pregnant women shows promising diagnostic value for significant proteinuria in suspected pre–eclampsia [5], a condition that complicates 2%–8% of all pregnancies [6]. However, racial differences in creatinine excretion have been reported [7] with Indo-Asians showing lower creatinine excretion and higher albumin/creatinine ratios [8]. Therefore, results obtained in one racial/ethnic group may not be applicable to others.

There is little data comparing protein excretion in the three trimesters of pregnancy [9]. We are unable to find literature comparing the urinary protein excretion in Indian non-pregnant and pregnant women. In the present study, performed on North Indian subjects, we have determined normal PCI in healthy non-pregnant women, compared it with that in pregnant women, and have compared the protein excretion and PCI in the three trimesters of pregnancy. We have also compared the routine qualitative tests and dipsticks with urinary PCI for the detection of proteinuria.

METHODOLOGY

The study was conducted during a period of 6 months (April–September, 2011) at Teerthanker Mahaveer Medical College and Research Centre, Moradabad, India, after obtaining permission from the Institute's Ethical Committee. Untimed urine samples were obtained from normal pregnant women (n=57) visiting the outpatient department of the Gynaecology and Obstetrics Department of the college. Urine samples from healthy non-pregnant women (n=80) of the same age group (between 18–36 years) were obtained similarly while the subjects were not in the menstrual phase of the cycle.

Qualitative and semi-quantitative analysis of urine for protein was performed using heat coagulation test, Heller's nitric acid test, and sulfosalicylic acid test [10] and by urinary dipsticks according to manufacturer's instructions. Quantitative colorimetric estimation of urinary protein was done by sulfosalicylic acid method [11] and of urinary creatinine by modified Jaffe's method [12]. Protein creatinine index of each urine sample was determined by the method of Shaw et al., [13].

Statistical Analysis: Normal range of urinary PCI was calculated from the data obtained from healthy non–pregnant subjects, using

non–parametric method as the frequency distribution was non-Gaussian. The 2.5th to 97.5th percentile (95%) of the frequency distribution curve was taken as the reference or normal range.

Data were compared by Student's t–test and by ANOVA. p–Values less than 0.05 were considered significant.

RESULTS

The modal range of urinary PCI in non-pregnant women (with maximum number of observations) was 60-80. The normal range of urinary PCI as determined by the non-parametric method was 30–150.

[Table/Fig-1] compares the urinary protein, creatinine, and PCI of normal non–pregnant and pregnant women. Significant differences were observed in all the three parameters. [Table/Fig-2] compares the urinary protein, creatinine, and PCI obtained in the three trimesters of normal pregnancy by ANOVA. Significant differences were observed in all the parameters.

A total of 125 subjects (non-pregnant and pregnant) had PCI value less than of 125, while 12 subjects had PCI values greater than or equal to 150. [Table/Fig-3] compares the results of the qualitative tests with the PCI values. The percentage of false positives was lowest with heat coagulation test (10.2%), while the percentage of false negatives was slightly higher with the heat coagulation test, compared to the other tests.

Parameter	Non-Pregnant (n = 80)	Pregnant (n = 57)
Protein (mg/dL)	61.88 ± 36.01	126.75 ± 64.06
Creatinine (mmol/L)	8.32 ± 3.45	10.26 ± 3.62
PCI	75.39 ± 28.76	116.58 ± 39.96

[Table/Fig-1]: Comparison of urinary protein, creatinine, and PCI of non pregnant and pregnant women

Parameter	1st Trimester (n=18)	2nd Trimester (n=20)	3rd Trimester (n=19)
Protein (mg/dL)	83.33± 38.35	122.5± 62.78	172.37 ± 55.84
Creatinine (mmol/L)	9.14± 3.14	9.39± 3.53	12.23± 3.48
PCI	88.67 ± 25.46	116.4± 33.42	143.2 ± 40.63

[Table/Fig-2]: Comparison of urinary protein, creatinine, and PCI in the three trimesters of pregnancy by ANOVA

Qualitative Test	Protein Creatinine Index					
	PCI<150 (n=125)			PCI>150 (n=12)		
	Negative	Positive	% False +ve	Positive	Negative	% False -ve
Heat Coagulation Test	111	14	10.2	9	3	2.3
Heller's Nitric Acid Test	63	62	45.9	10	2	1.5
Sulfosalicylic Acid Test	66	59	43.1	10	2	1.5
Urine Dipstick Test	69	56	40.9	10	2	1.5

[Table/Fig-3]: Correlation of qualitative tests results with urinary PCI in non-pregnant and pregnant women

PCI	PCR (mg/mg)	PCR (mg/m mol)	Reference
-	-	17-57	Cote et al., [19]
140	0.18	-	Khan et al., [20]
-	0.2	-	Shahbazian & Hosseini–Asl [21]
-	1.14	-	Aggarwal et al., [22]
-	0.19	-	Al et al., [23]
-	0.19	-	Dwyer et al., [24]
-	0.30	-	Leanos-Miranda et al., [25]

[Table/Fig-4]: Comparison of reported values of protein creatinine index (PCI) and protein creatinine ratio (PCR) for excluding proteinuria

DISCUSSION

The reference PCI range obtained from normal non-pregnant women in this study was 30–150. Shaw et al., [13] have reported a PCI of less than 125 in normal subjects (excreting 150 mg or less of urinary protein per day). Indians have less muscle mass and therefore excrete less creatinine than persons of Western origin, consequently showing slightly higher value of PCI. Gupta and Gupta [14] have reported a range of 37 to 247 in normal male and female subjects of Indian origin, of the age group 18 to 67 years. The higher values reported by them may be due to one or more of the following reasons:

1. Their study included subjects of both sexes. Usually, Indian males consume more protein than the females. Higher protein consumption is associated with increased excretion of urinary albumin [1], thus raising the PCI.
2. The subjects in our study were younger (18-36 year). The number of functional nephrons decreases with advancement of age, causing increased GFR in the remaining nephrons [15]. This might result in increased protein excretion leading to higher PCI.
3. Muscle mass decreases with advancement of age [16]. Decreased muscle mass results in decreased formation and excretion of creatinine, leading to higher PCI values.

Protein excretion increased significantly in pregnancy [Table/Fig-1]. The increase was slight in the first trimester and more in the second and the third trimesters. A prospective study by Higby et al., [17] on 270 gravidas showed the upper limit of urinary protein excretion of the 95% CI as 259.4 mg/d. They had further observed that proteinuria was greater in the second half of the pregnancy, as compared to the first. This observation matches ours, and was interesting as maximal increases in glomerular filtration have already appeared before mid pregnancy, and GFR might actually decline near term [18]. These observations suggest that either filtration or tubular reabsorption of proteins may change in late gestation [9].

Excretion of creatinine showed significant increase in pregnancy due to the increase in GFR. The PCI was significantly higher in pregnant state. The increase in PCI in the first trimester was not statistically significant. However, significant increases were observed in the second and third trimesters. The range of PCI observed in the pregnant women (44.6-228.7) corresponds to a urinary protein excretion of up to 250 mg/d. If the urine output was more than one litre, the diluted urine would give the same PCI.

When the upper limit of normal urinary PCI was taken as 150, a correlation of qualitative tests with urinary PCI [Table/Fig-2] showed the lowest rate of false positives by the heat coagulation test (10.2%), while the other tests (including dipsticks) showed much higher false positive results. False negative results varied from 1.5%–2.3%, with no discernible difference between different qualitative tests. It therefore appears that the heat coagulation test can be used to screen spot urine samples for the presence of protein. The PCI should be used as a confirmatory test, to rule out the possibility of false positives. Since the heat coagulation test may not be positive for the presence of small molecular weight proteins (as may be excreted in case of renal tubular disease), the merest hint of any renal disorder requires estimation of urinary PCI, using a protein detection method that is sensitive for the small molecular weight proteins also.

This study shows that the convenience of using only qualitative tests for urinary protein detection (dipsticks, sulfosalicylic acid test, Heller's nitric acid test, or heat coagulation test) may sacrifice the accuracy of diagnosis of proteinuria and consequently compromise the safety of the patient and her pregnancy. While these tests may be used in the routine examination of normal pregnant women, urinary PCI may be a better option in subjects in

whom early and definitive diagnosis of proteinuria is important e.g. in suspected cases of hypertensive pregnancies, preeclampsia, and HELLP (Hemolysis, Elevated Liver Enzymes, and Low Platelet Count) syndrome.

While searching literature related to PCI in pregnancy and renal diseases, a wide discrepancy in terms and values was observed. While Shaw et al., [13] have used the term protein creatinine index (PCI) and reported its value as less than 125 in normal subjects; other authors have used different terms with different formulae, presented in [Table/Fig-4]. Parag and Seedat [26] have calculated the PCI from the formula:

PCI=Spot urinary protein (mg/L)/Spot urinary creatinine (µmol/Lx 10-4)

They have obtained values upto 24000 in patients with proteinuria.

The use of different terms and units underlines the urgency of establishing a single unambiguous term for relating urinary protein and creatinine concentrations. Since the term protein creatinine index (as used by Shaw et al., [13]) appears to be unambiguous with no conflicting formulae for its calculation, we recommend its use for further studies.

CONCLUSIONS

The normal range of PCI in young adult (18–36 years) North Indian non–pregnant women is 30–150. Protein concentration in spot urine sample cannot be used to rule out proteinuria if urine output is more than 1 litre/day.

The use of qualitative tests for urinary protein detection should be restricted to routine pregnancy check up, and the patient should be questioned about the quantity and frequency of urine passed in a day (normal/approximately 1 litre or more). Since the heat coagulation test correlates best with urinary PCI in spot urine samples, this may be used for screening urine samples for the presence of protein. Samples testing positive with the heat coagulation test should be rechecked by establishing the PCI to rule out false positives.

Protein creatinine index should be obtained immediately from spot urine samples on suspicion of any abnormal condition during or after pregnancy.

There is an urgent need to establish a single, unambiguous term for relating urinary protein and creatinine concentrations.

REFERENCES

[1] Gosling P. In'Clinical Biochemistry. Metabolic and Clinical Aspects. 2nd Ed'. Editors: Marshall WJ, Bangert SK. Churchill Livingstone. *Elsevier.* 2008. Pp: 156-73.

[2] Maybury H, Waugh J. Proteinuria in pregnancy – Just how much is significant? *Fetal and Maternal Medicine Review.* 2004; 16 (1):71-95.

[3] Price CP, Newall RG, Boyd JC. Use of protein: creatinine ratio measurements on random urine samples for prediction of significant proteinuria: A systematic review. *Clinical Chemistry.* 2005; 51 (9): 1577-86.

[4] Agarwal I, Kirubakaran C, Markandeyulu S. Quantitation of proteinuria by spot urine sampling. *Ind J Clin Biochem.* 2004; 19 (2): 45-7.

[5] Morris RK, Riley RD, Doug M, Deeks JJ, Kilby MD. Diagnostic accuracy of spot urinary protein and albumin to creatinine ratios for detection of significant proteinuria or adverse pregnancy outcome in patients with suspected pre-eclampsia: systematic review and meta-analysis. *BMJ.* 2012; 345: E4342.

[6] Khan K, Wojdyla D, Say L, Gulmezoglu A, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet.* 2006; 367:1066-74.

[7] Mattix HJ, Hsu CY, Shaykevich S, Curhan G. Use of the albumin/creatinine ratio to detect microalbuminuria: importance of sex and race. *J Am Soc Nephrol.* 2002; 13: 1034-9.

[8] Cirillo M, Laurenzi M, Mancini M, Zanchetti A, De Santo NG. Low muscular mass and overestimation of microalbuminuria by urinary albumin to creatinine ratio. *Hypertension.* 2006; 47: 56-61.

[9] Lindheimer MD, Kanter D. Interpreting abnormal proteinuria in pregnancy. The need for a more pathophysiological approach. *Obstet Gynaecol.* 2010; 115: 365-75.

[10] Practical Clinical Biochemistry. Methods and Interpretations. 3rd Ed. Ranjna Chawla. Jaypee Brothers Medical Publishers (P) Ltd. 2003. Pp:58-9.

[11] Varley's Practical Clinical Biochemistry 6th Ed. Editors: Gowenlock AH, McMurray JR, McLauchlan DM. *CBS Publishers and Distributers.* 1988. Pp: 447-8.

[12] Practical Clinical Biochemistry. Methods and Interpretations. 3rd Ed. Ranjna Chawla. *Jaypee Brothers Medical Publishers (P) Ltd.* 2003. P 170.

[13] Shaw AB, Risdon P, Lewis-Jackson JD. Protein creatinine index and albustix in assessment of proteinuria. *BMJ.* 1983; 287: 929-32.

[14] Gupta RC, Gupta U. Urinary protein/ creatinine index. *Clin Chem.* 1986; 32 (5): 914.

[15] Guyton AC, Hall JE. Textbook of Medical Physiology, 12th Ed. *Elsevier Saunders Inc.* 2011. Pp: 303-22.

[16] Caruso LB, Silliman RA. Geriatric Medicine. In 'Harrison's Principles of Internal Medicine Vol 1, 17th Ed. Editors Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo *J. McGraw Hill.* 2008. Pp: 53-62.

[17] Higby K, Suiter CR, Phelps JY, Siler-Khodr T, Langer O. Normal values of urinary albumin and total protein excretion during pregnancy. *Am J Obstet Gynaecol.* 1994; 171:984-9.

[18] Conrad KP, Gaber LW, Lindheimer MD. The kidney in normal pregnancy and preeclampsia. In: Lindheimer MD, Roberts JM, Cunningham FG, editors. Chesley's hypertensive disorders in pregnancy. 3rd Ed. San Diego (CA): *Academic Press.* 2009. Pp 297-334.

[19] Cote AM, Brown MA, Lam E, von Dadelszen P, Firoz T, et al. Diagnostic accuracy of urinary protein: creatinine ratio for proteinuria in hypertensive pregnancy: Systematic review. *BMJ.* 2008;336(7651): 1003-6.

[20] Khan DA, Ahmad TM, Kureshi AH, Abdul H, Mumtaz A, et al. Assessment of proteinuria by using protein: creatinine index in random urine samples. *J Pak Med Assoc.* 2005; 55 (10): 428-31.

[21] Shahbazian N, HosseiniAsl F. A comparison of spot urine protein creatinine ratio with 24-hour protein excretion in women with preeclampsia. *Iranian J Kid Dis.* 2008; 2 (3): 127-31.

[22] Aggarwal N, Suri V, Soni S, Chopra V, Kohli HS. A prospective comparison of random urine protein-creatinine ratio vs 24-h urine proteinin women with preeclampsia. *Medscape J Med.* 2008; 10 (4): 98.

[23] Al RA, Baykal C, Karacay O, Geyik PO, Altun S, et al. Random urine protein-creatine ratio to predict proteinuria in new-onset mild hypertension in late pregnancy. *Obstet Gynecol.* 2004; 104: 367-71.

[24] Dwyer BK, Gorman M, Carroll IR, Druzin M. Urinalysis vs. urine protein-creatinine ratio to predict significant proteinuria in pregnancy. *J Perinatol.* 2008; 28: 461-7.

[25] Leanos-Miranda A, Marquez-Acosta J, Romero-Arauz F, Cardenas-Mondragon GM, Rivera-Leanos R, et al. Protein-creatinine ratio in random urine samples is a reliable marker of increased 24-hour protein excretion in hospitalized women with hypertensive disorders of pregnancy. *Clin Chem.* 2007; 53: 1623-8.

[26] Parag KB, Seedat YK. The protein/creatinine index. A semi-quantitative assessment of 24-hour protein excretion. *SAMT DEEL.* 1986; 69:42-3.

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