Comparison of Measurement of 24-Hour Urine Protein Excretion Versus Random Urine Protein-Creatinine Ratio in Pregnant Woman with Pre-eclampsia

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ABSTRACT:

BACK GROUND:

Measurement of protein excretion in a 24-hour urine collection has been the long-standing gold-standard but time consuming test for the quantitative evaluation of proteinuria induced by preeclampsia. An alternative method is the measurement of protein- creatinine ratio in a spot urine sample, which provides a more convenient and rapid method to assess protein excretion.

OBJECTIVE:

To assess the correlation between the spot urine protein- creatinine ratio and 24-hour urine protein excretion in women with preeclampsia and to use the urine protein- creatinine ratio as alternative to time-consuming 24-hour urine protein collection.

Study design: A cross sectional study.

Setting: Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital for a period of one year from Jan. 2013 to Jan. 2014

PATIENTSAND METHODS:

Eighty three singleton pregnant women suffering from pre-eclampsia with gestational age between 28 to 39 weeks were selected to participate in the study. They were divided into two groups: 61pregnant women with mild to moderate pre-eclampsia and 22 with severe pre-eclampsia.These women were prospectively studied for proteinuria. Urine protein- creatinine ratio was determined in a spot mid-stream urine sample, and the amount of protein excretion was measured in 24-hour urine collected on the subsequent day. The correlation between the two tests was assessed.

RESULTS :

Diagnostic value of protein/creatinine ratio was expressed in terms of specificity and sensitivity.There was significant correlation between protein/ creatinine ratio in a single void urine with 24 hr. urine collection for protein as the P value was (0.0001), The ROC curve analysis showed an area under the curve of (0.879), indicating that the urine protein: creatinine ratio can detect severe proteinuria at a cutoff point of 4.2 with a sensitivity of (81.8%) and specificity of (85.2%).

CONCLUSION:

There is a significant correlation between the spot urine protein/ creatinine ratio and 24-hour urine protein excretion in women with preeclampsia.

KEY WORDS: preeclampsia, protein, creatinine ratio,dipstick

INTRODUCTION:

Preeclampsia (PE) is a complication of pregnancy which is characterized by hypertension and proteinuria. It affects 2% to 5% of pregnancies and is a major contributor to fetal, neonatal, and maternal morbidity and mortality. Preeclampsia symptoms might be revealed from 20 weeks of gestation up to six weeks postpartum and is considered early onset before 34 weeks of gestation [1, 2].Proteinuria is one of the signs of preeclampsia which is defined as >300mg of protein in a 24- hour urine collection. This usually correlates with more than 30 mg /dl or >1+ reading in dip-stick in random urine specimen [3].

A value of (trace equal 20mg/dl), (1+ equal 30mg/dl), (2+ equal 100mg/dl), (3+ equal 300mg/dl), (4+ equal >1000mg/dl) [4].

The differentiation between no severe and severe gestational hypertension or preeclampsia can be misleading because what might be apparently

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mild disease may progress rapidly to severe disease [5].

Creatinine is a waste product from the normal breakdown of muscle tissue. As creatinine is produced, it's filtered through the kidneys and excreted in urine. The kidneys' ability to handle creatinine is called the creatinine clearance rate, which helps to estimate the glomerular filtration rate (GFR), the rate of blood flow through the kidneys. It is produced at a nearly constant rate and is excreted in the urine. Because of its constant rate of production, the amount of creatinine in the urine is an indirect measurement of kidney function. If kidney function is significantly reduced, the urine creatinine will fall. With more severe degrees of kidney failure, the creatinine clearance value decreases because the kidneys are filtering less creatinine out of the blood so the serum creatinine will eventually rise [6].

The detection of proteinuria is one of the criteria in differentiating those pregnancies with PE from those with gestational hypertension. The 24-hr urine sample is not practical as a routine test and so urine dipstick screening is employed [2].Visual dipstick reading is not very accurate [6], but the use of automated dipstick readers improves the accuracy of dipstick testing [7].

Quantification of proteinuria should follow the diagnosis by dipstick, this is by the 24-hour urine protein estimation, or the use of the protein to creatinine ratio (P/C), the later test is much quicker [8].

Diagnosis of significant proteinuria by using the urinary P/C is when the result is greater than 30mg/mmol which are corresponding to 0.3 mg/mg or a validated 24hr urine collection result shows greater than 300 mg protein and is considered to be severe when the excretion is greater than 5 g [7].

PATIENTS AND METHODS:

A cross sectional study was conducted at the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital for a period of one year from (Jan. 2013- Jan. 2014) & was approved by Iraqi Council of Medical Specialization (Iraqi Scientific Committee of Obstetrics and Gynecology).

Inclusion criteria include singleton viable pregnancy**,** gestational age between 28 and 39 weeks, confirmed by last menstrual period and early ultrasound. While exclusion criteria include: multiple pregnancies , diabetes mellitus , chronic hypertension, preexisting renal disease, abnormal fetus ,urinary tract infection , bladder cancer or tumors, congestive heart failure, multiple myeloma (cancer of the plasma cells) , lupus (an inflammatory autoimmune disease) & finally patients who did not comply with 24 hours urine collection.

Consent: Verbal consent was obtained from all pregnant women included in the study.

The study included 83 women with PE who were fulfilled the inclusion & exclusion criteria mentioned above & were divided into the following: 61 women with non-severe PE & 22 women with severe PE.

All patients (severe &non severe) were collected from the out patients clinics of Al-Yarmouk Teaching Hospital, were later admitted to the ward for further assessment.

Information's about the age of the patient, gestational age, parity, past medical, surgical and obstetric history were taken from all participants. General and obstetric examination was done.

Patient was diagnosed as having severe PE if their blood pressure was ≥160 /110 mmHg in addition to proteinuria more than +2 or at least, proteinuria of 5 g or more per 24 hours or if presented with other concurrent parameters: persistent headache, visual disturbances, epigastric pain or had maternal or fetal complications. While non severe PE were diagnosed if their blood pressure was less than 160/110mmHg in addition to proteinuria 2+ with absents of any maternal complications[2].

Patients were sent for midstream urine dipstick examination for albumin in urine; those who had ›+ albumin were further analyzed by: first P/C ratio & second by 24h urine.

The patients were given oral instructions about the proper way of urine collection, including perineal cleansing and avoiding skin contact with the collection specimen. The patients were given containers to collect the urine for 24hours. Each container was marked with the patient's name and the patients were told to put all the urine in the container and close it after each voiding. In some patients we use catheters for urine collection, as those with severe PE. The P/C ratio requires a single urine specimen, before start collection the 24 hr urine or after complete the 24hr urine collection. The National Kidney Foundation states that "first morning specimens are preferred, but random specimens are acceptable if first morning specimens are not available [9].

The predictive value of the random urinary P/C ratio for the diagnosis of significant proteinuria was estimated by using a 300-mg protein level within the collected 24-hour urine as the gold standard [1].

Patients were sent for other investigations including (full blood count, coagulation profile, liver function test, renal function test and abdominal ultrasound). Mid stream urine dipstick examination, for albumin in urine and patients who had more than +1 albumin in urine dipstick were included in the study, and further , evaluated firstly by P/C ratio & secondly by 24 hour urine collection for protein.

All the investigations were done at laboratories of Al-Yarmouk Teaching Hospital including creatinine concentration (mg/dl) which was determined on a Beckman Creatinine Analyzer II (Brea, California) with the modified Jaffe rate method. The test was based on the principle that at alkaline pH values, creatinine reacts with Picric acid to produce a coloured compound, creatine alkaline picrate, which can be photometrically measured. Regarding 24 hr. protein in urine we choose an a priori cut of value of more than 300mg/24hr to define significant proteinuria and more than 5000 mg/24hr.for severe proteinuria.

RESULTS:

At the beginning of the study we collect 100 pregnant women who were diagnosed as having PE , 17 of them were excluded from the study because (4 women had insignificant proteinuria (< 300mg) on the 24hour urine collection , 6 patients the 24-hr urine collection was not complete, 7 women had delivery before complete 24 hour urine collection). The remaining 83 were divided according to severity of preeclampsia into 61 patients had mild PE and 22 had severe PE.

The mean maternal age of patients with PE was (29.0±8.0) years and the range was (15-42).

While the mean parity was (1.9±2.0) and the range was (0-6) & the mean gestational age was (31.9±1.9) weeks and the range was (28-39) There was no significant difference regarding maternal age , parity, gestational age while regarding albumin in urine, it was significant since the (p=0.0001) as shown in table 1.

Table 1: The correlation between the demographic characteristics of the pregnant patients and the severity of PE in the study:

		Non severe PE(n=61)		Severe PE(n=22)		P value
		No	%	No	%	
Age (years)	<20	10	16.4	3	13.6	0.755
	20--24	7	11.5	3	13.6	
	25--29	12	19.7	7	31.8	
	30--34	14	23.0	3	13.6	
	=>35years	18	29.5	6	27.3	
Mean±SD(Range)		29.0±8.0		(15-42)		
Parity	P0	26	42.6	8	36.4	0.853
	P1	5	8.2	3	13.6	
	P2	11	18.0	3	13.6	
	P3	7	11.5	2	9.1	
	P4&more	12	19.7	6	27.3	
Mean±SD(Range)		1.9±2.0		(0-6)		
Gestational age (weeks)	<30	7	11.5	2	9.1	0.817
	30	6	9.8	3	13.6	
	31	12	19.7	2	9.1	
	32	9	14.8	5	22.7	
	33	12	25.5	5	22.7	
	34	6	9.8	3	13.6	
	35 37 39	3 2 1	4.9 3.25 1.62	2	9.1	
Mean±SD(Range)		31.9±1.9		(28-39)		
Albumin in urine	++	57	93.4	2	9.1	0.0001*
	+++	4	6.6	20	90.9	
*Significant positivity using Pearson Chi-square test at 0.05 levels.						

The presence of albumin in urine was (++) in 61 patients and (+++) in 22 patients.
The mean systolic blood pressure (SBP) in patients with PE was (150.8±7.2), while
The mean diastolic blood pressure (DBP) was (100.3±7.7).

Table 2: The albumin and blood pressure of the PE patients included in the study

	No	%
Albumin in urine		
++	61	74.5
+++	22	26.5
Blood pressure		
Systolic BP (mmHg) Mean±SD	150.8±7.2	(140-180)
Diastolic Bp (mmHg) Mean±SD	100.3±7.7	(90-120)

The mean for P/C ratio was (2.86±2.80) and the mean for 24hr urine collection for protein was (3572.08± 2825.27) as shown in table 3

Table 3: The distribution of protein/creatinine ratio and 24 hour urine collection for protein in the study.

	protein/creatinine ratio(mg/mg)	24 hour urine collection for protein(mg/24hr)
No	83	83
Mean±SD	2.86±2.80	3572.08±2825.27
Standard Error of Mean	0.308	310.114
Mode	1.4	2700
Range	0.2-7.7	550-8500
Percentile 05^{th}	0.2	558
25^{th}	0.3	1750
50^{th} (Median)	1.4	2690
75^{th}	6.3	6070
95^{th}	7.6	8400
99^{th}	7.7	8500

P=0.0001 (Significant using Students-t-test for difference between two independent means at 0.05 level)

In non severe PE the mean for P/C ratio was (1.80±2.12) and the range was (0.2-7.7).
In severe PE the mean for P/C ratio was (5.80-2.36) and the range was (1.2-7.7)
The median for both non severe and severe PE was1.3 and 7.1 respectively. So there was increase in the ratio of p/c ratio with the PE status.
There was significant difference between two independent means of P/C ratio between non severe and severe PE since the (p value = 0.0001) as shown in table 4.

Table 4: Distribution of protein/creatinine ratio by PE status.

		PE status	
		Non severe PE	Severe PE
protein/creatinine ratio	No	61	22
	Mean±SD	1.80±2.12	5.80±2.36
	Standard Error of Mean	0.271	0.503
	Mode	1.6	7.3
	Range	0.2-7.7	1.2-7.7
	Percentile 05^{th}	0.2	1.4
	25^{th}	0.3	4.3
	50^{th} (Median)	1.3	7.1
	75^{th}	1.6	7.3
	95^{th}	6.7	7.7
	99^{th}	7.7	7.7
P=0.0001 (Significant using Students-t-test for difference between two independent means at 0.05 level)			

According to the Pearson correlation test, P/C ratio was found to have a significant strong and moderate direct correlation with 24 hr urine collection for protein in non severe and severe PE as the correlation coefficient (r) =(0.911) and (0.562), respectively. As shown in figure 1

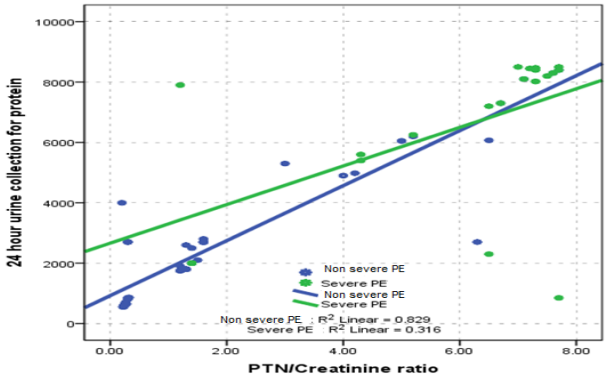

Figure 1: The correlation between P/C ratio with 24 hour urine collection for protein by PE status with (r=0.911 for non severe PE and 0.562 for Severe PE).

The ROC curve plot sensitivity (true positive rate) against 1 – specificity (true negative rate). The area under curve was calculated, it was 0.879 for P/C ratio with [95% confidence interval (95% CI), 0.798–0.959].

For 24 hr urine collection for protein, the area under the curve was 0.847 and with [95% confidence interval (95% CI), 0.747–0.947].The correlation was significant as the p value is (0.0001).

For random P/C ratio at various cut–offs for prediction of significant and severe proteinuria, the cutoff point in current study was (4.250), with sensitivity of (81.8%) and specify of (85.2%).

For 24 hrs. urine collection for protein, the result was (5500), with sensitivity of (72.7%) and specificity of (86.9%).

Analysis of the ROC curve indicated that a random urine P/C ratio of 4.250 was the best cutoff point to detect severe proteinuria in women with PE. As shown in figure2

DISCUSSION:

Preeclampsia is an endothelial disease that leads to glomeruloendotheliosis and in severe cases it may lead to renal impairment and failure. Increase permeability of the glomerular basement membrane leading to poteinuria [(10)].

The current study was conducted to evaluate the correlation between the 24hour urine collection for protein and single void of urine for P/C ratio in mild and severe PE.

The following studies agree with our study in demonstrating correlation between the 24 hours urine protein and the protein/creatinine ratio. The p values in these studies are also statistically very significant as it is <0.001 which is also seen in our study.

Eslamian L et al. 2011[(11)] reported in their study which include 100 hypertensive pregnant women, that the random urine p/c ratio was strongly correlated to 24h urine protein excretion with (r=0.777, *P*<0.001). A cut off value of 0.22mg/mg for P/C ratio best predicted significant proteinuria with sensitivity, specificity, of 87%, 92.6%, respectively. Urine protein and creatinine in their study were measured by Bio systems (Barcelona, Spain) , while our current study showed that there was significant correlation between P/C ratio in a single void urine with 24 hour urine collection for protein as the P value was (0.0001), The ROC curve analysis showed an area under the curve of (0.879), indicating that the urine P/C ratio can detect significant proteinuria, and to detect severe proteinuria at a cutoff point of 4.2 with a sensitivity of (81.8%) and specificity of (85.2%).

Sanchez-Ramos L et al. 2013[(12)] which included 186 participants who had mild and severe PE. Pooled sensitivities and specificities were 91.0% and 86.3% respectively. The cut off point for p/c ratio to detect proteinuria was (6.7). The study stated that random urine P/C ratio provided useful evidence to rule out the presence of significant proteinuria in patients at risk for PE.

Jung-Hwa Park et al. 2013 [(13)] their study agreed with the current study. In their study, random urine P/C ratio and 24-hr urine protein was performed on 79 patients with mild and severe PE to evaluate significant proteinuria.They found that random urine P/C ratio highly correlated

with 24-hr urine protein excretion. The optimal random urine P/C ratio cutoff points were 0.63 and 4.68 for 300 mg/24 hr and 5,000 mg/24 hr of protein excretion, respectively. With each sensitivity, specificity of 87.1%, 100%, and 100%, 85%, for significant and severe PE, respectively.

By Revankar Manohar Vijaya1 et al;2013 [14] study, which included 23 patients with mild preeclampsia and 15 patients with severe preeclampsia.It was noticed that there was correlation between the 24 hours urine protein and spot urine p/c ratio among 50 subjects which was statistically significant at $p<0.001$,a cut off point of p/c ratio was >3 for severe proteinuria in severe PE in their study.

Takahiro Yamada et al. 2013 [15] found in their study that a single void p/c ratio practically better than 24hour and more acceptable by the patients, we also found that in our study.

N.Aggrawal MD et al. 2008[16] found in their study which included 120 patients with PE constituted their study group,the area under the ROC curve was 0.79 (95% CI 0.67–0.91), with a cut off P/C ratio greater than 1.14 as a predictor of significant proteinuria, sensitivity and specificity were 72% and 75%, respectively, and the negative predictive value was 29.2% so they found that random urine P/C ratio was not a good predictor of significant proteinuria in patients with PE which disagree with our study.

R K Morris et al. 2012 [17] found in their study that the threshold values for P/C ratio ranged between 0.13 and 0.5, with estimates of sensitivity ranging from 0.65 to 0.89 and estimates of specificity from 0.63 to 0.87; the area under the summary receiver operating characteristics curve was 0.69.

In contrast to the current study, the following studies disagree with our probably because of the variability in laboratory methods for measuring proteinuria in different reported studies, several cutoff points and different units for the urinary P/C ratio have been reported, thereby precluding valid comparisons among such studies, and because of the study-to-study variability in laboratory methods for measuring proteinuria[11,12,16,17].

Regarding maternal age, parity, gestational age, the current study showed no significant effect of them on the correlation between P/C ratio and 24hr.urine collection for protein which was the same thing in all the above studies [11,12, 13, 14, 15, 16, 17].

CONCLUSION:

There is a significant correlation between the spot urine P/C ratio and 24-hr urine protein excretion in women with PE. & random urine P/C ratios can be used as a reasonable alternative to 24-hr urine protein excretion for exclusion of PE, especially in emergent situation.

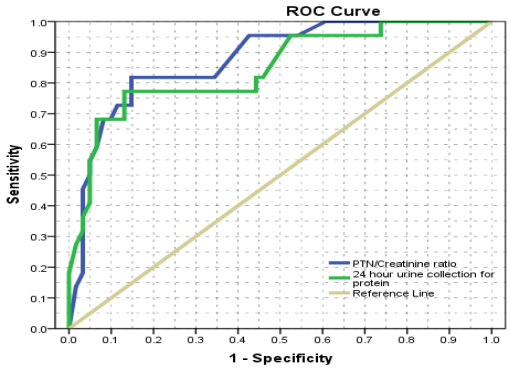

Figure 2: ROC curve showing the performance of random urine protein/creatinine for significant proteinuria.

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