Significance of Urine Kallikrein in the Prediction of Pregnancy-induced Hypertension

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Abstract : *A total of 158 urine kallikrein and creatinine levels were measured in 127 normotensive gravid women and 12 non-pregnant women and the kallikrein/creatinine ratio was evaluated to predict pregnancy induced hypertension. Urine kallikrein was examined using the MCA fluorescent method. Twenty-seven patients ultimately developed pregnancy-induced hypertension. In normotensive pregnant women, the urine kallikrein/creatinine ratio was significantly higher than that of non-pregnant women, especially during the first half of pregnancy. The mean urine kallikrein/creatinine ratio in patients destined to develop pregnancy-induced hypertension was lower than that of normotensive pregnant women at each gestational week and the difference was statistically significant at ≥ 32 weeks of gestation. When a kallikrein/creatinine ratio of less than 1.60 U/g creatinine was considered to represent a positive test, the predictive value of a positive test was high (71.4% at 32-35 weeks of gestation and 83.3% at ≥ 36 weeks of gestation), and there were no false nagative results at ≥32 weeks of gestation. The mean value of the latent period (interval between the time of a positive test result and the onset of pregnancy-induced hypertension) was 5.4 weeks. Evaluation of the urine kallikrein/creatinine ratio may be a useful screening tool in predicting the subsequent development of pregnancy-induced hypertension. (Thai J Obstet Gynaecol 1991;3:63-70.)*

Key words : pregnancy-induced hypertension, kallikrein

It has been reported that the urine content of kallikrein is decreased in patients with essential hypertension and glomerulonephritis, and is increased in patients with pheochromocytoma and primary aldosteronism[1-2]. Since the report by Elebute and Millo[3], there have been some studies of urinary kallikrein in normal pregnancy and in pregnancy-induced hypertension(PIH)[4-6]. In general, these studies found that urinary kallikrein activity in patients with PIH is significantly lower than in normal pregnant women. In this study, we used a fluorogenic substrate method[7] and estimated the amount of urinary kallikrein in uncomplicated pregnant

women at an early stage of pregnancy in an attempt to predict the onset of PIH.

Materials and Methods

A total of 127 pregnant women who were attending Kumamoto Municipal Women's Hospital for prenatal care and 12 volunteer non-pregnant women were entered in the study. Patients were selected randomly during gestation, and a total of 158 casual urine samples were collected. Women were excluded from the study if they had a history of chronic hypertension, diabetes or renal disease, or if at the time of entry into the study their blood pressure was ≥ 140/90 mmHg. They were also excluded if they had any evidence of proteinuria, as measured by the dipstick method. In all cases, gestation was estimated from the last menstrual period, and ultrasonic measurement of fetal crown-rump length was used early in pregnancy to confirm the gestational week. None of the subjects were taking medication, and their diets were unrestricted during the investigation.

After urine collection, the patients received routine prenatal care, and at the time of delivery their charts were reviewed for evidence of PIH. PIH was defined as a blood pressure of 140/90 mmHg or a rise of 30 mmHg in systolic pressure or 15 mmHg in diastolic pressure (measured twice, 6 hours apart at bed rest) with or without proteinuria and/or edema.

Urinary kallikrein was measured with a fluorogenic substrate method reported by Kato et al[7], using a fluorogenic peptide substrate, proly1-pheny1-arginine-4-methy1 coumary1-7-amide (Pro-Phe-Arg-MCA). Using 20 μl of urine, the kallikrein activity was quantitatively assayed by incubation with a final concentration of 10^{-4} M Pro-Phe-Arg-MCA at 37°C for 90 minutes. The reaction was terminated by adding 20 μl of 10% acetic acid. The amount of MCA liberated was measured using a fluorescence spectrophotometer (Hitachi, Model MPE-2A). Intra- and interassay coefficients of variation were 2.4 and 4.1% respectively. We also measured the urine creatinine concentration using the alkaline pyruvate method (intra- and interassay coefficients of variation were 0.8 and 1.2% respectively) and the kallikrein/creatinine ratio was calculated for the prediction of PIH, since the concentration of kallikrein may largely depend upon the urinary volume in a casual urine sample.

Comparison of the groups was made using the unpaired Student's t-test for non-paired data. Values are expressed as standard error of the mean (SEM). Significance was set at the level of $p<0.05$.

Results

Twenty-seven (21.3%) of 127 pregnant women ultimately developed PIH and the remainders (100 patients) remained normotensive. Table 1 shows the clinical data of patients who developed PIH. The mean age of the pa-

Table 1 The clinical data of patients who developed PIH

No.	Name	Age	Parity	BBP	Max. BP	ΔSBP	ΔDBP	P	E	Onset	Del	BBW	UKK/UCr (Latent time)		
1	M. M.	24	0G0P	106/66	150/100	44	34	−	+	33	34	2,140	0.95 (2)	0.78 (1)	
2	Y. Y.	31	2G1P	90/60	120/90	30	30	+	−	38	39	3,120	0.47 (22)	0.51 (18)	
3	K. Y.	31	0G0P	102/50	122/72	20	22	−	−	37	40	3,240	2.63 (18)	2.32 (14)	
4	Y. M.	26	0G0P	110/62	120/80	10	18	−	−	39	39	3,660	1.06 (13)	1.47 (9)	
5	M. M.	27	1G1P	100/60	130/78	30	18	−	+	36	38	2,890	0.74 (12)	0.49 (4)	
6	A. A.	35	1G1P	110/70	140/98	30	28	+	−+	37	39	2,490	0.49 (3)		
7	Y. F.	24	0G0P	102/60	126/80	24	20	3+		37	40	3,090	1.04 (25)		
8	T. N.	38	3G3P	100/60	160/92	60	32	+	2+	34	37	2,300	1.54 (18)	0.82 (13)	1.55 (8)
9	S. T.	30	0G0P	104/60	120/90	16	30	+	−−	40	42	2,940	1.89 (28)	2.42 (20)	
10	E. T.	26	0G0P	134/74	130/90	−4	16	−	−	35	37	2,400	0.69 (8)	0.49 (4)	0.33 (0)
11	M. D.	36	0G0P	110/60	118/80	8	20	−	−	37	38	2,580	0.47 (1)		
12	T. T.	28	1G1P	104/64	128/80	24	16	−	−	39	40	3,080	0.27 (1)		
13	A. S.	27	0G0P	110/60	130/80	20	20	−	−	37	38	3,080	2.62 (22)		
14	F. S.	30	0G0P	110/70	140/88	30	18	−	−	38	40	3,000	0.56 (6)		
15	Y. S.	27	1G0P	120/60	122/80	2	20	−	2+	39	41	3,020	0.17 (1)		
16	M. K.	36	1G1P	110/64	130/80	20	16	−	−	39	39	2,950	1.56 (0)		
17	K. K.	31	0G0P	104/52	132/86	28	34	+	+	37	40	3,320	0.72 (14)		
18	S. K.	26	1G0P	118/60	120/80	2	20	−	+	39	40	3,740	0.72 (10)	1.07 (5)	0.72 (1)
19	N. G.	25	0G0P	112/60	124/78	12	18	−	+	38	41	3,390	4.2 (21)	2.2 (16)	2.8 (14)
20	Y. K.	25	0G0P	110/50	120/78	10	28	−	−	38	38	2,550	0.50 (2)		
21	M. K.	30	0G0P	130/78	148/96	−	−	+	−	36	38	2,840	1.55 (4)	0.79 (2)	
22	Y. K.	23	0G0P	110/68	112/82	2	14	+	−	40	42	3,270	1.11 (2)		
23	M. K.	26	1G0P	116/60	120/80	4	20	+	−+	38	39	3,390	1.1 (9)	1.6 (6)	0.6 (2)
24	K. E.	30	1G1P	130/80	118/92	−12	12	−	−	40	42	3,550	1.15 (2)		
25	K. I.	29	1G1P	120/66	122/88	2	22	+	−−	37	40	3,790	1.68 (24)		
26	U. I.	25	0G0P	108/54	120/80	12	26	−	−	37	39	2,770	2.2 (17)	1.9 (13)	2.1 (9)
27	H. I.	22	0G0P	102/62	136/80	34	18	−	+	38	39	3,020	2.18 (22)		

BBP; basal blood pressure, Max. BP; Maximum blood pressure, ΔDBP; Max. BP-BBP, P; proteinuria, E; edema, Onset; gestational week at the onset of PIH, Del; gestational week at termination, BBW; weight of newborn, UKK/UCr; urinary kallikrein/urinary creatinine, Latent time; the interval from documentation of a low ratio of UKK/UCr to the onset of PIH (weeks).

tients was 28.4 years (range 22 to 38 years). There were 19 primigravid and 8 multiparous patients. Blood pressure measured early in pregnancy was recorded as basal blood pressure (BBP). Maximum BP indicates the maximum blood pressure recorded during pregnancy. All but one patient (case No.8) had mild hypertension, and an elevated diastolic blood pressure was observed in 25 patients. Furthermore, the onset of PIH occurred near term, so small for gestational age babies were rare.

The mean values of urinary kallikrein in normotensive subjects at each gestational week and puerperium are shown in Fig.1. Early in pregnancy, the urinary kallikrein content was significantly higher than in non-pregnant women ($p<0.05$). In the latter half of pregnancy, the kallikrein content gradually decreased and was rather low in puerperium.

The mean value of the urinary kallikrein/creatinine ratio in normotensive subjects at each gestational week and puerperium is shown in Fig.2. The urinary kallikrein/creatinine ratio was biphasic over the course of preg-

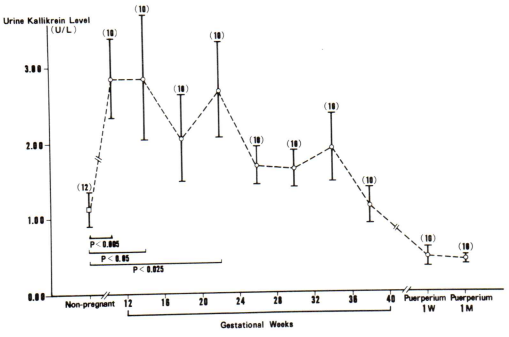


Fig. 1 Variation of urinary kallikrein levels during the course of normal pregnancy and puerperium. Values are expressed as Mean±SEM.

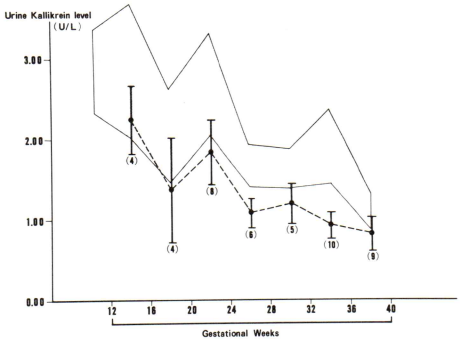


Fig. 3 Variation of urinary kallikrein before the onset of PIH in the women who finally developed PIH. Shaded area shows the range of normal pregnant women (Mean±SEM).

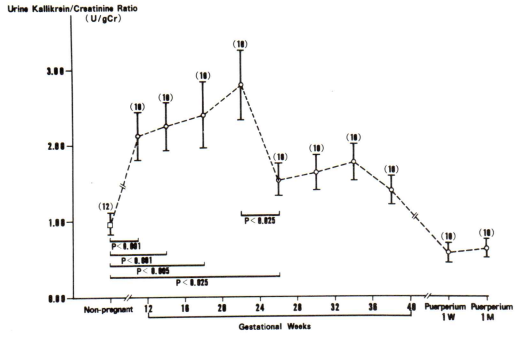


Fig. 2 Variation of the urinary kallikrein/creatinine ratio during the course of normal pregnancy and puerperium.

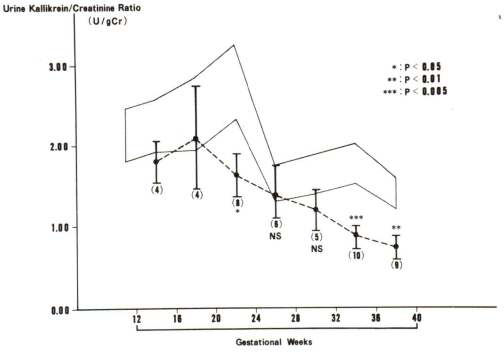


Fig. 4 Variation of the urinary kallikrein/creatinine ratio before the onset of PIH in the women who finally developed PIH. Shaded area shows the range of normal pregnant women (Mean±SEM).

nancy. Before 24 weeks of gestation, the ratios were significantly higher than in non-pregnant subjects. At around 24 weeks gestation, the ratio suddenly decreased to two thirds of that measured in the first half of pregnancy. This low value was maintained until near term.

The mean urinary kallikrein value in subjects who ultimately developed PIH is shown in Fig.3. The mean values in the PIH subjects were lower than those in normotensive subjects throughout pregnancy, but the differences were not significant.

The kallikrein/creatinine ratios

Table 2 Predictive parameters of PIH using the urinary kallikrein/creatinine ratio. PPV; Positive predictive value, NPV; negative predictive value

i) Cut-off value : 1.4 U/gCr

	sensitivity	specificity	PPV	NPV
GW 24～GW 27	4/6 (66.7%)	5/10 (50.0%)	4/9 (44.4%)	5/7 (71.4%)
GW 28～GW 31	3/5 (60.0%)	6/10 (60.0%)	3/7 (42.9%)	6/8 (75.0%)
GW 32～GW 35	8/10 (80.0%)	6/10 (60.0%)	8/12 (66.7%)	6/8 (75.0%)
GW 36～	8/9 (88.9%)	6/10 (60.0%)	8/12 (66.7%)	6/7 (85.7%)

ii) Cut-off value : 1.6 U/gCr

	sensitivity	specificity	PPV	NPV
GW 24～GW 27	4/6 (66.7%)	5/10 (50.0%)	4/9 (44.4%)	5/7 (71.4%)
GW 28～GW 31	4/5 (80.0%)	5/10 (50.0%)	4/9 (44.4%)	5/6 (83.3%)
GW 32～GW 35	10/10(100%)	6/10 (60.0%)	10/14 (71.4%)	6/6 (100%)
GW 36～	9/9 (100%)	2/10 (20.0%)	9/17 (52.9%)	2/2 (100%)

vi) Cut-off value : 1.8 U/gCr

	sensitivity	specificity	PPV	NPV
GW 24～GW 27	4/6 (66.7%)	3/10 (30.0%)	4/11 (36.4%)	3/5 (60.0%)
GW 28～GW 31	4/5 (80.0%)	3/10 (30.0%)	4/11 (36.4%)	3/4 (75.0%)
GW 32～GW 35	10/10(100%)	6/10 (60.0%)	10/14 (71.4%)	6/6 (100%)
GW 36～	9/9 (100%)	2/10 (20.0%)	9/17 (52.9%)	2/2 (100%)

in subjects who ultimately developed PIH are shown in Fig.4. The mean values in the PIH subjects were again lower than those in the normotensive subjects throughout pregnancy, and the differences were significant after 32 weeks ($p<0.01$).

From these results, we selected some appropriate cut-off values and gestational weeks for the prediction of PIH. Three cut-off values and four gestational periods were chosen and the sensitivity, specificity, positive predictive values and negative predictive values were calculated for each condition (Table 2). In general, for any cut-off value, each predictive parameter became more reliable as gestation advanced. However, those patients who are at risk for PIH must be recognized as early as possible. When a kallikrein/creatinine value of less than 1.60 U/g creatinine was taken as a positive test result, the predictive value was high (71.4% at 32-35 weeks of gestation and 83.3% at 36 or more weeks of gestation). Furthermore, there were no false negative results at 32 or more weeks of gestation. Therefore, we chose a kallikrein/creatinine ratio of 1.60 U/g creatinine as the cut-off value and 32 weeks gestation as the time of examination.

In this study, we regarded a kallikrein/creatinine ratio of less than one standard error below the mean as abnormal. We also defined the period from which the abnormal results were obtained to the onset of PIH as the latent time. In PIH subjects, this latent time ranged from a few days to 28 weeks, and the mean was 5.4 weeks (Talbe 1).

Discussion

There are many reports concerning the prediction of PIH, including the angiotensin sensitivity test (AST)[8-10], roll over test[11], hand grip test and biochemical parameters such as serum uric acid and hematocrit[12-13]. Among these trials, the AST has been recognized as the most reliable method. However, it has not been confirmed as a practical screening test because of its difficulty in out patient clinics[10]. In this study, we were able to predict the onset of PIH with a high positive predictive and negative values, using a casual urine sample. It serves as a useful predictive parameter of PIH in prenantal care practice.

The most sensitive and specific method reported so far for the estimation of kallikrein in urine is the radioimmunoassay method[14]. However, it is necessary to prepare the antibody against bradykinin and the purified kininogen for this assay method. It is often difficult to obtain specific antibody in high titers. Against this, kallikrein contents estimated by the fluorogenic method were comparable to those obtained by radioimmunoassay[7]. Furthermore, this method is simple and the substrate is commercially available. From these points of view, it may be a useful screening tool in prenatal care, especially when dealing with a large number of urine samples.

Although a 24-hour urine collection was obtained from each patient in most of the previous reports[1-6], a casual urine sample was used for the estimation of kallikrein in this study. It has been reported that the concentration of kallikrein in a casual urine sample correlates to that of a 24-hour urine collection and daily excretion of urine kallikrein is independent of the 24-hour urine volume[15]. Based on these observations, we used the kallikrein/creatinine ratio in casual urine samples for prenatal screening.

Our results confirm the findings of Elebute and Millo[3] with regard to the increase in urinary kallikrein during the first half of pregnancy and the low excretion of kallikrein in late pregnancy. They also suggested that pregnant women with PIH had lower excretions of urinary kallikrein compared with that in normotensive pregnant women throughout gestation. However, a change in urinary kallikrein before the onset of PIH has not been confirmed. From our results, pregnant women destined to develop PIH had a significantly lower excretion of kallikrein compared with normotensive pregnant women at late pregnancy.

Kallikrein activity in urine is renal in origin and its excretion reflects the renal kallikrein-kinin system[16]. Although the interactions among the renal kallikrein-kinin system, renin-angiotensin-aldosterone system and vasoactive intrarenal prostaglandins have been reported[17], they are complex and so far only partly understood. Urinary kallikrein, however, correlates closely with renal blood flow[18], and the vasoconstriction induced by angiotensin II is suppressed by the renal kallikrein-kinin system[19]. From these observations, the decrease of urinary kallikrein in pregnant women may play an important role in the development of PIH.

In conclusion, urine kallikrein content increases in early pregnancy. It peaks in the first or second trimester and tapers off thereafter. In subjects who later develop PIH, the urinary kallikrein content is significantly lower than in normotensive pregnant women. Estimation of urinary kallikrein content is useful in predicting the onset of PIH. We propose that a kallikrein/creatinine ratio of 1.60 U/g creatinine by the fluorogenic method at 32 weeks gestation is a predictor of PIH.

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