Urinary Thromboxane B2 and 11-dehydro-Thromboxane B2 in Normal and Pre-eclamptic Pregnancies

Yuen Tannirandorn MD,*
Mark HF Sullivan MRCOG,#
Murdoch G Elder FRCOG.#

*Department of Obstetrics and Gynaecology,
Faculty of Medicine, Chulalongkorn Hospital,
Chulalongkorn University,
Bangkok 10330, Thailand

#Institute of Obstetrics and Gynaecology,
Hammersmith Hospital,
London W 12 OHS, UK

Abstract: *The production of urinary thromboxane B2 (TXB2) and 11-dehydro-thromboxane B2 (11-dehydro-TXB2), a major urinary metabolite of TXB2, was determined throughout gestation in 25 normotensive pregnant women. These were compared with urinary levels of TXB2 and 11-dehydro-TXB2 in 20 women with mild pre-eclampsia. Urinary TXB2 and 11-dehydro-TXB2 levels did not change with gestation. In normal pregnancies urinary levels of TXB2 (geometric mean 0.66 ng/mg creatinine, 95% CI 0.57 to 0.75) were significantly lower ($p<0.0001$) than those of 11-dehydro-TXB2 (geometric mean 1.02 ng/mg creatinine, 95% CI 0.92 to 1.13). In women with pre-eclampsia, this was reversed in that urinary levels of TXB2 were higher (geometric mean 1.56, 95% CI 1.22 to 1.99) than those of 11-dehydro-TXB2 (geometric mean 0.68, 95% CI 0.52 to 0.87) ($p<0.0001$). TXB2 levels were higher ($p<0.0001$) and 11-dehydro-TXB2 levels were lower ($p<0.004$) in the women with pre-eclampsia than in the controls. This change in metabolism of TXB2 may be the result of an alteration in placental metabolism, since the enzyme responsible for the conversion of TXB2 to 11-dehydro-TXB2 is a placental enzyme. These changes are quite striking, considering that most of the women had mild pre-eclampsia, which settled on bed rest. (Thai J Obstet Gynaecol 1990; 2: 11-17.)*

Key words : urinary thromboxane B2, 11-dehydro-thromboxane B2, pre-eclamptic pregnancy

Pre-eclampsia is still a major cause of obstetric morbidity and mortality. Although its aetiology remains unknown, much recent work has focused on the role of prostaglandins in this disease[1]. Thromboxane A2, a major product of arachidonic acid metabolism in platelets and a powerful

vasoconstrictor inducing irreversible platelet aggregation, is thought to play a major role in the aetiology of pre-eclampsia[2,3]. Thromboxane A2 is labile and concerts rapidly into stable metabolite, thromboxane B2 (TXB2). The major urinary matabolite of TXB2 is 11-dehydro-thromboxane B2 (11-dehydro-TXB2)[4], and thus represents platelet turnover. Although the 11-dehydrogenase is found in the general vasculature, it is also present in the placenta[5], and the overall metabolism of TXB2 may therefore be altered during pregnancy. This prospective study was undertaken to assess urinary TXB2 and 11-dehydro-TXB2 levels throughout normotensive pregnancy. Urinary levels of TXB2 and 11-dehydro-TXB2 were also measured in cases of pre-eclampsia during the third trimester to determine whether this pathology affected TXB2 and 11-dehydro-TXB2 levels.

Materials and Methods

Subjects

Twenty five primigravidae were asked to collect urinary samples at the booking visit, between 16-20 weeks, 20-28 weeks, 28-36 weeks, and between 36 weeks to term and at 1-3 days after delivery to determine normal ranges of TXB2 and 11-dehydro-TXB2 in normal pregnancy. All women were healthy with certain dates confirmed on early ultrasound scans. Those with a previous history of diabetes or renal disease were excluded. These women took no drug (except iron and vitamin supplements), had normal blood pressure throughout gestation and gave birth to healthy infants at 37-42 weeks.

Twenty women with pre-eclampsia were also studied. All had a blood pressure of higher than 140/90 mmHg and proteinuria 1+(Dipstick) during the third trimester of pregnancy. They were entered into the study following the appearance of clinical symptoms of pre-eclampsia. All were admitted to hospital, and 2 received oral antihypertensive drug therapy (alpha-methyldopa); this treatment was started before collection of the first urine sample. Following hospitalisation, blood pressure fell in most patients, such that at the time of urine collection they were barely hypertensive (Table 1).

All infants survived and were discharged with the mothers.

Renal function, as determined by serum creatinine concentrations, was normal in all patients. All had normal blood pressure without proteinuria at 6-week postpartum check up.

No aspirin-like compounds were taken for two weeks prior to or during the collection periods.

Urine collection

Samples (15-20 ml) were collected after voiding and within 2 hours frozen at -20° C until analyzed. Storage did not alter the concentrations of these thromboxanes[4,6].

Metabolite assay

One ml of urine was acidified

to pH 3.0 with formic acid and passed through an ODS cartridge (Sep-Pak, Waters,UK) to extract the prostanoids as described previously[7].

TXB2 levels were determined by an "in-house" RIA, employing TXB2 specific antibody (ICN, Biomedical, UK), [3H]-TXB2 (Amersham, UK).

11-dehydro-TXB2 levels were determined by RIA kit (Amersham, UK). The 11-dehydro-TXB2 antibody had a cross-reactivity of 0.02% of TXB2.

The intraassay coefficient of variation was less than 8% for both assays and the interassay coefficient of variation was 10 and 7% for TXB2 and 11-dehydro-TXB2, respectively. The results of the TXB2 and 11-dehydro-TXB2 measurements are given as ng/mg creatinine, which was assayed by a routine method using quantitative, colometric determination at 500 nm (Sigma Diagnostics, MO, USA).

Statistical analysis

Descriptive parametric statistics were applied after logarithmic transformation to normalize distribution which were then verified using the Shapiro Francia W' test. Normally distributed parametric variables were analyzed by the Student's "t" test, while the Mann-Whitney test were used for nonparametric variables. Change in TXB2 and 11-dehydro-TXB2, (postdelivery level minus the last level during pregnancy) were analyzed by the paired Wilcoxon test.

Results

Clinical characteristics of the study groups are shown in Table 1. The patients in pre-eclamptic group were older than those in the normotensive group. Although gestational age at delivery was similar, mean birth weight in the pre-eclamptic group was significantly less ($p<0.008$) than in the normal group.

No correlation was found between urinary creatinine excretion and gestational age. Urinary creatinine excretion in pre-eclampsia did not differ from normal pregnancy (pre-eclampsia: geometric mean 0.69 mg/ml., 95% CI 0.52 to 0.93, normal pregnancy: geometric mean 0.74 mg/ml, 95% CI 0.66 to 0.84). Levels of urinary TXB2 were significantly higher ($p<0.0001$) in women with pre-eclampsia than in normotensive controls (Table 2). Urinary levels of TXB2 in both groups were unaffected by gestational age, and did not significantly during the three days after delivery. Levels of 11-dehydro-TXB2 in normal pregnancy were found to be significantly higher ($P<0.0001$)than those of TXB2 (Table 2), and were not affected by gestational age. In contrast, the levels of 11-dehydro-TXB2 were significantly lower than those of TXB2 ($P<0.0001$) in the women with pre-eclampsia (Table 2). In the immediate postpartum period levels of urinary 11-dehydro-TXB2 only fell significantly (mean change -0.37, $p=0.009$) in the women who had hypertensive pregnancies. Levels of urinary 11-de-

Table 1 Clinical characteristics of the study groups

	Normal pregnancy	Pre-eclampsia
No. of women	25	20
Age (year)	26.1	31.1*
	(CI 24.4-27.8)	(CI 28.6-33.6)
Parity:		
Nulliparous	25	18
Parous	0	2
Blood pressure:		
Systolic (mmHg)	112	139*
	(CI 110-114)	(CI 133-143)
Diastolic (mmHg)	69.8	90.6*
	(CI 68-71)	(CI 87-94)
Mode of delivery:		
Vaginal	22	16
Caesarean section	3	4
GA at delivery (week)	39.4	38.5
	(CI 38.8-39.9)	(CI 37.5-39.5)
Birth weight (Kg)	3.33	2.99**
	(CI 3.24-3.42)	(CI 2.76-3.22)

*p <0.001, **p <0.008 (unpaired t-test)
#Blood pressure at time of urine collection
CI = 95% confidence interval
GA = Gestational age

Table 2 Urinary excretion of TXB2, 11-dehydro-TXB2, and the TXB2:11-dehydro-TXB2 ratio in normotensive and pre-eclamptic women

	Normotensive women		Pre-eclampsia	
	Pregnant	Postpartum	Pregnant	Postpartum
Urinary TXB2				
(ng/mg Cr)				
geometric mean	0.66	0.89	1.56*	1.27
95% CI	0.57-0.57	0.61-1.29	1.22-1.99	0.91-1.79
Urinary 11-deH-TXB2				
(ng/mg Cr)				
geometric mean	1.02	0.89	0.68**	0.31
95% CI	0.92-1.13	0.58-1.36	0.52-0.87	0.21-0.43
Urinary TXB:11-deH-TXB2				
geometric mean	0.64	0.93	2.51*	3.81
95% CI	0.53-0.77	0.55-1.58	1.85-3.39	2.90-5.02

*P<0.0001, **P=0.004, compared to normotensive women (Mann-Whitney test)
Cr = Creatinine, TXB2 = Thromboxane B2, 11-deH-TXB2=11-dehydro-TXB2
CI = Confidence interval

Table 3 Accuracy of an elevated urinary TXB2:11-dehydro-TXB2 ratio of greater than 1.3 on consecutive occasions in identifying pre-eclampsia

	Urinary TXB2:11-dehydro-TXB2	
	<1.3	≥ 1.3
Normotensive women (n)	17	8
Pre-eclampsia (n)	1	19

Sensitivity 95%, Specificity 68%
Positive predictive value 70%
Negative predictive value 94%
Kappa index 0.61

hydro TXB2 were lower in women with pre-eclampsia than in women with normotensive pregnancies (Table 2).

The ratio of TXB2:11-dehydro-TXB2 was calculated and found to be very significantly different ($p<0.0001$) between women with pre-eclampsia and women with normotensive pregnancies (Table 2). The sensitivity and specificity of a TXB2:11-dehydro-TXB2 ratio of ≥ 1.3 on two consecutive measurements as a retrospective test for pre-eclampsia were calculated to be 95% and 68% respectively (Table 3).

Discussion

The analysis of urinary metabolites represents a noninvasive approach to the assessment of thromboxane biosynthesis in vivo and avoids potential artefacts associated with plasma measurements[8,9].

In normal pregnancy TXB2 and 11-dehydro-TXB2 concentrations did not change with gestation and the levels of 11-dehydro-TXB2 were higher than TXB2, confirming that 11-dehydro-TXB2 is the major urinary metabolite of TXB2 during pregnancy as well as in the non-pregnant state[4]. The major source of thromboxane in pregnancy is from platelet activation[8] and only a minority from the placenta[10] or the renal glomeruli[11].

The lack of a significant difference in urinary levels of thromboxane metabolites 1-3 days postpartum was not unexpected, since any change in platelets function during pregnancy would be expected to persist until a completely new population of platelets had been synthesised, which would require about 10 days[8]. Urinary TXB2 and 11-dehydro-TXB2 are known to have returned to pre-pregnancy levels 6 weeks postpartum [6,8].

It has been found that TXB2 levels in pre-eclamptic women were higher than in normotensive women[5, 6]. However, the levels of 11-dehydro-TXB2 in pre-eclamptic women have

not been clearly defined. Fizgerald et al[5] found that both plasma and urinary 11-dehydro-TXB2 levels in severe pre-eclampsia were elevated. In this study of relative mild pre-eclamptics urinary 11-dehydro-TXB2 levels in pre-eclamptic women were lower than in women with normal pregnancies. This was not due to reduced renal clearance of 11-dehydro-TXB2 since the urinary creatinine concentrations in both groups were similar. The enzyme responsible for the conversion of TXB2 to 11-dehydro-TXB2 is placental enzyme[5], so the placental damage present in pre-eclampsia may have decreased the activity of the enzyme. This is supported by the finding that although TXB2 levels were increased in the women with pre-eclampsia, 11-dehydro-TXB2 levels were decreased. This is consistent with placental damage inhibiting the activity of the 11-dehydrogenase enzyme. The finding of significantly decreased urinary levels of 11-dehydro-TXB2 after delivery, without any changes in TXB2 levels, also supports the role of a placental enzyme in the production of 11-dehydro-TXB2.

Despite the decrease in 11-dehydro-TXB2 levels, total thromboxane metabolite production (TXB2 + 11-dehydro-TXB2) was still higher in the women with pre-eclampsia, which has been reported elsewhere[2,3]. This elevation in platelets aggregration in pre-eclampsia is consistent with the known beneficial effects of low dose aspirin in pre-eclampsia[12-14].

Using a TXB2:11-dehydro-TXB2 ratio of $\geq$ 1.3, a sensitivity of 95% and specificity of 68% was obtained, although this is a retrospective analysis. The Kappa index of 0.61 suggests that this is not a chance finding. The clinical usefulness of this measurement in the prediction of pre-eclampsia needs to be assessed in a longitudinal study in pregnant women during the second and third trimesters.

In conclusion, this study documents normal levels of urinary TXB2 and 11-dehydro-TXB2 throughout pregnancy. In normal pregnancy 11-dehydro-TXB2 is the major metabolite for thromboxaneA2,whereas,in the women with pre-eclampsia the opposite applied and TXB2 predominated. The ratio of urinary TXB2 to 11-dehydro-TXB2 concentration might be a predictive test for pre-eclampsia, though further work is needed.

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