

The clinical utility of thrombin as predictor for preterm labour

Dr. Bushra Mohammed Majeed
Department of Obstetrics and Gynecology
College of medicine

Abstract

Back ground:

Preterm labour considered a major cause of perinatal death, and worldwide health and social problem. Many markers were investigated, but preterm birth still a management problem.

Objectives:-To investigate whether plasma level of Thrombin Anti Thrombin complex (TAT) in women with preterm labour is of potential clinical value in assessment risk of preterm birth.

Study design and setting: This is a case control study conducted on eighty five pregnant women attending Tikrit teaching hospital between (24-34+6weeks) of gestation. Sixty of them with preterm labour, all cases were admitted to hospital and followed up for any complication arising during their current pregnancy.

Twenty five low risk women were collected from the outpatient clinic with gestational age between (24-34+6weeks) as a control group which included patients with uncomplicated singleton pregnancies with no history of preterm labor. TAT value determined by an enzyme-linked immunoassay (ELISA).

Result

The study sample consisted of 85 pregnant women, 60 patients with diagnosis of threatened PTL, from 60 women 26 patients were delivered preterm birth within 3 weeks of admission, while 34 patients delivered after 3 week of admission.

The TAT level is significantly higher in patients with threatened preterm labor who delivered preterm. When the cutoff point of TAT level > 120 is used to detect delivery within 3 weeks. The sensitivity is 100% and specificity 98% and the accuracy is 99%

Conclusion:

The TAT levels were significantly higher in women with threatened preterm birth especially those who delivered within 3 weeks. TAT is highly sensitive and specific

Key word: Thrombin. Preterm Labour, Thrombin antithrombin

Introduction

Preterm labour is defined as the presence of uterine contractions of sufficient frequency and intensity to cause progressive effacement and

dilation of the cervix prior to gestation between (24- 37+6) weeks of gestation (1). According to aetiology, recurrence risk, and outcome preterm labour divided into three groups: mildly preterm at 32-36 weeks (incidence 5.5%), moderately preterm 28-32

weeks (incidence 0.7%) and extremely preterm birth at 24-28 weeks (incidence 0.4%) (1,2)

Normal pregnancy associated with excessive maternal thrombin generation [3] and platelets aggregation [4]. The increased thrombin generation in the maternal circulation has been reported in several obstetrical complications including preterm labor (PTL), preeclampsia, fetal growth restriction and preterm pre labor rupture of membranes (PROM) (5)

Thrombin, a component of the coagulation cascade, and potent uterotonic agonist. In vitro studies demonstrated that thrombin (at concentrations as low as 1 U/mL) increased myometrium contractions. (6) .And 1 mL of clotting blood generates 130 to 160 units of thrombin; so the potent effects of thrombin on uterine contractile activity are of physiological importance.

Small quantities of thrombin can cause uterine contractions, and subclinical decidual haemorrhage could be the explanation for "idiopathic" preterm labor. (7)

Those studies explained the increased myometrial activity observed in conditions associated with intrauterine haemorrhage, such as abruptio placenta. (6,7)

In this study we investigate whether plasma level of Thrombin Anti Thrombin complex (TAT) complex in women with preterm labour is of clinical value in predict risk of preterm birth.

Patients & Methods

This is a case control study conducted on eighty five pregnant women attending Tikrit Teaching Hospital between January 2013 and January 2014.

From the eighty five pregnant women, sixty patients (the study group) were admitted to the department of obstetrics and gynecology with a

diagnosis of threatened preterm labour between (24-34+6) weeks of gestation.

Twenty five low risk women (the control group) were collected from the outpatient clinic with gestational age between (24-34+6) weeks, the control group included patients with uncomplicated singleton pregnancies with no history preterm labor in current and previous pregnancies.

Pregnant women were between 18 and 45 years of age.

Demographic characteristics of patients were assessed; gestational age determination was based on a precisely recalled menstrual date as they were having regular menstrual cycles, and further confirmation by their first trimester or early second trimester ultrasound.

Inclusion criteria for study group were: singleton pregnancy without cervical cerclage, dilatation of the cervix must be < 3cm and membrane still intact.

Exclusion criteria: vaginal bleeding, signs of abruptio placenta premature rupture of membrane, Multiple pregnancies, diabetes and hypertension complicated pregnancy, history or suspicion of thrombo embolic disease, history of acute or chronic congestive heart failure, fetal intrauterine growth restriction, or anomalies

From patients in our study group full history was taken including [age, occupation, any history of medical illness, gynecological history, and Obstetrical history. Maternal vital signs was measured [blood pressure, temperature, pulse rate, respiratory rate). Obstetrical examination [abdominal, pelvic examination] was done with assessments of fetal heart and uterine contraction.

At time of admission investigations, haemogram, urine microscopy and abdominal US done. All patients were given oral tocolytic; Nifedipine in a dose of 10 mg 8 hourly. and Betamethasone, in dose two 12 mg in the first 24 h after admission.

For all study and control groups, 5 mL of venous blood was drawn into a tube contained the anticoagulant and sent to laboratory in Tikrit Teaching hospital . The plasma of this blood was centrifuged and placed in a -20°C freezer.

TAT levels measured for each specimen by enzyme-linked immunoassay the detection range Of TAT is 62.5 pg/ml-4000 pg/ml. With a minimum detectable dose of human TAT is typically less than 15.6pg/ml.

Observation chart was made for each patients including her PR,BP, uterine contraction ,fetal heart rate. The two groups were observed and followed up to identify the exact time of delivery, the patients contact with us in outpatient clinic for follow up and know her pregnancy out come and time of delivery.

Before discharging home, each patient was counseled about any new signs and symptoms like abdominal pain, discomfort, vaginal discharge or bleeding, fever and decrease fetal movement.

Statistical analysis and data management:

Statistical Package for Social Sciences (SPSS)and Chi (χ^2) square test was used for data analysis. Unpaired Student test, one -way and two way ANOVA, and Pearson correlation was used to compare means of numerical variables among the study groups, P value of ≤ 0.05 was regarded as statistically significant. ROC curve used to identify the cut-of points and sensitivity and specificity

Results

Table 1 shows the Mean TAT level among study groups which reveal that level in delivered within 3 weeks of admission is higher than that in patient with PTL who delivered after 3 weeks of admission their mean TAT level was

92.94 or control patient who delivered at term with TAT level 83.00.

Table 2 shows the distribution of study groups according to mode of delivery which reveal that 74 women (87.1%) of both study groups delivered by vaginal delivery and 11(12.9%) women delivered by C /S

Table 3 show The mean TAT level for patients who delivered vaginally was (107.97 ± 36.6) which is not differ significantly from TAT level for those who delivered by C /S their TAT level was 114.1 ± 39.64

Two way ANOVA test indicate significant variation for time of delivery on TAT level ($F=42.989$, $P = 0.00001 < 0.05$) , significant variation for blood group ($F= 2.495$, $P \text{ value} = 0.039 < 0.05$), and significant variation for interaction between blood group and time of labor ($F= 4.537$, $P \text{ value} = 0.0001 < 0.05$).

Pearson correlation show that, there is significant moderate negative linear correlation between TAT level and gestational age $r = - 0.622$, and with days spend to delivery $r = - 0.545$, which mean with low TAT the latency period between admission and delivery will increase.

We have 26 patient delivered within 3 week their TAT level >120 and one patient her TAT level >120 delivered after 3 week while 58 patient their TAT level <120 delivered after 3 week.

Discussion

Preterm labour considered as a great social problem as this may represent the end of the dream of many families to have a healthy offspring. So, it is important to determine the predictive factors of preterm labour. Many markers for risk of preterm birth have been investigated as predictors in the accurate diagnosis of preterm labour.

Because of the high rate of morbidity and mortality associated with preterm

delivery, and in the hope of delaying delivery and allowing time for corticosteroid administration, [8] as well as known morbidity from tocolytic drugs, [9] a test that can identify those patients who are truly at risk for preterm delivery would be of great clinical utility.

In our study TAT level was higher in patients who are delivered before term. There is significant relationship between TAT level and time of delivery with higher TAT level found in patient with PTL. The mean TAT level was significantly higher in patient who delivered within 3 weeks than control or those with PTL who deliver of 3 week after admission. There were no significant differences in TAT level between control who delivers at term and patients with PTL who deliver 3 weeks after admission.

when the Cut off point of TAT level > 120 is used to detect delivery within 3 weeks the sensitivity is 100% ,specificity 98% and the accuracy is 99% .

In our study we found that the TAT level have no effect on the mode of delivery, with non-significant variation in the mean TAT level between those who delivered vaginally or by C/S .

There was significant relation between TAT level and blood group and time of delivery with highest TAT level found in blood group B+ with mean TAT level(188.00)then followed by AB+ with mean TAT (186.50) in patient who delivered within 3 weeks of admission .

Several studies agree with our result like, Elovitz, et al 2001. (7)

and Rosen T et al 2001concluded that Second-trimester elevated plasma TAT concentrations are predictive of subsequent PPROM. These data provide further evidence that PPROM is associated with decidual thrombin activation. (10)

Chaiworapongsa et al 2002 also support our study, they perform a cross

sectional study to determine plasma concentrations of TAT complexes He Concluded that Preterm labor and preterm PROM are associated with an excess generation of thrombin.(11)

Rosen T et al 2002 .(12)and Offer Erez et al 2009 founded that thrombin have uterotonic properties and its activation associated with PTL.(13))

Our study is also supported by Charles J. Lockwood et al 2012who founded that thrombin formed from actively clotting blood had a strong uterotonic action that is independent of prostaglandin production (.14). So that uterine contraction increased in the presence of intrauterine hemorrhage that is consistent with the onset of preterm labor accompanying vaginal bleeding .

David N. et al 2010 dis agree with our study. He found that the TAT complexes, were significantly lower in the maternal plasma of patients who delivered at less than 37 weeks than in controls .(15)

TAT concentrations in asymptomatic patients may represent baseline production as opposed to occurrence of an acute hemorrhage.

Conclusion

TAT is highly sensitive and specific test and the TAT levels were significantly higher in symptomatic women with threatened preterm labour especially those who deliver within 3 weeks.

References

- Phillip Bennett: Preterm Labourin:D.Keith Edmonds. Dewhurst's Textbook of Obstetrics and Gynecology . 7th edition. UK, Blackwell Science Ltd; 2007: 177-191.
- David M. Luesly and Philip N Baker. An evidence-based text for MRCOGinObstetric and Gynecology,2006, 193: 287-96.
- Bellart J, Gilabert R, Miralles RM, Monasterio J, Cabero L. Endothelial cell markers fibrinopeptide A to D-dimer ratio as a measure of coagulation

- and fibrinolysis balance in normal pregnancy. *Gynecol Obstet Invest* 1998;46:17–21.
4. Sheu JR, Hsiao G, Luk HN, Chen YW, Chen TL, Lee LW, Lin CH, Chou DS. Mechanisms involved in the antiplatelet activity of midazolam in human platelets. *Anesthesiology* 2002;96:651–658.
 5. Elovitz MA, Baron J, Phillippe M: The role of thrombin in preterm parturition. *Am J Obstet Gynecol* 2001; 185:1059– 1063.
 6. Elovitz MA, Ascher-Landsberg J, Saunders T, Phillippe M. The mechanisms underlying the stimulatory effects of thrombin on myometrial smooth muscle. *Am J Obstet Gynecol* 2000;183: 674-81.
 7. Elovitz MA, Saunders T, Ascher-Landsberg J, Phillippe M. Effects of thrombin on myometrial contractions in vitro and in vivo. *Am J Obstet Gynecol* 2000;183:799-804.
 8. Kay HH, Bird IM, Coe CL, Dudley DJ. Antenatal steroid treatment and adverse fetal effects: what is the evidence? *J Soc Gynecol Investig* 2000;7:269-78.
 9. Gyetvai K, Hannah ME, Hodnett ED, Ohlsson A. Tocolytics for preterm labor: a systematic review. *Obstet Gynecol* 1999;94:869-77
 10. Rosen T, Kuczynski E, O'Neill LM, Funai EF, Lockwood CJ: Plasma levels of thrombin-antithrombin complexes predict preterm premature rupture of the fetal membranes. *J Matern Fetal Med* 2001;10:297–300.
 11. Chaiworapongsa T, Espinoza J, Yoshimatsu J, Kim YM, Bujold E, Edwin S, Yoon BH, Romero R: Activation of coagulation system in preterm labor and preterm premature rupture of membranes. *J Matern Fetal Neonatal Med* 2002;11:368–373.
 12. T. Rosen, F. Schatz, E. Kuczynski, H. Lam, A. B. Koo and C. J. Lockwood. Thrombin-enhanced matrix metalloproteinase-1 expression: a mechanism linking placental abruption with premature rupture of the membrane. *Journal of Maternal-Fetal and Neonatal Medicine*. 2002;11 :11-17.
 13. Offer Erez, Roberto Romero, Edi Vaisbuch, Tinnakorn Chaiworapongsa, Juan Pedro Kusanovic, Shali Mazaki-Tovi et al. Changes in amniotic fluid concentration of thrombin antithrombin III complexes in patients with preterm labor: Evidence of an increased thrombin generation. *Journal of Maternal-Fetal and Neonatal Medicine* 2009; 22: 971-982.
 14. Charles J. Lockwood, Umit A. Kayisli, Carlos Stocco, William Murk, Emre Vatandaslar, Lynn F. Buchwalder, and Frederick Schatz. Abruptio-Induced Preterm Delivery Is Associated with Thrombin-Mediated Functional Progesterone Withdrawal in Decidual Cells. *Am J Pathol* 2012; 181:2138–2148.
 15. David N Hackney DN, Catov JM, Simhan HN. Low concentrations of thrombin-inhibitor complexes and the risk of preterm delivery. *Am J Obstet Gynecol* 2010;203:184-6

The clinical utility of thrombin as predictor for preterm labour

Table 1 : The Mean TAT level among study group and control.

	N	Mean	Std Deviation	Minimum	Maximum	Median
Control	25	83.00	18.45	62.00	122.00	72.00
Delivered after 3 weeks	34	92.94	15.30	62.00	118.00	93.00
Delivered within 3 weeks	26	154.23	27.81	123.00	202.00	135.50
Total	85	108.76	36.86	62.00	202.00	103.00

F=91.737, df=2,P value=0.0001(<0. 05 significant)(one way ANOVA)

Table 2: The study groups according to mode of delivery

Mode of delivery	Study groups		Total
	control	Preterm	
Vaginal delivery	22 88.0%	52 86.7%	74 87.1%
Caesarean section	3 12.0%	8 13.3%	11 12.9%
Total	25 100.0%	60 100.0%	85 100.0%

$X^2 = 0.028$, df=1, pvalue=0.528 (> 0.05 not significant)

Table 3: The mean TAT level according to mode and time of delivery.

Mode of Delivery	Control		delivered after 3 weeks		Delivered within 3 weeks		Total	ANOVA p value
	No	Mean	No	Mean	No	Mean		
Vaginal Delivery	22	84.63±19.31	32	93.65±15.5	20	156.85±29.14	107.97±36.6	F=63.165,df=2 P=0.0001(<0.05significant)
Caesarean Section	3	73±1	2	81.5±0.7	6	145.5±2.8	114.1±39.64	F=14.5455,df=2, P=0.002 (<0.05significant)
In dependent t-test	25	T=2.72,df=23, p=0.013(<0.05 significant)	34	T=1.233,df=32, p=0.226(>0.05 not significant)	25	T=0.973,df=24, p=0.34(>0.05 not significant)	T=0.287,df=83, p=0.775(>0.05 not significant)	

Table 4: The Mean TAT level among different study groups according to blood group

Blood Group	Control		delivered after 3 weeks		Delivered within 3 weeks		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A+	73.00	1.00	105.25	13.31	129.50	0.71	101.54	21.13
B+	74.00	21.39	85.17	16.80	188.00	15.60	104.76	50.95
AB+	107.00	.			186.50	0.71	160.00	45.90
O+	80.56	16.18	91.17	13.08	147.00	23.86	110.57	34.63
B-	109.00	.	83.00	18.38			91.67	19.86
AB-	99.25	5.85			127.00		104.80	13.41
Total	83.00	18.45	92.94	15.30	154.23	27.81	108.76	36.86

The clinical utility of thrombin as predictor for preterm labour

Table 5: The sensitivity, specificity for the cut -off point > 120 to detect delivery within 3 weeks

TAT Test	Disease		Sensitivity	Specificity	False Positive	False Negative	accuracy	PPV	NPV
	Delivered within 3 week	Delivered after 3 week							
Positive	26	1	100%	98%	2%	0.00	99%	96.3%	100%
Negative	0	58							

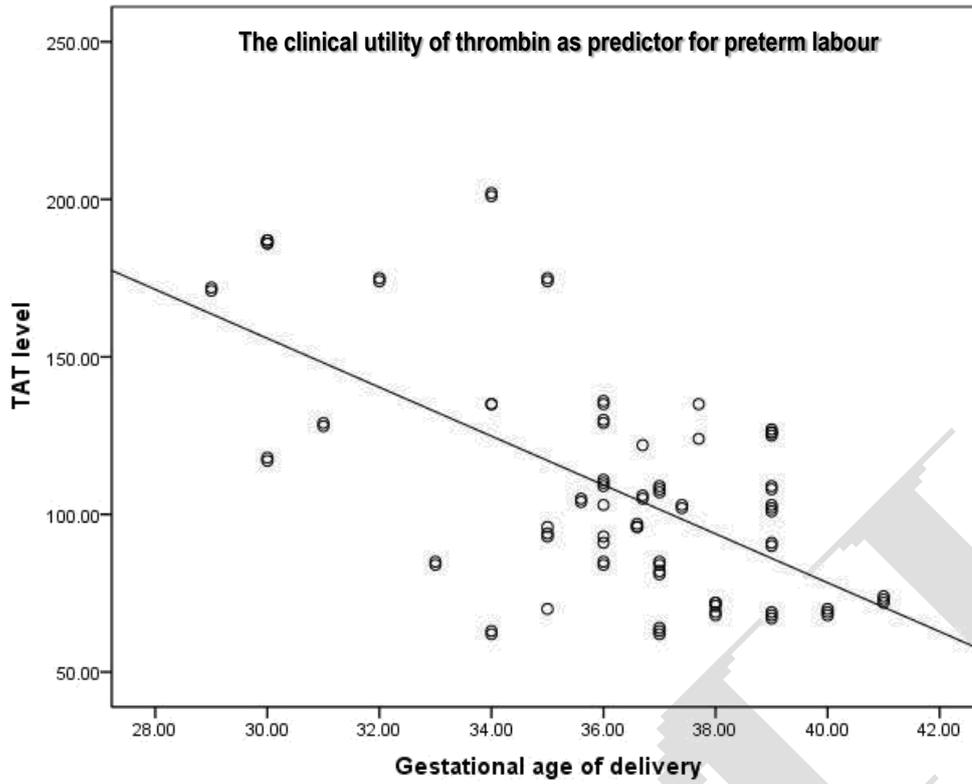


Figure 1:
The
linear
correlation
between

gestational age at delivery and TAT level

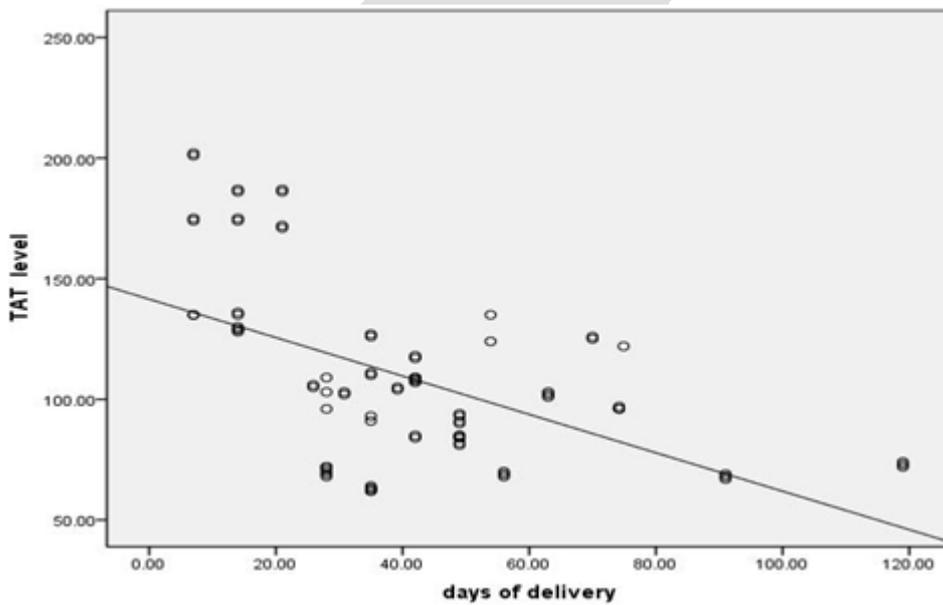


Figure 2: The linear correlation between days for delivery and TAT level

The clinical utility of thrombin as predictor for preterm labour

