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Characterization of antithrombin levels in pregnancy ☆

Andra H. James [a,*], Eleanor Rhee [a], Betty Thames [b], Claire S. Philipp [c]

[a] Department of Obstetrics & Gynecology, Duke University, Durham, NC, USA
[b] Department of Medicine, Duke University, Durham, NC, USA
[c] Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA

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ABSTRACT

Objective: To characterize antithrombin (AT) levels in normal pregnancy.
Methods: We performed secondary analyses with data from 3 studies. Using a single measurement from each subject in the first analysis (cross-sectional), we correlated AT levels with gestational age from the middle of the second trimester throughout the third trimester of pregnancy. Using serial measurements in a second analysis (cohort), we compared AT levels between the late first and second trimesters of pregnancy and baseline (the level at 6 weeks postpartum). Using serial measurements in a third analysis (cohort), we analyzed the pattern of change in AT levels in the immediate postpartum period. Assays of AT activity were performed using the Dade Behring (Siemens) Berichrom Antithrombin III Chromogenic Assay. AT levels were correlated with gestational age using the Pearson correlation coefficient and compared between the different time points using one-way ANOVA.
Results: Overall, AT levels were 20% lower than baseline during pregnancy ($p < 0.01$). There was no significant difference between AT levels obtained between late first trimester and late second trimester. From midtrimester to term, however, AT levels were negatively correlated with gestational age with a 13% drop during this period of time ($r = -0.26$ [−0.39, −0.11]; $p < 0.01$). Immediately after childbirth, AT levels fell precipitously to 30% below baseline ($p < 0.05$) and reached a nadir 12 hours postpartum before rising and returning to baseline by 72 hours postpartum.
Conclusion: It appears that antithrombin (AT) is consumed at the time of delivery. Our findings have implications for AT replacement or even anticoagulation at the time of delivery.

Introduction

Antithrombin (AT) is a natural anticoagulant which plays a potentially important role in whether women develop thromboembolism during pregnancy. Multiple reports have documented an association between inherited deficiency of AT and an increased rate of venous thromboembolism (VTE) [1]. In pregnancy, the rate of VTE is correspondingly higher. Historic series have reported rates as high as 18 to 70% [2–4]. Differences in these rates may be attributed to the relatively small numbers in each series, to selection bias and to the varied definitions of AT deficiency between series. A well-conducted study enrolled AT deficient female family members of AT deficient patients with a history of VTE. The subjects had no history of VTE prior to pregnancy. The incidence of pregnancy-related VTE among the subjects was 1/33 pregnancies or 3% [5]. This rate is not nearly as high as the historic series, but considerably higher than the background rate of pregnancy-related thrombosis which is 0.1 to 0.2% [6]. A systematic review including more recent studies (some with more liberal thresholds and not all requiring a positive family history) found a lower risk of VTE in pregnancy than the initial studies, but still found an odds ratio 4.76 [95% confidence interval 2.15, 10.57]). Recently, even mild AT deficiency has been found to be associated with an increased risk for VTE [7,8].

AT is the substrate for heparin, the preferred class of anticoagulant for the prevention and treatment of thrombosis in pregnancy [9]. Because women have a four- to five-fold increased risk of thromboembolism during pregnancy [10,11], women are not infrequently prescribed a heparin during pregnancy and and/or the postpartum period because of current thrombosis or because of a history of thrombosis. In the absence of heparin, AT has low inhibitory activity against thrombin. However, when heparin is bound to its binding site on AT, inhibitory activity is increased at least 1000-fold. [12] AT inactivates thrombin by binding to the active serine of thrombin and to the active serine of activated factor X (FXa) [1]. AT has the ability to inactivate other coagulation factors, including factors IXa, XIa, and XIIa [1]. Importantly, in the absence of AT, heparin has little to no effect.

AT concentrates have been available since 1979 [13] and are indicated for the treatment of women with AT during pregnancy or the

☆ This study was performed while Betty Thames with the Department of Obstetrics & Gynecology at Duke University.

* Corresponding author at: Department of Obstetrics & Gynecology, DUMC 3967, Durham, NC, USA 27710. Tel: +1 919 681 5220; fax +1 919 681 7861.
E-mail address: andra.james@nc.rr.com (A.H. James).

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postpartum period when anticoagulation is inadequate to prevent or treat thrombosis in women with AT deficiency or when anticoagulation is desired, but contraindicated [14]. AT concentrates have also been used in an attempt to prolong pregnancy in severe preeclampsia [15]. There are not only limited data about AT levels in patients with AT deficiency or severe preeclampsia during pregnancy, there are limited data about AT levels in normal pregnancy. Some investigators have found decreased AT levels in the third trimester of pregnancy or immediately postpartum and others have found no change [16–22]. The purpose of this study was to characterize AT levels in normal pregnancy.

Methods

We performed secondary analyses with data from three different studies to characterize AT levels. As the studies had other primary objectives, no sample size calculations were made for these secondary analyses. The studies from which the subjects were derived and the overall study of AT levels were approved by the Duke Institutional Review Board. Using a single measurement from each subject in the first analysis (cross-sectional), we correlated AT levels with gestational age from the middle of the second trimester throughout the third trimester of pregnancy. Using serial measurements from the same subjects in a second analysis (cohort), we compared AT levels between the late first trimester of pregnancy, the late second trimester of pregnancy and baseline (the level at 6 weeks postpartum). Again, using serial measurements from the same subjects in a third analysis (cohort), we analyzed the pattern of change in AT levels in the immediate postpartum period. AT levels were compared between the different time points using one-way ANOVA (JMP statistics software [SAS] v. 10.0.2).

Subjects in the first analysis (cross-sectional) comprised 172 pregnant women who were each sampled a single time from midtrimester throughout the third trimester of pregnancy. The study population was derived from normal control subjects who were enrolled at Duke University Medical Center as part of a multi-center study of thrombophilia and intrauterine growth restriction (IUGR) during the time period of August 2003 to December 2006. Mothers age 18 or older with normally-grown singleton gestations were enrolled between 25 and 40 weeks. Subjects with documented congenital or chromosomal anomalies, preeclampsia or prolonged premature rupture of the membranes (>4 weeks) were excluded. Subjects with a history of deep vein thrombosis or other thromboembolic event, or who were receiving anticoagulation therapy in the form of low molecular-weight-heparin (LMWH) or unfractionated heparin (UFH), were also excluded. A sample of approximately 25 cc of blood was collected by venipuncture in standard 4.5 mL 3.2% sodium citrate tubes from each subject. Within 4 hours of the blood draw, specimens were processed and plasma separated. Assays of AT activity were performed in real time using the Dade Behring (Siemens) Berichrom Antithrombin III Chromogenic Assay at the Duke Coagulation Laboratory. The normal range for AT levels at the time of the three studies was 73 to 132 IU/dL with a coefficient of variation of < 10% (2 to 8%). Demographic data including maternal age, race, gestational age, height, weight, medical comorbidities, and personal habits (i.e. tobacco use) were obtained at enrollment. A total of 182 potential subjects were identified. Ten were excluded after enrollment – 9 due to insufficient sample and one due to development of preeclampsia. AT levels were correlated with gestational age using the Pearson correlation coefficient (GraphPad Prism software).

Subjects in the second analysis (cohort) comprised 120 pregnant women who were participating in a study of hemostatic variables in pregnancy, were 18 years of age or older, were planning to deliver at Duke University Medical Center, were in their first trimester of pregnancy, were less than 14 weeks gestation and had documented fetal viability. Subjects who had multiple gestation, had a known bleeding disorder, were on anticoagulation or were on an antiplatelet agent were excluded. Samples were collected by venipuncture during the late first trimester (12-14weeks gestation), in the late second trimester (between 24 and 28 weeks gestation), and at 6 weeks postpartum (baseline). Samples were obtained and processed the same as for the subjects in the first analysis except that after processing samples were frozen and stored at −80° c. Assays were performed on batched samples, which had not been previously thawed, and were performed otherwise the same as for the subjects in the first analysis. AT levels were compared between the different time points using one-way ANOVA (JMP statistics software [SAS]).

Subjects in the third analysis (cohort) comprised 13 normal women without von Willebrand disease who were participating in a study of von Willebrand factor levels in pregnancy. Subjects were enrolled during the last trimester of pregnancy from obstetric clinics at Duke University. For each subject, an AT level was analyzed from the samples obtained at enrollment, on admission to the hospital for childbirth, and at 4 hours, 12 hours, 1 day, 2 days, 3 days, 7 days, 14 days, 21 days, 28 days, and 42 days postpartum. Samples were obtained, stored, processed and analyzed the same as for the subjects in the second analysis. AT levels were compared between the different time points using one-way ANOVA (JMP statistics software [SAS]).

Results

Secondary Analysis 1

The subjects comprised 172 pregnant women who were each sampled a single time between 25 and 40 weeks gestation. For the age, racial/ethnic distribution and parity see Table 1. Because there are data that suggest that African Americans have lower antithrombin levels [23], the racial/ethnic distribution was examined over the range of gestational ages studied and was found to be constant. Specifically, African Americans accounted for 49% of the subjects < 34 weeks gestation and 46% of the subjects ≥ 34 weeks gestation. Therefore, a greater proportion of African Americans later in pregnancy could not account for any observed decrease in AT levels with advancing gestational age. AT levels were negatively correlated with gestational age ($r = -0.26$ [−0.39, −0.11]; $p < 0.01$). As gestational age increased, AT levels decreased. See Fig. 1. There was a 13% drop from a predicted mean of 105 IU/dL at 25 weeks to 91 IU/dL at 40 weeks gestation.

Secondary Analysis 2

The subjects comprised 120 pregnant women. For the age, racial/ethnic distribution, parity and modes of delivery see Table 1. Serial AT levels were obtained in the late first trimester, in the late second trimester and at 6 weeks postpartum (the baseline level). By 12–14 weeks

Table 1
Characteristics of the Subjects.

	Secondary Analysis 1 (n = 172)	Secondary Analysis 2 (n = 120)	Secondary Analysis 3 (n = 13)
Mean Age (95% confidence interval)	33.9 (33.4, 34.3)	29.7 (28.6, 30.7)	31.9 (29.1, 35.4)
Race/ethnicity			
white	78 (45%)	47 (39%)	9 (69%)
African American	82 (48%)	54 (45%)	3 (23%)
Hispanic	7 (4%)	9 (8%)	-
Asian	4 (2%)	10 (8%)	1 (8%)
Unknown	1 (1%)	-	-
Parity			
0	37 (22%)	63 (53%)	3 (23%)
≥ 1	135 (78%)	57 (47%)	10 (15%)
Mode of delivery			
vaginal	73 (61%)		10 (77%)
cesarean	40 (33%)		3 (23%)
second trimester loss	3 (3%)		-
unknown	4 (3%)		-

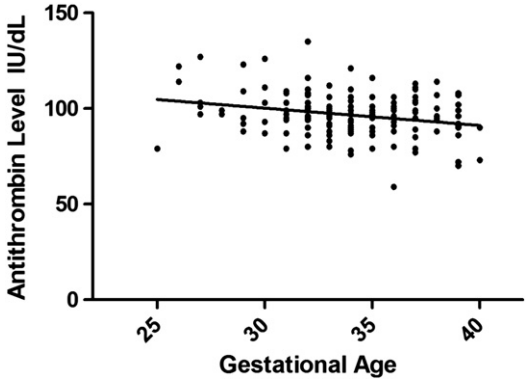

Fig. 1. Antithrombin levels by gestational age from 25 to 40 weeks in a cross-sectional sample of 172 women.

Fig. 3. Serial antithrombin levels from a single cohort (n = 13) sampled frequently during the postpartum period.

gestation the mean AT level was 79 IU/dL (95% confidence interval [CI] 75, 83). At 24–28 weeks the mean AT level was 82 IU/dL (95% CI 78, 87). As expected, by 6 weeks postpartum, the mean AT level was back to baseline at 100 IU/dL (95% CI 96, 105). The pregnancy levels, although not significantly different from each other, were both significantly lower than baseline levels by approximately 20% ($p < 0.01$). See Fig. 2.

Secondary Analysis 3

The subjects comprised 13 normal women. For the racial/ethnic distribution, parity and modes of delivery see Table 1.

The baseline AT level (at 6 wks postpartum) was 109 IU/dL (95% CI 93, 124) and was the same as the mean third trimester level (109 IU/dL [95% CI 97, 120]). The mean level on admission for childbirth was lower (93 IU/dL [95% CI 80, 107]). Immediately after childbirth, levels dropped precipitously and were significantly below baseline by 4 hours postpartum (88 IU/dL [95% CI 77, 99]) ($p < 0.05$) and reached a nadir at 12 hours postpartum (76 IU/dL [95% CI 53, 99]) ($p < 0.05$). The nadir was more than 30% below baseline. AT levels remained significantly below baseline at 24 hours postpartum (88 IU/dL [95% CI 67, 108]) ($p < 0.05$) before rising and returning to baseline by 72 hours postpartum (111 IU/dL [95% CI 92, 129]). Fig. 3 illustrates the nadir in relation to levels during pregnancy as well as the timing of recovery of AT levels after childbirth. AT levels, which returned to baseline at 72 hours, continued to rise slightly until 2 weeks PP (117 IU/dL [95% CI 104, 129], then declined to baseline and plateaued for the remainder of the postpartum period.

Discussion

We found that, overall, AT levels were 20% lower than baseline during pregnancy. Additionally from the second analysis (cohort) we learned that there was no difference between AT levels obtained between 12 to 14 weeks gestation (the end of the first trimester) and 24 to 28 weeks gestation (the end of the second trimester). Whether we would have found a difference between levels obtained at the end of the second trimester and levels obtained in the third trimester had we sampled then is unknown, but there was a remarkable difference between AT levels obtained during pregnancy and AT levels obtained at baseline (6 weeks postpartum). From the first analysis (corss-sectional) we learned that from midtrimester to term (25 to 40 weeks gestation), AT levels were negatively correlated with gestational age with a 13% drop during this period of time. From the third analysis (cohort), where frequent serial sampling was performed, we learned that immediately after childbirth, AT levels fell precipitously to 30% below baseline and reached a nadir 12 hours postpartum before rising and returning to baseline by 72 hours postpartum. This precipitous fall immediately after childbirth is a novel finding and has not been previously reported.

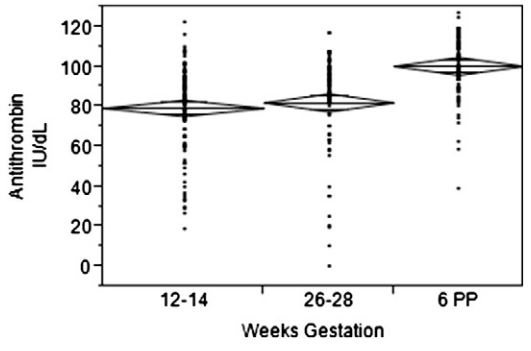

Fig. 2. Serial antithrombin levels from a single cohort (n = 120) sampled in the late first trimester, in the late second trimester and at 6 weeks postpartum (PP).

Previous investigators have found decreased AT levels in the third trimester of pregnancy or immediately postpartum and others have found no change [16–22]. This variation in results is likely because the decreases in AT levels are not large until the immediate postpartum period and then the dramatic decreases are fleeting. Depending on how the studies were conducted and how many subjects were sampled, the pattern of the changes in AT levels could have been missed. With subjects from three different secondary analyses and using frequent, serial sampling in the immediate postpartum period, we were able to elucidate the pattern of the changes.

The more dramatic changes observed in the immediate postpartum period may be easier to explain than the smaller changes observed during pregnancy. It is possible that the changes observed during pregnancy can be explained by hemodilution, altered synthesis, increased clearance or even a consumptive process. These were normal subjects who did not have renal disease or preeclampsia and would not have been expected to have lost antithrombin through urinary excretion. The precipitous drop in AT levels in the immediate postpartum period suggests a consumptive process consistent with thrombus formation. A recent study demonstrated that more than half of women have MRI evidence of non-occlusive thrombi in their pelvic veins 1–4 days after vaginal delivery [24]. The same investigators found a similarly high rate of non-occlusive thrombi at the time of discharge after cesarean delivery [25]. We have previously reported other biochemical evidence of this thrombotic process with consumption of fibrinogen [26] and factor VIII [27] at the time of delivery. It should be noted that while there were significant changes in AT levels during pregnancy and the postpartum period, most values remained in the normal range for the assay.

Limitations of this study are the lack of frequent, serial samples antepartum. In none of the studies were frequent, serial samples taken

early in pregnancy in order to establish the onset of the decrease in AT levels. Data from the second analysis (cohort) might have confirmed the finding from the first analysis (cross-sectional) of a 13% decrease in AT levels in the third trimester, but in the second analysis (cohort), frequent, serial samples were not obtained in the third trimester. Nonetheless, frequent, serial samples were taken in the third analysis (cohort) during the postpartum period leading to the discovery of the novel finding of the precipitous drop in AT levels during the immediate postpartum period. Unfortunately, there were insufficient subjects to elucidate whether there were differences in the pattern of AT levels between women who had a spontaneous vaginal delivery, a cesarean delivery before labor or a cesarean delivery after labor, nor did the study provide data on the pattern of AT levels in women with preeclampsia or AT deficiency. The response of AT levels in these other conditions and situations could have implications for AT replacement during different clinical scenarios.

In conclusion, we found that AT levels are 20% below baseline during pregnancy. It appears that this decrease is present by the end of the first trimester. There is a further decline of approximately 13% after midtrimester. Then, it appears that antithrombin is consumed at the time of delivery consistent with temporary thrombus formation. The mean antithrombin level drops 30% after delivery with a nadir at 12 hours postpartum before returning to baseline at 72 hours postpartum. These findings may have implications for the management of AT replacement in patients with either AT deficiency or an AT-deficient disease state such as severe preeclampsia and may have implications for anticoagulation for all patients at risk for pregnancy-related thrombosis.

Conflict of Interest Statement

Dr. James has received research support from Grifols. Dr. Philipp receives research support from Baxter. Dr. Rhee and Ms. Thames have no conflicts of interest relative to this paper.

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