Antithrombin III activity in healthy pregnant women seen at the University of Benin Teaching Hospital, Benin City

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Abstract

Background: Antithrombin (AT III) is a glycoprotein synthesized by the liver. It has anticoagulant and anti-inflammatory properties. Pregnancy is a hypercoagulable state due to increased synthesis of coagulation protein and depletion in the activity of natural anticoagulant due to consumption by activated coagulation proteins. Varying AT III activities have been reported in normal pregnancy but this has not been investigated in our environment.
Objective: This study aims to determine antithrombin (AT III) levels in normal pregnancy, to compare changes in second and third trimester and to correlate AT III levels with gestational age and haematological parameters.
Methods: This is a cross sectional study conducted at the University of Benin Teaching Hospital, Benin City. Eighty apparent normal pregnant women recruited at the antenatal clinic were evaluated for AT III activity using Technoclone chromogenic AT III kit and haematological parameters using automated Hematology Analyzer, ERMA INC (Tokyo model FCE 2.0). The data was analyzed using SPSS version 16.
Result: The average age of the women was 31.2 ± 4.3years at a mean gestational age of 28.1±5.5 weeks. Their mean haematocrit, white blood cell count, platelet counts and AT III activity were 33.6 ± 3.6%, 6.5 ± 2.0 x 10^9/L, 193.3 ± 48.7 x 10^9/L and 82.2±30.2% respectively. AT activity was significantly reduced in the third trimester ($p = 0.005$). A negative correlation coefficient was found between AT III activity and gestational age ($r = -0.337$; $p = 0.003$).
Conclusion: The mean AT III levels is within normal range in pregnancy however there is a significant decline in activity in the third trimester.

Keywords: Antithrombin III activity, Haematological parameters, Healthy Pregnancy, Benin City.

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Introduction

Normal pregnancy is associated with major changes in many aspects of haematological and haemostatic parameters all contributing to maintain placental function during pregnancy and to prevent excessive bleeding at child birth.[1, 2] The haemostatic balance is tilted in favour of a procoagulant state. Coagulation factors are widely reported to be elevated in pregnancy, fibrinolytic activity suppressed and some natural anticoagulant (protein C and S) levels reduced in pregnancy however controversial reports have been documented about antithrombin III (AT III).[3]

AT III is a glycoprotein synthesized in the liver, with a molecular weight of 58,000 and a plasma half-life of about 67 hour.[4] It inhibits action of thrombin and other activated coagulation factors namely Xa, IXa, XIa, XIIa, plasmin and kallikrein.[5] Its action is potentiated by heparin. It is the most critical modulator of coagulation and has potent anti-inflammatory properties independent of its effects on coagulation.

There is paucity of haemostatic studies in pregnant women in our environment. This study aims to investigate AT III activity in normal pregnancy, to compare changes in second and third trimesters and to correlate AT III activity with gestational age and haematological parameters.

Materials and Methods

This is a cross sectional study conducted at the University of Benin Teaching Hospital (UBTH) between August and December 2015. Eighty consenting apparently healthy pregnant women receiving antenatal care in the department of Obstetrics and Gynecology were recruited by systematic random sampling into the study.

Women with hypertension in pregnancy, proteinuria, diabetes mellitus and any chronic medical or previous obstetric adverse risk factors were excluded from the study.

The study was approved by the research and ethics committee of the University of Benin Teaching Hospital, Benin City. Informed consent was also obtained from the

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participants and confidentiality duly assured.

Seven millitres of venous blood was collected from each subject under aseptic technique. 2.5 mls was dispensed into an EDTA specimen bottle for determination of haematological parameters while 4.5mls was dispensed into a plain bottle containing 0.5 mls of 3.2% sodium citrate anticoagulant for the determination of AT III activity. The citrated sample was centrifuged at 2500RPM at room temperature for 20 minutes and the platelet poor citrated plasma was harvested and stored in plain tubes at -20^0 C for about 8 weeks before analysis.

The full blood count was analyzed using an automated Hematology Analyzer, ERMA INC (Tokyo model FCE 2.0). Antithrombin III activity was determined using Technoclone chromogenic AT III kit (Technozyme Lot no: 0551B00.02) produced by Technoclone Diagnostics, Vienna, Austria. Data was analyzed with statistical package for social science (SPSS) version 16. The results were presented as means, standard deviation and proportions as appropriate. Student t test was used to compare variables between women in their second and third trimesters. Pearson's correlation coefficient was used to correlate AT III activity with GA and haematological parameters. Statistical significance was set at 0.05.

Results

A total of 80 apparently healthy pregnant women participated in the study including 40 women each in their second and third trimester of gestation. The average age of the women was 31.2 ± 4.3 years at a mean gestational age of 28.1 ± 5.5 weeks.

Their mean haematocrit, white blood cell count and platelet counts were 0.34 ± 0.04, 6.5 ± 2.0 x 10^9/L and 193.3 ± 48.7 x 10^9/L respectively as shown in table 1. Their mean antithrombin III activity was 91.8 ± 22.5%. Ten (12.5%) of the women were anaemic, 2 (2.5%) had thrombocytopenia while 27 (33.8%) had antithrombin III deficiency.

Table 1: Gestational age and Laboratory Parameters of Pregnant women attending ANC of UBTH, Benin City between August and December 2015

	Mean ± SD	Median	Range
Age (yrs)	31.2 ± 4.3	31.5	20.0 - 41.0
Gestational age (wks)	28.1 ± 5.5	28.5	20.0 - 40.0
Haematocrit (%)	33.6 ± 3.6	33.8	20.4 - 44.1
WBC count (x 10^9/L)	6.5 ± 2.0	6.2	3.0 - 12.8
Platelet count (x 10^9/L)	193.3 ± 48.7	193	86.0 - 337.0
AT activity (%)	82.2 ± 30.2	94.6	8.6 - 121.5

Table 2: Comparison of Age and Laboratory Parameters of Pregnant women in 2nd and 3rd trimesters of gestation attending ANC at UBTH, Benin City between August and December 2015

Variable	2nd trimester Mean ± SD	3rd trimester Mean ± SD	T test	p value
Age (yrs)	31.2 ± 4.5	31.2 ± 4.2	-0.051	0.959
Haematocrit	33.7 ± 3.8	33.5 ± 3.5	0.176	0.861
WBC count (x 10^9/l)	6.2 ± 1.9	6.7 ± 2.1	-0.987	0.327
Platelet count (10^9/l)	194.9 ± 51.1	191.8 ± 46.7	0.283	0.778
AT activity (%)	91.8 ± 22.5	73.1 ± 33.8	2.865	0.005

Table 2 shows a comparison of age, haematological parameters and AT III activity between the pregnant women in their second and third trimesters. There was no significant difference in the age and haematological parameters in the women in each trimester however the AT activity was significantly reduced in the third trimester (p = 0.005).

Table 3: Correlation between GA and laboratory parameters of healthy pregnant women attending ANC at UBTH, Benin City between August and December 2015

Variable	AT III activity r	P value
Gestational age	-0.337	0.003
Haematocrit	0.139	0.226
WBC count	0.131	0.254
Platelet count	- 0.024	0.833

Table 3 shows the correlation coefficient between haematological parameters, antithrombin III activity and gestational age.

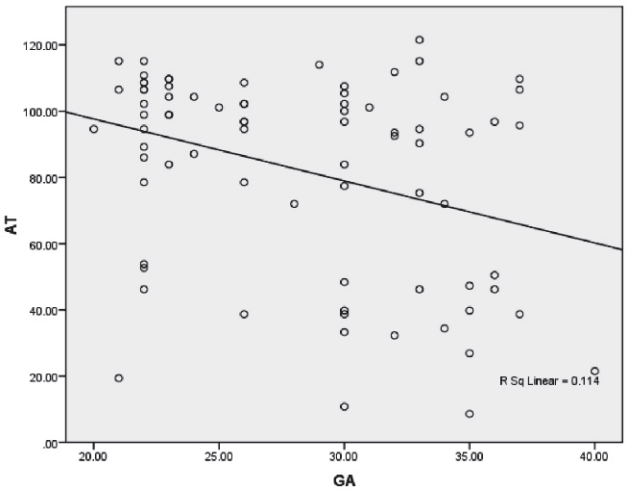

Figure 1: Correlation of AT III with gestational age in the second half of pregnancy in healthy pregnant women seen at UBTH, Benin City from

August to December 2015 (r = -0.337; p = 0.003)
A weak correlation was found between gestational age and AT III as well as between gestational age and platelet counts however only the former was statistically significant (r = -0.337; p = 0.003) (Figure 1).

Discussion

Natural anticoagulants including protein C and S are widely documented to decline in pregnancy but controversial reports have been reported about antithrombin III. Majority of authors opined that its level remain unchanged while a few others reported a decline in pregnancy.[6,7]

In this study we found that the mean AT III level was within the reference range (80 – 150%) however when our subjects were categorized into trimesters, there was a significant decline in AT III activity in the third trimester of pregnancy (p = 0.005). This is similar to the observations of Essien[6] who documented a significant decline in AT activity in pregnancy. Bremme et al[8] and Weiner[9] in separate studies noted that though AT III levels remain stable in most parts of pregnancy but there is a decline in the terminal parts of pregnancy. They however noted that this slight decline provided it remains within the reference range does not predispose to increased risk of thrombosis. The decline in AT III activity may be attributed to increased consumption in the bid to check the activity of the increasing coagulation proteins observed in pregnancy. van Wersch and Ubachs[10] reported increased thrombin-antithrombin (TAT) complex formation in pregnancy. Thus providing evidence of increased AT III utilization in pregnancy.

Wang et al[11] in a comparative study of AT III levels in the three trimesters of pregnancy, reported a progressive decline in AT III activity in each trimester however only the difference in mean activities between the first and second trimesters and the first and third trimesters were statistically significant. The difference in mean activity between the second and third trimesters was not statistically significant. Contrary to our observation, most researchers have reported that AT III levels remain stable throughout normal pregnancy. Imoru and Buseri[12] in a cross sectional studies among 150 normal pregnant women in Kano reported no significant change in AT III across the three trimester of gestation. Gatti et al[13] in a related longitudinal study reported no significant alteration in AT III activity.

We found that 33.7% of women with normal pregnancy had subnormal levels of AT III. There is paucity of data on the prevalence of AT III deficiency in pregnancy. The generality of studies report the mean values of AT III activity in their study populace but no information on prevalence of AT III deficiency. Functional deficiency is not unusual as the protein in addition to being a natural anticoagulant has anti-inflammatory activity.

We also found that there is a negative correlation between AT III activity with age in the second half of pregnancy. Similar to our observation, James et al[14] reported similar negative correlation with up to 13% reduction of AT activity in late pregnancy. The negative correlation in observed in the second half of pregnancy is due to the increased consumption of AT III by the increasing coagulation proteins with advancing gestational age.

In conclusion, our study showed that AT III declines in late pregnancy even significantly below the reference values that it may predispose to thrombotic tendencies. Its level also correlates negatively with gestational age in the second half of pregnancy.

We recommend further studies on AT activity in healthy pregnancy in our environment including investigating for evidence of clinical and subclinical thrombotic events in women with subnormal AT III activity.

Limitation of the Study: We could not establish if AT III deficiency was due to congential defect. This was because of lack of facility for genetic studies.

Conflict of Interest: None

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