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ORIGINAL ARTICLE

Evaluation of Coagulation Markers in Pregnant Women Attending Antenatal Care at a Teaching Hospital in Aba, Nigeria.

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ABSTRACT

Introduction: Pregnancy may alter the haemostatic system, which is directed at protecting the maternal subjects from haemorrhage during pregnancy and postpartum and the foetal subjects during implantation. This study was set to investigate the effect of pregnancy on the haemostatic system and possible predisposition to coagulopathy.

Materials and Methods: The study was conducted in Abia State University Teaching Hospital, Aba. A total of 72 healthy subjects, 40 pregnant women and 32 nonpregnant women that served as control, were recruited for the study. The subjects were between 20 – 45 years. The haemostatic parameters studied were Prothrombin time, Platelet count and Antithrombin III. The Prothrombin time was measured by manual method, Platelet count by auto hematology analyzer, and Antithrombin III using an Enzyme-linked immunosorbent assay technique. Data were analysed using Student's t-test, ANOVA, Pearson's correlation coefficient, and p values set at < 0.05.

Results: Mean±SD 12.34±1.27s, 173.95±50.22×10^9/L for Prothrombin time and Platelet count respectively were shortened and statistically significant when compared with 13.48±0.68s, 258.00±35.77 ×10^9/L for Prothrombin time and Platelet count respectively for the control ($p<0.05$). Mean±SD of Prothrombin time in 3rd trimester (11.44±1.17 s) was significantly shortened in comparison with 12.92±1.11s and 12.94±0.84s for 1st and 2nd trimesters respectively ($p<0.05$). Mean ±SD 1836.29±386.47ng/ml for Antithrombin III for 1st trimester was statistically lower when compared with 2590.59±739.78ng/ml, and 3772.01±902.11ng/ml for 2nd and 3rd trimesters, respectively. No correlation existed between Platelet count and Antithrombin III ($r = 0.30$, $p = 0.058$).

Conclusion: The findings of this study showed that pregnancy alters haemostatic indices but may not necessarily predispose to clinical coagulopathy.

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INTRODUCTION

Haemostasis is a physiological process that involves the tight and regulated interaction between the procoagulant and anticoagulant proteins, which results in the arrest of blood flow and repair of damaged vessels. The primary function of the haemostatic system is to respond quickly to vascular injury, stop bleeding, and heal the injured vessel. Under normal conditions, a delicate balance exists between the procoagulant and anticoagulant pathways (1).

The balance allows for effective control of bleeding and prevents the activation of the procoagulant activities beyond the injured or in the absence of bleeding. If this equilibrium is disrupted, it may lead to complications resulting in haemorrhage and thrombosis. This delicate balance is disrupted whenever there is an increase in the procoagulant activities or a decrease in the activity of natural inhibitors (2).

Pregnancy induces changes in the coagulation system, creating a natural hypercoagulable state. This includes increased levels of specific clotting factors, such as VIII, IX, and X. These changes promote clot formation, making pregnant and postpartum women susceptible to venous thrombosis (3).

In normal pregnancy, mean platelet count gradually decreases across the first, second, and third trimesters (4). The decrease is driven by various physiological changes, such as the dilution of platelets due to the expansion of maternal blood plasma volume and increased platelet sequestration and consumption in the placental system (5). In some cases, platelet counts fall below the normal range, known as gestational thrombocytopenia (4). During a healthy pregnancy, activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time decreased by 10%–20% from pre-conception levels to the third trimester (6). Antithrombin III (AT III) levels may remain stable. Antithrombin III is a plasma glycoprotein and the primary inhibitor of thrombin, as well as factors IXa, Xa, XIa and XIIa and prevents the excessive formation of fibrin clots. The enzymatic activity of AT III is enhanced in the presence of heparin (7,8).

This study aimed to determine the effect of pregnancy on prothrombin time, platelet count, and antithrombin III, with the aim of assessing any alterations in the haemostatic system associated with a predisposition to coagulopathy.

MATERIALS AND METHODS

Study area

This study was carried out at Aba. Aba is a city in Abia State, South East Nigeria and also the commercial center of Abia State. It is located at Latitude 5°7′0″ N and Longitude 7°22′0″ E, with a population estimate of 1,189,000 (2023). It is a cosmopolitan town in Abia State and South Eastern Nigeria's second largest commercial city. Based on population the area is ranked 9th in Nigeria.

Study Population: The subjects recruited for this study were apparently healthy pregnant and non-pregnant women between the ages of 20 and 45 in Aba, Abia State.

Study Design: This work's study design was a cross-sectional study that involved pregnant women of different gestational ages and non-pregnant women in Aba, Abia State.

Ethical Approval: Ethical approval for the study was obtained from the Faculty of Medical Laboratory Science ethics committee, Abia State University Uturu, Abia State. After due explanation of the study's nature, informed consent was obtained from all the participants.

Inclusion Criteria: This work includes pregnant women of various gestational ages between 20 and 45 years; only those who consented to participate in the study were included.

Exclusion Criteria: Women outside childbearing age and pregnant women with known haemostatic disorders such as thrombophilia, as well as subjects on anticoagulant therapy, were excluded from the study. Other subjects also excluded from the study were those who recently received blood transfusions or other forms of infusion. This was to avoid the effect of dilution.

Sample Collection

Two milliliters of blood (2ml) were collected in an EDTA bottle for the determination of Platelet count. The data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 25.0. Four milliliters of blood (4ml) were collected in a sodium citrate container for the determination of prothrombin time, whereas serum obtained from an anticoagulant-free tube was used for the estimation of antithrombin III by the use of the ELISA technique (URIT—660).

Statistical Analysis

The data analysis was entered in Statistical Package for Social Science (SPSS) version 25.0. The parameters were expressed as Mean and Standard Deviation. A comparison of groups was done using ANOVA and the student (t) test. Test of Association was carried out using Pearson's Correlation Coefficient. The level of significance was set at $p<0.05$, and values below 0.05 were considered statistically significant.

RESULTS

Table 1 shows the comparison of the Mean±SD of PT, PLC, and AT III between pregnant and nonpregnant women. The mean ± standard deviation for PT and PLT count (12.34±1.27s, 173.95±50.22×10⁹/L respectively) for pregnant women were shortened and statistically significant ($p<0.05$) when compared between the control (13.84±0.68s, 258.00±35.77×10⁹/L, respectively). The mean ± standard deviation for AT III (22855.71 ± 1086.60ng / ml) for Pregnant women had no statistically significant difference when compared with that of the control (2497.68±851.97ng/ml).

Table 2. The comparison of the Mean±SD of PT, PLC and AT III for the pregnant women by gestational age. The rationale for the table was to assess the effect of gestational age on

PT, PLC and AT III levels. The gestational age were grouped into the 1st , 2nd and 3rd trimesters.

Based on gestational age, the mean ± standard deviation for PT and AT III (12.92 ± 1.11s, 12.94 ± 0.84 s, 11.44 ± 1.17 s) and (1836.29 ± 386.47 ng/ml, 2590.59 ± 739.78 ng/ml and 3772.01 ± 902.11) respectively showed statistical difference when compared with the 1st, 2nd and 3rd trimesters respectively ($p < 0.05$). For PLC, the values were not statistically significant ($p < 0.05$).

Table 3 shows the comparison of the Mean±SD of PT, PLC and AT III for pregnant women by parity . The essence of the table was to assess if parity altered PT, PLC and AT III levels. The parity was grouped into 4. Group 1 were the pregnant women that were in their first pregnancy. Group 2 were pregnant women who has given birth to a child. Group 3 were pregnant women who has given birth to 2 children. Group 4 were pregnant women who has given birth to 3 children and above.

The mean ± standard deviation for PT, of women in group 2 (12.85±0.83s) were higher but not statistically significant [$p<0.05$] when compared with women in group 1,3 and 4 (11.94±1.32s,12.53±1.11s and 12.23±1.92s, respectively). For PLC, the mean ± standard deviation of women in group 3 ($205.2±41.58×10^9/L$) were higher but showed no statistical significance ($p<0.05$) when compared to that of women in group 1, 2 and 3 ($165.44±48.98×10^9/L$, $179.67±54.92×10^9/L$ and $166.8±52.15×10^9/L$, respectively). Also, the mean ± standard deviation for AT III in women in group 3 [3201±1266.85ng/ml] were higher but showed no statistical significance ($p<0.05$) when compared to women in group 1,2 and 4 (2711±967.49ng/ml, 2575.55±816.8ng/ml,3127.6±1550.9ng/ml).

Table 4 compares the Mean±SD of PT, PLC, and AT III by maternal age. The purpose of the table was to show whether maternal age affects PT, PLC, and AT III by maternal age. The maternal age was between 20 and 45 and was grouped into 5, as shown in the table below.

Based on maternal age, the mean ± standard deviation for PT in women of the 20-25 age group (13.27±0.66 s) was higher but not statistically significant ($p<0.05$) when compared with women of 26-30,31-35,36-40,41-45 age group (12.22±1.22s, 11.73±1.56s, 12.24±1.0 s and 12.5±1.56s, respectively). For PLC, the mean ± standard deviation of women of 31-35 age group ($195.5±35.61×10^9/L$) were higher but showed no statistical significance ($p<0.05$) when compared to women of 20-25,26-30,36-40,41-45 age group (163.57±63.71×109/L, 180.25±50.29×109/L, 127.6±34.80×109/L and 181.75±45.59×109/L, respectively). Similarly, the mean ± standard deviation for AT III in women of the 26-30 age group (3184.64±1218.81 ng/ml) were higher but showed no statistical significance($p<0.05$) when compared to women of 20-25,31-35,36-40,41-45 (2146.75±839.65 ng/ml, 3181.54±996.07ng/ml, 2773.88±1047.82 ng/ml and 2231.28±412.82 ng/ml, respectively).

Table I. Comparison of the Mean±SD of PT, PLC and AT III between pregnant and non pregnant women.

Haemostatic Parameters	Test (n= 40)	Control (n= 32)	p-value
Prothrombin Time (secs)	12.34 ± 1.27	13.84 ± 0.68	0.000067*
Platelet count(×10^9/L)	173.95 ± 50.22	258.00 ± 35.77	0.000019*
Antithrombin III (ng/ml)	2855.71 ± 1086.60	2497.68 ± 851.97	0.1316

Key : * denotes significance

Table 2: Comparison of the Mean±SD of PT, PLC and AT III for the pregnant women by gestational age using ANOVA & Tukey HSD posthoc

Haemostatic Parameters	1stTrimester	2ndTrimester	3rdTrimester	p-value
Prothrombin Time (secs)	12.92 ± 1.11 a,c	12.94 ± 0.84 a,c	11.44 ± 1.17 c	0.000463*
Platelet count(×10^9/L)	167.73 ± 52.06a	167.85 ± 48.29a	183.19 ± 52.28a	0.649
Antithrombin III(ng/ml)	1836.29±386.47c	2590.59±739.78b	3772.01 ±902.11a	0.000025*

Key : * denotes significance

Table 3: Comparison of the Mean±SD of PT, PLC and AT III for pregnant women by parity

Parity	Prothrombin time (secs)	Platelet count (x10^9/L)	Antithrombin III(ng/ml)
1	11.94 ± 1.32	165.44 ± 48.98	2711 ±967.39
2	12.85 ± 0.83	179.67 ± 54.92	2575.55±816.8
3	12.53 ± 1.11	166.8 ± 52.15	3201 ±1266.85
4	12.23 ± 1.92	205.2 ± 41.58	3127.6±1550.9
p-value	0.3525	0.452	0.5467

Table 4: Comparison of the Mean±SD of PT, PLC and AT III by maternal age.

Age Group	Prothrombin time (secs)	Platelet count (x10^9/L)	Antithrombin III(ng/ml)
20 - 25	13.27 ± 0.66	163.57 ± 63.71	2146.75 ± 839.65
26 - 30	12.22 ± 1.22	180.25 ± 50.29	3184.64 ± 1218.81
31 - 35	11.73 ± 1.56	195.5 ± 35.61	3181.54 ± 996.07
36 - 40	12.24 ± 1.0	127.6 ± 34.80	2773.88 ± 1047.13
41 - 45	12.5 ± 1.56	181.75 ± 45.59	2231.28 ± 412.82
p-value	0.212	0.17	0.16

Figure 1 (a, b, c). The Correlation scatter plot on the association between PT, PLC and AT III of the pregnant subjects. The figures assessed if any relationship existed between PT, PLC and AT III levels.

In the study (Fig 1A), AT III did not show any correlation with PT (r = -0.3, p = 0.06). Similarly (Fig 1b) no correlation existed between AT III and PLC (r = 0.3, p = 0.058). Again (Fig 1c) PLC showed no correlation with PT (r = -0.07, p = 0.65).

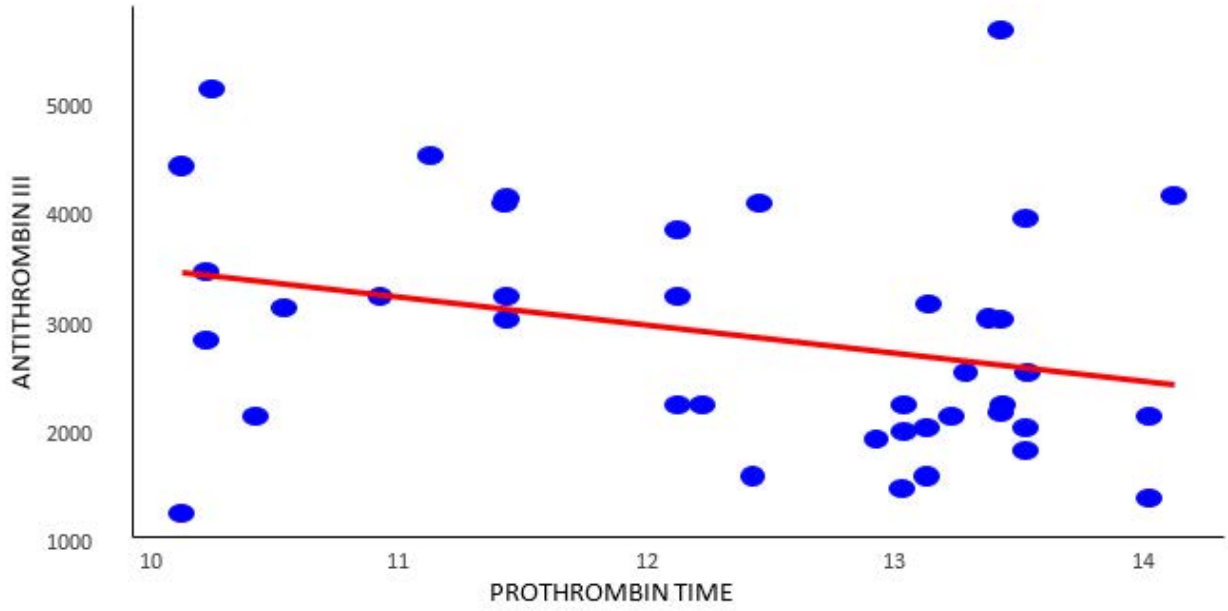

Fig 1a No correlation existed between AT III and PT (r = 0.3, p = 0.058)

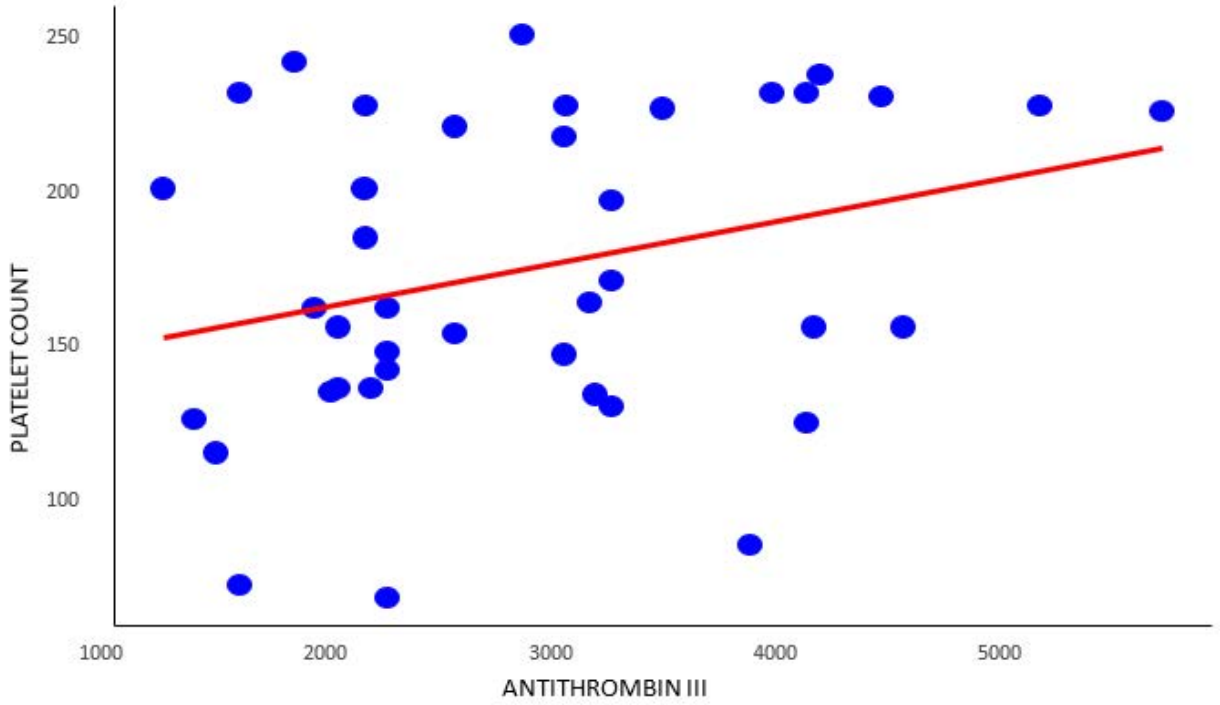

Fig 1b No correlation existed between AT III and PLC (r = 0.3, p = 0.058).

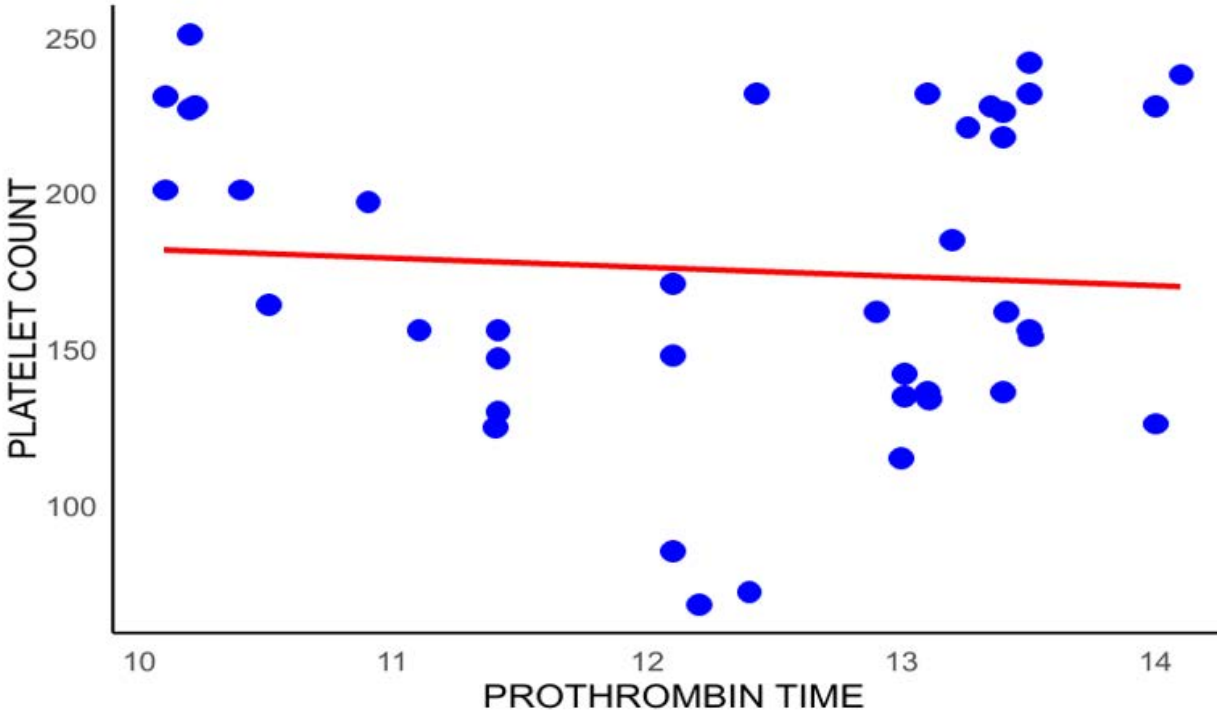

Fig 1c No correlation existed between PLC and PT (r = -0.07, p = 0.65).

DISCUSSION

Pregnancy induces changes in the haemostatic system. The changes are crucial for the tight and regulated balance in preventing excessive bleeding during delivery and minimizing the risk of thrombosis. Findings of this study showed that PT and PLC were lower during pregnancy than the non-pregnant conditions. The prothrombin time were found to be shortened in the pregnant than in the non-pregnant group. Studies by (9) and (10) found similar results. Various researchers posited different result of PT from the finding of this work which could be attributed to the various thromboplastin reagents having different sensitivities as well as concentration of the citrate anticoagulation and different assay methods (11). The PT was shortened as a result of the interference of some hormones such as oestrogen and progesterone that are required for the sustenance of pregnancy increases several folds and that oestrogen in particular stimulates hepatocyte for the synthesis and increased levels of some of coagulation factors thus, shortening the PT(s) in pregnant women (11). The study also revealed that the pregnant had lower platelet counts than the non-pregnant group. This finding is consistent with (13), (4) and (14). On the contrary, the finding differs from (11) and (15) that showed no statistical relevance in the Platelet count. The difference in platelet count in this study which did not agree with (11) and (15) could be attributed to the difference in the sampling procedures. Whereas used the manual method, (11, 15) used automated Haematology analyser. Platelet count could be decreased in pregnancy as a result of multiple factors, including hormonal changes, hemodilution, increased platelet turnover, and splenic sequestration (5). As for AT III, the finding of this study showed that AT III was higher among pregnant women than the control group. This is consistent with the finding of the study by (16). The increase of AT III level is as a result of the increased stimulation of the liver to synthesize AT III which is to prevent the hypercoagulability state and the risk of thrombosis in pregnancy.

As regards gestational age, the finding of this study revealed that there were no statistical differences in the platelet count within the three trimesters. This finding was consistent with the finding of (11). For PT, the finding of this study showed that PT was shortened. This was in accordance with the findings of (9,11,17). The decrease in PT in each trimester was due to increased procoagulant factors throughout the age of the pregnancy. On AT III, the finding of this study showed that AT III levels increased progressively with gestational age. This finding did not agree with the finding of (18) that reported no significant change in AT III across the three trimesters of gestation. Again, it differs with the finding of (16) who reported no significant alteration in AT III level. Moreso, this finding was not in accordance with the finding of (19) that posited a decrease in AT III in the trimesters. The difference in AT III reported in this study which did not agree with the findings of the researchers mentioned above could be as a result of the differences in sample size and study design. Whereas, this study used a sample size of 40, that of (18) was 150. On the other hand, this study adopted a cross-sectional study while (16) adopted a longitudinal study design. Pregnancy induces a hypercoagulable state which may be a risk factor to exacerbate adverse pregnancy conditions. On the other hand, increased synthesis of AT III by the liver is expected to regulate and mitigate any upregulated pregnancy induced hypercoagulable state.

With respect to parity and age, the data did not find any consistency and statistical relevance with parity and maternal age. The finding was in agreement with the finding of (11). It suggests that parity and maternal age are not conditions that provides any alteration in PT, PLC and AT III of the study subjects.

On the association between PT, PLC and AT

III Fig. (A, B, C), the study did not find any relationship among the three (3) parameters that were assessed. It suggests that pregnancy induces procoagulant factors as exemplified with the decrease in the PT which in turn favours the foetal implantation in the maternal subject. On the other hand, PLC was decreased and alongside the PT which was suggestive of the fact that the decreased PLC to a large extent probably with reduced platelet activation may dampen coagulation and its associated risks for an exacerbated vascular occlusion. Moreso, AT III was increased. The increase in AT III levels was to provide the haemostatic activity to regulate and mitigate, excessive procoagulant mechanisms. AT III serves as a major naturally occurring inhibitor of coagulation. It inhibits many serine protease enzymes such as thrombin and factor Xa, IXa, XIa and XIIa, by forming inactive complexes for each of the activated coagulation factors thus, regulating coagulation. The action of AT III is enhanced to about 1000folds on binding to heparin.

CONCLUSION

Pregnancy is considered a condition for maternofoetal adaptation during which the haemostatic system could be altered. The procoagulant activities were increased resulting in reduced PT. The PLT was decreased as a result of interplay of factors to prevent dysregulation of the haemostatic process. The AT III was increased to checkmate the possible procoagulant activities. The hallmark of these developments were to prevent the risks of hypercoagulable state thus ensuring normal pregnancy and successful delivery.

Abbreviations:

AT III…… Antithrombin III

BMI ..... Body Mass Index

EDTA…… Ethylenediamine Tetraacetic Acid

HSD ..... Honest Significant Difference

PLT ..... Platelet

PLT count ..... Platelet Count

PT ..... Prothrombin Time

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