

Protein C and Antithrombin III Activities in Healthy Nigerian Women

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ABSTRACT

AIM: In order to ensure that thrombin generation is limited and localised to prevent thrombosis after minor injury, coagulation inhibitors play a vital role. The study assessed protein C and antithrombin III levels in pregnant women in Kano, North-Western Nigeria.

MATERIALS AND METHODS: A total of two-hundred and fifty apparently healthy subjects (150 pregnant and 100 non-pregnant women), aged 17-40 years were recruited for the study in Aminu Kano Teaching Hospital, Kano between August 2010 and October 2011. Blood samples collected were analysed for Protein C and antithrombin III activities using standard laboratory procedures.

RESULTS: There was significantly lower value of proteins C activity in pregnant women compared to non-pregnant women ($P < 0.05$) while the values of antithrombin III in pregnant and non-pregnant women showed no statistically significant difference ($P > 0.05$). Different and fluctuated values of Protein C activities and antithrombin III activities with regard to first, second and third trimesters respectively, showed no significant differences ($P > 0.05$). Maternal age and parity had no significant influences on protein C and antithrombin III activities.

CONCLUSION: Pregnancy is associated with significantly lower

value of protein C activity while antithrombin level remained stable and unchanged. Gestational age, maternal age and parity showed no influences in protein C and antithrombin III activities. It is recommended that protein C activity be assessed in pregnant women to avoid thromboembolism.

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Key words: Protein C; Antithrombin III; Activities; Healthy Nigerian women

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INTRODUCTION

In order to ensure that thrombin generation is limited and localised to prevent thrombosis after minor injury, coagulation inhibitors play a vital role^[1,2]. The natural coagulation inhibitors can be of endothelial or hepatic group, based on their synthetic sites^[1].

Antithrombin III, heparin, heparin co-factor II, antitrypsin and tissue-factor pathway inhibitor (TFPI) are inhibitors of serine proteases of the coagulation cascade such as thrombin, Xa and TF-VIIa^[2]. Antithrombin III accounts for about 60% of plasma anticoagulant activities and it plays its inhibitory role by not only complexing with thrombin but also by inhibiting the clotting factors XIIa, XIa, IXa and Xa in the presence of heparin sulphate on the endothelial cell surface^[2-5].

Protein C, TM (thrombomodulin), protein S, C4b binding protein and APC (activated protein C) inhibitor are all components of the protein C system^[2]. Activation of this system occurs when thrombomodulin binds to thrombin to form activated protein C (APC) which rapidly degrades factors VIIIa and Va on the phospholipid surface of activated platelets and prevents the appropriate tenase and prothrombinase complexes, a reaction that increases 10-20 folds

when protein C combines with its co-factor protein S^[6-9].

Antithrombin concentration varies with race, age, sex and methodology used^[10,11]. Conflicting reports have been made on the levels of antithrombin III in pregnancy as reduced activity was documented by earlier author^[12], increased activity was reported by During *et al*^[13] while majority of the studies reported no significant changes^[14-16]. Earlier researcher documented reduced protein C activity during pregnancy^[17] while other studies showed stable and unchanged levels of protein C during pregnancy^[18-20].

The study was to determine protein C and antithrombin III levels in pregnancy as extremely scanty information on these naturally occurring anticoagulants is available in Nigeria.

MATERIALS AND METHODS

A total of two-hundred and fifty apparently healthy subjects (150 pregnant and non-pregnant women), aged 17-40 years were recruited for this study in Aminu Kano Teaching Hospital (AKTH), Kano between August 2010 and October 2011. Ethical approval and consent were obtained from the ethical committee of AKTH, Kano and the subjects respectively. Pregnant and non-pregnant women with histories of recurrent miscarriages, liver disease, renal disease, diabetes and hypertension and non-pregnant women who are on oral contraceptives were excluded from the study.

4.5 mL of venous blood sample was collected from each subject and mixed with 0.5 mL of 32.0 g/L trisodium citrate solution in the plain container. Blood samples in the citrated containers were centrifuged at 2,500 g for 15 minutes and the plasma separated into plastic containers for the determination of protein C and antithrombin III activities.

Protein C and antithrombin III activities were assayed according to the instructions of TEChron kits with reference numbers C1100-012 and C1000-010 respectively, manufactured in Germany and the clotting times determined using Cormay KG coagulometer manufactured in Poland.

Statistical analysis

The mean values and standard deviations of the parameters in the pregnant and non-pregnant women were assessed using student's *t*-test while the differences with regard to gestation period, maternal age and parity were assessed using one-way analysis of variance (ANOVA). *P*-value of ≤ 0.05 was considered significant.

RESULTS

Table 1 shows protein C and antithrombin III levels in pregnant and non-pregnant women. Pregnant women showed significantly lower value of protein C activity (55.4±29.0%) compared to non-pregnant women (86.0±32.7%) (*P*<0.05) while the values of antithrombin III in pregnant and non-pregnant women showed no statistically significant difference (*P*>0.05).

Changes in protein C and antithrombin III activities with gestation period (trimester) are summarised in table 2. Different and fluctuated values of protein C activities (52.9±28.4%, 60.0±31.4% and 51.3±26.1%) and antithrombin III activities (85.58±14.88%, 79.64±19.73% and 85.34±12.01%) with regard to first, second and third trimesters respectively, showed no statistically significant differences (*P*>0.05).

Table 3 indicates the effects of maternal age on protein C and antithrombin III activities. Different values of protein C activities (61.4±35.0%, 53.7±30.0%, 60.3±26.9% and 45.2±23.1%) and antithrombin III activities (78.34±22.96%, 82.7±15.26%, 85.37±13.47% and 74.81±18.14%) with regard to age-groups 17-22 years, 23-28 years, 29-34 years and 35-40 years respectively, showed no statistically significant differences (*P*>0.05).

85.37±13.47% and 74.81±18.14%) with regard to age-groups 17-22 years, 23-28 years, 29-34 years and 35-40 years respectively, showed no statistically significant differences (*P*>0.05).

Table 4 shows parity influences on protein C and antithrombin III activities in pregnant women. The levels of protein C (55.9±26.2%, 57.2±36.1% and 54.3±23.9%) and antithrombin III (78.07±20.94%, 79.42±17.36% and 84.98±13.17%) with regard to parity of 0, 1-2 and ≥ 3 respectively, showed no statistically significant differences (*P*>0.05).

Table 1 Protein C and antithrombin III levels in pregnant and non-pregnant women.

Parameter	Non-pregnant women (controls)	Pregnant women	<i>P</i> -value
Number of subjects	100	150	
Protein C activity (%)	86.0 ± 32.7	55.4 ± 29.0	<0.05
Antithrombin III activity (%)	78.8 ± 15.79	82.49 ± 16.67	>0.05

Table 2 Changes in protein C and antithrombin III activities with gestation period (trimester).

Parameter	First trimester	Second trimester	Third trimester	<i>P</i> -value
Number of subjects	24	66	60	
Protein C activity (%)	52.9 ± 28.4	60.0 ± 31.4	51.3 ± 26.1	<0.05
Antithrombin III activity (%)	85.58 ± 14.88	79.64 ± 19.73	85.34 ± 12.01	>0.05

Table 3 Effects of maternal age on protein C and antithrombin III activities.

Parameter	17-22 years	23-28 years	29-34 years	35-40 years	<i>P</i> -value
Number of subjects	24	67	48	11	
Protein C activity (%)	61.4±35.0	53.7±30.0	60.3±26.9	45.2±23.1	>0.05
Antithrombin III activity (%)	78.34±22.96	82.7±15.26	85.37±13.47	74.81±18.14	>0.05

Table 4 Parity influences on protein C and antithrombin III levels in pregnant women.

Parameter	0 Parity	1-2 Parity	≥ 3 Parity	<i>P</i> -value
Number of subjects	42	51	57	
Protein C activity (%)	55.9 ± 26.2	57.2 ± 36.1	54.3 ± 23.9	>0.05
Antithrombin III activity (%)	78.07 ± 20.94	79.42 ± 17.36	84.98 ± 13.17	>0.05

DISCUSSIONS

Deficiencies of naturally occurring anticoagulant proteins such as antithrombin III, protein C and protein S produce favourable medium for thrombus generation which has been linked to thromboembolic disease^[21,22].

This study has shown significantly lower value of protein C activity in pregnant women compared to the control subjects. This is in line with the previous authors^[17,23,24] but disagrees with other researchers who reported no significant change^[6,25]. Inconsistent reports of protein C levels during pregnancy by various authors might be associated with different sensitivities and specificities of protein C reagents

used, and assay techniques employed. The study further showed the stability of protein C levels within the gestation period which is consistent with other studies^[6,26,27], however, protein C deficiency has been associated with venous thromboembolism^[28,29]. Maternal age and parity had no significant effects on protein C activity within the period of pregnancy but showed significance when compared to non-pregnant status. However, maternal age of 35-40 years showed the least protein C level which could make an advanced maternal age a risk factor to thrombosis.

This study has further demonstrated that antithrombin III level showed no significant difference in pregnant women. This finding agrees with the majority of the earlier studies^[6,13,15] but disagrees with the significantly lower value reported by Essien^[12] probably due to the variation of sensitivities and specificities of antithrombin III reagents used. This study has further confirmed the earlier studies that gestational age had no effect on antithrombin III activity^[6,27]. Maternal age and parity showed no influences on antithrombin III level during pregnancy in this study. However, variation in antithrombin III levels in pregnancy as reported by various authors^[6,12,13] could be associated with methodology employed, poor storage of citrated plasma and reagents and sample size.

In conclusion, pregnancy is associated with significantly lower value of protein C activity while antithrombin III level showed no significant difference. Gestational age, maternal age and parity showed no influences on protein C and antithrombin III activities. It is recommended that protein C activity be assessed amongst other routine tests for pregnant women since its deficiency has been linked to thromboembolism.

CONFLICT OF INTERESTS

The author has no conflicts of interest to declare.

REFERENCES

- Walker CP, Royston D. Thrombin generation and its inhibition: a review of the scientific basis and mechanism of action of anticoagulant therapies. *Br J Anaesth* 2002; **88**(6): 848-863
- Holmes VA, Wallace JMW. Haemostasis in normal pregnancy: a balancing act? *Biochemical Society Transactions* 2005; **33**(2): 428-432
- Marcum JA, Rosenburg RD. Anticoagulant active heparin-like molecules from vascular tissue. *Biochemistry* 1984; **23**(8): 1730-1737
- Sheffield WP, Wu YI, Blajchman MA. Antithrombin structure and function. In: Molecular Basis of Thrombosis and Haemostasis. High KA and Roberts HR (eds). New York: Marcel Dekker Inc, 1995: 355-377
- Isobel DW. Inherited thrombophilia. In: Postgraduate Haematology, 5th Edition. Hoffbrand AV, Catovsky D, Tuddenham EGD (eds). London: Blackwell publications, 2001: 885-886
- Gatti L, Tenconi PM, Guaneri D, Bertulesi C, Ossola MW, Bosco P, Gianotti GA. Haemostatic parameters and platelet activation by flow cytometry in normal pregnancy: Research a longitudinal study. *International Journal of Clinical and Laboratory* 1994; **24**: 217-219
- Clark P, Brennard J, Conkie JA, McCall F, Greer IA, Walker ID. Activated protein C sensitivity, Protein C, protein S and coagulation in normal pregnancy. *Thrombosis and Haemostasis* 1998; **79**: 1166-1170
- Kjellberg U, Andersson NE, Rosen S, Tengborn L and Hellgren M. APC resistance and other haemostatic variables during pregnancy and puerperium. *Thrombosis and Haemostasis* 1999; **81**: 527-531
- Wickstrom K, Edelstam G, Lowbeer CH, Hansson LO, Siegbahn A. Reference intervals for plasma levels of fibronectin, von-Willebrand factor, free protein S and antithrombin during third trimester pregnancy. *Scand J Clin Lab Invest* 2004; **64**(1): 31-40
- Al-Mugeiren MM, Abdel-Gader AG, Al-Meshari AA, Al-Rasheed SA, Al-Jurayyan NA, Al-Hawasy MN. Normal levels of the natural anticoagulants (protein C and S and antithrombin III) and the fibrinolytic factors (tPA and PAI) in Arab children. *Ann Saudi Med* 1996; **16**(5): 501-504
- Mateo J, Oliver A, Borrell M, Sala N, Fortcuberta J. Laboratory evaluation and clinical characteristics of 2,132 consecutive unselected patients with venous thromboembolism: results of the Spanish Multicentric study on Thrombophilia (EMET study). *Thromb Haemost* 1997; **77**(3): 444-451.
- Essien EM, Changes in antithrombin III levels in pregnancy, labour and in women on the contraceptive pill. *Afr J Med Sci* 1977; **6**(3): 109-113.
- During R, Schwarzlos G. Antithrombin III level in normal pregnancy and patients with pregnancy-induced hypertension. *Zentralbl Gynakol* 1990; **112**(6): 351-354.
- Wallach J. Interpretation of Diagnostic Tests. 8th ed. Philadelphia PA: Lippincott Williams and Wilkins, 2007: 946-947
- Ghanavatti-Abbassi M, Greer LG, Cunningham FG. Pregnancy and Laboratory studies: a reference table for clinicians. *Obstet Gynecol* 2009; **114**(6):1326-1331
- Sarkar PD, Sogani S. Association of antithrombin III And platelet count with pregnancy induced-hypertension. *International Journal of Reproduction, contraception, Obstetrics and Gynaecology* 2013; **2**(3): 398-401
- Oruc S, Saruc M, Koyuncu FM, Ozdemir E. Changes in the plasma activities of protein C and S during pregnancy. *Aust N Z J Obstet Gynaecol* 2000; **40**(4): 448-450
- Lao TT, Yuen PMP, Yin IA. Protein S and C levels in Chinese women during pregnancy, delivery and the puerperium. *BJOG: An International Journal Obstetrics and Gynaecology* 1989; **96**: 167-170
- Warmick R, Hutton RA, Goff L, Letsky E, Heard M. Changes in protein C and free protein S during pregnancy and following hysterectomy. *J R Soc Med* 1989; **82**(10): 591-594
- Faught W, Garner P, Jones G, Ivey B. Changes in protein C and protein S levels in normal pregnancy. *Am J Obstet Gynecol* 1995; **172**: 147-150
- High KA. Antithrombin III, protein C and protein S. Naturally occurring anticoagulant proteins. *Arch Pathol Lab Med* 1988; **112**(1): 28-36.
- Egesel T, Buyukasik Y, Dundar SV, Gurgey A, Kirazli S, Bayraktar Y. The role of natural deficiencies and factor V leiden in the development of idiopathic portal vein thrombosis. *J Chin Gastroenterol* 2000; **30**(1): 66-71.
- Cerneca F, Ricci G, Simeon R, Malisamo M, Alberico S and Guaschono S. Coagulation and fibrinolysis changes in normal pregnancy. Pregnancy induce a hypercoagulable state combined with a reactive fibrinolysis. *European Journal of Obstetrics and Gynaecology Reproductive Biology* 1997; **73**: 31-36
- Uchikova EH, Ledjev II. Changes in haemostasis during normal pregnancy. *European Journal of Obstetrics and Gynaecology and Reproductive Biology* 2005; **119**: 185-188
- Szecs PB, Lorgensen M, Klainbard A, Andersen MR, Colov NP, Stender S. Haemostatic reference intervals in pregnancy. *Thrombosis and Haemostasis* 2010; **103**: 718-727
- Gilbert J, Fernandez JA, Espana F, Aznar J, Estelles A. Physiological coagulation inhibitors (protein S, protein C and antithrombin III) in severe preeclamptic states and users of oral contraceptives. *Thromb Res* 1988; **49**: 319-329

- 27 Prisco D, Ciuti G, Falciani M. Haemostatic changes in normal pregnancy. *Haematologica reports* 2005; **1(10)**: 1-5
- 28 Gladson CL, Scharrer I, Hach V, Beck KH, Griffin JH. The frequency of type I heterozygous protein S and protein C deficiencies in 141 unrelated young patients with venous thrombosis. *Thrombosis and Haemostasis* 1988; **59**: 18-22

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