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Effect of Hypercoagulability on Pregnancy Outcome

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Abstract

Pregnancy is a prothrombotic state, which is developed as a result of multifactorial reasons. Physiological changes that induce relative hypercoagulable state and physical changes leading to increased stasis. We aimed to assess possible thrombophilic parameters in women with a history of adverse pregnancy outcome. This cross-sectional study included 90 pregnant women during the second trimester of pregnancy divided into two groups. The Case group (n=45) had a history of intrauterine growth restriction (IUGR), intrauterine foetal death (IUFD), preterm labour or miscarriage. The Control group (n=45) included women with no history of adverse pregnancy outcome. The level of prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FBG), protein C (PC), protein S (PS), Antithrombin III (AT III), Lupus anticoagulant (LA), Anticardiolipin (IgM and IgG), Haemoglobin (Hb) and platelet (Plts) counts were measured. Patients with a history of bad pregnancy outcome were shown to have significantly higher levels of FBG, LA and anticardiolipin antibodies (IgM and IgG) and significantly lower levels of PS, PC and AT III compared to those with normal pregnancy outcome. There was no significant difference in PT and aPTT levels between the two groups. Patients with a history of adverse pregnancy outcome are at increased risk of hypercoagulable state.

Keywords: *Pregnancy, Miscarriage, Intrauterine fetal death, Intrauterine growth restriction, Hypercoagulability.*

Introduction

The process of haemostasis is complex and is further complicated during pregnancy due to the physiological changes (O'Riordan, *et al*., 2003). Pregnancy alters the haemostatic system into a hypercoagulable state and leads to thrombotic complications such as increased factors VII, VIII, X and von Willebrand factor activity in addition to the marked increase in fibrinogen (Greer, 1999).

Hypercoagulable state risks are due to increased thrombin generation markers such as prothrombin and decreased anticoagulant activities such as protein S and acquired activated protein C resistance (Anderson, *et al*., 2010, McLean, *et al*., 2012). Regulation of fibrinolysis occurs by plasminogen activator inhibitors (PAI-1). Fibrinolytic activity is decreased, while PAI-1 is increased by fivefold especially during the third trimester. These haemostatic system changes act as a physiological safety net for the postpartum period. However, these changes can predispose to early complications as recurrent pregnancy loss and late complications as placental vascular mediated problems as intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), placental abruption and pre-eclampsia (Simcox, *et al*., 2015).

The current study aimed to assess thrombophilic parameters in women who had a previous history of adverse pregnancy outcome.

Subjects and Methods

This study was performed in the Prenatal Diagnosis and Recurrent Pregnancy Loss Clinic, Prenatal Diagnosis and Fetal Medicine Department of the National Research Centre, Egypt and Faculty of Science, Fayoum University, Egypt from February 2015 to March 2018. The study involved two groups of pregnant women. The Case group consisted of 45 patients with a history of bad pregnancy outcome including preterm labour, IUGR, IUFD, and miscarriage. The Control group included 45 pregnant women with no history of obstetrical complications. All pregnant women were aware of the type, methodology and the value of the study and signed informed consents. The maternal age of the two groups ranged between 18 and 40 years. Pregnant women during the third trimester and those taking anticoagulant medications were excluded from the study.

Plasma levels of prothrombin time (PT), activated partial thromboplastin time (aPTT) and lupus anticoagulant (LA) were assayed immediately after collection of samples of blood on

sodium citrate anticoagulant. Prothrombin time and aPTT were assayed according to manufacturer instructions (Biomed Diagnostic, Hannover, Germany).

Plasma and serum were analysed immediately or stored at -20°C for determination of protein C (PC) (Reads, Corgenix, Broomfield, Colorado 80020, USA), protein S (PS) (Reads, Corgenix, Broomfield, Colorado 80020, USA), Antithrombin (AT) (R&D Systems, Inc. USA and Canada), fibrinogen (FBG) (Hyphen Biomed, France) and anticardiolipin (IgG and IgM) (ORGenTec, Mainz, Germany). FBG, PC, PS, AT and anticardiolipin were determined by Enzyme-Linked Immunosorbent Assay (ELISA) according to manufacturer instructions.

Statistical Methods

IBM© SPSS© Statistics version 22 was used for Statistical analysis (IBM© Corp., Armonk, NY, USA). Mean and standard deviation (SD) or median and range represent numerical data. Frequency and percentage represent Qualitative data. The relationship between qualitative variables was done by Chi-square test (Fisher's exact test). Mann-Whitney test was used for comparison between two groups (quantitative data. All tests were two-tailed. p-value less than 0.05 was considered significant.

Results

There was no significant difference between the two studied groups in maternal age and gestational age (Table 1).

Table 1: Maternal and gestational age of case and control groups

	Case group n=45	**Control Group n=45**	**p value**
Maternal Age (years)	28.3±6.0	30.3±6.1	0.213
Gestational Age (weeks)	18.7±4.0	17.9±3.8	0.301

Data are expressed as mean ± SD

Reasons for referral of the Case group to the National Research Centre (NRC) are shown in table 2. These include miscarriage (n=24), miscarriage + IUFD (n=13), IUFD (n=3), miscarriage + IUFD + IUGR (n=2) and one case of IUGR + IUFD, another case of miscarriage + preterm labour, and one case of IUGR + preterm labour.

Table 3 shows the levels of procoagulants in the two groups during the second trimester of gestation. There was no significant difference in the levels of PT, INR, aPTT between the two groups. As shown in figure 1, the fibrinogen level was significantly higher in the Case group ($p < 0.001$). A higher proportion of the Case group had hyperfibrinogenemia ($p < 0.001$).

Table 2: Detailed history of adverse pregnancy outcome in the Case group

Pregnancy outcome	**Times**				**Number**	**%**
	One	**Two**	**Three**	**Four or more**		
Miscarriage	4	7	6	7	24	53.0
Miscarriage + IUFD	7	3	1	2	13	29.0
IUFD	2	0	0	1	3	7.0
Miscarriage + IUFD + IUGR	2	0	0	0	2	4.4
IUFD + IUGR	1	0	0	0	1	2.2
Miscarriage + Preterm labour	0	1	0	0	1	2.2
IUGR + Preterm labour	0	1	0	0	1	2.2
Total					45	100

Table 3: Levels of procoagulants (PT, INR, aPTT and FBG) in Case and Control groups

	Case group n=45	**Control group n=45**	**p value**
Prothrombin time (sec.)	12.9±0.5	12.6±0.8	0.086
International normalized ratio (INR)	0.99±0.05	0.97±0.06	0.068
aPTT (sec.)	31.1±4.6	31.2±3.3	0.514
Fibrinogen (mg/dL)	536.8±84.5	279.8±79.4	< 0.001
Hyperfibrinogenemia (%)	42 (93.3%)	6 (13.3%)	< 0.001

Data are represented by mean ± SD or frequency and percentage,

aPTT: activated partial thromboplastin time

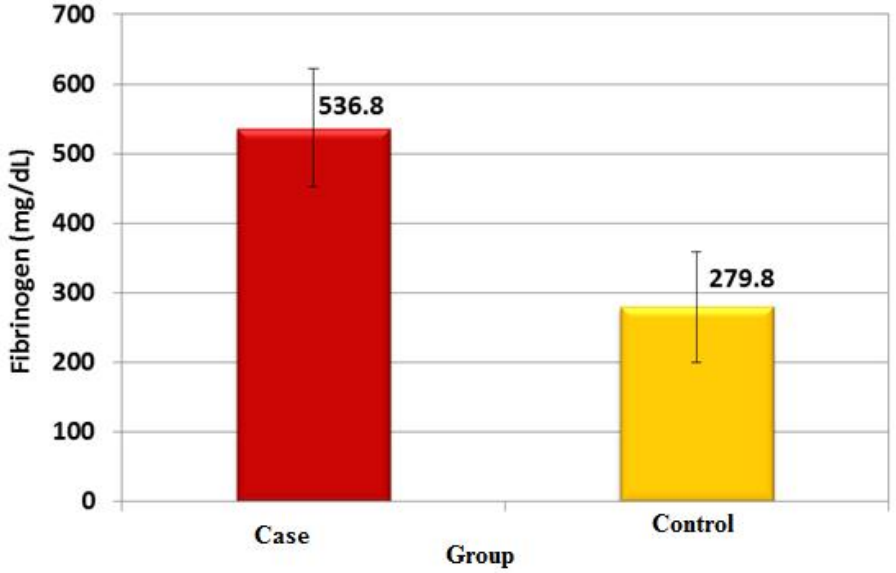

Figure 1: Fibrinogen levels for case and control groups during the second trimester of gestation

Table 4 and figures 2 to 4 shows the levels of AT III, PC and PS. The values of AT III, PC, and PS were significantly lower in the Case group ($p < 0.001$, for all).

Table 4: Concentrations of anticoagulants (AT III, PC, and PS) and the frequency of their deficiency in the two studied groups.

	Case group n=45	**Control Group n=45**	**p value**
Antithrombin III Activity (%)	65.7±21.9	95.1±30.5	< 0.001
Antithrombin III Deficiency (%)	34 (75.6%)	16 (35.6%)	< 0.001
Protein C level (%)	50 (8-130)	80 (46-190)	< 0.001
Protein C Deficiency (%)	34 (75.6%)	11 (24.4%)	< 0.001
Protein S level (%)	70 (10-200)	94 (60-230)	< 0.001
Protein S Deficiency (%)	16 (35.6%)	0 (0.0%)	< 0.001

Data are represented by mean ± SD, median (range) or frequency and percentage

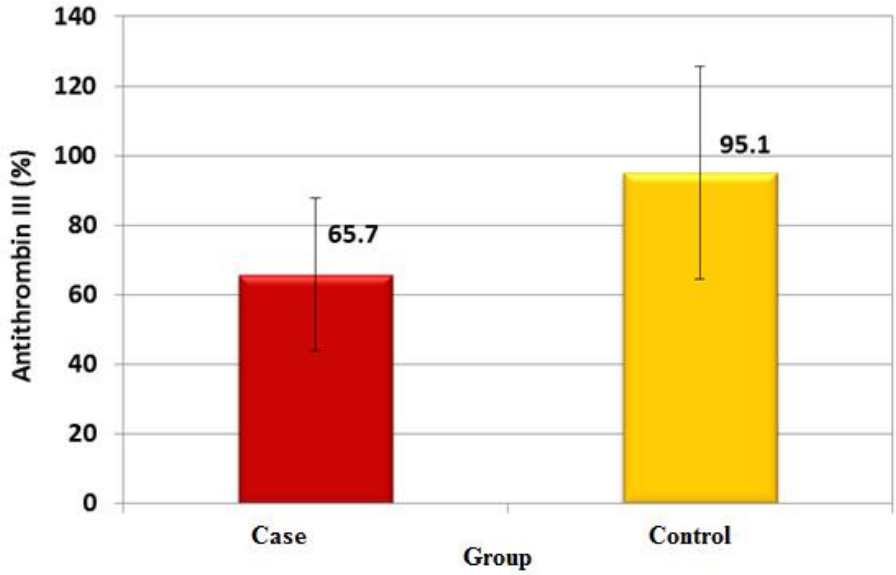

Figure 2: Antithrombin III activity in the case and control groups during the second trimester of gestation

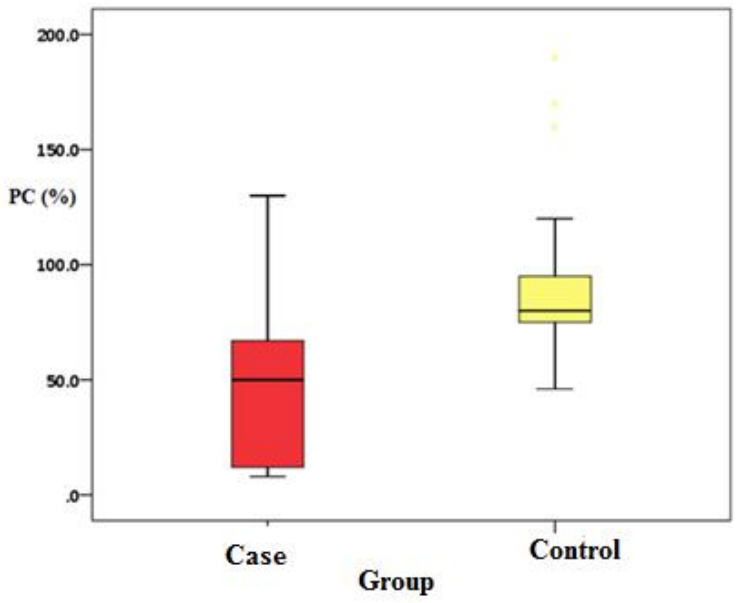

Figure 3: Protein C levels in the case and control groups during the second trimester of pregnancy

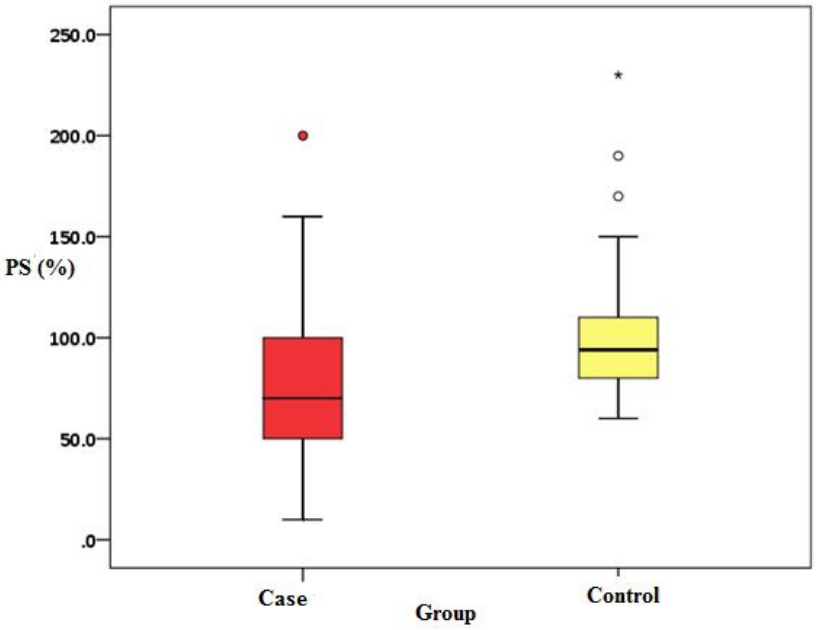

Figure 4: Protein S levels in the case and control groups during the second trimester of pregnancy

Haemoglobin concentration and platelet count were significantly lower in the Case group. Lupus anticoagulant and anticardiolipin (IgG and IgM) levels were significantly higher in the Case group (Table 5).

Table 5: Haemoglobin concentration, platelet count, lupus anticoagulant and anticardiolipin in the two studied groups

	Case group n=45	**Control Group n=45**	**p value**
Haemoglobin (g/dL)	10.6±0.4	12.1±0.7	< 0.001
Platelet count (x1000/mm^3)	183.2±32.5	257.6±50.8	< 0.001
Lupus anticoagulant (Sec.)	35.3±6.8	30.6±3.6	< 0.001
aCL			
IgM (MPL-U/ml)	7.9±1.3	4.8±3.1	< 0.001
IgG (GPL-U/ml)	5.6±1.5	3.3±1.9	< 0.001

Data are represented by mean ± SD, aCl : anticardiolipin.

Discussion

The physiological changes during pregnancy lead to the hypercoagulable state, which aid the placenta to perform its function well and reduce blood loss at delivery. The risk of thrombotic complications increases three to five times during pregnancy. The hypercoagulable state of pregnancy leads to pregnancy complications and adverse pregnancy outcome such as venous thromboembolism, pulmonary embolism, preeclampsia, miscarriage, IUFD, IUGR and placental abruption (Chunilal, *et al*., 2009, Vucić, *et al*., 2009).

The results of the current study demonstrated a hypercoagulable status in showed patients with history of bad pregnancy outcome compared to those with normal pregnancy. They showed significantly higher level of fibrinogen and significantly lower levels of PS, PC and AT III in patients with history of bad pregnancy outcome compared to those with normal pregnancy. Levels of anticardiolipin antibodies (IgG & IgM) were evaluated in 90 pregnant women including 45 pregnant women who had history of bad pregnancy outcome and 45 pregnant women as control, Significantly positive > 15 U/ml, Insignificantily positive 10-15 U/ml, Negative <10 U/ml (8)**.** Lupus anticoagulant and anticardiolipin antibodies (IgM and IgG) were significantly higher in the Case groups. On the other hand, there was no significant difference in PT and aPTT levels. Our results are consistent with the results of Preston *et al*., (1996) who showed that decreased levels of AT III, PC, and/or PS lead to the risk of bad pregnancy outcome such as stillbirths.

On the other hand, Keren-Politansky, 2014 showed that PT and aPTT were decreased in pregnant women with adverse pregnancy outcome, while there was no change in fibrinogen, and free protein S levels.

Another study reported decreased levels of PC, PS and AT III in association with obstetrical complications like IUGR, preeclampsia, placental abruption and late foetal loss (Keren-Politansky, *et al*., 2014). The mechanism of these complications was related to thrombosis of intervillous or spiral-artery leading to insufficient perfusion of the placenta.

Balasch *et al*., 1996 and Žigon *et al*., 2015 showed that pregnancy complications such as miscarriage, IUFD and/or intrauterine foetal death were associated with high level of PT, aCL and/or LA. Alfirevic *et al*., 2002 demonstrated that adverse pregnancy outcome such as foetal loss, intrauterine growth restriction and/or pre-eclampsia were associated with decreased level of PS and increased level of anticardiolipin IgG antibodies but were not associated with AT III and LA. These findings were consistent with the current results of decreased level of PS,

AT III. IgG aCL is more strongly associated with clinical events than is IgM aCL and the risk of thrombosis increases with higher titres > 40 U/ml (Silver, *et al*., 2013, Yelnik, *et al*., 2016). Poor trophoblast invasion and/or abnormal placental function are known to contribute to many pregnancy disorders including preeclampsia and intrauterine growth restriction. A number of factors are released into the maternal circulation due to cellular stress within the placenta. Some of these factors affect key physiological systems and contribute to disease development. These factors may be identified in physiological fluids and may serve as biomarkers of these pregnancy disorders (Cuffe, *et al*., 2017). Deficiency of AT, PC, and PS are defined as plasma levels below 75% for AT, below 69% for PC, and below 63% for PS (19).The presence of AT, PC, and PS deficiencies during the second trimester were found to be significantly connected to pregnancy complications such as recurrent pregnancy losses (Abraitis, *et al*., 2004, Mitic, *et al*., 2010).

Antiphospholipid antibodies syndrome (APS) is an acquired autoimmune disease of hypercoagulable state characterized by the existence of autoantibodies of different phospholipids or phospholipid-binding proteins directed against membrane anionic phospholipids or their associated plasma proteins. Lupus anticoagulant and anticardiolipin are antiphospholipid antibodies. These antibodies have procoagulant and anticoagulant effects, but the predominant effect is the procoagulant. The procoagulant effect leads to venous thrombosis, arterial thrombosis, and abortion. Lupus anticoagulants (LAs) do have an anticoagulant effect in vitro, LA syndrome manifests clinically with thrombotic rather than hemorrhagic complications. Antiphospholipid antibodies syndrome leads to localized thrombosis, weak perfusion of the placenta that lead to bad outcome of pregnancy such as RFL and uteroplacental insufficiency. Antiphospholipid antibodies affect the invasion of placental trophoblast layer and production of hormones (Levine, *et al*., 2002). Also, maternal anaemia leads to pregnancy complications such as preterm labour and low birth weight (Levy, *et al*., 2005).

In the current study, fibrinogen level and platelet count were consistent with the results of Huissoud *et al*., 2009 and Karlsson *et al*., 2014. They showed that fibrinogen level was increased significantly and platelet count decreased significantly during the second trimester which leads to strong clot formation. Hyperfibrinogenemia is the increased level of fibrinogen above 450 mg/dl and may contribute to the development of pathological thrombosis (Patel *et al*., 2016). The risk of thrombophilia during gestation was associated with pregnancy

complications such as miscarriage and complications due to defect of vascular system of placenta such as late foetal loss, pre-eclampsia, abruption of placenta and intra-uterine growth restriction (IUGR) (Simcox, 2015). Deficiency of any of the three natural anticoagulants was associated with an increased risk for venous thrombosis. Levels of PC and PS are lowered in conditions such as DIC, inflammatory states, acute thrombosis, and liver diseases.

Recommendations

Pregnant women that tested positive for hypercoagulability should undergo gestational follow-up, careful obstetric monitoring, proper delivery timing and should take optimal pharmacologic treatment especially anticoagulant medications to limit adverse pregnancy outcome.

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