Determination the Correlation between Estradiol Hormone and Some Clotting Factors in Pregnant Women

تحديد الارتباط بين هرمون الاستراديول وبعض عوامل التخثر لدى النساء الحوامل

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البحث مستل

Summary

The present study based essentially to estimate correlations of available clotting factors involved in extrinsic, intrinsic, and common pathway and estradiol hormones of pregnant women. Women were included, in this study of these, (60) women were pregnant and sub divided into three groups according to phases of pregnancy (20) women at first trimester, (20) women at second trimester, and 20 women at third trimester). Twenty five (25) women were also recruited in this study and used as control group (non-pregnant and non-lactating). All ages of women were ranged between 20–35 years old. Concerning estradiol hormone concentrations in pregnant women, it have been found that the levels of estradiol hormone were insignificantly increased ($p>0.05$) during first trimester and significantly elevated ($p<0.05$) throughout second and third trimester in a comparison with control group. Data obtained from the present study indicated a progressive elevation ($p<0.05$) in the levels of fibrinogen (FI) concentrations throughout pregnancy period, and its concentrations confirmed a significant positive correlation ($r = 0.825$) with estradiol concentrations. Levels of prothrombin (FII) activities in pregnant women were significantly higher ($p<0.05$) than of non-pregnant and these activities correlated positively ($r = 0.643$) with estradiol level. Results of factor FVII activities (major component of extrinsic pathway) were insignificantly elevated ($p>0.05$) during first and second trimesters and tend to be progressively increased ($p<0.05$) during third trimester and confirmed a positive correlation ($r = 0.523$) with estradiol levels.

Determination of major components of intrinsic pathway including FVIII and FIX. Their activities seen to be progressively increased ($p<0.05$) throughout periods of pregnancy and recorded a positive correlation ($r = 0.375$, $r = 0.538$, respectively) with levels of estradiol hormone. In conclusion, data obtained from this study and mentioned above may be due to increased efficiency of various clotting factors involved in different clotting pathway parallel with progress of gestation because of heightened levels of estradiol hormone, and these excessive activities tend to exert protective mechanism to prevent excessive blood loss during expulsion of placenta.

Key words: pregnancy, estradiol hormone, clotting factors.

الخلاصة

تضمنت الدراسة الحالية اختبار فعالية بعض عوامل تخثر الدم وارتباطها مع هرمون الاستراديول لدى النساء الحوامل. شملت الدراسة اختيار ستون (60) أمرأه من الحوامل وقد قسمت طبقا لأطوار الحمل الى ثلاثة مجاميع، المجموعة الأولى (20 أمرأه في الثلث الأول)، المجموعة الثانية (20 أمرأه في الثلث الثاني)، المجموعة الثالثة (20 أمرأه في الثلث الثالث).

شملت الدراسة ايضا اختيار 25 أمرأه غير حوامل وقد استخدمت كمجموعة سيطرة وتراوح معدل اعمار جميع النساء بين (20-35 سنة). بخصوص تركيز هرمون الاستراديول لدى النساء الحوامل، فقد بينت نتائج الدراسة الحالية حصول ارتفاع غير معنوي ($p>0.05$) في قيم هرمون الاستراديول خلال الثلث الاول وحصول ارتفاع معنوي ($p<0.05$) في قيم الاستراديول ايضا خلال الثلث الثاني والثالث عند المقارنة مع مجموعة السيطرة. بخصوص عوامل التخثر الموجودة في المسلك العام فقد بينت النتائج حصول ارتفاع معنوي ($p<0.05$) في قيم الفايبرينوجين (FI) خلال الطور الاول والثاني

والثالث من الحمل واعطت تراكيزه ارتباط ايجابي مع مستوى تراكيز هرمون الاستراديول (r = 0.825). اظهرت قيم فعالية عامل البروثرومبين (FII) ارتفاعاً معنوياً (p<0.05) خلال اطوار الحمل وارتبطت ايجابياً مع مستوى تراكيز هرمون الاستراديول (r = 0.643)خلال الحمل. وبخصوص فعالية العامل Stable factor (FVII) (العامل الاساسي في المسلك الخارجي) فقد أشّرت ارتفاعاً غير معنوي (p>0.05) خلال الثلث الاول من الحمل وارتفاعاً معنوياً (p<0.05) خلال الثلثين الثاني والثالث، واعطت ارتباطا ايجابياً(r = 0.523) مع مستوى تراكيز هرمون الاستراديول. وفيما يتعلق بعوامل المسلك الداخلي وبالخصوص FVIII و FIX فقد لوحظ ارتفاعاً معنوياً (p<0.05) في فعالية كل من FVIII و FIX على طول فترة الحمل وحصول ارتباط ايجابي بينهما وبين مستوى هرمون الاستراديول (r = 0.583 respectively, r = 0.375) عند مقارنتها مع مجموعة السيطرة.

يمكن ان نستنتج من الدراسة الحالية بأن هنالكَ حصول ارتفاع في فعالية اغلب عوامل التخثر خلال مراحل تقدم الحمل والتي تكون مرتبطة مع ارتفاع مستوى هرمون الاستراديول وهذا يؤدي الى حصول عملية وقاية لمنع فقدان الدم خلال مرحلة الولادة.

الكلمات المفتاحية : الحمل ، هرمون الاسترادايول، عوامل التخثر.

Introduction

Pregnancy results in multiple physiologic changes in different organ systems in the body mediated essentially by female sex hormones such as (Human chorionic gonadotropin, Luteinizing hormone, Follicular stimulating hormone, progesterone, and estrogen). Understanding of these normal changes is essential for providing quality care for pregnant women during pregnancy period [(1)]. Pregnancies have a complex hormonal alterations and interaction, which produce profound physiologic changes. Some of changes are more prominent than others. The changes that happen are a result of increasing maternal and fetal demands for the growth of the fetus and the stimulation of the mother for delivery. A rise in the production of female sex hormones, in particular, estrogen by 10-fold and progesterone by 30-fold, is important for the normal progression of pregnancy [(2)]. Haemostasis defined is the process by which bleeding is stopped and is also responsible for maintaining the stability of the blood vascular system. It has originated as the body's defense mechanism against ungoverned bleeding from locations of injuries. It includes the eventual formation of a blood clot that is confined to the open of injury. This is performed by an interaction of multiple mechanisms; vasoconstriction, platelet aggregation, coagulation and fibrinolysis system. Working together, these processes act to clot the blood will occur at the open where it is required, and stops it from extending beyond the location of damage or interruption of blood flow in the vascular system [(3)].

Subjects of study

The present study was carried out in AL-Hussein teaching hospital of Karbala province and collage of science for women in Babylon University. The present study was started at a period ranged between December 2013 to August 2014. Sixteen pregnant women were recruited in this study. These pregnant women were subdivided into three groups according to phases of pregnancy (20) women at first trimester, (20) women at second trimester, and (20) women at third trimester. Twenty five (25) women were recruited and served as a control group (non-pregnant women) and have a regular menstrual cycle. All ages of women were ranged between (20 – 35) years old. All women were free from chronic diseases such as hypertension, diabetes mellitus, thyrotoxicosis, nonsmokers, and without contraceptive drugs. All women were attended to health centers to check up their own health criteria.

Blood samples collection

The blood samples were collected in different health care centers of AL- Hussein teaching hospital in Karbala. Anticupital vein was employed. The arm should be warmed to activate blood circulation and engorged vein. Application of tourniquet was achieved directly around the skin of the arm (usually the left arm), nearly 8 cm above the location of collection. The skin was sterilized

with 70% ethyl alcohol and allowing to dry to prevent hemolysis. Needles used were 22 and 23 gauges. Three groups of labeled tubes were prepared; the first groups of tubes contain EDTA as anti- coagulants to prevent clotting of blood to be used for determination of blood parameters. The second group of tubes contain trisodium citrate (anti- coagulant) to prevent coagulation and to be used for preparing plasma involved in determination of clotting factors activities (I, II, VII, VIII, and IX), the tubes were centrifuged at 3000 rpm for 10 min, and plasma parts obtained immediately to perform the levels of clotting factor activities. The third group of tubes used without anti-coagulant (plain gel tubes) to be used for preparing sera, the blood samples were allowed for 5 minutes to clot and then transferred for centrifugation to obtained serum samples for future analysis of estrogen and progesterone hormones.

Evaluation of estradiol hormone

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen. A reaction results between the antigen and the antibody. The interaction is illustrated by the following equation: (according to Monobind Inc kit).

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

Measurement of Fibrinogen concentration (Factor I)

Method based on von clauses and al studies, validated by Destining F. and al. when an excess of thrombin is present, the pre-diluted plasma clotting time is reverse order proportional to the fibrinogen's concentration in the specimen. (According to Biolabo kit).

Determination of factor II activity

The assay consists of the measurement of clotting time, in the presence of the STA- Neoplastine reagent, of a system in which all the factors are present excess (supplied by STA – Deficient II) except factor II which is derived from the sample being tested (According to Diognostica Stago kit).

Determination of VII (Stable factor)

The assay consist of the measurement of the clotting time, in the presence of Neoplastine reagent, of a system in which all the factors are present, constant and in excess (supplied by STA-deficient VII) except factor VII which is derived from the sample being tested (According to Diognostica Stago kit).

Measurement of factor VIII activity

The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess (supplied by **STA-Deficient VIII**), except factor VIII which is derived from the sample being test(According to Diognostica Stago kit).

Determination of Factor IX activity

The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of system in which all the factors are present in excess (supplied by **STA-deficient IX)** except factor IX which is derived from the sample being tested(According to Diognostica Stago kit).

Statistical analysis

All values were expressed as mean ± stander deviation (SD). The data were analyzed by using of computer SPSS program, Low significant different (LSD) were used to explain the differences among tested groups and ($p<0.05$) was involved the lowest significant limit [(4)].

Results

Results of estrogen hormone concentration

The results which are mentioned in table 1 showed in-significant increase ($p>0.05$) of estradiol hormone in first trimester; but, there is a significant elevation ($p<0.05$) of estradiol hormone in second and third trimesters when compare to non-pregnant women group.

Results of fibrinogen concentration (Factor I)

Results which are illustrated in table 1 have a significant increase ($p<0.05$) of fibrinogen concentration of pregnant women in the first, second, and trimesters in a comparison with the non-pregnant women.

Levels of FVII activity

Results which are presented in table 1 tend to be in-significant increase ($p>0.05$) of FVII activity of pregnant women in first and second trimesters when compared with control group. The statistical analysis have a significant elevation ($p<0.05$) of factor VII activity of pregnant women in third trimester when compared with control group (non-pregnant women).

Results of FVIII activity

The results which are obtained from the presented study and presented in table 1 have a significant increase ($p<0.05$) of factor VIII activity of pregnant women throughout first, second, and third trimester when compared with control.

Results of FIX activity

The results that are illustrated in table 1 were a significantly higher ($p<0.05$) of factor IX activity of the pregnant women (first, second, and third trimesters) than control group.

Groups / Parameters	First trimester	Second trimester	Third trimester	Control group (non-pregnant)
FI (g/dL)	225.17±19.81*	265 ± 28.88*	361.17 ± 27.67*	238.44 ± 19.44
FII (%)	120.83 ± 3.79*	128.06 ± 1.76*	135 ± 4.70*	117.17 ± 3.11
FVII (%)	122.11 ± 4.71	122.72 ± 4.82	136± 5.29*	119.94 ± 4.04
FVIII (%)	125.61 ± 1.58*	118.11 ± 5.33*	136.78 ± 5.70*	113.50 ± 5.79
FIX (%)	117 ± 7.87*	118.61 ± 7.55*	136.56 ± 4.49*	103.78 ± 7.03
Estrogen (pg/mL)	405.03±166.60	2411.55±731.51*	3646.06±1303.6*	100.30±12.95

Table (1): The means of clotting factors: fibrinogen (FI g/dL), prothrombin factor (FII %), stable factor (FVII %), anti haemophilic factor (FVIII %), chrismas factor (FIX %) and estrogen concentration (pg/mL) in pregnant women (first, second, and third trimesters) and non-pregnant women (control group).

- Values are means ± SD.
- Means with a strik (*) are significantly different at $p<0.05$.

Correlation coefficient between levels of estrogen hormone and activity of clotting factors in pregnant women:-

The statistical analyses of correlation coefficient have been found the following facts:-

1. There is a significant positive correlation ($r = 0.825$) between FI concentration and estrogen concentration at the first, second, and third trimesters.
2. Results of correlation have been showed a positive correlation ($r = 0.643$) between FII activity and estrogen concentration in pregnant women (first, second, and third trimesters).
3. The present study confirmed a significant positive correlation ($r = 0.523$) between FVII activity and estrogen concentration in pregnant women.

4. The activity of FVIII also pointed out a positive correlation (r = 0.375) with estrogen concentration in pregnant women.
5. FIX activity confirmed a positive correlation (r = 0.583) with estrogen concentration in pregnant women.

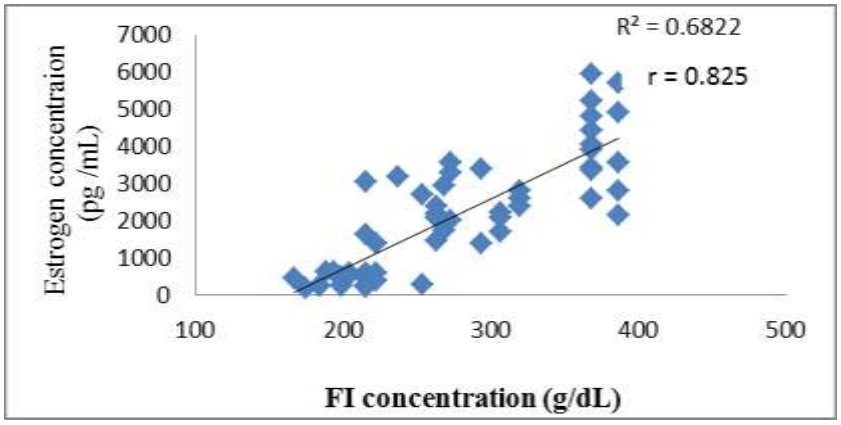

Fig. (1) Correlation coefficient between FI concentration and estrogen in pregnant women

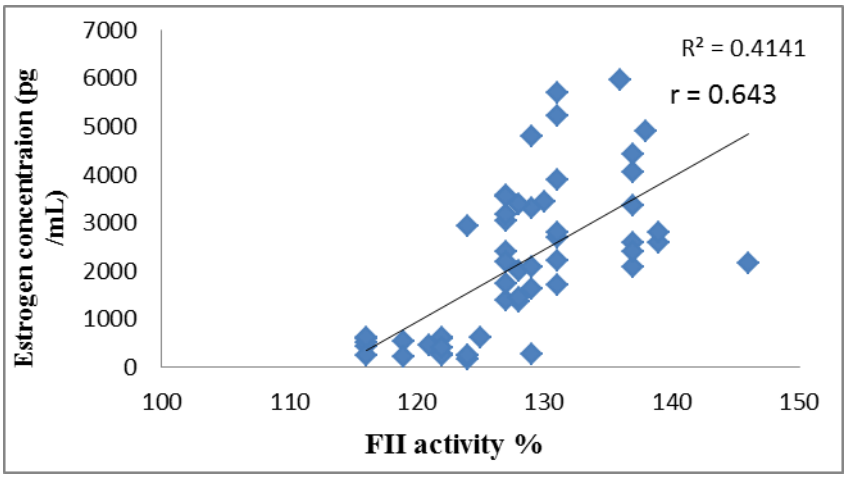

Fig. (2) Correlation coefficient between FII activity and estrogen in pregnant women

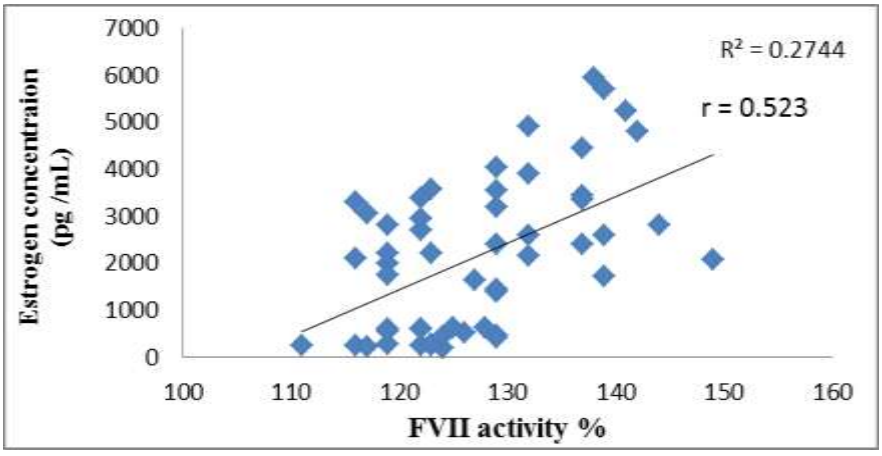

Fig. (3) Correlation coefficient between FVII activity and estrogen in pregnant women

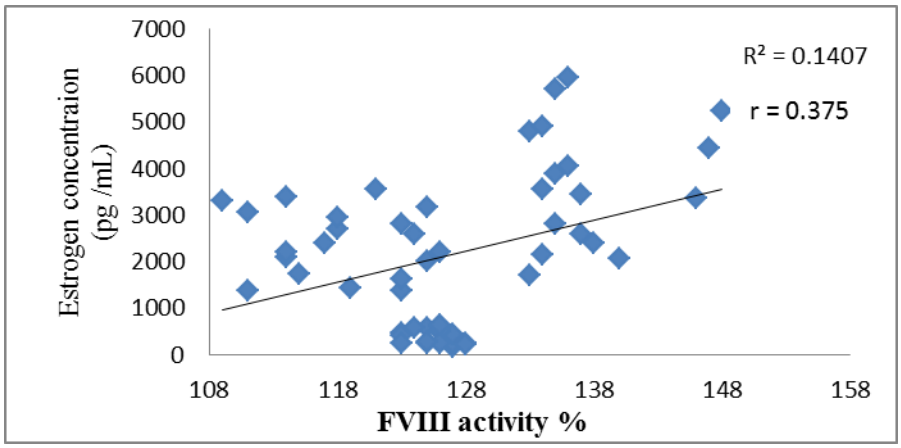

Fig. (4) Correlation coefficient between FVIII activity and estrogen in pregnant women

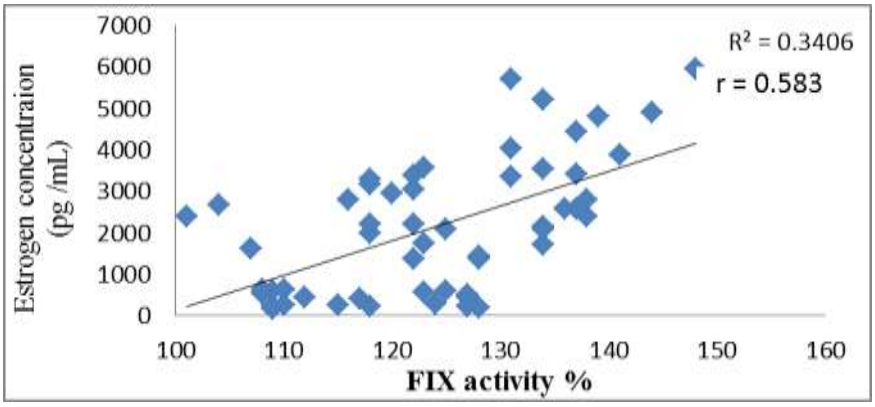

Fig. (5) Correlation coefficient between FIX activity and estrogen in pregnant women

Discussion

Fibrinogen (FI) concentration

In present study, fibrinogen concentration showed progressively increased ($p<0.05$) in pregnant women compare to non-pregnant women. Its concentration has correlation with estradiol level (r = 0. 825). During normal pregnancy, there are dramatic changes occur in the hemostatic system, coagulation factors activity increased physiologically in pregnancy and this is thought to be an evolutionary mechanism to avoid excessive blood loss at parturition [(5)]. The present study agrees with study of Greer who reported that during pregnancy the levels of coagulation factor (Fibrinogen) increase significantly [(6)]. Also, Choi and Pai showed there is steady elevation of factor I during pregnancy [(7)].

Study of walker *et al.*, indicated that fibrinogen levels increases from early pregnancy on wards to almost double its pre-pregnant value by term [(8)]. Normal pregnancy is related with a progressive rise in activities of many coagulation factors including fibrinogen [(9)]. It also have been found that fibrinogen clearance rate increase as the pregnancy progresses [(10)] and thus the levels of fibrinogen required to avoid placental abruption elevated with advancing gestation [(11)].

Factor II activity

Results obtained from the present study indicated that factor II activity was progressively increased ($p<0.05$) in pregnant women compare to non-pregnant women. The correlation coefficient of factor II with estrogen in pregnant women was ($r = 0.643$). Previous study of Clark *et al*., confirmed that FII activity was slightly increased or unchanged during normal phases of pregnancy [12]. Our data were consistent with Kadir who explained that Factor II (FII, Prothrombin) levels may elevated in early pregnancy but are normal by term [13]. Factor II activities in pregnancy trimesters have yielded in conclusive , reporting both an early elevated in pregnancy followed by sharp decreases back to non-pregnant activities over the course of pregnancy[14]. Changes occur in the coagulation system, with elevated procoagulant activity, dropped natural anticoagulant activity, and declined fibrinolysis, lead to prothrombotic state [15]. Thrombin synthesis is higher during pregnancy causing to increased fibrin production [16].

Factor VII activity

Statistical analyses of the present study showed that factor VII activities were insignificantly increased ($p>0.05$) in pregnant women during the first and second trimesters, and were indicated a significant increase ($p<0.05$) in the third trimesters compare to non-pregnant women. Correlation coefficient of factor VII with estrogen in pregnant women was $r = 0.523$. Our results were consistent with study of Donohoe *et al*., and study of Choi and pai., who showed that factor VII activity was increase step by step during trimesters of pregnancy also Hellgren., indicated that factor VII activity increase during normal pregnancy[9, 7, 17] . Previous study of Kadir *et al*., confirmed that the elevation in clotting factor VII is specifically more marked in the third trimester [13]. It has been found that normal pregnancy is associated with progressive rise in the activities of several clotting factors including FVII [18]. The study of Kadir *et al*., showed that gradually rise of factor VII activity was necessary to prevent threatend abortion [13]. Procoagulant factors such as FVII, FXIIa and vWF levels have been shown to be higher in maternal plasma [19]. There is also an elevated hindrance to endogenous anticoagulant factors such as (APC) [20].

Factor VIII activity

Results of F VIII activity which are illustrated in table 1 were significantly increased ($p<0.05$) at first, second, and third trimesters compare to non-pregnant. Correlation coefficient of FVIII with estrogen in pregnant women was $r = 0.375$. The present study agree with several previous studies with that activity of coagulation factor FVIII increased significantly during phases of pregnancy [5] . Also study of Thornton and Douglas; Oriodran and Higgins., indicated that factor (FVIII) increase during pregnancy [21, 22]. It was found that the increase in coagulation factors FVIII is almost mere markedly increase in the third trimester [13]. Factor VIII concentration and its coagulation activity were increased progressively during trimester [12, 23, 24]. Also, study of Prisco *et al*., indicated that factor VIII levels were increased in late pregnancy when coagulation activity is about double that in the non-pregnant women. So, these mechanisms consider evolutionary mechanism in human and other mammals to prevent blood loss during parturition [26].

FIX activity

In present study, FIX activity was progressively heightened ($p<0.05$) in first, second, and third trimester compare to non-pregnant. Correlation coefficient of FIX with estrogen in pregnant women was $r = 0.583$. The elevation in factor IX concentration during pregnancy is reported by several authors to be small [26]**.** Data of the present study were disagree with study of Clark *et al* ., which indicated that factor IX activity are slightly increased or remained with normal during normal pregnancy[12]. The present data are consistent with Donohoe *et al*., who indicated that activity of factor IX increased progressively during pregnancy [9]. Also, the present studies agree with Greer who confirms elevation of FIX during pregnancy [25]. From physiological point view, normal pregnancy is related with several changes in different mechanism of hemostasis processes

take part to maintain placental function during trimesters and to prevent excessive blood loss in delivery. Most changes in clotting and fibrinolysis mechanism lead to a stable of hypercoagulability [(26)]. Coagulability was essential to prevent bleeding [(27)].

References

1. Sifakis S., Pharmakides G. (2000). Anemia in pregnancy. Ann N Y Acad Sci; 900: 125-36.
2. Casanueva E., Pfeffer, F; Fernandez-Gaxiola, A.C; Gutierrez- Valenzuela, V; and Rothenberg, S.J. (2003). Iron and folate status before pregnancy and anemia during pregnancy. Ann Nutr Metab; 47: 60-3.
3. Saja M. (2009). Coagulation Inhibitors in Liver Disease. Chapter 1: 1-2.
4. Daneil, W.W. (1999). Biostatisttics: afoundation for analysis in the health sciences. 7th ed. John Wily. Philadelphia. 8.
5. Ganchev R.V. and Ludlam C.A. (2004). Acquired and Congenital Hemostatic Disorders in Pregnancy and the Puerperium. Thromb Res; *114*: 409- 14.
6. Greer, I.A. (1994). Hemostasis and thrombosis in pregnancy. Hemostasis and thrombosis (3rd ed.), Churchill Livingstone , Edinburgh ; 987-1015
7. Choi, J and Pai, S.H. (2002). Change in erythropoiesis with gestational age during pregnancy. Ann. Hematol., 80: 26.31 .
8. Walker, I.D.; Wallker, J.J.; Colvin, B.T.; Letsky, E.A.; Rivers, R.; and Stevens, R. (1994). Investigation and management of haemorrhagic disorders in pregnancy. J Clin Pathol; 47:100-108.
9. Donohoe, S.; Quenby, S.; and Mackie, I. (2002). Fluctuation in levels of antiphospholipid antibodies and increased coagulation activation marker in normal and heparin treated antiphospholipid syndrome. Lupus; *11*:11-20.
10. Roque, H.; Stephenson, C.; and Lee, M.J. (2004). Pregnancy related thrombosis in a women with congenital afibrinogenaemia : a report of two successful Pregnancies. Am J Hematol; *76*: 267-70.
11. Kobayashi, T.; Kanayama, N.; Tokunaga, N.; Asahina, T.; and Terao, T. (2000). Prenatal and Peripartum management of congenital afibrinogenaemia. Br J Haematol; *109*: 364-6.
12. Clark, P.; Brennand, J.; Conkie, J.A.; McCall, F.; Greer, I.A., and Walker, I.D. (1998). Activaed protein C sensitivity, protein S and coagulation in normal pregnancy. Thromb Hemost; *79*: 1166-70.
13. Kadir, R.; Chi, C.; and Bolton-Maggs, P. (2009). Pregnancy and rare bleeding disorders. Haemophilia; *15*, 990–1005.
14. Holmes, V.A and Wallace, J.M.W. (2005). Haemostasis in normal pregnancy: a balancing act? Biochemical Society Transaction; *33*, part 2.
15. Connors, J.M. (2011). Hematologic Disorders in Pregnancy. Hematology/Oncology Clinics of North America; *25* (2), Pages xiii-xiv.
16. Lopez, Y.; Paloma, M.J.; Rifon, J.; Cuesta, B.; and Paramo, J.A. (1999). Measurement of prethrombotic markers in the assessment of acquired hypercoagulable states. Thromb Res; *93*:71-78.
17. Hellgren, M. (2003). Haemostasis during normal pregnancy and puerperium. Semin Thromb Hemost; *29*: 125-30.
18. Uchkova, E.H; and Ledjev, I.I. (2005). Changes in haemostasis during normal pregnancy .European Journal of Obstetrics and Gynecology and Reproductive Biology; *119*: 185-188.
19. Wickstrom, K.; Edelstam, G.; Lowbeer, C.H.; Hansson, L.O.; and Siegbahn, A. (2004). Reference intervals for plasma levels of fibronectin, von Willebrand factor, free protein S and antithrombin during third-trimester pregnancy. Scand J Clin Lab Invest; *64*:31-40.

20. Peek, M.J.; Nelson-Piercy, C.; Manning, R.A.; de Swiet, M.; and Letsky, E.A. (1997). Activated protein C resistance in normal pregnancy. Br J Obstet Gynaecol; *104*:1084-1086.
21. Thornton, P.; and Douglas, J. (2010). Coagulation in pregnancy. Best practice and Research Clinical Obstetrics and Gynaecology; *24*:339-352.
22. O'Riordan, M.N.; and Higgins, J.R. (2003). Haemostasis in normal and abnormal pregnancy. Best PractResClinObstetGynaecol; *17*: 385–396. 5
23. Blomback , M. (1991). Adv.Contracept. 259-272.
24. Kjellberg, U.; Andersson, N.E.; Rosen, S.; Tengborn, L.; and Hellgren, M. (1999). APC resistance and other haemostatic variables during pregnancy and puerperium. Thromb Haemost; *81*:527-531.
25. Greer, I.A. (1998). The special case of venous thromboembolism during pregnancy. Haemostasis; *28* (suppl 3):22-34.
26. Prisco, D.; Ciuti, G.; and Falciani, M. (2005). Hemostatic changes in normal pregnancy. haematologica reports ; *1*:1-5 .
27. Riddle, J.P.; Aouizerat, B.E.; Miaskowski, C.; and Lillicrap, D. (2007). Theories of Blood Coagulation. Journal of Pediatric Oncology Nursing; *24*: Pp 123-131 .