

Estimation of the Effects of Estradiol Hormone Concentrations on the Activities of Some Clotting Factors (FI, FII, VII, VIII, IX) Involved in Extrinsic, Intrinsic, and Common Pathways During Pregnancy

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Abstract

The present study was conducted to confirm the effects and correlations of estradiol hormone on some major clotting factors (fibrinogen FI , prothrombin FII , stable factor F VII , antihemophilic factor FVIII , and Christmas factor IX) during pregnancy phases. Seventy five (75) pregnant women at different stages of pregnancy and were sub- divided into three groups according to period of gestational phases , included twenty five (25) pregnant women in each group (first, second, third trimesters). Twenty (20) volunteer non-pregnant non-lactating women were involved in this study and used as a control group. All ages of tested women (pregnant and non- pregnant) were between 25 year to 35 year old . Regarding levels of estradiol hormone , they were showed insignificant elevation ($p > 0.05$) during first trimester and remarkable increase ($p < 0.05$) during second and third trimesters when matched with their counterparts of non- pregnant group. About concentrations of fibrinogen factor (FI) , they were indicated a significant heightening ($p < 0.05$) during all phases of pregnancy in matching with control group, and these concentrations have a positive correlation ($r = 0.714$) with levels of estradiol. Activities of prothrombin factor (F II) were significantly elevated ($p < 0.05$) throughout pregnancy phases than of non- pregnant women , and have a significant positive correlation ($r = 0.532$) with estradiol hormone . Levels of stable factor (F VII) activity indicated insignificant increase ($p > 0.05$) in first and second trimesters and its activities become increased ($p < 0.05$) at third trimester in a comparison with those of control group, and pointed out a significant positive correlation ($r = 0.412$) with estradiol level . Estimation of intrinsic pathway activities (antihemophilic factor FVIII and Christmas factor FIX) were showed a progressive elevation ($p < 0.05$) during first, second, and third trimesters in a comparison with control group, and these activities confirmed a significant positive correlation ($r = 0.260$, $r = 0.632$, respectively) with estradiol.

Key words: pregnancy, estradiol hormone, clotting factors, bleeding.

الخلاصة

وضعت الدراسة الحالية لمعرفة تأثيرات وارتباطات تراكيز هرمون الاستراديول على بعض تراكيز وفعاليات عوامل التخثر الرئيسية (الفايبرينوجين FI والبروثرومبين FII والعامل الثابت FVII والعامل المضاد للنزف FVIII وعامل كريسماس FIX) خلال مراحل الحمل . تضمنت الدراسة اختبار 75 امرأة من الحوامل خلال مراحل مختلفة من الحمل , وقد قسمت النساء الحوامل الى ثلاث مجاميع طبقا لمراحل الحمل , اذ شملت المجاميع 15 امرأة في كل مجموعة من الطور الاول والثاني والثالث للحمل . كذلك شملت الدراسة اختبار 20 امرأة غير حوامل وغير مرضعات كمجموعة سيطرة. تراوحت جميع اعمار النساء في الدراسة الحالية بين 25 و 35 سنة . بينت تراكيز هرمون الاستروجين ارتفاعا غير معنوي ($p < 0.05$) خلال الطور الاول من الحمل واعطت ارتفاعا معنويا ($p < 0.05$) خلال الطور الثاني والثالث من الحمل عند المقارنة مع مجموعة السيطرة (غير الحوامل) . اما فيما يخص عوامل التخثر , فقد سجلت تراكيز الفايبرينوجين ارتفاعا معنويا ($p < 0.05$) مع تركيز هرمون الاستروجين في جميع مراحل الحمل وبينت تراكيزه ارتباطا ايجابيا مقداره $r = 0.714$ مع تراكيز هرمون الاستراديول .

لوحظ وجود ارتفاع معنوي ($p < 0.05$) في قيم فعاليات عامل البروثرومبين في جميع النساء الحوامل واطهرت فعالياته ارتباطا ايجابيا مقداره $r = 0.523$ مع تركيز هرمون الاستروجين . اظهرت قيم فعاليات العامل الثابت وجود ارتفاع معنوي ($p < 0.05$) خلال الطور

الاول والثاني والثالث من الحمل كما بينت ارتباط ايجابي $p=0.412$ مع تركيز هرمون الاستراديول . اما بخصوص فعاليات عوامل المسار الداخلي (العامل المضاد للنزف وعامل كريسما س) فقد لوحظ وجود ارتفاع معنوي ($p<0.05$) في جميع النساء الحوامل واعطت ارتباط ايجابي $p=0.632$ مع قيم هرمون الاستراديول .
الكلمات المفتاحية: الحمل , هرمون الاستراديول , النزف , التخثر .

Introduction

Many physiological changes and fluctuations in different organs and systems of the body occur during pregnancy, these changes are achieved by essential sexual hormones of female particularly human chorionic gonadotropin (hCG), progesterone, estrogens, follicular stimulating hormone (FSH), luteal hormone (LH), human placental lactogen (hPL), to perform specific care during pregnancy, one should be complete understanding about these fluctuations of these hormones and other physiological changes occurring in other parts of the body (Sifakis and Pharmakides 2000). Also, pregnancy characterized with several complex of hormones and many interactions among hormone and their activities that lead to large physiological alterations, many of these alterations seem remarkable than that occur in other organs, it is well known that these physiological alterations result from elevation of fetal requirements for development and preparing different maternal organs responsible for delivery and feeding of fetus, one of the most hormonal changes that appear more prominent and ensure normal pregnancy are more production of female sexual hormones, especially, estrogens and progesterone (Casanueva *et al.*, 2003). Human and other mammalian animals develop specific mechanism to ensure prevent loss of their blood by injuries and maintain fluidity of blood within vascular system, these mechanism called hemostasis. Formation of blood clot is more limited to enhance closure of wound opening, and blood clot produced as a result of interaction and synergism among different components of blood circulation which include blood vasculature, blood platelets, clotting factors, fibrinolysis. Together, hemostasis components play a critical role to stop bleeding by formation of clot at injury and prevent disturb of blood flow beyond site wound (Saja, 2009).

Women of the study

The present work was performed in Babylon and Karbala teaching hospitals / Iraq, during the period ranged from January 2016 to June 2016. Seventy five (75) women were pregnant and enrolled, pregnant women were subdivided into three subgroups (first group included 25 women at first trimester, second group included 25 women at second trimester, third group included 25 women at third trimester). Twenty (20) women were non-pregnant and non-lactating with normal menstrual cycle were used a control group. Ages of women (pregnant and non-pregnant) in this study were 25 years to 35 years old. Including criteria of women were normal menstrual cycle, normal pregnancy, timing of trimesters. Excluded criteria involved hypertension, diabetes mellitus, bone diseases, thyrotoxicosis, smoking, preeclampsia, contraceptive drugs, polycystic ovary, and recurrent abortion.

Collection of Blood Samples:-

The Collection of blood samples from studied women in this study was performed in paternity hospitals in Babylon and Baghdad provinces. Anticubital vein of left arm was selected and warmed to improve blood flow and swollen of targeted vein and then a elastic tourniquet was applied above the collection site and skin was sterilized with alcohol (70 %). Two groups of labeled tube were selected to collect the blood

samples, the first group of tubes contained tri-sodium citrate as anti-coagulant to impair blood coagulation that used for estimation of clotting factor activities. These tubes were transferred to centrifuge at 3000xg for at least 10 minutes, then plasma parts were readily transferred for measurement of clotting factor activities. The second group of labeled tubes were without anticoagulant (plain gel tubes) and blood samples in these tubes were left for about 10 minutes to permit blood to clot and then transferred for centrifugation at 2500 xg to obtain the sera for measurement of estradiol hormone.

Determination of estradiol concentration

Enzyme immunoassay was used and its reagents include (antibodies, conjugation of enzyme- antigen and native antigen). The interaction among these components is depicted by the following reaction: (according to commands of Monobind Inc kit).



Estimation of Fibrinogen concentration (Factor I)

According to Biolabo Kit based on von clauses validated by Destining, an excess amount of thrombin factor is present, the pre-diluted plasma clotting time is reverse order proportionate to the fibrinogen levels in that sample. (According to Biolabo kit).

Measurement of prothrombin factor(F II) activity

The method is based on determination of clotting time of specimen. According to kit supplied by Diagnostic Stago that involve in the presence of the STA-Neoplastine reagent, of a system in which all the clotting factors are present in excess (supplied by STA-Deficient II) with exception of prothrombin factor II which can be derived from the sample that already tested (According to Diagnostica Stago kit).

Determination of Stable factor (F VII)

Also, the assay is depend on the determination of the clotting time, in the presence of Neoplastine reagent of a system that has all the clotting factors, constant and in excess (supplied by STA- deficient VII) except stable factor(F VII) which is presented in the sample that prepared for testing (According to Diagnostica Stago kit).

Measurement of factor VIII activity:-

The method based on the estimation of the clotting time, in the presence of cephalin and activator. In exception of factor VIII that is released from sample, all the other factors are present in excess (Supplied by STA- Dificient VIII) (According to Diagnostica Stago kit).

Determination of Factor IX activity:-

This assay is involved the measurement of the clotting time of blood sample according to presence of cephalin and activator. All clotting factors are present in excess (supplied by **STA-deficient IX**) with exception of Christmas factor(F IX) which can be extracted from the sample preparing to test (According to Diagnostica Stago kit).

Statistical analysis

The results of the present study were showed as means \pm stander deviation (SD). All results were completely analyzed by computer program (SPSS). To explained the differences among tested groups, the low significant different (LSD) was used and $p < 0.05$ were used as a lower significant difference (Daneil, 1999).

Results

All results of this study are illustrated in following table and included on the results of estradiol concentration , clotting factor activities (FI, FII, FVII, FVIII, FIX)

Estradio hormone concentration

The values of estradiol concentration were insignificantly increased ($p > 0.05$) in first group (first trimester) and indicated a remarkable heightening ($p < 0.05$) in both second and third groups (second and third trimester) of pregnant women in matching with non- pregnant women (control group).

Levels of fibrinogen concentration (F I)

Fibrinogen concentrations are markedly elevated ($p < 0.05$) in all pregnant women (first , second , and third trimesters) when compared with those non- pregnant women.

Activities of prothrombin factor (F II)

Activities of FII are significantly increased ($p < 0.05$) throughout period of pregnancy in all pregnant women groups in a comparison with those counterparts of non-pregnant women

Activities of stable factor(FVII)

From data mentioned in below table , the levels of FVII activities were showed an insignificant increase ($p > 0.05$) during first and second trimesters and they indicated a significant increase ($p < 0.05$) in third trimester when compared with control group .

Activities of antihemphilic factor (FVIII) :-

The activities of FVIII are significantly elevated ($p < 0.05$) in all groups of pregnant women in a comparison with those non- pregnant women .

Activities of Christmas factor(FIX) activity :-

These activities are illustrated in below table and indicated a significant increase ($p < 0.05$) in all trimesters of pregnancy of women when matched with those non-pregnant women .

Table :- Shows the means of estradiol concentration (pg / ml) and clotting factors: fibrinogen concentration (FI g/dL), prothrombin factor (FII %), stable factor (FVII %), anti haemophilic factor (FVIII %), and christmas factor (FIX %) during first, second, and third trimesters of pregnancy and non-pregnant women (control group).

Groups Parameters	First group	Second group	Third group	Control group
Estradiol concentration (pg/mL)	422.47±144.87	2506.33±634.92*	3639.12±17392*	122.1 ±34.12
FI activities (g/dL)	250.98±14.56*	276 ± 24.57*	367.18 ± 23.55*	212.45 ± 18.13
FII activities (%)	122.81 ± 2.99*	129.15 ± 2.86*	136 ± 3.91*	113.28± 2.33
FVII activities (%)	123.55 ± 3.28	124.32 ± 4.82	137± 4.33*	115.78 ±5.23
FVIII activities (%)	126.39 ± 2.87*	119.56 ± 3.49*	137.59 ±4.67*	112.92 ± 4.36
FIX activities (%)	118 ± 6.44*	120.23 ±6.24*	135.24 ± 3.98*	104.56 ± 8.44

- Results illustrated above are means ± stander deviation.
- Means having a mark * are significantly different($p < 0.05$).

Correlations among levels of estrogen hormone and activities of clotting factors in pregnant and non- pregnant women

1. A significant positive correlation ($r = 0.714$) is found between FI concentrations and estradiol concentration during pregnancy (first, second, and third trimesters).
2. A positive correlation ($r=0.532$) has been found between FII activities and estradiol concentration in pregnant women
3. A significant positive correlation ($r = 0.412$) indicated between FVII activities and estradiol concentration during pregnancy phases .
4. A significant positive correlation ($r=0.260$) is confirmed between estradiol concentration and FVIII activities in pregnant women.
5. A significant positive correlation ($r=0.632$) is established between estradiol concentrations and activities of IX during pregnancy.

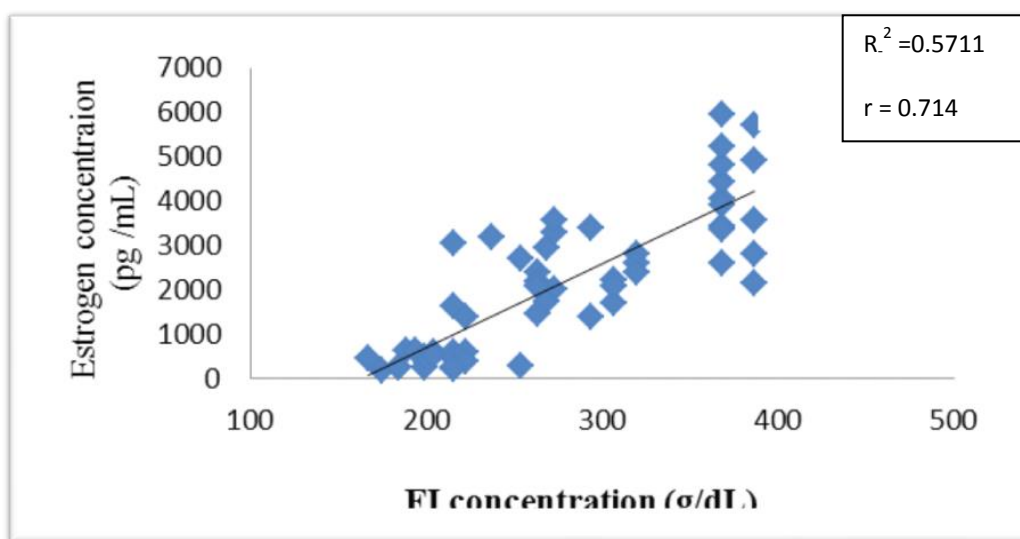


Fig. (1):- Shows correlation between estradiol hormone (pg/ml) and FI concentrations (g/dL) during pregnancy trimesters

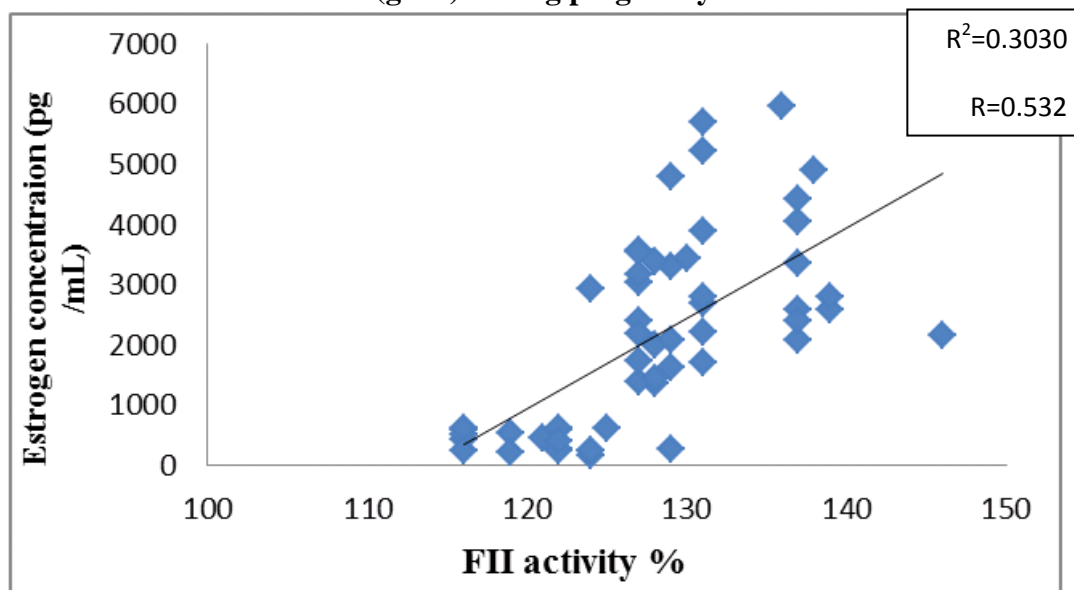


Fig. (2) :- Shows correlation between FII (%) activities and estradiol hormone (pg /ml) during pregnancy trimesters

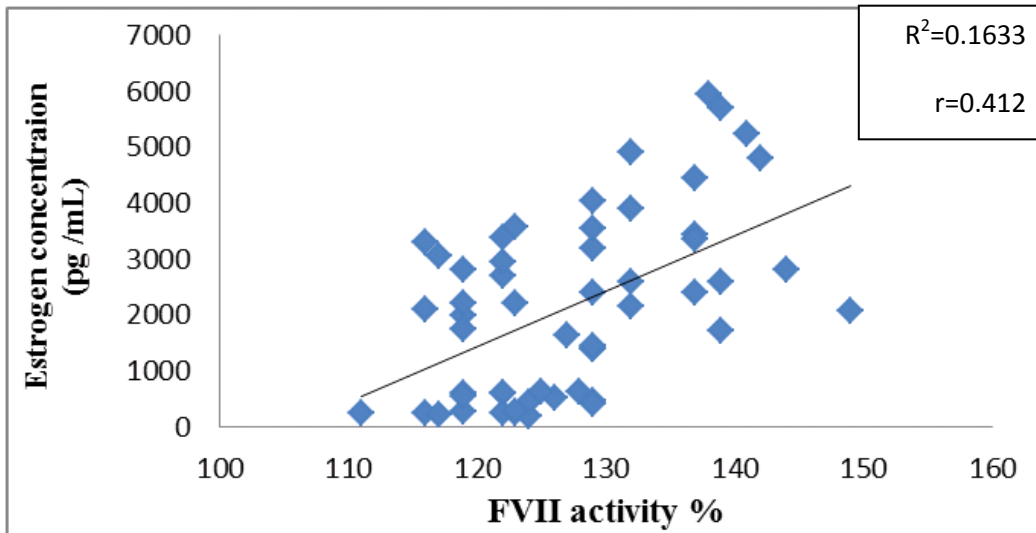


Fig. (3):- Shows correlation between FVII activities (%) and estradiol concentrations (pg / ml) during pregnancy trimesters

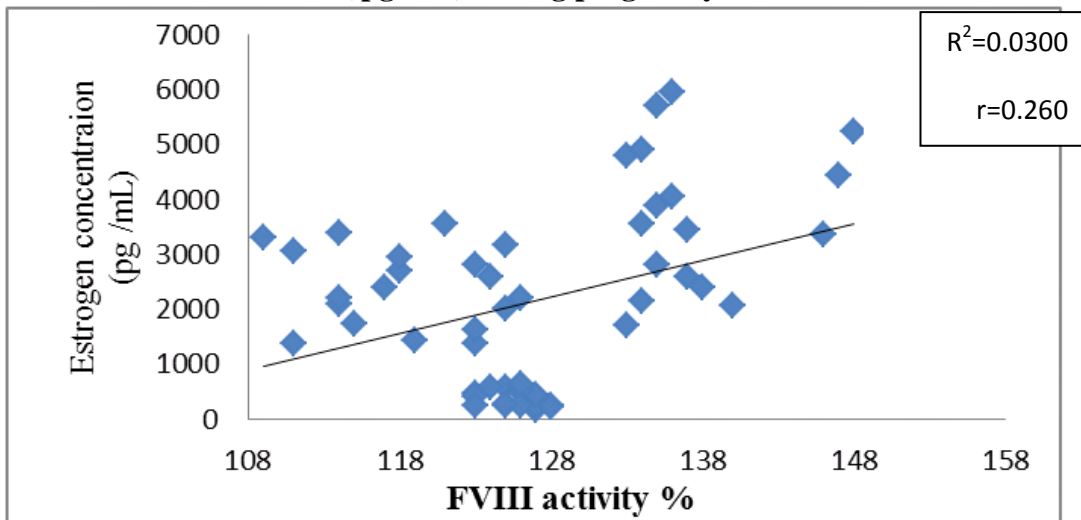


Fig. (4):- Shows correlation between FVIII activities and estradiol hormone (pg / ml) during pregnancy trimesters

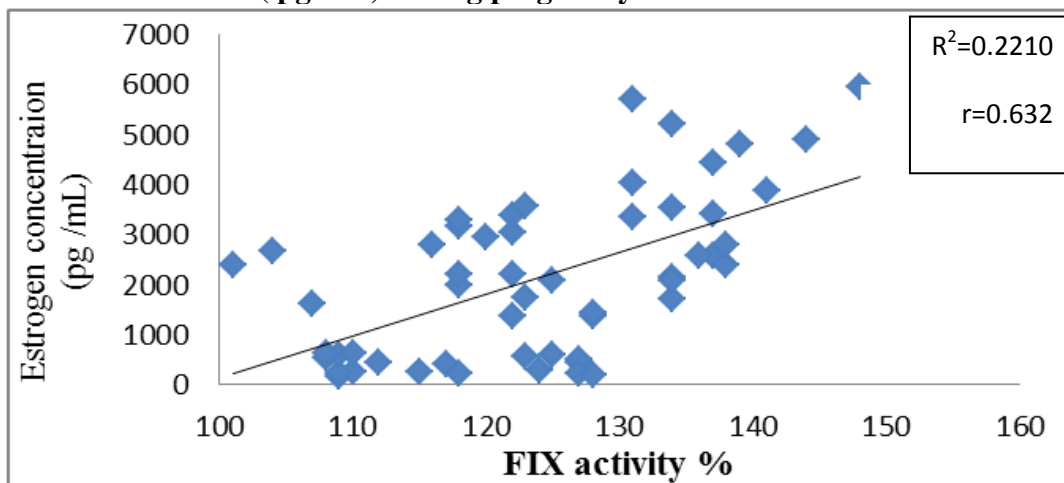


Fig. (5) :- Shows correlation between FIX(%) activities and estradiol hormone (pg /ml) during pregnancy trimesters

Discussion

Fibrinogen (FI) concentration

In normal pregnancy, bodies of pregnant women have multiple alterations, especially in mechanisms responsible for maintaining of hemostatic pathways, many physiological changes happen during pregnancy and involved, particularly in activities of many clotting factors and these changes are believed as developmental pathways of pregnant females to prevent excessive bleeding during labour (Ganchev and Ludlam 2004). Data of this work are consistent with Greer (1994) who indicated an increase of fibrinogen concentrations in pregnancy. The present study agrees with previous studies study that reported during pregnancy the levels of coagulation factor (Fibrinogen) increase significantly (Greer, 1994, Choi and Pai, 2002).

Walker *et al.* (1994). showed increase of fibrinogen is started from first stages of gestation and progress in double toward final stages of term . It is well documented that the activities and concentrations of many clotting factors progressively increase ,fibrinogen ,in particular (Donohoe *et al.*,2002). Studies also indicated that increase fibrinogen clearance with advanced pregnancy (Roque *et al.*,2004) and these levels of fibrinogen are important to prevent abruption of placenta that increased with progress of pregnancy(Kobayashi *et al.*,2000).

Activities of Factor II

Previous work indicated a moderate increase in the activities of FII during pregnancy or tend to remain within normal range (Clark *et al.*,1998). The present data disagree with previous study that mentioned prothrombin activity can to increase during first stage of pregnancy and return to normal limits at time of labou(Kadir *et al.*,2009) and also it found that in pregnancy phases the activities of FII tended to elevate during early phase of pregnancy and then return to levels as in non- pregnant women on progress of pregnancy (Holmes and Wallace 2005). During pregnancy, there are many changes occurring in mechanisms and components of hemostatic system, including increase of procoagulants, lowering of anticoagulants,and depressed fibrinolysis, these changes render prothrombotic events are more occurring(Connors, 2011). It is well founded that thrombin production mechanism become increased in particular in the final stages of pregnancy (Lopez *et al.*,1999).

Activities of FVII

These data are appeared to agree with previous studies which confirmed that stable factor activity gradually increase when pregnancy become progressed, and also another studies showed a progress heightening of FVII activity within gestational phases (Donohoe *et al.*, 2002; Choi and Pai ,2002; Hellgren, 2003). It is found that a marked increase in FVII concentrations during third trimester(Kadir *et al.*,2009). A remarkable rise in many clotting factors is related with pregnancy , FVII in particular (Uchkova and Ledjev 2005). Increase FVII activity has more importance to impair threatened abortion(Kadir *et al.*,2009). Levels of many clotting factors , including FVII, FXIIa, and vWF are found to rise in blood of pregnant women and have been shown to be higher in maternal plasma and associated with impairment to intrinsic production of anticoagulant including APC Wickstrom *et al.*,2004; Peek *et al.*, 1997).

Activities of FVIII

The present data are consistent with previous research which pointed out an elevation the clotting factor (FVIII) at all phases of pregnancy (Ganchev and Ludlam

2004). More ever, other previous studies of Oriodran and Higgins (2003); Thornton and Douglas (2010) are also consistent with present data and indicated an increase of FVIII in pregnant women. There is a higher elevation in the levels factor VIII concentrations and its clotting activity at trimesters of pregnancy (Clark *et al.*,1998; Blomback, 1991; Kjellberg *et al.*,1999). Also, There is a double increase of FVIII in particular at third trimester in comparison with that of first trimester and these changes explain an evolutionary process in pregnant females to increase defense mechanisms against excess bleeding at labour(Prisco *et al.*,2005).

Activities of FIX

There is found the elevation of FIX concentrations and efficiency of intrinsic pathways during trimesters are at a lower level (Prisco *et al.*,2005). Study of Clark *et al.*, (1998) showed there is a moderate increase or remain at normal limit of FIX during pregnancy. The present results obtained from this study appear consistent with study of Donohoe *et al.*, (2009) which confirmed that activity of stable factor(FIX) become progressively increased during gestation. Also, a study of Greer (1998) which indicated that there is an elevation of FIX in trimesters. According to physiological principals, There are several changes occurring during pregnancy and associated with various hemostatic mechanisms that are necessary to increase defense mechanisms to prevent excessive bleeding and to keep normal functions, parts of these changes involve increase clotting activities and decrease fibrinolysis to ensure hypercoagulability states that are essential to stop of bleeding at expulsion of placenta (Prisco *et al.*,2005; Riddle *et al.*,2007).

Conclusion

The possible explanation of these data can be return to physiological facts that explain increased activities and concentrations of different clotting factors involved in clotting pathways that continue with progress of pregnancy, and these activities may be resulted because of a higher levels of estradiol hormone that may be exerted a stimulatory effect to enhance concentrations and activities of different clotting factors to ensure a defense role that prevent excessive blood loss during parturition .

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