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RESEARCH ARTICLE

Correlation between estradiol deficiency and clotting mechanism in postmenopausal women

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Abstract

Aging in women is associated by a major loss in ovarian hormone function and consequently of menopause around the age of 50 years old. The present study was conducted essentially to evaluate the concentrations of some clotting factors and biochemical parameters in postmenopausal women.A total number of women was 125, of those, 100 women were post menopause and subdivided into four subgroups according to their ages: First group (51-55 years), Second group (56-60 years), Third group (61-65 years), and Fourth group (66-70 years). Twenty five (25) subjects were premenopausal women, their ages ranged between 20-30 years old and served as a control group.Results of estradiol hormone (E2) were significantly lowering ($p<0.05$) in all groups of postmenopausal women in a comparison with premenopausal women. Values of Prothrombin time (PT) recorded a significant heightening ($p<0.05$) in first and fourth groups, and these values tend to be non-significantly different ($p>0.05$) in the remaining tested groups when compared with control group. Regarding the results of the activated partial thromboplastin time (APPT) were insignificantly different ($p>0.05$) in most postmenopausal women compared to control group.About concentrations of clotting factors, it have been found a progressive elevation ($p<0.05$) in concentration of fibrinogen (FI) of aging women as compared to the control group and its concentration recorded a negative correlation ($r = 0.645$) with estradiol hormone level in all the tested group. On the other hand, the concentrations of stable factor (F VII) and anti-hemophilic factor (F VIII) were significantly dropped ($p<0.05$) in all tested group of postmenopausal women and their concentration indicated a positive correlation ($r = 0.423$, $r = 0.344$ respectively) with levels of estradiol hormone in all tested groups.

We can be suggested that a deficiency of estradiol hormone is associated with disturbances of hemostatic mechanism of postmenopausal women.

INTRODUCTION

Menopause is defined as the time when menstrual cycles permanently stop because of the complete depletion ovarian oocytes from aging. The diagnosis is markedly made after the women have missed menstruation for 12 consecutive months. Menopause indicates the permanent finish of fertility and the average age of menopause which is approximately 51 years (Santoro and Randolph , 2011). The menopause phenomenon , as a physiological time in life of women , causes alterations of the clotting factors (an

elevation of the fibrinogen (I) , factor VII , and suppressor of plasminogen-activator) and all of these variations increase incidence of cardiovascular risks . HRT causes an up regulation of the local prostaglandin secretion , and it appears that this is because of its positive influences of hemostasis , is well understood (Ylikorkala, 1992). The coagulation system is changed by the menopause age , with up regulations in many clotting factors which are defined to be related with incidence of vascular risk (Tait *et al* . , 1993 ; Lowe *et al* . , 1997). HRT can decrease factor VII levels . By contrast, oral HRT also inhibits plasma concentration of the normally anticoagulant protein S , (Post *et al.* , 2003; Salpeter *et al.* , 2006), in spite a parallel effect on inhibiting anti thrombin levels has not been estimated (Borgfeldt *et al.* , 2004).

2.Material and methods

The present study was carried out in general AL-Qasim hospital of Babylon and collage of science for women in Babylon University.

The present study was started at a period ranged between December 2014 to August 2015. One hundred twenty five women(postmenopausal and premenopausal)were recruited in this study. Of these , 100 women were postmenopausal and subdivided into four groups according to their age (51-55 years) , (56-60 years) , (61-65 years) , and (66-70 years) . Twenty five (25) women were enrolled and used as a control group, their ages ranged between 20-30 years old. The blood samples were taken from control group during follicular phase of menstrual cycle to estimate the high levels of estrogen. All the women were free from chronic diseases such as hypertension , diabetes mellitus , thyrotoxicosis , nonsmokers and without hormone replacement therapy . All the women attend health centers to check up their own health criteria.

Estradiol hormone level

The assay principle combination is based on a competition method with a final fluorescent detection(ELFA).The Solid Phase Receptacle (SPR) is used as the solid phase in adding the pipetting device for the assay. Reagents involved in the assay are ready-to be used and pre dispensed in the sealed reagent strips. All of the assay steps are usually performed automatically by the instrument device. The reaction medium was cycled in and out of the SPR many times.

The sample was transferred into the well containing the conjugate, which was an alkaline phosphatase-labeled estradiol derivative. The estradiol of the serum and the estradiol derivative in the conjugate competed for the anti-estradiol specific antibody location coated to the inner surface of the SPR.

Unbound elements are released through the washing steps. During the final detection stage, the substrate (4-Methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme mediates the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which was determined at 450 nm. The density of the fluorescence was inversely proportional to the concentration of antigen present in the sample. At the final step of the assay, results were automatically analyzed by the instrument in relation to the calibration curve retained in memory, and then printed out of instrument.(Estradiol was estimated according to kit supplied by Biomerieux company with using ELIZA test).

Prothrombin time test (PT)

The clotting time was measured at 37ºC in the presence of tissular thromboplastin and calcium. The measured PT (in sec.) was converted into PT (%).(According to Biolabo company).

Activated partial thromboplastin time test (APTT)

The BIO CK reagent involves recalcification of plasma in the presence of standardised amount of cephalin (platelet substitute) and a factor XII activator (Kaolin). The use of kaolin minimizes reading time and optimizes the optical detection. (According to Biolabo company)

Fibrinogen levels (FI)

The method was essentially based on Von Claus and al studies, validated by Destaing F and al. When a high amout of thrombin is presented in the sample the pre-diluted plasma. Clotting time was in reverse order proportional to the fibrinogen's concentration in the specimen.(According to Biolabo company).

Factor VII level (FVII)

This ELISA kit applies to the in vitro quantitative determination of human FVII concentrations in serum , plasma and other biological fluids(According to Elabscience company).

Factor VIII level (FVIII)

This ELISA kit applies to the in vitro quantitative determination of human FVII concentrations in serum , plasma and other biological fluids(According to Elabscience company).

Statistical analysis

All values were expressed as mean ± stander deviation (SD). The data were analyzed by using of computer SPSS program , Students-Test was used to explain the difference between groups and ($p<0.05$) was represented the lowest significant limit (Daneil , 1999).

3.Results

Estradiol hormone level (E2)

The results of the estradiol , which were illustrated in Figure (4-1) pointed out a significant decrease ($p<0.05$) in the estradiol levels in all groups of postmenopausal women (28.61 ± 9.63 , 29.87 ± 9.25 , 29.29 ± 9.93 , 30.35 ± 13.26 pg / ml., respectively) in comparison with those of the control group (216.37 ± 66.54 pg / ml

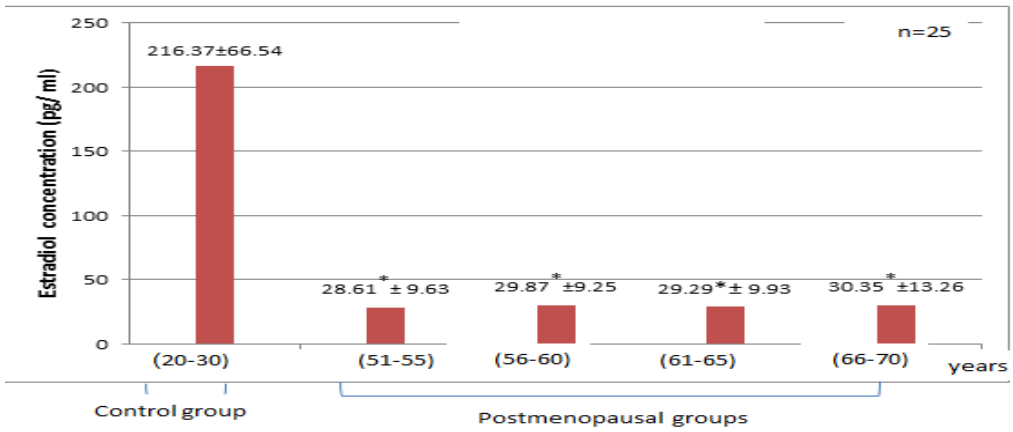

Figure (4-1) The means of estradiol levels (E2) (pg / ml) in postmenopausal women and premenopausal women.

- n = number of women in one group.
-Values are means ± SD.
-Means with astrik (*) are significantly different at $p<0.05$.

4.2 The results of prothrombin time (PT)

The levels of prothrombin time (PT) in Figure (4-2) were significantly increased ($P<0.05$) in group (51-55 years) and group (66-70 years) (11.41 ± 0.73 , 11.98 ± 0.47 sec., respectively) when compared with the control group (10.45 ± 1.14 sec.), while, these results were non-significantly different ($P>0.05$) of (PT) in group (56-60 years) and group (61-65 years) (10.64 ± 0.57 , 10.93 ± 1.31 sec., respectively) when compared with the control group (10.45 ± 1.14).

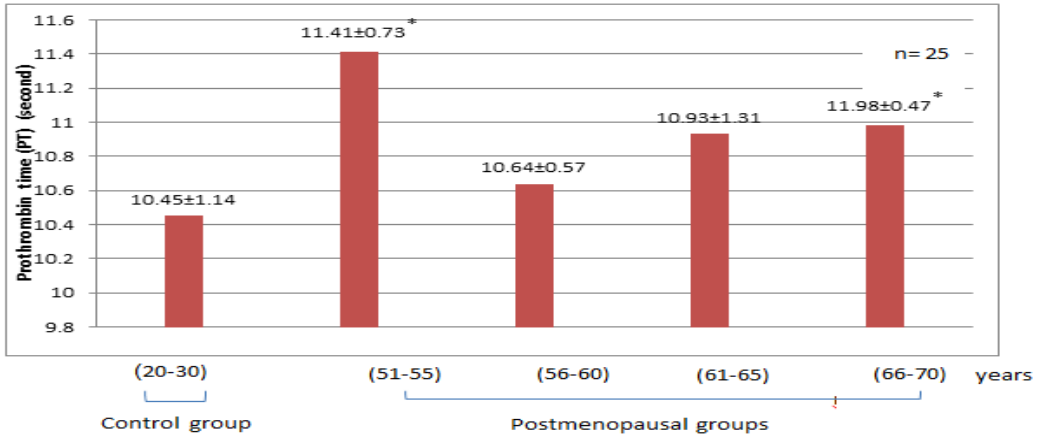

Figure (4-2) The means of prothrombin time (PT sec.) in postmenopausal and premenopausal women (control group).

-n = number of women in one group.
-Values are means ± SD.
-Means with a strik (*) are significantly different at $p<0.05$.

4.3 Results of activated partial thromboplastin time (APTT)

The results of APTT in Figure (4-3) were non-significantly different (p>0.05) of menopausal women in groups (51-55 , 56-60 , 61-65 years, respectively (30.22 ± 0.64 , 30.05 ± 1.57 , 29.65 ± 1.77 sec., respectively) when compared with the control group (29.65 ± 1.52 sec.). In contrast, it had been found that there was a significant increase (p<0.05) of APTT level of menopausal women in group (66-70) years (31.05 ± 1.56 sec.) in comparison with the control group.

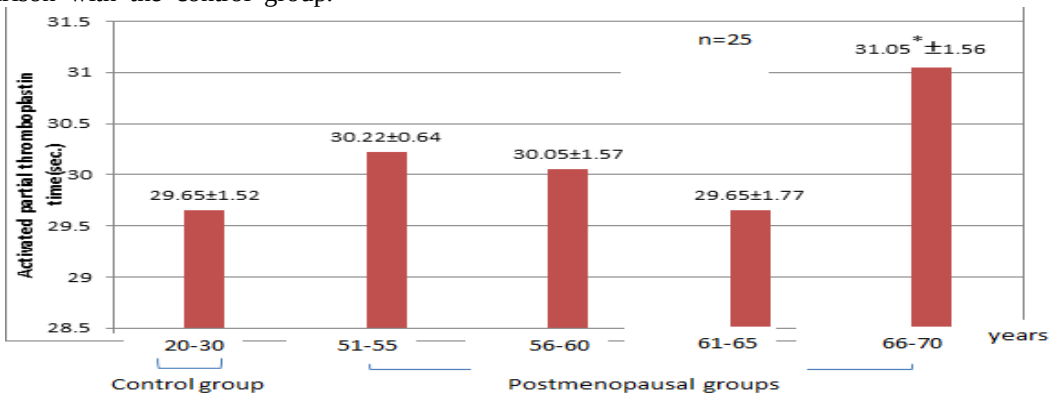

Figure (4-3)The means of activated partial thromboplastin time (APTT sec.)in postmenopausal and premenopausal women (control group).

-n = number of women in one group.

-Values are means ± SD.

-Means with astrik (*) are significantly different at p<0.05.

4.4 Levels of fibrinogen concentration (Factor I)

The levels of fibrinogen which illustrated in Figure (4- 4) pointed out a significant elevation (p<0.05) in all groups of postmenopausal women(333.4 ± 39.13 , 335.5 ± 10.39 , 336.3 ± 28.21 , 382.5 ± 52.77 mg / dl., respectively) in comparison with those of the control group (256.4 ± 23.35 mg / dl).

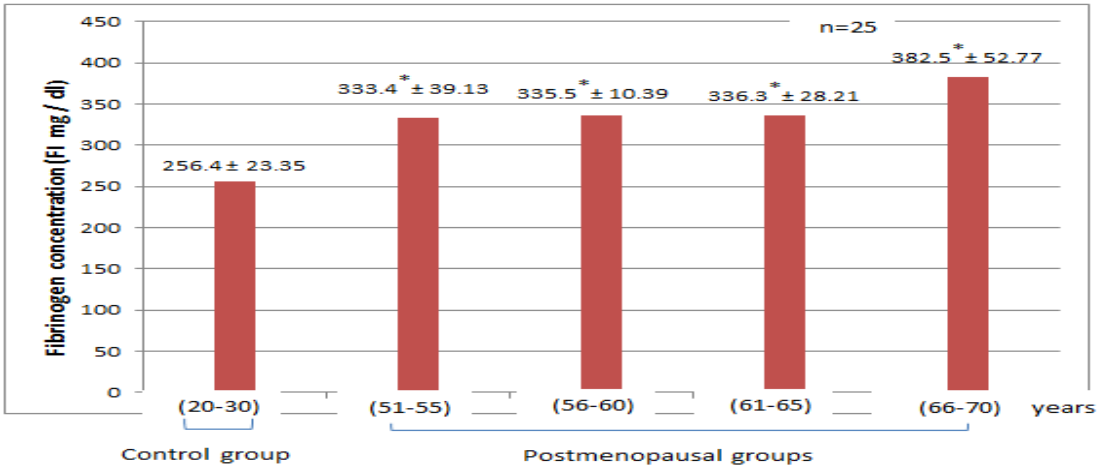

Figure (4-4) The means of fibrinogen concentration (F I mg / dl) of postmenopausal women and premenopausal w _ en (control group).

-n = number of women in one group

-Values are means ± SD.

-Means with astrik (*) are significantly different at p<0.05.

4.5Correlation coefficient between fibrinogen level and estradiol hormone in premenopausal and postmenopausal women

There is a significant negative correlation (r = 0.645) between fibrinogen concentration (FI) and estradiol concentration of premenopausal and postmenopausal women (Fig 4-5)

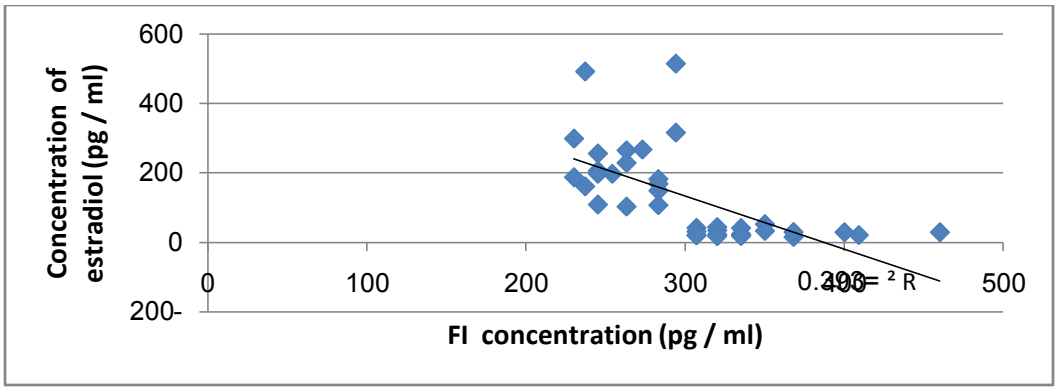

Fig. (4-5) Correlation coefficient between FI concentration and estradiol hormone levels in premenopausal and postmenopausal women.

4.6 The concentration of stable factor (FVII)

The results obtained and elaborated from the present study in Figure (4-6) showed a significant drop ($p<0.05$) in factor VII concentration of postmenopausal women groups (140.46 ± 28.51 , 148.87 ± 29.97 , 139.6 ± 25.98 , 156.88 ± 16.85 pg / ml., respectively) when matched with those of the control group (199.3 ± 48.48 pg / ml).

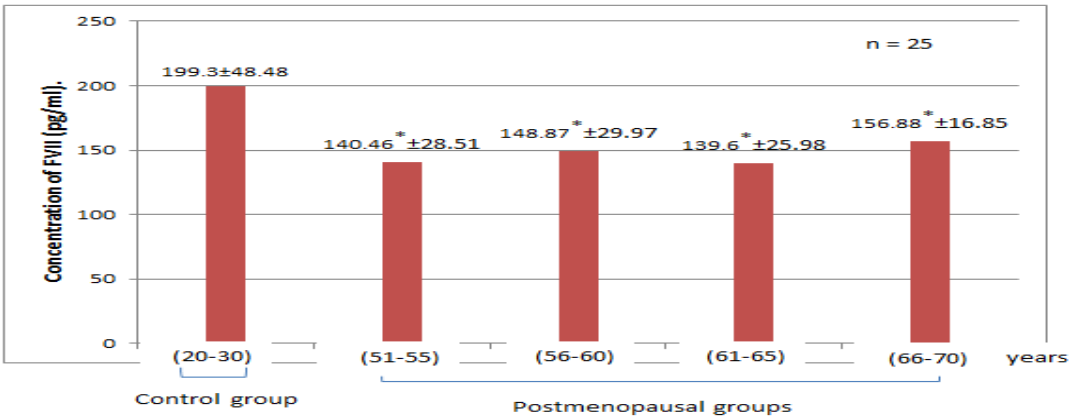

Figure (4-6) The means of stable factor (FVII mg / ml) in postmenopausal and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

-Means with astrik (*) are significantly different at $p<0.05$.

4.7The correlation coeffecient between FVII level and estradiol hormone in premenopausal and postmenopausal women

The results of correlation in Figure (4-7) showed a positive correlation (r = 0.423) between FVII concentration and estradiol concentrations of premenopausal and postmenopausal women.

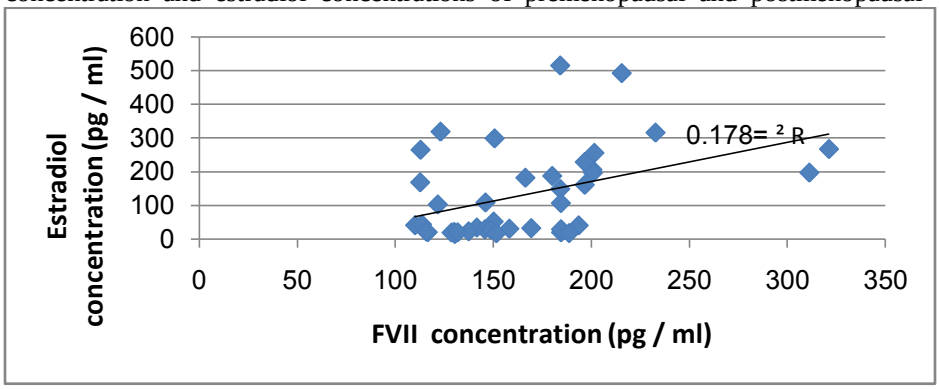

Fig. (4-7) :- Correlation coefficient between FVII concentration and estradiol level in premenopausal and postmenopausal women .

4.8Values of antihemophilic concentration factor(FVIIIpg/ml)

The results presented in Figure (4-8) tended to be non- significantly different ($p>0.05$) in FVIII concentration of postmenopausal women groups (51-55 years) and (56-60 years) (2.40 ± 0.89 , 2.33 ± 1.32 pg / ml , respectively) when compared with control group (3.20 ± 1.54 pg / ml). While the results in groups (61-65 years) and (66-70 years) showed a significant fall ($p<0.05$) of FVIII concentration (2.09 ± 0.69 , 1.8 ± 0.86 pg / ml, respectively) when compared with the control group (3.20 ± 1.54 pg / ml).

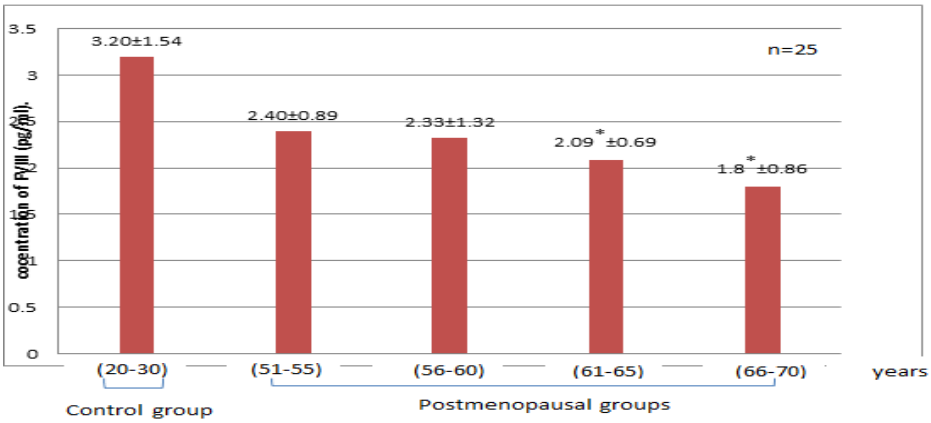

Figure (4-8) The means of anti- hemophilic factor (FVIII pg/ml) in postmenopausal women and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

-Means with astrik (*) are significantly different at $p<0.05$.

4.9Correlation coefficient between FVIII level and estradiol hormone in premenopausal and postmenopausal women

The present study confirmed a significant positive correlation ($r = 0.344$) between FVIII concentration and estradiol concentration in premenopausal and postmenopausal women (Fig 4-9).

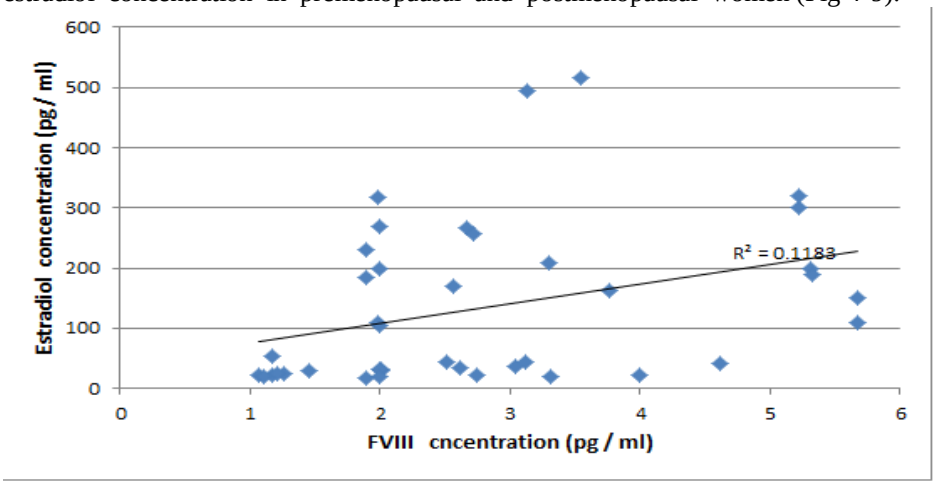

Fig.(4-9) Correlation coefficient between FVIII concentration and estradiol levels in premenopausal and postmenopausal women.

4.Discussion

Estradiol hormone

The present study showed a significant decrease ($p < 0.05$) of estradiol hormone level in groups of postmenopausal women compared to that of premenopausal women (control group).

As mentioned previously, menopause is defined as the point in life when menstrual flow continuously stop because of the complete missing of ovarian oocytes . Women became menopause when their menses

stop at least one year. Being a permanent cease of fertility and menopause occurs at age 51 years old (Santoro and Randolph , 2011).

The ovary of females releases three types of sexual steroid hormones including : estrogens , progesterone , and androgens . Synthesis of sex hormones varies with ovarian activities . Hormonal secretions in the menstrual cycle involve increasing 17β-estradiol (E2), but decrease progesterone concentrations during follicular phase, and upper plasma 17β-E2 and progesterone levels during luteal phase. If the concentration occurs, corpus leutum is developed and 17β-E2 and progesterone concentration continue high. At the age of menopause with the exhaustion of ovarian follicles , sex hormone levels fall to minimum levels (Bouman *et al.* , 2005).

Prothrombin time (PT)

In the present study , (PT) values were significantly increased ($p< 0.05$) in the first groups (51-55 years) and (66-70 years) of postmenopausal women. It is found that there are non- significant differences in other groups of postmenopausal women .

Our study is in consistent with the study of Kamal *et al* . , (2007) that indicated a decrease or inhibition of clotting factors that mediate the extrinsic and final common pathway leading to prolongation of the PT. Prothrombin time (PT) determines the sufficient of the extrinsic and the common clotting pathway . Insufficient of factor VII (stable factor) or vitamin k and anticlotting warfarin therapy can cause a prolongation of the PT (Gadag *et al* . , 2010).As previously illustrated , the PT test reflects concentration and normal activity of coagulation factors in extrinsic pathway which include the following factors (Fibrinogen , FII , FV , and FVII). All of these factors are manufactured in hepatocytes (Chambrlain , 1995).

The present study agrees with the study of Arslan *et al*.,(2011) who reported that the prolongation of prothrombin time (PT) in postmenopausal. Pasa *et al*., (2010) who reported the hormone replacement therapy elevation fibrinogen and d-dimer levels because of the increase in its clearance, but PT and APTT did not reach normal levels.

These results may be attributed as results of estrogen deficiency that can cause insufficient clotting factors production involved in extrinsic or common pathway of blood coagulation.

Active partial thromboplastin (APTT)

The data of the present study indicate that APTT values were non-significantly different ($p > 0.05$) in most postmenopausal women groups except the fourth group, they tend to be increased significantly ($p<0.05$).

The APTT reagent is named partial thromboplastin due to tissue factor which is not included in association with the phospholipid substance as it is in the PT reagent (Bajaj *et al* . , 1999). As previously illustrated, the APTT test gives the concentration and normal activity of coagulation factors in intrinsic pathway such as (FIX , FVIII , FXII , FX , and FXI) (Lewis , *et* al. ; 2006) . Absence or inhibition of coagulation factors within the intrinsic and final common pathway leads to the increase of the APTT (Kamal *et al* . , 2007).

Smith and Morrissey (2004) revealed that the a PTT is elevated at the upper limit of normal range with genetic or acquired intrinsic factor disorders < 40 % (factor VIII: c , factor XI , factor XII , and von Willebrand) lupus anticoagulants or other inhibitors of the intrinsic cascade of clotting factors . Turgeon , (1999) showed that the APTT may be increased because either a factor drop or the presence of circulating anticoagulant.

The deficiencies PT and APTT can be associated with elevated levels of coagulants including fibrinogen and fibrin degradation products (d-dimer), which leads to its lowered clearance (Pasa *et al.* ; 2010). It may be because estrogen hormone exerts anabolic effect on protein synthesis in liver, and thus aging and deficiency of estrogen leading to impair clotting factor synthesis implicated in prolongation of APTT.

Fibrinogen (F I)

The results obtained from this research indicated that fibrinogen concentrations reported a progressive increase in postmenopausal women ($p<0.05$) as compared to that of premenopausal women and its concentrations has a negative correlation with estradiol hormone ($r = 0.645$) .

FI is a liver-synthesized proteins which is the primary precursor of fibrin in the clotting process . Levels of fibrinogen are more in women than of in men, and primarily in postmenopausal women (Fischbach , 2004) . Cushman *et al* . , (1999) explained that fibrinogen dropped with the use of postmenopausal estrogen therapy . Fibrinogen (FI) appeared in atherosclerotic lesions (Topol , 2002) , and more level are associated with cardiovascular risks (Kofoed *et al.* , 2003).

Meade *et al* . , (1993) showed a significant positive correlations of fibrinogen and stable factor (FVII) with ischemic cardiac disease. Fibrinogen is the major known clotting factor and has been implicated to

carotid atherosclerosis events (Chapman *et al.* , 2004) . Also Bonithon-Kopp *et al.* , (1991) explained a positive association between carotid atherosclerosis disease and fibrinogen level included only in women. The present data agreed with study of Vigen *et al* . , (2007) who indicated that FI levels rise with postmenopausal age . Also Falsom *et al.* , (1991) and Lip *et al.* , (1997) demonstrated that plasma fibrinogen levels elevate with aging and depresses with administration of hormone replacement therapy (HRT).

It has been found that plasma levels of fibrinogen generally elevated with age (Tarallo *et al* . , 1992 and Ishikaws *et al* . , 1997). This age-related up regulation of plasma fibrinogen level could be because of as minimum rate of degradation of fibrinogen substance , rather than an elevated synthesis rate (Fu and Sreekumaran Nair , 1998). Hepatic expression of the genes activity for many clotting and fibrinolytic protein factor is also maintained by estrogen through estrogen receptors on hepatocytes . Plasma fibrinogen concentration are down by prolongation of estrogen therapy (Salomaa *et al.* , 1995).

Koh *et al* . , (1997) revealed that estrogen also inhibits plasma levels of the anti-fibrinolytic protein factors such as plasminogen activator inhibitor type I , also , Gebara *et al* . , (1995) explained that more serum estrogen levels are related with an elevated special potential for fibrinolysis process.

From these results, it is suggested that the production of fibrinogen by hepatocytes is independent on estrogen hormone stimulation or degradation process of fibrinogen that is depressed with aging.

Stabile factor (FVII)

Statistical analyses of the present study showed that factor VII concentrations were significantly decreased ($p<0.05$) in postmenopausal women compared to premenopausal women , and have a positive correlation coefficient with estradiol hormone ($r = 0.423$).

FVII takes part to the change of fibrinogen (inactive) to fibrin (Fishbach , 2004) . High FVII levels are associated to elevate risk factor of cardiovascular disease (Meade *et al.* , 1993). FVII concentration are found to be elevated with administration of estrogen therapy in post menopause (Cushman *et al.* , 1999).

FVII and fibrinogen can be synergize to atherosclerosis events , marked by thrombotic precipitation located in atherosclerotic lesions (Hansson , 2001). Estrogen therapy in post menopause is also linked with increased FVII (Cushman *et al.* , 1999). FVII is minor in premenopausal than of in postmenopausal women and lower in women compared to men (Scarabin *et al.* , 1996). Factor VII and TFPI are essential components of the clotting mechanism , and when tissue factor (TF) is emerged to circulating blood , it links and stimulates factor VII (Rapaport and Rao , 1995).

The central key of factor VII is provided by epidemiological studies which hypothesized that the clotting activity of factor VII is a risk marker of ischemic heart disease (Junker *et al.* , 1997). Factor VII activity is attacked by polymorphism in the factor FVII gen(De Maat *et al.* , 1997), as well as environmental stress , e.g. diet , weight loss , and exercise (Connelly *et al.* , 1992 ; Markmann *et al.* , 1998). Moreover , factor VII become elevated with age (Scarabin *et al.* , 1996 ; Wright *et al.* , 1997) and is altered by hormonal fluctuation , i.e. gestation , menopause , and hormone replacement therapy (Dalaker , 1968 ; Scarabin *et al.* , 1996; Falsom *et al.* , 2000).

Our study is not in consistent with Kroon *et al.* , (1994) ; Salomaa *et al.* , (1995) ; and De Valk-de Roo *et al.* ,(2000) who showed the elevation in protein concentration . Kroon *et al.* , (1997) reported a drop in factor VII activity with HRT accompanying transdermal estradiol and oral med Roxy progesterone acetate (MPA) in normal healthy women. This fall in FVII is likely resulted by the oral norethisterone substance , as both transdermal estradiol and oral estradiol component elevate factor VII potency . The study of Kroon *et al.* ,(1994), also explained that the FVII heightening is most prominent with oral intake HRT; but also transdermal estrogen therapy up regulates factor VII .

Anti-hemophilic factor (FVIII)

The results of FVIII which are illustrated in fig.(4-8) were significantly decreased ($p<0.05$) in postmenopausal women groups in a comparison to that of premenopausal women and have a positive correlation coefficient with estradiol hormone ($r = 0.344$). Previous study recorded elevated levels of factor VIII activity as an independent risk factor in the incidence of venous thromboembolism risk (VTE) (Kyrle *et al.* , 2000).

Also , these maximum levels of factor VIII appear independent of a potential acute phase reactant increase in factor VIII activity anticipated following development of acute deep vein thrombosis (DVT) (Kamphuisen *et al.* , 1999) and remain independent of other essential markers of inflammation process (O'Donnel *et al.* , 2000). The present data disagrees with study of Wells *et al* . , (2005) that identified an elevated risk for primary idiopathic and recurrent idiopathic thrombosis in patients with increased factor VIII activities .

Balleisen *et al* . , (1985) explained that levels of factor VIII do not vary by intake of hormones or oral contraceptives drugs , and suggested that levels of factor VIII may be associated to age and illustrated

factor VIII levels were progressively increased in postmenopausal women when compared to premenopausal women, and this study is not in consistent with present data.

de Visser *et al* . , (2003) showed that factor VIII levels is lowered after disruption of anticoagulants therapies, but Palaret *et al* . , (1995) demonstrated that this fall was in a short time. Study of Koster *et al* . , (1995) and Gonzalez *et al* . , (1999) pointed out that subjects with non – O blood types have maximum levels of factor VIII than of those with type O. Heightened levels of clotting factors , such as fibrinogen , factor VIII , factor VII , tissue factor (TF), and von Willebrand (vWF) had been linked to elevated cardiovascular disease risk (CVD) (Morange *et al*. , 2007 ; Speil *et al*. , 2008).

A lot of studies had been designed to explain the effects of wholediest therapies on incidence of cardiovascular risk factors (Zarraga and Schwarz , 2006 ; Giugliano and Esposito , 2008). Passaro *et al* . , (2008) showed that FVII and FVIII activity were clearly lowered , whereas fibrinogen levels did not show prominent alteration during this study. These data elucidate differential influences of diet on those blood clotting factors that are essentially synthesized by the hepatocyte , a major organ associated with metabolic activities.

These results show that estrogen hormone is an activator factor in the stimulation of expression of some clotting factors affecting protein synthesis mechanism and this stimulation is selective on most if not all of clotting factors.

Conclusions

From the obtained results , it is concluded that :

1- There is a positive effect of estrogen deficiency on blood clotting mechanism through the depression of clotting factors production and so prevention of blood thrombosis.

Recommendations

The present study recommends to perform further studies in :-

1- Investigating concentrations and activities of other clotting factors involved in intrinsic, extrinsic, and common pathway of blood coagulation.
2- Performing experimental study on ovariactomized animals and no-ovariactomized animals to estimate clotting mechanisms.

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