

Assessment of Clotting Factors (F I, FVII, FVIII, and FIX) and Estradiol Hormone Deficiency in Postmenopausal Women

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Abstract

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Women complained from several physiological and biochemical disturbances when they become aged and loss adequate amount of estradiol hormone secreted from ovarian follicles. The present study was conducted to evaluate the concentration of some available clotting factors and feminizing hormone (estradiol hormone) in menopausal women . A total number of participants postmenopausal women were sixty (60) women and their ages were 50 to 69 years old and fifteen (15) premenopausal women, their ages (control group) that they have normal menstruation cycle and whose ages ranged between 20 – 25 years old. The aging women (menopause) were subdivided according to their ages periods into four groups, the first group (50 – 54 years), second group (55 – 59 years), third group (60 - 64 years), and fourth group (65 – 69 years). Values of estradiol hormone recorded a significant drop in all groups of postmenopausal women in matching with counter parts of premenopausal women. About prothrombin time (PT), its results showed a remarkable elevation ($p < 0.05$) in all groups of menopausal women when compared with control group. Values of intrinsic pathway test (activated partial thromboplastin time) indicated remarkable increase ($p < 0.05$) in all tested groups (menopausal women) compared to control group. About clotting factors, levels of fibrinogen (FI) concentrations did significantly progressive elevated ($p < 0.05$) in all postmenopausal women groups in matching with those of control group. Also, concentrations of stable factor (VII) were significantly increased ($p < 0.05$), whereas antihemophilic factors (VIII) and Christmas factor (IX) pointed out a significant progressive fall ($p < 0.05$) of (Any groups of aging women should be determined) aging women in a comparison with premenopausal women. In conclusion, deficiency of estradiol hormone in postmenopausal women appears associated with fluctuation of clotting factor concentrations and these disturbances can give an indicator that estradiol hormone maintain hemostatic mechanism to prevent risk of cardiovascular problems.

Keywords: Estradiol Hormone, Clotting Factors, Menopause

INTRODUCTION

Menopause, a physiological phenomenon results from aging that associated with absent of ovarian follicles and stop of menstrual cycles. Generally, when women do not menstruate for at least one year, they become menopausal women and this event often occurs of most women at age 50 years old therefore women become infertile (Santoro and Randolph, 2011). The aging that associated with menopause is dramatic change affects most if not all physiological functions of female body systems. Among these systems, hemostatic system and whose components are clotting factors which can be fluctuated and become predisposing risk factors for incidence of cardiovascular diseases (Ylikorkala, 1992). hormone replacement therapy (HRT) affects pro coagulant and pro anticoagulant lead to increase some

activity of clotting factors (Lowe et al., 1997), therefore the menopause is implicated in different complications of cardiovascular system and influence psychological and emotional disturbances and it represent a critical event of women life and consider a risk factor for incidence of many metabolic and hemostatic disturbances (Cowman et al., 2015).

MATERIALS AND METHODS

Women of the study

The present research was carried out throughout the period from October 2017 to April 2018 in college of

medicine / university of Baghdad, Marjan teaching hospital / Babylon, and other private clinical laboratories. Sixty (60) menopausal (The researcher mentioned in the abstract postmenopausal women, while in the Materials and methods are mentioned menopausal women. I hope uniformity in all research) women were recruited in this study that they are finished their menstrual cycles about six month ago and free from chronic diseases and of their ages ranged between 50 to 69 years old, and they are divided according to their ages into four groups (50 -54 years, 55 – 59 years, 60 – 64 years, and 65 – 69 years old). Twenty five (25) premenopausal women that they had normal regulatory menstrual cycle(without pregnancy, no contraceptive drugs, no lactation, without ovarian cysts, non- smoker) and their ages were limited from 20 to 25 years old they were included in this study to serve as a control group .

Collection of blood samples

The blood samples were collected from 8 to 10 am. Woman was asked to set on a chair at least 10 minutes before collection. Anticubital vein was employed to take the blood samples. The site of skin was clean with alcohol solution (70 %) to prevent contamination and warmed to improve blood perfusion. Needless with gauges 22 were used. After that, the blood directly transferred to tubes containing sodium citrate anticoagulant to prevent coagulation and then the samples were centrifuged at 3000 rpm for 15 minutes to obtain plasma. The plasma was immediately pipetted to eppendorf tubes to perform all tests within one hour after

blood collection time.

Estimation of estradiol hormone levels

Estradiol hormone concentration was estimated according to instructions of Biomerieaxu company / France that based on ELISA test.

Determination of Prothrombin time (PT) test

PT test was evaluated by principles instructed by Biomerieaxue Company / France. The test was carried out at 37 C with presence of tissue thromboplastin reagent and calcium solution. The reaction was complete within seconds (formation of clot) when tubes containing samples were put in a water path at 37 C .

Determination of activated partial thromboplastin time (APTT)

The reagents involved to determination of APTT were Cephalin and Kaolin. The reaction performed when samples – reagents mixture put within water bath at 37 C (Biomeriaxue company / France) and unit of this test are seconds when clot starts.

Measurement of fibrinogen concentration

Fibrinogen (FI) level was determined by instruction of Biolabo Company that based on thrombin amount converting fibrinogen to fibrin and clotting time was inversely proportionated to concentration of fibrinogen concentration.

Levels of stable factor (FVII)

Concentration of FVII was determined by principles of Elabscience Company that based on ELISA kit to estimated levels of FVII in vitro.

Concentration of antihemophilic factor (FVIII)

The quantitative method by Elabscience company was conducted to evaluate FVIII in vitro according to ELISA kit technique supplied by those company.

Measurement of Christmas Factor (FIX) concentration

According to ELISA kit technique supplied by Elab-

Table 1. Shows the results of estradiol hormone, prothrombin time (PT), activated partial thromboplastin time (APTT), stable factor (FVII), antihemophilic factor (FVIII), and christmas factor(FIX) in postmenopausal and young women (control group).

Groups		Control group (20-25years)	First group (50-54 years)	Second group (55- 59years)	Third group (60-64years)	Fourth group (65-69years)
Parameters						
Estradiol	hormone	270±50.71	40± 9.521 *	43±10.26*	39.8±8.67*	35.77±5.98*
	pg/ml					
PT (second)		11±1.1	13± 1*	14±2*	14±1*	15±2*
APTT (second)		28±3	33±2*	35±2*	34±1*	35±1*
FI (mg/dl)		280.3±15.91	350±22.85*	325.18.72*	315±17.48*	300±10.56*

FVII (pg/ml)	124.2±30.12	150.7± 25.24*	160.44±19.38*	167.4±27.34*	145.67±23.19*
FVIII (pg/ml)	5.2±1.34	3.5±1.91*	2.7±1.88*	2.51±1.51*	2.8±1.95*
FIX (pg/ml)	5.5 ± 1.29	3.5± 0.91*	4.1±0.5*	3.22±1.22*	3.9±1.47*

-Values are means ± SD .

- Values with a strike are significantly different at $p < 0.05$ compared to control group.

science Company, concentration of FIX was carried out in vitro of plasma specimens that derived from a whole blood samples.

Statistical analysis

All values of the present study were shown as means ± Standard deviation (SD). the results were analyzed by ANOVA according SPSS program and students t-test was used to explain the differences among each tested groups and control group, the $p < 0.05$ was represented as a lowest significant difference (Daniel, 1999).

RESULTS

Results that are obtained from the present study and illustrated in the following Table 1 indicated the different changes as follow:-

- The levels of estradiol hormone were markedly drop ($p < 0.05$) in all age groups of menopausal women when compared with those control group (premenopausal women).
- Results of PT indicated a significant heightening ($p < 0.05$) in all tested groups when they compared with their counterparts of control women.
- Concerning values of APTT were also showed significantly increased ($p < 0.05$) in all groups of menopausal women in matching with control group.
- In regard to fibrinogen concentration (FI), all postmenopausal women groups pointed out a remarkable increase ($p < 0.05$) compared to those of control group.
- About concentration of stable factor (FVII), its results had been also a significant increase ($p < 0.05$) in all tested groups of aging women in matching with young women (control group).
- As far to FVIII concentration, it had been found a significant drop ($p < 0.05$) in all postmenopausal women groups in a comparison with those of young women.
- Regarding of Christmas factor (FIX) concentration were significantly fall ($p < 0.05$) of all aging women compared to control group.

DISCUSSION

It is not surprising that exhaustion of all ovarian follicles (a major source of estradiol) is associated with sharp drop of estradiol hormone. Women and all females of other species of animal have limited number of oocytes that tend to decrease systematically during menstrual cycles because of no renewal of these cells (Bouman et al., 2005).

Thrombin time test results obtained from this study were consistent with previous study of (Arslan et al., 2011) who confirmed an increase of thrombin time of menopausal women. However , it was found women who had administered hormone replacement therapy (HRT) , a combination of estrogen compounds , lead to increase concentration of fibrinogen but the level of prothrombin time and activated partial thromboplastin time remain within normal values (Pasa et al., 2010). It is well found that inhibition of any clotting factor within intrinsic and common pathway causes prolongation of APTT (Kamal et al., 2007). APTT increases as a results of defect or drop the activity or concentration of clotting factor (Turgeon, 1999). It may be that estrogen deficiency implicated in the prolongation time of PT and APTT when women become old.

Cardiovascular risk factors in women who are found to be associated with increase fibrinogen levels as results atherosclerosis diseases that seem more prominent in postmenopausal women and indicated that women have significant level of fibrinogen in matching with men (Kofoed et al., 2018). Furthermore, administration of estrogen replacement therapy can inhibit fibrinogen production in postmenopausal women (Cushman et al., 1999). There is found that a relation between fibrinogen (FI) and FVII associated with increasing incidence of cardiovascular problems (Mead et al., 1993). The present data agree with study carried out by (Vigen et al., 2007) they confirmed elevation level when women become menopause, moreover, another study showed increase fibrinogen with advanced age without administration HRT of postmenopausal women (Lip et al., 1997). In fact, hepatocytes express many genes encoding clotting factors and fibrinolytic factors become down when administration of estrogen therapy (Salomaa et al., 1995).

It is well documented that stable factor (FVII) increases with aging, exercise, type of diet, and body weight loss (Markmann et al., 1998). The present data are supported by (Devalk et al., 2000) who indicated increase FVII and other protein with aging. In addition, experimental study showed that administration of transdermal estrogen therapy leads to drop of FVII levels (Kofoed et al., 2018). Study of (Wright et al., 1997) illustrated up regulation of FVII when women become aging, menopause, and gestation.

Concerning the level of anti – hemophilic factor (VIII), the present findings showed decrease FVIII in all postmenopausal women. (21) pointed that increase activity of anti-hemophilic factor prove increase incidence of deep venous thrombosis .In fact, it was found that level of anti-hemophilic factor independent to acute phase protein and other inflammatory (Odonnell et al., 2000), whereas (Palareti et al., 1995) and his colleagues confirmed that this drop is short time, Moreover , it is confirmed that

subjects who have O – blood group also they have a high levels of FVIII comparing with those non O – blood group (Gonzalez et al., 1999). The present study can be concluded a stimulating role to induce production of FVIII when women have normal menstrual cycles.

Previous study indicated that endogenous estrogen hormone has ability to suppress or delay cardio – vascular diseases, it conducts to modulate fibrinolytic mechanism with no effects on clotting factors and inflammatory markers (Sowers et al., 2005). Study carried out by (Bucciarelli and Mannucci, 2009) showed old aged humans are complained from alteration of hemostatic mechanism in different pathways including blood coagulation factors, fibrinolysis components, platelet functions, and endothelium of vascular system. This study concluded that with aging increase incidence of thrombosis and increase hypercoagulable state and thus can conflict with present data

CONCLUSION

In conclusion, it appear clearly that estradiol hormone has effective modulating role in regulation of clotting factor concentrations and their activities during premenopausal period and this regulatory mechanism

tend to disturb when estradiol hormone become deficient when menstrual cycle stops at menopause

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