The effects of Estradiol Deficiency on the Level of Lipids and Proteins of Postmenopause 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 .

This study included125women, of those, 100 women were post menopause aged between(51-70),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.

The results of estradiol hormone (E2) were significantly decrease (p<0.05) in all groups of postmenopausal women in comparison with premenopausal women.

Concerning biochemical alterations, especially lipid profile, they showed a significant increase (p<0.05) in the levels of cholesterol, triglycerides, and low density lipoprotein (LDL) concentrations in all aging women groups in comparison with premenopausal women. In contrast, levels of high density lipoprotein (HDL) were progressively declined in all aging women compared to the control group.

Regarding to the results of protein electrophoresis, it is found that the levels: albumin, beta globulin, and gamma globulin were significantly increased (p<0.05) in all groups of postmenopausal women. Values of alpha 1 and alpha 2 globulin pointed out a significant lowering (p<0.05) in most groups of postmenopausal women when matched with their counterparts of premenopausal women (control group).

Key words: Estradiol, lipids, protein electrophoresis.

الخلاصة

يتميز التقدم بالعمر (Aging) لدى النساء بأنخفاض شديد في وظيفة المبايض الأفرازية للهرمونات الجنسية وتدعى هذه الفترة من العمر بسن اليأس Menopause والتي تبدء بوصول المرأة لعمر 50 سنة .

صممت الدراسة الحالية بصورة أساسية لتقييم تراكيز بعض عوامل التخثر clotting factors والعوامل الكيموحيوية لدى النساء في سن اليأس. كان العدد الكلي لأشخاص الدراسة 125 أمرأة ، كان منها 100 أمرأة في سن اليأس وقد قسمت إلى أربع مجاميع طبقاً للفئات العمرية ، المجموعة الأولى (51–55 سنة)، المجموعة الثانية (56–60 سنة)، المجموعة الثالثة (61–65 سنة)، والمجموعة الرابعة (66–70 سنة). أختيرت 25 أمرأة كمجموعة سيطرة تراوحت أعمارهن بين 20–30 سنة. بينت قيم هرمون الإستراديول (E2) إنخفاضاً معنوياً (P<0.05) لدى جميع النساء بعد سن اليأس عند المقارنة مع مجموعة السيطرة بشأن التغيرات الحاصلة في مستوى الدهون، بينت الدراسة الحالية حصول إرتفاع معنوي (P<0.05) في قيم كل من الكولستيرول والدهون واطئة الكثافة (LDL) وثلاثي الكليسيرايد عند جميع النساء بعد سن اليأس في حين لوحظ حصول إنخفاض معنوي (P<0.05) في قيم الدهون عالية الكثافة (HDL) لدى النساء المسنات عند مقارنتها مع مجموعة السيطرة. أظهرت النتائج المستحصلة من الترحيل الكهربائي لبروتينات الدم Protein electrophoresis وجود إرتفاع معنوي (P<0.05) في قيم كل من الألبومين وبيتا كلوبيولين وكاما كلوبيولين لدى جميع النساء المسنات عند مقارنتها مع مجموعة السيطرة، في حين تبين وجود إنخفاض معنوي (P<0.05) في قيم كل من ألفا 1 وألفا 2 عند النساء بعد سن اليأس عند مقارنتها مع النساء قبل سن اليأس (مجموعة السيطرة).

الكلمات المفتاحية: الاستراديول، الدهون، الترحيل الكهربائي للبروتينات

Introduction

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).

Serum Protein Electrophoresis determine patients with multiple myeloma syndrome and other serum protein disturbances . A homogeneous spike-like peak in a focal area of the gamma-globulin zone explains a monoclonal gammopathy. Monoclonal gammopathies are related with a clonal process that is malignant or seriously malignant, including multiple myeloma,Waldenstrom's macroglobulinemia, solitary plasmacytoma smoldering multiple myeloma, monoclonal gammopathy of undetermined significance, plasma cell leukemia, heavy chain disease, and amyloidosis (O'Connell *et al*.,2005).

The figure of serum protein electrophoresis products is based on the fraction of two common types of protein: albumin and globulins. Albumin , the major protein constituent of serum , is manufactured by the hepatocytes under normal physiologic states. Globulins constitute a much smaller amount of the total serum protein content. The subtypes of these proteins and their relative quantity are the primary aim of the interpretation of serum protein electrophoresis (Jacopy and Cole , 2000).

Albumin represents the largest peak , and lies closely to the positive electrode. The next five components (globulins) are labeled $alpha^1$, $alpha^2$, $beta^1$, $beta^2$, and gamma. The peaks for these components located toward the negative electrode , with the gamma peak fraction being nearly to that electrode (O'Connell *et al* . ,2005).

It is incontrast to its popular image as a potent dangerous factor of health and longevity. It is primarily an essential substance that exerts several vital functions in the body. Cholesterol is required the manufacturing bile acids , which are necessary for the absorption of fats from GIT, and synthesis of many hormones such as androgens , estrogen , progesterone and hormones of adrenal cortex (Colpo , 2005).

In human body, high concentrations of triglycerides in the blood circulation have been associated to atherosclerosis and , by extension , the risk of heart disease and stroke incidence (Drummond, 2014). Diets with high refined carbohydrates , with carbohydrates representing more than 60% of the total energy intake by organism, can elevate triglycerides concentration. The inquire is how the correlation is stronger for those with higher BMI (28+) and insulin increases tance (more common among over weight and obese)which is an early suspected reason of the feature of carbohydrate-activated hyper triglyceridemia (Parks , 2002).

Aerobic exercise activity has been shown more significantly increase 9% HDL-c levels and leading to statistically down regulation in blood triglycerides 11% (Kelley *et al*. , 2006). It has also been shown that male and female patients are affected with low HDL-c level and associated with normal total cholesterol levels and have more predisposing for cardiovascular events such as heart attacks and unstable chest pain when compared to their adult counterparts with high HDL-c levels (Eapen *et al*. , 2009).

Particularly , elevated plasma levels of LDL have a risk factor to disease. Moreover, disturbances of LDL , because of the abnormalities in LDL

composition, have been determined as independent markers of risk factor for coronary heart diseases (Packard *et al.* , 2000 ; Packard *et al.* , 2006).

Materials and methods
Subjects of the study

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 starts at a period ranged between December 2014 to August 2015. One hundred and 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 the 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,the 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 Elisa test).

The protein electrophoresis on Agarose Gel

Hellabio Agarose Gels for protein electrophoresis are intended to be fluids. The clinical use of electrophoresis in protein analysis generally is based on the simple electrophoretic separation of proteins according to their relative mobility and molecular weight ,into albumin , $\alpha1, - \alpha2- \beta(\beta1,\beta2)-$,and Υ- globulin's in spite of the knowledge that each of the classical electrophoretic zones may contain which is more than one major proteins.(According to Hellabio company).

The determination of cholesterol level

The enzymatic method described by Allain and al., saying that the reaction scheme is as follows: (According to Biolabo company).

The determination of triglycerides

Fossati and Prencipe method is associated with Trinder reaction. Reaction scheme is as follows:(According to Biolabo company).

The determination of HDL cholesterol

Low density lipoproteins are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions.
The HDL fraction remains in supernatant and this is determined by cholesterol assay(According to Fortress company).

The determination of LDL cholesterol

Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (PH 5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.(According to Biorex company).

The 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).

The results

Estradiol hormone levels (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).
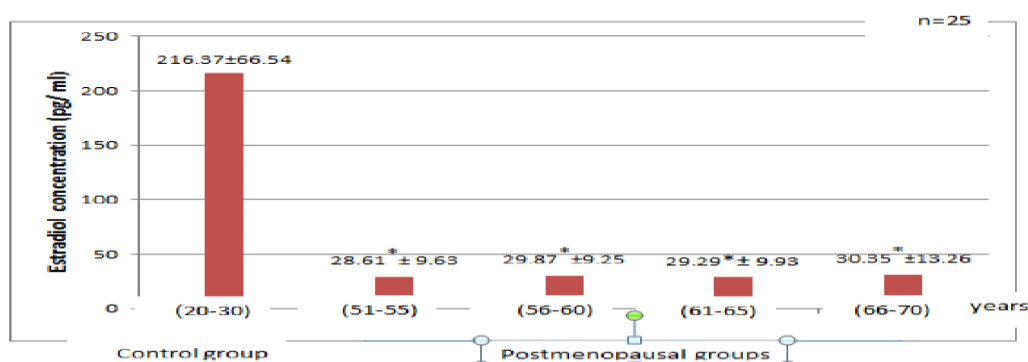

Figure (4-1) The means of estradiol levels (E2) (pg / ml) in postmenopausal women and premenopausal women.

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

The levels of protein electrophoresis

The result of albumin levels

The result which are illustrated in Figure (4-10) indicated a progressive lowering ($p < 0.05$) of albumin levels in all groups of postmenopausal women (55.37 ± 4.00 , 53.94 ± 2.79 , 55.11 ± 5.35 , 54.80 ± 3.92 % , respectively) when compared with the control group (59.48 ± 3.36 %).
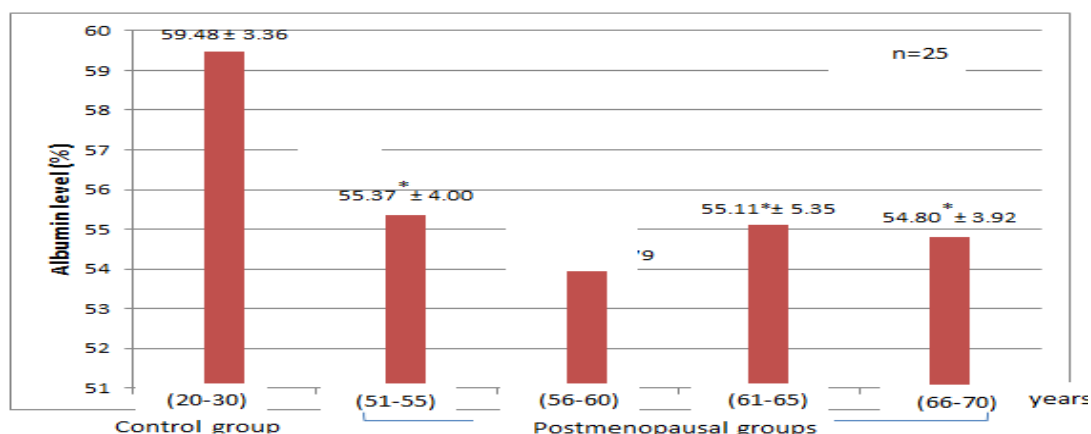
Figure (4-10) The means of albumin level (%) in postmenopausal and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

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

The values of alpha 1 levels

The resluts, which are obtained from this study and presented in Figure (4-11), recorded significant decrease (p<0.05) in alpha 1 levels of aging women groups (51-55 , 56-60 , and 61-65 years respectively) (3.50 ± 0.49 , 3.57 ± 0.58 , 3.41 ± 0.48 % , respectively) when compared with those of the control (4.12 ± 0.71 %); while the fourth group (66-70 years) pointed out non-significant fall (p>0.05) (3.61 ± 0.70 %) when compared with those of the control group (4.12 ± 0.71 %).
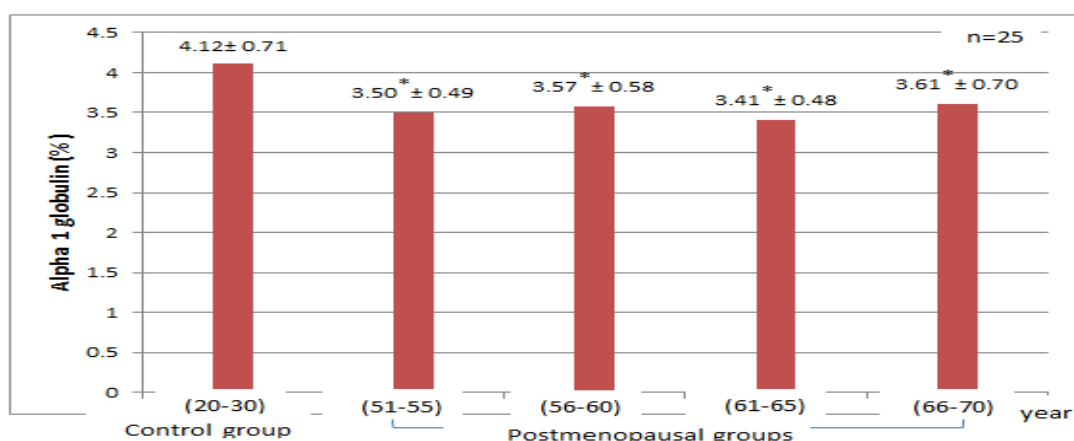

Figure (4-11) The means of alpha 1 level (%) in postmenopausal and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

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

The levels of alpha 2 globulin

The results, which are found in Figure (4-12), showed a significant elevation (p<0.05) of alpha 2 level only in the first group (51-55 years) (9.53 ± 0.91 %) when compared with the control group (8.07 ± 1.68 %) and showed non-

significant elevation in the remaining groups of postmenopausal women (8.77±1.13, 8.86 ± 1.84 , 8.77 ± 1.36 % . , respectively) comparison with the control
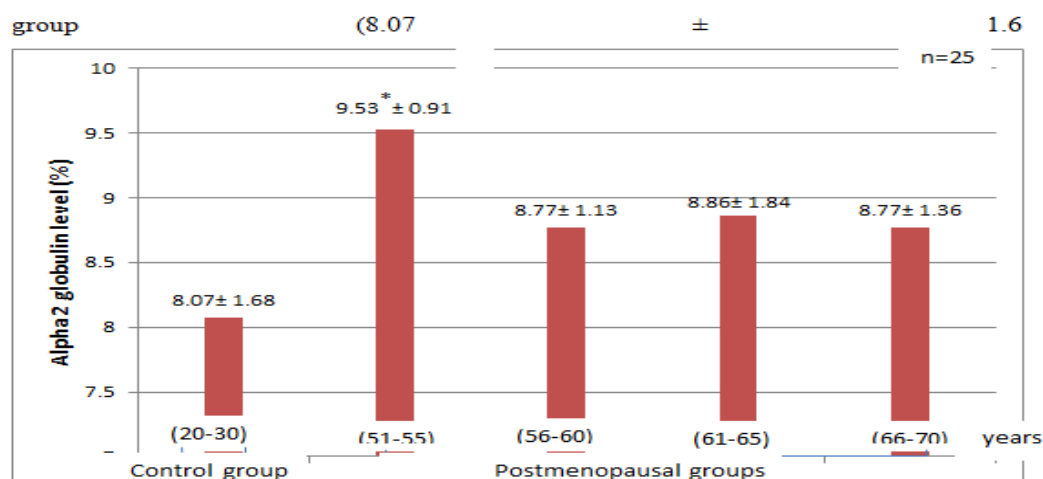
Figure (4-12) The means of alpha 2 level (%) in postmenopausal and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

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

Levels of beta globulin

In Figure (4-13) , it had been found that there was a non-significant heightening (p>0.05) of beta globulin level in the first group of post menopause women (51-55 years) (10.92 ± 1.07 %) when compared with the control group (9.86 ± 0.98 %), and found a progressive heightening (p<0.05) of beta globulin level of postmenopausal women in other groups (56-60 , 61-65 , and 66-70 years respectively) (11.64 ± 0.56 , 11.93 ± 2.06 , 12.68 ± 1.85 % , respectively) when compared with that of the control group (9.86 ± 0.98 %).
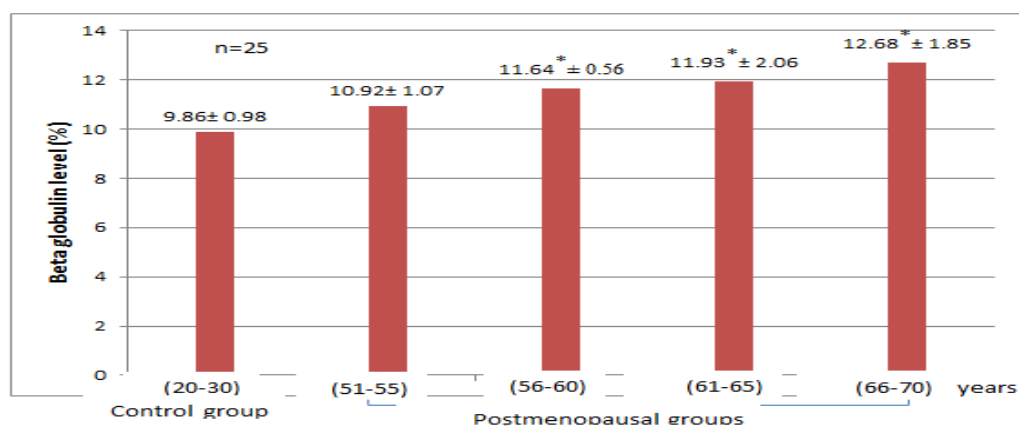
Figure (4-13) The means of beta level (%) in postmenopausal women and premenopausal women (control group).

-n = number of women in one group

-Values are means ± SD.

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

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The results of gamma globulin level

The results that are found in the Figure (4-14) showed a significant elevation (p<0.05) of gamma globulin level in postmenopausal women (20.40 ± 1.32 , 22.35 ± 1.49 , 21.71 ± 2.89 , 22.84 ± 1.62 % , respectively) when matched with that of the control group (17.98 ± 0.50 %).
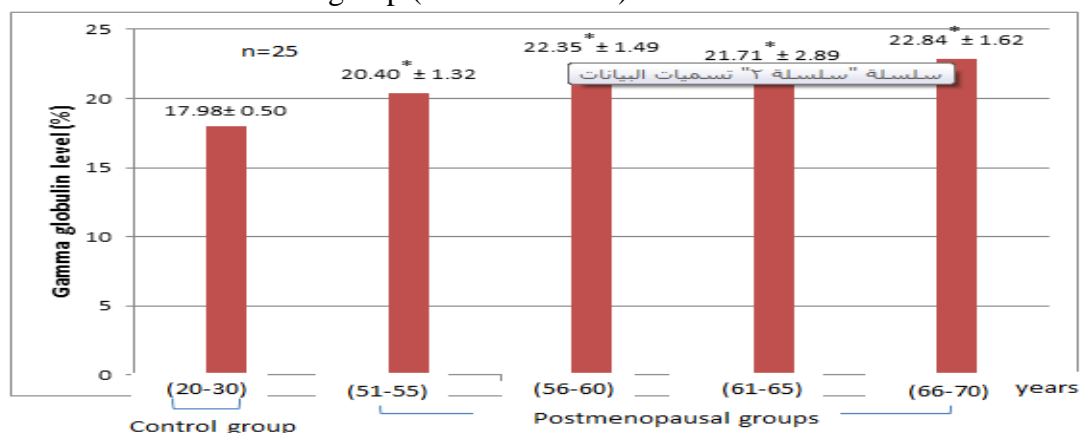

Figure (4-14) The means of gamma level (%) in postmenopausal women and premenopausal women (control group).

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

Lipid profile Testes
The Results of cholesterol level

The results, which are presented in Figure (4-15), revealed a significant elevation (p<0.05) in cholesterol level of all groups of postmenopausal women (5.64 ± 0.41, 5.92 ± 0.35 , 6.04 ± 0.23 , 6.11 ± 0.07 mmol /L, respectively) when compared with premenopausal women (5.39 ± 0.27 mmol / L).
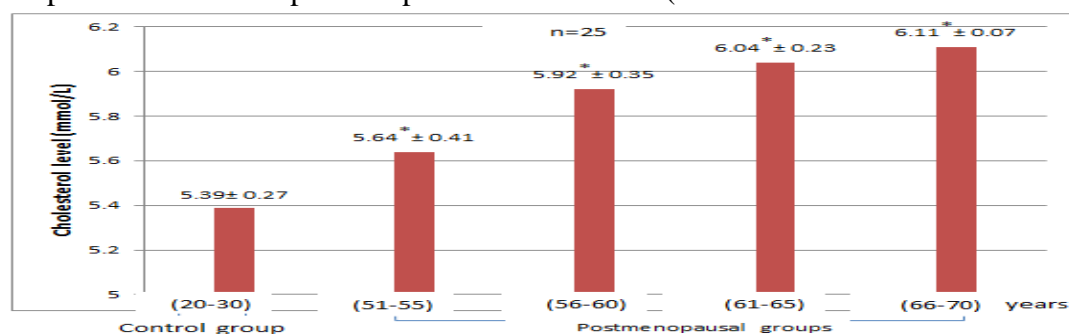

Figure (4-15) The means of cholesterol level (mmol / L) in postmenopausal and premenopausal women (control group).

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

The levels of triglycerides

The results illustrated in Figure (4-16) were significantly higher (p<0.05) of triglyceride levels in all groups of postmenopausal women (1.50 ± 0.29 , 1.40 ± 0.26 , 1.45 ± 0.31 , 1.29 ± 0.08 mmol / L , respectively) than of the control group (1.06 ± 0.17 mmol / L).
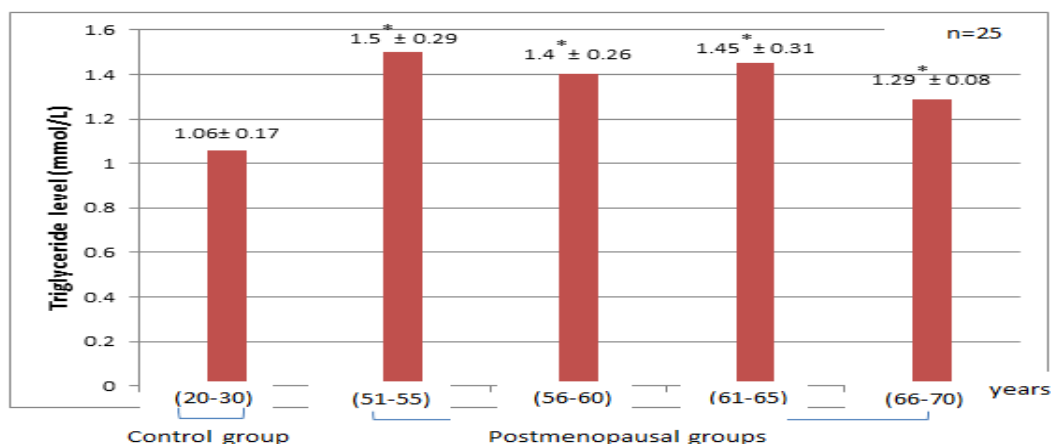
Figure (4-16) The means of triglyceride level (mmol / L) in postmenopausal and premenopausal women (control group).

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

High-density lipoprotein levels (HDL)

The results, explained in Figure (4-17), pointed out a significant lowering (p<0.05) in high-density lipoprotein level of all groups of postmenopausal women (0.78 ± 0.07 , 0.92 ± 0.15 , 0.76 ± 0.09 , 0.63 ± 0.73 mmol / L , respectively) when matched with those of the control group (1.28 ± 0.12 mmol / L).
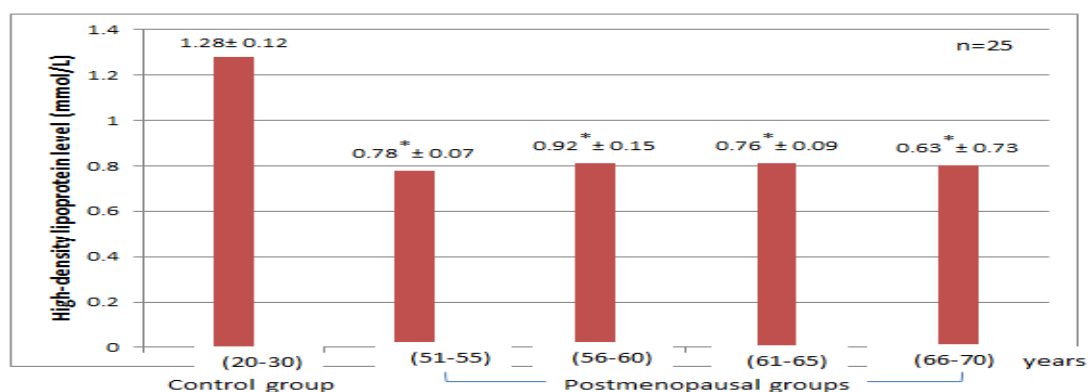

Figure (4-17) The means of high-density lipoprotein (HDL mmol / L) in postmenopausal women and premenopausal women (control group).

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

Low-density lipoprotein levels (LDL)

The results, listed in Figure (4-18), explained a significant elevation (p<0.05) in low-density lipoprotein of postmenopausal women in groups (3.72 ± 0.45 , 3.62 ± 0.49 , 3.51 ± 0.57 , 3.67 ± 0.38 mmol / L , respectively) when matched with those of the control group (2.23 ± 0.20 mmol / L) .
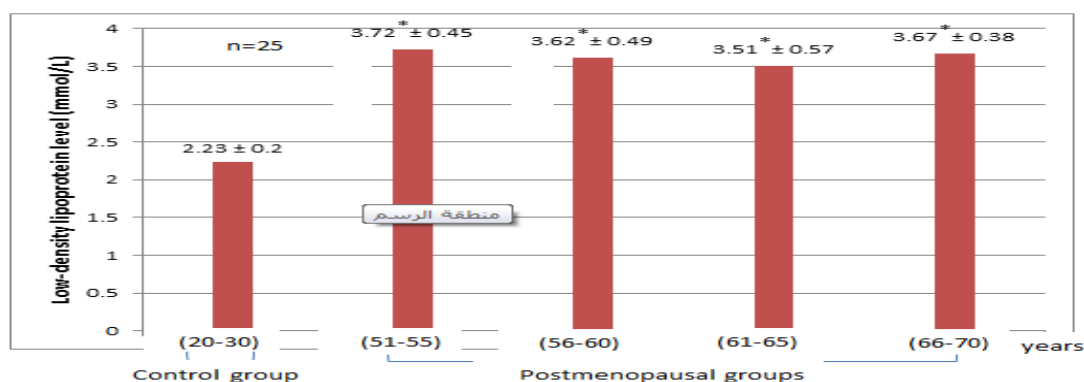
Figure (4-18) The means of low-density lipoprotein level (LDL mmol / L) in postmenopausal and premenopausal women (control group).

-n = number of women in one group.

-Values are means ± SD.

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

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).

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 decreasing 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 are lowered to minimum levels (Bouman *et al*. , 2005).

Protein electrophoresis

The albumin level

The present study is in consistent with the previous study of Kumari *et al* . , (2010) who found albumin concentration drop in postmenopausal women compared to that of premenopausal women , because in some inflammatory states the secretion of tumor necrosis factor (TNF) depresses albumin manufacturing but stimulates the production of proteins involved in the acute phase response.

Low level of serum albumin was found to link incidence of cardiovascular disease and all-reason of mortality , but the mechanisms are not well-known (Djouss , *et al*. , 2002).Our study also agree with Vigen *et al* . , (2007) that explained the albumin values that is depressed with age, production of albumin drops with infection or inflammation processes , suggesting that albumin component may be considered a marker for inflammation-related cardiovascular disease rather than a cause.

The study of Devi and Kumar , (2012) pointed out the minimum levels of albumin in females could lower the activity of hepatic protein production in the fairer sex. The present study is also in consistent with the previous studies of Seve *et al* . , (2006) ; Gupta *et al* . , (2009) that proved a low reduction serum

albumin in menopausal women and showed that the decline serum albumin (SA) is served as an independent prognostic marker in several diseases, such as tumors.

It is well recognized that in advanced age, there is an upregulation of oxidative stress and this state is related with estrogen deficiency as anti-oxidant factor. So, the elevation of free radical levels may enhance deleterious of albumin and some protein components of the body. Moreover, estrogen is anabolic hormone and enhance protein synthesis within the body and its deficiency can lead to depress protein production.

Globulin fraction

The present study agrees with the study of Fatima *et al* . , (2013) who found albumin concentration decline in postmenopausal women is compared to premenopausal women , while globulins levels showed a progressive increase and gamma globulin values showed excessive increase in postmenopausal women in comparison to premenopausal women.

Al-Joudi (2005) found that an elevation in serum globulin levels often follows drop of serum albumin level in breast carcinoma . Also , Al-Joudi and Wahab , (2004) pointed out in progressive metastatic stage , a partial compensation (elevation) of globular protein components to albumin has been found explaining the impairment of lymphocytes to increase globulins to the values that are largely enough to abolish lowered albumin levels in serum. This is occurred because, in metastatic step, the protein production is decreased and protein degradation has facilitated . This implies that the fall in human serum albumin level is further amplified by the globulin levels to compensate .

Ragno and Delmas , (1999) indicated a lower serum albumin , total protein , and globulin values in postmenopausal women groups compared to premenopausal women.

Devi and Kumar (2012) hypothesized that the globulin levels do not base only on the protein production , but they are changed by the immunoglobulin levels , as a result of which the globulin values in ageing human and gender cannot be elaborated by the protein metabolism process .

It is well established that estrogen directly maintains vasomotor tone of blood vessels through both short-term and long-term actions . Long-term intake of estrogen is related to lowered plasma levels of renin (Schunkert *et al* . , 1997) , angiotentensin converting enzyme (ACE), and endothelin-1 (ET-1) (Yilkorkala *et al*. , 1995) and depressed vascular expression of the gene for angiotensin II receptor type I (Nickenig *et al*. , 1998) as well as an elevated ratio of nitric oxide (NO) to endothelin-1(ET-1) in plasma (Best *et al*. , 1998).

From these results, it can be hypothesized that aging is related to increasing the inflammatory and catabolic process. These results indicated that estrogen hormone can be exerted inhibitory effects on inflammatory reactions.

Lipids profile

The present study explained a significant heightening (p<0.05) of total cholesterol and triglyceride levels and LDL-C in postmenopausal women , while there was a significant drop (p<0.05) of HDL-C in postmenopausal women groups in comparison to the control group .

The previous researches had shown that the women are at less exposure to the risk of developing CAD than of men , but this feature is disappeared after 60 years of age (Rich-Edward *et al*. , 1995 ; Couderc and Machi , 1999). It is well indicated that maximum values of LDL and minimum levels of HDL in postmenopausal women are largely related with the incidence of CAD (Mc

Namara *et al*., 1992). Plasma triglycerides levels also have a determinative effect on the levels of small dense LDL elements in normal healthy population (Mendelsohn and Karas , 1999).

After the age of menopause , there is a suppression of ovarian physiology. This leads to adverse alterations such as glucose and insulin hormone metabolism, body fat distribution, coagulation , fibrinolysis , and vascular endothelial abnormalities (Spencer *et al*., 1977). There is also disturbance of lipoprotein level independent of age (Bales , 2000). The study of Taddec *et al*., (1996) showed that loss of estradiol is a necessary factor in this mechanism.

Our results were inconsistent with the findings of other previous studies: Jensen *et al*., (1990) ; Igweh *et al*., (2003) . These researchers explained no significant alterations in total cholesterol levels but there was a significant fall in HDL-C and a progressive elevation in LDL-C. Many previous studies had shown the important functions of hormonal replacement therapy (HRT) on the lipid profile of menopausal women (Stampfer, 1991).

It is well recognized that ERT in postmenopausal women regulated up to HDL-C level (Kim *et al*., 1994). These observations do not explain that HDL-C level is decreased after menopause . HDL-C values progressively increased with age especially to the sixth decade and then fall in women (Johuson *et al*. , 1993).

The present findings were also in consistent with study of the Kim *et al*., (2000) who indicated that the lipid profile is regulated up with aging. Eapen *et al*., (2009) showed the risk for the incidence of coronary heart diseases fall 2% for every 1mg/dl elevated in HDL in males and 3% in females. However, premenopausal women have more HDL-C levels than of men of the similar age because of higher levels of estrogen.

Other researches stopped to support any marker for an independent activity of estradiol levels in estimating CHD in postmenopausal females (Lee *et al*., 2010 and Chen *et al*., 2011), and also other studies even confirmed a positive correlation of endogenous estradiol hormone level with the incidence of CHD among female above 65 years of age (Scarabin-Carre *et al*., 2012).

It can be suggested that estrogen enable the body tissues to clear the LDL through the induction of several enzymatic reaction involved in metabolism of lipid, also , largely increased efficiency of hepatocytes to elevate HDL components.

Conclusions

From the obtained results , it is concluded that :

1- There is a negative effect of estrogen deficiency on synthesis of essential protein components, albumin particularly, and increase production of inflammatory proteins, globulins in particular, that are markers of inflammatory process with aging.

2- High levels of estrogen hormone have a positive effects on producing a good lipid (HDL) and low levels of estrogen exerts stimulatory effects on the expression of bad lipids (Cholesterol, Triglycerides, and LDL).

Recommendations

The present study recommends to perform further studies in :-

1- Performing a comparative study between women with HRT and without HRT.

2- Investigating oxidative stress in aging women and their relations with clotting mechanism.

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