STUDY OF ENDOGENOUS SEX HORMONES AND INFLAMMATION IN ELDERLY WOMEN WITH DIABETES MELLITUS

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ABSTRACT

Background .C-Reactive Protein (CRP), an acute phase reactant has long been considered as a classic marker of inflammation. Ageing is characterized by a chronic, low-grade inflammatory status, the so-called "inflame-ageing". Diabetes is known to be also associated with low-grade inflammation .The normal process of reproductive ageing is characterized by marked hormonal changes, during menopause, estradiol (E2) level decreases dramatically, whereas testosterone level presents a small decline or remain unchanged. A large body of clinical data suggests the important roles of endogenous sex hormones in the pathogenesis of type 2 diabetes**.** In many studies in women, plasma estradiol and testosterone have been positively associated with glucose intolerance and insulin resistance, independently of adiposity**. Objective .**The aim of this study was to assess serum levels of endogenous sex hormones (estrogen and testosterone) as well as high sensitive C - Reactive protein (hs-CRP) in elderly women with and without type 2 diabetes.

Research Design. This study was done in the period between august 2009 to august 2011, The study population included a total number of 60 post menopausal elderly females, 20 healthy non diabetic elderly females as control group and 40 diabetic females. The diabetic females were further divided into two groups according to presence or absence of complications cardiovascular diseaes complicated group and non complicated group. They were also divided into two groups according to glycosylated hemoglobin level (HbA1c) to controlled group and non controlled group.

Methods. Total testosterone, total estradiol, hs-CRP and HbA1c were measured in diabetic and non diabetic elderly women.

Results. As regard hs-CRP there was a high significant difference between patients(2,2±,56 mg/L) and control group(1.60 ± 0.39 mg/L) and also there was a significant difference between complicated(2.16±0.51 mg/L) and non complicated group(2.08±0.76 mg/L) and no significant difference among diabetics whether controlled or non controlled . As regard the serum estradiol, there was a high significant difference between patients (22.5±13.9 pg/ml) and control group (12.9±2.71 pg/ml). While there was no significant difference as regard presence or absence of complication and diabetes control. As regard the serum testosterone, there was no significant difference between patients and control group, presence or absence of complication and control of diabetes.

Conclusion. In type 2 diabetic postmenopausal elderly women there was significant association between inflammation and serum estradiol while there was no association between inflammation and serum testosterone. As regarding presence or absence of cardiovascular complication and diabetes control neither of two hormones were associated with inflammation.

Keywords: Sex hormones, Postmenopausal women, Inflammation, Elderly, Diabetes.

INTRODUCTION

Diabetes mellitus in (DM) in the elderly population may ultimately prove to be the most important epidemic of the 21st century. By the age of 75, approximately 20% of the elderly population are afflicted with this illness[(1)]. Diabetes mellitus, together with its complications, is one of the most prevalent chronic diseases and its prevalence rises considerably by ageing[(2)]**.** Ageing is characterized by a mild pro inflammatory state[(3)]**.** It is now commonly accepted that diabetes is associated with low-grade inflammation[(4)]**.** The rising incidence of coronary artery disease (CAD) during menopause occurs in parallel with an increase in the incidence of both type 2 diabetes mellitus (T2DM) and hypertension[(5)]**.** Inflammation is a critical process in atherogenesis. Early stages of atherosclerosis involve an inflammatory process consisting of migration of leukocytes and monocytes into the sub endothelium[(6)]. Inflammatory markers also have been shown to be important and independent risk factors for cardiovascular disease in postmenopausal women[(7)]. Several circulating markers of inflammation, including high sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6), are associated with increased cardiovascular events in this population[(8)]. The normal process of reproductive ageing is characterized by marked hormonal changes[(9)]**.** A large body of clinical data suggests the important roles of endogenous sex hormones in the pathogenesis of type 2 diabetes[(10)]**.** In many studies in women, plasma estradiol and testosterone have been positively associated with glucose intolerance and insulin resistance, independently of adiposity[(11)]**.** Similar association of free testosterone and C-reactive protein have

also been reported in women[12]. The purpose of this study was to determine the serum levels of endogenous sex hormones, estrogen and testosterone, as well as hs-CRP in elderly women with and without type 2 diabetes, and to study the association between them. Also, the relation between endogenous sex hormones and CRP and factors modulating the course of DM, such as body mass index (BMI) and glycosylated hemoglobin, were studied.

RESEARCH DESIGN AND METHODS

Study population. Our study included a total number of 60 post menopausal elderly females, 20 healthy non diabetic elderly females as control group and 40 diabetic females, all patients and control were postmenopausal elderly females above (65 years). All patients were diagnosed as diabetic (type 2 diabetes mellitus) according to ADA criteria[13]. The diabetic females were divided into two groups according to presence or absence of complications (cardiovascular diseaes) into two groups,complicated group (29 patients) and non complicated group (11 patients). cardiovascular diseaes was defined as evidence of ischemic heart disease according to clinical history and electrocardiogram. They were also divided into two groups according to glycosylated hemoglobin (HbA1c) to controlled group(17 patients) and non controlled group (23 patients). Patients were excluded if they were taking any medications known to affects sex hormones concentrations such hormonal replacement therapy. Also if they were suffering from any diseases that affect the level of C-reactive protein such as presence of infectious or inflammatory adverse events like pneumonia, rheumatic fever, recent surgery, liver or renal disease and recent intake of drug that might affect CRP.

METHODS

This work had been done in the Departments of Internal Medicine, and Medical Biochemistry, Faculty of Medicine, Zagazig University Hospitals in the period from August 2009 to August 2011. Full history with special stress on age of the patients, smoking, cardiovascular disease such as angina, myocardial infarction, hypertension. A detailed gynecological and reproductive history concerning a detailed gynecological and reproductive history concerning age of menopause and duration of menopause and thorough clinical examination including anthropometric measures, height and weight were measured. Body mass index (BMI), calculated as weight (kg)/height (m^2), was used as a measure of obesity and waist circumference (WC) was measured at the umbilicus level while the subject was in a standing position using a fiberglass measuring tape at the end of a normal expiration. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor. Measurement should be repeated twice; if the measurements are within 1 cm of one another, the average measurement was used for the analysis[14]. Systolic and diastolic blood pressure women were measured in the sitting position after the subjects had rested for at least 5 min with standard mercury sphygmomanometer and blood pressure was calculated as the mean of two measurements[5]. We defined hypertension as systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg or taking anti hypertension medication baseline, or both[15].

Biochemical samples and analysis Fasting blood samples were obtained by venipuncture. Serum samples, extracted by centrifugation and were kept frozen at -20 until analysis of total estradiol and total testosterone levels. Serum hormone concentrations were measured from stored samples by enzyme linked fluorescence assay (ELFA) by using an automatic analyzer (VIDAS) by kits obtained from Diagnostic Products of (Biomerieux company, France). As regard high sensitivity C-reactive protein (hs-CRP), serum samples were extracted by centrifugation and were kept frozen at -20 until analysis by enzyme linked immunosorbent assay (ELISA). HbA1c was analyzed by colorimetric method by using device (NycoCard reader II system).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) program was used for statistical analysis and statistical significance was accepted at $P<0.05$. Descriptive statistics were used to characterize the population. All continuous variables were presented as means ±standard deviation (SD). Differences in continuous variables between groups were analyzed using one-way analysis of variance (ANOVA). Comparisons between the two groups were done using the student *t* test.

RESULTS

General and biochemical characteristics

There was no significant difference as regard Age (years), age at menopause and years since menopause between diabetic and non-diabetic women. Diabetic patients had significantly higher WC , HC,WHR, systolic and diastolic blood pressure and significant difference as regard BMI. They also had significantly higher HbA1c,hs-CRP and serum estrasdiol and no significant difference was obeserved as regard serum testoestrone level as showed in (Table 1)

Table (1): Mean values ± SD and range of the anthropometric and biochemical parameters in the groups of diabetic and non-diabetic women.

	Control (N=20)	Case (N= 40)	t	P
Age (years)	63.6 ± 2.8 (65-73)	67.9± 4.03 (65 – 85)	0.67	0.50 NS
Age at menopause (years)	49.8±0.8 (48 – 51)	49± 0.9 (48 – 51)	1.72	0.09 NS
Years since menopause	18.1 ± 2.4 (15-23)	17.6 ± 2.6 (13-30)	0.72	0.47 NS
WC (cm)	90.3±7.3 (80- 100)	104 ± 14.7 (80-143)	4.39	0.00 HS
HC (cm)	95±4.2 (91.5 – 100.2)	111.8 ± 13.5 (90-140)	6.4	0.00 HS
WHR	0.82±0.04 (0.75-0.90)	0.87± 0.04 (0.8 – 0.95)	5.35	0.00 HS
BMI(kg / m2)	27.9±1.7 (24- 32)	29.3±2.9 (25-40)	2.27	0.03 S
Systolic Bl.p (mmHg)	127.2±4.8 (120-140)	147.5 ±15.0 (120-170)	7.07	0.00 HS
Diastolic Bl. p (mmHg)	83.0±3.8 (80- 90)	93.7 ± 6.3 (80-100)	7.68	0.00 HS
HbA1c (%)	5.7± 0.43 (5-6.4)	8.8±2.3 (5.4-10,4)	8,08	0.00 HS
S.estradiol (pg/ml)	12.9±2.71 (9.5-17)	22.5±13.9 (9-75.5)	4.27	0.00 HS
S. testosterone (ng/ ml)	0.46±0.11 (0.28- 0.66)	0.41±0.25 (0.01- 0.86)	1.04	0.30 NS
hs-CRP(mg/l)	1.60 ± 0.39 (1.008–2.19)	2,2±,56 (,68 – 2,68)	4.33	0.00 HS

When the diabetic women were separated according to presence and absence of cardio vascular complications into two groups, complicated and non complicated group ,there was no significant difference as regard HbA1c,serum estrasdiol and serum testoestrone level, while there was significant difference as regard the hs-CRP between the two groups as showed in table(2).

Table (2): Mean values ± SD and range of the biochemical parameters in complicated and non complicated group.

	Non complicated (11)	Complicated (29)	t	P
HbA1c (%)	9.13±1,8 (5,4-13,6)	8.6±2.57 (5.4-13.6)	o.64	0.54 NS
hs-CRP(mg/L)	2.08±0.76 (0.68-2.83)	2.16±0.51 (.68-2.727)	10.6	0.04 S
S.estradiol (pg/ml)	17.9±13.5 (9-56.2)	24.2±13.9 (9-75.5)	0.67	0.79 NS
S. testosterone (ng/ ml)	0.34±0.28 (0.02- 0.86)	0.43±0.23 (0.01- 0.82)	0.97	0.33 NS

When the diabetic women were separated according to HbA1c into two groups, controlled and non controlled group. There was a high significant difference as regard HbA1c,a significant difference as regard serum testostrone level and no significant difference as regard hs-CRP and serum estradiol level as showed in table(3) .

Table (3): Mean values ±SD and range of the biochemical parameters in controlled and non controlled groups.

	Non controlled No = 23	Controlled No = 17	t	P
HbA1c (%)	10.4±1,6 (7.6-10.4)	6.46±0.68 (5.4-7.5)	10.5	0.00 HS
hs-CRP(mg/L)	2.17±,55 (1.17-2,7)	2.09±0.60 (0.678-2.984)	6.36	0.72 NS
Serum estradiol(pg/ml)	25.2±17.3 (9-75)	18.9±6.47 (10.4-35.4)	1.43	0.16 NS
Serum testosterone (ng/ ml)	0.33±0.26 (0.1-0.82)	0.50±0.20 (0.1-0.86)	2.25	0.03 S

There were statistically significantly positive correlations between serum estradiol and systolic blood pressure, serum testosterone, HbA1c and years since menopause, while there were no significant correlation between S. estradiol, age, age at menopause, BMI, WC, HC, WHR, diastolic blood pressure, hs-CRP as showed in table(4) and figure (1-4)

Table (4): Correlation between serum estradiol and the studied parameters in all patients:

	r	P
Age	0.06	0.64
Age at menopous	-0.00	0.96
Years since menopause	0.26	0.04 S
Systolic blood pressure.	0.37	0.00 HS
Diastolic blood pressure	0.27	0.06
BMI	-0.39	0.77
WC	-0.077	0.94
HC	-0.15	0.36
WHR	0.140	0.28
HbA1c	0.43	0.00 HS
S.testosterone	0.31	0.00 HS
hs-CRP.	0.08	0.55

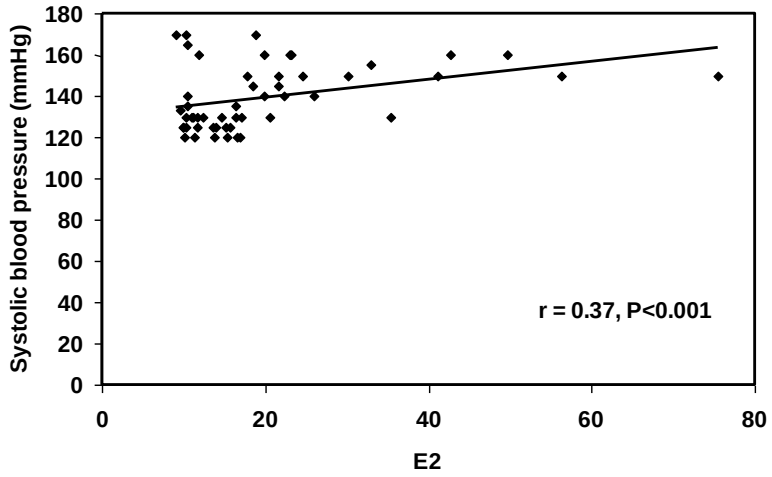

Fig. (1): Correlation between serum estradiol and systolic blood pressure.

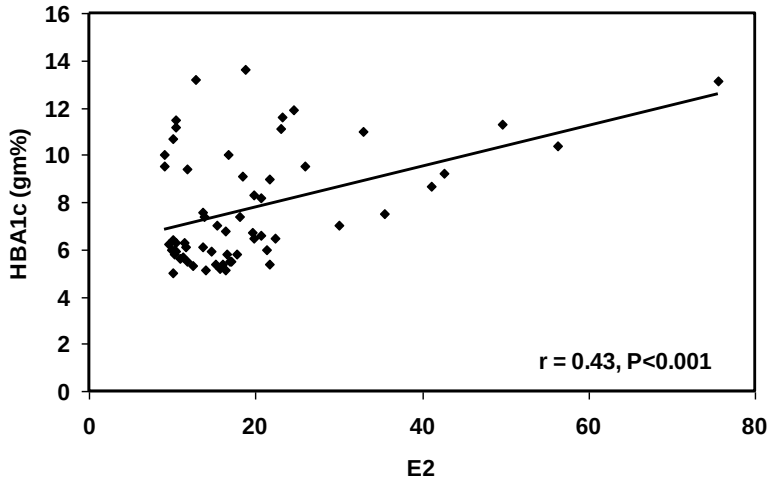

Fig. (2): Correlation between serum estradiol and HbA1c

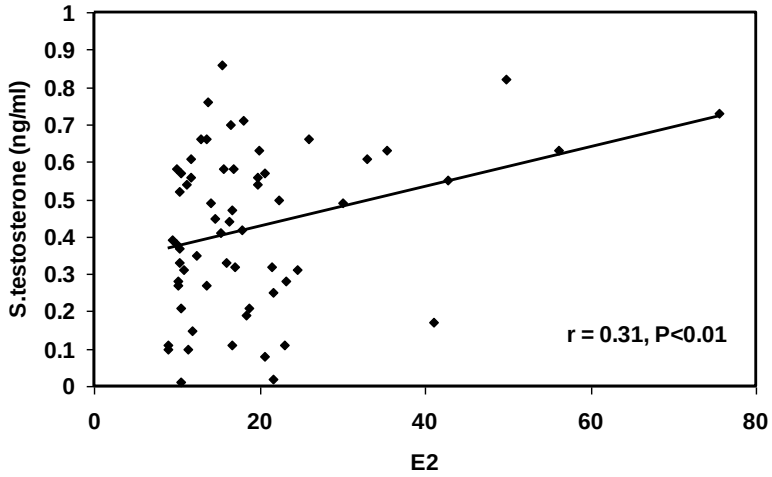

Fig. (3): Correlation between serum estradiol and serum testosterone.

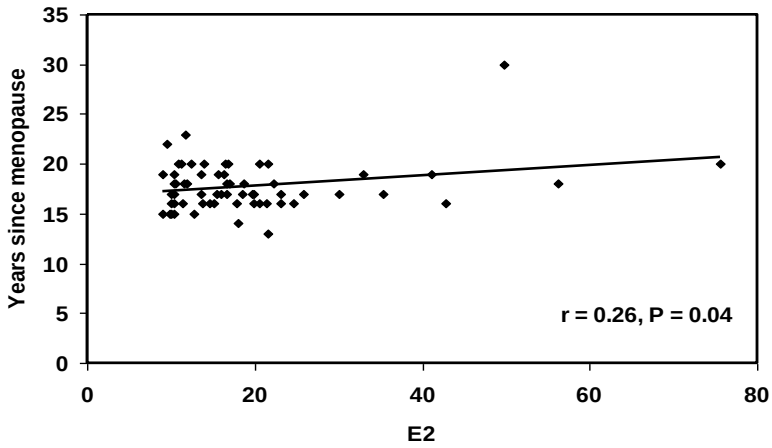

Fig. (4): Correlation between serum estradiol and years since menopause.

Table (5) and figures (5,6) show statistically significantly negative correlation between serum testosterone and systolic blood pressure, while there were positive correlations between serum testosterone ,WC and serum estradiol .There was no significant correlations between serum testosterone and age, age at menopause, years since menopause, BMI,HC,WHR ,diastolic blood pressure, hs-CRP and HbA1c .

Table (5): Correlation between serum testosterone and the studied parameters in all patients:

	R	P
Age	0.76	0.21
Age at menopous	-0.13	0.30
Years since menopaus	0.13	0.31
Systolic blood pressure.	-0.34	0.01HS
Diastolic blood pressure.	-0.23	0.11
BMI	-0.13	0.32
WC	0.40	0.00 HS
HC	0.29	0.08
WHR	0.14	0.29
HBA1C	-0.14	0.28
Serum estradiol	0.31	0.01 HS
Hs-CRP.	-0.09	0.50

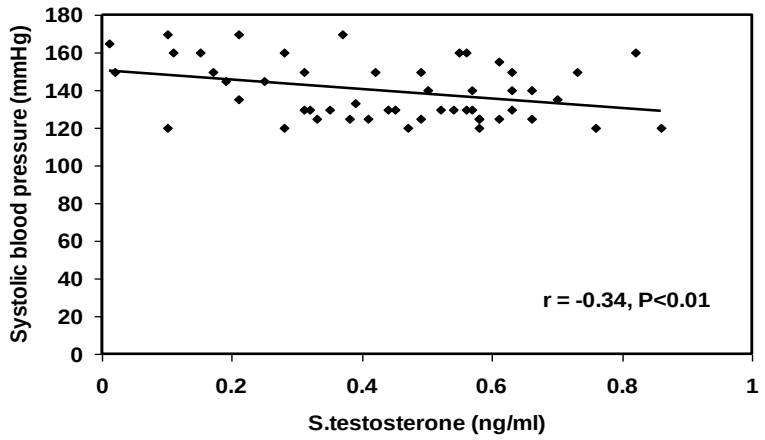

Fig. (5): Correlation between serum testosterone and systolic blood pressure.

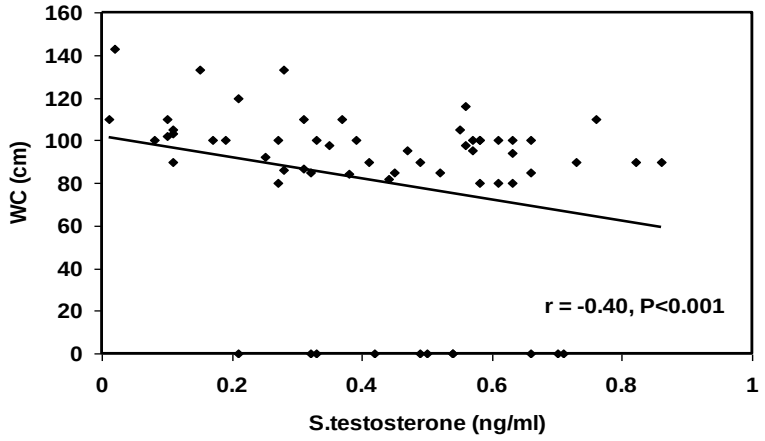

Fig. (6): Correlation between serum testosterone and waist circumference.

DISCUSSION

In our study, we found a significant increase in mean ±SD of hs-CRP in patient group (2.2±0.56 mg/l) in comparison to control group (1.60± 0.39) (p<0.001). This result was matched with results of recent study of **Casula et al.**[16] who found in the cohort of Italian adults (40–79 years of age), the median CRP concentration was

1.30 mg/L. CRP levels increased with age, was higher in women than in men, in hypertensive or diabetic subjects and was lower in physically active people and CRP levels considerably increased with BMI. Also **Goto et al.**[14] and **Kalyani et al.**[17] who found that there was a significant difference in hs-CRP in diabetic postmenopausal women compared to non diabetics women, diabetics patients tended to have higher BMI, waist circumference , HbA1c, fasting glucose levels and fasting insulin levels than controls. This is in line with result of **DeRekeneire et al.**[18] who found that diabetic patients had higher levels of hs-CRP than non-diabetics, and suggested that the inflammation is strong predictor to development of diabetes and thus the link between diabetes and inflammation could be due to reciprocal process, in that inflammation may contribute to diabetes onset and diabetes may then contribute to continued inflammation. In addition, hyperglycemia is known to mediate formation of advanced glycosylation end-products. This advanced glycosylation end-products may also contribute to inflammation, producing a chronic stimulation for secretion of cytokines. This result on contrary to, a longitudinal study of **Luigi et al.**[19] who found that there was no significant difference in hs-CRP between patients with and without diabetes and this might be resulted from small number of the patients enrolled in the study and short follow-up, which did not allow evaluation of the long-term prognostic role of hs-CRP in type 2 DM. Also in studies like European Prospective Investigation of Cancer (EPIC) **Lee et al.**[20], Insulin Resistance Atherosclerosis Study (IRAS) **Festa et al.**[21] and Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) and Augsburg Cohort Study **Thorand et al.(**[22], even though CRP was associated with diabetes, the association became non-significant after adjustment for body mass index, smoking, and systolic blood pressure.

In our study, we found a significant differences in mean ± SD of hs-CRP in complicated group (2.16 ±0.51 mg/l) in comparison with non complicated group (2.08 ±0.76 mg/l) (p= 0.04). This was in agreement with **Dai et al.**[23] and **Kim et al.**[24] who found that serum level of elevated hs-CRP was higher in elderly females complicated with CHD than non complicated subjects .This result was not far away from the result of **Tabak et al.**[25] who found that hs-CRP levels were higher among participants who developed fatal cardiovascular disease and suggested that low-grade inflammation may be 'common soil' for the development of type 2 diabetes and CVD. On the other hand, **Rao et al.**[26] did not find a significant difference in baseline levels of hs-CRP between CAD affected and unaffected subjects even after adjusting for gender, BMI, and statin therapy. This may possibly be attributed to therapeutic modifications by drugs used by affected subjects such as (aspirin), PPRγ antagonists that are known to possess anti-inflammatory properties in addition to their primary effect. Moreover lifestyle and diet modifications play a significant role in overall risk reduction. Our study was in contrast to the finding of Honolulu Heart Program **Naveed and Arron**[27] who found that associations between hs-CRP and risk of thromboembolic or myocardial infarction were generally weaker and not significant in diabetic subjects compared with non-diabetic subjects. Also, **Lynne et al.**[28] who found that CRP was not associated with coronary artery disease and this may be attributed to the age group in these studies was lower than our group.

In our study, we found no significant differences in mean ± SD of hs-CRP between controlled (2.09±0.60 mg/l) group and non controlled group (2,17±,55 mg/l) (p=0.72).This result was in agreement to **Fousteris et al.**[29] who found that mean ± SD of hs-CRP of poorly controlled diabetic subject elevated than well-controlled diabetic subjects. This was not in line with the result of **Tan et al.**[30] and **Rodriguez-Moran and Guerrero-Romero**[31] who showed that inflammatory parameters in diabetic subjects with good glycemic control remained significantly increased because some of subjects might have undiagnosed atherosclerosis, and they did not perform any surrogate measures of atherosclerosis, such as carotid intima media thickness.

In our study, we found a high significant increase in mean ± SD of HbA1c in patient group(8.8± 2.3 %) in comparison with control group (5.7 ± 0.43%) ($p < 0.001$).This result also went with result obtained from many studies of **Goto et al.**[14], **Koshikaw et al.**[32], **Fazulul and Saifuddin**[33], **Abdulla Jarai**[34], **Huffman et al.**[35], **Dale et al.**[37], **Goodarzi et al.**[38], **Eric and John**[39] and **Cosin et al.**[40] who found significantly elevated HbA1c plasma levels in type 2 diabetes compared to healthy non-diabetic subjects.

In our study, we found no significant differences in mean ±SD of HbA1c (9.13±1.8%) in complicated group in comparison with non complicated group (8.6±2.57%) (p= 0.54).This result was in agreement to **Lu et al.**[41] and **Yoo et al.**[42] who found that serum HbA1c was not

statistically significant between T2DM patients with and without CAD and among CAD subgroups this was due to CVD severity was not different between the two groups, suggesting that even asymptomatic patients with few risk factors could have severe CAD while **Dale et al.**[37] who found that diabetics elderly patients complicated with CAD had elevated HbA1c and had four times higher risk of death from IHD compared with matched group without diabetes and diabetic patients with good diabetes control and this difference from our result may be due to age in non complicated group was higher than the age in the study of dale.

In our study, we found a significant increase in mean ± SD of estradiol in patient groups (22.5±13.9 pg/ml) in comparison with control group (12.9±2.71 pg/ml) ($p < 0.001$). This result was in agreement to **Oh et al.**[43] *who* did not find a significant correlation of E2 with age-adjusted fasting glucose levels and other measures of insulin resistance. Although other studies of **Shaw et al.**[11]**, Goodma-Gruen & Barrett-Connor**[44]**,** and **Korytkowski et al.**[45] who found that in diabetics postmenopausal women had higher E2 level than normal glucose tolerance .Also **Kalyani et al.**[17] who found that E2 was positively associated with incident diabetes in multivariable analysis and this association was only partially explained by adiposity and insulin resistance and inflammatory marker such as CRP .Also **Ding et al.**[46] who found that high endogenous estradiol levels may be associated with a higher risk of type 2 diabetes postmenopausal women. From this point of view **Golden et al.**[47] and **Gooding et al.**[48] they found that *estradiol* levels were positively associated with the presence of diabetes. They found that serum level of estradiol was higher in postmenopausal type 2 diabetic women compared to non-diabetic and the source of estrogen was produced by the aromatization of androgens by the enzyme aromatase. The source of aromatase in type 2 diabetic postmenopausal women was adipose tissue and this was supported by the fact that BMI was higher in postmenopausal type 2 diabetic women than in healthy women.

In our study, we found no significant differences in mean ± SD of estradiol (24.2±13.9 pg/ml) in complicated group in comparison with (pg/ml) non complicated subjects (17.9±13.5 pg/ml) ($p= 0.79$).Previous reports of an association between endogenous steroid hormone levels in older individuals and cardiovascular disease (CVD) or common carotid artery (CCA), intima-media thickness (IMT) measurements have been contradictory **The North American Menopause Society**[49]. This result was not matched to the result of **Naessen et al.**[50] who reported that in elderly females complicated with CHD had higher levels of estradiol and lower level of total testosterone and higher E 2 /T ratio (representive of aromatase activity) may be protective mechanism against atherosclerosis as estrogen may play central role in protection against CHD .The source of estradiol was due to increased aromatase enzyme activity .Aromatase enzyme is present in most tissue, including the arterial wall .Furthermore, aromatase enzyme is present in the coronary artery wall and located in the vicinity of atherosclerotic plaques. Local aromatization of androgens to estrogens therefore seems important for protection against atherosclerosis and estrogens moderate the inflammatory response. Also **Naessen et al.**[51] recently suggested that rather than being a causative factor in the development of atherosclerosis, the hormone profile with higher endogenous estrogens in older men and women with prevalent CVD might reflect an endogenous attempt to counteract the ongoing atherosclerotic process. More recently, another study of **Chen et al.**[52] who reported that increased risk of CHD among women with high estradiol concentrations. Nevertheless, this association disappeared after adjustment for BMI and other cardiovascular risk factors**.** On the same line was the result of **Scarabin-Carr´ et al.**[15] who found that serum level of estradiol was high in elderly females with CHD and stroke and mentioned that the cause of this might be attributed to that the source of estradiol was adipose tissue. Adipose aromatization of testosterone represents the main source of estradiol production in women after cessation of ovarian activity. Therefore, obese women were more likely than lean ones to present high levels of estradiol. Both adipose tissue and high levels of endogenous estradiol have been associated with a low-grade inflammation state that can be a mechanism for mediating the association of estradiol with ischemic arterial disease. On the other hand **Dai et al.**[23] found that elderly females with CHD had higher level of serum testosterone and lower level of total estradiol. The balance of the serum E 2 /T ratio was broken in the women with CHD and an imbalanced E 2 /T ratio showed a strong association with cardiovascular risk factors in postmenopausal women with CHD. Also **Rexrode et al.**[53] mentioned that among postmenopausal women who was suffering from combined ischemic arterial disease, no association was found between estradiol level and cardiovascular risk. Also **Nilsson et al.**[54] who evaluated 195

women aged 71–80 years followed for 8 years; who found no difference in estradiol levels with respect to the development of CVD and mentioned that increased physiologic concentrations of progesterone were found to be associated with an increased prevalence of CHF, independent of inflammatory factors, markers of renal function, and insulin metabolism.

In our study, we found no significant differences in mean ± SD of testosterone in patient group (0.47±0.25 ng /ml) in comparison with control group (0.46±0.11 ng /ml) (p= 1.04). This result was matched with result of **Goto et al.**[14] who found that serum level of total testosterone was not significantly different between diabetic postmenopausal group and control group in the same line went the result of **Golden et al.**[47], who found that after adjustment for BMI, race/ethnic differences total and bioavailable testosterone were no longer significant between the diabetic and healthy postmenopausal women**.** Also **Phillips et al.**[55] and **Oh et al.**[43] found that total testosterone levels were insignificantly associated with the risk of developing DM, even after adjustment for other metabolic parameters. On the other hand our result was not matched with result of **Golden et al.**[56] who found that women in the highest FAI quartiles had fivefold greater odds of meeting the criteria of metabolic syndrome compared with those in the lowest quartiles .Also **Ding et al.**[10] indicated that high testosterone levels in women and conversely, low testosterone levels in men are associated with a higher risk of type 2 diabetes. This result was not in agreement with the result of The Invecchiarenel CHIANTI Study **Maggio et al**.[57] who reported that older women with metabolic syndrome had higher, total T levels**.** .In similarly designed study of **Kalyani et al.**[17] women in the higher quartiles of bioavailable testosterone and estradiol were at an increased risk of DM development. After adjustment for BMI and insulin resistance, however, this relationship remained significant for estradiol but not for testosterone. Also **Patel et al.**[58] who reported that in older women with metabolic syndrome had higher total T levels for each criterion, as well as for metabolic syndrome as a whole.

In our study, we found no significant differences in mean ± SD of testosterone in complicated group (0.43±0.23 ng/ ml) in comparison with non complicated subjects (0.34±0.28 ng/ ml). This result was in agreement with the result of **Stanczyk et al.**[59] who found that neither low nor high total T was independently related to future cardiovascular events in the Women's Health Study of elderly postmenopausal women. On contrary to **Golden et al.**[60] who found in elderly postmenopausal women from ARIC (Atherosclerosis Risk in Communities Study) had lower testosterone levels in comparison with control group. Also **Debing et al.**[61] who found that in patients undergoing carotid artery endarterectomy total testosterone and D4-androstenedione levels were lower in the patients' group compared with the controls. The inverse association of circulating androgens with carotid atherosclerosis remained significant even after controlling for classical CVD risk factors . These result was reproduced in the same year by **Montalcini et al.**[62] and in a study of MESA (Multi-Ethnic Study of Atherosclerosis) **Ouyang et al.**[63], who found in patients with severe carotid atherosclerosis (by intima-media thickness) had lower testosterone levels in comparison with control group.

In our study, we found a significant increase in mean ± SD of BMI in patient groups (29.3±2.9 kg / m^2) in comparison with control subjects (27.9±1.7 kg / m^2) (p= 0.03).This result was matched with the results of **Goto et al.**[14] and **Huffman et al.**[35]**, Oh et al.**[43]**, Maggio et al.**[57]**, Gooding et al.**[48], **Ding et al.**[46] and **Kalyani et al.**[17] who found that elderly diabetic postmenopausal females had higher WC and BMI than non diabetic females.

In our study, we found no significant difference in mean ± SD of BMI in complicated group (29.3±3.2 kg / m^2) in comparison with non complicated group (29.3± 2.2kg / m^2) (p= 0.97).This result was in agreement with the result of **Yoo et al.**[42] who found no significant difference in mean ± SD of BMI in the elderly diabetics females complicated with cardiovascular disease and non complicated diabetics females. On contrary to **Scarabin-Carr´et al.**[15] who found that elderly females complicated with cardiovascular disease and cerebrovascular disease had higher BMI than non complicated group.

In our study, we found a high significant difference in mean ± SD of BMI in controlled group (27.8±1.82 kg / m^2) in comparison with non controlled group (30.6±3.17 kg / m^2) (p < 0.001). This result was in agreement with the result of **Bi et al.**[64] who found that diabetic individuals with greater control of glycated hemoglobin had lower BMI (p = 0.005 for HbA1c <6.5%, whereas **Sone et al.**[65] who found no relationship in type 2 diabetic subjects with good diabetes control HbA1c <7.0% .

Our result showed statistically significantly positive correlation between estradiol and systolic blood pressure, and no significant correlation was observed as regard diastolic blood pressure. This result was matched with results of **Shelley et al.**[66], **Szmuilowicz et al.**[67] and **Masi et al.**[68] who reported that estradiol was not significantly related to diastolic BP values. On the other hand **Shakir et al.**[69] who found that in women with metabolic syndrome, estradiol negatively related to diastolic BP and mentioned an inverse relationship in absence of metabolic syndrome.

Our result showed no statistically significantly correlation between estradiol and BMI. This result was in agreement with result of **Chen et al.**[52] who found no statistically significant correlation between BMI and estradiol between complicated and non complicated postmenopausal women. While our result wasn't also in line with the results of **Scarabin-Carr et al.**[15], **Kalyani et al.**[17], **Mahabir et al.**[70] and **McTiernan et al.**[71] who reported statistically significantly correlation between estradiol and BMI in diabetics elderly postmenopausal women and reported that the adipose tissue may be a source of estradiol from peripheral aromatization of testosterone.

Our result showed no statistically significantly correlation between serum estradiol and CRP, a result that mimicked that of **Sowers et al.**[72] who reported estradiol was not associated with high-sensitivity C-reactive protein (CRP). While our result was not matched with result of **Maggio et al.**[73] who found that total testosterone, and E2 were all significantly associated with CRP in elderly postmenopausal women. Also **Stork et al.**[74] `who found a positive association between estradiol and CRP concentrations in postmenopausal women. And mentioned that the source of the CRP from adipose tissue and due to ageing and menopause was a natural protective mechanism reducing the inflammatory potential by decreasing the level of circulating estrogens and androgens and substitution of hormones using hormone replacement therapy may be not be a favorable strategy for improving the cardiovascular risk profile of postmenopausal women. Also **Folsom et al.**[75] who reported a positive association of estrone levels with CRP concentration in a study on postmenopausal women; however, controlling for obesity attenuated the findings.

Our result showed statistically significantly negative correlation between serum testosterone and systolic blood pressure and no significant correlation as regard diastolic blood pressure.This result was not matched with result of **Lambrinoudaki et al.**[76] who found no association between testosterone and diastolic blood pressure while **Maturana et al.**[77] who showed an association between testosterone, systolic and diastolic blood pressure. And attributed that testosterone may influence blood pressure through a direct effect on the renin–angiotensin–aldosterone system (RAAS).

Our result showed statistically significantly positive correlation between serum testosterone and waist circumference. This result was matched with results of **Baglietto et al.** [78], **Maturana et al.**[77] and **Phillips et al.**[79] who found that total testosterone correlated with waist circumference in postmenopausal women with metabolic syndrome. On contrary to **McTiernan et al.**[71] who found that total testosterone did not correlated with waist circumference in postmenopausal women.

Our result showed no statistically significantly correlation between serum testosterone and BMI. This result was matched with result of **Goto et al.**[14] who showed no association between testosterone and BMI in diabetic's elderly women. Also **McTiernan et al.**[71], **Kalish et al.**[80] and **Oh et al.**[43] they found no association between testosterone, BMI and WHR in diabetics postmenopausal women. On the other hand **Baglietto et al.**[78], **Stork et al.**[74] and **Ding et al.**[81], showed strong positive association between testosterone and BMI.

The relationship between androgenicity and glycemia may have an important influence on cardiovascular disease (CVD) risk in postmenopausal women **Page-Wilson et al.**[82]. In the present study, we did not observe significant association between total testosterone level and HbA1c.The absence of an association is consistent with other studies that failed to identify a relationship between total testosterone and impaired fasting glucose, insulin resistance, and incident T2DM **Goto et al.**[14], **Golden et al.**[47], **Kalish et al.**[80] and **Oh et al.**[43].

In the present study, we did not observe significant association between total testosterone levels and CRP. This result was in agreement with result of **Kaczmarek et al.**[83] who found no association between CRP and testosterone in postmenopausal women referred for coronary angiography**.** On contrary to **Bell et al.**[84] who found a positive association between total testosterone and CRP in healthy postmenopausal women**.** Also **Joffe et al.**[12] who found in women referred to coronary angiography, with low testosterone levels being associated with an increase in CRP levels. Interestingly, this inverse

association with testosterone was not present in women who remained CVD-free. These findings suggest that the association between testosterone and CRP depends on CVD status, with a potential confounding effect of subclinical CVD and this may be due to early atherogenic changes may affect testosterone production by the ovaries and adrenals through restriction of the blood supply. This may explain the presence of the reverse association among women with subclinical CVD.

We conclude that; In type 2 diabetic postmenopausal elderly women there was significant association between inflammation and serum estradiol while there was no association between inflammation and serum testosterone. As regarding presence or absence of cardiovascular complication and diabetes control neither of two hormones were associated with inflammation .Further studies are recommended to clarify the relation between diabetes control and sex hormones and the role of anti-inflammatory drugs in diabetics elderly females and its relation to sex hormones.

Limitations

We acknowledge that our study has limitations.. No information on estrone (the principal estrogen in postmenopausal women) and androstenedione was available for analysis. The assay method used for testosterone is not considered the absolute gold standard, especially in postmenopausal women who have very low levels.

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