STUDY OF SERUM ESTROGEN AND TESTOSTERONE LEVELS IN ADULT AND ELDERELY MEN WITH TYPE 2 DIABETES MELLITUS .

Nermin S.Ghanem, Nabila A. Hussein, Imam M. Ismail, Shereen EL-Tarhony

Internal medicine department , Medical Biochemistry, faculty of medicine, Zagazig University, Egypt.

ABSTRACT

Objective: Measurement of serum levels of estrogen and testosterone in adult and elderly men, Evaluation of the relationship between these two hormones and major cardiovascular risk factors ; Their relation with retinopathy as an index of diabetic microangiopathy and myocardial infarction as an index of diabetic macroangiopathy was also measured and Studying of the effect of normalization of glycosylated hemoglobin A_{1c} (HbA_{1c})and plasma lipids on serum levels of estrogen and testosterone by re-measuring their level after normalization.

Research Design and Methods: We studied from august 2008 to august 2010, The study population included 150 subjects, I)120 patients with type 2 diabetes, were selected so that both adult and elderly men as two main groups;1&2 each (*n*=60). Each group of patients included four subgroups each one (*n*=15): Subgroup A: well controlled non complicated diabetics, Subgroup B: poorly controlled non complicated diabetics, Subgroup C: patients with diabetic retinopathy as an index of diabetic microangiopathy complications Subgroup D: patients with myocardial infarction as an index of diabetic macroangiopathy complications. II) The study also included two groups of healthy subjects, each (*n*=15), as control groups. Each one was matched with the age of its corresponding group of patients (adult and elderly).

Results: S.free testosterone is statistically significant predictors of CVD (B ±SE =0.116 ±0.062 ,P=0.011), statistically significantly positive correlation between S. estradiol and HDL(r= +0.33,P< 0.o1), statistically significantly negative correlation between S. estradiol and TC(r= -0.23,P< 0.o5), statistically significantly negative correlation between serum free testosterone and age, BMI , SBP, DBP, SBP, HbA_{1C}, TC ,LDL and TG, (r= - 0.21, - 0.33, - 0.72, - 0.67, - 0.59, - 0.79, - 0.75, - 0.77 respectively ,P< 0.o1, P< 0.oo1, P< 0.oo1,P < 0.oo1, P< 0.oo1 ,P< 0.oo1) statistically significantly positive correlation between serum free testosterone and HDL (r=+ 0.78, P< 0.oo1), while there was no significant correlation between serum free testosterone and S.estradiol.

Conclusions: In type 2 diabetic men , serum free testosterone was significantly associated with cardiovascular disease ,while there was no association of s.estradiol with CVD and both of them were not associated with diabetic retinopathy.

Keywords: serum estrogen, serum free testosterone, cardiovascular disease , elderly.

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Corresponding author: Nermin Ghanem e-mail dr_Nermin5@yahoo.com mobile: 0165683366

INTRODUCTION

Male sex is an independent risk factor for cardiovascular disease (CVD) [(1).] Scientists have postulated that the 5- to 10-year lag period in CVD incidence in women (compared with men) may be related to differences in endogenous sex hormones [(2,3,4)]. Indeed, substantial evidence suggests that sex hormones (testosterone, estrogen, and dehydroepiandrosterone sulfate [DHEA-S]) influence traditional and newer CVD risk factors [(2,3,4).] Interest in the role of sex hormones in the pathogenesis of CVD has been rekindled by the observation that men with genetic defects of estrogen synthesis[(5)] or action[(6)] develop premature atherosclerosis.

In contrast to the aforementioned data, prospective studies relating circulating sex hormone levels to incident CVD in men have been inconclusive. For example, low serum testosterone levels have been associated with greater progression of subclinical atherosclerosis in a previous investigations [(7)], but other studies have reported no association of testosterone levels with CVD events [(8,9)]

It is demonstrated that serum testosterone is decreasing with aging [10] so the increased risk for CVD could be partially mediated through low concentration of testosterone [11] ,however some investigators have found no association between testosterone concentration and the prevelance of CVD in diabetic men [9] .Interestingly ,a very recent study found that oestradiol ,but not testosterone is related to CAD in men [12] In addition, genetic variation in estrogen receptor has been associated with prevalent CVD [13], and androgen and estrogen receptor expression in coronary arteries has been reported to influence coronary atherosclerosis in men [14]. **Fukui et al. [15]** postulated that, serum estradiol concentration is inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated intima media thickness(IMT) in men with type 2 diabetes mellitus. Mean while **Tivesten et al. [16]** suggested that increased estradiol levels are associated with lower extremity peripheral vascular disease (PAD),adding to dilemma ,**Oh et al [17]** reported no significant association between serum estradiol levels and type 2 diabetes in older men**.**

SUBJECTS AND METHODS

The ethical committee of our institution approved this study to be conducted at diabetes and endocrinology outpatient clinic of Internal Medicine and Biochemistry Departments, Faculty of Medicine, Zagazig University Hospitals.In the period from august 2008 to august 2010.

The study population included 150 subjects, I)120 patients with type 2 diabetes, were selected so that both adult and elderly men as two main groups;1(adults <65years)&2 (elderly >65 years), each (*n*=60). Each group of patients included four subgroups each one (*n*=15): Subgroup A: well controlled non complicated diabetics, Subgroup B: poorly controlled non complicated diabetics, Subgroup C: patients with diabetic retinopathy as an index of diabetic microangiopathy complications Subgroup D: patients with myocardial infarction as an index of diabetic macroangiopathy complications. II)The study also included two groups of healthy subjects, each (*n*=15), as control groups. Each one was matched with the age of its corresponding group of patients (adult and elderly).

The patients were among patients attending diabetes and endocrinology outpatient clinic of Zagazig university hospital .Written informed consent was obtained from all participants .

Exclusion criteria:

Patients were excluded if they had been castrated for treatment of testicular or prostate cancer or if they were taking any medications known to affect sex hormone concentrations (e.g, antiandrogenic agents for prostate cancer).

Methods:

All members of the study were subjected to the following: Full history and thorough clinical examination,The auscultatory method of blood pressure measurement ,routine investigations: (Complete blood picture , Liver function tests, Renal function tests ,Resting 12 leads electrocardiographic tracing).Calculation of the body mass index (B.M.I),Lipid profile included:(total cholesterol (TC),low density lipoprotein(LDL)- cholesterol, high density lipoprotein(HDL)- cholesterol, Serum triglycerides(TG)), HbA1c was measured by affinity chromatography, Free testosterone: working solutions were prepared of the free testosterone-HRP conjugate and wash buffer, pipette 25 ul of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate , pipette 100 ul of the conjugate working solution into each well , the plate was gently shaked for 10 seconds ,the plate was incubated at 37 c for one hour, wash the wells were washed 3 times wit 300 ul of diluted wash buffer per well ,pipette 150 ul of TMB substrate into each well at timed intervals ,the plate was incubated at 37 c for 10-15 minutes, pipette 50 ul of stopping

solution into each well at the same timed intervals ,the plate on was read a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution .Expected normal values :3.84-34.17 pg/ml, Serum Estradiol :working solutions were prepared of estradiol-biotin avidin -HRP conjugate and wash buffer,pipette 50 ul of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate , pipette 100 ul of the conjugate working solution into each well ,was incubated 0n plate shaker (approximately 200 rpm)for one hour at room temperature, the wells were washed 3 times wit 300 ul of diluted wash buffer per well and tap the plate firmly against absorbent paper ,pipette 150 ul of TMB substrate into each well at timed intervals, the plate shaker was incubated for 10-15 minutes at room temperature, pipette 50 ul of stopping solution into each well at the same timed intervals ,the plate was read on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution Expected normal values :<100 pg/ml

STATISTICAL ANALYSIS

Data are presented as means ± SD comparisons between groups were performed by analysis of variance (ANOVA),paired t-test, Pearson's correlation analyses, and logistic regression analyses were performed using statistical package for social sciences (SPSS) for windows version 17.

RESULTS

Study of serum estrogen and free testosterone in different groups shown in table (1) there were highly statistically significant decrease of mean values ± SD of S.free testosterone($F=34.8, P< 0.001$) between control group and patients group(A1,B1,C1 and D1), while there was no significant difference ($F=1.75, P > 0.05$) of mean values ± SD of S.estradiol ,and table(2) there were highly statistically significant decrease of mean values ± SD of serum free(S.free) testosterone($F=55.1, P< 0.001$) between control group and patients group(A2, B2,C2 and D2), while there was While there was no statistically significant difference ($F=0.61, P > 0.05$) of mean values ± SD of S.estradiol .our results showed statistically significant increase of S.free testosterone ($t=7.78, P< 0.001$) using paired t-test for comparison of group B1 before and after glycemic and blood pressure control ,while there was no statistically significant difference ($t=0.66, P > 0.05$) of mean values ± SD of S.estradiol , statistically significant increase of mean values ± SD of S.free testosterone ($t= 3.74, P< 0.001$) after glycemic and blood pressure control of group B2 ,while there was no statistically significant difference ($t=1.46, P > 0.05$) of mean values ± SD of S.estradiol .

Table (1): Comparison of mean values±SD of S. Free testosterone and S. estradiol between control group and patients group(A1,B1,C1 and D1) .

	Control	A1	B1	C1	D1	F	P
S.Free testosterone (pg/ml)	31.1±3.7	26.4±3.6	18.7±3.5	25.6±3.9	14.5±6.2	34.8	<0.001 HS
S.estradiol (pg/ml)	33.9±2.2	34.8±1.7	33.3±2.7	34.4±1.4	35 ±1.8	1.75	0.14 NS

Table (2): Comparison of mean values ± SD of S.Free testosterone and S. estradiol between control group and patients group(A2,B2,C2 and D2).

	Control	A2	B2	C2	D2	F	P
S.Free testosterone (pg/ml)	27.7±1.7	24.3±3.8	14.1±4.3	21.8±3.8	10.3±4.4	55.1	<0.001 HS
S.estradiol (pg/ml)	34.3±2.1	33.8±2.3	33.1±2.1	33.4±2.1	33.6±2.1	0.66	0.61 NS

Table (3): show logistic **r**egression analysis for predictors of cardiovascular disease.

	B ±SE	Walid test	P
S.Free testosterone(pg/ml)	0.116 ±0.062	2.576	0.011 S.D
Mean blood pressure(mmHg)	0.298 ± 0.012	3.06	< 0.003 H.S
TC(mg/dl)	0.352 ±0.103	5.29	<0.001 H.S
LDL(mg/dl)	0.652 ±0.012	5.56	<0.001 H.S
HDL(mg/dl)	1.118 ± 0.034	7.015	<0.001 H.S

Using logistic regression analysis as shown in table (3),Our results also showed that S.free testosterone (B ±SE =0.116 ±0.062,P< 0.05) , mean blood pressure(B ±SE=0.298 ± 0.012,p< 0.003) ,TC(B ±SE =0.352 ±0.103, p< 0.001),LDL(B±SE=0.652±0.012, p< 0.001) and HDL (B ±SE =1.118 ± 0.034 p< 0.001)statistically significant independent predictors of CVD. In comparison between adults and elderly regarding serum FT and estradiol using unpaired-t test our results showed no statistically significant difference of mean values ± SD of S.free testosterone and S.estradiol between group A1 and group A2 (t=1.53,P > 0.05;t=1.45, P > 0.05) respectively, statistically significant decrease of mean values ± SD 0f S.free testosterone between group B1 and group B2 (t=3.21,P< 0.05) and there was no statistically significant difference of mean values ± SD of S.estradiol (t=0.07, P > 0.05), statistically significant decrease of mean values ± SD 0f S.free testosterone between group C1 and group C2 (t=2.62,P< 0.05) and there was no statistically significant difference of mean values ± SD of S.estradiol (t=1.62, P > 0.05), statistically significant decrease of mean values ± SD 0f S.free testosterone between group D1 and group D2 (t=2.14,P< 0.05) and there was no statistically significant difference of mean values ± SD of S.estradiol (t=1.93, P > 0.05).

Also our results showed as in table(4) ,statistically significantly positive correlation between S. estradiol and HDL (r=+0.33,P< 0.01) , statistically significantly negative correlation between S. estradiol and TC(r=-0.23,P< 0.01), while there was no significant correlation between S. estradiol and age(r=-0.16,P > 0.05),BMI (r=-0.13,P > 0.05),systolic blood pressure (SBP)(r=-0.1,P > 0.05), diastolic blood pressure (DBP)(r=- 0.14,P > 0.05) , TG (r=-0.12,P> 0.05), LDL(r=- 0.08,P> 0.05) and HbA_{1C}(r=-0.13,P> 0.05),and as shown in table(5) Shows statistically significantly negative correlation between serum free testosterone and age(r=- 0.21,P< 0.01), BMI (r=- 0.33,P< 0.001), SBP(r=- 0.72,P< 0.001), DBP(r=- 0.67,P< 0.001), HbA_{1C}(r=- 0.59,P< 0.001), TC(r=- 0.79,P< 0.001) ,LDL(r=- 0.75,P< 0.001) and TG(r=- 0.77,P< 0.001), statistically significantly positive correlation between serum free testosterone and HDL(r=+ 0.78,P< 0.001) , while there was no significant correlation between serum free testosterone and S.estradiol(r=+0.12,P> 0.05)

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

Item	r	p	sig
Age(years)	-0.16	> 0.05	N.S
BMI (kg/m2)	-0.13	>0.05	N.S
SBP(mmHg)	-0.1	>0.05	N.S
DBP(mmHg)	- 0.14	>0.05	N.S
HbA_{1C}(%)	-0.13	>0.05	N.S
TC(mg/dl)	-0.23	<0.05	S.D
HDL (mg/dl)	+0.33	<0.01	H.S
LDL(mg/dl)	-0.08	>0.05	N.S
TG(mg/dl)	-0.12	>0.05	N.S

Table (5): Correlation between serum free testosterone and other parameters in all patients:

Item	r	p	sig
Age(years)	- 0.21	< 0.01	H.S
BMI(kg/m2)	- 0.33	< 0.oo1	H.S
SBP(mmHg)	- 0.72	< 0.oo1	H.S
DBP(mmHg)	- 0.67	< 0.oo1	H.S
HbA_{1C}%	- 0.59	< 0.oo1	H.S
TC(mg/dl)	- 0.79	< 0.oo1	H.S
HDL (mg/dl)	+ 0.78	< 0.oo1	H.S
LDL(mg/dl)	- 0.75	< 0.oo1	H.S
TG(mg/dl)	- 0.77	< 0.oo1	H.S
S.estradiol	+0.12	> 0.05	N.S

Our results showed statistically significant increase (t=2.33,P< 0.05) in mean values ± SD of duration of treatment between patients with simple diabetic retinopathy (SDR) and proliferative diabetic retinopathy(PDR) in group C1,while there was no statistically significant difference (t=0.1 ,t=0.54
,t=1.66 ,P > 0.05) respectively ,of mean values ± SD of HbA_{1C} , S.free testosterone , S.estradiol. and there was highly statistically significant increase (t=4.74 ,P< 0.001) in mean values ± SD of duration of treatment between patients with SDR and PDR in group C2,while there was no statistically significant difference (t= 0.2,t=0.92,t=2.0,P > 0.05) respectively,of mean values ± SD of HbA_{1C} , S.free testosterone , S.estradiol.

DISCUSSION

Androgen deficiency is emerging as a primary risk factor for a host of disorders including obesity, metabolic syndrome, dyslipidemia, endothelial cell dysfunction, vascular disease, insulin resistance and diabetes [18,19,20], Atherogenic lipoprotein profiles, characterized by high LDL and triglyceride levels are associated with reduced testosterone concentrations in plasma [4]. The link between androgen deficiency and atherosclerosis has been noted in a number of studies [21,22,23,24] . The Multi-Ethnic Study of Atherosclerosis found that testosterone levels were associated with less atherogenic VLDL profile in men [25].

Until recently, male gender was considered a major contributor to risk of atherosclerosis and this was attributed to the adverse effects of testosterone (T) on the vascular system. Now new emerging evidence points to androgen deficiency, henceforth referred to as testosterone deficiency (TD), is more likely to be

associated with atherosclerosis than gender per se. TD alters carbohydrate, lipid and protein metabolism, thus contributing to oxidative stress, endothelial dysfunction and increased production of pro-inflammatory factors, in turn promoting the pathogenic process leading to atherosclerosis[15] .

In agreement with this data ,in this work TD was independent risk factor of CVD ;similar findings were reported by **Rosano et al.**[26] showed that in 138 patients, the coronary artery score increased with decreased T levels and concluded that men with coronary artery disease(CAD) had significantly lower T levels. Further, it was demonstrated recently that low T levels are associated with cardiovascular events independent of coronary risk factors and endothelial function [27]. Despite several reports suggesting that low concentrations of testosterone are associated with an increased risk of CVD in men, some investigators have found no significant association between T-tes concentration and the prevalence of CVD [9], **Fukui et al.** [28] demonstrated that serum testosterone concentrations are not significantly different between patients with or without CVD, And also a very recent study found that oestradiol ,but not testosterone is related to CAD in men [12]

In this work , low testosterone level was associated with atherogenic lipid profile as it was negatively correlated with TC, LDL, TG level and positively correlated with HDL level.

Similarly, other investigators reported that Low levels of testosterone characterized by high LDL and triglyceride levels [4]. Several studies have suggested that reduced testosterone levels are associated with increased total cholesterol and LDL cholesterol [9,29]. Further, testosterone therapy was shown to reduce TC and LDL levels [30,31]. Interestingly, a positive association between HDL levels and testosterone was noted in a number of studies ([31,32].However,discrepancies between HDL levels and testosterone concentrations have been noted in observational studies, interventional studies with testosterone therapy as well as with androgen deprivation therapy and anti-androgen therapy. In most studies, increased HDL levels were associated with normalizing testosterone [30,32], however, other studies showed no change in HDL levels [32] or even reported reduced HDL levels [34].

Therefore, the noted changes in HDL concentrations associated with increased levels of androgens [35] could be accompanied with an improvement of the atheroprotective potential of HDL, including antioxidant behavior and increased efficiency of reverse cholesterol transport and may not necessarily imply an increased risk of atherosclerosis and cardiovascular disease.

Alternative explanations may be attributed to differences in estrogen plasma levels. It has been suggested recently [36] that estradiol and testosterone have opposite effects on lipid profiles, in particular HDL-C and estrogens are associated with unfavorable lipid profile in men.

Advanced age is one of the strongest predictors for coronary artery disease. The Telecom Study demonstrated a significant decrease in testosterone concentration with each decade of life [37]. The decrease in testosterone concentration with age may partly explain the greater risk of CVD with advancing age,this is consistent with our results as serum testosterone was significantly negatively correlated with age as well as serum free testosterone was significantly lower in comparing adult and elderly in all subgroups.

Testosterone (T) is another hormone of major interest in the discussion of the pathogenesis of insulin resistance. In males, too low and too high concentrations of T are followed by insulin resistance. When men with abdominal obesity and low T levels are given T to normal for age concentrations, insulin resistance improves

markedly, and insulin sensitivity approaches normal values. Furthermore, visceral fat mass is specifically diminished, as well as blood pressure and plasma lipids [38].

The inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β have been shown to reduce hypothalamic gonadotrophin releasing hormone (GnRH) and luteinizing hormone(LH) secretion in animals and *in vitro* [39]. Furthermore, high plasma estradiol level derived from insulin resistance and hyperglycemia is associated with inhibition of gonadotrophins via hypothalamic estrogen receptors [40] Testosterone also inhibits the differentiation of 3T3-L1 preadipocytes into adipocytes *in vitro* [41]. Furthermore, effects of testosterone on expression of the insulin-receptor substrate (IRS)-1 and glucose transporter (GLUT)-4 genes have been observed *in vitro* [42]. These studies suggest that testosterone may promote insulin sensitivity and, while deficiency of testosterone induces obesity and insulin resistance.

These results are in accordance with our results as s. free testosterone was negatively correlated with BMI .

Also, our results showed significant decrease in serum free testosterone in all groups of both adult and elderly diabetic patients ,compared to healthy control group ,this decrease was most evident poorly controlled groups and complicated groups than well controlled group as well as serum testosterone was negatively correlated with HbA1c i.e the degree of glycemic control. similar results were postulated by **Brand et al.** [43]who found that in middle aged and older men ,low endogenous testosterone and sex hormone binding globulin (SHBG) levels are associated with glycemia measured by HbA1c.

In this study there was significant improvement of serum testosterone after glycemic control .this in accordance with **Kopoor et al.** [61] who showed that rosiglitazone increased bioavailable ,free ,total testosterone and SHBG levels in hypogonadal men with type 2 diabetes.

Hypertension has also been associated with hypogonadism, as well as long being known to have an affiliation with cardiovascular risk. **Mulligan et al** [44] have shown that more men with hypertension have low T levels than have normal T levels.**Smith et al** [45] showed that androgen deprivation in men with prostate cancer could also induce hypertension and arterial stiffness, even after only several months.

These previous data were matched with our results ,as serum free testosterone was also negatively correlated with SBP and DBP .

As regard serum estradiol , studies examining associations between serum estradiol concentrations and CVD risk in men have been inconclusive. Despite several reports suggesting that low concentrations of estradiol are associated with an increased risk of CVD in men [46], some investigators have found no significant association between estradiol concentration and the prevalence of CVD [47].

Possible mechanisms underlying the inconclusive data concerning the associations between serum estradiol concentrations and CVD in men include the fact that testosterone is aromatized into estradiol at the cellular level. Plasma serum estradiol concentrations do not necessarily reflect tissue-level activity, as peripherally formed estradiol is partially metabolized in situ; thus, not all enters the general circulation. **Fukui et al.** [28] has demonstrated an inverse association between serum testosterone concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus. **Nathan et al** [60] demonstrated that testosterone may attenuate early atherogenesis at least in part by being converted to estradiol by the enzyme aromatase, which is also expressed in endothelial cells.

In addition Estrogen may lower CVD risk in men through beneficial effects on CVD risk factors, including blood pressure, lipid levels, and glycemia [48]) In this work , there was no statistically significant difference between s.estradiol in all groups of adult and elderly men and healthy control group. Our results failed to find association between s.estradiol and CVD,this was similar to the findings of **Fukui et al.** [15]who stated that there was no difference between patients with CVD and patients without CVD**.**

Similarly **Small et al.** [49] concluded that ,it is unclear whether hyperestogenemia could be regarded as a risk factor of MI in men, while **Arnlov et al.** [46] mentioned that serum estradiol was associated with a lower risk of CVD in elderly men.

In contrast to these results , **Muller et al.** [7] have investigated the relationship between T and E2 and progression of carotid atherosclerosis in 195 elderly men and demonstrated that higher serum total and free E2 levels were related to progression of intima media thickness (IMT) ' a marker of atherosclerosis. **Tivesten et al.** [50] demonstrated that circulating levels of endogenous estradiol are strong predictor of progression of carotid artery IMT in health middle aged men.

In this work there was no statistically significant difference in s.estradiol when both adult and elderly were compared in all subgroups , as well as there was no statistically significant correlation between s.estradiol and age

So in contrast to some studies [51,52]**,** but in accordance with others [3]**,** s.estradiol did not significantly change with age

Muller et al . [53]) failed to detect a significant association between E2 and metabolic syndrome(MS) in adult men, although E2 levels were positively associated with central obesity and triglycerides. **Kiel et al** [54] found strong relationships of total and free E2 with total and HDL-cholesterol. In a representative sample of older Italian men, while **Maggio et al.** [55] founda positive independent relationship of total and free E2 with MS.

In this study, serum estradiol was significantly negatively correlated with serum TC and positively correlated with HDL cholesterol but no significance was associated with BMI, HbA1c, systolic and diastolic blood pressure , LDL nor TG. While **Fukui et al.** [15] showed an inverse correlation between serum estradiol concentration and plasma triglyceride concentration, while no significant correlations was found between serum estradiol concentration and age, BMI, HbA1c, plasma total cholesterol, HDL cholesterol, blood pressure.

In contrast with these results strong association between circulating concentrations of estrogens and lipid profiles in 933 young men was recently reported by **Tomaszewski et al.** [36]**.** Total cholesterol and HDL-cholesterol levels were associated with E2 and TC and LDL levels were associated with E1 levels. It was concluded that increased estrogen levels are associated with unfavorable lipid profile in young healthy men.

In this work s.estradiol was differed insignificantly different after glycemic control of poorly controlled group , so it was not affected by diabetes status ,this is in accordance with **Barrett-Connor et al.** [58]but against **Colangelo et al.** [59]who concluded that S.estrdiol was associated positively with IGF and diabetes in men. In this study both serum free testosterone and serum estradiol were not associated with diabetic retinopathy nor the stage of the disease either SDR or PDR ,results similar to that reported by **Fukui et al.** [15,28]**.**

Several factors were found to influence diabetic retinopathy including long duration of the disease, age, level of hyperglycemia control, level of blood pressure control, puberty, Pregnancy, hyperlipidemia, hyperviscosity, renal failure and anemia. Hyperviscosity of the blood due to any cause such as dehydration

and polycythemia may influence the diabetic retinopathy[56].

In this work ,duration of diabetes was statistically significantly higher in patients with PDR than SDR in both adult and elderly men,a result near to that reported by **Niazi et al.** [57] who concluded that there was a strong association between the duration of diabetes and severity of retinopathy and this emphasized the need for regular screening of diabetic individuals to detect retinopathy in early stage**.**

IN conclusion,in type 2 diabetic men ,serum free testosterone was significantly associated with cardiovascular disease ,while there was no association of s.estradiol and CVD and both of them were not associated with diabetic retinopathy.

REFERENCES

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults(2001): Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA.;285: 2486–2497.
2. Liu PY., Death AK. and Handelsman DJ.(2003) :Androgens and cardiovascular disease. *Endo Rev*.;24:313–340.
3. Muller M., van der Schouw YT., Thijssen JH. ,et al. (2003): Endogenous sex hormones and cardiovascular disease in men, *J Clin Endocrinol Metab* ;88 , 5076–5086
4. Wu FC. and von Eckardstein A. (2003): Androgens and coronary artery disease.Endocr Rev;24:183–217.
5. Maffei L., Murata Y., Rochira V., et al. (2004): Dysmetabolicsyndrome in a man with a novel mutation of the aromatase gene:effects of testosterone, alendronate, and estradiol treatment. *J Clin Endocrinol Metab*.;89:61-70.
6. Sudhir K., Chou T.M., Chatterjee K.,et al. (1997): Premature coronary artery diseaese associated with a disruptive mutation in the oestrogen receptor gene in a man. *Circulation*; 96, 3774–3777.
7. Muller M., van den Beld A., Bots M., et al.(2004): Endogenous sex hormones and progression of carotidatherosclerosis in elderly men. Circulation.;109:2074–2079
8. Phillips GB., Yano K. , Stemmerman, GN. (1988) : Serum sex hormone levels and myocardial infarction in the Honolulu heart program. Pitfalls in prospective studies on sex hormones. *J Clin Epidemiol* ; 41, 1151–1156.
9. Barrett-Connor E. and Khaw, K.T. (1988) :Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. *Circulation* ; 78: 539–545.
10. Simon D., Preziosi P., Barret-Connor E., et al (1992): The inluence of aging on plasma sex hormones in men: the Telecom study. *Am J Epidemiol* ; 135, 783–791.
11. Stellato RK., Feldman HA., Hamdy O., et al.(2000):Testosterone, sex hormone-binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. *Diabetes Care*;23:490–494
12. Emmanuela QC.,Francisco CF.,Rebeca DS., et al.(2011): Endogenous oestradiol but not testosterone is related to CAD in men Sex hormones and CAD. *Clin Endocrinol*,DOI :10.1111/j.1365-2265
13. Schuit SC., Oei HH., Witteman JC.,et al.(2004) :Estrogen receptor alpha gene polymorphisms and risk of myocardial infarction. *JAMA*;291:2969-77.
14. Liu PY., Christian RC., Ruan M., et al(2005):. Correlating androgen and estrogen steroid receptor expression with coronary calcification and atherosclerosis in men without known coronary artery disease,J Clin Endocrinol Met.;90:1041-6.
15. Fukui M., Kitagawa Y., Kamiuchib K., et al. (2008): Association between serum estradiol concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus .*Met Clin Experiment* ;57 : 285–289.
16. Tivesten A., Mellström D., Jutberger H., et al. (2007): Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. The MrOS Study in Sweden, *J. Am. Coll. Cardiol*. ;50 ,1070–1076.
17. Oh JY., Barrett-Connor E., Wedick NM., et al.(2002): Endogenous sex hormones and the development of type 2 diabetes in older men and women; the Rancho Bernardo study. *Diabetes Care;*25:55–60.
18. Traish AM, Saad F, Guay AT. (2009): The dark side of testosterone deficiency: II. Type 2 diabetes and insulin resistance. *J Androl*;30(1):23–32.
19. Kalyani RR. And Dobs AS.. (2007): Androgen deficiency, diabetes, and the metabolic syndrome in men. *Curr Opin Endocrinol Diabetes Obes;*14:226–234.
20. Kapoor D. and Jones T.H. (2008): Androgen deficiency as a predictor of metabolic

syndrome in aging men: an opportunity for investigation?, *Drugs Aging* ;25 , 357–369.

21. Hak A., Witteman J., de Jong F., et al.(2002): Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Met*;87:3632–3639
22. Debing E., Peeters E., Duquet W.,et al. (2008): Men with atherosclerotic stenosis of the carotid artery have lower testosterone levels compared with controls, *Int Angiol*; 27, 135–141
23. Demirbag R., Yilmaz R., Ulucay A. ,et al. (2005): The inverse relationship between thoracic intima media thickness and testosterone level, *Endocr Res*; 31: 335–344.
24. Van den Beld AW., Bots ML., Janssen J.A. ,et al. (2003):Endogenous hormones and carotid atherosclerosis in elderly men, *Am. J. Epidemiol*. ;157, 25–31.
25. Vaidya D., Dobs A., Gapstur S.M. ,et al. (2008): The association of endogenous sex hormones with lipoprotein subfraction profile in the Multi-Ethnic Study of Atherosclerosis, *Metabolism* ;57, 782–790.
26. Rosano I. Sheiban R. Massaro P.et al.(2007): Low testosterone levels are associated with coronary artery disease in male patients with angina, Int. J. Import. Res, 19 , pp. 176–182.
27. Akishita M., Hashimoto M., Ohike Y., et al. (2010):Low testosterone level as a predictor of cardiovascular events in Japanese men with coronary risk factors, *Atherosclerosis* ;210:232–236.
28. Fukui M., Kitagawa Y., Nakamura N., et al. (2003) :Association between serum testosterone concentration and carotid atherosclerosis in men with type2 diabetes. *Diabetes Care* ;26:1869-73.
29. Haffner SM., Mykkanen L., Valdez RA. ,et al. (1993) : Relationship of sex hormones to lipids and lipoproteins in nondiabetic men *J Clin Endocrinol Met*; 77, 1610–1615.
30. Saad F., Gooren L., Haider A., et al.(2007): An exploratory study of the effects of 12 month administration of the novel long-acting testosterone undecanoate on measures of sexual function and the metabolic syndrome. *Arch Androl*;53:353–357.
31. Saad F., Gooren LJ., Haider A. ,et al. (2008): A dose–response study of testosterone on sexual dysfunction and features of the metabolic syndrome using testosterone gel and parenteral testosterone undecanoate, *J. Androl*.; 29 ,102–105.
32. Zitzmann M. and Nieschlag E. (2007): Androgen receptor gene CAG repeat length and body mass index modulate the safety of long term intramuscular testosterone undecanoae therapy in hypogonadal men. *J Clin Endocrinol Metab*;92:3844–3853.
33. Uyanik BS., Ari Z., Gumus B., et al. (1997): Beneficial effects of testosterone undecanoate on the lipoprotein profiles in healthy elderly men, Jpn. Heart J. ;38 , 73–82.
34. Bagatell CJ., Heiman JR., Matsumoto AM., et al. (1994): Metabolic and behavioral effects of high dose exogenous testosterone in healthy men, *J. Clin. Endocrinol. Met*.; 79: 561–567
35. Langer C., Gansz B., Goepfert C.,et al. (2002): Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages, *Biochem. Biophys. Res. Commun*. ;296 : 1051–1057.
36. Tomaszewski M., Charchar F.J., Maric C. et al., (2009):Association between lipid profile and circulating concentrations of estrogens in young men, *Atheroscl* ;203 , 257–262.
37. Simon D., Preziosi P., Barret-Connor E., et al (1992): The inluence of aging on plasma sex hormones in men: the Telecom study. *Am J Epidemiol* ; 135, 783–791.
38. Marin P., Holmang S., Gustafsson C., et al. (1993): Androgen treatment of abdominally obese men. *Obes Res*;1:245–251.
39. Watanobe H. and Hayakawa Y. (2003) :Hypothalamic interleukin-1 beta and tumor necrosis factor-alpha, but not interleukin-6, mediate the endotoxin-induced suppression of the reproductive axis in rats. Endocrinology;144, 4868-4875.
40. Dandona P., Dhindsa S., Chaudhuri A. ,et al. (2008): Hypogonadotrophic hypogonadism in type 2 diabetes. *Aging Male* ;11, 107-117.
41. Singh R., Artaza JN., Taylor WE.,et al. (2006): Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinol*; 147,141-154.
42. Zitzmann M. (2009) :Testosterone deficiency, insulin resistance and the metabolic syndrome. *Nat Rev Endocrinol*; 5, 673-681.
43. Brand JS.,Wareham NJ.,Dowsett M.,et al.(2011): Assocition of endogenous testosterone and SHBG with glycated haemoglobin in middle aged and older men .*Clinic Endocrinol* ,74(5):572-578.
44. Mulligan T., Frick MF., Zuraw QC.,et al. (2006): Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *Int J Clin Pract;*60,762–769.
45. Smith MR., Lee H., Nathan DM. (2006):Insulin sensitivity during combined androgen blockade for prostate cancer. J Clin Endocrinol Metab;91,1305–1308.
46. Arnlov J., Michael J P, Amin S., ,et al. (2006): Endogenous Sex Hormones and Cardiovascular Disease Incidence in Men. *Ann Intern Med.*; 145:176-184.

47. Makinen J, Jarvisalo M, Pollanen P, et al.(2005):Increased carotid atherosclerosis in andropausal middle-aged men. *J Am Coll Cardiol;*45:1603–1608.
48. Komesaroff PA., Fullerton M., Esler MD., ,et al. (2001): Low-dose estrogen supplementation improves vascular function in hypogonadalmen. *Hypert*.;38:1011-6.
49. Small M., MacRury S., Beastall GH., et al. (1987): Oestradiol levels in diabetic men with and without a previous myocardial infarction.*QJMed*; ;64(243),617-23.
50. Tivesten A., Hulthe J., Wallenfeldt K. ,et al. (2006): Circulating estradiol is an independent predictor of progression of carotid artery intima–media thickness in middle-aged men, *J Clin Endocrinol Met*; 91,4433–4437.
51. Bjornerem A., Straume B., Midtby M., ,et al. (2004): Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromso Study. *J Clin Endocrinol Met*.;89,6039 –6047.
52. Orwoll E., Lambert LC., Marshall L., ,et al. (2006): Testosterone and estradiol among older men. *J Clin Endocrinol Metab.*; 91, 1336 –1344.
53. Muller M., Grobbee DE., den Tonkelaar I. ,et al. (2005): Endogenous sex hormones and metabolic syndrome in aging men. J *Clin Endocrinol Met*.; 90,2618 –2623.
54. Kiel DP.., Baron JA, Plymate SR., et al. (1989): Sex hormones and lipoproteins in men. *Am J Med*.; 87, 35 –39.
55. Maggio M., Lauretani F., Ceda GP., ,et al. (2010): Estradiol and metabolic syndrome in older italian men: The InCHIANTI Study. *J Androl*.;Mar;31(2):155-62.
56. Aiello L.P., Edwards A.R., Beck R.W. ,et al. (2010): Diabetic retinopathy clinical research network factors associated with improvement and worsening of visual acuity 2 years after focal/grid photocoagulation for diabetic macular edema, *Ophthalmology* ;117: 946–953.
57. Niazi MK., Akram A., Nazm A., et al.(2010): Duration of diabetes as a significant factor for Retinopathy .*Pak J Ophthalmpl*,26(4):182-186
58. Barrett-Connor E., Khaw KT. and Yen SS.(1990): Endogenous sex hormone levels in older adult men with diabetes mellitus. Am J Epidemiol: Nov;132(5):895-901.
59. Colangelo LA., Ouyang P., Liu K. ,et al. (2009): Association of endogenous sex hormones with diabetes and impaired fasting glucose in men: multi-ethnic study of atherosclerosis. *Diabetes Care*.;32(6):1049-51.
60. Nathan L., Shi W., Dinh H. ,et al. (2001): Testosterone inhibits early atherogenesis by conversion to estradiol: critical role of aromatase.*Proc Natl Acad Sci U S A* ;98,3589-93.
61. Kapoor D., Channer KS., Jones TH.(2008): Rosiglitazone increases bioactive testosterone and reduces waist circumference in hypogonadal men with type 2 diabetes. *Diab Vasc Dis Res*;6(1):51.