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Estimation of sex hormones in type 2 diabetes patients

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Abstract : The present study is aimed to determine whether sex hormones predict an association with type 2 diabetes using ELISA. The results of this study showed that patients with type 2 diabetic of both male and female have significant elevation ($p \leq 0.05$) insulin hormone levels , insulin resistance, and fasting blood glucose (FBG) than control group. While the testosterone levels decreased significantly in male and increased significantly in female of patients group than control groups. Also, estradiol levels decreased significantly in females but it elevated significantly in males when compared with control group. Insulin sensitivity show significant decrease in diabetic group when compared with control group. According to the gender the results of testosterone level show significant differences between males and females. The division according to the duration detected that at the period (> 15) Estradiol levels showed significant elevation in males while it decreased significantly in females while insulin sensitivity values showed a significant decrease in both genders when compared with other duration. With the increase in the duration of diabetic, the fasting blood glucose (FBG) values showed a significant increase than the lower duration of diabetic. The correlation analysis showed inverse correlation between testosterone and insulin, FBG and insulin resistance in males and positive correlation with insulin sensitivity while in females there were positive correlation between testosterone and insulin sensitivity. There were positive correlation between estradiol and insulin resistance and FBG in male and while in females the correlation is negative and positive correlation with insulin sensitivity.
Conclusions: Disorders in sex hormones are associated with insulin resistance and development of type 2.
Key Words: Type2 diabetes, testosterone, estradiol, Sex hormones.

Introduction

Diabetes is a complex disease represented the major deadly global health problem and it is defined as a group of chronic metabolic disease by high levels of blood glucose[1]. It is occurring as a result of impaired in insulin action, secretion, or both, and this may result from autoimmune destruction of β-cells of the pancreas that's it lead to deficiencies in insulin secretion, while the impaired insulin action may result from decrease responses of tissues to the insulin at the pathway of insulin action[2]. Normal glucose homeostasis result from a balance between insulin secretion and action that's it any dysregulation in this balance responsible for the initiation of type 1 diabetes and insulin resistance the character of type 2 diabetes, which represent the main types of diabetes[3]. The main symptoms of this disease, including polyuria, polydipsia, increased hunger, loss of weight, fatigue and vision disrupt[4]. If untreated, chronic hyperglycemia in diabetes may lead several complications, especially dysfunction and failure in heart, blood vessels, nerves, kidney and eyes[2].

Sex hormones represented by testosterone and estradiol are synthesis by the gonads (ovaries or testes), [5]by adrenal glands, or in other tissue such as liver or fat by conversion from other sex steroids. Sex hormones have direct effect on sexual differentiation and reproduction, and also, influence the immune system and their levels are affected by several condition and diseases[6,7].The present study is aimed to determine whether sex hormones predict an association with type 2 diabetes and to detect the whether the effect is same in males and females.

Material and methods

The study subjects comprised from 80 patients suffer from type 2 diabetes without any complications selected from AL-Sader Teaching Hospital (male and female) with different duration of disease range from (1-20 years), the control group study included 40 people apparently healthy and this group matched with patient group. All subjects in this study were taken consent before participation in this study.Venous blood samples were drawn from patient and control subjects by using disposable syringes (5mL) in the sitting position. Five ml of blood were obtained from each subject by vein puncture pushed slowly into disposable tubes containing separating gel was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 2000 × g for approximately 10-15 minutes then the sera were obtained and stored at -20˚C until analysis (hormonal assayed).

Determination of fasting blood glucose (FBG)

The RanDox kit was used to determine serum Glucose levels. It is based on the PAP enzymatic determination of glucose [8].

Hormonal assay

Hormones (Testosterone, Estradiol and Insulin) are assayed by using the Monobind ELISA Kit which was based on standard sandwich enzyme-linked immune-sorbent assay technology

Determination of insulin resistance and insulin sensitivity

Insulin resistance is evaluated by determination of homeostasis model assessment of insulin resistance (HOMA-IR) [9,10] and calculate by using the following equation

$IR_{HOMA} = (I_0 \times G_0) / 22.5$
Were : IR_{HOMA} : insulin resistance according to homeostasis model assessment.
I_0: Fasting insulin level.
G_0: Fasting glucose level.

The **quantitative insulin sensitivity check index** (***QUICKI***) is derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose[11].

1 / (log(fasting insulin µU/mL) + log(fasting glucose mg/dL)

7. Statistical Analysis

All statistical analysis were performed by using SPSS 17 version. Data were expressed as (mean ± SD) by using T-test. The normality of the distribution of all variables was assessed by the student's ANOVA test and Pearson correlation analyses that have been used to determine the significant difference between the groups [12].

Results

The levels of some hormones and some physiological parameters in both gender of patients with diabetic only and control group

The statistical analysis of this study shows significant differences between patients with diabetic only and control groups. Patients with diabetic of both male and female have significant elevation ($p \leq 0.05$) insulin hormone levels , insulin resistance , and fasting blood glucose (FBG) than control group. While the testosterone levels decreased significantly ($p \leq 0.05$) in male and increased significantly ($p \leq 0.05$) in female of patients group than control groups. In diabetic group, estradiol levels decreased significantly($p \leq 0.05$) in females but

it elevated significantly($p \leq 0.05$) in males when compared with control group. Insulin sensitivity show significant decrease in diabetic group when compared with control group.

According to the gender the results of testosterone level show significant differences ($p \leq 0.05$) between males and females as shown in table (1).

Table(1) The levels of some hormones and some physiological parameters in both gender of patients with diabetic and control group

	Male	**Female**	**Male**	**Female**		
Testosterone (ng/ml)	5.62± 0.24	0.44± 0.25	3.27±0.83	0.84±0.16	0.04*	0.05*
Estradiol (Pg /ml)	49.87± 8.24	104.44±11.45	66.23±7.63	72.38±4.25	1.03	0.007 *
Insulin (µIU/ml)	8.46±3.40	8.39±2.97	13.81±2.49	13.34±2.209	0.62	0.05*
FBG(mg/dl)	93.25±5.11	89.42±6.21	263.25±9.92	201.33±6.97	0.3	0.01*
Insulin resistance	2.2±0.49	2.27±0.6	8.69±2.81	6.42±2.53	0.06	0.02*
Insulin sensitivity	0.39±0.02	0.41±0.1	0.15±0.06	0.17±0.09	0.11	0.03*

t-test at $P \leq 0.05$
(Mean ± SD): Mean± Standard Deviation

Correlation between Testosterone hormone and some hormonal and physiological parameters in type 2 diabetic patients

In diabetic patients the correlation analysis showed inverse correlation between testosterone and insulin , FBG and insulin resistance in males and positive correlation with insulin sensitivity while in females there were positive correlation between testosterone and insulin sensitivity as shown in table (2) .

Table (2) Correlation analysis between Testosterone hormone (ng/ml) and some parameters of type 2 diabetic group.

	Male		**Female**	
	r	**P value**	**r**	**P value**
Estradiol (Pg /ml)	0.05	0.36	0.35	0.08
Insulin (µIU/ml)	-0.52	0.01*	0.24	0.06
FBG(mg/dl)	-0.45	0.04*	0.11	0.34
Insulin resistance	-0.42	0.05*	0.38	0.07
Insulin sensitivity	0.37	0.05*	0.49	0.02*

Correlation coefficient (r)

* significant $P \leq 0.05$

Correlation between Estradiol hormone and some hormonal and physiological parameters in type 2 diabetic patients

In diabetic patients the correlation analysis showed positive correlation between estradiol and insulin resistance and FBG in male while in females the correlation is negative and it has positive correlation with insulin sensitivity as shown in table (3).

Table (3) Correlation analysis between Estradiol (Pg /ml) hormone and some parameters of type 2 diabetic group

	Male		Female	
	r	P value	r	P value
Insulin (µIU/ml)	0.21	0.13	-0.30	0.01*
FBG(mg/dl)	0.44	0.01*	-0.39	0.04*
Insulin resistance	0.33	0.02*	-0.36	0.05*
Insulin sensitivity	0.07	0.91	0.43	0.01*

Correlation coefficient (r)

* significant $P \leq 0.05$

The levels of some hormones and some physiological parameters in patients with Type 2 diabetic among duration of disease and between gender

The statistical analysis of some hormonal and physiological parameters in diabetic only patients according to the duration of disease showed significant differences among the duration for both males and females where Estradiol levels showed significant elevation($p \leq 0.05$) in males while it decreased significantly($p \leq 0.05$) in females in the fourth duration (> 15) years of disease when compared with other duration.With the increase in the duration of diabetic , the fasting blood glucose (FBG) values showed a significant increase($p \leq 0.05$) than the lower duration of diabetic.Insulin sensitivity values showed a significant decrease($p \leq 0.05$) in the fourth duration (> 15) years of disease when compared with other duration.Other parameters show no significant differences with the progress of the duration as shown in table (4) .

Table(4) The levels of some hormones and some physiological parameters in patients with Type 2 diabetic among duration of disease and between gender

	1-5 (years)		> 5 (years)		> 10 (years)		> 15 (years)	
	Male	Female	Male	Female	Male	Female	Male	Female
Testosterone (ng/ml)	5.11±1.25	0.64±0.21	5.05±0.86	0.84±0.19	3.13±0.32	0.87±0.35	2.01±0.59	0.76±0.32
Estradiol (Pg /ml)	55.23±1.69	85.4±6.43 ^{d}	59.73±2.16	76.14±9.74	66.67±4.42	69.01±8.05	80.6±1.65	60.49±6.13^{a}
Insulin (µIU/ml)	12.4±1.84	13.39±2.68	14.34±0.24	12.24±0.76	11.22±3.71	12.65±2.73	15.29±2.92	14.63±1.98
FBG (mg/dl)	188±6.64	158.25±5.42^{bcd}	218.33±4.48	201.4±6.54^{ad}	266±7.21	229.1±6.75^{a}	238±10.53	359±7.01^{ab}
Insulin resistance	6.4±2.00	5.22±2.41	7.04±3.61	6.63±0.01	8.44±2.35	7.15±1.64	8.82±3.67	8.68±2.87
Insulin sensitivity	0.23±0.08	0.24±0.05^{d}	0.19±0.07	0.21±0.05	0.11±0.06	0.18±0.08	0.05±0.01	0.01±0.002^{a}

(Mean ± SD): Mean± Standard Deviation

a : significant difference with first duration ; b : significant difference with second durationc : significant difference with third duration ; d : significant difference with fourth duration

Discussion

The results of this study demonstrate that Testosterone levels significantly lower in males while it significantly higher in females of type 2 diabetes than control group and the estradiol levels significantly lower in females while it significantly higher in males of type 2 diabetes than control group as shown in table (1) and these results are in agreement with [13,14] who found that type 2 diabetes is associated hypogonadism in males as a result of hormone synthesis alteration while in female type 2 diabetes is associated hyperandrogenism as a

consequence of hypothalamic-pituitary axis alteration. In addition this alteration in the levels of testosterone in males and females appear to be linked with insulin resistance [15].

Several studies show that testosterone and estradiol present sex-dimorphic profile were contribute with increase testosterone levels in women and decrease in males and the patients tend to be obese [16,17,18] .

A study was found the presence of inverse correlation between testosterone levels and BMI [19] where aromatase enzyme expressed by adipose tissue which its function to convert testosterone to estradiol and this lead to believe that an excessive levels of aromatase depend the conversion of testosterone to estradiol[20]. The pathogenesis of decrement of testosterone may explained by that elevation of estradiol may lead to suppression of gonadotropin releasing hormone from hypothalamus and secretion of gonadotropin from the pituitary gland[21].Another study indicate that the use of letrozole an aromatase inhibitor in men with low testosterone will lead to decrease estradiol and increase in testosterone levels[22].

In women the exact mechanisms that associated with the testosterone levels elevation is not entirely illustrated . It is believed that the levels of testosterone increased in women who have low levels of sex hormone binding globulin (SHBG) which is plasma protein that act on regulation of circulating testosterone [23] .Testosterone is documented to be play important role in obesity ,homeostasis of glucose and metabolism of lipid which may have an etiology role in diabetes and thus higher testosterone levels in women may lead to insulin resistance[24].According to available studies this may be real for women with hypergonadism where find that insulin resistance is improved when high levels of testosterone is corrected [25,26].In diabetic women [27] indicate decrease in the levels of sex hormone binding globulin (SHBG) will lead to increase testosterone levels in females and this prove the distinct role of androgens in insulin resistance regulation in women and the effect of its elevation in the pathogenesis of diabetes and its cardiovascular risk.

In the current study we have found an elevation of estradiol levels in males and it is decrease in females with type 2 diabetes and this agreed with the study of Hu *et al.,*[28] who found that increase of estradiol levels in males may related to decrease in the levels of sex hormone-binding globulin (SHBG) and the men who have high estradiol level and low levels of SHBG had develop type 2 diabetes risk 20-fold greater than men with high levels of SHBG. Other study indicate that diabetes occurrence was associated directly with estradiol and inversely with testosterone in men but have no correlation in women[29].

In women , the decrease of estradiol levels in the current study is in agreement with finding of [30] of that premenopausal non –obese women with type 2 diabetes expressed low level of estradiol and this may result from the weak ability of ovaries in women with type 2 diabetes to convert androgen to estrogen because the reduction of aromatase activity in the ovaries .In addition , [31,32] indicate that the diabetes may be sex specific and characterized by abnormal levels of sex hormones when they performed a study in the female STZ-induced diabetic rat and found reduced in the estradiol levels and increase in the progesterone and testosterone levels.

According to the duration of the disease there is no significant difference in testosterone levels among diabetic group for both gender while estradiol level show significant elevation in males and significant decrease in females in the fourth duration of disease when compared with the first and second duration as shown in table (4) .The results of testosterone agreed with the study of Dandona*et al.,*[33]who found that the duration have no effect on testosterone levels.Other studies indicate that elevation of testosterone levels in females and its decreamet in males with type 2 diabetes are associated with increasing of C-peptide and the C-peptidewas noticed to be increased with progress of duration of type 2 disease[34,35].

The elevation of estradiol levels in males with progress of duration in the current study is agreed with the study of Mascarenhas *et al.,*[36] who notice elevation of estradiol levels in males with type 2 diabetes in the duration (more than 10 years) and suggest that estradiol levels elevation in the male may play a protection role to mechanisms of bone loss in diabetic male.

The results of the present study show that patients with type2 diabetes ,whether males or females , have significantly higher insulin hormone levels than control group table (1) and these results are in agreement with the study of Mamza*et al.,*[37] were they found elevation of insulin hormone levels in patients with type2 diabetes and this elevation associated with insulin resistance status. Also, the global diabetes community[38] explain the role of insulin resistance in developing of hyperinsulinemia with the compensating of pancreas in producing

more insulin and this will lead to development of type 2 diabetes and reported that hyperinsulinemia associated with several risks such as atherosclerosis, high levels of triglycerides and uric acid, hypertension and obesity.

Hyperinsulinemia may result from reduced the levels of leptin which participate in inhibition of insulin production where found that the inhibition of leptin receptors on pancreatic β-cell in the knockout mice lead to overproduction of insulin[39,40].

Several studies found that impairment of insulin sensitivity may result from hyperglycemia and hyperinsulinemia themselves[41]. Insulin Resistance is defined as a condition in which target tissues have decreased sensitivity to insulin, leads to elevated both blood insulin and glucose levels [42].

The body become more resistant to insulin as the duration of disease are increase, so that high or normal levels of insulin but the available insulin is insufficient [43]and this agreed with our finding as shown in table(4).

The results of this study showed significant elevation of FBG in type 2 diabetic group than control and this agreed with the former studies of [44;35].

Hyperglycemia is the main feature of diabetic and its elevation may associated with the elevation of glucagon level which involve in hepatic glucose production ,the major factor that participate in fasting and postprandial hyperglycemia[45].

Other studies indicate that hyperglycemia may result from elevation of cortisol levels which is ensure the elevation of blood glucose levels and in diabetic state it have undiserable role as it tend to sustain hyperglycemia[46,47].The physiological mechanism that possibly responsible for elevation of FBG in males than females may be related to differences in the distribution of fat in the body, where the men have large waist circumference[48]. Increase the abdominal fat lead to decrease in the metabolism of glucose and this associated with insulin resistance which have been found to be lower in males than in females[49,50]. Other studies found the presence of an association between increase glucose production and elevation of free fatty acids that seen more in males than in females[51,52].

According to the duration of disease the levels of FBG was significantly higher as the duration increase as shown in table(4) and this may be due to increase of insulin resistance and impaired the glycemic control and this related with longer duration of diabetes[53,54,55].

The correlation analysis show that the correlation between testosterone and estradiol is non-significant in males and females as shown in table(2) and this mean that the increase in the estradiol levels in men with type 2 diabetes is not dependent on decrease testosterone levels which represent the substrate for estradiol ,where several studies improved that estradiol levels is decreased and testosterone elevated in male with low testosterone levels and eugonadal men when used aromatase inhibitors and this indicate that not estradiol cause of low testosterone [56,57].Other study ,indicated that the mechanism that reduced testosterone levels in type 2 diabetes is more complicated and not estrogen cause it but insulin is seems to be responsible on this event because insulin act at brain level and may lead to defect in testosterone production in male and females[58].

The results of the current study show the presence of negative correlation between testosterone hormone and insulin and insulin resistance in males while the correlation is positive in females while testosterone has positive correlation with insulin sensitivity in males and females and this agreed with several researches that found the presence of inverse correlation between testosterone and insulin and insulin resistance in males independent to age ,smoking and alcohol consumption, that's it the negative correlation between testosterone and insulin resistance may results from the inverse correlation between insulin and SHBG where they found that men who have low testosterone levels is associated with significantly higher insulin levels and low SHBG[59,60,61,62]. Reduced testosterone levels cause muscle mass decrease and this lead to increase in free fatty acid that participate in insulin resistance development that end with type 2 diabetes[63,64]. Other study indicate that decreased testosterone levels release the normally suppressed ER beta expression and results in the down regulation of GLUT4 with resultant insulin resistance[65].

The positive correlation between testosterone and insulin and insulin resistance in female in the current study is agreed with the study of [66]. Hyperinsulinemia in diabetic patients lead to decrease SHBG levels and this

lead to increase levels of testosterone in females and insulin resistance cause elevation in LH which stimulate theca cells to production of testosterone in females[67,68,69]. Another study indicating that healthy women who administrated of androgen will developed insulin resistance and this mean that elevation of testosterone in female associated directly with insulin resistance[70].

The positive correlation between testosterone and insulin sensitivity in the current study agreed with study of Pitteloud *et al.,*[62]who found the presence of positive correlation between insulin sensitivity and testosterone and SHBG which mediated this correlation and it represent a good marker of insulin resistance [71] and the improved of insulin resistance after administration of testosterone lead to decrease in insulin sensitivity and thus prevention of type 2 diabetes in males while in female the reduction of androgens will lead to improve insulin sensitivity[70 ;58].

The correlation analysis show that the correlation between testosterone and FBG is inversely significant in males while the correlation is positive in females and this agreed with the studies of[17;72 ; 62]. The levels of testosterone have been reported to be decreased in patients with type 2 diabetes and found the presence of reduction in leydig cells in diabetic animals and proposed that hyperglycemia which cause the dysfunction of testes and then found that after testosterone administration in men with low testosterone lead to improve FBG and consequently improve insulin resistance, while in females atherosclerosis has been found to be associated strongly with hyperinsulinemia and hyperglycemia[73,74,75].

The results of the current study show the presence of negative correlation between estradiol hormone and insulin and insulin resistance and positive correlation with insulin sensitivity in females while the correlation is positively significant in males with insulin resistance as shown in table (3) and this agreed with study ofMatsui *et al.,*[76] who found the presence of an inverse correlation between estradiol and insulin and insulin resistance in women with type 2 diabetes.

Livingstone and Collison[77], suggested that estradiol at physiological levels keep the function of pancreaticβ-cell and its abnormal levels lead to developing of insulin resistance i.e (Matsui *et al.,*[76]) found that insulin resistance may developed in females who exposed to exogenous estradiol that was at inappropriate concentration.

It is seems that estradiol have a protective role in females from diabetes developing where it make body cells more sensitive to insulin[78,79]. This may explain the reduction of insulin sensitivity side by side with reduction of estradiol levels in our results. Insulin sensitivity is a key determinant of T2D risk and overall cardiometabolic health and studies indicate that estradiol directly impacts insulin action. Estradiol mediated effects on insulin action may be one mechanism by which hormone therapy reduces the incidence of T2D in women [80].

In healthy males, small amount of estrogen is produced by ledig cells and testosterone act on suppress the estrogen receptors while in patient with type 2 diabetes, the increased estradiol concentrations influence both of the estrogen receptors (alpha and beta), but specifically intensify the metabolic effects of ER beta because of its released suppression, a consequence of diminished testosterone concentrations. These dual actions then combine to amplify the mechanisms that lead to disordered glucose homeostasis and insulin resistance under these conditions[81;65], although the study of (pitteloud*et al.,*[62]) found that the correlation between estradiol and insulin sensitivity is non significant while the correlation is significant between testosterone and insulin sensitivity in males with type 2 diabetes.

In males and females the imbalance in the sex hormone occur with progress of age and this lead to developed of insulin resistance in both gender and this may explain developed of insulin resistance with duration of disease.

The correlation analysis show the presence of inverse correlation between estradiol and Fasting Blood glucose in females while the correlation is positive in males and this correlation increase with duration of disease. Estradiol hormone has an important role in glucose metabolism in females where several studies indicate that estradiol hormone is associated with decrease blood glucose by acting on reduce the production of hepatic glucose by inhibiting hepatic glucose-6-phosphatase activity[82,83]. Estrogen has special receptors that located in liver act on regulation of glucose homeostasis and deficiency of these receptors lead to inability of insulin to suppress the production of liver glucose and this cause hepatic insulin resistance[84]. Other study

indicate that using of estradiol as hormonal therapy in rats with induced type 2 diabetes will lead to improvement of damaged pancreatic cells and decrease glucose levels[85] and this may agree with our study where the estradiol level is decreased while FBG is elevated.

In males, the presence of positive correlation between estradiol and FBG in the current study is agreed with finding of (Jee-Young*et al.,*[17]) who found that elevation of estradiol in males associated with hyperglycemia and hyperinsulinemia.Other studies indicate that low levels of testosterone is secondary to increase of estradiol levels and poor glycemic control and polymorphisms in estrogen receptors genes lead to development of type 2 diabetes [86-87].

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