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ENDOGENOUS SEX HORMONES LEVELS AND THEIR ASSOCIATION WITH TYPE 2 DIABETES IN NORTH INDIAN MEN AND WOMEN

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ABSTRACT

Objective

To study endogenous sex hormones serum levels and their effects in Indian men and women having Type 2 Diabetes

Research Design and Methods

For the analyses, (n=200) subjects including (n=94) males and (n=106) females, out of which 100 diagnosed cases and 100 age and sex matched healthy controls were studied. Only diagnosed cases of diabetes type 2 (50 men and 50 women) aged 45–75 years undergoing glucose profile testing in outdoor clinics in the hospital PGIMS, Rohtak (2011-2013) were included following a detailed protocol. Patients with acute complications like coma and acidosis, pregnant women, postmenopausal women on hormone replacement therapy, use of steroids since past six months, type 1 diabetes were excluded. Early morning fasting samples were collected and serum analysed for testosterone, estrogen, fasting blood glucose and HbA1c. Serum testosterone levels [normal= males 20-39 years: 241–827 ng/dL, 40-89 years: 141-703 ng/dL; Adult Females:>19 years:<77ng/dL] were measured using direct chemiluminescent technology on ADVIA Centaur autoanalyser. **S**erum estrogen (normal in males- 10-36 pg/ml, females-Premenopausal:13-191 pg/ml, Postmenopausal:11-65 pg/ml) and HbA1c levels (normal=4-5.6% in normal people, <6.5% -target for control in diabetics) were measured on Autoanalyser via Immunoassay Kits. The results were analysed and compared.

Results

Overall analysis showed that diabetic men had low testosterone values (287.50±61.09) ng/dL as compared to controls (409.38 ±113.23) ng/dL ($p<0.001$) and raised HbA1c, whereas diabetic women had raised testosterone (52.35 ± 41.09) ng/dL values ($p<0.001$) and raised HbA1c as compared to controls (25.00±16.99) ng/dL ($p<0.001$). Diabetic Women had mean estradiol levels (47.00 ±53.36) pg/ml lower as compared to control females (69.31±57.51) pg/ml, ($p<0.05$), also they negatively correlated with HbA1c. Men showed no significant difference in estradiol levels in diseased and controls and showed no correlation between estradiol and HbA1c levels.

Conclusion

In North India -Diabetes type 2 is associated with Low Testosterone levels in Males, low estradiol and High Testosterone levels in Females, which in turn is associated with poor glycemic control in Diabetes type 2. Such

associations suggest possible clinical applications of sex hormone biomarkers in potentially adding prospective risk information. More prospective studies are needed to better define risk levels.

KEYWORDS: Coma and Acidosis, Pregnant Women, Postmenopausal Women on Hormone Replacement Therapy, Use of Steroids

INTRODUCTION

Hyperandrogenicity is well known to be correlated to insulin resistance in the polycystic ovarian syndrome and in nondiabetic women with abdominal obesity.[1]Androgen administration has been shown to induce insulin resistance and impaired glucose tolerance in women.[2]

The relation between testosterone and insulin sensitivity in men has been studied much less. Exposure to excess androgens seems to be followed by insulin resistance in some studies.[3] Also population studies of men confirm that low testosterone values are associated with insulin resistance in men with abdominal obesity4, 5. The low testosterone concentrations suggest that NIDDM in men is associated with a relative hypogonadism.

Further research in this area is important because of the therapeutic implications for NIDDM, where insulin resistance is a common phenomenon that hampers effective therapy. Furthermore, hyperandrogenicity, is a strong, independent risk factor for NIDDM in women[6], which raises interesting possibilities for screening women at risk for NIDDM. Sex-dependent relationships may also exist for estradiol and risk of diabetes. Several studies have observed positive associations between estradiol and insulin resistance in women but not in men, while results from other studies were conflicting.[7-9]

From a clinical perspective, the consistent findings among both men and women of significant associations for testosterone and estradiol, suggest possible clinical applications of sex hormone biomarkers in potentially adding predictive risk information. More prospective hormonal investigations are needed to better define risk levels More prospective hormonal investigations are needed to better define risk levels. Furthermore, the potential adverse clinical diabetes risk associated with antiandrogen therapy for men and testosterone therapy for women should also be carefully considered.

For inconsistency observed in the studies so far, the present study has been planned to study Testosterone, Estrogen and HbA1c levels in the patients of Type 2 DM and find the correlation with glycemic control and complications.

RESEARCH DESIGN AND METHODS

The study was conducted in the hospital PGIMS, Rohtak (2011-2013) in department of biochemistry in collaboration with department of medicine. Only diagnosed cases of diabetes type 2 undergoing glucose profile testing in outdoor clinics were included following a detailed protocol. 100 patients with Type 2 DM and 100 age and sex matched healthy controls were taken. Out of 100 cases, 55 were males and 45 were females whereas in 100 controls 51 were males and 49 females. Patients of age group 45-75 years were included in the study. Patients with acute complications like hyperglycaemic hyperosmolar coma, comorbid conditions like testicular tumor, prostate or breast cancer, lipidemias, PCOS (polycystic ovarian syndrome) and CAH (congenital adrenal hyperplasia), Insulin therapy,

Intake of drugs (known to interfere with HPA axis or with autonomic nervous system) like β-blockers, α-blockers,

and cholinergic agonists and antagonists; hormone-modulating therapies or topical/systemic glucocorticoids within 3 months, chronic debilitating disease such as severe depression or psychiatric illness, head trauma, renal failure, haemochromatosis, cirrhosis, hepatitis C, HIV, congenital hypogonadotropic hypogonadism or panhypopituitarism, pregnant and lactating women were excluded.

History was taken from all diabetic patients and control subjects and complete general and systemic physical examination was performed. All patients and controls were subjected to anthropometric measurements, routine and special investigations. Anthropometry included measurement of weight, height, waist circumference, hip circumference, BMI and waist hip ratio. Informed consent was taken from all subjects and all hazards were explained. The study was approved by ethical committee of the University of health Sciences, Rohtak where the study was carried out. Routine investigations included haemoglobin, total leukocyte count, blood urea, serum creatinine and fasting blood glucose levels. Special investigation performed were glycosylated haemoglobin, serum estrogen and serum testosterone

5ml overnight fasting blood sample was collected from the antecubital vein aseptically without anticoagulant and allowed to clot. Serum was separated by centrifugation of the sample and was used for the assays (sample were stored at 2-8°C for 1day, and at -20°C if storage was required for more than 1 day).1 ml blood sample was collected in EDTA vial separately irrespective of time and meal for estimation of glycosylated haemoglobin. All the patients with diabetes mellitus type-2 as well as control were subjected to serum investigations.

Glycosylated Haemoglobin was determined by ion exchange chromatography as described by Goldstein et al, using ion exchange chromatography kits.[10]

Testosterone was measured using the ADVIA Centaur Testosterone assay which is a competitive immunoassay using direct chemiluminescent technology.11,12 Reference normal serum testosterone levels in males 20-39 yrs: 241–827 ng/dL **;**40-89 yrs:141-703 ng/dL and in adult females >19 years: < 77 ng/dL.

Serum Estradiol was measured via ELISA kit, a solid phase enzyme linked immunosorbent assay, based on the principle of competitive binding. Reference range being: Males- 10-36 pg/ml; Females-Premenopausal: 13-191 pg/ml and Postmenopausal: 11-65 pg/ml.

Serum hormone levels were measured in an biochemistry laboratory and pathology blood transfusion laboratory by chemiluminiscence and Elisa techniques using first-thawed specimens from the 2011 to 2013 venipuncture during 2011-2013. Free testosterone and estradiol were thus determined.

Data were analyzed using simple statistical techniques. Analyses were performed using mean values and bar diagrams. Unadjusted associations between hormone levels and diabetes were evaluated using Student's *t* test and χ^2 test and calculating p values.

RESULTS

Baseline Characteristics and Diabetes

Mean fasting blood glucose levels (149.46±29.28 mg/dL) were significantly higher in men ($P < 0.001$) and women ($P < 0.01$) with diabetes compared with persons without diabetes (95.72±6.21 mg/dL). (Table 1)

Table 1: Fasting Blood Glucose and Glycosylated Hemoglobin Levels in Cases and Controls

Parameter	Cases	Controls	p value
Fasting blood glucose (mg/dl)	149.46± 29.28	95.72±6.21	<0.001
HbA1c (%)	9.32±2.85	4.37±0.845	<0.001

The mean levels of glycosylated haemoglobin in diabetic and control group were 9.32±2.85% and 4.37±0.845% respectively, and the difference was statistically highly significant (p<0.001). (Table 1)

No differences were observed for age and sex (Table 2)

Table 2: Age and Sex Wise Distribution of Cases and Controls

	Cases (n=100)	Controls (n=100)
Mean age	53.73±11.30	51.43±14.11
Range	31-78	24-80
Male	50 (50%)	50(50%)
Female	50 (50%)	50 (50%)

Diabetes had significantly higher mean waist circumference, BMI (Table 3), triglycerides. (Table4) and HbAlc (Table 2) Mean total testosterone (Table 5), estradiol (Table 6) and HDL-cholesterol levels were lower

Table 3: Body Mass Index (BMI) and Waist Hip Ratio (W/H R) in Cases and Controls (All Values are in Mean±SD)

	Cases	Controls	p value
BMI (kg/m^2)	29.17±6.50	25.66±5.07	<0.001
W/H Ratio (Waist Hip Ratio)	0.951±0.022	0.934±0.073	<0.001

Table 4: Lipid profile in cases and controls (mean±SD)

	Cases	Controls	p-value
TC (mg/dl)	200.97±40.14	170.78±50.66	<0.001
TG (mg/dl)	170.74±44.18	151.09±83.91	<0.001
HDL-C (mg/dl)	42.73±18.24	47.78±5.40	<0.001
VLDL-C (mg/dl)	34.14±8.83	30.21±16.78	<0.001
LDL-C (mg/dl)	111.86±48.42	101.21±32.03	<0.05

Sex Hormones and Diabetes

Diabetic men had significantly lower (p<0.001) mean testosterone levels (287.50±61.09 ng/dl) than men without diabetes (409.38±113.23 ng/dl) and diabetic women had mean testosterone levels (52.35±41.09 ng/dl) significantly higher (p <0.001) compared to women without diabetes (25.008±16.99 ng/dl). (Table 5)

Table 5: Serum Testosterone Levels in Cases and Controls

	Diabetes	Control	P value
Serum Testosterone levels (M) (ng/dl)	287.50±61.09	409.38±113.23	<0.001
Serum Testosterone levels (F) (ng/dl)	52.35±41.09	25.00±16.99	<0.001

Table 6: Estradiol (E2) Distribution Sex wise in Cases and Controls

	Diabetes	Control	P value
Female estradiol levels (pg/ml)	47.00±53.36	69.31±57.51	<0.05
Male estradiol levels (pg/ml)	20.94±9.23	22.73±13.97	0.470

Mean Estradiol (E2- pg/ml) levels were significantly ($p < 0.05$) lower in diabetic women (47.00±53.36 pg/ml) as compared to women without diabetes (69.31±57.51 pg/ml). The difference in men was insignificant ($p = 0.470$). (Table 6)

CONCLUSIONS

In present study, diabetic males had mean testosterone levels 287.50±61.09 ng/dl whereas in the control group males it was 409.38±113.23 ng/dl. The difference in levels of serum testosterone in diabetic males and control male group was statistically highly significant ($p<0.001$). The diabetic females had mean testosterone levels of 52.35±41.09 ng/dl whereas in the control group females it was 25.008±16.99 ng/dl. The difference in levels of serum testosterone in diabetic females and control female was statistically highly significant ($p <0.001$). The mean testosterone levels in diabetic males were lower as compared to control males and in diabetic females were higher than control females.

This was in corroboration with various studies by Grossman, Dhindsa, Atlantis and Kapoor which were done in males with diabetes type 2. They found that testosterone levels were frequently low in type 2 diabetic men and are partially influenced by insulin resistance. In other words, testosterone was inversely and independently associated with DM prevalence and they have symptoms of hypogonadism.[13-16] It is further supported by other studies conducted by Ding and Young. They found that low testosterone levels in men and high in women are associated with higher risk of type 2 diabetes.[17,18]

It has also been reported in the literature that Insulin diminishes SHBG production in the liver. Therefore, hyperinsulinemia may be associated with low SHBG levels, which in turn affects levels of testosterone.[19,20] Testosterone may also influence glucose control through increasing lean body mass in men.[21] Patients had raised insulin levels, thus decreasing testosterone levels in men. This is further supported by a study carried out by Jones et al. They found that testosterone replacement therapy (TRT) was associated with beneficial effects on insulin resistance, total and LDL-cholesterol and sexual health in hypogonadal men with type 2 diabetes.[22]

Estrogen might play an important role in the pathogenesis of diabetes mellitus type 2. It has been suggested that estrogens inhibit diabetes via distinct mechanisms particularly by reducing both hyperglycemia and plasma insulin levels. One mechanism can be via its receptors as estrogen exerts its physiological effects mainly through estrogen receptors including α and β types, as they are found in many tissues that participate in the pathogenesis of type 2 diabetes.[23]

We found that estradiol (E2) levels were significantly lower in female cases as compared to controls ($p <0.001$) (Table 9). This is affirmed by study that estrogen replacement therapy on postmenopausal women was shown to lower glycosylated hemoglobin levels.24

Our results are in concordance with a study conducted by Koh and associates. They assessed the effects of estrogen on homeostatic functions in type 2 diabetic postmenopausal women. They found that estrogen tended to lower low-density lipoprotein (LDL) cholesterol and glycosylated hemoglobin levels whereas increase high-density lipoprotein (HDL) cholesterol and triglyceride levels. The decrease in LDL levels results from accelerated LDL catabolism; the increase in triglyceride levels results from increased production of large, triglyceride-rich VLDL.[25]

To conclude, patients with DM type 2 have abnormalities in various hormone levels. These associations may be considered in the pathogenesis of the disease and should be taken into account for the treatment of patients of DM type 2.

SUMMARY AND CONCLUSIONS

Serum testosterone levels in diabetic males were lower as compared to control males ($p<0.001$) whereas in diabetic females were higher than control ($p<0.001$) and Serum estrogen levels were significantly lower in diabetic females as compared to controls ($p <0.000$).

In conclusion, patients with DM type 2 have abnormalities in testosterone and estrogen levels. These associations may be considered in the pathogenesis of the disease and should be taken into account for the treatment of patients of DM type 2.

Moreover, from a clinical perspective, the consistent findings among both men and women of significant associations for testosterone and estradiol, suggest possible clinical applications of sex hormone biomarkers in potentially adding predictive risk information. More prospective investigations are needed to better define risk levels.

Furthermore, the hormone therapy may have a role to play. Therefore, the potential adverse clinical diabetes risk and other risks associated with various hormone replacement therapies like antiandrogen therapy for men, testosterone therapy for women, estrogen replacement therapy for postmenopausal women should also be carefully considered.

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