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RESEARCH ARTICLE

Stress hormone- cortisol and testosterone hormone levels and their role in North Indian Men and Women with Diabetes mellitus type 2.

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Abstract

Objective: To study stress hormone - cortisol and testosterone hormone serum levels and their role in north 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 cortisol, testosterone, 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 1,2, serum Cortisol levels3,4 (Reference range: 50-230 ng/ml) were done using DRG Cortisol ELISA kit, a solid phase enzyme linked immunosorbent assay, and HbA1c levels (normal=4 - 5.6% in normal people, <6.5% - target for control in diabetics) were measured on Autoanalyser via Immunoassay Kits5. 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 ($p<0.001$) . Mean cortisol levels (106.07 ±46.57) ng/ml were signiicantly higher in diabetic cases as compared to controls (84.32±54.84) ng/ml, ($p <0.05$).

Conclusion: In North India -Diabetes type 2 is associated with Cortisol levels significantly higher in cases as compared to controls, also fasting blood glucose and HbA1c levels were higher in diabetics irrespective of sex. Serum testosterone levels were lower in diabetic males as compared to controls whereas in diabteic females were higher than controls. Such associations suggest possible clinical applications of hormone biomarkers in potentially adding prospective risk information. More prospective studies are

needed to better define risk levels.

Cortisol directly plays role in glucose metabolism and affects insulin's actions as well. Cortisol stimulates gluconeogenesis (formation, in the liver, of glucose from certain amino acids, glycerol, lactate and/or propionate). Cortisol counteracts insulin, contributes to hyperglycemia - causing hepatic gluconeogenesis and inhibits the peripheral utilization of glucose (insulin resistance) by decreasing the translocation of glucose transporters (especially GLUT4) to the cell membrane.[15]However, cortisol increases glycogen synthesis (glycogenesis) in the liver.

Chiodini (2007) found that in type 2 diabetic subjects, hypothalmic-pituitary-adrenal activity is enhanced in patients with dia betic complications and the degree of cortisol secretion is related to the presence of diabetes complications. They evaluated cortisol secretion in hundred and seventy, type 2 diabetic subjects. They evaluated the presence of chronic complications (incipient nephropathy, asymptomatic neuropathy, background retinopathy, and silent macroangiopathy) and found that cortisol levels were higher in diabetics with complications than without them and controls.[3]

Reynolds(2010) analysed serum cortisol levels in type 2 DM cases and found higher fasting cortisol levels to be associated with greater estimated cognitive decline. Therefore, strategies targeted at lowering cortisol action may be useful in ameliorating cognitive decline in individuals with type 2 diabetes.[16]

Also, population studies of men confirm that low testosterone values are associated with insulin resistance in men with abdominal obesity. The low testosterone concentrations suggest that NIDDM in men is associated with a relative hypogonadism.10,17 Hyperandrogenicity is well known to be correlated to insulin resistance in the polycystic ovarian syndrome and in nondiabetic women with abdominal obesity.[4]Androgen administration has been shown to induce insulin resistance and impaired glucose tolerance in women.[13]

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

For inconsistency observed in the studies so far, the present study has been planned to study cortisol, testosterone 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 cortisol 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.[8]

Serum Cortisol 18,14 levels were measured using DRG Cortisol ELISA kit – a solid phase enzyme linked immunosorbent assay, based on the principle of competitive binding.

Serum Testosterone was measured using the ADVIA Centaur Testosterone assay which is a competitive immunoassay using direct chemiluminescent technology.2,5 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 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 cortisol and testosterone levels 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 calculationg 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).

Age and Sex wise distribution:-

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%)

Body Mass Index (BMI) and Waist Hip Ratio (W/H R):-
Diabetes had significantly higher mean waist circumference and BMI (Table 3),

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

Lipid profile and Diabetes :-
Diabetes had significantly higher triglycerides. (Table4) and HDL-cholesterol levels were lower in cases.

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

Fasting Blood Glucose and Glycosylated Hemoglobin:-
The subjects in the diabetic group had mean fasting blood glucose levels of 149.46±29.28 mg/dL whereas in the control group it was 95.72±6.21 mg/dL. The difference in levels of FBG in diabetic and control groups was statistically highly significant (p<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 5:- 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

Cortisol and diabetes:-
Cortisol was significantly higher in diabetic cases as compared to controls, (p =0.000) which is statistically significant.

Table 6:- Mean Cortisol levels in cases and controls.

parameter	**Cases**	**Controls**	**P value**
Cortisol levels (ng/ml)	106.07±46.57	84.32±54.84	<0.001

Testosterone 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 7:- 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

Conclusions:-

In present study, serum cortisol levels were significantly higher in cases as compared to controls (p <0.001). This is in accordance with literature which reveals that cortisol directly plays role in glucose metabolism and affects insulin as well. Cortisol counteracts insulin, contributes to hyperglycemia-causing hepatic gluconeogenesis and inhibits the peripheral utilization of glucose (insulin resistance) by decreasing the translocation of glucose transporters

(especially GLUT4) to the cell membrane.[15]

Our finding is further supported by previous studies carried out by,Chiodini(2007) and Reynolds (2010) which suggest that high cortisol levels in diabetes type 2 are positively associated with derranged glycosylated haemoglobin, blood pressure changes, relative abdominal mass, severity of clinical features and complications.[3,16]

In present study, 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 (2008), Dhindsa (2004), Atlantis(2011) and Kapoor (2007) 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.[9,6, 1, 12]It is further supported by other studies conducted by Ding (2006) and Young (2002). They found that low testosterone levels in men and high in women are associated with higher risk of type 2 diabetes.[7,11]

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.

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

Furthermore, the hormone therapy may have a role to play. The role of various strategies targeted at maintaining cortisol levels in improving health profile of DM 2 patients, might be considered.

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:-

Mean cortisol levels were significantly higher in diabetics.

Serum testosterone levels in diabetic males were lower as compared to control males ($p<0.001$) whereas in diabetic females were higher than control ($p<0.001$)

In conclusion, patients with DM type 2 have abnormalities in lipid profile, cortisol and testosterone 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.

The role of various strategies targeted at maintaining cortisol levels in improving health profile of DM 2 patients, might be considered.

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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