CLINICAL STUDY OF INSULIN RESISTANCE IN DIABETIC PATIENTS WITH SUBCLINICAL AND CLINICAL HYPOTHYROIDISM

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ABSTRACT

The relationship of insulin resistance in subclinical and clinical hypothyroid diabetic patients has been elucidated. This study was conducted in Regional Hospital, Hamirpur, Himachal Pradesh, India. 117 patients (aged 20-50, male 23, female 94) were selected randomly. 100 age and sex matched control subjects were also included. The concentration of thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), insulin were evaluated by Enzyme Linked Immunoassay (ELISA) and glucose was estimated by auto analyser. Insulin resistance and β -cell function were assessed by Model Assessmen. Diabetic patients had significantly (P<0.0001) higher level of TSH, Insulin and glucose. The levels of FT3 and FT4 were significantly (P<0.0001) lower. The level of HOMA-IR and HOMA- β were significantly higher in diabetic patients. We concluded that insulin resistance and hypothyroidism were present in diabetic patients.

Key Words: Diabetes, Hypothyroidism Insulin Resistance

INTRODUCTION

Diabetes mellitus and thyroid disorders are the two most common endocrinopathies seen in the adult population. Excess or deficiency of either insulin or thyroid hormones can result in functional abnormalities of one another, as both of them are closely involved in cellular metabolism (Satish and Mohan, 2003). Possibly, thence, diabetes and thyroid disorders have a propensity to appear together in patients (Wu, 2000). Thyroid hormones play an indispensable role in various metabolic processes in the human body (Fernandez-Real *et al.*, 2006). Hypothyroidism and hyperthyroidism are the main clinical conditions that affect the basal metabolic rate.

Type 2 diabetes accounts for most individuals with non-autoimmune forms of diabetes. The spectacular increase in the prevalence of type 2 diabetes worldwide is well documented. Patients with type 2 diabetes commonly display the symptoms of hypothyroidism, and symptoms of hyperthyroidism have been documented in the patients with type 1 diabetes (Wu, 2000).

Insulin resistant diabetes mellitus is the commonest form which accounts for 90% of the diabetic population in the world (Wild *et al.*, 2004). Insulin resistance is the central pathophysiological phenomenon of metabolic syndrome (Eckel, 2005). Thyroid disorders in terms of subclinical and overt hypothyroidism have also been associated with defective insulin secretion, hyperinsulinemia, altered peripheral glucose disposal, and insulin resistance (Maratou *et al.*, 2009).

Aim of the study

In the light of the above facts, the present study elucidates the relationship of thyroid hormones and insulin secretions in glucose homeostasis.

MATERIALS AND METHODS

For the present study, 117 diabetic patients of both sexes (males-23, females-94) affected with thyroid dysfunction, in the age group of 20-50 years (Mean age 37.82 ± 8.90 years) from Regional Hospital Hamirpur, Himachal Pradesh, India were randomly selected. 100 age and sex matched euthyroid non-diabetic controls were also included in the study.

The study protocol was approved by the Institutional Human Ethical Committee of Punjabi University Patiala, India. Full informed written consent was obtained from all the patients. Overnight fasting venous

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blood samples were withdrawn with the help of trained laboratory technician. Blood samples were collected and centrifuged for 4 minutes at 3000 rpm. The serum samples were assayed for the estimation of thyroid stimulating hormone, free triiodothyronine, free thyroxine, insulin. Insulin resistance was assessed by Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and β -cell function was assessed by HOMA- β .

HOMA-IR = Fasting Insulin \times Fasting Glucose/22.5 and β -cell function by HOMA- β (20 \times Fasting insulin/fasting glucose -3.5%)

Statistical Analysis

Data were analysed using SPSS for windows version 16 and Statistica. Demographic data, distribution of patients was assessed by Fishers exact test, nutritional status and socioeconomic status was compared by using chi-square test. TSH, FT3, FT4, insulin, glucose, HOMA-IR and HOMA- β were compared by ANOVA with post hoc Tuckey's multiple comparison tests.

RESULTS AND DISCUSSION

Results

In the present study, the number of patients with thyroid dysfunction and their respective prevalence were: subclinical hypothyroidism 57 (48.71%) and clinical hypothyroidism 60 (51.28%). The incidence of thyroid disorders was highest in clinical hypothyroid females. Present study revealed the female predominance of developing type 2 diabetes and hypothyroidism simultaneously was higher in women (80.34%) and in men (19.65%).

Thyroid dysfunction was significantly ($\chi^2 = 6.359$, P<0.0416, Figure 1) higher in diabetic patients with poor nutritional status and was lowest (24.79%) in the group with good nutritional status. The impact of thyroid dysfunction in diabetics was significantly ($\chi^2 = 16.667$, P<0.0002, Figure 2) highest in the low socio-economic group (50.43%), than in the middle socio-economic group. The effect of thyroid dysfunction was lowest (20.51%) in diabetic patients with high socio-economic status.

The mean value of basal metabolic rate was significantly (F = 177.07, P<0.0001) declined in the subclinical and clinical hypothyroid diabetics (Figure 3). Post hoc Tukey's multiple comparison test after one way ANOVA described a significant (F = 1479, P<0.0001 q =33.17 to 76.73, 95%CI -6.188 to - 12.230, Figure 4) increase in body mass index in subclinical and clinical hypothyroid diabetics as compared to non-diabetic control.

Thyroid Hormones Function Parameters

There was a significant (f=1014, P<0.0001) elevation in the mean level of thyroid stimulating hormone (TSH) in both sub clinical and clinical hypothyroid diabetic patients in comparison to non diabetic control. Post hoc tuckey's multiple comparison test after one way ANOVA described a significant (P<0.05) elevation in mean level of TSH, in control vs subclinical hypothyroid (q= 35.98, 95%CI- 10.62 to -8.806) and clinical hypothyroid (q= 62.11, 95% CI -17.39 to -15.60) Figure 5.

FT3 concentration in hypothyroid diabetic sub clinical and clinical groups were significantly (F=3488, q= 11.39 to 113.5, 95% CI 0.1285 to 1.838, P<0.0001, Figure 6) lower than the non diabetic control group. The mean serum concentration of FT4 was significantly (F= 1830, q= 7.374 to 81.94, P<0.0001) decreased in sub clinical and clinical hypothyroid diabetic than non diabetic control (figure 7). The ratio of FT3/FT4 showed significant (t= 6.353, P<0.001) decrease in the clinical hypothyroid diabetic patients (Figure 8).

Insulin

Pot hoc multiple comparison test after one way ANOVA revealed that the mean serum level of insulin (fasting) was significantly (F= 2462, P<0.0001, figure 9) increased in the both subclinical (q= 50.87, 95% CI -10.54 to -9.235, mean difference -9.887, P<0.05) and clinical (q= 97.97, -19.38 to -18.09 mean difference -18.740, P<0.05) hypothyroid patients as compare to non diabetic control. In sub clinical vs clinical group there was also a significant (q= 40.85, 95% CI 8.122 to 9.575, mean difference 8.848) increase in the level of serum insulin (fasting).

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Glucose

Fasting serum glucose level were significantly (F= 521.4, P<0.0001, q= 31.62 to 42.29, 95% CI -50.98 to -55.85, figure 10) elevated in diabetic patiets with thyroid hypofunction.

Insulin-Resistance and β-cell functiom

Level of insulin resistance was increased (F=1980, q=45.38 to 87.90 95% CI -5.042 to -8.606, P<0.0001, Figure 11) in sub clinical and clinical hypothyroid diabetic patients. The mean level of HOMA- β was significantly (F= 566.590, q= 12.07 tp 28.09, 95% CI -2871.53 to 2785.47, P<0.0001 Figure 12) increased in the hypothyroid diabetic patients in comparison to non diabetic control.



Figure 1: Nutritional status in diabetic hypothyroid patients



Figure 2: Socio-economic status in diabetic patients affected with hypothyroidism





Figure 3: Basal metabolic rate in diabetic patients



Figure 4: Body mass index in diabetic patients affected with hypothyroidism





Figure 5: Serum concentration of TSH in diabetic patients



Figure 6: Serum concentration FT3 in diabetic patients





Figure 7: Serum concentration FT4 in diabetic patients



Figure 8: Ratio of FT3/FT4 in diabetic hypothyroid patients





Figure 9: Serum fasting insulin level in diabetic patients with hypothyroidism



Figure 10: Mean fasting blood glucose in diabetic hypothyroid patients

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Figure 11: Insulin resistance (HOMA-IR) level in diabetic patients with hypothyroidism



Figure 12: Level of HOMA-β in diabetic hypothyroid patients

Correlation Analysis

Pearson's bivariate correlation revealed a significant (Y = $-2.825x + 5.4594^*$, r = 0.82264, P<0.05 Figure 13) positive relation between insulin (fasting) and glucose (fasting). Pearson's bivariate correlation revealed that TSH positively correlated with insulin fasting (Y= $23.701x + 0.29993^*$, r = 0.47569, P<0.05 Figure 14) in hypothyroid patients. There was significant (P<0.05) negative correlation between FT3 vs insulin (fasting) and (Y = $40.924x - 10.14^*$, r = -0.6886 figure 15). However the correlation between FT4 vs insulin (fasting) (r = -0.2190, P = 0.082) was statistically non significant.

Relationship of TSH, FT3 and FT4 with Fasting Serum Glucose Groups

Pearson's bivariate correlation and linear regression revealed a positive correlation of TSH with different fasting serum glucose (FSG) groups (Y = 3.498x + 8.505, $R^2 = 0.9839$, r = 0.96, P<0.01). Pearson's bivariate correlation revealed a negative correlation of FT3 vs different fasting serum glucose (FSG)

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groups (Y = 2.8894x -0.535, $R^2 = 0.8132$, r = -0.92, P<0.01) and FT4 vs different fasting serum glucose (FSG) groups (Y = 1.4531x -0.494, $R^2 = 0.8959$, r = -0.94, P<0.001) and a significant (Y = -0.221x + 1.32, $R^2 = 0.942$, r = -0.92, P<0.01) correlation of FT3/FT4 vs different fasting serum glucose (FSG) groups Figure 16.



Figure 13: Scatterplot showing correlation between serum insulin (fasting) and glucose (fasting) in hypothyroid diabetic patients



Figure 14: Scatterplot showing correlation between serum TSH and insulin (fasting) in hypothyroid diabetic patients

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Figure 15: Scatterplot showing correlation between serum FT3 and insulin (fasting) in hypothyroid diabetic patients



Figure 16: Correlation and regression analysis of serum fasting glucose with FT3 and FT4

Discussion

Present study revealed the female predominance of developing type 2 diabetes and hypothyroidism simultaneously was higher in women (80.34%) than in men (19.65%).

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In the present study, body mass index (BMI) was increased in diabetic patients affected with hypothyroidism as compared to non-diabetic control. The value of BMI was highest in the clinical hypothyroid diabetic patients. Our observations are in consonance with the study of Hettihewa (2007) and Thakkar and Jain (2011).

Thyroid hormones have metabolic functions that serve to control the basic hormone metabolic rate. In the present study the basal metabolic rate (BMR) was decreased in the subclinical and clinical hypothyroid diabetic patients as compared to control. Basal metabolic rate has been reported to be decreased in hypothyroidism (Jennal and Johnson, 2006). BMR in hypothyroid patients may also have been responsible for the increased body mass index in the study population. Nonetheless, obesity is a major risk factor for type 2 diabetes and hypothyroidism (Singh *et al.*, 2008).

The present study, reported high incidence of abnormal thyroid hormone level in diabetic patients. Subclinical and clinical hypothyroidism was reported in the diabetic population. Our observations are in agreement with study of Udoing *et al.*, (2007).

Diabetes mellitus appears to influence thyroid function in at least two sites, one at the level hypothalamic control of TSH release and the other at the conversion of T4 to T3 in the peripheral tissue. Present study highlights the changes in TSH levels in normal ranges are significantly associated with insulin resistance. The level of TSH was altered in the diabetic patients in the present study. This study was supported by a report with high prevalence of abnormal TSH concentration. Our results showed that the mean serum FT3 was lower, this is in consistence with the mean serum FT3 in patients was significantly lower than controls (Islam *et al.*, 2008). Only 0.4% T3 and 0.04% T4 dynamically escape binding and, since free to interact directly with peripheral organs and tissues, are called free T3 (FT3) and free T4 (FT4) and are the only fraction believed to be metabolically active (Gershengorn *et al.*, 1980). In the present study FT3/FT4 ratio was decreased in clinical hypothyroid diabetic patient in comparison to non diabetic controls.

In the present study, the level of TSH according to different ranges of glucose was increased and the level of FT3 and FT4 were decreased in the diabetic hypothyroid patients. The ratio FT3/FT4 was also decreased according to different ranges of glucose. The serum levels of insulin fasting were increased in hypothyroid patients. Thyroid hormones are insulin antagonists, both insulin and thyroid hormones are involved in cellular metabolism and excess and deficit of any one can result in functional derangement of the other. The physiological and biochemical interrelationship between insulin and the influence of both insulin and iodothyronines on the metabolism of carbohydrates, proteins and lipid have been recorded (Dias et al., 1995). In some study population it was found that insulin resistance modifies the relationship between thyroid function and insulin sensitivity. It is known that thyroid hormone can stimulate the expression and activate a number of proteins that are candidates for regulating insulin sensitivity (Kleiverik et al., 2009). Glycemic status is influenced by insulin, which is known to modulate TSH levels. In the present study the level of glucose was decreased in the hypothyroid diabetic patients. Many studies revealed that T3 and insulin both stimulate the expression of hexokinase and glycogen synthase which are respectively responsible for uptake and disposal of glucose via formation of glucose-6 phosphate and glucose-1 phosphate (Chidakel et al., 2005). The rate of hepatic glucose output is decreased probably due to reduced gluconeogenesis. Insulin resistance is defined as an inability of insulin to produce its biological effects at physiological concentrations and is a cardinal feature of type 2 diabetes mellitus (Ferrannini, 2004). It is characterized by the impaired ability of insulin to inhibit hepatic glucose output and to stimulate glucose uptake into skeletal muscle. Insulin resistance is assessed by the calculation of HOMA-IR. Homeostatic model assessment (HOMA) of β -cell function and insulin resistance (IR) is a method for assessing β-cell function and IR from basal glucose and insulin or C-peptide concentration. In recent study it was found that insulin resistance was comparable in both subclinical and clinical hypothyroidism (Maratou, et al., 2009). The present study reported that the insulin resistance by HOMA-IR was present in the diabetic patients affected with hypothyroidism both in subclinical and clinical conditions. HOMA-IR was significantly raised in clinical hypothyroidism as compared to controls and subclinical hypothyroidism.

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Consistent hyperglycemia in the hyperinsulinemic and insulin resistance subjects might be attributed to this disturbed balance between insulin and T3, which fail to maintain normal glycemia. The results of the present study showed that the homeostasis relationship of insulin and T3 is gradually lost with the parallel development of insulin resistance and insufficiency of insulin to the cells.

Homeostatic model assessment (HOMA) of β -cell function and insulin resistance (IR) is a method for assessing β -cell function and IR from basal glucose and insulin or C-peptide concentration (Wallace *et al.*, 2004). In present study it was found that insulin resistance was comparable in both subclinical and clinical hypothyroidism (Maratou, *et al.*, 2009). Thyroid hormones increase beta-cell apoptosis and that this could be one major element responsible for deterioration of glucose tolerance in thyrotoxicosis. Long term thyrotoxicosis has been shown to cause beta-cell dysfunction resulting in reduced pancreatic insulin content, poor insulin response to glucose and decreased rate of insulin secretion (Bech *et al.*, 1996). Records indicate that iodothyronine are insulin antagonists with high levels being diabetogenic, while absence of iodothyronines inhibits the development of diabetes (Udiong *et al.*, 2007).

The present study demonstrates that the insulin resistance by HOMA-IR was present in the diabetic patients affected with hypothyroidism both in subclinical and clinical conditions. HOMA-IR was significantly raised in clinical hypothyroidism as compared to controls and subclinical hypothyroidism. This is in agreement with the study of Bakker et al., (2001) which attributed the main pathophysiological basis underlying glucose intolerance, dyslipidemia, abdominal obesity and hypertension to insulin resistance. In type 2 diabetes mellitus subjects, lower levels of T3 and higher levels of insulin failed to maintain normoglycemic condition and this further enhanced the hyperinsulinemia. Consistent hyperglycemia in the hyperinsulinemic and insulin resistance subjects might be attributed to this disturbed balance between insulin and T3, which fail to maintain normal glycemia. The results of the present study showed that the homeostasis relationship of insulin and T3 is gradually lost with the parallel development of insulin resistance and insufficiency of insulin to the cells. Insulin resistance with the hyperglycemia, hyperinsulinemia, and the altered natures of skeletal muscle fibres with lower ratio of oxidative to glycolytic enzymes, reduced the glucose disposal via reduced oxidative of glucose (Shmiokawa et al., 1997, Crunkhorn and Petti, 2008). Insulin resistance is a pathological situation characterized by a lack of physiological response of peripheral tissues to insulin action, leading to the metabolic and hemodynamic disturbances known as the metabolic syndrome.

The relationship between glucose and insulin in the basal state reflects the balance between hepatic glucose output and insulin secretion, which is maintained by a feedback loop between the liver and β -cells (Turner *et al.*, 1979). Recently, it has been reported that oxidative stress leads to development of late complications of diabetes and also participates in beta cell dysfunction or insulin resistance (Sarabesh, 2006).

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