Screening for Thyroid Dysfunction
in the Diabetic/Non-Diabetic Population

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Abstract. The objective of this study was to investigate the effect of diabetes mellitus on thyroid hormone levels and other biochemical variables. The study population consisted of 200 subjects divided into two groups: diabetic (n=100) and non-diabetic (n=100). In order to clarify the mechanism of impaired thyroid hormone levels in patients with diabetes, blood glucose, glycosylated hemoglobin (HbA1C), lipids, proteins, urea, microalbuminurea, thyroid stimulating hormone (TSH), total thyroxine (T_4), total triiodothyronine (T_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) were examined. A significant increase in the levels of blood glucose, HbA1C, serum cholesterol, triglyceride, low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), urea, creatinine, and microalbuminurea was observed in diabetic patients compared to non-diabetic subjects. On the other hand, the levels of total protein, albumin, and high-density lipoprotein (HDL-C) were significantly decreased in diabetics. The level of TSH was significantly decreased whereas the levels of T_4 and FT_4 were significantly increased in diabetic patients compared to control subjects. However, the T_3 and FT_3 levels did not differ significantly between groups. Out of 100 diabetic patients studied, 28% had low plasma thyroid hormone levels, 17% had high thyroid hormone, and 55% had euthyroid levels. This study has shown a high incidence of abnormal thyroid hormone levels among the diabetics. In conclusion, our findings demonstrate that detection of abnormal thyroid hormone levels in the early stage of diabetes mellitus will help the patients to improve quality of life and reduce the morbidity rate.

Keywords. Diabetes mellitus • Glycosylated hemoglobin • Thyroid hormones • Serum lipids

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Introduction

Diabetes mellitus is an important health problem affecting major populations worldwide. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological system to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia.[1][2][3]

Epidemiological studies and clinical trials strongly support the notion that hyperglycemia is the principal cause of diabetic complications. Effective blood glucose control is the key for preventing or reversing complications and improving the quality of life for patients with diabetes. Thus, sustained reduction of hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications.[4][5]

A recent study by the World Health Organization (WHO) estimated that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million or more by 2030. The adoption of a sedentary lifestyle, the consumption of non-traditional foods, and a genetic predisposition to the disease are thought to be the major underlying causes of the epidemic.[6] Despite great strides that have been made in the understanding and management of diabetes, the disease and its related complications are increasing unabated.

The influence of endocrine and non-endocrine organs other than the pancreas on diabetes mellitus is documented. Occasionally, other endocrine disorders such as abnormal thyroid hormone levels are found in diabetes.[7] The major alterations in thyroid hormone system are a reduction in the TSH stimulation of the thyroid gland, probably caused by central hypothyroidism, and in the peripheral generation of T_3 from T_4. In chemically induced diabetic animals, the alterations in the hypothalamo-pituitary-thyroid axis in diabetic rats are numerous. Hypothalamic and plasma TRH, pituitary and plasma TSH, as well as TSH secretion rate are all reduced, and the TSH response to TRH is decreased despite normal peripheral TSH metabolism. T_3 and T_4 production and iodide uptake by the thyroid are diminished. There are also important structural changes in the thyroid gland and pituitary that are accompanied by marked alterations in their secretory activity. In addition, T_4 deiodination to T_3 in peripheral tissues is decreased.[8][9] The physiological and biochemical interrelationship between insulin and the influence of both insulin and iodothyronines on the metabolism of carbohydrates, proteins, and lipids are recorded.[10] As Udiong et al. said, "Such records indicate that iodothyronines are insulin antagonists with high levels being diabetogenic, while absence of iodothyronines inhibits the development of diabetes."[9] Since thyroid hormone abnormalities are frequently associated with diabetes, the present investigation was to assess the adverse effects of diabetes on thyroid hormone levels and other biochemical variables in diabetic patients.

Materials and Methods

Study Population
The study population consisted of 200 subjects (age- and sex-matched) divided into two groups: diabetic (n=100; 15 type 1 and 85 type 2) and non-diabetic (n=100). All the subjects under study were recruited from K.G. Hospital and Postgraduate Medical Institute, Coimbatore, Tamil Nadu, India, from January, 2007 to March, 2008. General health characteristics such as age, sex, smoking status, menopausal status, alcohol consumption, and dietary habits (particularly as related to preference) were investigated by a self-administered questionnaire. All the patients in the diabetic group were confirmed diabetics who previously had fasting blood glucose levels >120 mg/dL on more than two occasions, and who were receiving treatment such as insulin, glybenicide, glucophage, or physical exercise therapy for diabetes mellitus. The initial criteria used in separating type 1 from type 2 subjects were the physician classifications based on the age of onset of diabetes and dependence on insulin therapy alone to achieve normal plasma concentrations. We classified diabetics who met the above criteria and had fasting C-peptide levels of less than 3.8ng/mL as type 1. Diabetics whose fasting C-peptide concentrations were above 3.8ng/mL and who were responding to other therapeutic measures or agents were classified as type 2. This was based on a C-peptide reference range of 0.8-3.8 ng/mL that established using the same reagent kits for the 100 non-diabetic subjects of the same community.

Each diabetic and non-diabetic subject was physically examined to rule out thyroid disorders. In addition, none of the subjects had a history of previous thyroid disease. Age- and sex-matched healthy volunteers without a history of diabetes were considered to be control subjects. All subjects were informed about the objectives of the study and what roles they were expected to play. The study excluded very ill patients with complications of diabetes mellitus and those with a known history of thyroid dysfunction. All samples were specimens taken from subjects who fasted for at least 8 hours before the blood collection.

Classification of the values into high, low, or normal thyroid hormone levels was based on the following criteria. Subjects classified as having high levels of thyroid hormones had FT_4 values >1.6 ng/L or TSH<0.4 µIU/mL or both. Those classified as having low levels had FT_4 values <0.68 ng/mL or TSH values >5.0 µIU/mL or both. Subjects grouped as normal had FT_4 and TSH values within the range >0.68-1.6 ng/mL and 0.4-5.0 µIU/mL, respectively.

Biochemical Investigation
The levels of serum thyroid stimulating hormone (TSH), total triiodothyronine (T_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) were measured by a Microparticle Enzyme Immunoassay (MEIA) on AXSYM System (Abbott Laboratories, Abbott Park, USA); while serum total thyroxine (T_4) was measured by the Fluorescence Polarization Immunoassay (FPIA) method on AXSYM System using the standard laboratory methodologies. Blood glucose, HbA1C, lipids, urea, creatinine, proteins,

and microalbuminurea were determined using a fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany).

Statistical Analysis

All data were expressed as mean ± SD of the number of experiments. The statistical significance was evaluated by Student's t-test using SPSS version 10.0 (SPSS, Cary, NC USA).

Results

Information about the demographic characteristics of the study population is shown in Table 1. The mean age of diabetic patients was 52 ± 9 and that of control subjects was 47 ± 7. The body mass index of diabetic patients (39 ± 6.2 kg/m^2) was significantly increased compared with that of control subjects (32 ± 5.9 kg/m^2).

Table 1. Demographic characteristics of non-diabetic and diabetic subjects.

	STUDY GROUP	
Parameter	**Non-diabetic subjects**	**Diabetic patients**
Age (years)	47 ± 7	52 ± 9*
Sex-Males	50%	50%
Females	50%	50%
Smokers	15%	20%
Alcohols	12%	17%
Hypertension	12%	25%
Diabetes mellitus	0%	100%
Body mass index (kg/m^2)	32 ± 5.9	39 ± 6.2**

Values are given as mean ± SD from 100 subjects in each group.
Diabetic patients compared with non-diabetic subjects *p<0.05, **p<0.01.

Table 2 shows the levels of various biochemical parameters in diabetic and non-diabetic subjects. Blood glucose fasting and postprandial, HbA1C, serum triglyceride, VLDL-C, urea, and microalbuminurea were significantly increased while the levels of total protein, albumin, and HDL-C were significantly decreased in diabetic patients when compared with non-diabetic subjects. Total cholesterol, LDL-C, and creatinine in diabetic and non-diabetic subjects did not differ significantly. Table 3 illustrates the levels

of serum thyroid hormones in diabetic and non-diabetic subjects. The level of TSH was significantly decreased whereas the levels of T$_4$ and FT$_4$ were significantly increased in diabetic patients compared to control subjects. However, the levels of T$_3$ and FT$_3$ in diabetic and non-diabetic subjects did not differ significantly.

Table 2. Comparison of biochemical changes in non-diabetic and diabetic subjects.

	STUDY GROUP	
Parameter	**Non-diabetic subjects**	**Diabetic patients**
Blood glucose		
Fasting (mg/dl)	92 ± 13	195 ± 37***
Postprandial (mg/dl)	125 ± 25	373 ± 30***
HbA1C (%)	3.2 ± 1.7	11.3 ± 2.01***
Microalbuminurea		
(mg/l)	9 ± 7	42 ± 20***
Urea (mg/dl)	21 ± 15	30 ± 20*
Creatnine (mg/dl)	0.6 ± 0.3	0.7 ± 0.4 NS
Total cholesterol		
(mg/dl)	165 ± 15	164 ± 17 NS
Triglyceride (mg/dl)	120 ± 30	165 ± 40**
HDL-C (mg/dl)	45 ± 7	37 ± 12*
LDL-C (mg/dl)	75 ± 15	79 ± 20 NS
VLDL-C (mg/dl)	32 ± 10	42 ± 17*
Total protein (g/dl)	7.5 ± 0.6	6.8 ± 1.7*
Albumin (g/dl)	4.1 ± 1.2	3.3 ± 1.5 *

Values are given as mean ± SD from 100 subjects in each group.
Diabetic patients compared with non-diabetic subjects *p<0.05, **p<0.01, ***p<0.001, NS (not significant).

Table 4 shows the distribution of diabetics with high, low, and euthyroid thyroid hormone levels. Out of 100 diabetic subjects studied, 28% had low thyroid hormone, 17% had high thyroid hormone, and 55% had euthyroid hormone levels. The incidence of low hormone levels was higher in females (19%) than in males (9%), while the number with high hormone levels was higher in males (10%) than in females (7%).

Discussion

India has the dubious distinction of being home to the largest number of people suffering from diabetes in any country. In theory, treating diabetes should be simple: just prevent hyperglycemia from

causing damage to organs and not allow hypoglycemia to cause coma. In practice, it does not work that way. Glucose fluctuations occur all the time. One way to assess the mean levels is to monitor the HbA1c, which gives the average blood glucose level of the preceding 2-3 months. In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins, including hemoglobin and a-crystalline of the lenses. HbA1C was found to increase in patients with diabetes to approximately 16%, and the amount of increase was directly proportional to the fasting blood glucose level. During diabetes, the excess glucose present in blood reacts with hemoglobin.[11][12] In the present study, we noticed a marked increase in HbA1C levels in diabetic patients, which could be due to excessive glycosylation of hemoglobin. Diabetes is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs. Increased blood urea production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins.[13] Diabetic hyperglycemia induces elevation of the plasma levels of urea and creatinine, which are considered significant markers of renal dysfunction. The decrease in total protein and albumin may be due to microproteinuria and albuminuria, which are important clinical markers of diabetic nephropathy and may be due to increased protein catabolism.

Diabetes has been shown to be associated with numerous thrombotic, atherosclerotic, and cardiovascular diseases. Cholesterol has been singled out as the cause of atherosclerosis. However, other lipids, such as triglycerides and phospholipids, also show similar correlations.[14][15][16] In our study, the levels of serum lipids were found to be elevated in diabetic patients. The abnormally high concentration of serum lipids in diabetes is mainly a result of the increase in mobilization of free fatty acids from peripheral depots. This happens because reduced insulin levels increase the activity of the hormone-sensitive lipase. On the other hand, glucagons, catecholamines, and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots.[17] The increase and fall in the individual lipoprotein levels is a reflection of the total serum cholesterol levels; that is, the levels of VLDL-C, LDL-C, and HDL-C increase or decrease with the level of total serum cholesterol, and it is their ratio that determines the pathophysiology of lipoprotein metabolism.[15][18]

Table 3. Serum thyroid hormone levels in non-diabetic and diabetic subjects.

STUDY GROUP		
Parameter	Non-diabetic subjects	Diabetic patients
TSH (mIU/ml)	2.44 ± 1.23	1.98 ± 1.01*
T_4 (μg/dl)	8.21 ± 2.10	10.56 ± 4.76**
T_3 (ng/ml)	0.95 ± 0.35	1.00 ± 0.58 NS
FT_4 (ng/ml)	0.26 ± 0.71	1.68 ± 0.85*
FT_3 (pg/ml)	2.87 ± 0.85	3.00 ± 0.75 NS

Values are given as mean $\pm$ SD from 100 subjects in each group.
Diabetic patients compared with non-diabetic subjects *p<0.05, **p<0.01, NS (not significant).

As Johnson et al. said, "The thyroid hormones, triiodothyronine and tetraiodothyronine, are insulin antagonists that also potentiate the action of insulin indirectly."[19] TRH synthesis decreases in diabetes, and this could be responsible for the occurrence of low thyroid hormone levels in diabetics.[20] In our study, the TSH was significantly lower in diabetics than in non-diabetics (1.98 ± 1.01 μIU/mL vs 2.44 ± 1.23 μIU/mL, p<0.05). Among the 100 diabetic subjects investigated, 28% had low levels of thyroid hormone while 17% had high levels. All the non-diabetic subjects had euthyroid levels except for one male subject who had a high level. These findings show a high incidence of abnormal thyroid hormone levels (both low and high) in the diabetic population. Our observation is in agreement with the reports of Suzuki et al.[20] and Smithson,[21] who in separate studies found altered thyroid hormone levels of different magnitudes in diabetic patients. The abnormal thyroid hormone levels may be the outcome of the various medications the diabetics were receiving. For example, it is known that insulin, an anabolic hormone, enhances the levels of FT_4 while it suppresses the levels of T_3 by inhibiting hepatic conversion of T_4 to T_3. On the other hand, some of the oral hypoglycemic agents such as the phenylthioureas are known to suppress the levels of FT_4 and T_4, while raising the levels of TSH.[19] Some of the type 2 di-

abetics were on oral hypoglycemic agents alone and some were on both insulin injections and oral hypoglycemic agents. The type 1 subjects were treated with insulin injections.

Table 4. The distribution of subjects with high, low, or euthyroid thyroid hormone levels.

	STUDY GROUP					
	Non-diabetic subjects			Diabetic patients		
	Total	Male	Female	Total	Male	Female
Total	100%	50%	50%	100%	50%	50%
Low level	00%	00%	00%	28%	9%	19%
High level	1%	1%	00%	17%	10%	7%
Euthyroid level	99%	49%	50%	55%	31%	24%

These factors may explain the findings of low or high thyroid hormone levels in diabetic subjects. Nevertheless, the diabetics in our study do not seem to follow the pattern previously recorded in other non-thyroidal diseases such as liver diseases and Cushing's syndrome where low thyroid hormone levels were recorded.[22] The presence of both high and low levels of thyroid hormones in diabetics in this study may also be due to modified TRH synthesis and release and may depend on the glycemic status of the diabetics studied. Glycemic status is influenced by insulin, which is known to modulate TRH and TSH levels.[23]

The TSH level in diabetic males was significantly lower than the level in females (1.68 ± 0.97 μIU/mL vs 2.10 ± 1.05 μIU/mL, p<0.05). The incidence of hyperthyroidism was lower in females than in males, but the number of subjects in a hypothyroid state was higher in females than in males. This finding is probably associated with the higher prevalence of obesity recorded in female diabetics. Insulin, which is used in treating diabetes and is produced in normal quantities or in excess, has been associated with increased anabolic activity.[24] Recently, C-peptide has also been shown to enhance Na^+/K^+-ATPase activity, an action that may also increase protein synthesis. Such an action would induce increased turnover of TSH, a protein hormone.[9]

The bulk of the hormones secreted by follicular cells of the thyroid gland are released in the free form into plasma where they become largely bound to thyroid binding globulin (TBG) and to some extent to pre-albumin and albumin. A small fraction circulates free in plasma (FT_4 and FT_3). Suzuki et al.[20] attributed the abnormal thyroid hormone levels found in diabetes to the presence of thyroid hormone binding inhibitor (THBI), an inhibitor of the extrathyroidal conversion enzyme (5'-deiodinase) of T_4 to T_3, and dysfunction of the hypothalamo-pituitary-thyroid axis. These situations may prevail in diabetes and would be aggravated in poorly controlled diabetics. Stress, which is associated with diabetes, may also cause changes in the hypothalamus-anterior pituitary axis in these diabetics. It appears that the presence of subclinical hypothyroidism and hyperthyroidism may result from hypothalamus-hypophyseal-thyroid axis disorders as suggested by Celani et al.[25] Failure to recognize the presence of abnormal thyroid hormone levels in diabetes may be a primary cause of poor management often encountered in some treated diabetics. There is therefore need for the routine assay of thyroid hormones in diabetics, particularly in those patients whose conditions are difficult to manage.

This study has shown a high incidence of abnormal thyroid hormone levels among diabetic patients. In conclusion, our findings demonstrate that detection of abnormal thyroid hormone levels in addition to other biochemical variables in the early stage of diabetes will help patients improve their health and reduce their morbidity rate.

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