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EVALUATION OF THYROID FUNCTION IN DIABETES MELLITUS IN CALABAR, NIGERIA

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ABSTRACT

The prevalence of abnormal thyroid hormone levels in diabetes mellitus in Nigeria is not well described. To determine the incidence of abnormal thyroid hormone levels in diabetics in Calabar, Nigeria, fasting blood samples from 161 diabetic subjects and 105 non-diabetic controls were analysed. Free thyroxine (FT_4), thyroid stimulating hormone (TSH), total triiodothyronine (T_3) and total thyroxine (T_4) kits obtained from Biomerica Inc. of USA were used for the analysis. TSH levels (1.80 $\pm$ 1.62) in diabetics were significantly lower ($p = 0.016$) than the level in non-diabetic controls (2.34 $\pm$ 1.24). Male diabetics had lower ($p < 0.05$) levels of TSH (1.192 $\pm$ 0.68 miu/ml) than diabetic females (1.90 $\pm$ 1.70 mIu/ml). The level of T_3 in diabetic males (125 $\pm$ 97ng/ml) was higher than the level in females (98 $\pm$ 75ng/dl). TSH ($F = 2.74$, $p = 0.049$), T_4 ($F = 56.87$, $p = 0.O01$), T_3 ($F = 56.44$, $P = 0.001$) in diabetics and FT_4 ($F = 5.74$, $p = 0.002$) in controls showed significant variation with the ages of the subjects. Out of 161 diabetics subjects studied 26.6% had low plasma thyroid hormone levels (FT_4>2.01ng/dl), 19.8% had raised plasma thyroid hormone levels ($FT_4 < 2.01$), and 54% was euthyroid ($FT_4$0.78 - 2.01ng/dl). This study has shown a high incidence (46.5%) of abnormal thyroid hormone levels among the diabetics in Nigeria (hypothyroidism 26.6%, hyperthyroidism, 19.9%). The prevalence of hypothyroidism was higher in women (16.8%) than in men (9.9%), while hyperthyroidism was higher in males (11%) than in females (8%). This study has defined thyroid function status of diabetics in Calabar, Nigeria probably the first of such work in Africa.

KEY WORDS

Thyroid hormones, diabetes mellitus, diagnosis.

INTRODUCTION

The influence of other endocrine and non-endocrine organs other than the pancreas on diabetes mellitus is documented (1,2,3). Occasionally other endocrine disorders such as abnormal thyroid hormones levels are found in diabetes mellitus (4,5). The physiological and biochemical inter-relationship between insulin and the influence of both insulin and iodothyronines on the metabolism of carbohydrates, proteins and lipid are recorded (6,7). Such records indicate that iodothyronines are insulin antagonist with high levels being diabetogenic while absence of the hormone inhibits the development of diabetes (7). Diabetes mellitus and hyperthyroidism are metabolic disorders that affect the levels of carbohydrates, proteins and lipids

Investigation of the thyroid functions which in most cases refers to follicular cell function, includes measurement of secretions (hormones) of the gland such as iodothyronine, carrier protein levels, trophic hormone such as thyroid stimulating hormone (TSH) and releasing hormone e.g. thyroxine releasing hormone (TRH). The effects of iodothyronine on the various metabolic pathways are assessed by specific tests, such as TSH, FT_4 and FT_3.

This study was carried out in Calabar city, which lies within the tropical rain forest of West Africa. The dietary components of the inhabitants of the area are highly leafy and may exhibit goitrogenous effect on the thyroid and/or diabetogenic effect on diabetes or vice-versa. However, low prevalence of diabetes mellitus was reported in some parts of Nigeria among the low-income group who relied mostly on native unprocessed food for their existence (8). Data on the actual prevalence, incidence or mortality of diabetes mellitus and thyroid disorder

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is scanty if not non-existing in the area. These facts warrant further investigations into the relationship between diabetes mellitus and thyroid follicular cell function.

MATERIALS AND METHODS

Blood specimens were collected from 161 (18 Type 1 and143 type 2) diabetic patients who were attending the University of Calabar Teaching Hospital, and the University of Calabar Medical Centre. All the diabetic subjects were confirmed diabetics, who previously had fasting plasma glucose levels of 6.1mmol/l (110mg/dl) at more than two occasions and were receiving treatment such as insulin, glybenicide, glucophage, and or physical exercise therapy for diabetes mellitus. Among the diabetics studied 143 were Type 2, 42 of who were on both insulin and oral hypoglycaemic agents. 92 subjects were on oral hypoglycaemic agents only while the remaining 11 subjects were on diet and physical exercise. All the 18 Type 1 diabetics were on insulin treatment

The initial criteria used in separating Type 1 from Type 2 subjects were the physician classifications based on age of onset of diabetes (less than 35 years), 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 0.38ng/ml as Type 1. Diabetics, whose fasting C-peptide concentration was above 0.38ng/ml and were responding to other therapeutic measures or agents were classified as Type 2 diabetics. This was based on C-peptide reference range of 0 3.8ng/ml.that established using the same reagent kits for 100 non–diabetic subjects of the same community

All the subjects under study (diabetics and non-diabetics) were Nigerians residing in Cross River State and the neighbouring states. There were no diabetics of other races. 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. Blood specimens from age and sex matched non-diabetic volunteers without history of diabetes mellitus, whose FPG was less than 6.1mmol/l (110mg/dl) on two occasions, were the control samples. 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 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 raised, low or normal thyroid hormone levels were based on the following criteria. Subjects classified as having raised level of thyroid hormones: had FT_4 values > 2.01ng/l or, TSH < 0.4miu/ or both, those classified as having Low level had FT4 values < 0.68ng/ml, or TSH values > 5.4miu/ml, or both. Subjects grouped as normal had FT_4 and TSH values within the range > 0.68 - 2.01ng/ml, and

Table 1 : Comparison of thyroid hormones in diabetic and non-diabetic subjects using t-test (Values are Mean ± SD)

Test	Diabetics (n=161)	Non-diabetics (n=105)	P value
TSH mIU/ml	1.80 ± 1.62	2.84 ± 1.24	0.51
FT_4 ng/l	1.62 ± 0.72	1.19 ± 0. 46	0.015*
T_4 mg/dl	9.09 ± 5.19	7.68 ± 2.05	0.01**
T_3 ng/dl	112 ± 88	110 ± 46	0.277

* significant; ** highly significant.

Table 2 : Comparison of thyroid hormones of diabetics and non-diabetics at different age intervals (Values are Mean ± SD)

Age in years	Diabetic subjects					Non-diabetic subjects				
	Number of Subjects	TSH μ.IU/ml	FT_4 ng/dl	T_4 μg/dl	T_3 ng/ml	Number of Subjects	TSH μ.IU/ml	FT_4 ng/dl	T_4 μg/dl	T_3 ng/ml
25 to ≤ 35	16	2.17 ± 1.47	1.18 ± 2.17	6.93 ± 3.66	90 ± 54	25	2.38 ± 1.35	1.25 ± 0.45	7.65 ± 2.17	106 ± 41
35 to ≤ 45	43	2.11 ± 1.67	1.22 ± 1.42	8.91 ± 3.86	115 ± 69	33	2.49 ± 1.19	1.29 ± 0.40	7.69 ± 1.65	120 ± 55
45 to ≤ 55	50	1.96± 1.57	1.54 ± 1.63	8.72 ± 3.97	106 ± 78	15	2.37 ± 1.21	1.08 ± 0.55	7.47 ± 2.06	99 ± 25
55 to ≤ 65	30	2.02 ± 1.59	1.74 ± 1.62	8.54 ± 3.57	148 ± 132	13	1.94 ± 1.01	0.98 ± .36	9.56 ± 1.03	139 ± 34
65 & above	15	2.01 ± 2.22	1.16 ± 0.99	11.87 ± 10	95 ± 80	11	2.02 ± 1.12	0.92 ± 0.22	9.61 ± 1.12	121 ± 67
P-value		O.049*	0.304	<0.001**	<0.001**		0.582	0.00l**	.772	0.620

* significant, ** highly significant

0. 4 – 5.4miu/ml respectively.

Of the 7ml of venous blood drawn from each subject, 2ml was dispensed into fluoride oxalate bottles for plasma glucose estimation and the rest of the blood sample was discharged into a plain sample bottle and allowed to clot. We separated the serum from cells, divided it into three aliquots and stored them frozen and thawed only when required for used. We also separated plasma from fluoride/oxalate samples and stored them at -20°C until needed for use. Plasma glucose was determined on the same day while we carried out all other tests within 2 weeks of collection. Fasting plasma glucose estimation was by the method of Trinder (9), and TSH, FT_4, T4 and T3 by enzyme immunoassay (EIA) kit method using commercial kits from Biomerica Inc., California USA. We adhered strictly to the manufacturer instructions on the procedures. All analysis was done in duplicate and the average of the duplicate data used for calcualations.

DATA ANALYSIS

Comparison of paired data from the three groups of subjects was done using T-test (t), while correlations between groups were analyzed using Pearson correlation coefficient (r) formula. SPSS and Microsoft excel programmes were used for T-test and correlation coefficient calculations respectively. Variation of grouped data was assessed by two-way analysis of variance (ANOVA = F) using SPSS programme. A two-tailed p-value of <0.05 was considered indicative of a statistically significance difference.

QUALITY CONTROL OF ANALYTICAL PROCEDURES

Commercial quality control sera provided by kits manufacturers were used to monitor the performance of the procedures. Initially, recovery experiments were performed on each of the parameters TSH, FT_4, T_4, and T_3 to assess the accuracy of the methods. The precision, the within and between batch variations were also determined, the results of which are tabulated below.

RESULTS

Table 1 shows that the levels of FT_4 and T_4 in diabetic subjects were statistically higher than the levels in the control subjects ($p = 0.015$, 0.01 respectively), but the levels of T_3 in the diabetic and control subjects did not differ significantly. The level of TSH in the diabetics was not significantly different from the level in non-diabetic controls ($p = 0.051$).

The levels of TSH, T_4 and T_3 varied significantly with age in diabetics ($p = 0.049$, 0.001, 0.001) respectively (Table 2). There was no such variation in the non-diabetic except FT_4 levels that varied significantly with age in the non-diabetics controls ($p = 0.002$).

Table 3 shows that of 161 diabetic subjects studied, 26.6% had low, 19.8% had raised levels of thyroid hormone, and 54% had euthyroid levels. The incidence of low levels was higher in women (16.8%) than in men (9.9%), while the number with raised level was higher in males (11%) than in females (8%). Table 4 shows that the levels of FT4 and TSH in each group of diabetics (low, normal or raised levels of thyroid hormones) differed significantly from one another ($p < 0.001$) except the level in euthyroid subjects, which was similar to the level in all the diabetic subjects ($p > 0.05$).

DISCUSSION

The thyroid hormones, tri-iodothyronine and tetraiodothynine are insulin antagonists that also potentiate the action insulin indirectly (7). TRH synthesis decreases in diabetes mellitus (10,11). These facts could be responsible for the occurrences of low thyroid hormone levels in some diabetics. The level of TSH in our study was not clinically significant in diabetics than in non-diabetics. This finding is not consistent with the report of Celani (12), Smithson (13) who recorded varied levels of thyroid hormones in diabetic subjects. The large SD observed among the diabetics may be due to the influence of the oral hypoglycaemic agents some of the diabetics were receiving since some of the diabetics were Type 2 subjects. Among the diabetics studied 143 were Type 2, and 18 were Type 1 diabetics. And all the Type1 subjects were on insulin treatment

Table 3 : The distribution of diabetics with raised, low and euthyroid thyroid hormone levels.

Subjects	Diabetics			Non-diabetics		
	Total Subject	Male	Female	Total Subject	Male	Female
Total	161 (100%)	81 (50.3%)	80 (49.68%)	105 (100%	49(46.81%	56 (53.31)
Low levels	43 (26.6%)	16 (9.9%)	27 (16.8%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Raised levels	32 (19.8%)	19 (1%1)	13 (8%)	2(1.9%)	2(1.9%)	0(0.00%)
Euthyroid levels	87 (53.6%)	46 (28.6%)	41 (25.5%)	103(97.1%)	47(49.8%	56(53.3%)

Table 4 : Comparison of thyroid hormones in hyperthyroid, hypothyroid, euthyroid and all diabetic subjects

Diabetic group	FT_4	TSH
Euthyroid vs all diabetic subjects	1.45 ± 0.91 vs. 1.62 ± 0.72	1.99 ± 1.32 vs. 1.80 ± I.62
Euthyroid vs Hyperthyroid	1.45 ± 1.99 vs. 3.77 ± 2.14	1.99 ± I.3 vs. 0.17 ± 0.01
Euthyroid vs Hypothyroid	1.45 ± 1.99 vs. 0.45 ± 0.13	1.99 ± I.32 vs. 6.13 ± 0.17
All diabetic subjects vs Hyperthyroid	1.62 ± 0.72 vs. 3.77 ± 2.14	1.80 ± I.62 vs. 0.17 ± 0.01
All diabetic subjects vs Hypothyroid	1.62 ± 0.72 vs. 0.45 0.13	1.80 ± I.62 vs. 6.13 ± 0.17
Hyperthyroid vs Hypothyroid	3.77 ± 2.14 VS 0.45 ± 0.I3	0.17 ± 0.01 vs. 6.13 ± 0.1

Values are mean ± SD

of. Apart from all the Type 1 diabetics on insulin treatment some Type 2 subjects were also on insulin that is capable of raising the levels of TSH and suppressing the levels of T_3. (14) Apart from the physiological impact of diabetes on TSH insulin administration could also have influenced its levels

Among the 161 diabetic subjects, investigated 26.6% had low levels of thyroid hormone while 19.9% had raised levels. All the non-diabetic subjects showed euthyroid status. These findings show a high incidence of abnormal thyroid hormone levels (low and raised levels) in diabetics population apart from the under study. Our observations is in agreement with the report of Smithson (12); Suzuki *et al*, (11); and Celani *et al,* (12) who in separate studies found altered thyroid hormone levels of different magnitudes in diabetic patients. The abnormal thyroid hormones levels may be the outcome of the various medications the diabetics were receiving. For example, it is known that insulin (14), 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 hypoglycaemic agents such as the phenylthioureas are known to suppress the levels of FT_4 and T_4, while causing raised levels of TSH (15,16) Some of the type 2 diabetic were on oral hypoglycaemic agents alone and some were on both insulin injections and oral hypoglycaemic agents .The Type 1 subjects were treated with insulin injections

These situations may explaian the finding of low or raised thyroid hormone levels in some of the euthyroid diabetics. Never the less the situation in these diabetics does not seem to follow the pattern previously recorded in other non-thyroidal diseases such as liver diseases and Cushing syndrome where low thyroid hormone levels were recorded (15,17,18). The presence of both raised and low levels of thyroid hormones levels in diabetics in this study may also be due to modified TRH synthesis and release (10) and may depend on the glycaemic status of the diabetics studied. Glycaemic status is influenced by insulin, which is known to modulate TRH and TSH levels (19).

The TSH level in diabetic males was significantly lower than the level in females. The incidence of hyperthyroidism was lower in females (8%) than in males (11%), but the number of subjects in hypothyroid state was higher in females (16.8%) than in males (9.9%). These observations agree with the report of Radetti, (1994); Sacks, (18) and Celani *et al*(12). The finding is probably associated with the higher prevalence of obesity recorded in female diabetics (18). Insulin, which is used in treating Type 1 diabetes mellitus and is produced in normal quantities or in excess in Type 2 diabetics, has been associated with increased anabolic activity (20). Recently, C-peptide has been shown to enhance Na^+ /P^+-ATPase activity, an action that may also stimulate increase protein synthesis. Such an increase would induce increase turn- over of TSH, a protein hormone.

The bulk of the hormones secreted by follicular cells of the thyroid gland are released in the free form into plasma where it becomes largely bound to thyroid binding globulin (TBG) and to some extent to pre-albumin and albumin. A small fraction circulates free in plasma (free T_4, FT_4). Suzuki *et al* (11) attributed the abnormal thyroid hormone levels found in diabetes to the presence of Thyroid Hormone Binding Inhibitor (THBI), an inhibitor of extra thyroidal conversion enzyme of T_4 to T_3, and dysfunction of the hypothalamus-hypophyseal-thyroid-axis. These situations may prevail in diabetics and would be aggravated in poorly controlled diabetics. Stress, which is associated with diabetes mellitus, may also cause changes in the hypothalamus anterior-pituitary axis in these diabetics. It appears that the presence of sub-clinical hypothyroidism and hyperthyroidism may result from hypothalamus-hypophyseal-thyroid-axis disorders as suggested by Celani *et al*(12). Suggestion was made that the finding of definite hypothyroidism or hyperthyroidism be given adequate attention and treatment of the thyroid disorder appropriately undertaken (21). Failure to recognize the presence of these abnormal thyroid hormone levels in diabetics may be a primary cause of poor management often encountered in some treated diabetics. There is therefore need

for routine assay of thyroid hormone on diabetics, particularly those whose conditions are difficult to manage.

The high prevalence of abnormal thyroid hormone levels in connection with the local diets of the people of Calabar require further research attention as most foods are leafy. These local diets may also influence thyroid hormone levels at the thyroid, hypothalamus or insulin levels. Further studies are necessary to elucidate the cause and the role of abnormal thyroid hormone levels in this population in which the incidence of diabetes mellitus seem low.

In conclusion, this study has shown that 46.5% of diabetic in Calabar, Nigeria had abnormal thyroid hormone levels. Twenty six per cent of the diabetics had low levels of thyroid hormones, while 19.9%.had raised levels. This study, the first of its kind in Nigeria, which may also serve as an example of the situation in sub-Saharan Africa, shows high incidence of abnormal thyroid hormone level higher than what is recorded in Europe. We recommend routine assessment of thyroid hormone levels in diabetics, particularly the difficult to manage cases and caution in the treatment of subjects with sub-clinical thyroid hormone levels.

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