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Assessment of Thyroid Hormone Levels in Diabetes Mellitus Subjects Resident in Port Harcourt Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DO and DGT designed the study and wrote the protocol, author TSN wrote the first draft of the manuscript and managed the biochemical and statistical analyses and the literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to assess thyroid hormone levels in among diabetics in Port-Harcourt of Rivers State in Nigeria
Study Design: This study is a cross-sectional study.
Place and Duration of Study: This study was conducted at Chemical Pathology Department, Rivers State University Teaching Hospital, Port Harcourt, Nigeria, between December, 2019 and February, 2020.
Methodology: A total of 224 subjects were recruited for the study, diabetic parents (132 subjects), non-diabetic parents (112 subjects. Five (5) ml of blood sample was collected and used to analyse for serum thyroxine (T_4), tri-iodothyronine (T_3) and thyroid stimulating hormone (TSH) using Enzyme-linked immunoasorbent assay. Data were analyzed statistically with SPSS version 22.0 and values considered significant at $p< 0.05$.
Results: The mean ± S.D of serum TSH were 1.28± 0.6u/ml (control) and 1.84±1.05 u/ml (subject), ($p=0.023$). T_4 value were 2.49 ± 0.86u/ml (control) ($p=0.024$) and 3.90 ±1,62u/ml (subjects) and T_3 values were 1.89±0.41u/ml ($p=0.0001$).

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Conclusion: The evaluation of thyroid hormones (TSH, T_4 and T_3) among diabetes with the age ranges of population studied shows that there was altered thyroid hormone in diabetes mellitus individuals.

Keywords: Thyroid hormones; diabetes mellitus; Port Harcourt.

1. INTRODUCTION

Thyroid hormones are hormones produced by the thyroid gland. They are thyroxine (T_4), tri-iodothyronine (T_3). These hormones mediate the regulation of glucose metabolism, stimulate the enhancement of insulin-dependent entry of glucose into cells and also increase gluconeogenesis and glycogenolysis to generate free glucose [1]. A variety of separate associations have been identified between thyroid function and glucose metabolism, and these provide common pathways through which thyroid dysfunction and diabetes can develop [2]. Also because the pathways between the hypothalamic-pituitary axis and T_3 receptor in thyroid cells are abnormal in patients with diabetes not managed at the initial stage alter TSH level and interfere with the conversion of T_4 toT_3 in peripheral tissues [3]. Diabetic individual have high level of TSH and low level T_3 as a result of hyperglycemia [4]. Diabetes worsen thyroid disease and vice versa on the other hands anti-diabetes drugs also alters functions [4].

Diabetes a metabolic disease and thyroid hormones play important part in glucose metabolism shared the same hypothalamic pituitary-axis there was need to check diabetes effects on thyroid hormone among patients that comes to endocrinology unit of the RSUTH, Port-Harcourt in Nigeria. Therefore, the aim of this study was to assess thyroid hormone levels in among diabetics in Port- Harcourt of Rivers State in Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design/ Area/Subject Characteristics

This was a cross-sectional study carried out in Rivers State University Teaching Hospital (RSUTH) on diabetic subjects and healthy individual in Port Harcourt.

The subjects recruited for the study were diabetic and non-diabetic subjects that visited Rivers State University Teaching Hospital (RSUTH) from Endocrinology, unit of the hospital. Also, offspring of diabetics and non-diabetic subjects were recruited for the study. A total of 224 subjects were recruited for the study, diabetic parents (132 subjects), non-diabetic parents (112 subjects).

2.1.1 Inclusion criteria

All diabetic subjects and Non diabetic subjects within age ranges 30 -70years.

2.1.2 Exclusion criteria

Individual with thyroiditis, diabetes subjects, that were smokers, as those could affect the measured parameters.

2.2 Sample Size Calculation

The sample size was determined using

Lesli Kish's Formula: $N = Z^2.d.q/ D^2$

Where N – minimum number of subjects

Z - 1.96

p- Prevalence of Diabetes Mellitus in Nigeria (2018)

q – 1- p

D – Confidence level = 0.05

The prevalence of type 2 diabetes mellitus and type 1 diabetes mellitus diagnosed were 7% and 4% respectively in Nigeria. So this was used to calculate the sample size [5].

Total sample size =74

2.3 Sample Collection and Laboratory Analysis

2.3.1 Collection of blood sample

Prior to sample collection participant consent was obtained after thorough explanation of research purpose and its importance at the endocrinology, obstetrics and gynecology out subjects waiting hall. After this sample bottles were labelled systematically and carefully. Confidence was then built in subjects while

seated to prevent panic or fear. Prominent vein selected, a tourniquet tied around the arm and skin cleaned with cotton wool soaked in methylated spirit with cotton wool to disinfect the area. Patient made a fist. Five (5) ml syringe with 21 or 18 Gauge needle was inserted in the ante-cubital fossa vein and blood drawn. Punctured area was wiped with dried cotton wool and pressure exerted over site to avoid thrombosis and further bleeding. Blood withdrawn was dispensed into K_2 EDTA and plain bottles in volumes of two (2) ml and three (3) ml respectively.

K_2EDTA samples were taken immediately for HbA1c analysis while the plain blood samples were allowed to clot and then centrifuged at 12,000 rpm for 5 minutes. Serum was obtained and stored at 4^0c temperature in a refrigerator until the day laboratory analysis was performed. Prior to sample analysis, serum samples were allowed to thaw and mixed properly for homogeneity.

2.3.1 Determination of fasting blood sugar (FBS) [6]

Fasting blood sugar was determined by glucose oxidase method using URIT auto-analyzer Laboratories Inc Company, USA. Glucose in plasma is catalysis by glucose oxidase, oxidation of glucose produces hydrogen peroxide and gluconic acid, hydrogen peroxide in present of peroxidase with 4-aminophenazone and phenol produce a pink colour.

2.3.2 Determination of thyroid hormones [7]

Thyroxine, tri-iodothyronine and thyroid stimulating hormone were analyzed. Serum obtained was analyzed for these hormones using ELISA Plate reader. An Enzyme-linked immunosorbent assay Kit by Perkin Elmer Health Science Inc, Haywand U.S.A, following manufacturer instructions briefly, desired number of coated wells were secured in a holder and data sheet with sample identification made. 25ul of standard sample and control were dispensed into appropriate wells -100µl of enzyme conjugate reagent was dispensed into each well and thoroughly mixed for 10 seconds and was incubated at room temperature for an hour. Microtiters well were washed with washing buffer for 5tmes. Well were strikes sharply onto absorbant paper to remove all residual water droplet. 100µl of TMB substrate was dispensed into each well and gently mixed for 5seconds.Well incubated at room temperature for 20minutes without shaking. 100µl stop solution is added into each well to stop the reaction and gently mixed for 5 seconds. Absorbance read at 480 mm with a microtiter well reader.

2.3.3 Determination of C-peptides [7]

C-peptide was determined using ELISA Plate reader and ELISA Kits by Perkin Elmer Health Science Inc, Haywand U.S.A following manufacturer instruction briefly prior 50ul of calibrators, control and samples were dispensed into the assay wells. 100ul of the C-peptide enzyme reagent to each well, and wells covered then mixed gently for 20-30 mins on ELISA plate shaker (500 shaker per minute). Then incubated for 2 hours at room temperature ($20\text{-}25^0$c).well contents was discarded, washed, tapped, blotted and dried with absorbent paper. 350ml buffer was added to the wells, after which well contents discarded, washed three times then was blotted with absorbent paper. 100ul of working solution was added to wells. Then incubated at room temperature for 15 minutes. And finally 50ul stop solution was added to each well and mixed gently for 15-20seconds. Absorbance was read at 450nm in microplate reader.

2.3.4 Determination of glycated haemoglobin

Glycated haemoglobin (HbA1c) was determined with Ion exchange resin using Sysmex auto analyzer model KV -21n Kobe, Japan with the method of Nathan et al, [8].

Step1: Hemolysate preparation 0.5ml lysing reagent was dispensed into tubes labeled as Control (c) and Test (T) .0.1ml of the reconstructed control and well mixed blood sample were added and was mixed until complete lysis is evident, then allowed to stand for 5 minutes.

Step 2; Glycosylated hemoglobin (GHb) separation Cap was removed from the non-Exchange Resin tubes and label as control and test.

0.1ml of the hemolysate made in step A, was dispensed into the appropriately labeled ion-Exchange Resin tubes. A resin separator was inserted into each tube so that the rubber sleeve is approximately 1cm above the liquid level of the resin suspension. Tubes were mixed on vortex mixer continuously for 5 minutes. Resin was allowed to settle then the resin separator was

pushed into the tubes until the resin was firmly packed. Each supernatant was aspirated directly into a cuvette and each absorbance was measured against distilled water.

Step 3: Total Hemoglobin (THb) Fraction: 5.0ml of distilled water was dispensed into tubes labeled as Control and Test. 0.2ml of hemolysate from step 1 was added into appropriately labeled tube and was mixed well. Absorbance read at 520nm each against distilled water.

2.4 Statistical Analysis

The data obtained from subjects via the questionaire and results were analysed using, Excel program, Statistical Package for Social Sciences (SPSS) version 22.0 and expressed as mean ± standard deviation. Differences between two means were analysed using independent sample t-test with p-values less than 0.05 being considered significant. Pearson correlation was also used to compare glycemic parameters and thyroid hormones.

3. RESULTS AND DISCUSSION

There was a significantly reduced level of Connecting-peptide (C-peptide) in diabetes mellitus than in non-diabetes subjects (Table 1). The reason being that lower C-peptide level are observed in both type 1 or type 2 diabetes [9]. An individual with type 1 diabetes typically present lower C-peptide levels than individuals with type 2 diabetes since more insulin are produced in response to glucagon [9]. In DM there is a decreased production of endogenous insulin and reduced plasma level of C-peptide because of beta cell destruction [10]. C-peptide levels are not influenced by exogenous insulin levels or insulin antibodies, but may over estimate beta-cell function in case of chronic kidney disease, diabetes nephropathy, or when using an insufficiently specific assay, in case of elevated levels of intact proinsulin or its conversion intermediates [11]. Insulin and C-peptide are secreted in equimolar amounts by the β-Cells of the pancreas but the metabolic clearance of insulin is much more rapid than that of C-peptide [12]. Therefore, C-peptide has a longer half-life and is present in peripheral blood in higher molar concentrations than insulin, making it less prone to marked fluctuations. Consequently, the measurement of plasma C-peptide concentrations may be more reliable as an indication of endogenous insulin production. However, as C-peptide is cleared by the kidneys, raised concentrations may occur in renal impairment. [13]. C-peptide is a prominent factor in the pathogenesis of micro-vascular complications in TIDM [14].

Significantly higher level of HbA1c was observed in diabetes than in non-diabetes subjects. HbA1c provides a reliable measure of chronic hyperglycemia and correlates well with the risk of long-term diabetes complications and it is a reliable biomarker for the diagnosis and prognosis of diabetes, [15]. The result of this study is consistence with the finding on Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients in which it was revealed that HbA1c is an effective tool in establishing the diagnosis of diabetes, especially in low- and middle-income (developing) countries and hard-to-reach (poor) populations. Also, HbA1c has been endorsed for diagnosis of diabetes and HbA1c test should be implemented as part of the diagnostic and prognostic tool, for better patient care and successful clinical outcomes.

Table 1. Glycemic parameters of diabetic and non - diabetic subjects

Subject	N	FBS (mmo/l) M±SD	C-peptide (U/ml) M±SD	HbA1c% M±SD
Diabetic Subject	132	10.31 ± 6.72	1.56 ± 0.67	6.41 ± 2.20
Control Subject	112	5.55 ± 1.60	1.71 ± 0.88	5.08 ± 0.71
P-value		0.001	0.001	0.004
Remark		S	S	S

N – Number of subjects, S – Significant

Table 2. Thyroid hormones of diabetic and non-diabetic (control) subjects

	N	TSH (U/ml) M±SD	T_3 (U/ml) M±SD	T_4 (U/ml) M±SD
Diabetic Subject	132	1.84 ± 1.05	1.71 ± 0.25	3.90 ± 1.62
Control Subject	112	1.28 ± 0.86	1.89 ± 0.41	2.49 ± 0.86
P-value		0.023	0.024	0.000
Remark		S	S	S

N- Number of subjects, s-subjects

Table 3. Glycemic parameter correlated with thyroid hormones of non-DM subjects

Parameters		TSH (Ul/ml)	T_3 (Ul/ml)	T_4 (Ul/ml)
BMI (kg/m^2)	r value	-0.446*	-0.234	0.383
	p-value	0.022	0.250	0.054
FBS (mmol/L)	r value	0.071	-0.207	-0.390*
	p-value	0.729	0.310	0.049
C-peptide (Ul/ml)	r value	-0.400*	-0.296	-0.280
	p-value	0.043	0.142	0.166
HbA1c (%)	r value	-0.189	-0.498*	0.054
	p-value	0.364	0.011	0.799

- : negative correlation
** : Correlation is significant at the 0.05 level*

Table 4. Correlation glycemic parameters vs thyroid hormones (DM Parents)

Parameters		TSH (Ul/ml)	T_3 (Ul/ml)	T_4 (Ul/ml)
BMI (kg/m^2)	r value	-0.086	-0.101	0.140
	p-value	0.570	0.503	0.354
FBS (mmol/L)	r value	0.347*	-0.416*	-0.220
	p-value	0.018	0.004	0.142
C-peptide(Ul/ml)	r value	-0.066	0.130	-0.266
	p-value	0.663	0.388	0.074
HbA1c (%)	r value	0.232	-0.102	-0.197
	p-value	0.121	0.502	0.189

**. Correlation is significant at the 0.05*

From this study there was significant higher levels of thyroid stimulating hormones (TSH) and thyroxine (T_4) in diabetic than non-diabetic subjects (Table 2). Thyroid gland plays a central regulatory role in metabolism so if the initial stage of diabetes was not managed properly it may altered TSH level and induces low T_3 since T_4 cannot be converted to T_3 in peripheral tissues. This finding is consistent with the work of Ray & Ghosh, [16] in which it was revealed that hyperthyroidism are common in patients with hyperglycemia. The work of Nair et al. [17] on prevalence and association of hypothyroidism in Indian patients with T2DM also revealed that diabetic mellitus alters TSH level and impaired the conversion of thyroxine to tri-iodothyronine in peripheral tissue hence chronic hyperglycemia have cumulative effect on thyroid function. Hage et al. [4] revealed that hyperthyroidism is associated with poor glycemic control and worst glycemic control are observed in diabetic patients with hyperthyroidism.

In the study of Wang [1] about the relationship between T2DM and thyroid diseases, it was observed that hyperthyroidism is enhanced by the production of endogenous glucose. The increase in TSH and T_4 levels could be as a result of diabetic alteration of TSH level and interference with the conversion of T_4 to T_3 in the peripheral tissue. It could also be due to loss of nocturnal TSH peak as TSH response to Thyroid Releasing Hormone (TRH) is impaired (suppressed) in diabetic patients. T_3 and T_4 are the two main hormones of the thyroid while TSH is a pituitary hormone that regulates T_3 and T4. The pathways between the hypothalamic-pituitary axis and the T_3 receptor in thyroid cells are abnormal in patients with diabetes. Therefore, a variety of separate associations have been identified between thyroid function and glucose metabolism, and these provide common pathways through which thyroid dysfunction and diabetes can develop [18]. Thyroid hormones mediate the regulation of glucose metabolism and can increase the production of glucose transport -2 (GLUT-2) which is the main glucose transporter in the liver. An increased level of GLUT -2 causes an increased hepatic glucose output and alters glucose metabolism and impairs glucose tolerance.

There was significant reduction in T_3 in diabetic subjects when compared with non-diabetic subjects from this study (Table 2). The reason being that hypothyroidism is associated with higher BMI and this is a risk factor for diabetes. Hypothyroidism is associated with decreased insulin sensitivity and glucose intolerance that is

commonly observed in uncontrolled diabetes, and poorly managed, uncontrolled diabetes that progresses to diabetic ketoacidosis lowered T_3. This report is consistent with the work of [15] in which it was revealed that poorly managed diabetes mellitus induces low serum T_3, because hyperglycemia induces the inhibition of peripheral deionization of T_4 to T_3. The work of Shuai et al. [18] on thyroid dysfunctions and T2DM; screening strategies and implication for management, revealed that hypothyroidism was observed in T2DM patients and thyroid disease worsens T2DM, and vice versa. On the other hand the presence of thyroid dysfunction in DM is a risk factor of cardiovascular disease. The report of Mirboluk et al. [19] while testing thyroid hormone to ascertain thyroid function in diabetic patients with ketoacidosis observed that diabetes ketoacidosis and poorly managed, uncontrolled diabetes that progresses to diabetic ketoacitosis lowered T_3. The work of Ogbonna et al. [20] on the association between glycemic status and thyroid dysfunction with T2DM patients shows that T_3 was significantly lowered in T2DM patients than in non T2DM individuals because of the effect of chronic hyperglycemia on peripheral de-iodinization of T_4 to T_3 or nocturnal peak in TRH secretion. Thus the raised TSH and lower T_3 levels observed in this study could be is an indication of low thyroid function.

In the study of Wei et al. [21] observing the association between glycemic status and thyroid dysfunction in T2DM patients, reported that higher TSH level and lower level of T_3 were observed in T2DM because the rate of peripheral transformation of T_4 to T_3 increases with excessive energy intake, so peripheral de-iodinase activity is affected.

Correlation of glycemic parameter with thyroid hormones of non-diabetes mellitus subjects revealed that BMI, FBS, C-peptide and HbA1c had negative correlation with TSH, T_4, TSH and T_3 respectively (Table 3) Also correlation of glycemic parameter with thyroid hormone of diabetes mellitus subjects that only FBS has positive correlation with TSH and negative correlation with T_3 (Table 4).

4. CONCLUSION

The evaluation of thyroid hormones (TSH, T_4 and T_3) among diabetes with the age ranges of population studied shows that there was altered thyroid hormone in diabetes mellitus individuals.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this manuscript. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Ethical approval was obtained from Ethics and Research Committee of Hospital Management Board, Port- Harcourt of Rivers State. Sample collection approval was obtained from various Heads of Department and finally from the Chief Medical Director (C.M.D) of RSUTH, Port Harcourt, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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