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Evaluation of Some Thyriod Hormones in Diabetics Attending Abia State University Teaching Hospital

Ahiara C.O.[1], Onyeakolam I.F.[2], Nwosu D.C.[1], Nwanjo H.U.[1], Chima C.O.[1], Anyanwu G.O.[1], Nwokorie E. A.[2], Emengaha F. C.[1], Onu I. O.[1] and *Emmanuel Ifeanyi Obeagu[3]

'Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria.
[2]Department of Medical Laboratory Science, Rhema University, Aba, Abia State, Nigeria.
3Department of Medical Laboratory Science, Kampala International University, Uganda.
***Corresponding author: Emmanuel Ifeanyi Obeagu. Department of Medical Laboratory Science, Kampala International University, Uganda, emmanuelobeagu@yahoo.com.**
ORCID: 0000-0002-4538-0161

ABSTRACT

The evaluation triiodothyronine, thyroxine, thyrotropin, c-reactive protein, interleukin-6 and tumor necrosis factor-α in diabetic patients attending Abia State Teaching Hospital Aba, was carried out. Venous blood samples were collected from 150 participants who gave consent. This comprises 100 diabetic patients as test and 50 healthy subjects as control. The serum Triiodothyronine, thyroxine, thyrotropin was determined using Fincare™ quantitative assay method. Data from this study were analyzed using statistical package for the social sciences (SPSS). Result shows that thyroxine (8.97±1.28µg/dl), thyrotropin(2.08±0.85µiu/ml) respectively) ($p<0.05$ in each case). The serum triiodothyronine (0.89±0.38ng/ml), was lower in diabetic patients, compared with control (1.04±0.33ng/l) ($p<0.05$). From the findings, management of conditions related to cardiovascular disease, atherosclerotic disease, anemia and stress in diabetics may benefit patients if thyroid hormones are included as part of their routine laboratory investigations.

Keywords: thyroid hormones, cardiovascular disease, *diabetes mellitus*, atherosclerotic disease

INTRODUCTION

Diabetes mellitus (DM), is a group of metabolic disorders in which there is high blood sugar level over a prolonged period and it is commonly referred to as diabetes [1-10]. Frequent urination, increased thirst, and increased hunger are symptoms of high blood sugar [11]. Many complications are resulted as a cause of untreated diabetes [12]. Diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death are as a result of acute complications. However, cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes are included as long-term complication [13-20]. There is evidence that each of these dyslipidemia features is associated with increased risk of cardiovascular disease, the leading cause of death in patients with type 2 diabetes [20-27]. Numerous studies have demonstrated an association between LDL size or density and coronary artery disease (CAD). Moreover, recent reports have indicated that LDL particle concentrations, and specifically levels of small dense LDL, are predictive of coronary events and that this is independent of other coronary disease risk factors [20-27].

MATERIALS AND METHODS

STUDY AREA

The study was carried out at Abia State University Teaching Hospital (ABSUTH),Aba city in Abia state,South East of Nigeria.It lies on coordinates of 5° 57′ 0”North and 8° 55′ 0” East.It is bordered to the North by Enugu state,to the north-east by Ebonyi state,to the west by Imo state,to the east by Cross-river state,to the south-east and south by Akwa ibom and Rivers states.

ADVOCACY, MOBILIZATION AND PRE-SURVEY CONTACT

With the letter of introduction from the Head of Department,Medical Laboratory Science of Imo State University,I met the gate keeper of Abia State University Teaching Hospital Aba, who helped me to see the Chief Medical Director of the institution,to whom I submitted the letter and he reffered me to the ethical committee of Abia State University Teaching Hospital(ABSUTH) Aba, where I also submitted my research proposal, and after their consideration, approval was obtained.Several meetings were held with the nurses incharge of the diabetic clinic, and clinic days were chosen as the days of sample collection. Consent was sought and obtained from recruited subjects after explaining the purpose of the research to them.

STUDY POPULATION

The size of population was calculated using the method of Aroye 2004 with the formula $n=(z^2pq)/d^2$), and one hundred and fifty (150) subjects were recruited into the study.This comprises fifty(50)non-diabetic subjects as control and one hundred(100) diabetic subjects as test subjects.

SELECTION CRITERIA

1. INCLUSION;Those selected are;

(i) Male and Female subjects of age 18 years to 74years,

(ii) Diabetic patients with blood sugar 10mmol/l and above,

(iii) The subjects that gave their consent.

EXCLUSION;The excluded subjects are;

(i) Male and Female subjects below the age of 18 years

(ii) Subjects of blood sugar below 10mmol/l

(iii)The subjects that did not give consent.

LABORATORY PROCEDURES

$$\text{Conc. of glucose} = \frac{\text{Abs. of test}}{\text{Abs. of Std}} \times \text{conc. of Std (mmol/l)}$$

Abs=absorbance

Conc.=concentration

Std=standard.

The reagents were commercially purchased and the manufacturers' standard operating procedures (S.O.P) were strictly adhered to.

(**Determination of plasma glucose (enzymatic method) of Trinder [21].**

Assay procedure; 1000µl of glucose solution and 10µl of sample, standard and control were added into test tubes respectively, and incubated for 10minutes at 37°c.The absorbance of standard and sample was measured(read) against reagent blank within 60 minutes at 500nm wavelength.

DETERMINATIONOF THYROXINE(T4) AND TRIIODOTHYRONINE (T3)

[Microplate enzyme immunoassay method] of Eva, E. and Perlmann [22]. **Assay procedure**;50µl (for t3) and 25µl (for t4) of serum, standard and control were pipetted into appropriate designated wells.100µl of t3/t4 working enzyme reagent was added and swirled for 30 seconds for mixing. The mixture was incubated for 60 minutes at room temperature. After incubation the content was decanted and 350µl of wash buffer was used to wash the micro-plate wells respectively for three (3) times. This was followed with the addition of 100µl of working substrate solution to all wells. For 15 minutes without shaking the plates were incubated at room temperature, after which 50µl of stop solution was added and the plates were mixed by shaking gently. The absorbance was read at 450nm using micro-plate reader within 30 minutes. Concentrations of t3/t4 was obtained by plotting absorbance versus concentrations of each sample, standard and control.

DETERMINATION OF THYROTROPIN (TSH)

[Microplate enzyme immunoassay method] of Eva, E. and perlmann [22].

Assay procedure

50µl of serum, standard and control were pipetted into appropriate designated wells.100µl of TSH enzyme reagent was added and swirled for 30 seconds for mixing. The mixture was incubated for 60 minutes at room temperature. After incubation the content was decanted and 350µl of wash buffer was used to wash the micro-plate wells respectively for three (3) times. This was followed with the addition of 100µl of working substrate solution to all wells. For 15 minutes without shaking the plates were incubated at room temperature, after which 50µl of stop solution was added and the plates were mixed by shaking gently. The absorbance was read at 450nm using micro-plate reader within 30 minutes. Concentrations of TSH was obtained by plotting

absorbance versus concentrations of each sample, standard and control.

Statistical Analysis

All data generated from this study were subjected to statistical analysis using statistical package for the social sciences (SPSS) version 20. Values were expressed as Mean ± SD, at 95% confidence limit. Results are presented in tables.

RESULTS

Table 1: Mean ± SD values of T3, T4 AND TSH of the studied population

Parameters	Diabetic patients n=100	controls n=50	t-value	p-value
Triiodothyronine (ng/ml)	0.89±0.38	1.04±0.33	-2.462	0.015
Lowe r95% C.I	-0.28	-0.27		
Upper 95% C.I	-0.03	-0.03		
Thyroxine(µg/dl)	8.97±1.28	7.53±1.73	5.750	0.001
Lowe r95% C.I	0.94	0.89		
Upper 95% C.I	1.94	1.99		
Thyrotropin(µIU/ml)	2.08±0.85	1.73±0.80	2.375	0.019
Lowe r95% C.I	0.05	0.06		
Upper 95% C.I	0.63	0.62		

The serum level of T3 was significantly lower (p=0.015) in the study population compared with control. The serum levels of T4 and TSH were significantly higher (p=0.001 and p=0.019 respectively) in the study population compared with control (Table 1).

Table 2: Mean ± SD values of T3, T4 AND TSH of the studied population in relation to sex

Parameters	Male patients (n=56)	Female patients (n=44)	t-value	p-value
Triiodothyronine(ng/ml)	0.95±0.37	0.82±0.38	1.736	0.086
Lower 95% C.I	-0.01	-0.01		
Upper 95% C.I	0.28	0.28		
Thyroxine(µg/dl)	8.82±1.39	9.12±1.13	-1.164	0.247
Lower 95% C.I	-0.81	-0.80		
Upper 95% C.I	0.21	0.19		
Thyrotropin(µIU/ml)	2.10±0.81	2.09±0.93	0.052	0.956
Lower 95% C.I.	-0.33	-0.34		
Upper 95% C.I.	0.35	0.36		

The serum T3 and TSH levels were not significantly higher (p=0.086 and p=0.959 respectively) in male studied population compared with female studied population. The serum T4 level was not significantly lower (p=0.247) in male studied population compared with female studied population (Table 2).

Table 3: Mean ± SD values of T3, T4 AND TSH of the studied population in relation to age group

Parameter	35-44 (years)	45-54 (years)	55-64 (years)	65-74 (years)	f-value	p-value
Triiodothyronine(ng/ml)	0.69±0.22	0.77±0.25	0.93±0.39	1.00±0.45	2.844	0.042
Lower 95% C.I	0.53	0.65	0.78	0.84		
Upper 95% C.I	0.84	0.87	1.06	1.15		
Thyroxine(µg/dl)	10.04±0.82	8.80± 1.14	9.21±0.84	8.57±1.61	4.277	0.007
Lower 95% C.I	9.45	8.31	8.90	8.01		
Upper 95% C.I	10.62	9.29	9.50	9.12		
Thyrotropin(µIU/ml)	2.88±0.87	2.17±0.64	2.06±0.83	1.81±0.87	4.638	0.005
Lower 95% C.I.	2.25	1.89	1.75	1.51		
Upper 95% C.I.	3.50	2.44	2.36	2.10		

There was statistical progressive increase (p=0.042) in T3 levels of the studied population in relation to age groups. There were statistical progressive decrease (p=0.007 and p=0.005 respectively) in T4 and TSH levels of the studied population in relation to age groups. (Table 3).

Table 4: Mean ± SD values of T3, T4 AND TSH of the studied population in relation to weight

Parameter	53-64kg (n=25)	65-74kg (n=75)	t-value	p-value
Triiodothyronine(ng/ml)	0.87±0.38	0.89±0.38	-0.254	0.800
Lower 95% C.I	-0.19	-0.19		
Upper 95% C.I	0.15	0.15		
Thyroxine(µg/dl)	9.16±1.24	8.91±1.30	0.860	0.392
Lower 95% C.I	-0.33	-0.33		
Upper 95% C.I	0.84	0.84		
Thyrotropin(µIU/ml)	2.07±0.88	2.08±0.84	-0.054	0.957
Lower 95% C.I.	-0.40	-0.40		
Upper 95% C.I.	0.38	0.38		

The serum T3 and TSH levels were not significantly lower (p=0.800; p=0.957, respectively) in 53-64kg weight when compared with 65-74kg weight of the studied population. The serum T4 level was not significantly higher (p=0.392) in 53-64kg weight when compared with 65-74kg weight of the studied population (Table 4).

Table 5: Mean ± SD values of T3, T4 AND TSH of the studied population according to duration of disease

Parameters	1 year (n=37)	2years (n=18)	3years (n=28)	4years (n=17)	f-value	p-value
Triiodothyronine (ng/ml)	0.92±0.41	1.01±0.44	0.90±0.38	0.76±0.32	1.191	0.317
Lower 95% C.I	0.78	0.78	0.75	0.59		
Upper 95% C.I	1.05	1.22	1.05	0.92		
Thyroxine(µg/dl)	8.81±1.04	8.97±1.55	9.10±1.34	9.19±1.47	0.443	0.723
Lower 95% C.I	8.46	8.19	8.57	8.43		
Upper 95% C.I	9.15	9.73	9.61	9.95		
Thyrotropin(µIU/ml)	2.09±0.80	2.05±0.90	2.17±0.79	2.00±1.05	0.150	0.929
Lower 95% C.I.	1.82	1.60	1.85	1.45		
Upper 95% C.I.	2.35	2.49	2.47	2.54		

There was no progressive decrease (p=0.317) in T3 level from 1st year to 4th year duration of disease. The serum T4 and TSH levels showed no statistical progressive increase (p=0.723 and p=0.929 respectively) from 1st year to 4th year duration of disease (Table 5).

Table 6: Mean ± SD values of T3, T4 AND TSH of the studied population according to drug administered

Parameters	Glucophage only (n=28)	Glucophage and Clamide (n=30)	Insulin only (n=42)	f-value	t-value
Triiodothyronine(ng/ml)	0.93±0.42	0.95±0.35	0.83±0.38	1.098	0.338
Lower 95% C.I	0.76	0.82	0.70		
Upper 95% C.I	1.08	1.08	0.94		
Thyroxine(µg/dl)	8.80±1.09	8.87±1.28	9.14±1.42	0.725	0.487
Lower 95% C.I	8.37	8.38	8.70		
Upper 95% C.I	9.21	9.34	9.58		
Thyrotropin(µIU/ml)	1.94±0.82	2.24±0.77	2.05±0.93	0.891	0.413
Lower 95% C.I.	1.62	1.94	1.75		
Upper 95% C.I.	2.26	2.52	2.33		

There was no progressive increase (p=0.338; p=0.487; p=0.413 respectively) in T3, T4 and TSH levels of the studied population according to drug administered (Table 6).

DISCUSSION

In this present work, the serum T4, TSH, CRP, IL-6 and TNFα levels were higher in studied population compared with controls. There was significant progressive increase in T3 according to age groups. The statistically significant higher level of T4 (Table 1), could be due to the fact that during hyperthyroidism, the half-life of insulin is reduced most likely secondary to an increased rate of degradation and an enhanced release of biologically inactive insulin precursors. In untreated Graves' disease, increased pro-insulin levels in response to a meal were observed in a study by Beck *et al* [23]. In addition, untreated hyperthyroidism was associated with a reduced C-peptide to pro-insulin ratio suggesting an underlying defect in pro-insulin processing. Another mechanism explaining the relationship between hyperthyroidism and hyperglycemia is the increase in glucose gut absorption mediated by the excess thyroid hormones. Endogenous production of glucose is also enhanced in hyperthyroidism via several mechanisms. Thyroid hormones produce an increase in the hepatocyte plasma membrane concentrations of GLUT2 which is the main glucose transporter in the liver, and consequently, the increased levels of GLUT-2 contribute to the increased hepatic glucose output and abnormal glucose metabolism. Additionally, increased lipolysis is observed in hyperthyroidism resulting in an increase in free fatty acid (FFA) that stimulates hepatic gluconeogenesis. The increased release of free fatty acid could partially be explained by an enhanced catecholamine-stimulated lipolysis induced by the excess thyroid hormones. Moreover, the nonoxidative glucose disposal in hyperthyroidism is enhanced resulting in an overproduction of lactate that enters the Cori cycle and promotes further hepatic gluconeogenesis. The increase in growth hormone, glucagon and catecholamine levels associated with hyperthyroidism further contributes to the impaired glucose tolerance.

CONCLUSION

From the study since thyroxine, thyroid stimulating hormone were higher in studied population and are associated with risk factors as coronary artery disease, cardiovascular disease, atherosclerotic diseases, atherothrombotic complications and deficiency of vitamin k, care has to be taken in managing the disease. Furthermore, individuals are advised to go for regular laboratory investigation to ascertain their health conditions.

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