CORRELATION OF THYROID HORMONES BETWEEN TYPE 2 DIABETES MELLITUS PATIENTS AND NORMAL INDIVIDUALS

Internship Training Report

Submitted to

Lovely Professional University, Punjab

In partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Biochemistry

Submitted by:
Arun Acharya
(Reg. No. 11403865)

SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA
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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Ekta Chitkara (Internal supervisor), Lovely Professional University and Mr. Prithvi Bahadur Shah (External Supervisor). This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Arun Acharya

Date: 06-05-2016

Place: Lovely Professional University, Punjab

CERTIFICATE

This is to certify that **Mr.** ***Arun Acharya*** bearing **Registration Number** 11403865has completed his Master of Science in Clinical Biochemistry internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

Internal Supervisor

Date: 06/05/2016

External Supervisor

Date: 29/04/2016

Lovely professional University
Punjab.

Norvic International Hospital
नर्भिक इन्टरनेशनल हस्पिटल

Ref. No.:- HR 108-072/073

29th April 2016

TO WHOM IT MAY CONCERN

This is to certify that **Mr. Arun Acharya** (Reg. No. 11403865), a student of Lovely Professional University, Punjab, India, has successfully completed a four months training program (1st January 2016 to 29th April 2016) from this hospital. During this period, he has been exposed to sample collection, sample receiving, sample processing, and handling of all the instruments available in Biochemistry department. He has also successfully completed the project on **"CORRELATION OF THYROID HORMONES BETWEEN TYPE 2 DIABETES MELLITUS PATIENTS AND NORMAL INDIVIDUALS".**

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Dr. Sabina Shrestha

Lab Director

ABSTRACT

The aim of this study is to correlate thyroid hormones between type 2 diabetes mellitus patients and normal individuals. The study was conducted among 30 patients attending in-patient and out-patient department at Norvic International Hospital, Nepal. According to American Diabetes Association, Diabetes mellitus is defined as a group of metabolic disorder characterized by hyperglycemia that result either from defects in insulin secretion, insulin action or the combination of both . WHO estimated worldwide prevalence of 170 million diabetes in 2002, with the number predicted to grow to 366 million or more by 2030. Diabetes mellitus is considered as one of the emerging disease that has made its mark among general population in Nepal. According to the survey conducted in urban Nepal between 2001 and 2002, 10.8 and 13.2 % of males were suffering from diabetes and prediabetes respectively. Similarly 6.9% and 10.2 % of female individuals suffered from diabetes and pre diabetes respectively. According to World health organization (WHO) diabetes affects more than 436,000 people in Nepal, and this number is expected to rise to 1,328,000 by 2030. Statistically significant differences were observed in the mean FT3 activity of controls (4.44 ± 0.82) and type 2 diabetes mellitus patients (3.37 ± 1.14) (p=0.000). Statistically significant differences were also observed in the mean FT4 activity of controls (1.45 ± 0.39) and type 2 diabetes mellitus patients (1.1± 0.335) (p=0.002), which was also higher in normal individuals. Statistically differences in the mean TSH activity were also observed between controls (3.56 ± 2.50) and type 2 diabetes mellitus patients (5.52 ± 2.41) (p=0.003), where mean value of TSH in type 2 diabetes mellitus patients is high. The association between diabetes and thyroid dysfunction had been recognized since late 90's and this has emphasized the importance of screening of diabetic patients to identify thyroid diseases. A number of the studies have reported the prevalence of thyroid dysfunction among diabetes patients to be between 2.2 to 17%. However, few studies have observed the very high prevalence of thyroid dysfunction in diabetes i.e.

31 % and 46.5% respectively. Diabetes patients have a higher prevalence of thyroid disorders than the normal population. Therefore, this study suggests high prevalence of hypothyroidism in type 2 diabetes mellitus patients as compared to normal individuals.

ACKNOWLEDGEMENT

First and foremost I would like to thank **Dr. Sabina Shrestha**, Pathologist, and Director of the laboratory, for giving me permission to work in the lab. Without it, my work would not have been possible.

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Next, I would like to thank **Mr. Prithvi Bahadur Shah**, (Sr.Biochemist) my mentor who has enlightened me with the working of a clinical laboratory. He has helped me understand the role of a Clinical Biochemist and encouraged to better it. It is his support and guidance that made it possible to work on this particular topic. He has helped me run the tests and interpret them relating it to the quality assessment and maintenance of biochemistry analyzers.

I would like to extend my thanks to the entire family of Norvic International Hospital, pathology lab for supporting me in every step. They have provided me with an ambience that has made it possible to conduct my study smoothly. I thank **Mrs. Anita kuikel** and **Mrs. Amanta Dahal**, as they have taught me various aspects of a lab. They have encouraged me to achieve more.

Most importantly, I would like to thank my parents, especially my brother to whom I will be forever indebted. They have been the backbone of my journey till this stage. It is their vision that has made me able to excel in my education.

Finally, I thank the **ALMIGHTY** for giving me hope and strength to complete my work.

Arun Acharya

Reg No: 11403865

TABLE OF CONTENTS

LIST OF TABLES

TABLE NO **PAGE NO**

LIST OF FIGURES

FIGURE NO **PAGE NO**

LIST OF APPENDICES

INTRODUCTION

The term diabetes is derived from the Greek word [*Dia*; pass through and *bainein*; to go]. Hence, diabetes literally means to pass through. In this condition, the body is unable to utilize carbohydrate, mostly glucose as an energy source leading to increased glucose level in the blood and is characterized by chronic hyperglycemia associated with disturbances in protein and lipid metabolism on account of absolute or relative deficiency or inefficiency of insulin (1). The rate of diabetes among the general population is constantly increasing and has reached an alarming state of concern.

Diabetes mellitus is broadly classified into two main types;

- Type I diabetes mellitus: It is also known as immune mediated diabetes, insulin-dependent diabetes or juvenile mediated diabetes. It accounts for 5-10% of diabetes mellitus and results due to autoimmune destruction of pancreatic B-cells.
- Type II diabetes mellitus: It is also known as non-insulin dependent diabetes or adult onset diabetes. It accounts for 90-95% of diabetes mellitus and occurs as a result of insulin resistance and progressive insulin deficiency (2).

International Diabetes Federation (IDF) had estimated a 72.1 million people in South East Asia had diabetes in 2014 and this number is expected to increase up to 123 million by 2040. Also according to the data compiled in IDF, sixth edition, 2013, Diabetes caused 5.1 million deaths in 2013. This corresponds to the death of a person every six seconds due to diabetes (3).

Diabetes cases are increasing in the modern world due to an increasing prevalence of obesity and sedentary lifestyle (4). Type II Diabetes mellitus results as a result of insulin resistance combined with β- cell dysfunction. However, β- cells are initially functional and the level of insulin in the

blood may vary from above normal to below normal (2). Metabolic changes observed in patients with T2DM are mostly due to insulin resistance in liver, muscle and adipose tissue. Hyperglycemia is observed due to an increased glucose production from liver and decreased peripheral use as a result of insulin resistance (5). Type 2 Diabetes Mellitus develops gradually without any specific symptoms and is characterized late only once the other conditions affecting various organs of an individual (6). Individuals with diabetes mellitus are presented with classical symptoms of frequent urination, thirst, and hunger (7).

On the other hand thyroid diseases had also became common among general population affecting 750 million people worldwide according to the data provided by World health organization (8). Thyroid hormones are produced by a butterfly- shaped thyroid gland located in the lower anterior neck (9). Thyroxine (T4) is the primary hormone secreted by the thyroid gland which is relatively inactive and is converted to the highly active form triiodothyronine (T3) by the enzyme thyroxine 5- deiodinase (10).

The thyroid hormones are insulin antagonists and influence the action of insulin indirectly which could be responsible for the occurrences of low thyroid hormone levels in diabetic mellitus patients **(**11**).** Insulin, an anabolic hormone has been found to enhance the levels of FT4 and suppresses the levels of FT3 by inhibiting hepatic conversion of T4 to T3. Therefore, this may be the reason for low FT3 in type 2 diabetes mellitus patients**.** Diabetes mellitus influence thyroid function mainly at two sites; first, at the level of hypothalamic control of thyroid-stimulating hormone release and second, at the conversion of T4 to T3 in the peripheral tissue **(**12**).**

A study by Panneerselvam et al. showed that serum levels of T3, T4, FT3 and FT4 were significantly lower in diabetic subjects as compared to the non-diabetic subjects while serum

level of TSH was found to be significantly higher in type 2 diabetes mellitus patients as compared to normal individuals **(**11**).**

Another study by Islam S et al. showed serum level of FT3 was significantly lower in type 2 diabetic patients as compared to the non-diabetic individuals. While FT4 and TSH level did not show any statistical difference between type 2 diabetic patients as compared to normal individuals **(**13**).**

Therefore, this study is an effort to evaluate thyroid hormones; FT4, FT3, and TSH between type II diabetes mellitus patients and normal individuals.

AIMS AND OBJECTIVE

1. To evaluate thyroid hormones (T3, T4) and TSH with HBA1C in type 2 diabetes mellitus patients.
2. To evaluate T3, T4, and TSH between type 2 diabetes mellitus patients and normal individuals.

REVIEW OF LITERATURE

1. VENKATACHALAM RAMESH, RAJAGOPALAN GEETHA, DEVARAJ ANITHA, NRVK SWAMY, THANGARAJAN THANGA PANNEERSELVAM, 2015.

Type 2 diabetes mellitus is commonly associated with altered thyroid function. Out of the 50 type 2 diabetes mellitus patients, 25.11% showed abnormal thyroid function (21.90 % had hypothyroidism and 3.21% had hyperthyroidism) and 74.89 % showed normal thyroid hormone level. The thyroid hormones are insulin antagonists that also potentiate the action of insulin indirectly. These facts could be responsible for the occurrences of low thyroid hormone levels in some diabetes patients. The serum levels of T3, T4, FT3 and FT4 were significantly lower in diabetic subjects as compared to the non-diabetic subjects while serum level of TSH was found to be significantly higher in type 2 diabetes mellitus patients as compared to normal individuals (14).

2. ATHANASIA PAPAZAFIROPOULOU, ALEXIOS SOTIROPOULOS, ANTHI KOKOLAKI, MARINA KARDARA, PETROULA STAMATAKI, STAVROS PAPPAS, 2010.

In total 1,092 patients with type 2 diabetes mellitus 12.3 % had thyroid dysfunction. Higher prevalence of thyroid dysfunction among diabetic females was reported. A study by Smithson et al. showed a prevalence of 10.8% of thyroid dysfunction in diabetes mellitus patients (15). Another study by Perros et al. in a randomly selected group of 1,310 diabetic adults estimated that the prevalence of thyroid dysfunction was found to be 13.4 % (16). A recent study reported that thyroid dysfunction was present in 16% of Saudi type 2 diabetes mellitus patients (17). Also, a study in Jordan showed that the overall prevalence of thyroid dysfunction was 12.5% in type 2 diabetes mellitus patients (18).

3. SUMIT SOHAL, AANCHAL WATS, CHITTARANJAN VIJ, 2016.

Thyroid hormones action has long been recognized as an important determinant of glucose homeostasis. Diabetes and thyroid disorders have a propensity to appear together in patients. Out of 30 patients 26 were diagnosed as hypothyroid and 4 were diagnosed as hyperthyroid. HbA1c levels were found to be elevated in patients of thyroid disorders (hypothyroidism and hyperthyroidism) as compared to control. HbA1c levels in hypothyroid patients were found to be in a range of 7.2-7.79% with a mean of 7.41 ± 0.15% against the range of 5.89-7.2% with the mean of 6.69 ± 0.34% in controls. The difference was found to be highly significant ($p<0.001$). HbA1c levels in hyperthyroid patients were found to be in a range of 7.23-7.89% with a mean of 7.51 ± 0.23%, which was significantly ($p<001$) greater than the controls. HbA1c levels are increase in both hypothyroid and hyperthyroid patients. Both the disorders have increased levels of HbA1c due to differential actions of thyroid hormones on liver, skeletal muscles, and adipose tissue. Many of these actions are complex and based on gene expression changes brought about by thyroid hormones (19).

4. DR. M. ANITA DEVI, L. SAMANANDA SINGH, DR. N. SASHIKANTA SINGH, 2013.

Diabetic patients are highly associated with thyroid disorders, it may disrupt in the treatment of diabetes. Association of thyroid hormone disorders in type 2 diabetes mellitus patients is very common (20). The level of T3 and T4 in type 2 diabetes mellitus patients was significantly low as compared to normal individuals whereas TSH was significantly high in type 2 diabetes mellitus patients as compared to normal individuals. Higher TSH level in diabetic patients indicates hypothyroidism in type 2 diabetes mellitus patients (21). Abnormal thyroid hormones in type 2 diabetes mellitus may also depend on the glycemic status of the patients. Glycemic

status is influenced by insulin, which modulates Thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH) level (22, 23). It has also been reported that in type 2 diabetes, abnormal level of thyroid hormone was found due to the presence of thyroid hormone binding inhibitor (THBI) which is an inhibitor of the extra thyroidal conversion of enzyme T4 to T3 and also the dysfunction of hypothalamic-pituitary-thyroid axis (24).

5. VIBHA UPPAL, CHITTRANJAN VIJ, GURDEEP KAUR BEDI, ANIL VIJ, BASU DEV BANERJEE, 2013.

Diabetes and thyroid disorders have been shown to mutually influence each other and an association between both conditions has been reported in literature (25). Insulin and thyroid hormones are intimately involved in cellular metabolism and thus excess or deficit of either of these hormones result in functional derangement of the other (26). Excessive thyroid hormones increase the rate of digestive tract absorption and increase insulin resistance and insulin degradation. In hypothyroidism, liver secretion of glycogen decreases, so does degradation, leading to increased levels of glycogen (27). Hyperthyroidism impairs glycemic control in type 2 diabetes mellitus patients, while hypothyroidism increases susceptibility to hypoglycemia thus complicating diabetes management (25). Among 120 type 2 diabetes mellitus patients studied, 24.5% patients showed thyroid disorder (28).

6. SRINIDHI RAI, ASHOK KUMAR, PRAJNA K, SHIBITH KUMAR SHETTY, TIRTHAL RAI, SHRINIDHI et al., 2013.

Diabetic patients have higher prevalence of thyroid disorder when compared with the normal individuals, with hypothyroidism being the most common disorder (29). Serum T3 and serum T4 levels were found to be decreased while serum TSH levels were increased in type 2 diabetes

mellitus patients as compared to normal individuals. A study by Islam S et al. showed that the levels of FT3 was significantly lower in type 2 diabetes mellitus patients as compared to normal individuals. Similarly TSH did not show any statistically significant difference between type 2 diabetes mellitus patients and normal individuals (30).In diabetes mellitus there are alterations in the hypothalamus-pituitary-thyroid axis. Hypothalamic and plasma TRH, pituitary and plasma TSH, as well as TSH secretion rated are reduced, and the TSH response to TRH is decreased. There are important structural changes in the thyroid gland and pituitary that are accompanied by marked alterations in their secretory activities (31, 32).

7. MIRELLA HAGE, MIRA S. ZANTOUT, SAMI T. AZAR, 2011.

Thyroid diseases and diabetes mellitus are the two most common endocrine disorders encountered in clinical practice. Diabetes and thyroid disorders have been shown to mutually influence each other and association between both conditions have been long reported (33, 34). On one hand, thyroid hormones contribute to the regulation of carbohydrate metabolism and pancreatic function, and on the other hand, diabetes affects thyroid function tests to variable extents. Thyroid hormones affect glucose metabolism via several mechanisms. Hyperthyroidism has long been recognized to promote hyperglycemia (35). Another mechanism explaining the relationship between hyperthyroidism and hyperglycemia is the increase in glucose gut absorption mediated by the excess thyroid hormones (36, 37). 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 (38, 39).

8. RAGHAD A. MOHSIN, SABAH N. ALWACHI, KHALID I. Al- LEHIBI, 2013.

Hypothyroidism is one of the most common functional disorders of the thyroid gland (40). Glucose intolerance is associated with hyperthyroidism, and more recently it was shown that hypothyroidism is associated with insulin resistance (41), which is a pathological state in which the target cell fails to respond to ordinary levels of circulating insulin resulting in failure to maintain normal glucose and lipid levels in circulation (42).The result of the correlation between TSH and HbA1c agreed to a study carried out on patients with subclinical hypothyroidism, where it found that there is a positive relationship between the level of TSH and the level of HbA1c (43).

9. WILMA DELPHINE SILVIA CR, GANDHAM RAJEEV, VENKATA BHARAT KUMAR PINNELLI, 2013.

Type 2 diabetes mellitus patients have a higher prevalence of thyroid disorders than the normal population. The correlation between type 2 diabetes mellitus and thyroid disorder is poorly understood and are associated with vascular complications and responsible for high mortality and morbidity (44). The association between diabetes and thyroid dysfunction had been recognized since 1979 and emphasized the importance of screening of thyroid disorders in diabetes mellitus patients (31, 45). Diabetes mellitus appears to influence thyroid function in at least two sites, one at the level of hypothalamic control of thyroid stimulating hormone (TSH) release and the other at the conversion of thyroxine (T4) to 3,5,3’-triiodothyronine (T3) in the peripheral tissue (46). Altered thyroid hormones have been described in patients with type 2 diabetes mellitus

especially those with poor glycemic control. In type 2 diabetes mellitus patients, the nocturnal TSH peak is blunted or abolished, and the TSH response to TRH is impaired (47).

10. C.M. BENNETT, M. GUO, S.C. DHARMAGE, 2007.

The high prevalence of diabetes mellitus has emerged as a worldwide public health problem in the past 20 years. Type 2 diabetes mellitus is the most common form of diabetes, estimated to account for 85-90% of diabetes (48). Type 2 diabetes mellitus is often asymptomatic in its early stages and can remain undetected for several years (49). Early diagnosis of type 2 diabetes mellitus of the condition is important as careful diabetes management can reduce long term complications, such as blindness, kidney failure, and cardiovascular disease and limb amputation (50-52).

MATERIALS AND METHODS

MATERIALS

The materials used for the analysis of various required biochemical parameters are as follows:

FOR THE ESTIMATION OF PLASMA GLUCOSE

Two different working solutions were used for the estimation of Serum glucose

Reagent 1:

MES (2-(N-morpholino) ethanesulfonic acid) buffer: 5.0 mmol/L

- pH 6.0
- Mg^{2+} : 24 mmol/L
- ATP : ≥ 4.5 mmol/L
- NADP : ≥ 7.0 mmol/L
- Preservative

Reagent 2:

HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer: 200 mmol/L

- Ph 8.0
- Mg^{2+} : 4 mmol/L
- HK (yeast) : ≥ 300 µkat/L
- G-6-PDH (E. coli) : ≥ 300 µkat/L
- Preservative

MES buffer occupied position B whereas HEPES buffer occupied position C

FOR THE ESTIMATION OF HbA1c

For the measurement of HbA1c, two different reagents were used:

R1/Reagent 1:

Glycinamide buffer containing dye bound boronic acid and detergents.

R2/Washing Solution:

Morpholine buffer of sodium chloride (Nacl) and detergent.

Apart from the reagents, the materials used were Capillary tube (5 µL), pipette (25µL) and Nycocard Reader II.

ESTIMATION OF THYROID HORMONES

Three different reagents were used for the measurement of thyroid hormones; FT4, FT3, TSH. Determination of thyroid hormones was done with the help of a specific antibody labeled with Ruthenium complex. Streptavidin- coated micro particles (0.72ng/mL) was used as a common reagent for all the three tests.

FOR THE ESTIMATION OF SERUM FT3

Reagent 1:

Monoclonal anti-T3 antibody labeled with ruthenium complex (10ng/mL)

Phosphate buffer: 100 mmol/L

pH 7.0

Reagent 2:

Biotinylated T3 (2 ng/mL)

Phosphate buffer: 100 mmol/L

pH 7.0

FOR THE ESTIMATION OF SERUM FT4

Reagent 1:

Polyclonal anti-T4 antibody (sheep) labeled with ruthenium complex (50ng/mL)

PHOSPHATE BUFFER: 100 mmol/L

Ph 7.0

Reagent 2:

Biotinylated T4 (2.5ng/mL)

Phosphate buffer: 100 mmol/L

pH 7.0

FOR THE ESTIMATION OF TSH

Reagent 1:

Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L

Phosphate buffer: 100 mmol/L

pH 7.2

Reagent 2:

Monoclonal anti- TSH antibody (mouse/human) labeled with ruthenium complex (1.2 mg/L)

Phosphate buffer: 100 mmol/L

pH 7.2

METHODS

SUBJECT SELECTION

The study was conducted at Norvic International hospital located at Thapathali, Kathmandu, Nepal. Study was conducted among fifty patients attending Inpatient and Outpatient department. Serum free thyroxine (FT4), free tri-iodothyronine (FT3) and thyroid stimulating hormone (TSH) were estimated as a part of thyroid hormone assay. Glycosylated hemoglobin (HbA1c) and Random plasma glucose were estimated to test diabetes.

Inclusion Criteria:

In the study we have included only those patients who are having random plasma glucose levels > 200 mg/dl and HbA1c level more than 6.5 %.

Exclusion Criteria:

Known diabetic patients who were under insulin therapy or those taking oral hypoglycemic drugs.

SAMPLE COLLECTION

Blood samples were collected through antecubital vein from all subjects into following vials for various biochemical tests:

1. Fluoride oxalate vial for random plasma glucose estimation
2. EDTA vial for Glycosylated hemoglobin (Hb_1Ac) estimation.
3. Silica gel vial for TSH, FT3, and FT4 estimation.

PROCEDURES

ESTIMATION OF PLASMA GLUCOSE (53, 54)

METHOD: HEXOKINASE (KINETIC)

PRINCIPLE:

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose + ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate + ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{Glucose-6-phosphate + } NADP^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-phosphate + NADPH + } H^{+}$$

ESTIMATION OF HbA1C (55)

METHOD: BORONATE AFFINITY CHROMATOGRAPHY

PRINCIPLE:

The reagent contains agents that lyse erythrocytes and precipitate hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diols of glycated hemoglobin. When blood is added to the reagent, erythrocytes immediately lyse. All hemoglobin precipitates. The boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin.

An aliquot of reaction mixture is added to the test device, and all the precipitated hemoglobin, conjugate-bound and unbound, remains on top of the filter. Any excess of colored conjugate is

removed with the washing solution. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) color intensity with the Nycocard READER II, the ration between them being proportional to the percentage of Hb_1Ac in the sample.

NYCO CARD ASSAY:

Nyco card HbA1c is a boronate affinity assay. The kit contains test devices with a porous membrane filter, test tubes pre-filled with reagent and a washing solution.

Precipitation of hemoglobin

5µL whole blood was added to the test tube pre-filled with R1/Reagent. Mixed well and then tube was left for two to three minutes.

Application of sample

Test tube was remixed to obtain a homogenous suspension. 25 µL of the reaction mixture was applied to a Test Device by holding the pipette approximately 0.5cm above the test well and pipette was emptied quickly in the middle of the test well. Allow the reaction mixture to soak completely into the membrane (approx. 10 mins).

Application of R2/Washing solution

25 µL R2/Washing solutions were applied to the Test Device. Washing solution was allowed to soak completely into the membrane and then waited for minimum 10 seconds.

Test result measurement

The test result was read within 5 minutes using Nycocard READER. Color intensity is directly proportional to the percentage of Hb_1Ac.

ESTIMATION OF FT3

PRINCIPLE:

FT3 estimation is based on competitive ELISA principle and the total duration of assay is 18 minutes.

PROCEDURE:

- First incubation of 15 µL sample and an anti-T3-specific antibody labeled with a ruthenium complex.
- After Second incubation with Biotinylated T3 and Streptavidin-coated microparticles the remaining free binding sites of the labeled antibody becomes occupied and forms an antibody-hapten complex.
- The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.
- Unbound substances are then removed by treatment with ProCell.
- Application of voltage to the electrode then induces chemiluminiscent emission which is measured by a photomultiplier.
- Final results are determined via a calibration curve.

ESTIMATION OF SERUM FT4

FT4 is also estimated by competitive ELISA assay and the duration of test is 18 minutes.

PROCEDURE:

- First incubation of 15 µL sample and an anti-T3-specific antibody labeled with a ruthenium complex.
- After Second incubation with Biotinylated T3 and Streptavidin-coated microparticles the remaining free binding sites of the labeled antibody becomes occupied and forms an antibody-hapten complex.
- The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.

- Unbound substances are then removed by treatment with ProCell.
- Application of voltage to the electrode then induces chemiluminiscent emission which is measured by a photomultiplier.
- Final results are determined via a calibration curve.

ESTIMATION OF SERUM TSH

PRINCIPLE:

TSH assay is based on sandwich ELISA principle and the total duration of assay is 18 minutes similar to that of other two thyroid hormones.

PROCEDURE:

- First incubation consists of 50 µL samples with Biotinylated monoclonal TSH- specific antibody and a monoclonal TSH-specific antibody labeled with ruthenium complex to form a sandwich complex.
- After Second incubation with Streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and Streptavidin.
- The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.
- Unbound substances are then removed by treatment with ProCell.
- Application of voltage to the electrode then induces chemiluminiscent emission which is measured by a photomultiplier.
- Final results are determined via a calibration curve.

STATISTICAL ANALYSIS:

Mean ± SD were calculated for all the parameters and were compared by Student's t-test and correlated by calculating Pearson's correlation coefficient using SPSS calculator. P-values considered significant were as follows:

$P < 0.05$ – Significant

$P < 0.01$ – Highly Significant

RESULTS

Table 1: Comparison of serum free tri-iodothyronine (FT3) activity between controls and diabetes mellitus patients by Student's t-test.

Parameter	Control Group (n=30) Mean ± SD	Diabetic patients (n=30) Mean ± SD	p-value
Serum FT3 activity (pmol/L)	4.44 ± 0.82	3.37 ± 1.14	0.000***

P < 0.001 *** P < 0.01 ** P < 0.05 * NS = Non significant

Statistically significant differences were observed in the mean serum FT3 activity of controls (4.44 ± 0.82) and type 2 diabetic patients (3.37 ± 1.14). (p=0.000) when two tailed test was applied.

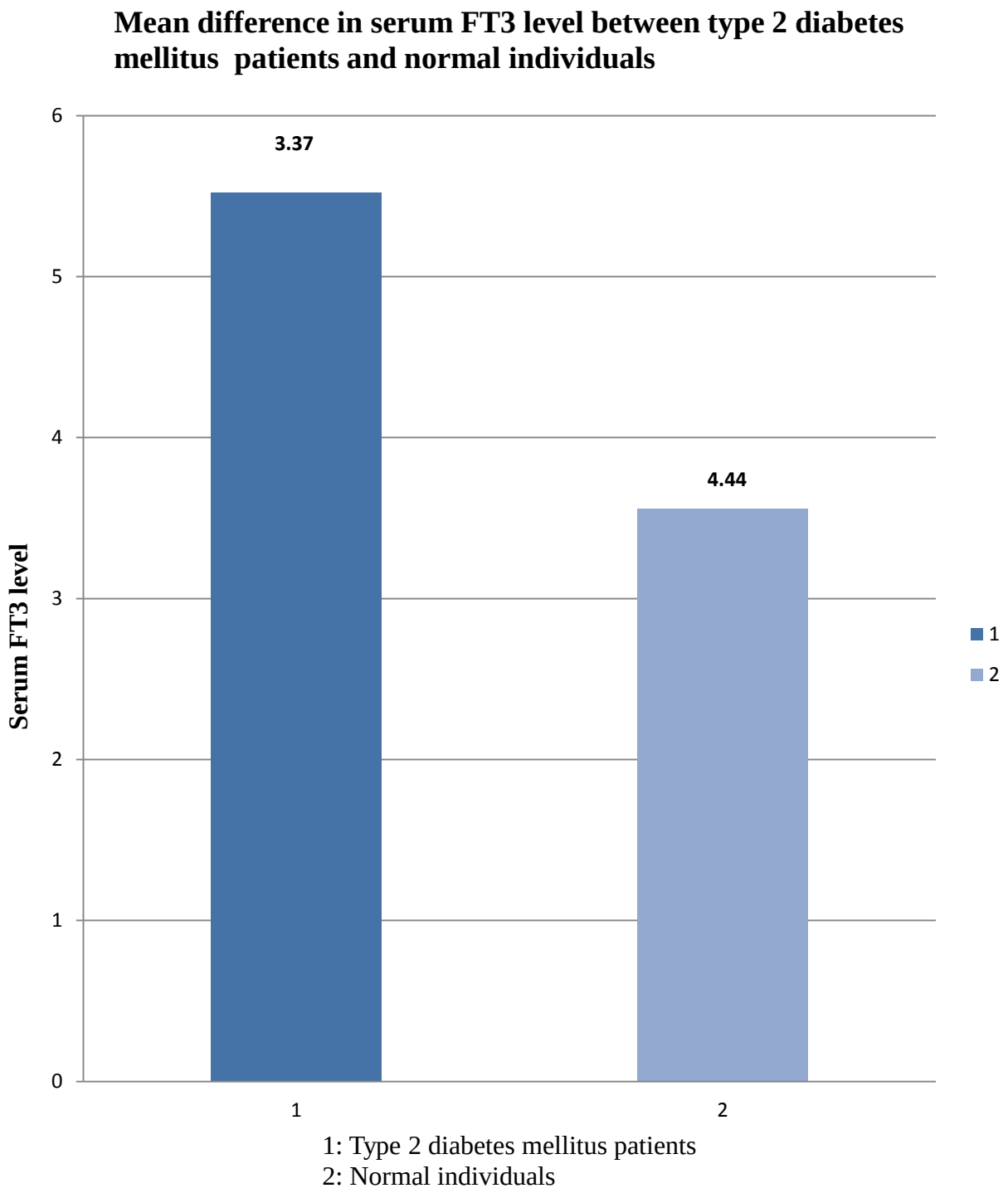

Fig 7: Figure showing mean difference in serum FT3 level between normal individuals and type 2 diabetes mellitus patients.

Table 2: Comparison of serum free tetra-iodothyronine (FT4) activity between controls and diabetes mellitus patients by Student's t-test.

Parameter	Control Group (n=30) Mean ± SD	Diabetic patients (n=30) Mean ± SD	p-value
Serum FT4 activity (ng/dL)	1.45 ± 0.39	1.1± 0.335	0.002**

P < 0.001 *** P < 0.01 ** P < 0.05 * NS = Non significant

Statistically significant differences were observed in the mean serum FT4 activity of controls (1.45 ± 0.39) and type 2 diabetic patients (1.15 ± 0.33). (p=0.002) when two tailed test was applied.

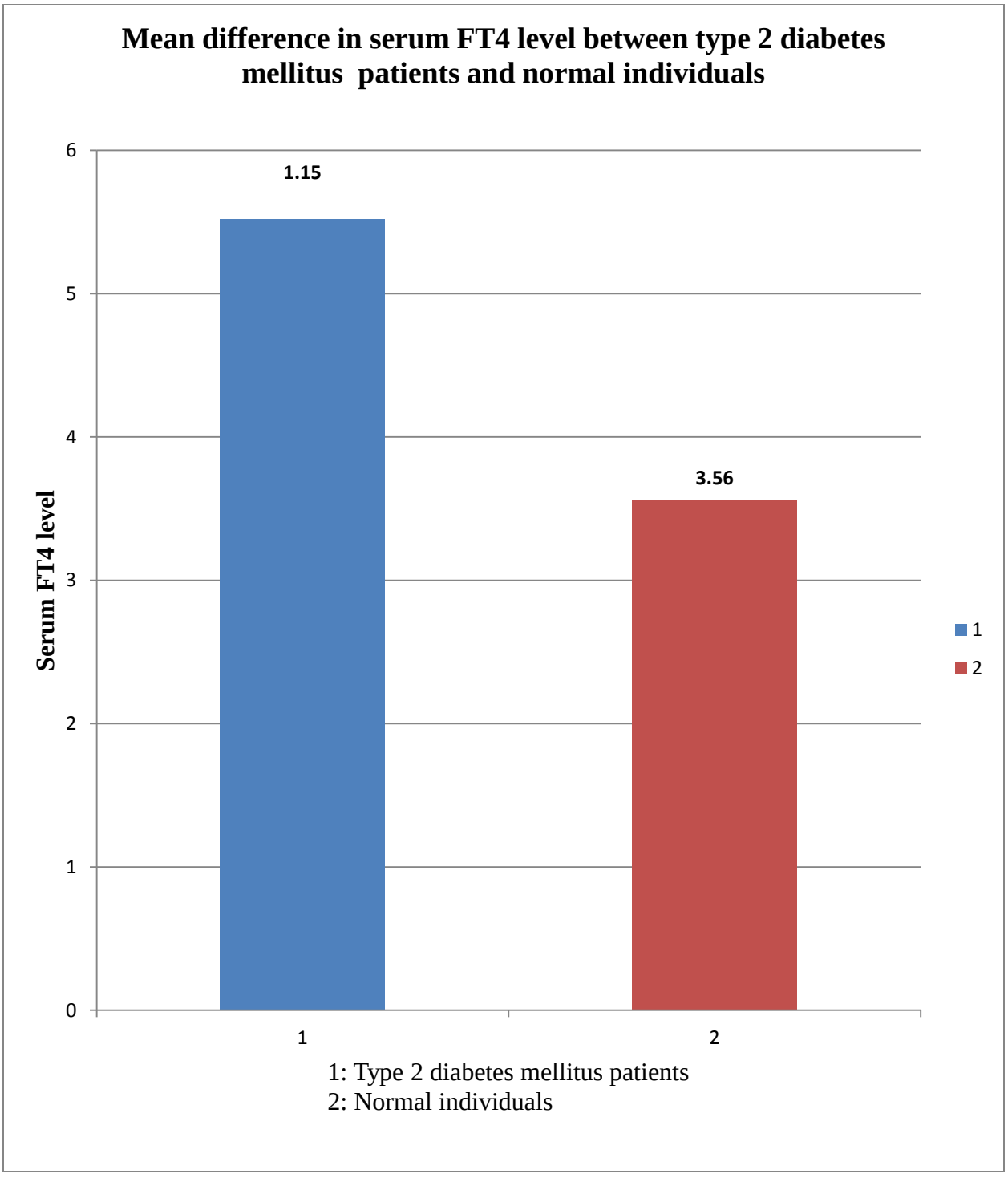

Fig 8: Figure showing mean difference in serum FT4 level between normal individuals and type 2 diabetes mellitus patients.

Table 3: Comparison of serum Thyroid Stimulating Hormone (TSH) activity between controls and diabetes mellitus patients by Student's t-test.

Parameter	Control Group (n=30) Mean ± SD	Diabetic patients (n=30) Mean ± SD	p-value
Serum TSH Activity (uIU/L)	3.56 ± 2.50	5.52 ± 2.41	0.003**

P < 0.001 *** P < 0.01 ** P < 0.05 * NS = Non significant

Statistically significant differences were observed in the mean serum TSH activity of controls (3.56 ± 2.50) and type 2 diabetic patients (5.52 ± 2.41). (p=0.003) when two tailed test was applied.

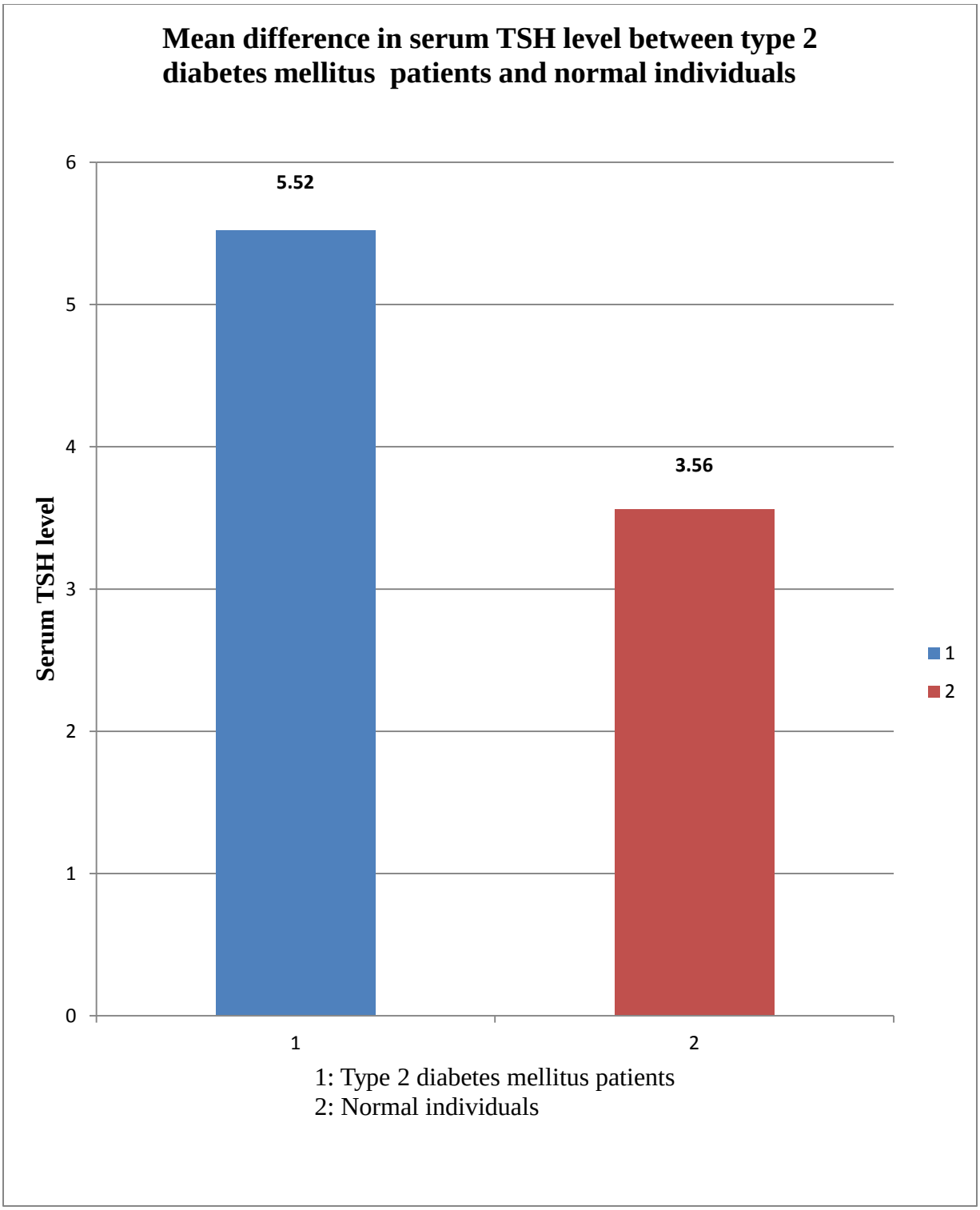

Fig 9: Figure showing mean difference in serum TSH level between normal individuals and type 2 diabetes mellitus patients.

Table 4: Tabular representation of different parameters among diabetes mellitus patients showing Pearson correlation coefficient (r) and p value.

Parameters	r-value	p-value
FT3- HbA1c	-0.508	0.004
TSH- HbA1c	0.397	0.03

After applying Pearson's correlation coefficient it was found that there is a negative correlation between serum FT3 activity and HbA1c (r = -0.508) and positive correlation between serum TSH activity and HbA1c (r = 0.397).

DISCUSSION

This study was done with the purpose to evaluate the levels of serum FT3, FT4, and TSH in type 2 diabetes mellitus patients.

Diabetes mellitus is a common health problem affecting millions of population worldwide. The root cause of diabetes mellitus is defective production or ineffective action of insulin that controls glucose, fat and amino acid metabolism (56). Adoption of a sedentary lifestyle, the consumption of non-traditional foods, and a genetic predisposition to the disease are some other factors contributing to the development of diabetes mellitus (57).

Glycosylated hemoglobin, also known as hemoglobin A1c, HbA1c, A1C or Hb1c, is a form of hemoglobin used primarily to identify the average plasma glucose concentration over a prolonged period of time (58). During the normal 120-day life span of the red blood cell (RBC), glucose molecules react with hemoglobin, forming glycated hemoglobin. Once a hemoglobin molecule is glycated, it remains in this form. Red blood cells (RBCs) that contain the hemoglobin circulate in the bloodstream for three to four months before being broken down and replaced. A buildup of glycated hemoglobin within the red blood cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life cycle. Thus, A1C readings higher than about 6% indicate higher than normal amounts of glucose roaming the blood stream in the past 120 days (59).

Thyroid gland is one of the largest endocrine gland in the body. It is a butterfly shaped organ made up of two lobes connected via the isthmus. It is situated on the anterior side of the neck and around trachea and larynx (33). Thyroid hormone is produced by the thyroid gland, which consists of follicles in which thyroid hormone is synthesized through iodination of

tyrosine residues in the glycoprotein thyroglobulin (60, 61). Thyroid stimulating hormone (TSH), secreted by the anterior pituitary in response to feedback from circulating thyroid hormone, acts directly on the TSH receptor on thyroid gland to produce thyroid hormones (T3 and T4) (62). Thyroid hormone is essential for normal development, growth, neural differentiation, and metabolic regulation in mammals (63-65).

The association between diabetes and thyroid dysfunction had been recognized since late 90's and this has emphasized the importance of screening of diabetic patients to identify thyroid diseases (66, 67). A number of the studies have reported the prevalence of thyroid dysfunction among diabetes patients to be between 2.2 to 17% (68). However, few studies have observed the very high prevalence of thyroid dysfunction in diabetes i.e. 31 % and 46.5% respectively (69). Diabetes patients have a higher prevalence of thyroid disorders than the normal population (70). Varieties of thyroid abnormalities are known to co-exist and interact with diabetes mellitus. The frequency of hyperthyroidism and hypothyroidism in patients with diabetes has varied from 3.2 % to 4.6 % and 0.7 % to 4.0 % respectively (71). In hyperthyroidism, increased levels of serum FT3 and FT4 is observed while serum TSH is significantly low. Similarly hypothyroidism is characterized by decreased level of serum FT3 and FT4 with an increase in the level of serum TSH.

This study was conducted among 30 known type 2 diabetes mellitus patients who were not under any oral hypoglycemic drugs or insulin therapy and 30 normal individuals taken as controls. The mean values of serum FT3 in our present study was found to be (3.37 ± 1.14) in type 2 diabetes mellitus patients. Similarly the mean value of serum FT3 in controls was found to be (4.44 ± 0.82). Therefore, our present study suggests significantly low level of serum FT3 activity in type 2 diabetes mellitus patients as compared to normal individuals (p=0.0000). A

study by Islam S et al (13), Singh G et al. (72) also shows level of FT3 to be significantly lower in type 2 diabetic mellitus patients when compared to normal individuals.

Mean values of serum FT4 in our present study was found to be (1.1± 0.335) in type 2 diabetes mellitus patients and (1.45 ± 0.39) in normal individuals (p=0.002).Our result suggests significantly lower level of serum FT4 activity in type 2 diabetes mellitus patients as compared to controls. This is comparable to the previous study by Singh G et al. (72) where serum FT4 level was significantly lower in type 2 diabetes mellitus patients as compared to normal individuals. However, a study by Islam S et al. (13) shows no statistically significant difference in serum FT4 level between type 2 diabetic individuals and normal individuals.

Similarly, mean values of serum TSH in our study was found to be (5.52 ± 2.41) in type 2 diabetic patients and (3.56 ± 2.50) in normal individuals (p=0.003). This statistical value suggests significantly higher level of serum TSH activity in type 2 diabetes mellitus patients as compared to controls. A study by M Anita Devi et al. (73) also showed the level of TSH to be significantly higher in diabetes mellitus patients indicating hypothyroidism in the diabetic patients which agrees to our findings. A study by Panneerselvam et al. (11) showed significantly higher level of TSH in type 2 diabetes mellitus patients than in controls. In contrast to our finding, a study by Islam S et al. (13) and Udiong CEJ et al. (74) showed no statistically significant difference between type 2 diabetics and controls.

On the other hand, Pearson's correlation coefficient of free triiodothyronine (FT3) with HbA1c showed inverse correlation (-0.508). A study by Panneerselvam et al. (11) also showed a negative correlation between FT3 and HbA1c level which indicates significantly lower level of serum FT3 in type 2 diabetes mellitus patients as compared to controls. Another study by Islam S

et al. (13) also showed significant inverse correlation between serum FT3 levels and HbA1c levels.

Similarly, in this study Thyroid stimulating hormone (TSH) showed positive correlation with HbA1c (0.397). A study by Velija-Asimi et al. (75) and Billic-Komarica et al. (76) also shows positive correlation between serum level of TSH and the level of HbA1c, which agrees to our findings.

Therefore, this study suggests high prevalence of hypothyroidism in type 2 diabetes mellitus patients as compared to normal individuals. Thus finding in our study is limited as thyroid hormones have different normal ranges between males, females, pregnant women, and non-pregnant women. Therefore, further studies needs to be carried out for the interpretation of thyroid hormone in type 2 diabetes mellitus patients.

SUMMARY

Diabetes mellitus and thyroid disorders are one of the most common endocrine disorders. Thyroid hormones and hyperglycemia are known to be related and their inter-connection is well established. Studying the levels of thyroid hormones in patients with type2 diabetes mellitus can help in the diagnosis of various thyroid disorders and can manage the complications. Aim of this study is to evaluate thyroid hormones (FT3, FT4) and TSH in type 2 diabetes mellitus patients.

This study was conducted on 30 type 2 diabetes mellitus patients who were not under insulin therapy and taking any oral hypoglycemic drugs. Random blood sample was taken from each type 2 diabetes mellitus patient and normal individuals and analyzed for random plasma glucose and HbA1c. Fasting sample from same patients were taken for the estimation of FT3, FT4, and TSH. Glucose was estimated by hexokinase method and HbA1c by boronate affinity chromatography using Nyco card reader. Thyroid hormones and TSH were estimated using Electro-chemiluminescence immunoassay (ECLIA). Statistical analysis was done by using SPSS 16.

Statistically significant differences were observed in the mean FT3 activity of controls (4.44 ± 0.82) and type 2 diabetes mellitus patients (3.37 ± 1.14) (p=0.000). Mean FT3 levels was higher in normal individuals as compared to type 2 diabetes mellitus patients. Statistically significant differences were also observed in the mean FT4 activity of controls (1.45 ± 0.39) and type 2 diabetes mellitus patients (1.1± 0.335) (p=0.002), which was also higher in normal individuals. Statistically differences in the mean TSH activity were also observed between controls (3.56 ± 2.50) and type 2 diabetes mellitus patients (5.52 ± 2.41) (p=0.003), where mean value of TSH in type 2 diabetes mellitus patients is high.

After applying Pearson's correlation coefficient it was found that there is a positive correlation between TSH and HBA1c (0.397). Therefore, this implies that value of TSH increases with an increase in the levels of HbA1c. Hence, diabetes mellitus patients have more than normal levels of Thyroid stimulating hormone as compared to controls. Similarly a negative correlation was found between FT3 and HbA1c (-0.508). Hence, the level of serum FT3 decreases with severity of diabetes mellitus.

From this study, it can be concluded that level of thyroid hormones in type 2 diabetes mellitus are deranged with an increase in TSH and decrease in FT3 and FT4. Therefore this study suggests hypothyroidism in type 2 diabetes mellitus patients.

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APPENDIX

Appendix I

LIST OF ABBREVIATIONS

WHO	World Health Organization
T2DM	Type 2 Diabetes mellitus
FT4	Free thyroxine
FT3	Free triiodothyronine
TSH	Thyroid Stimulating Hormone
GAD_{65}	Glutamic acid decarboxylase
MODY	Maturity Onset Diabetes Mellitus
TPO	Thyroid Peroxidase
DIT	Di-iodothyronine
MIT	Mono-iodothyronine

TBG	Thyroxine Binding Globulin
TBPA	Thyroxine-binding prealbumin
OGTT	Oral Glucose Tolerance Test
FPG	Fasting Blood Glucose
HbA1c	Glycosylated Hemoglobin
IDF	International Diabetes Federation
AMP	Adenosine Monophosphate
TCA	Tri-carboxylic acid cycle
DCTT	Diabetes Control and Complication Trial
MES	2-(N-morpholino) ethanesulfonic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

TRH	Thyroid hormone releasing hormone
THBI	Thyroid hormone binding inhibitor