

## RESEARCH ARTICLE

**Sub-clinical Hypothyroidism Fundamentally Associates with Type-II Diabetic Patients: A Clinical Concern to Care Eastern Nepalese People****Rajneesh Jha<sup>1\*</sup>, Shripad J. Walavalkar<sup>2</sup>, Apeksha Niraula<sup>3</sup>, Ram L. Mallick<sup>3</sup>**<sup>1</sup>*Department of Medicine, Birat Medical College and Teaching Hospital, Biratnagar, Nepal;*<sup>2</sup>*Department of Pathology, Birat Medical College and Teaching Hospital, Biratnagar, Nepal;*<sup>3</sup>*Department of Biochemistry, Birat Medical College and Teaching Hospital, Biratnagar, Nepal***Received 18 April 2017; Revised 11 May 2017; Accepted 16 May 2017****ABSTRACT**

The prevalence of thyroid disease, a general health-problem among people, increases as age advances. Hypothyroidism is the most common thyroid disorder among adults and older women. The diabetic patients have higher prevalence of thyroid disorders compared to normal population. Additionally, hyperthyroidism correlates to worsening of diabetes. Unmanaged pro-diabetics, of both types-I and-II, induce a 'low T<sub>3</sub> state' characterized by reduced serum total and free T<sub>3</sub> levels. The relation between type-2 diabetes and thyroid dysfunction has been a less explored domain. It is, therefore, regular screening for associated thyroid abnormalities in all diabetic patients is essential. This approach will allow practitioner to early treat the correlating thyroid-related problems among diabetics in locality. However, in spite of its vital clinical concern, none of the studies has reported yet about incidences of thyroid abnormalities among diabetics in eastern Nepal. We, therefore, investigated the status of thyroid abnormalities in type-II diabetic patients in this area. In present study, we observed the significant prevalence of sub-clinical hypothyroidism as 40 %, followed by overt hypothyroidism, sub-clinical hyperthyroidism and frank hyperthyroidism as 12, 8, and 6 %, respectively. Hence, our data reflects the fact to care for occurrences of thyroid abnormalities among type-II diabetic patients in locality.

**Key words:** Sub-clinical hypothyroidism, Prevalence, Eastern Nepal**INTRODUCTION:**

Thyroid disease is common in general population and prevalence of it increases as age advances<sup>[1]</sup>. Hypothyroidism is by far the most common thyroid disorder among adults and older women<sup>[3]</sup>. Approximately, 4 million people in the United States suffer from hypothyroidism. Diabetic patients have a higher prevalence of thyroid disorders compared to normal population<sup>[4, 5]</sup>. Because patients with one organ-specific autoimmune disease are at risk of developing other autoimmune disorders, and thyroid disorders are more common, it is not surprising that more the type-1 diabetic patients have thyroid disease<sup>[6, 7]</sup>. According to investigation carried out earlier by Collier and Hoggins, hyperthyroidism associates with worsening of diabetes<sup>[8]</sup>. It was shown in their study that surgical removal of the parts of thyroid glands exerts ameliorative effect on restoration of glucose tolerance in hyperthyroid patients suffering from coexisting diabetes.

Unmanaged pro-diabetics, both type-1 and type-2, can induce a 'low T<sub>3</sub> state' characterized by reduced serum total and free T<sub>3</sub> (FT<sub>3</sub>) levels. A deep underlying relation thus exists between diabetes mellitus and thyroid dysfunction. The relation between type-2 diabetes mellitus and thyroid dysfunction has been a less explored domain. It can behold the answers to various facts of metabolic syndrome<sup>[9]</sup>. Several studies have reported about higher incidence of thyroid abnormalities in type-2 diabetes, with hypothyroidism being most common<sup>[4, 10]</sup>. The presence of thyroid dysfunction may affect diabetes control. Hyperthyroidism typically associates with worsening glycemic control and increased insulin requirements<sup>[4, 11]</sup>. Indeed, thyrotoxicosis may unmask the latent diabetes<sup>[12]</sup>. In practice; several implications exist for patients with both diabetes and hyperthyroidism<sup>[13]</sup>. First, in hyperthyroid patients, the diagnosis of glucose intolerance needs to be considered

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cautiously because hyperglycemia may improve with treatment of thyrotoxicosis. Second, the underlying hyperthyroidism should be considered in diabetic patients with unexplained worsening hyperglycemia. Third, in diabetic patients with hyperthyroidism, physicians need to anticipate possible deterioration in glycemic control and adjust treatment accordingly. Restoration of euthyroidism will lower blood glucose level. It is, therefore, regular screening for associated thyroid abnormalities in all diabetic patients is essential. This approach, therefore, allows practitioner to early treat the correlating thyroid-related problems in diabetics in locality.

However, in spite of the vital clinical importance, none of the studies in eastern Nepal has reported yet about incidences of thyroid abnormalities in diabetic patients. We, therefore, investigated the status of thyroid abnormalities in type-II diabetic patients. In present study, we observed the significant prevalence of sub-clinical hypothyroidism as 40 %, followed by overt hypothyroidism, sub-clinical hyperthyroidism and frank hyperthyroidism as 8, and 6 %, respectively. Our study, thus reflects the concern to take care for occurrences of thyroid abnormalities in patients suffering from type-II diabetes mellitus in locality.

## MATERIALS AND METHODS

### Study design and enrolment criteria:

This was hospital-based retrospective study conducted in the Department of Diagnostic Laboratory at Birat Medical College and Teaching Hospital (BMCTH), Biratnagar, Morang, Nepal. This is a cross-sectional and observational descriptive study. A total of 100 type-II diabetic patients aged > 40 years. Written informed consent was taken from all patients and the study was approved by Institutional Ethical Committee. All the participants had undergone detailed history, clinical examination and laboratory investigations using Performa designed for this study. All patients were subjected to following investigations: Fasting blood sugar (FBS), Post-prandial blood sugar (PPBS), free triiodothyronine (FT<sub>3</sub>), free Tetraiodothyronine (FT<sub>4</sub>) and thyroid stimulating hormone (TSH).

### Sample collection and serum preparation:

Venipuncture was performed to collect blood samples under universal attentiveness as described in manufacturer's protocol<sup>[14]</sup>. Antecubital venous blood was collected from patients in plain vial

with informed written consent, strictly as per the norms, recommendations and approval of the Institutional Ethical Committee. Blood samples were allowed to clot for 5 min and centrifuged at 3000 rpm for 10 min in order to separation of serum. Sera were stored at - 20 °C until assessment. All steps were carried out sterile conditions and precautions were taken to prevent blood samples from hemolysis, as described earlier<sup>[14, 15]</sup>.

### Determination of glucose in serum:

The determination of glucose levels in serum was done using spectrophotometer and glucose oxidase-peroxidase assay kit by Tindler's method<sup>[16]</sup>. The unhemolyzed serum samples and normal as well as abnormal controls were used while estimation, according to the instructions provided by manufacturers.

### Estimation of FT<sub>3</sub>:

FT<sub>3</sub> immunoassay was performed using direct chemiluminescent technology (ADVIA Centaur FT<sub>3</sub> assay kit) according to instruction of the manufacturers. ADVIA Centaur XP system was employed while performing assay. System was performed after loading reagent packs (containing monoclonal mouse anti-FT<sub>3</sub> antibody). Instrumentation had automatically performed actions that include: dispensing 50 µl serum into cuvette, adding 100 µl Lite reagent (containing 0.1 % sodium azide) followed by incubation at 37 °C for 5 min, supplementing 450 µl solid phase reagent and incubating at 37 °C for 2 min 30 s. System had separated, aspirated and washed the cuvettes with reagent water. Following incubation, system had dispensed 300 µl of acid and base reagents to initiate chemiluminescent reaction. Results were obtained according to selection option as stated in system operating instructions. As on setting up of the assay, system had reported FT<sub>3</sub> results in pg/ml.

### Estimation of FT<sub>4</sub>:

FT<sub>4</sub> immunoassay was performed using direct chemiluminescent technology by FT<sub>4</sub> assay kit (from manufacturer as mentioned under estimation of FT<sub>3</sub>). With application of an analyzer (that was employed above for estimation of FT<sub>3</sub>) and after system was loaded with reagent packs (containing monoclonal mouse anti-FT<sub>4</sub> antibody), estimation based on chemiluminescent reaction was carried out as discussed. System was set to receive FT<sub>4</sub> results in ng/dl.

**Estimation of TSH:**

TSH immunoassay was performed using direct chemiluminescent technology by TSH assay kit as mentioned above and according to manufacturers. After loading an analyzer with reagent packs (containing monoclonal mouse anti-TSH antibody), experiment was performed. System dispensed itself 200 µl serum in cuvette, added 50 µl Lite reagent and 225 µl Solid Phase followed by incubation at 37 °C for 7 min 30 s. Separation, aspiration, washing of cuvettes and chemiluminescent reaction steps were in accordance with FT<sub>3</sub> and FT<sub>4</sub> assays. Assessment was fixed to acquire TSH results in mIU/L.

**Data interpretation:**

The validity and reliability of test results were determined using control sera (from *BIO-RAD*). Data were analyzed under Software Package for Social Science version 16 (SPSS 16).

**RESULTS:**

**Strategy to investigation of thyroid status in type-II diabetic patients:**

Strictly as per the rules prescribed by American Diabetic Association and the guidelines provided by WHO, all the participants had undergone through detailed history and clinical examination [17, 18]. A total of 100 patients aged > 40 years was enrolled for investigation. All the contestants were subjected to investigation for FBS and PPBS. These individuals, after showing FBS > 7.1 and PPBS > 15.2 mmol/L, were registered as type-II diabetics (1<sup>st</sup> & 2<sup>nd</sup> bars, respectively, in figures 1, 2, 3, 4 & 5) in the present study. All of them were then subjected to investigations of FT<sub>3</sub>, FT<sub>4</sub> and TSH to rule out the occurrence of thyroid abnormalities.

**Majority of Type-II Diabetics suffered from Sub-clinical Hypothyroidism:**

Sub-clinical hypothyroidism is prevalent in population without thyroid disease [19]. As thyroid abnormality can associate with diabetes [20], we collected antecubital venous blood and separated sera to determine thyroid status in these individuals. The higher serum TSH despite to normal values of thyroxin distinguishes sub-clinical hypothyroidism. Thus we carried out FT<sub>3</sub>, FT<sub>4</sub> and TSH immunoassay. As expected, control sera confirmed 1.4 - 4.2 pg/ml, 0.8 - 2.0 ng/dl and 0.4 - 6.1 mIU/L as reference ranges for FT<sub>3</sub>, FT<sub>4</sub> and TSH, respectively (data not shown). Out of 100 number of total type-II diabetic patients in

locality, major portion (*n*=40) showed FT<sub>3</sub> and FT<sub>4</sub> levels within reference limits ranging from 1.9 - 3.7 pg/ml and 0.8 - 2.0 ng/dl, respectively (Figure 1; 3<sup>rd</sup> & 4<sup>th</sup> bars). In contrast, we observed the raised TSH levels such as 6.5 - 10.1 mIU/L (Figure 1; 5<sup>th</sup> bar) in these patients; thus suggestive of significant association of sub-clinical hypothyroidism with type-II diabetics.

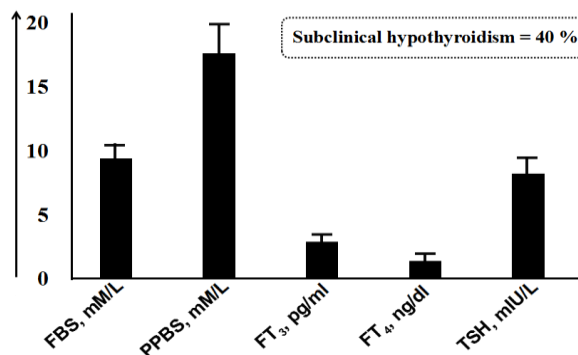


Figure 1. Bar diagram demonstrating 40 % percent distribution of type-II diabetics suffering from subclinical hypothyroidism

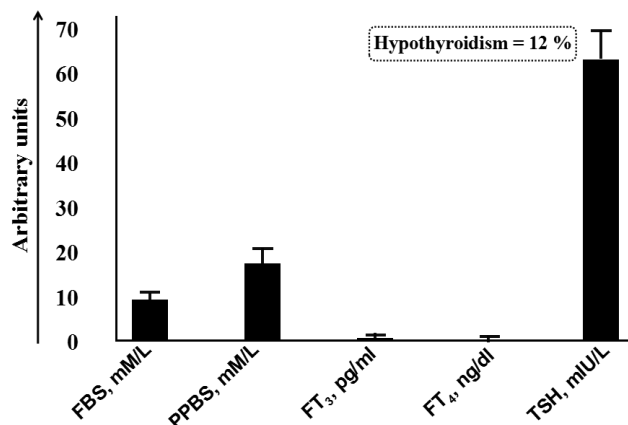


Figure 2. Bar diagram demonstrating 12 % percent distribution of type-II diabetics suffering from overt hypothyroidism

**Minor portion of type-II diabetics suffered from overt hypothyroidism:**

Hypothyroidism is a clinical syndrome related to elevate TSH and decreased FT<sub>3</sub> & FT<sub>4</sub> values [21]. As abnormality in carbohydrate metabolism is one of its underlying cause [21, 22], we subordinated the type-II diabetic patients into a group of overt hypothyroidism. In total figure of 100 diabetics (type-II) who visited the hospital, minor but significant number of patients (*n*= 12) had the reduced FT<sub>3</sub> and FT<sub>4</sub> levels as 0.7 - 1.2 pg/ml and 0.4 - 0.7 ng/dl, respectively (Figure 2; 3<sup>rd</sup> & 4<sup>th</sup> bars). In addition, these patients were having the raised TSH levels as 55.3 - 71.8 mIU/L (Figure 2; 5<sup>th</sup> bar); thus suggesting that statistically significant part of type-II diabetics suffered from overt hypothyroidism.

**Few patients with Type-II diabetes mellitus had sub-clinical hyperthyroidism:**

Sub-clinical hyperthyroidism affects both the psycho & somatic components of well-being and thereby reduces the quality of life [23]. Thus we categorized the part incidence of sub-clinical hyperthyroidism in type-II diabetics. Among 100 number of these patients, only eight individuals showed serum levels of FT<sub>3</sub> and FT<sub>4</sub> within reference range as 2.6 - 4.1 pg/ml and 1.3 - 2.0 ng/dl, respectively (Figure 3; 3<sup>rd</sup> & 4<sup>th</sup> bars). In contrast, we observed the reduced values of serum TSH like 0.1 - 0.4 mIU/L in these patients (Figure 3; 5<sup>th</sup> bar); thus indicating that occurrence of sub-clinical hyperthyroidism in type-II diabetics was little and statistically insignificant.

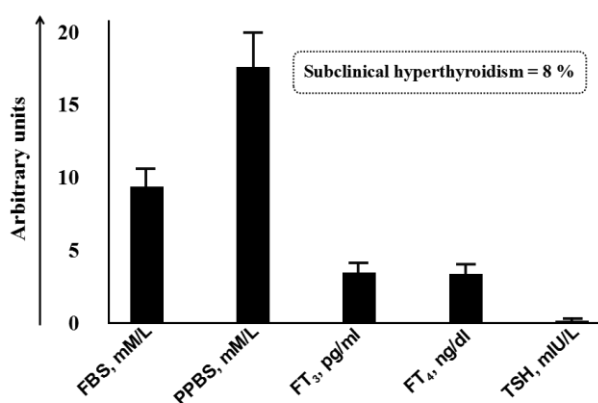


Figure 3. Bar diagram demonstrating 8 % percent distribution of type-II diabetics suffering from subclinical hyperthyroidism

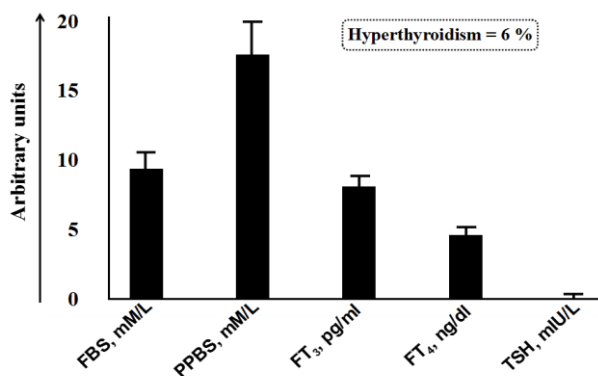


Figure 4. Bar diagram demonstrating 6 % percent distribution of type-II diabetics suffering from overt hyperthyroidism

**Minor portion of type-II diabetics were sufferers of overt hyperthyroidism:**

Hyperthyroidism is a clinical syndrome of thyrotoxicosis associated with elevated serum levels of FT<sub>3</sub> and FT<sub>4</sub> with suppression of TSH [24]. As existence of overt hyperthyroidism correlates with diabetes [24, 25], we analysed our findings to rule out the corresponding incidence. We observed that six number from 100 enrolled type-II diabetics had the increased levels of FT<sub>3</sub> & FT<sub>4</sub> as 6.2 - 10.1 pg/ml & 2.4 - 7.2 ng/dl,

respectively (Figure 4; 3<sup>rd</sup> & 4<sup>th</sup> bars). We further found that these individuals were having negligible serum TSH values ranging from 0.05 - 0.1 mIU/L (Figure 4; 5<sup>th</sup> bar); thus suggesting that very less number of type-II diabetic patients in locality had suffered from overt hyperthyroidism.

**Residual group of diabetics had normal thyroid functions:**

In a total of 100 enrolled patients, the remaining 34 individuals showed the serum levels of FT<sub>3</sub>, FT<sub>4</sub> and TSH within reference range as 1.4 - 4.2 pg/ml, 0.8 - 2.0 ng/dl and 0.4 - 0.61 mIU/L, respectively (Figure 5; 3<sup>rd</sup>, 4<sup>th</sup> & 5<sup>th</sup> bars); thus indicating that the smallest group of these people in our study had normal thyroid status in locality.

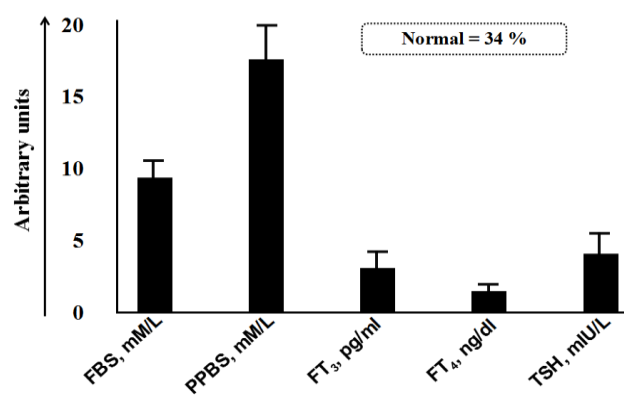


Figure 5. Bar diagram demonstrating 34 % percent distribution of type-II diabetics having no significant thyroid disorders

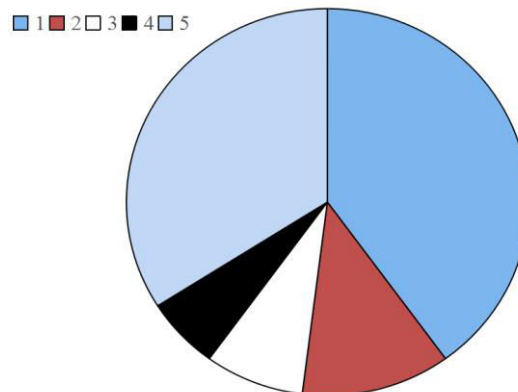


Figure 6. Pie-chart showing percent distribution of thyroid disorders (1. sub-clinical hypothyroidism, 2. Hypothyroidism, 3. Sub-clinical hyperthyroidism, 4. Hyperthyroidism & 5. Normal thyroid functions) in a total of 100 diabetic patients

**Association of sub-clinical hypothyroidism with type-II diabetes was common:**

As enrolled diabetic patients displayed both normal and abnormal status of thyroid, we compiled the whole data to analyze their distributive pattern. Following compilation, we observed that 40 % type-II diabetics suffered from sub-clinical hypothyroidism (Plot-1; Figure 6), ensued by 12, 8 & 6 % having overt hypothyroidism, sub-clinical hyperthyroidism &

hyperthyroidism (Plots 2, 3 & 4; Figure 6), respectively. Our findings, therefore, was indicative to establishment of the association of thyroid disorders (especially sub-clinical hypothyroidism) with type-II diabetes among locales. In addition, a set of numerous type-II diabetic people in locality had normal thyroid function and their group stood next to that of sub-clinical hypothyroidism.

#### DISCUSSION:

In the present study, we observed that sub-clinical hypothyroidism is predominantly significant in 40 % of type-II diabetic patients who attended the hospital in eastern part of Nepal (Figure 1). Based on the few studies carried out relatively, sub-clinical hypothyroidism was shown to be more common among thyroid abnormalities in diabetics [26, 27, 28]. Our observation is in accordance with these reports. By contrast, in eastern part of this nation, frequency of various thyroid abnormalities (such as sub-clinical hypothyroidism, hypothyroidism, sub-clinical hyperthyroidism and hyperthyroidism) was unknown. Thus we screened the local diabetic individuals to investigate existence of associated thyroid dysfunction in this area and observed preponderant significance of sub-clinical hypothyroidism (Figure 1).

Thyroid dysfunction is a common endocrine disorder with variable prevalence [29, 30]. According to the survey carried out by Wickham, prevalence of thyroid dysfunction among male adults in England was 6.6%<sup>[31]</sup>. According to Colorado prevalence study, 9.5% of participants had the elevated TSH, while 2.2% showed a low TSH<sup>[32]</sup>. According to the National Health and Nutrition Examination Survey-III (NHANES III) Study, hypothyroidism and hyperthyroidism were reported in 4.6% and 1.3% among total participants, respectively<sup>[33]</sup>. The prevalence of subclinical hypothyroidism was 4 to 8.5 percent and high as 20 percent. The prevalence of subclinical hyperthyroidism is reported to be approximately 2%. In the present study, we observed that following the highest prevalence of sub-clinical hypothyroidism, type-II diabetics were sufferers of hypothyroidism (12 %), sub-clinical hyperthyroidism (8 %) and hyperthyroidism (6 %). In contrast, 34 % local type-II diabetics show their biochemical thyroid parameters within reference range and these patients were devoid off having thyroid abnormalities. It is plausible to mean that these

local type-II diabetic patients would have the underlying cause or complications other than thyroid abnormalities.

In demarcation, we observed that the existence of sub-clinical hypothyroidism was 40 %. This striking prevalence among type-II diabetics appears to be a matter to burden in locality and thus has to be ruled out in medicine. The present hospital-based retrospective study, therefore, put forward a point of concern to essentially screen for the occurrence of thyroid abnormalities, if any.

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