HOSPITAL-BASED ANALYTICAL STUDY OF ANTI-THYROID PEROXIDASE TITRE AMONG TYPE-2 DIABETIC PATIENTS SUFFERING FROM THYROID DISORDERS

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ABSTRACT
Introduction
Thyroid hormones control growth, development and metabolic processes. Either excessive or deficient secretion of these hormones interfere with metabolism. Thyroid hormones, therefore, can alter a person’s blood sugar. Relatively, thyroid peroxidase enzyme plays a key role in thyroid hormone production. The individuals who suffer from autoimmune thyroid diseases produce auto antibodies against thyroid peroxidase (called anti-TPO).

Objectives
We aimed to investigate an association of anti-TPO titer among type-2 diabetic patients suffering from thyroid disorders attending the local hospital.

Methodology
Based on findings of thyroid variables [such as total triiodothyronine (TT₃), thyroxine (T₄) and thyroid stimulating hormone (TSH)], type-2 diabetic patients were categorized into Euthyroidism, Subclinical hypothyroidism and Hypothyroidism. In this hospital-based prospective study, venipuncture was performed to collect anti-cubital venous blood samples (n=100) from January to December, 2018. After separation of sera, estimation of certain parameters such as TT₃, T₄, TSH and anti-TPO were carried out for correlative analyses. In this aspect, based on WHO guidelines, the patients who had fasting as well as post-prandial blood sugar levels > 126 and 200 mg/dl were confirmed for diabetes mellitus. In addition, if these patients exhibited HbA₁C levels > 10.8 %, they were enrolled into the study. The patients who were hyperglycemic (with fasting as well as post-prandial blood sugar levels < 126 and 200 mg/dl) were excluded.

Results
In the three groups as stated above, we observed that more a rise in level of glucose, greater were the values of TSH and anti-TPO.

Conclusion
Such findings emphasize and put an impact in situations of thyroid abnormality among type-2 local diabetic population

KEYWORDS
Anti-TPO, thyroid stimulating hormone, total triiodothyronine, thyroxine, type-2 diabetes.
INTRODUCTION
Thyroid peroxidase (TPO) exists as a key enzyme in thyroid hormone biosynthesis. The individuals suffering from autoimmune thyroid diseases produce auto antibodies against TPO (called anti-TPO) which, therefore, are very important in diagnostic point of view as well as clinical outcomes. Anti-TPO antibodies arise against a transmembrane protein of thyrocytes involved in thyroid hormone biosynthesis. Thyroid hormones regulate several biological processes such as growth, development and metabolism. Either excessive or deficient secretion of thyroid hormones interferes with metabolism in an individual. Thyroid hormones, therefore, can alter a person’s blood sugar. So, such association increases the risk to develop diabetes and can make the situation tough to manage blood sugar if the condition is left untreated. We, therefore, aim to study about association of anti-TPO titer among type-2 diabetic patients who visited in local hospital. In present study, based on findings of thyroid variables (such as total triiodothyronine (TT3), thyroxine (T4) and thyroid stimulated hormone (TSH), type-2 diabetic patients were categorized into three states to suffer from Euthyroidism, Subclinical hypothyroidism and Hypothyroidism. In spite of well known fact behind correlation of diabetes with thyroid abnormality, the association of glucose levels with TSH and anti-TPO in Euthyroidism, Subclinical Hypothyroidism and Hypothyroidism states still remained obscure. Our study, therefore, aims to emphasize and put an impact in situations of thyroid abnormality among type-2 local diabetic population.

METHODOLOGY
Study design and enrolment criteria:
This was a hospital-based prospective study carried out in the Departments of Internal Medicine and Diagnostic Laboratory at Birat Medical College Teaching Hospital (BMCTH) Biratnagar, Morang, Nepal. In addition to estimation of fasting as well as post-prandial blood glucose levels and HbA1C to rule out that participants had suffered from diabetes mellitus, we analyzed the circulating levels of total triiodothyronine (TT3), thyroxine (T4) and thyroid stimulating hormone (TSH) as well as anti-TPO at a duration between January and December, 2018. Repetition of a similar participant was excluded. The inclusion criteria were made in accordance with the guidelines for assessment of thyroid function tests, as described earlier. The patients who had fasting as well as post-prandial blood sugar levels > 126 and 200 mg/dl were confirmed for diabetes mellitus. In addition, if these patients exhibited HbA1C levels > 10.8 % were enrolled in this study. The patients who were hyperglycemic (with fasting as well as post-prandial blood sugar levels < 126 and 200 mg/dl) had been excluded.

Sample collection and serum preparation:
Venipuncture was performed to collect blood samples (n=100) under universal attentiveness as described previously. After having informed written consents from patients, antecubital venous blood samples were collected strictly as per the norms and approval of the Institutional Ethical Committee. Blood samples were allowed to clot for five minutes and centrifuged at 3000 rpm for 15 min to separate serum. Diabetic patients suffering from hypothyroidism were the study population in present study. In our study duration, approximately 1000 number of diabetic patients had visited to the hospital. Among these, around 400 diabetics had met the inclusion criteria (related to fasting as well post-prandial blood sugar levels and HbA1C parameters) as mentioned above. Out of these 400 attendees, 100 patients were found to have thyroid disorders as well and so they were our study group.

Determination of serum blood sugar at Fasting (FBS) and Post-prandial (PPBS) states:
The circulating levels of blood sugars were analysed using spectrophotometer during fasting and post-prandial states. A known 100 mg/dl of glucose standard was used on regular basis in each lot of blood sugar estimation.

Determination of HbA1C using Dry-chemistry immunoassay analyzer:
Glycated haemoglobin (HbA1C) was analyzed using a Fully-automated Dry-chemistry Immunoassay Analyzer Prior to performing the test, a standard curve was constructed using a set of calibrators (provided from manufacturer). As stated, 10 µl whole blood from EDTA vial was hemolyzed with adding 200 µl of hemolysing solution (provided with the kit) and instrument employed 50 µl hemolyzed serum for every assessment and results were then displayed after 10 minutes of incubation time. Based on standards set by precision of an instrument, the reference range of HbA1C was 4.7 %.

Determination of TT3, T4 and TSH using Dry-chemistry immunoassay analyzer:
TT3, T4 and TSH were analyzed using a fully-automated dry-chemistry immunoassay analyzer Prior to performing the test, a standard curve was constructed using a set of calibrators (provided from manufacturer). As stated, the instrument employed 50 µl serum for every assessment and results were then displayed after 10 minutes of incubation time. Based on standards set by precision of an instrument, the reference range of TT3, T4 and TSH are 0.79-1.59 ng/ml, 4.9-11 µg/dl and 0.38-4.31 mIU/ml, respectively.

Determination of serum levels of anti-TPO by Enzyme-linked immunosorbant assay (ELISA):
Anti-thyroperoxidase (Anti-TPO) was assayed using human sandwich ELISA method, as described earlier. The reference range for anti-TPO was <40 IU/ml as described in manufacturer’s protocol.

Determination of serum levels of an-TPO:
An-thyroperoxidase (An-TPO) was assayed using human linked immunosorbant assay (ELISA): Determination of serum levels of an-TPO by Enzyme-linked immunosorbant assay (ELISA):
An-thyroperoxidase (An-TPO) was assayed using human linked immunosorbant assay (ELISA):
Data interpretation:
The validity and consistency of test results were determined using standards supplied with a kit. The Data that had been both continuous and categorical were presented as mean±SD, analyzed under Software Package for Social Sciences version 18 (SPSS 18).

RESULTS
Following collection of antecubital venous blood, we separated sera and evaluated serum levels of glucose in fasting (FBS) and post-prandial (PPBS) states along with HbA1C. A person having FBS, PPBS and HbA1C more than 140 mg/dl, 250 mg/dl and 10 %, respectively, were registered in the present study. In a total of 110 participants, mean value for FBS, PPBS and HbA1C were 146±1.5 mg/dl, 273±3.4 mg/dl and 11.2±0.4 %, respectively; indicating that these subjects had been the confirmed suffers of type-2 diabetes mellitus (Figure-1).

We observed that variables of thyroid function tests (such as TT3, TT4 and TSH) varied in these diabetics. So, based on values of TT3, TT4 and TSH, they were separated into three states as 'Euthyroidism', 'Subclinical Hypothyroidism' and 'Hypothyroidism' which constituted 20, 12 and 68 number of patients, respectively. Our data, therefore, indicated that 68 % of type-2 diabetics in locality suffered from hypothyroidism (Figure-2).

Under physiological circumstances, an enzyme called 'peroxidase' plays a role in production of thyroid hormones. In pathologic events of thyroiditis, thyroid gland produces antibodies against peroxidase which the human biologists term as 'anti-thyroidperoxidase' or 'anti-TPO'. So, we assessed anti-TPO in type-2 diabetics who had 'Euthyroidism', 'Subclinical Hypothyroidism' and 'Hypothyroidism'.

In Euthyroidism, the circulating levels of TT3, TT4, and TSH were 1.24±0.21 ng/ml, 6.4±1.2 µg/dl and 4.81±0.71 µIU/ml. However, TSH level among participants in this group remained within reference interval (Figure-3). In next set of subclinical hypothyroidism, the numerals of TT3, TT4 and TSH were in an increasing order with only a rise of TSH. Interestingly, anti-TPO was found to be elevated more than those under a set of Euthyroidism (Figure-4).

Figure-4. Bar diagram showing concentration of thyroid variables with anti-TPO status in serum of type-II diabetics who suffered from subclinical hypothyroidism.
Figure-5 depicts for the values of thyroid variables including anti-TPO in a majority of people who suffered from hypothyroidism. Here, the sufferers had negligible presence of both TT and T3, but the extent of elevation of TSH was up to 50 µIU/ml. In contrast, these participants were having anti-TPO more than 350 IU/ml.

The occurrence of type-2 diabetes positively associates with thyroid disorders. However, statistical correlation of diabetes with the above mentioned three states of thyroid status still remains obscure. We, therefore, analyzed our data for their probability of correlation. On comparing certain variables such as FBS, PPBS, TSH and anti-TPO in the above mentioned thyroid states, the pattern of elevation was gradually increasing. Our data therefore were suggestive of the fact that more a rise in level of glucose (both FBS & PPBS) greater will be the values of TSH as well as anti-TPO (Figure-6).

DISCUSSION

In spite of having association between type-2 diabetes and thyroid abnormality, clinicians still use anti-TPO to lesser extent for diagnostic purpose and clinical outcomes. Researchers have recently reported that anti-TPO positively correlate with autoimmune disorders related to thyroïditis. Based on certain studies, one can use anti-TPO as predictor of thyroid abnormality in relation to autoimmune disorders. So evaluation of anti-TPO can play a significant role in determining appropriate diagnosis and clinical outcomes.

Nowadays clinicians in our locality commonly advise TT, T3 and TSH in assessing the thyroid function test (TFT) of patients but still many of them don’t have habit of investigating the underlying cause of thyroid abnormalities(such Hashimoto’s thyroiditis which is prevalent). So advising anti-TPO titers in this group of patients becomes useful as it is not just for diagnosis but it also influences future prognosis of patients of autoimmune disorders.

In thyroid abnormality, researchers have shown the highest correlation of blood sugar levels with it. Based on certain studies, a clinician can use either high or low TSH value in prediction of thyroid abnormality, but it does not appear as accurate predictor as scientists have not shown a detailed analysis of it in accordance with autoimmune disorders of thyroid glands.

Based on few studies, combination of TSH and anti-TPO can be employed to predict thyroid abnormality in type-2 diabetics. The clinical usefulness of anti-TPO has been confirmed earlier in numerous studies.

Previous studies which have shown the prevalence of thyroid autoantibodies in diabetic patients reported that they had a high frequency of autoimmune thyroiditis, but these studies also demonstrated a very wide range of prevalence. Lee et al. also evaluated the prevalence of autonimmune thyroiditis in 139 diabetic patients and 38.8% of these patients had at least one of the thyroid autoantibodies. A similar study reported that thyroid autoantibodies positivity was detected in 26% of diabetics at the initial diagnosis. In the general population, the rate of autoimmune thyroiditis ranges from 0.1% to 2%, but its prevalence in diabetic adolescents is higher than in the nondiabetic general population of the same age group. Moreover, diabetic persons have a 24-fold risk of developing thyroid disease in a nationwide cohort study. Hence, the American Diabetic Association recommend the screening of thyroid function and autoantibodies at the initial diagnosis, and the regular screening of thyroid function even in asymptomatic patients.

Many studies have demonstrated the duration of disease, severity and the presence of beta cell autoimmunity are associated with autoimmune thyroiditis in diabetic patients. Our study results in similar ways have shown on the relevance of measuring anti-TPO titres in diabetic individuals with thyroid abnormalities. We have found the increasing anti-TPO titres in diabetics with higher blood sugar levels, also the titres were found to be statistically significant in patients with hypothyroidism as compared to euthyroid and subclinical hypothyroid states.

To the best of our knowledge, this is first study to report on correlation between blood sugar levels and TSH as well anti-TPO concentrations in eastern Nepal. In present study, our data support clinically the valuable meaning of anti-TPO as we can consider it along with TSH as useful marker for thyroid abnormality in the diabetic patients suffering from thyroid disorders.

RECOMMENDATIONS

There have been few studies to demonstrate correlative analysis of anti-TPO in a selected population of type-2 local diabetics. The strength of our study is that we comparatively investigated correlation of anti-TPO in the thyroid states as mentioned above. It would be more imperative if we in addition had included a group of hyperthyroidism in present study.

CONCLUSION

A significant positive correlation exists between anti-TPO and thyroid variables. In addition, we found remarkable correlation between anti-TPO & increasing levels of blood glucose at fasting as well as post-prandial states. These findings, therefore, suggest that a clinician could use anti-TPO as marker in type-2 diabetics who will have a probability for existence of hypothyroidism.

LIMITATIONS OF THE STUDY

The limitation could be as we just evaluated correlation of anti-TPO among type-2 diabetics suffered from hypothyroidism. In the present study, we found a lot of diabetic patients suffering from hypothyroidism. Relatively the cases of hyperthyroidism were negligible. So we omitted to include the cases of hyperthyroidism. Further if the investigations combined with our findings performed in the cases of hyperthyroidism as well, it will insight extensively to the whole of thyroid disorders.

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Conflict of Interest

The authors declare no conflict of interest.

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