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ORIGINAL RESEARCH

Higher anti-TPO antibody titers are associated with greater thyroid-related symptomatology

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ABSTRACT

The thyroid peroxidase (TPO) is a 105 kDa glycoprotein enzyme and the main antigen of the thyroid microsomal fraction. It catalyses iodine oxidation and thyroglobulin tyrosyl iodination reactions in the thyroid gland. Anti-TPO antibodies activate complement and are thought to be significantly involved in thyroid dysfunction and the pathogenesis of hypothyroidism. These antibodies are significantly present in patientsof Hashimoto's thyroiditis,Gravesdisease and even in non-thyroidal conditions such as diabetes. In sub-clinical hypothyroidism (SCH), presence of these antibodies is associated with increased risk of developing overt hypothyroidism (OH). In the present study, anti-TPO antibodies were tested on 33 individuals, all of whom reported thyroid stimulating hormone (TSH) levels > 6μIU/mL. These included 18 with SCH and 15 with OH.Anti-TPO ab positivity (levels > 28.4 IU/ml) was observed in 17 patients (51.5%) which included 5 with SCH and 12 with OH. There was greater prevalence of positivity in OH compared to SCH (80% vs 27.8%). 8 of 33 subjects (24%) had significantly raised anti-TPO ab (>200 IU/ml). The Billewicz scoring system was used for assessment of clinical features of hypothyroidism with value ≥25 strongly suggestive of OH. 9 of 33 subjects had scores ≥25 and out of these, 7 had positive anti-TPO ab.Pearson correlation revealed the serum TSH and anti-TPO levels to be strongly positively correlated, r(32) = .7902, p <0.0001.The Billewicz diagnostic score was also correlated with anti-TPO levels, r(32) = .4107 which was significant at p<0.05.Our results show that higher anti-TPO ab and TSHlevels are associated with higher symptom scores, indicating underlying pathophysiological and immunological processes and we suggest that antibodies against TPO should be routinely assessed in patients presenting with either elevated TSH levels or with symptoms suggestive of thyroid dysfunction.

Key Words:- Thyroid Peroxidase, Anti-TPO antibodies, Hypothyroidism, subclinical hypothyroidism, Billewicz scores, Thyroid stimulating hormone

Introduction

Thyroid hypofunction, typically known as hypothyroidism, is a common thyroid disorder, which may result in serious symptoms if ignored or inadequately treated. The course of symptoms mimics several other disorders as symptoms are mostly non specific and shared with several other diseases. Nonetheless, classic symptomatology of a low metabolic state, such as lethargy, weight gain and cold intolerance is often seen, often accompanied by several other symptoms. Treatment with levothyroxine often restores thyroid function. The large variations observed in symptomatology have played a role in increasing reliability on biochemical testing for thyroid stimulating hormone (TSH). Hypothyroidism has often a strong autoimmune component and the prevalence is thus often high in other autoimmune diseases. In areas with sufficient iodine in the diet, Hashimoto's thyroiditis is the commonest cause of hypothyroidism, with patients demonstrating high levels of anti-thyroid antibodies. Patients with elevated antibody levels early in the course of the disease have increased risk of progression to overt hypothyroidism. It has also been observed that 11% of the general population has raised thyroid peroxidase antibodies (Anti-TPO). [1]AA cross-sectional, multi-centre, epidemiological study of 8 indian studies showed a hypothyroidism prevalence of 10.95% and a sub-clinical hypothyroidism (SCH)prevalence of 8.02 %. [2]

The putative underlying mechanism of development of SCH and overt hypothyroidism (OH) involves a combination of genetic predisposition, environmental triggers, immune infiltration by T helper type 1 (TH1) cells resulting in apoptotic destruction of thyroid cells eventually leadingto dysfunction.[3]

Thyroid peroxidase (TPO) is a 105kDa glycoprotein enzyme also known as thyroid microsomal antigen that catalyzes iodine oxidation and tyrosine residues iodination.

Antibodies to TPO (mostly IgG) are the most common of all thyroid auto-antibodies and are better predictors of dysfunction than anti-thyroglobulin antibodies.Apart from a diagnostic role, Anti-TPO antibodies are involved in the pathogenesis of hypothyroidism as well. They fix complement resulting in possible damage to the thyroid gland. In vitro studies have shown that anti-TPO antibodies inhibit activity of TPO.[4]

They are the serological hallmark of auto-immune thyroid disease (AITD) and are used to monitor disease progression as well as response to treatment. High levels of these anti-bodies are seen in Hashimoto's thyroiditis, Graves disease, Diabetes Mellitus. However, routine antibodies are checked only after abnormal TSH, thyroxine (T4) results are obtained. It is well known that appearance of anti-TPO antibodies may predate abnormal thyroid function tests and subsequent development of hypothyroidism/hyperthyroidism. Thus, early measurement of anti-TPO may be beneficial so that early treatment decisions are taken to potentially protect against cardiovascular ill-effects.[5]Studies have shown antibodies against thyroid may be present in 15% of euthyroid subjects. One study reported a prevalence of 21.85% in a population containing both euthyroid and dysthyroid subjects. For the prediction of development of OH, Anti-TPO Ab in combination with TSH are used. In the earlier stages of thyroid disease, Anti-TPO levels maybe elevated even before any changes are seen in the routine thyroid panel (T3,T4 and TSH). In such patients who show raised anti-TPO levels, routine follow ups are required so that disease treatment is initiated at an early stage. [6][2]In cases with mildly elevated TSH, TPO-Ab titers maybe strong prognostic predictors. In such cases, normal TPO-Ab titers maybe associated with spontaneous disease remission while elevated titers may be associated with progression to OH.[7]

Materials and methods

The study, conducted in the month of February 2016, in the endocrine laboratory of the department of biochemistry, of MMIMSR, Ambala, Haryana, India, included patients who had been advised thyroid testing by their physicianswith any of the complaints of easy fatigability, nonspecific muscle/joint aches, depression, constipation, cold intolerance, excessive sleepiness. All patients' TSH records were retrieved. A cut-off level of TSH of 6 µIU/mL was used for selection of patients in the study. The included patients had no prior history or awareness of having any thyroid disorder/abnormal thyroid test and all were newly diagnosed cases of SCH/OH.Samples of all patients with TSH levels above the cut-off range were retrieved from the laboratory sample storage and retested for anti-TPO levels.The normal reference range of Anti-TPOab is 6.8-28.4 IU/ml.Anti-TPO Measurements were performed on the MonobindAutoplex™ ELISA+ CLIA workstation utilizing Enzyme linked immunosorbent assay (ELISA) method. The Autoplex™ has an internal ELISA optical reader. The procedure involves adding of controls and samples to microplate wells followed by addition of biotinylated thyroid Peroxidase antigen. A complex forms between the TPO antibodies present in the specimen and the biotinylated antigen which remains attached to the wells after successive decantation and washing steps. Addition of anti-human IgG conjugate enzyme followed by its substrate results in a spectrophotometrically quantifiable reaction which, after construction of the standard curve using several calibrators of known values, helps in arriving at the value of anti-TPO in the unknown sample.

Tri-iodothyronine (T3) and TSH were also performed on the MonobindAutoplex™ ELISA+ CLIA workstation but utilized similar variations of a chemiluminescence method. T4 utilised a Competitive Enzyme Immunoassay method on the same workstation.

The expected normal adult range for T3, T4 and TSH as per manufacturer was 0.52-1.98 ng/ml, 4.3-11.9 µg/dl and 0.4-6.02 µIU/mL respectively.

For assessment of hypothyroidism,the Billewicz diagnostic index was used which utilizes a scoring system based on the following symptoms and signs. (**Table 1)**A score of +25 or more is strongly suggestive of hypothyroidism while the maximum score which can be reached is +67.[8]

Symptom/Sign	Present	Absent
Reduced sweating	+6	-2
Cold intolerance	+4	-5
Dry Skin	+3	-6
Weight increase	+1	-1
Constipation	+2	-1
Hoarseness	+5	-4
Deafness	+2	0
Slow movements	+11	-3
Coarse skin	+7	-7
Cold skin	+3	-2
Periorbital puffiness	+4	-6
Pulse rate	+4	-4
Ankle jerk	+15	-6

Table 1-Billewicz scoring system for detection of hypothyroidism.

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Statistical analysis

Results were analysedutilising Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA) version 23.0. Results of the various parameters were expressed as mean values with standard deviation in the case of continuous variables while categorical variables were illustrated as frequencies and percentages.Karl Pearson's correlation coefficient was utilised to determine the correlation of TSH with anti-TPO and that of anti-TPO with the Billewicz diagnostic scores. A P-value less than 0.05 was used for determining statistical significance.

Results

A total of 236 patients suffering from varying numbers of the non-specific symptoms mentioned previously were initially assessed for TSH levels. Of these, it was discovered that 33 (13.98%) had TSH levels> 6 µIU/mL with mean values of 52.83 ± 75.82 µIU/mL. Of these, 18 had SCH (High TSH with normal T3,T4) while 15 had overt hypothyroidism (raised TSH and low T3,T4). In 17 (51.5%) of the 33 patients, anti-TPO levels were above the reference range (>28.4 IU/ml),with a range of 3.18 IU/ml (lowest) to 416 IU/ml (highest) and mean value of 94.61 ± 111.87 IU/ml.**(Figure 1)**These included 5 cases with SCH and 12 cases with overt hypothyroidism.Thus, while 80% of hypothyroid subjects had a positive (i.e>28.4 IU/ml) anti-TPO Ab level, only 27.8% of those with SCH had raised levels. (**Figure 2**)8 out of 33 (24%) had levels above 200 IU/ml.Assessment of symptoms and signs as per the Billewicz diagnostic scoring revealed that 9 of 33 had values ≥25 which supported a strong confirmation for the diagnosis of hypothyroidism. Pertinent to mention that among these 9 patients, all but 2 had anti-TPO ab levels in the positive range.**(Figure 3)**Pearson correlation revealed the serum TSH and anti-TPO levels to be strongly positively correlated, $r(32) = .7902$, $p < 0.0001$.(**Figure 4**) The Billewicz diagnostic score was also correlated with anti-TPO levels, r(32) = .4107 which was significant at p<0.05.**(Figure 5)**

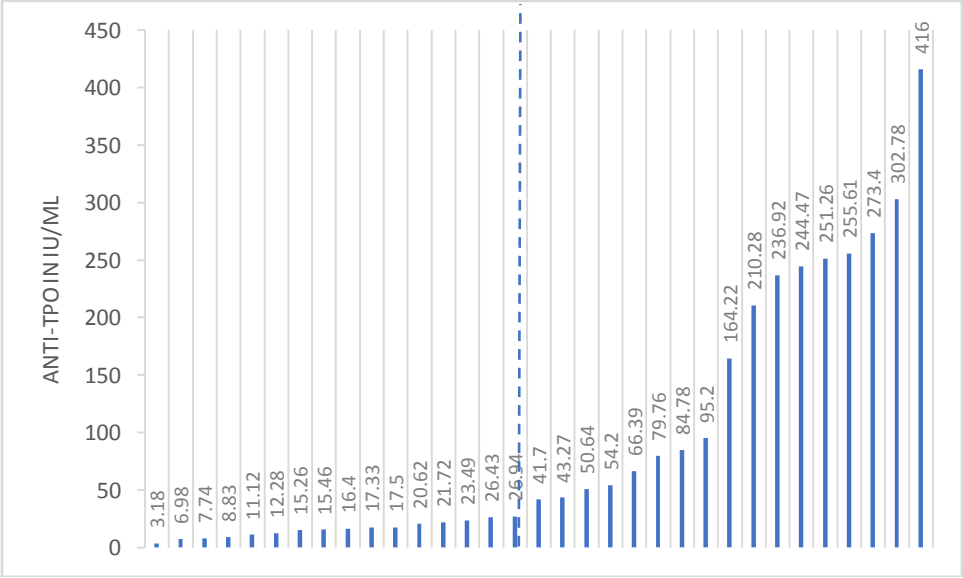

Figure 1- Individual serum anti-TPO values of all the patients with TSH >6 µIU/mL. The dotted bar shows the upper limit of normal level of serum anti-TPO.

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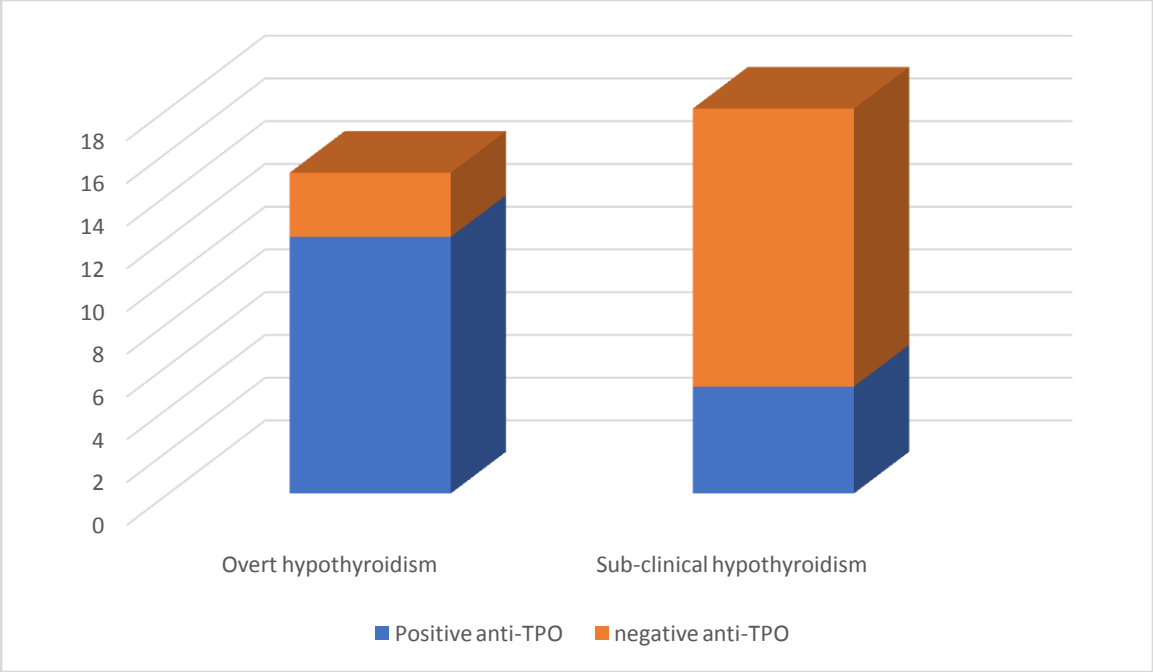

Figure 2 - Relative distribution of raised anti-TPO ab levels among overt and subclinical hypothyroidism subjects.

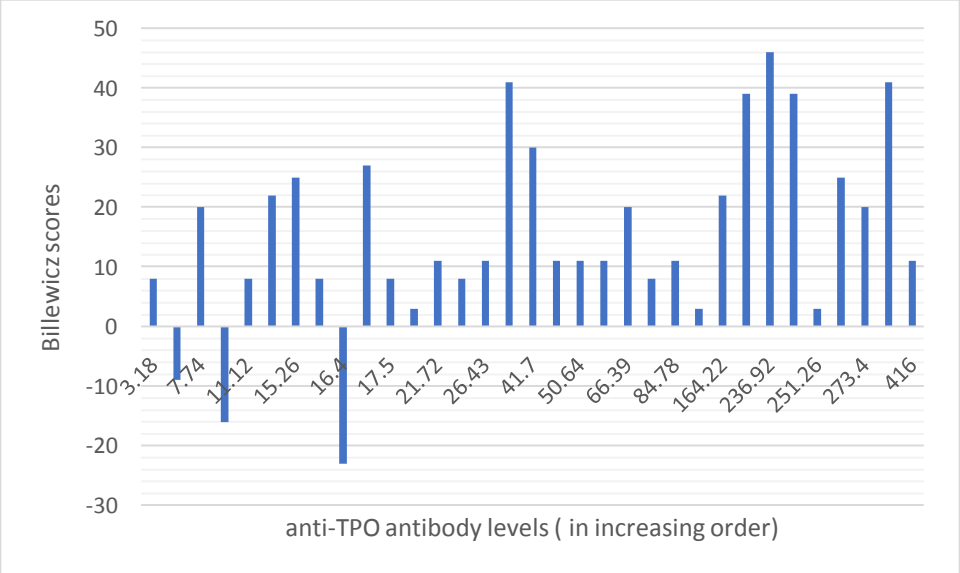

Figure 3- Billewicz scores of patients arranged in increasing order of TPO ab values

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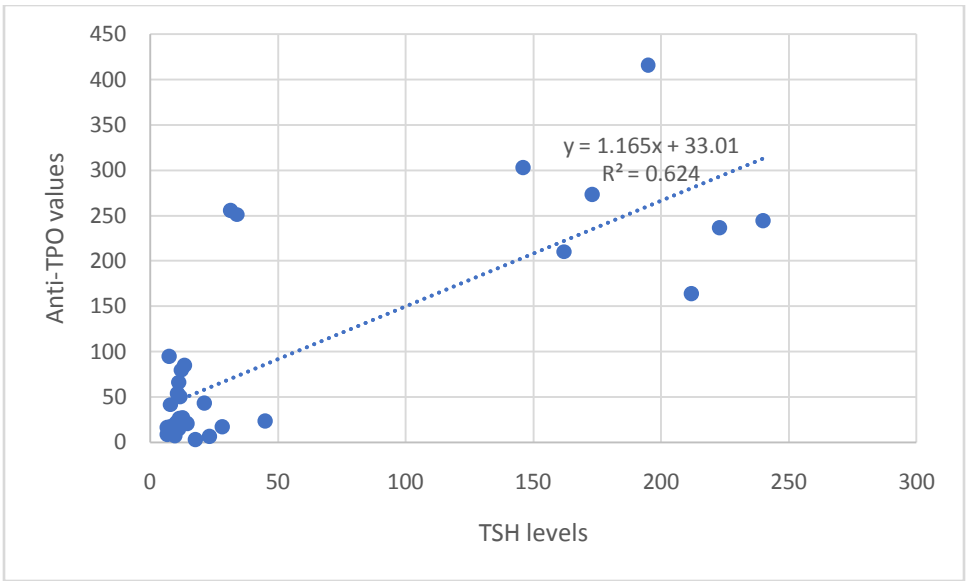

Figure 4 - Scatter plot showing TSH and anti-TPO values of patients with TSH > 6 µIU/mL.

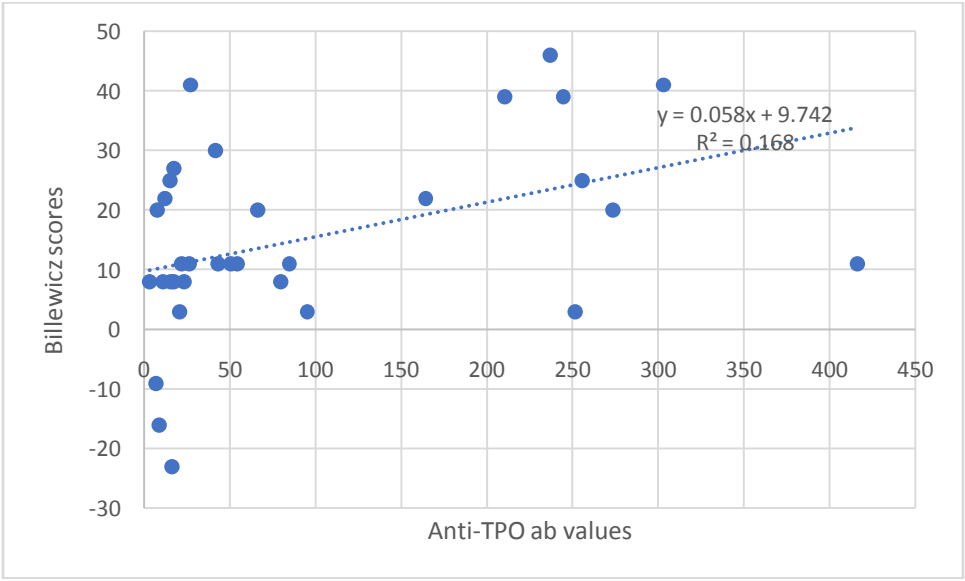

Figure 5 – Scatter plot showing Billewicz scores and anti-TPO values of patients with TSH > 6 µIU/mL.

Discussion

That, more than half of patients with raised TSH levels had a positive anti-TPO value established the fact that autoimmune thyroid disease is a predominant contributor to thyroid hypofunction and that raised antibody levels against thyroid peroxidase are significantly prevalent in those with a deranged thyroid profile. In our study, an overwhelming number of OH subjects had a positive anti-TPO value, while the number was lesser in SCH subjects. These findings maybe compared with those of a Bangladeshi study wherein 45% of SCH patients were positive for multiple anti-thyroid ab, of which 16.7% had exclusive ab against TPO only while 23.3% had Ab against both TPO as well as thyroglobulin.[9] Another study also pointed out that thyroid tests

are often deranged significantly in antibody-positive group.[10] Deranged TSH itself also increases the probability that anti-TPO antibody levels will be raised.[6] Our findings also revealed that presence of observable clinical signs and symptoms was associated with anti-TPO positivity. An earlier study also reported increased occurrence of typical symptoms characteristic of hypothyroidism in antibody-positive postnatal females even if they were euthyroid on biochemical testing compared to anti-TPO ab negative controls. [11] The observation of a significant correlation of anti-TPO with TSH levels in our study was seen earlier in different studies.[10] An Iranian study reported that each unit increase in TSH was associated with a 1% increase in TPO-Ab positivity. [12] Another Iranian study reported that anti-TPO ab levels in OH were, on the whole significantly higher than those seen in SCH. It also reported a positive association between both hypothyroidism as well as hyperthyroidism and positive TPO ab levels. Raised anti-TPO antibodies maybe correlated with pathological evidence of lymphocytic infiltration and immune mediated damage. [13] Earlier studies have also shown that even in the absence of clinical or biochemical evidence of thyroid dysfunction, positive thyroid antibody titers maybe indicative of focal lymphocytic infiltrations with higher titers pointing to correspondingly higher degrees of inflammation. [14]

Conclusion

In more than half of patients with thyroid hypofunction, we observed TPO positivity which was directly correlated with TSH levels. Generally, antibody levels were higher in OH compared to SCH. Our results strongly indicate that patients with higher anti-TPO ab and TSH levels generally have higher symptom scores as well , possibly due to underlying pathophysiologicaland immunological causes and we suggest that anti-TPO levels should be routinely assessed in patients either presenting with raised TSH or with symptoms suggestive of thyroid dysfunction.

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Conflicts of Interests: None

Authors' Contribution: Tousief Irshad Ahmed (First Author)-70% Introduction, discussion, performance of diagnostic tests and conclusion.SummaiyaIrshad (Second author)- 30%- statistics and results.

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