

Prevalence of Antithyroid Antibodies in Newly Diagnosed Subclinical Hypothyroidism in Relation to Type 2 Diabetes

K. G. Sunitha¹, Padmini Ekambaram²

¹Research and Development Centre, Bharathiar University, 641046, Tamilnadu, India

²P. G. Department of Biochemistry, Bharathi Women's College,
Affiliated to University of Madras, Chennai-600108, Tamilnadu, India

Abstract: Autoimmune thyroid disorders are the commonest form of thyroid disease. The presence of autoantibodies against thyroid antigens mainly thyroid peroxidase (anti-TPO) and thyroglobulin (anti-Tg) are the hallmarks for autoimmune thyroid disease. Thyroid disorders and diabetes mutually influence each other but the role of type 2 diabetes in the development of autoimmune thyroid disease is not well studied. The study was designed to investigate whether diabetes had any role in triggering the occurrence of thyroid autoantibodies. Newly diagnosed Subclinical hypothyroid patients were categorized in relation to diabetes and their antithyroid antibodies were studied, the results revealed that diabetes was not a prime factor in the development of autoimmune thyroid disease. Moreover, the prevalence of autoimmune thyroid disease were more in female compared to male like other autoimmune disease, and anti-TPO were more common compared to anti-Tg in both male and female patients with or without thyroid disorder irrespective of being diabetic or normal. The present work can be extended to a larger population to gain a better understanding of this concept.

Keywords: autoimmune thyroid disease, thyroid peroxidase, thyroglobulin, antithyroid antibodies, diabetes Mellitus

1. Introduction

Thyroid hormones (TH) are key regulators of metabolism and development and are known to have multiple effects on many different organs. Thyroid dysfunction causes a wide spectrum of clinical symptoms, depending on onset and severity of TH deficiency or TH excess. Diseases of thyroid gland are amongst the most abundant endocrine disorder in the world after diabetes mellitus (1). According to various reports, thyroid burden in India is estimated to be about 42 million (2). Thyroid disease like other endocrine disorders presents with symptoms due to either underproduction of hormone or excess hormonal activity with a swelling, due to a neoplastic process or due to the pressure effects on surrounding structures. Hypothyroidism is a clinical syndrome which is caused due to the deficiency of thyroid hormones, resulting in a generalized slowing down of the metabolic process. The incidence of hypothyroidism varies, depending on geographical and the environmental factors such as dietary iodide, goitrogen intake, the genetic characteristic of the population and the age distribution of the population.

Sub-clinical hypothyroidism, the most prevalent form of thyroid diseases, is more common in females and in the elderly, reaching a prevalence of up to 20% in women over 60 years old (3). This increased prevalence in the elderly was questioned by Surks *et al.* (4) whose re-analysis of the NHANES data revealed that TSH serum values might be shifted toward higher levels with increasing age. Autoimmunity to thyroid antigens is the most common cause of thyroid diseases including Hashimoto's thyroiditis (HT), Graves' disease (GD), and primary myxedema (PM) (5). Autoimmune thyropathies are chronic inflammatory thyroid diseases that are caused by dysregulation of specific immune defences (B-cells and T-cells) (6). Prevalence rate of autoimmune mediated

hypothyroidism is about 0.8 per 100 and 95% among them are women (7). Moreover, autoimmune thyroid disease (AITD) prevalence increases with advancing age, with more than 10% of subjects over 75 yr of age having biochemical evidence of mild (subclinical) hypothyroidism, the majority of which is due to autoimmune disease (3, 8). Autoimmune thyroid diseases are characterized by the presence of autoantibodies to multiple thyroid antigens (9) including thyroglobulin (TG), thyroid peroxidase (TPO), and the thyroid stimulating hormone receptor (TR). They result from interactions between genetic and environmental factors.

Diabetes and thyroid disorders have been shown to mutually influence each other and associations between both conditions have been reported (10, 11). A large number of studies have evidenced an array of complex interwining biochemical, genetic and hormonal malfunctions mirroring this pathophysiological association (12, 13). TH tend to regulate carbohydrate metabolism and pancreatic function whereas diabetes affects thyroid function tests to variable extents. Hypothyroidism (Hashimoto's thyroiditis) or thyroid over activity (Graves' disease) has been investigated to be associated with diabetes mellitus. A meta-analysis reported a frequency of 11% thyroid dysfunction in the patients of diabetes mellitus (14). Autoimmunity has been implicated to be the major cause of thyroid-dysfunction associated diabetes mellitus (15-17). Subclinical thyroid diseases should be considered when evaluating patients with autoimmune diseases (18). Hence the study was designed to investigate the prevalence of anti TPO and anti Tg in newly diagnosed Subclinical hypothyroid patients in relation to type 2 Diabetes.

2. Materials and Methods

Patients between the age group of 25 yrs to 65 yrs with the mean age of 48yrs were selected. Clearance was obtained from Institute Ethical Committee (IEC NO:0169/S/2014) prior to the commencement of study .The patients were screened for Diabetes and Thyroid diseases by assessing their fasting glucose and fT3, fT4, TSH .Glucose was determined by Glucose Oxidase peroxidase method and Thyroid parameters were analyzed by Chemiluminescence method. Patients with normal levels of fT3 and fT4 but raised TSH (above 5.5 μ IU/ml) and with fasting glucose values more than 120 mg/dl, were categorized into Sub clinical Hypothyroid diabetic and those patients with normal fasting glucose values and normal fT3 and fT4 and raised TSH as sub clinical hypothyroid patients without diabetes. HbA1C was checked in patients whose Fasting blood sugar was more than 120 mg/dl .These patients were then screened for antithyroid antibodies (anti-TPO and anti-Tg) using Biochip technology (Euroimmun ,Germany). Controls were run simultaneously.

The third category of patients ie Subclinical hypothyroid with diabetes consisted of around 60 patients with 32 female and 28 males while the second category Subclinical hypothyroid patients without diabetes consisted of 60 patients with 30 females and 20 males and the controls consisted of 50 patients with 25 male and 25 females.

Serum samples were diluted in 1:10 dilution with Phosphate buffer saline and Tween20 and vortexed. Samples were applied to the reaction fields of the reagent tray. The biochip slides consist of a substrate combination of Thyroid gland (monkey) and Kidney (rat). As the fluid was confined to a closed space, there was no need to use a conventional "humidity chamber". The samples which showed characteristic pattern with thyroid gland and negative for rat kidney were considered positive for antithyroid antibodies. 25 μ l of diluted sample was applied to each reaction field of the reagent tray without any air bubbles. Then the Biochip slide was fitted into the corresponding recesses of the reagent tray. The samples were incubated at room temperature for 30 minutes. Then the Biochips were rinsed with a flush of Phosphate buffer saline (PBS)-tween using a beaker & then it was immersed in a cuvette containing PBS-tween for 5 minutes.

20 μ l of fluorescein-labelled anti-human globulin was added to each reaction field of a clean reagent tray. Then Biochip slide was removed from the cuvette & within five seconds the back and the long sides of the Biochip slide was blotted with a paper towel and immediately the Biochip slide was put into the recesses of the reagent tray. Again it was incubated at room temperature for 30 minutes. Then again the Biochip slides were rinsed with a flush of PBS-tween using a beaker & then it was immersed in a cuvettes containing PBS-tween for 5 minutes. 10 μ l embedding medium (Glycerol) was placed on to a cover glass. Then biochip was removed from the cuvette & it was dried & the biochip facing downwards

was put on to the cover glass. Then the fluorescence was read with the microscope in objective 20 \times and then in objective 40 \times .

3. Results

The present study on the prevalence of auto antibodies in Newly diagnosed subclinical Hypothyroid patients in relation to Diabetes reveals that 60% of hypothyroid patient with diabetes showed presence of autoimmune thyroid disorder of which 86% of the positive cases had autoantibodies against TPO and rest were against Tg, but 48% of hypothyroid patients without Diabetes also showed presence of autoimmunity against thyroid with 90% against TPO and 7% against Tg and 3% also showed mixed pattern with anti- TPO and anti -Tg (Tables 1 and 4).

Table 1: Prevalence of autoimmune thyroid disease in different categories

Sr. No.	Category	% of Positive Cases	% of Positive Male	% of Positive Female
1	I	8	25	75
2	II	48	17	83
3	III	60	20	80

Notes: I –Normal controls; II – Subclinical hypothyroid without diabetes; and III – Subclinical hypothyroid with diabetes

The study of healthy patient i.e. non diabetic, euthyroid patient revealed 8% positive for autoimmune thyroidism of which 75% were cases of anti-TPO and 25% showed a mixed pattern of anti- TPO and anti-Tg.

This study was carried out to correlate the prevalence of thyroid auto antibodies in patients with newly diagnosed subclinical hypothyroid patients with diabetes and without diabetes. The study was also analyzed taking gender of patients into account. In the first category of patients i.e. subclinical hypothyroid diabetic patients,80% of female patients showed the presence of autoimmune thyroid disease and 20% of males showed the presence of autoimmune thyroid disease with 86% prevalence of anti-TPO in females the rest for Tg but in male patients 100% had anti-TPO with no cases of anti-Tg. In the second category, i.e. Subclinical hypothyroid non diabetic patients ,83% female patients showed auto immune thyroid disease with 90% prevalence of anti-TPO and rest for anti-Tg. Whereas only 17% male patients showed the presence of auto antibodies with 80% for TPO and 20% for mixed pattern. In normal healthy individuals, only 8% of the population showed the presence of antithyroid antibodies, of which 75% were female and 25% were male with 67% prevalence for anti-TPO in females and 33% with mixed pattern for anti-TPO and anti-Tg, whereas only positive male showed only anti- TPO antibodies (Tables 2-4 and Figure 1).

Table 2: Prevalence of thyroid autoantibodies (anti-TPO and anti-Tg) in autoimmune thyroid male patients

Sr. No.	Category	% of TPO	% of Tg	% of Mixed
1	I	100	-	-
2	II	80	-	20
3	III	100	-	-

Notes: I –Normal controls; II – Subclinical hypothyroid without diabetes; and III – Subclinical hypothyroid with diabetes

Table 3: Prevalence of thyroid autoantibodies (TPO and Tg) in autoimmune thyroid female patients

Sr. No.	Category	% of TPO	% of Tg	% of Mixed
1	I	83	17	-
2	II	92	8	-
3	III	67	-	33.3

Notes: I –Normal controls; II – Subclinical hypothyroid without diabetes; and III – Subclinical hypothyroid with diabetes

Table 4: Prevalence of thyroid autoantibodies (TPO and Tg) in autoimmune thyroid patients.

Sr. No.	Category	% of TPO	% of Tg	% of Mixed
1	I	75	-	25
2	II	90	7	3
3	III	86	14	-

Notes: I – Normal controls. II – Subclinical hypothyroid without diabetes; and III – Subclinical hypothyroid with diabetes;

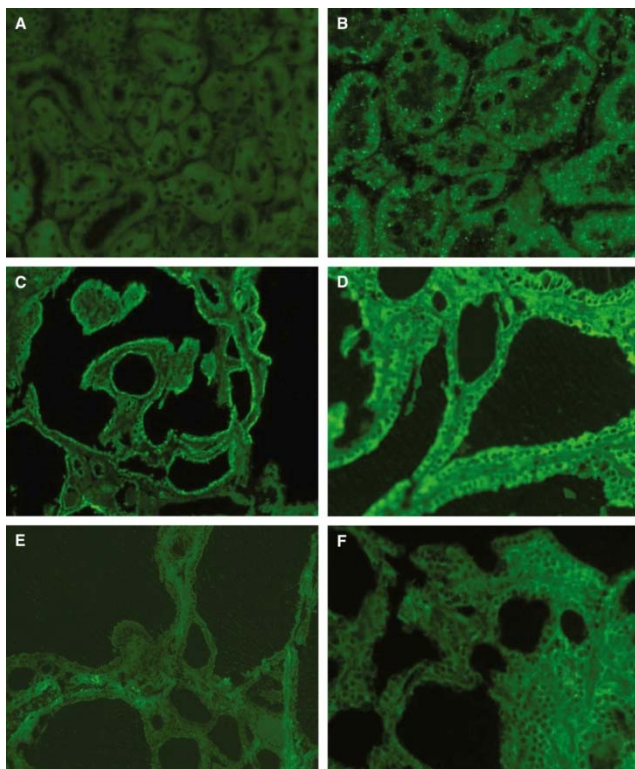


Figure 1: A. Negative - AMA (anti mitochondrial antibody), B. Positive -AMA, C. Positive anti -TPO, D. Mixed Pattern (anti- TPO, anti-Tg), E. Positive anti-Tg, F. Negative Thyroid Control.

4. Discussion

Thyroid disorders are common endocrine disorder with variable prevalence. The prevalence of subclinical hypothyroidism is reported to be 4 to 8.5% and may be as high as 20% in women older than 60 yrs (19). Thyroid autoantibodies are the markers of autoimmunity in AITD. The presence of thyroid autoantibodies contributes to the pathogenesis of a number of thyroid disorders, such as Hashimoto's thyroiditis, primary myxoedema, Graves' disease, as well as thyroid cancers (20). Moreover studies by Bryhni et al., 1972 suggest that thyroid autoantibodies are found more frequently in females and prevalence increases with age (21). Studies by Feely et al., 1979 show that diabetes and thyroid disorders mutually influence each other, thyroid hormones regulate carbohydrate metabolism and pancreatic function while diabetes alters thyroid functions. Many reports documented a higher than normal prevalence of thyroid dysfunction in the diabetic patients.

Perros et al., 1995 demonstrated an overall prevalence of 13.4% thyroid diseases in diabetics with the highest prevalence in type 1 female diabetics (31.4%) and lowest prevalence in type 2 male diabetics (6.9%). Studies on Greek type 2 diabetic patient revealed 12.3% of patients had thyroid dysfunction and a similar study in Saudi patient revealed 16% patient with thyroid dysfunction (22). Radaideh et al., 2004 reported that thyroid dysfunction was present in 12.5% of type 2 diabetic patients (T2DM).(23)

The above studies reveal a strong link between diabetes and thyroid disorders. In our study, an attempt was made to investigate the possible link between autoimmune thyroid diseases in diabetic patients with sub clinical hypothyroidism. Our study suggests that type 2 diabetes does not have a significant role in producing antithyroid antibodies as both categories of patients showed the presence of TPO and Tg antibodies and the prevalence of TPO antibodies was higher than Tg in both the cases. 60 subclinical hypothyroid diabetic patients were studied of which 36 showed positive for antithyroid antibodies of which 80% were females and 20% were males. Among the reported autoimmune thyroid patients, auto antibodies against TPO was more (86%) compared to that of Tg (14%) these reports were coherent with earlier reports by Bryhni et al., 1996 and Nystrom et al., 1996 (24,25). Our results also indicate that all autoimmune thyroid males showed 100% prevalence to antibodies against TPO. Whereas females showed 83% against TPO and 17% against Tg. Kotani 1998 reported that anti-thyroid peroxidase antibody is important in diagnosing autoimmune thyroid disease and for judging treatment efficacy (26). Moreover reports by Yoshida et al., 1978 suggest that serum anti-TPO antibody concentrations are positively correlated with the activity of chronic autoimmune thyroiditis (27). Mariotti, S. et al 1987 also has reported that more patients with thyroiditis have high serum anti-TPO than anti-Tg antibody concentrations (28). In the second category of Subclinical hypothyroid patients without diabetes, 48% showed the presence of autoimmune thyroid disease with 90% showing antibodies

against TPO and 7% against Tg and 3% had mixed pattern with 80% prevalence in females and just 20% in males. A similar observation with anti TPO, 4-5 times more was reported by Bjoro *et al.*, 2000 in a health survey in Norway, in which the prevalence of pathological anti-TPO was 13.9% in females and 2.8% in males (29). This is similar to the study done by Swain *et al.*, 2005, which showed that the prevalence of anti-TPO is about 7 times more in females than in males. Our study shows that subclinical hypothyroid patients with or without diabetes exhibit presence of antithyroid antibodies, which reveals that diabetes is not an important criteria for the production of antithyroid antibodies. The study shows that only 12% increase in autoantibodies against thyroid antigens when diabetic hypothyroid patients are compared to non-diabetic hypothyroid patients. Normal healthy individuals showed 8% positive for antithyroid antibodies with more prevalence in females than males. Marcocci *et al.*, 2000 has reported that anti-TPO is also found in sera of about 10% of normal adults, with an increasing prevalence (up to 30%) in older adults (30). The prevalence of antithyroid antibodies among patients with thyroid disorders in the current study was greater than in the non-thyroid group and they are in close agreement with previous reports of Benvenga *et al.*, 1987 and Benvenga *et al.* 1997(31,32). When gender specific prevalence of thyroid problems in total population were compared, it showed that, thyroid autoimmunity were more common in females than in males irrespective of the fact that they are diabetes or nondiabetic similar to the report by Usha Menon 2011(33). The presence of autoantibodies against thyroid antigens in normal individuals suggests that hypothyroidism cannot be concluded as the only triggering factor for the production of antithyroid antibodies. In all the categories the presence of antithyroid antibodies is much higher in female population as reported by Hollowell *et al.*, (2002) and Canaris *et al.*, (2000) (34)

5. Conclusion

Diabetes and thyroid disorders are two most common endocrine disorders in adult population worldwide. The relationship between diabetes and thyroid is complex and interdependent. The present study reveals that autoimmune thyroid disorder is more prevalent in females like other autoimmune disorder. It is also noted that irrespective of diabetic or non-diabetic, hypothyroid patients were more prone to autoimmune thyroid disorder especially with the dominance of anti -TPO diagnostic marker. The occurrence of autoantibodies is multi factorial. In most autoimmune diseases, however, it has not been determined whether autoantibodies cause or contribute to disease or are merely a secondary consequence of the underlying clinical condition. In order to have a clear idea it is important to extend the same to a bigger population and also consider the contribution of anti-TRABs as well.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Author Profile

K. G. Sunitha Ph.D Research Scholar, Research & development centre, Bharathiar University, Coimbatore.

Dr. E. Padmini received M.Sc., M. Phil., Ph.D. in Biochemistry from University of Madras in 1983, 1984, 1988, respectively and awarded the Post Doctoral Research Associate (D.Sc FABMS) from the Department of Pharmacology, University of Tennessee, USA in the year 1993. In addition to this, completed PG Diploma in Bio-Informatics in the year 2004. Currently working as faculty in the Department of Biochemistry, Bharathi Women's College, Chennai- 600 108, Tamilnadu, India since 1988